

**FORMULARIO UNICO DE SOLICITUD DE PATENTE
PCT
ENTRADA EN FASE NACIONAL**

① **SOLICITUD DE:**
☒ Patente de Invención ☐ Patente de Modelo de Utilidad

☐ Capítulo I. ☒ Capítulo II.

② **SOLICITANTE
(71)**

Nombre: WYETH
Dirección: Five Giralda Farms
Madison, New Jersey 07940
Nacionalidad o Domicilio: Madison,
New Jersey
Lugar de Constitución: Estado de Delaware,
Estados Unidos de América
Teléfono: 3284270 Fax: 6089701
E-mail: jllcco@lloredacamacho.com

IDENTIFICACION

C.C. ☐ NIT ☐
C.E. ☐ Otro ☐

Cual _____

Número _____

③ **REPRESENTANTE
O APODERADO**

Nombre: JOSE ANTONIO LLOREDA
Dirección: Calle 72 No. 5-83 Piso 5o.
Teléfono: 3284270 Fax: 6089701
E-mail: jllcco@lloredacamacho.com

C.C. ☒ NIT ☐
C.E. ☐ Otro ☐
Cual _____

Número 17.159.881 TP 10.784

④ **INVENTOR(ES)
(72)**

Nombre: PICKAR, James, H.
Dirección: 816 Crum Creek Road, Springfield, PA 19084
Nacionalidad o Domicilio: ESTADOS UNIDOS DE AMERICA

⑤ **PCT (86)**

Solicitud Internacional No. PCT/US01/40302

Fecha: Marzo 16, 2001

Publicación Internacional No. WO 01/70208

Fecha: Septiembre 27, 2001

⑥ **Título (54) "TERAPIA DE REEMPLAZO DE HORMONAS EMPLEANDO UNA
COMBINACION DE ESTROGENOS CONJUGADOS Y ACETATO DE MEDROXIPROGESTERONA "**

⑦ **Clasificación Internacional (51) A61K31/57**

⑧ **Prioridad**

(33) País de Origen

(32) Fecha

(31) Número de Solicitud

SI ☒ NO ☐

US
US

Marzo 20, 2000
Febrero 14, 2001

60/190,630
60/268,607

⑨ **Comprobante de Pago No. 02-57,011 Fecha: Agosto 28, 2002**

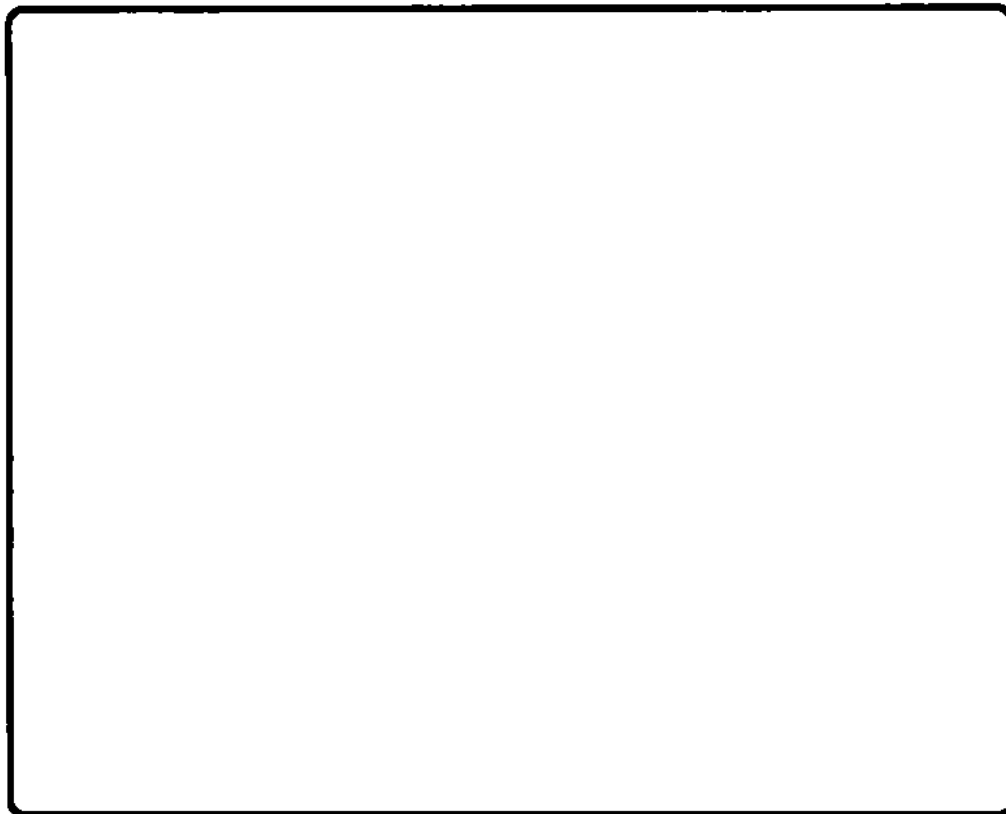
10

ANEXOS

- ☒ Comprobante de pago de la tasa de presentación de la solicitud
- ☒ Documento que acredite la existencia y representación legal cuando el solicitante sea persona jurídica
- ☒ Documento de Representación Legal
- ☐ Documento de cesion del inventor al solicitante o a su causante
- ☐ Declaraciones
- ☐ Copia de la solicitud internacional (Resumen descripción reivindicaciones dibujos)
- ☒ Traducción de la solicitud internacional al castellano (Resumen descripción reivindicaciones dibujos)
- ☒ Copia del examen preliminar internacional efectuado por la Autoridad Internacional de Examen Preliminar (IPEA)
- ☒ Traducción del examen preliminar internacional efectuado por la IPEA
- ☐ De ser el caso copia del contrato de acceso
- ☐ De ser el caso documento que acredite la licencia o autorización de uso de conocimientos tradicionales de las comunidades indígenas
- ☐ Arte Final 12X12
- ☐ De ser el caso información sobre otras solicitudes de patente o títulos obtenidos en el extranjero por el mismo titular o su causante relacionados parcial o totalmente con la invención de esta solicitud
- ☐ De ser el caso modificaciones efectuadas a la solicitud internacional
- ☒ Otros Informe Búsqueda Preliminar Publicación con sus traducciones Dibujos solicitud de cambio de nombre del solicitante bajo la Regla 92 bis del PCT Notificación de un cambio y Dos documentos de traspaso junto con las traducciones oficiales correspondientes

11

FIGURA CARACTERISTICA



12

Solicito la concesión de la patente

NOMBRE

JOSE ANTONIO LLOREDA

FIRMA

CC 17 189 881 88 TP 10 784



Industria y Comercio

SUPERINTENDENCIA

NTT 000176089 2

SUPERINTENDENCIA Y COMERCIO

Radicación: 00000000000000000000 Folios: 113
 Fecha (MM): 2002-09 10 1 3 22
 Trámite: 011 PCT PATENT 379 FASE INCI 411 PRESENTAC
 Dependencia: 2020 DIVISION DE NUEVOS CREACIONES

RECIBO OFICIAL DE CAJA 02 57 011
 FECHA AGOSTO 28 DE 2002

***** CONSIGNACION *****

DEPOSITANTE	TIPO PAGO	BANCO	CUENTA	Nº PAGO	FECHA PAGO	Vr PAGO
LLORENA Y CIA S A	CONSIGNACION	BANCO POPULAR	050 00110 6	2404993	28/08/2002	2,736 900 00

***** CONCEPTO *****

CANT	IDENTIFIC	CONCEPTO	TOTAL CONCEPTO
1	50005 01 01	SOLICITUDES	
		1 TRAMITES DE SOL DE PATENTE DE IN	370 000 00
		TOTAL	370 000 00

SON TRESCIENTOS SETENTA Y OCHO MIL PESOS

RESPONSABLE

RECIBO DE CAJA APLICADO AL EXPEDIENTE Nº

Carrera 13 No 27 00 Pisos 5 7 y 10
 Conmutador 382 0840
 Fax 350 5220
 E mail info@sic.gov.co
 www.sic.gov.co
 Bogotá D C Colombia

3

ANEXO

El documento que acredita la calidad de REPRESENTANTES LEGALES en Bogota de los doctores JOSE ANTONIO LLOREDA y/o GUSTAVO TAMAYO y/o ANGELA MARIA RESTREPO y/o ALICIA LLOREDA RICAURTE para TODOS LOS ASUNTOS RELACIONADOS CON LA PROPIEDAD INDUSTRIAL de la sociedad peticionaria que faculta a cada uno de dichos abogados para actuar individualmente se esta presentando con esta solicitud En consecuencia ruego a usted reconocer la personeria de cada uno de dichos Representantes Legales para actuar dentro del presente expediente a fin de que cualquiera de ellos pueda intervenir en cualquier tiempo respecto de la solicitud en el contenida



JOSE ANTONIO LLOREDA

T P No 10 784

Codigo 231

TRADUCCION OFICIAL No. 2002-07-03-411
DE UN DOCUMENTO PRESENTADO EN INGLES

PODER

Legalización de un Poder de Representación Legal conferido a: JOSE ANTONIO LLOREDA, y/o GUSTAVO TAMAYO, y/o ANGELA MARIA RESTREPO, y/o ALICIA LLOREDA, por WYETH.

Documento escrito simultáneamente en inglés y en español, dado y firmado este día 7 de junio de 2002.

(firma: ilegible)

William H. Calnan; Subsecretario

Certificado Notarial (corporación), documento también escrito en inglés y en español, firma ilegible por Michelle L. Kalbfell.

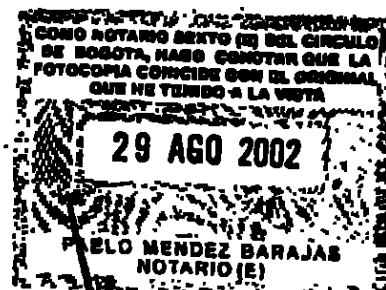
Hay un sello Notarial que dice:

MICHELLE L. KALBFELL

Notario Público, Estado de New Jersey

No. 2108903

La Comisión Expira el 25 de octubre de 2005



A large, stylized handwritten mark, possibly a signature or initials, written in black ink. It consists of a large, sweeping 'N' shape with a horizontal bar at the top.

APOSTILLA

(Convención de La Haya del 5 de octubre de 1961)

- 1. País: ESTADOS UNIDOS DE AMERICA
- 2. Este documento público ha sido firmado por:
MICHELLE L. KALBFELL
- 3. actuando como:
NOTARIO PÚBLICO DE NEW JERSEY
- 4. lleva el sello/estampilla de
MICHELLE L. KALBFELL, NOTARIO

CERTIFICADO

- 5. en TRENTON, NEW JERSEY
 - 6. el día 10 de junio de 2002
 - 7. por: John E McCormac, CPA; TESORERO DE NEW JERSEY
 - 8. NO. A152470
 - 9. Sello/Estampilla
 - 10. Firma: (ilegible)
- Hay un sello dorado.

MINISTERIO DE RELACIONES
EXTERIORES
4 18911
CIVIL FIRMAS APARECE EN EL
DOCUMENTO DESAPARECE
LAS FUNCIONES INDICADAS

NO SE ASUME
RESPONSABILIDAD DEL TEXTO
2002 JUN -4 P 12:01
JEFE DE LEGALIZACIONES
SANTAFE DE BOGOTA D.C.

Traducido a mi mejor saber y entender, este día 3 de julio de 2002, en la ciudad de Santafé de Bogotá, por Anita Escobar de Tamayo, Traductora Oficial, Res. 2177 de 1989, Ministerio de Justicia, República de Colombia.

TRADUCTOR OFICIAL
RESOLUCION 2177
Anita Escobar de Tamayo
ANITA ESCOBAR DE TAMAYO

COPIA NOTARIAL (DE EL CIRCULO)
DE BOGOTA, HAGO CONSTAR QUE LA
FOTOCOPIA CONCORDA CON EL ORIGINAL
QUE NO FUE A LA VISTA

29 AGO 2002

PAULO BERNAL
NOTARIO

APOSTILLE

(CONVENTION DE LA HAYE DU 5 OCTOBRE 1961)

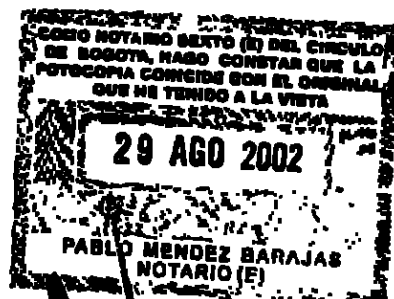
1. COUNTRY: UNITED STATES OF AMERICA
2. THIS PUBLIC DOCUMENT HAS BEEN SIGNED BY:
MICHELLE L KALBFELL
3. ACTING IN THE CAPACITY OF:
NOTARY PUBLIC OF NEW JERSEY
4. BEARS THE SEAL/STAMP OF:
MICHELLE L KALBFELL, NOTARY

CERTIFIED

5. AT TRENTON, NEW JERSEY
6. THE 10TH DAY OF JUNE 2002
7. BY: John E McCormac, CPA, NEW JERSEY TREASURER
8. NO. A152470
9. SEAL/STAMP:
10. SIGNATURE



John E McCormac



Los abajo firmados,

Wyeth
Organizada y Existente de Conformidad
con las leyes del Estado de Delaware,
Estados Unidos de América

con domicilio en

Five Giralda Farms
Madison, New Jersey 07940
Estados Unidos de América

nombramos por el presente a JOSE ANTONIO LLOREDA y/o GUSTAVO TAMAYO y/o ANGELA MARIA RESTREPO y/o ALICIA LLOREDA, domiciliados en Bogotá, Colombia, con oficinas en dicha ciudad, Calle 72 No. 5-83, piso 6º., nuestros representantes legales y verdaderos en Bogotá, con facultades para recibir notificaciones y nombrar apoderados judiciales o extrajudiciales, con el fin de dar cumplimiento al parágrafo 1o. del artículo 543 del decreto 410 de 1971.

Los doctores JOSE ANTONIO LLOREDA y/o GUSTAVO TAMAYO y/o ANGELA MARIA RESTREPO y/o ALICIA LLOREDA, o los apoderados que cualquiera de ellos designe, quedan especialmente facultados para representarnos ante las autoridades colombianas, administrativas, judiciales o de policía, en todos los trámites relativos a la propiedad industrial, a cuyo efecto les facultamos para dar ante dichas autoridades todos los pasos necesarios al objeto indicado; elevar solicitudes, declaraciones, reclamos y recursos; formular descripciones, enmiendas, oposiciones y apelaciones; instaurar cancelaciones y amparos administrativos; abonar todos los impuestos y cuotas y efectuar todo otro pago determinado por la ley; recibir todos los documentos y valores, dando el descargo respectivo; llenar cualesquiera otros requisitos y tomar, en fin, todas las medidas que creyeren conducentes al resguardo de nuestros intereses; y en caso de producirse oposición, pasando los antecedentes a los tribunales, quedan facultados para tomar intervención como demandantes o demandados ante los jueces que sean competentes, pudiendo transar, someter a árbitros, desistirse, percibir y apelar, con todas las demás facultades que resulten necesarias, y por el presente declaramos desde ahora válido y bueno cuanto dichos representantes y/o los apoderados que cualquiera de ellos nombre hicieren en nuestro beneficio.

Asimismo, facultamos a los doctores JOSE ANTONIO LLOREDA y/o GUSTAVO TAMAYO y/o ANGELA MARIA RESTREPO y/o ALICIA LLOREDA, para solicitar en la Oficina de Propiedad Industrial la protocolización de este documento y de cualquier poder que cualquiera de ellos otorgue a nombre nuestro.

Dado y firmado

William H. Calnan; Secretario Asistente

The undersigned,

Wyeth
Five Giralda Farms
Madison, New Jersey 07940
United States of America

of

Organized and existing under
the laws of Delaware, United
States of America.

hereby appoint JOSE ANTONIO LLOREDA and/or GUSTAVO TAMAYO and/or ANGELA MARIA RESTREPO and/or ALICIA LLOREDA, of Bogotá, Colombia, with offices in said town, Calle 72 No. 5-83, Sixth Floor, our true and legal representatives in Bogotá, with power to receive notifications and to appoint judicial or extrajudicial agents, so as to comply with paragraph 1o. of article 543 of decree 410 of 1971.

Doctors JOSE ANTONIO LLOREDA and/or GUSTAVO TAMAYO and/or ANGELA MARIA RESTREPO and/or ALICIA LLOREDA, or the agents by any one of them appointed, are especially empowered to represent us before the administrative, judicial or police authorities of Colombia, in all proceedings pertaining to industrial property, for which purpose we authorize them to take before the said authorities all the necessary steps for the said end; to file petitions, declarations, claims, recourses, cancellations and administrative injunction proceedings; to draw up specifications; to file amendments, oppositions and appeals, to pay all installments and effect all other payments prescribed by law; to receive proper receipts, to comply with all other requirements and, in short, to take all those measures which they may deem fit for the protection of our interests; and in case of opposition, where the proceedings be passed over to the courts, we authorize said agents to act on our behalf, as complainants as well as defendants, before the competent judges, with full powers to make arrangements, to submit to arbitrators, to desist, to collect, to appeal and, in short, with all such further powers as may be required, and we hereby declare from now on ~~as our true and legal representatives~~ said representatives and/or the agents appointed by any one of them appointed may do for our benefit.

29 AGO 2002

We also authorize doctors JOSE ANTONIO LLOREDA and/or GUSTAVO TAMAYO and/or ANGELA MARIA RESTREPO and/or ALICIA LLOREDA, to apply for protocolization of this document and of any power of attorney by any one of them conferred in our name, before the Office of Industrial Property.

Given and signed this 7th day of June 2002


William H. Calnan; Assistant Secretary
NOTARY SHOULD EXECUTE OTHER SIDE

CERTIFICADO NOTARIAL (INDIVIDUO)

Estados Unidos de América
Estado de {ss
Condado de

A los días del mes de de 20
compareció personalmente ante mí, Notario
Público en y por el condado antes citado,

a quien doy fe de conocer y me consta
que es/son la persona descrita y firmante del
documento que precede, declarando ante mí que
otorga el mismo para los fines y propósitos que en él
se expresan.

Notary Public (Notario Público).

NOTARIAL CERTIFICATE (INDIVIDUAL)

United States of America
State of {ss
County of

On this day of , 20 , personally
appeared before me, a Notary Public, in and for the
above mentioned county,

to me known to be the person described in and
who executed the foregoing document, and who duly
acknowledged to me that executed same for the
uses and purposes therein expressed.

CERTIFICADO NOTARIAL (INDIVIDUO)

Estados Unidos de América
Estado de {ss
Condado de

A los días del mes de de 20
compareció personalmente ante
mí, Notario Público en y por el condado antes citado,

a quien doy fe de conocer y me consta
que es/son la persona descrita y firmante del
documento que precede, declarando ante mí que
otorga el mismo para los fines y propósitos que en él
se expresan.

Notary Public (Notario Público).

NOTARIAL CERTIFICATE (INDIVIDUAL)

United States of America
State of {ss
County of

On this day of , 20 , personally
appeared before me, a Notary Public, in and for the
above mentioned county,

to me known to be the person described in and who
executed the foregoing document, and who duly
acknowledged to me that executed same for the
uses and purposes therein expressed.

CERTIFICADO NOTARIAL (SOCIEDAD)

Estados Unidos de América
Estado de New Jersey {ss
Condado de Morris

Hoy 7 de Junio de 2002, compareció
William H. Calnan, a quien conozco y
de quien me consta que firmó el anterior documento,
y quien debidamente juramentado declaró y dijo que
éles Secretario Asistente

de Wyeth
la compañía nombrada en
el documento que antecede, una sociedad
debidamente organizada y existente conforme a las
leyes del Estado de Delaware, Estados
Unidos de América, domiciliada en la ciudad de
Madison, Condado de Morris,
Estado de New Jersey, Estados Unidos de
América, legalmente constituida para un período de
duración que aún no ha terminado, que ejerce
legalmente su objeto conforme a las leyes del Estado
de Delaware, Estados Unidos de América; que el
declarante está autorizado por dicha Compañía
para conferir este poder, que el sello estampado por
él al pie de tal poder, en nombre y en representación
de la expresada Compañía, es el sello oficial de ella;
y que el objeto para el cual se confiere este
instrumento está comprendido dentro del de la
sociedad.

El suscrito Notario ha visto los Estatutos de
incorporación firmados en Madison,

Estado de New Jersey, con fecha Abril 25, 2002,
y certifica que de conformidad con la legislación de
dicho Estado la anterior declaración juramentada es
prueba suficiente de que los hechos sobre que ella
versa son ciertos o verdaderos, de lo cual da fe.

Notary Public (Notario Público).

NOTARIAL CERTIFICATE (CORPORATION)

United States of America
State of New Jersey {ss
County of Morris

On this 7th day of June, 2002,
personally

came William H. Calnan
to me known, and known to me to be the person who
signed the foregoing instrument, who being by me
duly sworn did depose and say that he is the
Assistant Secretary of Wyeth

, the company named
within, a Corporation organized and existing under
the laws of the State of Delaware, United States
of America, domiciled at the City of Madison,
County of Morris, State of New Jersey,
United States of America, legally constituted for a
period of tenure not yet expired, and who exercises its
corporate purposes according to the laws of the State
of Delaware, United States of America; that the
deponent is authorized by said Corporation to confer
this power of attorney, that the seal affixed hereunto
by the deponent in the name and on behalf of said
Corporation is the corporate seal thereof, and that the
purpose for which this instrument is granted is
included within the corporate purposes of the
Company.

The undersigned Notary Public has seen the articles
of Incorporation of said Corporation executed at
Madison, State of New Jersey, under date of
April 25, 2002, and hereby
certifies that under the laws of said State the
foregoing acknowledgment is sufficient proof that the
facts therein contained are true, which he attests.

Michelle L. Kalbfell
MICHELLE L. KALBFELL
NOTARY PUBLIC, State of New Jersey
No. 2108903
Qualified in Morris County
Commission Expires Oct. 25, 2005

TRATADO DE COOPERACION DE PATENTES PCT REPORTE DE BUSQUEDA INTERNACIONAL

(PCT Artículo 18 y Reglas 43 y 44)

Registro de referencia del solicitante o agente AM10226	PARA FUTURAS ACCIONES ver Notificación o Transmisión del Reporte de Búsqueda Internacional (Forma PCT/ISA/220) así como, donde aplique, el ítem 5 a continuación.	
Solicitud internacional No. PCT/US 01/40302	Fecha de registro internacional (día/mes/año) 16/03/2001	Fecha de Prioridad (Primera) (día/mes/año) 20/03/2000
Solicitante AMERICAN HOME PRODUCTS CORPORATION		

Este Reporte de Búsqueda Internacional ha sido preparado por esta Autoridad de Búsqueda Internacional y es transmitido al solicitante Según el Artículo 18. Una copia será transmitida a la Oficina Internacional.

Este Reporte de Búsqueda Internacional consta de un total de 3 hojas.

☒ También está acompañado de una copia de cada documento técnico anterior citado en este reporte.

1. Bases del reporte

a. Respecto al lenguaje, la búsqueda internacional se llevó a cabo según la solicitud internacional en el lenguaje en el cual se presentó, a menos que se indique de otro modo en este ítem.

☐ la búsqueda internacional se llevó a cabo según la traducción de la solicitud internacional proporcionada a esta Autoridad (Regla 23.1(b)).

b. Respecto a una secuencia de ácido nucleótico y/o amino revelado en la solicitud internacional, la búsqueda internacional se llevó a cabo según la lista de la secuencia:

☐ contenido en la solicitud internacional en forma escrita.

☐ presentada junto con la solicitud internacional en forma legible por computador.

☐ proporcionada subsiguientemente a esta Autoridad en forma escrita.

☐ proporcionada subsiguientemente a esta Autoridad en forma legible por computador.

☐ se ha proporcionado la afirmación de que el listado de secuencias escrito proporcionado subsiguientemente no va más allá de la revelación en la solicitud internacional según se presentó.

☐ se ha proporcionado la afirmación de que la información registrada en forma legible por computador es idéntica al listado de la secuencia escrita.

2. ☐ Ciertas reivindicaciones no pudieron localizarse (Ver Casilla I).

3. ☐ Falta unidad de invención (ver Casilla II).

4. Respecto al título,

☐ El texto es aprobado según lo presenta el solicitante.

☒ Esta Autoridad ha establecido que el texto se lee así:

TERAPIA DE REEMPLAZO DE HORMONAS EMPLANDO UNA COMBINACION DE ESTROGENOS CONJUGADOS Y ACETATO DE MEDROXIPROGESTERONA

5. Respecto al resumen,

☒ El texto es aprobado según lo presenta el solicitante.

☐ Esta Autoridad ha establecido el texto de acuerdo con la Norma 38.2(b), según aparece en el Recuadro III. El solicitante puede, un mes después de la fecha de haber enviado por correo este reporte de búsqueda internacional, presentar comentarios a esta Autoridad.

6. La figura de los dibujos que se publicará con el resumen es la Figura No.

☐ como lo sugirió el solicitante.

☐ porque el solicitante no sugirió una figura.

☐ porque esta figura caracteriza mejor a la invención.

☒ Ninguna de las figuras.

REPORTE DE BUSQUEDA INTERNACIONAL

Solicitud Internacional No.
PCT/US 01/40302

A. CLASIFICACION DE MATERIA IPC 7 A61K 31/57 A61P5/30 //(A61K31/57,31:565) Según la Clasificación de Patentes Internacional (CPI) o según la clasificación nacional y la CPI		
B. CAMPOS BUSCADOS Documentación mínima buscada (sistema de clasificación seguido de símbolos de clasificación) IPC 7 A61K Documentación buscada fuera de documentación mínima hasta donde dichos documentos se incluyan en los campos buscados Base de datos electrónica consultada durante la búsqueda internacional (nombre de la base de datos y, donde sea práctico, buscar términos usados) EPO-Internal, PAJ, WPI Data, BIOSIS, CHEM ABS Data, EMBASE		
C. DOCUMENTOS CONSIDERADOS RELEVANTES		
Categoría *	Citación de documento, con indicación, donde sea apropiado, de los textos relevantes	Relevante a la reivindicación No.
X	EP 0 309 263 A (JENCAP RESEARCH) Marzo 29, 1989 (1989-03-29) reivindicaciones 1,2,5,9,10,13 columna 3, línea 59 -columna 4, línea 32 columna 5, línea 28-48 columna 11, línea 35-37	1-4,6,7, 9,11-23, 25,26, 40-43, 45,46, 48,50-56

☒ En el recuadro C se enumeran más documentos.		☒ En el anexo se enumeran miembros de la familia de patentes.	
* Categorías especiales de documentos citados: 'A' documento que define el estado general de la técnica el cual no se considera de relevancia particular 'E' documento anterior pero publicado en o después de la fecha de presentación internacional 'L' documento que puede arrojar dudas sobre reivindicaciones de prioridad o que se cita para establecer la fecha de publicación de otra citación u otra razón especial (según se especifica) 'O' documento que se refiere a una revelación oral, uso, exhibición u otros medios 'P' documento publicado antes de la fecha de presentación internacional pero después de la fecha de prioridad reivindicada		'T' documento posterior publicado después de la fecha de presentación internacional o fecha de prioridad y que no está en conflicto con la solicitud pero citado para entender el principio o teoría que sustenta la invención 'X' documento de relevancia particular; la invención reivindicada no puede considerarse nueva o no puede considerarse que comprende un paso inventivo cuando el documento se toma solo 'Y' documento de relevancia particular; la invención reivindicada no puede considerarse que comprende un paso inventivo cuando el documento se combina con uno o más documentos, siendo dicha combinación obvia para una persona experta en la técnica 'Z' documento miembro de la misma familia de patentes	
Fecha de la terminación real de la búsqueda internacional Febrero 13, 2002		Fecha de envío por correo del reporte de búsqueda internacional 27/02/2002	
Nombre y dirección de correo de ISA European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. No. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax No. (+31-70) 340-3016		Funcionario autorizado Peeters, J.	

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11

REPORTE DE BUSQUEDA INTERNACIONAL

Solicitud Internacional No.
PCT/US 01/40302

C. (Continuación) DOCUMENTOS CONSIDERADOS RELEVANTES		
Categoría *	Citación de documento, con indicación, donde sea apropiado, de los textos relevantes	Relevante a la reivindicación No.
X	B.M.WOLFE, M.W. HUFF: "Efectos de la terapia continua de reemplazo hormonal de baja dosificación sobre el metabolismo de las lipoproteínas en mujeres postmenopáusicas" METABOLISM CLINICAL & EXPERIMENTAL, Vol. 44, no. 3, 1995, páginas 410-417, Página 410	1,2,4,6 7,9, 11-15, 19,20, 22,25, 26,40, 41,43, 45,46,
X	---	
	H. SUMINO E.A.: "El nivel sérico del factor de crecimiento endotelial vascular se disminuye por la terapia de reemplazo hormonal en mujeres postmenopáusicas sin hipercolesterolemia" ATHEROSCLEROSIS, Vol. 148, no. 1, 2000, páginas 189-195, XP001053480 Página 189 - página 190	1,2,6,9, 13-15, 19-22, 25-26, 40,41,45

X	G.G. GIRAUD E.A.: "Efectos de estrógeno y la progestina sobre el tamaño y la deformación aórtica en mujeres postmenopáusicas" AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, Vol. 174, no. 6, 1996, páginas 1708-1718, XP001053521 página 1708 -página 1709	1,2,6, 13-15, 19-23, 25-26, 40,41,45

REPORTE DE BUSQUEDA INTERNACIONAL

Informe sobre miembros de familia de patentes

Solicitud Internacional No.
PCT/US 01/40302

Documento de patente citado en el reporte de búsqueda	Fecha de publicación	Miembro(s) de la familia de patentes	Fecha de publicación
EP 309263 A	29/03/1989	CA 1332227 A1	04-10-1994
		CA 1332228 A1	04-10-1994
		AT 102484 T	15-03-1994
		AT 209919 T	15-12-2001
		AU 630334 B2	29-10-1992
		AU 2276088 A	06-04-1989
		AU 3044892 A	11-02-1993
		CN 1041528 A,B	25-04-1990
		DE 3856508 D1	17-01-2002
		DE 3888269 D1	14-04-1994
		DK 529688 A	25-05-1989
		EP 0309263 A2	29-03-1989
		EP 0559240 A2	08-09-1993
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		FI 884378 A	25-03-1989
		FI 972370 A	04-06-1997
		HU 50043 A2	28-12-1989
		JP 1132523 A	25-05-1989
		JP 3208482 B2	10-09-2001
		JP 11286446 A	19-10-1999
		KR 170764 B1	01-02-1999
		NO 301689 B1	01-12-1997
		NZ 226316 A	25-10-1991
		US 5382573 A	17-01-1995
		US 5422119 A	06-06-1995
		US 5585370 A	17-12-1996
		US 5108995 A	28-04-1992
		US 5276022 A	04-01-1994
		US 5256421 A	26-10-1993
		ZA 8807127 A	28-06-1989

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference AM100226	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, Item 5 below.	
International application No. PCT/US 01/40302	International filing date (day/month/year) 16/03/2001	(Earliest) Priority Date (day/month/year) 20/03/2000
Applicant AMERICAN HOME PRODUCTS CORPORATION		

This International Search Report has been prepared by the International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

2. ☐ Certain claims were found unsearchable (See Box I).

3. ☐ Unity of invention is lacking (see Box II).

4. With regard to the title,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

HORMONE REPLACEMENT THERAPY USING A COMBINATION OF CONJUGATED ESTROGENS AND MEDROXYPROGESTERONE ACETATE

5. With regard to the abstract,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 01/40302

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/57 A61P5/30 //(A61K31/57,31:565)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data bases consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, WPI Data, BIOSIS, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 309 263 A (JENCAP RESEARCH) 29 March 1989 (1989-03-29) claims 1,2,5,9,10,13 column 3, line 59 -column 4, line 32 column 5, line 28-48 column 11, line 35-37 -/-	1-4,6,7, 9,11-23, 25,26, 40-43, 45,46, 48,50-56

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *A* document member of the same patent family

Date of the actual completion of the international search

13 February 2002

Date of mailing of the international search report

27/02/2002

Name and mailing address of the ISA

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Fax (+31-70) 340-2016

Authorized officer

Peeters, J

INTERNATIONAL SEARCH REPORT

Wyeth
C-AM100226 1/15

International Application No

PCT/US 01/40302

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	B.M.WOLFE, M.W.HUFF: "Effects of continuous low-dosage hormonal replacement therapy on lipoprotein metabolism in postmenopausal women" METABOLISM CLINICAL & EXPERIMENTAL, vol. 44, no. 3, 1995, pages 410-417, XP001053539 page 410	1,2,4,6, 7,9, 11-15, 19,20, 22,25, 26,40, 41,43, 45,46
X	H.SUMINO E.A.: "Serum level of vascular endothelial growth factor is decreased by hormone replacement therapy in postmenopausal women without hypercholesterolemia" ATHEROSCLEROSIS, vol. 148, no. 1, 2000, pages 189-195, XP001053480 page 189 -page 190	1,2,6,9, 13-15, 19-22, 25,26, 40,41,45
X	G.G.GIRAUD E.A.: "Effects of estrogen and progestin on aortic size and compliance in postmenopausal women" AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, vol. 174, no. 6, 1996, pages 1708-1718, XP001053521 page 1708 -page 1709	1,2,6, 13,15, 19-23, 25,26, 40,41,45

INTERNATI NAL SEARCH REPORT

International Application No

PCT/US 01/40302

A9

16

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	B.M.WOLFE, M.W.HUFF: "Effects of continuous low-dosage hormonal replacement therapy on lipoprotein metabolism in postmenopausal women" METABOLISM CLINICAL & EXPERIMENTAL, vol. 44, no. 3, 1995, pages 410-417, XP001053539 page 410	1,2,4,6, 7,9, 11-15, 19,20, 22,25, 26,40, 41,43, 45,46
X	H.SUMINO E.A.: "Serum level of vascular endothelial growth factor is decreased by hormone replacement therapy in postmenopausal women without hypercholesterolemia" ATHEROSCLEROSIS, vol. 148, no. 1, 2000, pages 189-195, XP001053480 page 189 -page 190	1,2,6,9, 13-15, 19-22, 25,26, 40,41,45
X	G.G.GIRAUD E.A.: "Effects of estrogen and progestin on aortic size and compliance in postmanopausal women" AMERICAN JOURNAL OF OBSTETRICS AND GYNECOLOGY, vol. 174, no. 6, 1996, pages 1708-1718, XP001053521 page 1708 -page 1709	1,2,6, 13,15, 19-23, 25,26, 40,41,45

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 01/40302

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 309263	A	29-03-1989	CA 1332227 A1 04-10-1994
			CA 1332228 A1 04-10-1994
			AT 102484 T 15-03-1994
			AT 209919 T 15-12-2001
			AU 630334 B2 29-10-1992
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			CN 1041528 A , B 25-04-1990
			DE 3856508 D1 17-01-2002
			DE 3888269 D1 14-04-1994
			DK 529688 A 25-05-1989
			EP 0309263 A2 29-03-1989
			EP 0559240 A2 08-09-1993
			ES 2061672 T3 16-12-1994
			FI 884378 A 25-03-1989
			FI 972370 A 04-06-1997
			HU 50043 A2 28-12-1989
			JP 1132523 A 25-05-1989
			JP 3208482 B2 10-09-2001
			JP 11286446 A 19-10-1999
			KR 170764 B1 01-02-1999
			NO 301689 B1 01-12-1997
			NZ 226316 A 25-10-1991
			US 5382573 A 17-01-1995
			US 5422119 A 06-06-1995
			US 5585370 A 17-12-1996
			US 5108995 A 28-04-1992
			US 5276022 A 04-01-1994
			US 5256421 A 26-10-1993
			ZA 8807127 A 28-06-1989



Europäisches Patentamt
European Patent Office
Office européen des brevets

Publication number:

0 309 263
A1

EUROPEAN PATENT APPLICATION

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Hormone composition and use.

This invention is concerned with a contraceptive formulation and a method of contraception which employs a combination of estrogen and progestin and wherein a short period of relatively dominant estrogenic activity alternates with a short period of relatively dominant progestagenic activity. The invention also concerns a hormonal replacement formulation and method for use in menopausal or castrate women which employs a similar combination of estrogen and progestin.

EP 0 309 263 A1

Bundesdruckerei Berlin

Description

HORMONE COMPOSITION AND USE

This invention is concerned with a contraceptive formulation and a method of contraception which employs a combination of estrogen and progestin and wherein a short period of relatively dominant estrogenic activity alternates with a short period of relatively dominant progestagenic activity. Also described are formulations which employ similar hormone combinations for hormonal replacement therapy for menopausal or castrate women.

In the luteal phase of the menstrual cycle, serum progesterone levels increase and progesterone mediated secretory changes occur in the uterine endometrium. The presence of progesterone receptors has been shown to be a necessary prerequisite for progesterone action in the endometrium (see Walters, M.R. and Clark, J.H. Relationship between the quantity of progesterone receptors and the antagonism of estrogen-induced uterotrophic response *Endocrinology* 105:382, 1979) and it is well documented that estrogen priming in the follicular phase of the cycle is responsible for the development of both estrogen and progesterone receptors (see Bayard, F., Damilano, S., Robel, P. and Baufien, E.E. Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium, *J. Clin Endocrinol Metab.* 46:335, 1978). On the other hand, progesterone exerts a negative feedback effect on its own receptor (see Tsang, L. and Gurpide, E. Effects of progestins on estradiol receptor levels in human endometrium, *J. Clin Endocrinol Metab.* 41:402, 1975) and also acts to downregulate endometrial estrogen receptors possibly by induction of an estrogen receptor regulatory factor (see Leavitt, W.W., Okulicz, W.C., McCracken, J.A., Schramm, W.S. and Robidoux, W.F. Jr. Rapid recovery of nuclear estrogen receptor and oxytocin receptor in the ovine uterus following progesterone withdrawal, *J. Steroid Biochem.* 22:686, 1985).

These physiologic changes can be reproduced pharmacologically as shown by the induction of estrogen and progestin receptors in postmenopausal women by the administration of ethinyl estradiol (see Kraftmann, B., Bugat, R. and Bayard, F. Estrogen and Progestin Regulation of the Progesterone Receptor Concentration in Human Endometrium, *J. Clin Endocrinol Metab.* 49:926, 1979). Neumannova et al (see Short-Term Effects of Tamoxifen, Medroxy-progesterone Acetate, and Their Combination on Receptor Kinetics and 17beta-Hydroxysteroid Dehydrogenase in Human Endometrium, *Obstet. Gynecol.* 66:695, 1985) have also demonstrated that administration of medroxy-progesterone acetate in estrogen-primed women decreases the concentration of endometrial progestin receptors while at the same time increasing the activity of 17beta-hydroxysteroid dehydrogenase, an enzyme which is responsible for metabolism of estradiol to the less potent estrone.

A complex interaction occurs between estrogen and progesterone or progestin in the human endometrium with the progestins acting as anti-estro-

gens. Estrogen and progestin interactions are also dynamic. For example, estrogen administration increased the concentration of both estrogen and progestin receptors to peak levels, 7 times above baseline, within 3 days (see Ekert, R.L. and Katzenellenbogen, B.S. Human Endometrial Cells in Primary Tissue Culture: Modulation of the Progesterone Receptor Level by Natural and Synthetic Estrogens *In Vitro, J. Clin Endocrinol Metab.* 52:699, 1981). A three-fold increase in receptor concentrations occurred within one day. Normal physiologic levels of progesterone in the first 3 days of the luteal phase resulted in a rapid and significant decrease in estrogen receptor number (see Kraftmann-Glimbal, B., Bayard, F., Nixon, W.E. and Hodgen, G.D. Patterns of Estrogen and Progesterone Receptors in Monkey Endometrium During the Normal Menstrual Cycle, *Steroids* 35:471, 1980). Exogenous administration of progesterone to cynomolgous macaques significantly suppressed estrogen receptors within 1 to 2 days (see West, N.B. and Brenner, R.M. Progesterone-Mediated Suppression of Estradiol Receptors in Cynomolgous Macaque Cervix, Endometrium and Oviduct During Sequential Estradiol-Progesterone Treatment, *J. Steroid Biochem.* 22:29, 1985) and medroxy-progesterone acetate was able to significantly suppress progestin receptor levels in premenopausal women within 4 hours (see Neumannova M., Kaupplia, A., Kivinen, S. and Vihko, R. Short-Term Effects of Tamoxifen, Medroxy-progesterone Acetate, and Their Combination on Receptor Kinetics and 17beta-Hydroxysteroid Dehydrogenase in Human Endometrium, *Obstet. Gynecol.* 66:695, 1985). In contrast, progesterone withdrawal in the presence of constant estrogen levels has been shown to result in rapid (8 to 12 hours) recovery of nuclear estrogen receptors in sheep endometrium, associated with an estrogen induced biological response, i.e. production of oxytocin receptors (see Leavitt, W.W., Okulicz, W.C., McCracken, J.A., Schramm, W.S. and Robidoux, W.F. Jr. Rapid recovery of nuclear estrogen receptor and oxytocin receptor in the ovine uterus following progesterone withdrawal, *J. Steroid Biochem.* 22:686, 1985). A similar phenomenon occurs in pregnant guinea pigs when estrogen levels rise relative to progesterone levels prior to parturition (see Alexandrova, M. and Soloff, M.S. Oxytocin receptors and parturition in the guinea pig, *Biol. Reprod.* 22:1106, 1980).

Therefore, it appears that estrogen acts to stimulate both estrogen and progestin receptor concentrations and to induce sensitivity of the endometrium to both estrogen and progestin. Progesterone or progestin exerts an anti-estrogen action by decreasing estrogen receptors and by increasing 17beta-hydroxysteroid dehydrogenase activity in endometrial tissue. However, it appears that the stimulatory effects of progesterone on human endometrial function are of short duration probably because of a self-provoked downregula-

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tion of progestin receptors and estrogen receptors. For example, the effect of progesterone on 17 β -hydroxysteroid dehydrogenase peaks at 3 days and is then followed in 2 to 3 weeks by suppression of the enzyme (see Whitehead, M.I., Townsend, P.T., Pryce-Davies, J. et al. Effects of estrogens and progestins on the biochemistry and morphology of the postmenopausal endometrium, N. Engl. J. Med 305:1599, 1981).

Currently on the market there are a number of contraceptive formulations which can be classified readily into several general types. The first of these are known as monophasic formulations. These contain a constant amount of estrogen and progestin. Nuisance side effects with these pills depend on the balance between the estrogen and progestin component of the pill. For example, with a relatively dominant progestin pill, the formulation will, over time, result in a depletion of both estrogen and progestin receptors. The result which might be expected is an understimulated or atrophic endometrium which may eventually cause either on-pill amenorrhea or breakthrough bleeding or spotting due to poor epithelialization. On the other hand, with a relatively dominant estrogenic preparation, it is possible that prolonged use could result in endometrial growth with the development of unsupported fragile stroma and subsequent spotting or breakthrough bleeding.

Newer formulations known as triphases have varying levels of estrogen and progestin; in most cases consisting of relatively constant levels of estrogen with a step-wise increase in progestin throughout the cycle. This pattern of estrogen and progestin administration results in a relatively dominant estrogenic formulation at the beginning of the package with increasing progestagenic activity toward the end of the package. Endometrial stability may be better with these pills since the estrogenic activity at the beginning of the package induces both estrogen and progestin receptors making the endometrium sensitive to the increased levels of progestin towards the end of the package. The progestin activity produces denser, more stable endometrial stroma although the relatively long duration of progestin exposure, toward the end of the package, may still lead to decreased estrogen and progestin receptors and activity. A significant problem with this type of formulation is the low dose of steroids at the beginning of the package which makes these pills vulnerable to drug interactions or missed pills which may lead to breakthrough ovulation. The beginning of the package is the critical time in terms of breakthrough ovulation since the user has just completed a 7 day drug-free interval during which follicular development may begin. Even if pregnancy does not occur, breakthrough ovulation can lead to poor cycle control.

Estrogen replacement therapy is warranted in menopausal women for several reasons. Estrogen replacement will relieve hot flashes and this relief of flashes and night sweats improves sleep patterns and contributes to the patient's general feeling of well-being (see Campbell S., Whitehead M.I. Estrogen therapy and the menopausal syndrome, in

Clinics in Obstetrics and Gynecology: Volume 4. The Menopause. Edited by R.B. Greenblatt, J.W.W. Studd, London, W.B. Saunders, 1977, pages 31-47; Erlik Y., Tatarsky I.V., Meldrum D.R. et al. Association of waking episodes with menopausal hot flashes. JAMA 24:1741, 1981). Estrogen replacement protects against postmenopausal loss of calcium from the skeleton, especially from vertebral bodies, preventing crush fractures and loss of body height (see Lindsay R., Hart D.M., Forrest, C. et al. Prevention of spinal osteoporosis in oophorectomized women. Lancet 2:1151, 1980). Several studies have now reported that long-term estrogen therapy is also associated with a reduction in the incidence of classical osteoporotic fractures of the forearm and hip (see Hutchinson, T.A., Polansky, S.M., Finestain, A. Postmenopausal estrogens protect against fractures of hip and distal radius. Lancet 2:708, 1979; Paganini-Hill, A., Ross, R.K., Gerkens, V.R., et al. A case control study of menopausal estrogen therapy in hip fractures. Annals of Internal Medicine 95:29, 1981; Weiss N.S., Ure G.L., Ballard J.H. et al. Decreased risk of fractures of the hip and lower forearm with postmenopausal use of estrogen. New England Journal of Medicine 303:1195, 1980). Another beneficial effect of long-term estrogen use is the reduction of the risk of death from ischemic heart disease probably mediated by changes in blood lipoprotein concentrations (see Ross R.K., Paganini-Hill A., Mack T.M. et al. Menopausal estrogen therapy and protection from ischemic heart disease. Lancet 1:858, 1981). Estrogen replacement has also been shown to improve the vascularity and health of the vaginal mucosa and urinary tract. The only major risk factor associated with estrogen administration in the doses required to relieve menopausal symptoms, is hyperstimulation of the endometrium and an increased risk of endometrial cancer (see Cramer D.W., Knapp R.C. Review of epidemiologic studies of endometrial cancer and exogenous estrogen. Obstetrics and Gynecology 54:521, 1979; Shapiro S., Coughman D.W., Sloan D., et al. Recent and past use of conjugated estrogens in relation to adenocarcinoma of the endometrium. New England Journal of Medicine 303:485, 1980).

Estrogens predispose to cancer of the endometrium by stimulating cell mitosis and proliferation and increasing the levels of DNA synthesis and nuclear estradiol receptors in the endometrium (see Whitehead M.I., Townsend P.T., Pryce-Davies J., et al. Effects of estrogens and progestins on the biochemistry and morphology of the postmenopausal endometrium. New England Journal of Medicine 305:599, 1981; Whitehead M.I., Townsend P.T., Pryce-Davies J., et al. Actions of progestins on the morphology and biochemistry of the endometrium of postmenopausal women receiving low dose estrogen therapy. American Journal of Obstetrics and Gynecology, 142:791, 1982).

The addition of a progestin for 13 days each month has been demonstrated to protect the endometrium from these stimulatory effects of estrogen (see Gambrell R.D., Jr., Massey F.M., Castaneda et al. Use of the progestogen challenge test to reduce the risk

of endometrial cancer. *Obstetrics and Gynecology* 55:732, 1980; Studd J.W.W., Thom M.H., Patterson M.E.L., Wade-Evans T. The prevention and treatment of endometrial pathology in postmenopausal women receiving exogenous estrogens. In: Pasetto N., Paoletti R., Armbus J.L., Editors. *The menopause and postmenopause*. Lancaster MPT Press. 127, 1980).

The addition of a progestin protects the endometrium by reducing nuclear estradiol receptor concentration and thereby decrease nuclear estrogen bioavailability resulting in an antimitotic effect and lowering DNA synthesis. Progestins also increase the activity of endometrial estradiol-17 β -dehydrogenase, an enzyme which metabolize estradiol to estrone, a less potent estrogen (see Whitehead M.J., Townsen P.T., Pryce-Davies J. Effects of estrogens and progestins on the biochemistry and morphology of the postmenopausal endometrium. *New England Journal of Medicine*. 305:1599, 1981; King R.J.B., Townsen P.T., Sittle N.C., et al. Regulation of estrogen and progesterone receptor levels in epithellum and stroma from pre and postmenopausal endometria. *Journal of Steroids and Biochemistry*, 16:21, 1982; Gurpide E. Enzymatic modulation of hormonal action at the target tissue. *Journal of Toxicology and Environmental Health*, 4:249, 1978). The addition of progestin to estrogen replacement therapy may also result in an increase in bone mass when started within 3 years of the menopause (see Nachtigall L.E., Nachtigall R.H., Nachtigall R.D., et al. Estrogen replacement therapy: A 10 year prospective study in relationship to osteoporosis. *Obstetrics and Gynecology* 58:277, 1979; Lindsay R., Hart D.M., Forrest C., et al. Prevention of spinal osteoporosis in oophorectomized women. *Lancet* 2:1151, 1980). However, concerns have been expressed about the potential adverse effects of progestin in suppressing high density lipoprotein cholesterol concentrations (see Hirvonen E., Malkonen M., Manninen V. Effects of different progestogens on lipoproteins during postmenopausal replacement therapy. *New England Journal of Medicine* 304:580, 1981). This cholesterol fraction appears to have a protective effect against ischemic heart disease and atherosclerosis. The lowering of HDL cholesterol by progestin could negate the long-term beneficial effects of estrogen in reducing the incidence of myocardial infarction. Other side effects of progestins include acne, breast tenderness, depression and irritability (see Barranco V.P. Effect of androgen dominant and estrogen dominant oral contraceptives on acne. *Cutis* 14:384, 1974; Royal College of General Practitioners. *Oral Contraceptives and Health: An Interim Report*. New York: Pitman, 1974). Since the side effects of progestins appear to be dose dependent, the dose of progestin used with postmenopausal estrogen replacement should be the minimum necessary to achieve endometrial protection. (see Padwick M.L., Pryce-Davies J., Whitehead M.J. A simple method for determining the optimal dosage of progestin in postmenopausal women receiving estrogens. *New England Journal of Medicine* 315:930, 1986).

The biological effects of both estrogen and progestin in target tissues such as the endometrium

are dependent on the levels of estrogen and progestin receptors. Both estrogen and progestins exert a modulating influence on the levels of their own receptors. For example, in the luteal phase of the menstrual cycle, serum progesterone levels increase and progesterone mediated secretory changes occur in the uterine endometrium. The presence of progesterone receptors has been shown to be a necessary prerequisite for progesterone action in the endometrium (see Walters M.R. and Clark J.H. Relationship between the quantity of progesterone receptors and the antagonism of estrogen-induced uterotrophic response. *Endocrinology* 105:382, 1979) and it is well documented that estrogen priming in the follicular phase of the cycle is responsible for the development of both estrogen and progesterone receptors (see Bayard F., Damilano S., Robel P. and Baulieu E.E. Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium. *Journal Clinical Endocrinology and Metabolism* 46:635, 1978). On the other hand, progesterone exerts a negative feedback effect on its own receptor (see Tseng L. and Gurpide E. Effects of progestins on estradiol receptor levels in human endometrium. *Journal Clinical Endocrinology and Metabolism* 41:402, 1975) and also acts to downregulate endometrial estrogen receptors possibly by induction of an estrogen receptor regulatory factor (see Leavitt W.W., Okulicz W.C., McCracken J.A., Schramm W.S. and Robidoux W.F., Jr. Rapid recovery of nuclear estrogen receptor and oxytocin receptor in the ovine uterus following progesterone withdrawal. *Journal Steroid Biochemistry* 22:688, 1985).

These physiologic changes can be reproduced pharmacologically as shown by the induction of estrogen and progestin receptors in postmenopausal women by the administration of ethinyl estradiol (see Kreitmann B., Bugat R. and Bayard F. Estrogen and progestin regulation of the progesterone receptor concentration in human endometrium. *Journal Clinical Endocrinology and Metabolism* 49:926, 1979). Neumannova et al. (see Short-term effects of tamoxifen, medroxyprogesterone acetate, and their combination on receptor kinetics and 17 β -hydroxysteroid dehydrogenase in human endometrium. *Obstetrics and Gynecology* 66:695, 1985) have also demonstrated that administration of medroxyprogesterone acetate in estrogen-primed women decreases the concentration of endometrial progestin receptors while at the same time increasing the activity of 17 β -hydroxysteroid dehydrogenase, the enzyme which is responsible for metabolism of estradiol to the less potent estrone.

A complex interaction occurs between estrogen and progesterone or progestin in the human endometrium with the progestins acting as anti-estrogens. Estrogen and progestin interactions are also dynamic. For example, estrogen administration increased the concentration of both estrogen and progestin receptors to peak levels, 7 times above baseline, within 3 days (see Ekert R.L. and Katzenellenbogen B.S. Human endometrial cells in primary tissue culture: Modulation of the progesterone receptor level by natural and synthetic estrogens in

vitro. *Journal Clinical Endocrinology and Metabolism* 52:899, 1981). A three-fold increase in receptor concentrations occurred within one day. Normal physiologic levels of progesterone in the first 3 days of the luteal phase resulted in a rapid and significant decrease in estrogen receptor number (see Kraftmann-Glimbal B., Bayard F., Nixon W.E. and Hodgen G.D. Patterns of estrogen and progesterone receptors in monkey endometrium during the normal menstrual cycle. *Steroids* 35:471, 1980). Exogenous administration of progesterone to cynomolgous macaques significantly suppressed estrogen receptors within 1 to 2 days (see West N.B. and Brenner R.M. Progesterone-mediated suppression of estradiol receptors in cynomolgous macaque cervix, endometrium and oviduct during sequential estradiol-progesterone treatment. *Journal Steroid Biochemistry* 22:29, 1985) and medroxyprogesterone acetate was able to significantly suppress progesterin receptor levels in premenopausal women within 4 hours (see Neumannova M., Kauppila A., Kivinen S. and Vihko R. Short-term effects of tamoxifen, medroxyprogesterone acetate, and their combination on receptor kinetics and 17beta-hydroxysteroid dehydrogenase in human endometrium, *Obstetrics and Gynecology* 66:895, 1985). In contrast, progesterone withdrawal in the presence of constant estrogen levels has been shown to result in rapid (8 to 12 hours) recovery of nuclear estrogen receptors in sheep endometrium, associated with an estrogen induced biological response, i.e. production of oxytocin receptors (see Laevitt W.W., Okulicz W.C., McCracken J.A., Schramm W.S. and Robidoux W.F., Jr. Rapid recovery of nuclear estrogen receptor and oxytocin receptor in the ovine uterus following progesterone withdrawal. *Journal Steroids and Biochemistry* 22:686, 1985). A similar phenomenon occurs in pregnant guinea pigs when estrogen levels rise relative to progesterone levels prior to parturition (*Biology and Reproduction* 22:1108, 1980).

Therefore, it appears that estrogen acts to stimulate both estrogen and progesterin receptor concentrations and to induce sensitivity of the endometrium to both estrogen and progesterin. Progesterone or progesterin exerts an anti-estrogen action by decreasing the concentration of estrogen receptors and by increasing 17beta-hydroxysteroid dehydrogenase activity in endometrial tissue. However, it appears that the stimulatory effects of progesterone on human endometrial function are of short duration probably because of a self-provoked downregulation of progesterin receptors (see Neumannova M., Kauppila A., Kivinen S. and Vihko R. Short-term effects of tamoxifen, medroxyprogesterone acetate, and their combination on receptor kinetics and 17beta-hydroxysteroid dehydrogenase in human endometrium, *Obstetrics and Gynecology* 66:895, 1985; Whitehead M.J., Townsend P.T., Pryce-Davies J. et al. Effects of estrogens and progestins on the biochemistry and morphology of the postmenopausal endometrium. *New England Journal of Medicine*, 305:1599, 1981). For example, the effect of progesterone on 17beta-hydroxysteroid dehydrogenase peaks at 3 days and is then followed in 2 to 3 weeks by suppression of the enzyme (see White-

head M.J., Townsend P.T., Pryce-Davies J. et al. Effects of estrogens and progestins on the biochemistry and morphology of the postmenopausal endometrium. *New England Journal of Medicine* 305:1599, 1981).

Current hormonal replacement consists of continuous (daily or cyclic) (example day 1-25 of each month) estrogen administration with the addition of a progestin for 10-13 days (example days 13-25) each month. This type of replacement regimen is effective in preventing menopausal symptoms and at the same time, protects the endometrium against the development of hyperplasia or adenocarcinoma. However, the cyclic administration of a progestin leads to a scheduled withdrawal bleed or period in 65-75% of women (see Hellberg D., Nilsson S. Comparison of a triphasic estradiol/norethisterone acetate preparation with and without estriol component in the treatment of climacteric complaints; *Maturitas* 5:233, 1984; Christensen M.S., Hagen C., Christiansen C., Transbol I. Dose response evaluation of cyclic estrogen/progestagen in postmenopausal women: Placebo controlled trial of its gynecologic and metabolic actions. *American Journal of Obstetrics and Gynecology*, 144:573, 1982). This withdrawal bleeding is usually not welcomed by the patient and can lead to problems with compliance. Also because the progestin administration is preceded by up to 13-16 days of unopposed estrogen therapy with endometrial proliferation and estrogen and progesterin receptor induction, it is possible that a high dose of progestin is required to antagonize these effects resulting in a greater chance of side effects and adverse metabolic effects. Newer continuous low dose estrogen and progestin regimens for hormonal replacement may avoid the problem of withdrawal bleeding (see Magos A.L., Brincatt M., O'Dowd T., et al. Amenorrhea and endometrial atrophy following continuous oral estrogen and progestogen therapy in postmenopausal women. *Maturitas* 6:145, 1984). However, daily administration of a progestin in these regimens induces depletion of both estrogen and progesterin receptors resulting in endometrial atrophy which may be associated with breakthrough bleeding. Since abnormal bleeding in a postmenopausal woman is known to be associated with endometrial carcinoma, it must be investigated by endometrial sampling for hypertrophy usually by D&C. Daily administration of a progestin also raises the concern that the favourable effects of estrogen on HDL cholesterol metabolism will be adversely affected with a fall in HDL cholesterol (see Notalovitz M., Gudat J.C., Ware M.D., Dougherty M.C. *British Journal of Obstetrics and Gynecology*, 90:171, 1983).

The present invention provides a product containing estrogen and progestin as a combined preparation for administering a plurality of unit dosages of a relatively dominant estrogen activity combination and for administering a plurality of unit dosages of a relatively dominant progestin activity combination. In general, in the product the unit dosages are arranged for the administration alternately of a number of unit dosages of the relatively dominant

estrogen activity combination and of a number of unit dosages of the relatively dominant progestin activity combination, each said number of unit dosages preferably consisting of 1 to 5 unit dosages, more preferably 2 to 4 and most preferably 3 unit dosages, the product preferably being presented in the form of packages each containing 20 to 35 unit dosages.

In one aspect the product is for administration to females of child bearing age as a contraceptive and in another aspect is for hormone replacement therapy for administration to a female in need of such treatment, preferably wherein the female is of child bearing age or older in whom ovarian estrogen and progesterone production has been interrupted either because of natural menopause, surgical, radiation or chemical ovarian ovisation of extirpation or premature ovarian failure.

The invention also provides the new use of estrogen and progestin in the manufacture of the new product. The invention also provides new methods of treatment comprising administering two types of hormone-containing dosage combinations.

The present invention provides a pharmaceutical preparation for administration to a female of child bearing age for contraceptive purposes, which comprises a total of twenty to thirty-five unit dosages, each unit dosage for consecutive daily administration; each unit dosage comprising a combination of estrogen and progestin selected from a relatively dominant estrogen activity combination and a relatively dominant progestin activity combination, with a plurality of dominant estrogen activity dosages being alternated with a plurality of dominant progestin dosages; and each unit dosage also comprising a pharmaceutically acceptable inert carrier when required.

In another aspect, the invention provides a method of contraception which comprises administering to a female of child bearing age, in daily sequence, twenty to thirty-five unit dosages; each unit dosage comprising a combination of estrogen and progestin and being selected from a relatively dominant estrogen activity combination and a relatively dominant progestin activity combination, a plurality of dominant estrogen activity unit dosages being alternated with a plurality of dominant progestin activity unit dosages.

In a preferred form of the invention, dominant estrogen activity dosages are used to begin and end the twenty to thirty-five unit dosages.

Preferred methods of contraception involve twenty-one and twenty-four unit dosages.

In another aspect of the invention, there may be included an additional seven or four unit dosages in the pharmaceutical preparation which may comprise a placebo or any other hormone-free agent. These are usually taken at the completion of the twenty-one or twenty-four unit dosages.

Thus, in the present disclosure, a formulation is described that is better able to protect the endometrium against the estrogen related risk of endometrial hyperplasia and adenocarcinoma with a lower dose of progestin by administering progestin for a short period of time alternating with a short period of

absent or reduced progestin. It has been demonstrated that a protective effect of progestin is related to the duration of administration with 12-13 days per month appearing to be the minimum required for greatest protection. The present formulation administers a low dose of progestin intermittently throughout the month for a minimum of 15 days exposure.

The present invention provides a pharmaceutical preparation for administration to a female of child bearing age or older in whom ovarian estrogen and progesterone production has been interrupted either because of natural menopause, surgical, radiation, or chemical ovarian ablation or extirpation or premature ovarian failure, which comprises a plurality of unit dosages, each unit dosage for continuous consecutive daily administration and comprising combinations of estrogen and progestin selected from a combination with relatively dominant estrogen activity and a combination with relatively dominant progestin activity, with a plurality of dominant estrogen dosages being alternated with plurality of dominant progestin dosages, and each unit dosage also comprising a pharmaceutically acceptable inert carrier when required.

In another aspect, the invention provides a method of hormonal replacement therapy for administration to a female in need of such treatment, in particular to a female of child bearing age or older to whom ovarian estrogen and progesterone production has been interrupted either because of natural menopause, surgical, radiation, or chemical ovarian ablation or extirpation or premature ovarian failure, which comprises administering continuously and consecutively, in daily sequence, a plurality of daily unit dosages comprising combinations of estrogen and progestin, selected from a combination with relatively dominant estrogen activity and a combination with relatively dominant progestin activity, a plurality of the dominant estrogen activity unit dosages being alternated with a plurality of the dominant progestin activity unit dosages.

The contraceptive formulation of the present invention results in better cycle control. Intermittent increases in estrogen activity stimulate endometrial growth and progestin receptors. This makes the endometrium more sensitive to subsequent progestin activity which limits growth by decreasing estrogen receptors and increasing 17beta-hydroxysteroid dehydrogenase. Interaction of progestin with progestin receptors induces secretory changes in the endometrium which results in a denser stroma and endometrial stability. A return to relatively dominant estrogenic activity then again stimulates estrogen and progestin receptors and renews endometrial sensitivity to progestin. This push/pull activity keeps endometrial activity within a narrow range depending on the number of days of estrogenic and progestagenic activity.

The design of the present contraceptive invention avoids low levels of steroids present during the first part of the triphasic package which makes the triphasic formulations more sensitive to drug interactions and missed pills. As a result, less pill failures in terms of pregnancy occur and also cycle control is

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better because of fewer breakthrough ovulations.

The current contraceptive formulation allows better progestational effect with less progestin. With the current formulation the dose of progestin is significantly decreased compared to most monophasic preparations and a total steroid dosage is achieved which is lower than that of the present triphasics and yet the present formulation offers better cycle control and efficacy. A reduction in progestin dosage results in less negative impact on HDL cholesterol levels. HDL cholesterol has been shown to be protective against development of atherosclerosis and its concentration is increased by estrogen and decreased by progestin.

Alternatively, the reduction in progestin dose possible with the subject contraceptive formulation results in a pill that also has good estrogenic effect. Therefore, this formulation is a good one for the management of acne, oily skin and hirsutism and also has less chance of on-pill amenorrhea.

It is thought that the current contraceptive formulation is better able to inhibit ovulation with lower doses of steroids, since it has been demonstrated that estrogen priming increases progesterone receptor concentration in the hypothalamus and anterior pituitary gland in a number of animal species (see Katzenellenbogen, B.S. Dynamics of steroid hormone receptor action, Annual Rev. Physiol. 42:17, 1980). Therefore, by allowing intermittent estrogenic priming to occur by administering the preparation of estrogen and progestin in the alternating fashion of the present method, it is possible to potentiate the central negative feedback actions of both progestin and estrogen.

The hormonal replacement formulation of the present invention results in the absence of withdrawal bleeding; intermittent increases in estrogen activity; and stimulation of endometrial growth and progestin receptors. This makes the endometrium more sensitive to subsequent progestin activity which limits growth by decreasing estrogen receptors and increasing 17 β -hydroxysteroid dehydrogenase. Interaction of progestin with progestin receptors induces secretory changes in the endometrium which results in a denser stroma and endometrial stability. A return to relatively dominant estrogenic activity then again stimulates estrogen and progestin receptors and renews endometrial sensitivity to progestin. This push/pull activity keeps endometrial activity within a narrow range depending on the number of days of estrogenic and progestagenic activity and maintains a stable endometrium resulting in the absence of breakthrough or withdrawal bleeding.

This hormonal replacement formulation allows better progestational effects with less progestin. With the current formulation the dose of progestin is significantly decreased compared with a preparation containing constant daily administration of a progestin. A total steroid dosage is achieved which is similar to or even lower than that of the present cyclic method of administering estrogen and progestin for hormonal replacement therapy of ovarian failure. A reduction in progestin dosage results in less negative impact on HDL cholesterol levels. HDL

cholesterol has been shown to be protective against the development of atherosclerosis. The concentration of HDL cholesterol is increased by estrogen and decreased by progestin.

The estrogens which may be employed as a component in the regimens of this invention may be any of those conventionally available. Typically, the estrogen may be selected from the group comprising synthetic and natural estrogens. The synthetic estrogens may be selected from, for example, ethinyl estradiol, mestranol and quinestranol. Particularly of interest are 17 α -ethinylestradiol and esters and ethers thereof. The preferred estrogen is 17 α -ethinylestradiol. The natural estrogens may include, for example, conjugated equine estrogens, estradiol-17 β , estradiol valerate, estrone, piperazine estrone sulphate, estriol, estriol succinate, desogestrel and polyestrol phosphate.

The progestin component may be any progestationally active compound. Thus, the progestin may be selected from progesterone and its derivatives such as, for example, 17-hydroxy progesterone esters, 19-nor-17-hydroxy progesterone esters, 17 α -ethinytestosterone and derivatives thereof, 17 α -ethinyl-19-nor-testosterone and derivatives thereof, norethindrone, norethindrone acetate, ethynodiol diacetate, dydrogesterone, medroxy-progesterone acetate, norethynodrel, allylestrenol, lynoestrenol, fulgestanol acetate, medrogestone, norgestrel, dimethilderone, ethisterone, cyproterone, lavo-norgestrel, d-norgestrel, di-norgestrel, d-17 α -acetoxy-13 β -ethyl-17 α -ethinyl-gon-4-en-3-one oxime, cyproterone acetate, gestodene and norgestimate. Preferred progestins are norethindrone, d-norgestrel and norgestimate.

In a preferred form of the invention, the plurality of dosages may comprise from one to five unit dosages, but preferably three unit dosages are employed. Thus, in a preferred form of the invention, three unit dosages of relatively dominant estrogen activity are alternated with three unit dosages of relatively dominant progestin activity and so on for a total of twenty-one or twenty-four unit dosages. Four or seven unit dosages which are free of hormone are included to approximate the natural twenty-eight day menstrual cycle of the female. These pills may comprise a placebo or any other hormone-free agent. Examples of suitable alternative agents include vitamins, such as iron supplements. Where the total unit dosages do not comprise multiples of three, an appropriate number of hormone-free unit dosages may be included to make up the total required units.

Generally, the quantities of estrogen and progestin incorporated in the formulation of the invention are dependent on the type of estrogen or progestin selected. However, the quantities employed are generally less than those used in the currently marketed formulations for reasons mentioned earlier. In the present formulation, the estrogen level is kept constant, while the progestin level is adjusted up or down to produce the required estrogen or progestin dominance. The selection of quantity is dependent on the type of estrogen or progestin since each hormone has its own specific activity.

Typically for the contraceptive formulation, the amount of estrogen per unit dose may range from a minimum of about 0.020 mg. to a maximum of about 0.050 mg. of 17alpha-ethinylestradiol or its equivalent dosage in other synthetic or natural estrogens and the amount of progestin per unit dosage may range from a minimum of about 0.00 mg. of norethindrone or its equivalent in a synthetic or natural progestin to a maximum of about 1.00 mg. Thus, the maximum amount of hormone over the 21 days of administration may range from about 6.72 mg. to about 22.05 mg. (These amounts generally refer to oral dosages.)

Some preferred combinations include the following:

1. Three unit dosages of 0.035 mg. of 17alpha-ethinyl-estradiol with 0.5 mg. of norethindrone, alternating with three unit dosages of 0.035 mg. of 17alpha-ethinylestradiol with 0.75 mg. of norethindrone for a total of 7 groups of three, beginning and ending with the 0.5 mg. of norethindrone combination.

2. Three unit dosages of 0.035 mg. of 17alpha-ethinyl-estradiol and 0.5 mg. of norethindrone alternating with three unit dosages of 0.035 mg. of 17alpha-ethinyl-estradiol and 0.35 mg. of norethindrone, beginning and ending with the 0.5 mg. of norethindrone combination.

Typically in the hormone replacement formulations, the amount of estrogen per unit dose may range from a minimum of about 0.3 mg of estrone sulphate or its equivalent to a maximum of about 2.5 mg of estrone sulphate or its equivalent. The amount of progestin per unit dosage may range from a minimum of 0 mg to a maximum of about 5 mg of norethindrone or its equivalent. (These amounts generally refer to oral dosages.)

Some preferred combinations include the following:

1. Three units dosages of 0.75 mg piperazine estrone sulphate alternating with three unit dosages of 0.75 mg of piperazine estrone sulphate with 0.35 mg of norethindrone.

2. Three unit dosages of 0.75 mg piperazine estrone sulphate and 0.15 of norethindrone alternating with three unit dosages of 0.75 mg of piperazine estrone sulphate and 0.35 mg of norethindrone.

The above combinations may also be grouped into three's and four's, starting with either three or four day groups and ending with the other. For the normal replacement therapy it may be advantageous for the groups of dominant estrogen activity combination to comprise one more unit dosage than the dominant progestin activity combination unit dosages, or vice versa, for instance the numbers in the groups being 2 and 3 or 3 and 4.

The formulations of the invention may be administered orally, preferably in tablet form, parenterally, sublingually, transdermally, intravaginally, intranasally or buccally. The method of administration determines the types of estrogens and progestins useful in the formulation, as well as the amounts per unit dosage.

Methods for transdermal administration including

the associated methods for manufacturing such systems are well known in the art. In this connection, reference may be had to U.S. Patents Nos. 4,752,478, 4,885,911, 4,438,139 and 4,291,014.

Generally speaking, the formulations are prepared according to conventionally known procedures in accordance

with the method of administration. Thus, the active ingredients are prepared according to known methods in a pharmaceutically acceptable form for administration. These ingredients, in their required quantities are combined with the appropriate pharmaceutical carriers such as additives, vehicles and/or flavour ameliorating substances. These substances may be referred to as diluents, binders and lubricants. Gums, starches and sugars are also common terms. Typical of these types of substances or excipients are pharmaceutical grades of mannitol, lactose starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate and the like. The active ingredient(s) may comprise from about 0.01% by weight to about 99.99% by weight of the total formulation and the remainder comprises the pharmaceutically acceptable carrier. The percentage of active ingredient(s) may vary according to the delivery system or method of administration and is chosen in accordance with conventional methods known in the art.

Thus, the active ingredients are compounded with the chosen carrier and in for example the case of a tablet form, placed in a tablet molding apparatus to form the tablets which are subsequently packaged in accordance with the chosen regimen.

In the oral form of the formulation, the contraceptives are preferably produced in the form of a pharmaceutical kit or package, with the daily dosages arranged for proper sequential administration. Thus, in another aspect, the present invention also provides a pharmaceutical package which contains combination-type contraceptives in multiple dosage units in a synchronized, fixed sequence, wherein the sequence or arrangement of the dosage units corresponds to the stages of daily administration. This can be known as a multi-preparation pack.

Preferably, such packages are in the form of a transparent package with twenty-eight dosage units arranged sequentially and consisting of twenty-one or twenty-four tablets containing the combined estrogen/progestin formulation set up for the cyclical regimen of the invention and seven or four placebos thereafter.

Preferably the placebo tablets and tablets containing the hormones are different colours or shapes. Data indications may be provided on the packaging. The packaging may be a tube or box or a strip. The box may be circular, square, or otherwise shaped with the tablets being accommodated separately therein for ease of administration. Data indications may appear adjacent each tablet corresponding with the days on which each tablet is to be taken. Some indication of the sequence in which the tablets are to be taken preferably appears on the packaging regardless of its form.

In the following examples, specific embodiments

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of the present invention are set forth. These are meant to be illustrative of the invention and are not meant to limit it in any way. All parts and percentages are by weight, unless indicated otherwise.

EXAMPLE 1

Three-day phases of unit dosages of 17alpha-ethinyl-estradiol (EE) 0.035 mg. and norethindrone (NET) 0.5 mg. alternating with three-day phases of unit dosages of EE 0.035 mg. and NET 0.75 mg. for a total of 7 phases (21 days or 21 unit dosages) beginning and ending with the NET 0.5 mg. combination.

EXAMPLE 2

Three-day phases (unit dosages of EE 0.035 mg. and NET 0.5 mg.) alternating with three-day phases of EE 0.035 mg. and NET 0.35 mg., beginning and ending with the 0.5 mg. combination

EXAMPLE 3

Two-day phases of unit dosages of EE 0.035 mg alternating with unit dosages of EE 0.035 mg and NET 0.35 mg, beginning and ending with the first unit dosage and running for 24 days total.

EXAMPLE 4

Three-day phases of unit dosages of EE 0.035 mg and NET 0.15 mg alternating with EE 0.035 mg and NET 0.35 mg and running for 24 days total.

EXAMPLE 5

Three-day and four-day phases of each of the above combinations as set forth in Examples 1 and 2, starting with either 3 or 4-day phases and ending with the other.

EXAMPLE 6

Four-day and three-day phases of each of the above are prepared, starting with a four-day unit dosage of 0.5 mg. NET and 0.035 mg. EE and ending with 0.75 mg. NET and 0.035 mg. EE.

EXAMPLE 7

Three-day and four-day phases of each of the

above formulations starting with a three-day phase of 0.35 mg. NET with 0.035 mg. EE and ending with a four-day phase of 0.5 mg. NET and 0.035 mg. EE.

EXAMPLE 8

One-day alternating phases using the unit dosages set forth in Examples 1 and 2.

EXAMPLE 9

Two-day alternating phases ending or beginning with a single 3-day phase, using the unit dosage formulations set forth in Examples 1 or 2.

EXAMPLE 10

Three-day phases of EE 0.035 mg. and levonorgestrel (D-norgestrel) 0.05 mg. alternating with 3-day phases of EE 0.035 mg. and levonorgestrel 0.075 mg.

EXAMPLE 11

Three-day phases of EE 0.035 mg. and norgestimate 0.05 mg. alternating with EE 0.035 mg. and norgestimate 0.075 mg.

EXAMPLE 12

Three-day phases of EE 0.035 mg. and norgestimate 0.05 mg. alternating with EE 0.035 and norgestimate 0.035 mg.

EXAMPLES 13 & 14

One formulation was administered to 2 women for a total of 3 cycles to establish that cycle control, in terms of breakthrough bleeding, is acceptable. The test formulation consisted of three unit dosages of 0.035 mg. of 17alpha-ethinylestradiol and 0.5 mg. of norethindrone alternating with three unit dosages of 0.035 mg. of 17alpha-ethinylestradiol and 0.75 mg. of norethindrone for a total of 7 groups of three, beginning and ending with the 0.5 mg. of norethindrone combination.

EXAMPLE 13

A 23 year old nulliparous woman who had not taken any hormonal formulation including oral con-

traceptives for three months agreed to take the test formulation of the invention for two cycles. The subject was in good health and did not smoke. The subject had no contraindications to the use of oral contraceptives and her menstrual cycles were regular. The subject started the test formulation on the fifth day of her cycle (onset of menstruation is considered day 1) for 21 consecutive days (first cycle), followed by a 7 day interval which was free of any hormone and then restarted the test formulation for another 21 days (second cycle). In the first cycle the subject had no bleeding or spotting while taking the test formulation and had a withdrawal bleed starting on the second day of the hormone free interval. The withdrawal bleed lasted for 5 days and was lighter than a normal menstrual period consisting of reddish-brown spotting. There was no discomfort associated with the withdrawal bleeding. In the second cycle, the subject was also free of bleeding or spotting while taking the test formulation and again had a brownish, very light withdrawal bleed which began two days after stopping the test formulation and lasted for 6 days. The subject experienced no side effects during the two test cycles.

EXAMPLE 14

The subject was a healthy, 27 year old nulliparous woman who was currently taking a commercially available oral contraceptive formulation containing 17alpha-ethinyloestradiol and di-norgestrel (Triphasil (Trade mark of Wyeth Pharmaceuticals)). The subject agreed to take the test formulation of the invention for one cycle. The subject started the test formulation after a 7 day hormone free interval following the last Triphasil tablet. The test formulation was taken for 21 days followed by a 7 day drug free interval. The subject had no spotting or bleeding during the time she took the test formulation and experienced a withdrawal bleed which began two days after stopping the test formulation. The withdrawal bleeding lasted four days, was painless and was the same amount and colour as a normal menstrual period for the subject. The subject had no side effects during the test formulation. Both subjects found the test formulation to be acceptable in terms of cycle control, side effects and menstrual bleeding.

Examples illustrating the Hormone-Replacement Therapy.

EXAMPLE 15

Three-day phases of unit dosages of 0.75 mg piperazine estrone sulphate alternating with three-day phases of unit dosages of estrone sulphate 0.75 mg and NET 0.35 mg given continuously and orally.

EXAMPLE 16

Three-day phases (unit dosages of estrone sulphate 0.75 mg and norethindrone 0.15 mg) alternating with three-day phases of estrone sulphate 0.75 mg and norethindrone 0.35 mg given continuously and orally.

EXAMPLE 17

Three-day phases of oral micronized 17beta-estradiol 1 mg alternating with three-days of 17beta-estradiol 1 mg and norethindrone .35 mg given continuously.

EXAMPLE 18

Three-day phases of transdermal 17beta-estradiol (100 ug/day) alternating with three-days of transdermal 17beta-estradiol (100 ug/day) and transdermal norethindrone (.35 mg/day) given continuously.

EXAMPLE 19

Three-day phases of estrone sulphate 1.25 mg alternating with three-day phases of estrone sulphate 1.25 mg and norethindrone 0.35 mg given continuously and orally.

EXAMPLE 20

Three-day phases of estrone sulphate 1.25 mg alternating with three-day phases of estrone sulphate 1.25 mg and norethindrone 0.5 mg given continuously and orally.

EXAMPLE 21

One-day or two-day alternating phases using the unit dosages set forth in Examples 13 and 14 given continuously and orally.

EXAMPLE 22

Three-day phases of estrone sulphate 0.75 mg alternating with three-day phases of estrone sulphate 0.75 mg and norgestimate 0.050 mg given continuously and orally.

EXAMPLE 23

Three-day and four-day phases of each of the combinations as set forth in Examples 13 and 14, starting with either a 3- or 4-day phase and given continuously and orally.

EXAMPLE 24

Two-day and three-day phases of each of the combinations as set forth in Examples 13 and 14, starting with either a 2- or 3-day phase and given continuously and orally.

While the present invention has been described with reference to specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material or composition of matter, process, process step or steps, or then present objective to the spirit of this invention without departing from its essential teachings.

Claims

1. A product containing estrogen and progestin as a combined preparation for administering a plurality of unit dosages of a relatively dominant estrogen activity combination and for administering a plurality of unit dosages of a relatively dominant progestin activity combination.

2. A product according to claim 1 in which the unit dosages are arranged for the administration alternately of a number of unit dosages of the relatively dominant estrogen activity combination and of a number of unit dosages of the relatively dominant progestin activity combination, each said number of unit dosages preferably consisting of 1 to 5 unit dosages, more preferably 2 to 4 and most preferably 3 unit dosages, the product preferably being presented in the form of packages each containing 20 to 35 unit dosages.

3. A product according to claim 1 or claim 2 which comprises a plurality of hormone-free unit dosages for administration after the hormone-containing unit dosages, the product preferably being presented in the form of packages, each containing 28 unit dosages in total of which suitably 21 or 24 are hormone-containing unit dosages and 7 or 4 are hormone-free unit dosages.

4. A product according to any preceding claim which is for administration to females of child bearing age as a contraceptive.

5. A product according to any of claims 1 to 3 which is for hormone replacement therapy for administration to a female in need of such treatment, preferably wherein the female is of child bearing age or older in whom ovarian estrogen and progesterone production has been interrupted either because of natural menopause, surgical, radiation or chemical ovarian ablation of extirpation or premature ovarian failure.

6. Use of estrogen and progestin in a method of manufacturing a product characterised in that the product is as defined in any preceding claim.

7. Method of treatment of a female which comprises administering consecutively and in daily sequence a plurality of daily unit dosages each unit dosage being selected from a relatively dominant estrogen activity combination and a relatively dominant progestin activity combination characterised in that a number of the dominant estrogen activity combination unit dosages are alternated with a number of the dominant progestin activity unit dosages, preferably starting and more preferably also finishing with a number of dominant estrogen activity combination unit dosages.

8. Method according to claim 7 having the further characteristic features of any of claims 2 to 5.

9. A product according to any of claims 1 to 5, a use according to claim 6 or a method according to claim 7 or claim 8, in which the estrogen is selected from synthetic and natural estrogens, preferably selected from 17alpha-ethinyloestradiol and esters and ethers thereof or from natural estrogens selected from conjugated equine estrogens, 17beta-estradiol, estradiol valerate, estrone, estrone sulphate, piperazine estrone sulphate, estril, estril succinate and polyestril phosphate or synthetic estrogens comprising ethinyl estradiol, mestranol and quinestranol.

10. A product according to any of claims 1 to 5 and 9, a use according to claim 6 or 9 or a method according to any of claims 7 to 9 in which the progestin is selected from progesterone, 17-hydroxy progesterone esters, 19-nor-17-hydroxy progesterone esters, 17alpha-ethinyl-testosterone, 17alpha-ethinyl-19-nor-testosterone and derivatives thereof, norethindrone, norethindrone acetate, ethynodiol diacetate, dydrogesterone, medroxyprogesterone acetate, norethynodrel, allylestrenol, lynoestrenol, quingestanol acetate, medrogestone, norgestrienone, dimethisterone, ethisterone, cyproterone, cyproterone acetate, levonorgestrel, d-norgestrel, dl-norgestrel, d-17beta-acetoxy-13beta-ethyl-17alpha-ethinylgon-4-en-3-one oxime, gestodene, norgestimate and desogestrel, and is preferably norethindrone or norgestimate or for a contraceptive product is dl-norgestrel or, for a hormonal replacement product, is progesterone.

11. A product according to any of claims 1 to

4, 9 and 10, a use according to any of claims 6, 9 and 10 or a method according to any of claims 7 to 10 which is a contraceptive in which the amount of estrogen per unit dosage ranges from a minimum of about 0.020 mg. to a maximum of about 0.050 mg. of 17alpha-ethinylestradiol or its equivalent dosage in another synthetic or natural estrogen and the amount of progestin per unit dosage may range from a minimum of about 0.00 mg, preferably about 0.2 mg, to a maximum of about 1.00mg. of norethindrone or its equivalent in a synthetic or natural progestin.

12. A product, use or method according to claim 11, in which the dominant estrogen combination unit dosages contain 0.035 mg 17alpha-ethinylestradiol and 0.35-0.5 mg norethindrone and the dominant progestin combination unit dosages contain 0.035 mg 17alpha-ethinylestradiol and 0.5-0.75 mg norethindrone, and in which the unit dosages are preferably for administration in alternating groups of three of each type of hormone containing unit dosage.

13. A product according to any of claims 1 to 3, 5, 9 and 10, a use according to any of claims 6, 9 and 10 or a method according to any of claims 7 to 10 which is for hormonal replacement therapy in which the amount of estrogen per unit dosage ranges from a minimum of about

0.3 mg to a maximum of about 5.0 mg preferably to a maximum of about 2.5 mg of piperazine estrone sulphate or its equivalent dosage in another synthetic or natural estrogen and the amount of progestin per unit dosage may range from a minimum of about 0.0 mg to a maximum of about 5.0 mg of norethindrone or its equivalent in a synthetic or natural progestin.

14. A product, use or method according to claim 13 in which the unit dosages are for oral administration and in which the dominant estrogen combination unit dosage containing 0.75 mg piperazine estrone sulphate and 0.015 mg norethindrone and the dominant progestin combination unit dosages contain 0.75 piperazine estrone sulphate and 0.35 mg norethindrone and in which the unit dosages are preferably for administration in alternating groups of three of each type of hormone-containing unit dosage.

15. A product, use or method according to claim 13 in which the unit dosages are for transdermal administration and are for administration in alternating three day periods of 17beta-estradiol at 0.1 mg/day plus norethindrone at 0-0.15 mg/day and three day periods of 17beta-estradiol at 0.1 mg/day plus norethindrone at 0.35 mg/day.

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⑨ Hormone composition and use.

⑩ This invention is concerned with a contraceptive formulation and a method of contraception which employs a combination of estrogen and progestin and wherein a short period of relatively dominant estrogenic activity alternates with a short period of relatively dominant progestagenic activity. The invention also concerns a hormonal replacement formulation and method for use in menopausal or castrate women which employs a similar combination of estrogen and progestin.

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shall be considered, for the purposes of subsequent
proceedings, as the European search report

Application number

EP 88 30 8840

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	US-A-3 568 828 (L.J. LERNER) * Column 1, line 55 - column 2, line 38 *	1-6	A 61 K 31/565
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X	ROTE LISTE, 1987, Editio Cantor, Aulendorf, Württemberg, DE * No. 75 157, "Synphasec" *	1-6	
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A	ROTE LISTE, 1987, Editio Cantor, Aulendorf, Württemberg, DE * No. 75 154, "Perikursal 21"; no. 75 156, "Sinovula" *	1-6	
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A	UNLISTED DRUGS, vol. 38, no. 1, January 1986, page 2, Chatham, New Jersey, US * Page 2, ref. o, "7/7/7" *	1-6	

			TECHNICAL FIELDS SEARCHED (Int. Cl.4)
			A 61 K
INCOMPLETE SEARCH			
The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims. Claims searched completely: 1-6 Claims searched incompletely: 7-15 Claims not searched: 7-15 Reason for the limitation of the search: Method for treatment of the human or animal body by surgery or therapy (See art. 52(4) of the European Patent Convention)			
Place of search		Date of completion of the search	Examiner
The Hague		09-12-1988	BRINKMANN
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Effects of Continuous Low-Dosage Hormonal Replacement Therapy on Lipoprotein Metabolism in Postmenopausal Women

Bernard M. Wolfe and Murray W. Huff

The effects on lipoprotein metabolism of female hormone replacement therapy (HRT) for 7 weeks with combined low dosages of a widely used oral progestin and estrogen combination, medroxyprogesterone acetate (MPA) 2.5 mg/d and conjugated equine estrogen (CEE) 0.625 mg/d, were studied in six postmenopausal women. To investigate the mechanism of the reduction of low-density lipoprotein (LDL) cholesterol by HRT, the kinetics of very-low-density lipoprotein (VLDL) and LDL apolipoprotein (apo) B turnover were studied by injection of autologous ^{125} I-labeled VLDL and ^{125} I-labeled LDL under control conditions and again in the fourth week of HRT. HRT induced (1) a $12\% \pm 4\%$ ($P < .02$) reduction of the cholesterol content of LDL of 8, 0-12, which was attributable to a $15\% \pm 5\%$ decrease in the mean ratio of cholesterol to apo B (1.3 ± 0.1 v 1.5 ± 0.1 , $P < .025$), and (2) a $12\% \pm 4\%$ increase in the mean fractional catabolic rate (FCR) of LDL apo B (0.34 ± 0.03 v 0.30 ± 0.02 pools/d, respectively, $P = .05$). However, there were no significant changes in mean values for (1) pool size (42 ± 4 v 43 ± 3 mg/kg) or production rate (14 ± 0.5 v 12 ± 0.8 mg/kg/d, $P > .1$) of LDL apo B or (2) pool size (2.5 ± 0.9 v 2.8 ± 0.8 mg/kg), FCR (8.0 ± 2.0 v 6.1 ± 1.7 pools/d) or production rate (18 ± 4 v 10 ± 2 mg/kg/d, $P > .04$) of VLDL apo B. HRT increased high-density lipoprotein (HDL) cholesterol concentration significantly by 18% to 19%, $P < .05$, whereas the mean ratio of plasma total cholesterol to HDL cholesterol decreased by $19\% \pm 3\%$ ($P < .005$). HRT favorably influenced the overall plasma lipoprotein lipid vascular risk profile while significantly altering both the composition and fractional catabolism of LDL.

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APPROXIMATELY 36 million American women currently lack estrogen and progesterone as a result of menopause.^{1,2} Their numbers are expected to increase rapidly, so that by the year 2006 approximately 70 million Americans and 700 million women world wide will be of menopausal age.^{1,3} Coronary heart disease (CHD), the major mortality risk of postmenopausal women, has a mortality rate that markedly exceeds that of either breast cancer or hip fracture by an order of magnitude.⁴ Elevated plasma cholesterol^{5,6} tends to accompany the postmenopausal increase in CHD^{7,8} and contributes to the loss of relative immunity from CHD enjoyed by premenopausal women as compared with men of similar age.⁹ Estrogen ($\pm$ progestin) replacement appears to reduce all-cause mortality by 11% to 20% and CHD mortality by 50%.¹⁰⁻¹² However, prolonged estrogen therapy that is unopposed by natural or synthetic progestin increases the risk of uterine neoplasia by 1.7-fold to eightfold.^{13,14} Although intermittent administration of progestin together with estrogen reduces the relative risk of endometrial cancer to ≤ 1.0 ,^{14,15} such therapy tends to result in poor patient compliance¹⁴ because of bothersome and often painful scheduled vaginal withdrawal bleeding,^{13,14,17} premenstrual-like symptoms, and anxiety about uterine cancer and/or return of fertility.^{14,16,18} However, continuous daily administration of hormone replacement therapy (HRT) induces endometrial atrophy and largely eliminates vaginal bleeding (usually immediately or over time), which facilitates compliance and long-

term prophylaxis against CHD and osteoporosis.¹⁹⁻²³ Estrogen/progestin replacement therapy appears to decrease the risk of CHD by various mechanisms, including reductions in plasma low-density lipoprotein (LDL) cholesterol²⁴ and increases in high-density lipoprotein (HDL) cholesterol,^{25,26} as well as other mechanisms.²⁴⁻³¹ Although the combination of continuous low dosages of medroxyprogesterone acetate (MPA) and conjugated equine estrogen (CEE) has recently been recommended for HRT in menopause,³² the mechanism whereby the combination influences the metabolism of major apolipoprotein (apo) B-containing lipoproteins and in particular decreases LDL cholesterol²⁴ has not been investigated. We have therefore studied the effects of continuous oral low-dosage MPA + CEE on the kinetics of apo B metabolism of plasma LDL and very-low-density lipoprotein (VLDL) in estrogen-deficient postmenopausal women.

SUBJECTS AND METHODS

Subjects

Six participants who had experienced typical menopausal symptoms, were amenorrheic for at least 6 months, and had serum follicle-stimulating hormone levels greater than 70 U/L before entering the study were recruited from University Hospital outpatient clinics (Table 1). Baseline fasting plasma cholesterol concentrations during phase I American Heart Association diets before entry into the study were ≥ 200 mg/dL, the cutoff point of eligibility for dietary treatment recommended by the National Cholesterol Education Program.³³ One subject was treated with L-thyroxine 0.15 mg/d for primary hypothyroidism. Two subjects were treated throughout the entire study with antihypertensive medication, one with tramterene 50 mg and hydrochlorothiazide 25 mg daily and the other with hydrochlorothiazide 50 mg and amlodipine 5 mg daily. Subjects received no other medications known to affect lipid metabolism, and intake of ethanol was less than 30 mL/d for the month preceding each turnover study. All subjects had normal fasting concentrations of serum glucose, glutamic oxaloacetic transaminase, thyroxine, creatinine, calcium, albumin, sodium, potassium, chloride, and bicarbonate. Hematologic and urine analyses were normal. The experimental protocol

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Table 1. Characteristics of Postmenopausal Subjects

Subject No.	Age (yr)	Height (cm)	Weight (kg)	Cholesterol (mg/dL)		Triglycerides (mg/dL)		Lp(a) (mg/dL)	
				Control	Treated	Control	Treated	Control	Treated
1	38	163	65	255	248	88	86	12	13
2	68	176	78	182	178	138	148	3.4	2.6
3	65	165	60	238	213	181	188	23	20
4	61	161	78	283	232	292	224	6.2	3.8
5	71	180	52	232	244	120	120	18	18
6	71	166	67	221	205	112	120	33	27
Mean $\pm$ SE	61 $\pm$ 5	162 $\pm$ 3	67 $\pm$ 4	232 $\pm$ 12	220 $\pm$ 11	155 $\pm$ 30	151 $\pm$ 21	18 $\pm$ 5	14 $\pm$ 4

NOTE. Values for each subject are the mean of four fasting blood plasma samples obtained over 3 weeks during a low-fat, low-cholesterol diet for each control and treatment period.

approved by The Standing Committee on Human Research of The University of Western Ontario was explained to each subject, and informed consent was obtained in writing.

Subjects were maintained on moderately low-fat, low-cholesterol diets²⁴ that contained approximately 27% of energy from fat (mean ratio of polyunsaturated:monounsaturated:saturated fat, ~0.7:1.0:1.0; 150 mg cholesterol/d), 21% from protein, and 52% from carbohydrate. During hospitalization for the first day only of each turnover study, the diet was unchanged except that the carbohydrate content was increased at the expense of fat to maintain caloric intake. Fat intake was reduced to less than 5 g/d to eliminate the contribution of intestinal lipoproteins to plasma triglyceride-rich lipoproteins.²⁵

Samples of venous blood for determination of plasma total and HDL cholesterol, total triglyceride, and lipoprotein(a) [Lp(a)] concentrations were obtained from a forearm vein (after a 12-hour fast) from each subject on four occasions at approximately weekly intervals: (1) during the initial 3-week control period before initiation of HRT and (2) during HRT (weeks 3 to 6 of the 7-week course of MPA 2.5 mg/d + CEE 1.625 mg/d). Subjects underwent studies of the kinetics of VLDL and LDL apo B turnover during the control period and again during the last 16 days of treatment with MPA + CEE. All subjects received potassium iodide 300 mg/d for 3 days before and 13 days after resection of the radiolabeled lipoproteins.

Preparation of Labeled Lipoproteins and Kinetic Studies

Fasting blood plasma (1.5 mg EDTA/mL) for isolation of VLDL and LDL was obtained from each subject 6 days before each turnover study. Procedures used for preparation of labeled lipoproteins have been previously described.²⁶⁻²⁸ VLDL (S₁ 60-400) and LDL (S₁ 0-12) were radiolabeled with ¹²⁵I (Merck-Frost Canada, Point Claire-Dorval, Quebec) and ¹²⁵I (Amersham, Oakville, Ontario, Canada), respectively. Ninety-two percent of the radiolabel ¹²⁵I was bound to VLDL protein, 2% was associated with lipids, and 5% remained unbound; VLDL apo B accounted for 37% of ¹²⁵I bound to VLDL protein. Ninety-eight percent of the radiolabel ¹²⁵I was bound to LDL protein, 1% was associated with lipids, and 1% remained unbound; LDL apo B accounted for 92% of ¹²⁵I bound to LDL protein.

Experimental Protocol

Each recipient was fasted 12 hours before receiving serial bolus injections of ¹²⁵I-labeled VLDL 30 μ Ci and ¹²⁵I-labeled LDL 100 μ Ci in 2 to 3 mL normal saline via a hand or forearm vein. Venous blood samples (each 25 mL, 1.5 mg EDTA/mL) were obtained over 16 days after injection at 0.08, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72, 120, 216, 288, and 384 hours and placed on ice before immediate separation of plasma. Plasma VLDL (S₁ 60-400),

intermediate-density lipoprotein (IDL; S₁ 12-40), and LDL (S₁ 0-12) were fractionated,^{27,41} apo B was isolated from each lipoprotein fraction by isopropanol precipitation, and specific activity was determined as previously described.⁴²

Analysis

The structure of the kinetic model and equations used to calculate kinetic parameters have been described previously.²⁸ The principal assumptions of this model are that (1) apo B is an integral part of VLDL, IDL, and LDL, is nonexchangeable, and therefore provides an accurate measure of metabolism of these lipoproteins; (2) metabolism of the tracer is identical to that of the tracee; (3) production rate or flux of VLDL apo B represents input of apo B primarily into the larger, rapid-turnover, primary metabolic compartment (pool 1); (4) all apo B catabolism occurs from pool 1 either directly or after passage through pool 2; (5) all subpopulations of VLDL are isolated for labeling, and the initial injected activity is proportional to the mass of apo B in each population; (6) the two-pool model for LDL apo B is characterized by a plasma compartment and extravascular exchange compartment; and (7) at the time of injection all VLDL and LDL apo B radioactivity is introduced into the plasma compartment, and irreversible loss of LDL apo B occurs from the same compartment.

Specific-activity curves for apo B of reinjected VLDL and LDL were analyzed and kinetic parameters calculated as described in earlier studies in humans.^{27,28,43-45} This yielded values for flux rates through pool 1, irreversible fractional catabolic rate ((FCR) = k_A , assuming that catabolism from other pools is negligible),^{44,45} and mass in pool 1. The intravascular content of VLDL apo B was also calculated independently from the plasma concentration of VLDL apo B multiplied by the plasma volume taken as 45 mL/kg body weight.⁴⁶ The amount of LDL apo B derived from IDL apo B was calculated as the area under the ¹²⁵I-LDL apo B specific-activity curve divided by the area under the ¹²⁵I-IDL apo B specific-activity curve.^{28,47}

Plasma VLDL and IDL apo B concentrations were measured immunoturbidimetrically using a Tina-quant apo B kit obtained from Boehringer Mannheim Canada (Laval, Quebec). The assay was standardized to LDL isolated by ultracentrifugation (d 1.040 to 1.063) in which it was determined that apo B was the only protein present. Protein content was determined by the modified Lowry procedure.⁴⁸ Lp(a) level was measured using a Macra Lp(a) Kit obtained from Terasco Medical (Elkton, MD). Intraassay coefficients of variation for apo B and Lp(a) were 1.3% and 4.7%, respectively. Concentrations of cholesterol and triglycerides in the chloroform-methanol extract of whole plasma were determined as previously described,²⁸ except that diagnostic kits from Boehringer Mannheim Diagnostics, Montreal, Quebec (C-system Kit for cholesterol and Test Combination Kit for triglycerides), were used

Table 2. Effects of HRT on Cholesterol Levels (mg/dL) of Plasma Lipoproteins in Fasted Subjects During Turnover Studies

Subject No.	Whole Plasma		VLDL		IDL		LDL		HDL	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
1	252	252	1.0	3.0	18	21	178	162	64	68
2	174	178	8.9	8.9	17	22	113	104	35	43
3	238	201	17	16	24	27	180	113	35	48
4	263	217	20	13	42	28	162	141	39	35
5	247	248	8.0	2.1	18	12	148	140	74	83
6	208	187	3.0	2.8	12	16	185	134	39	43
Mean $\pm$ SE	220 $\pm$ 14	216 $\pm$ 12	11 $\pm$ 4	7.4 $\pm$ 2	23 $\pm$ 4	21 $\pm$ 2	151 $\pm$ 8	132 $\pm$ 9 ^a	48 $\pm$ 6	54 $\pm$ 8 ^a

NOTE: Values for each subject were based on fasting blood plasma samples obtained immediately before injection of autologous labeled ¹²⁵I-VLDL and ¹²⁵I-LDL at the beginning of each turnover study.

^aDiffers from control, $P < .025$.

^bDiffers from control, $P < .05$.

to measure cholesterol content of fasting plasma VLDL, IDL, and LDL and triglyceride content of VLDL during the turnover studies because of the small amounts of material available for assay. HDL cholesterol level was measured following precipitation with heparin-manganese chloride.²⁰

Values from control and treatment periods were compared using Student's paired two-tailed t test.²¹ Variance was expressed as standard error of the mean.

RESULTS

Concentrations of Lipoprotein Lipids and Apo B

The mean plasma concentration of cholesterol transported in plasma LDL (S₁ 0-12) decreased significantly by 12% $\pm$ 4% during HRT as compared with the control value ($P < .025$; Table 2). However, the mean concentration of plasma total cholesterol decreased by only 5% to 6% because of the significant 16% $\pm$ 6% (54 $\pm$ 9 v 46 $\pm$ 6 mg/dL, $P < .05$) increase in HDL cholesterol. There was a significant 19% $\pm$ 3% decrease in the mean ratio of total cholesterol to HDL cholesterol (4.2 $\pm$ 0.5 v 3.1 $\pm$ 0.5, $P < .005$, HRT v control) and a 26% $\pm$ 5% decrease in the ratio of conventional LDL (S₁ 0-20) cholesterol to HDL cholesterol (2.9 $\pm$ 0.4 v 3.8 $\pm$ 0.4, $P < .005$) during weeks 3 to 6 of HRT versus control conditions. The 10% $\pm$ 2% reduction in the mean cholesterol concentration of S₁ 0-20 lipoproteins during HRT versus control (149 $\pm$ 11 v 166 $\pm$ 11 mg/dL, $P < .02$) was similar to the 12% $\pm$ 4% reduction observed for S₁ 0-12 (Table 2). The mean concentration of Lp(a)¹³⁷ decreased by 9% $\pm$ 5% during HRT versus control (14 $\pm$ 4 v 16 $\pm$ 5 mg/dL, $P > .2$). The mean ratio of cholesterol to apo B of plasma LDL (S₁ 0-12) was significantly lower for HRT than for control by 15% $\pm$ 5% (1.3 $\pm$ 0.1 v 1.5 $\pm$ 0.1, $P < .025$), whereas the ratio of triglyceride to apo B was 18% $\pm$ 9% higher (0.31 $\pm$ 0.03 v 0.27 $\pm$ 0.03, .05 $< P < .10$). Mean values for the ratio of cholesterol to apo B in plasma VLDL or IDL were not significantly different during HRT versus control (2.4 $\pm$ 0.6 v 3.3 $\pm$ 1.2 and 1.4 $\pm$ 0.1 v 1.4 $\pm$ 0.1, respectively, $P > .2$), nor were those for the ratio of triglycerides to apo B in VLDL and IDL (29 $\pm$ 8 v 25 $\pm$ 5 and 2.5 $\pm$ 0.2 v 2.5 $\pm$ 0.4, respectively, $P > .4$). Mean values for the concentration of apo B in plasma VLDL, IDL, and LDL were similar during HRT versus control (2.2 $\pm$ 0.4 v 2.2 $\pm$ 0.3, 12 $\pm$ 1 v 12 $\pm$ 1, and 81 $\pm$ 5 v 76 $\pm$ 5 mg/dL, respectively; Table 3). The

intravascular content of the VLDL apo B pool calculated from the plasma concentration of VLDL apo B multiplied by estimated plasma volume yielded values that were less than those obtained for the mass of VLDL apo B in pool A, in agreement with results reported by Reardon et al.³⁷

The mean plasma concentration of triglycerides transported in lipoproteins of S₁ 0-12 during turnover studies was significantly greater during HRT versus control (30 $\pm$ 2 v 22 $\pm$ 3 mg/dL, $P < .05$). However, mean values for plasma total triglycerides (143 $\pm$ 20 v 155 $\pm$ 37 mg/dL), VLDL triglycerides (64 $\pm$ 16 v 83 $\pm$ 28 mg/dL), and triglycerides transported in lipoproteins of S₁ 12-60 (IDL, 34 $\pm$ 4 v 32 $\pm$ 8 mg/dL) were not different during HRT versus control ($P > .5$).

Consistent with a steady state, there were no systematic changes during turnover studies in plasma concentrations of the most readily quantified indices, namely total protein content of LDL (mean coefficient of variation of 8.1%, corresponding to a mean SD of 7.0 mg/dL, $n = 6$ paired studies) and total protein content of VLDL (mean coefficient of variation of 16%, corresponding to a mean SD of 1.3 mg/dL, $n = 6$ paired studies).

Metabolism of Lipoproteins

Values for specific activities of ¹²⁵I-labeled apo B in VLDL, IDL, and LDL over 72 hours after injection of

Table 3. Effects of HRT on Apo B Levels (mg/dL) of Plasma Lipoproteins in Fasted Subjects During Turnover Studies

Subject No.	VLDL ^a		IDL ^a		LDL ^b	
	Control	Treated	Control	Treated	Control	Treated
1	1.0	1.3	17	11	85	83
2	2.8	2.9	19	11	86	72
3	3.4	3.7	12	14	78	84
4	2.1	2.8	15	14	91	87
5	1.8	1.5	11	8.3	88	83
6	1.8	1.5	8.4	11	77	79
Mean $\pm$ SE	2.2 $\pm$ 0.3	2.2 $\pm$ 0.4	12 $\pm$ 1	12 $\pm$ 1	78 $\pm$ 5	81 $\pm$ 5

^aMean values based on plasma mass determined immunochemically on fasting specimens obtained daily during first 4 days of each turnover study.

^bMean values based on plasma mass determined by isopropanol precipitation on fasting specimens obtained daily during first 4 days of each turnover study.²⁰

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¹²⁵I-labeled VLDL during HRT versus control conditions for a representative subject are shown in Fig 1. Kinetic parameters of VLDL turnover calculated from ¹²⁵I-labeled VLDL apo B decay curves are listed in Table 4. During the treatment versus control periods, there were no significant changes in FCR, production rate, or pool size of VLDL apo B (*P* > .4).

Kinetic parameters of LDL turnover calculated from ¹²⁵I-labeled LDL decay curves are listed in Table 5. FCR of LDL apo B was increased significantly by an average of 13% ± 4% during HRT versus control (0.34 ± 0.03 v 0.30 ± 0.02 pools/d, *P* < .05). Figure 2 illustrates the effect of HRT on the percent decrease of ¹²⁵I-labeled LDL apo B specific activity from the plasma for a representative subject. Enhancement of LDL apo B catabolism was not accompanied by any systematic change in pool size of LDL apo B, because LDL apo B production rate also tended to increase, although not significantly, during HRT versus control (14 ± 0.5 v 13 ± 0.9 mg/kg/d, *P* > .1).

Evaluation of relationships³³ between lipoprotein fractions indicated that VLDL apo B was the sole precursor of IDL apo B (Fig 1). Comparisons of the areas under specific-activity curves for LDL apo B and IDL apo B, as described by Goldberg et al,⁴⁷ indicated that LDL production derived from plasma VLDL via IDL accounted for a similar proportion of total LDL apo B production during HRT versus control (30% v 29%, respectively). This is

Table 4. Kinetic Parameters of Human ¹²⁵I-VLDL Apo B Turnover in HRT-Treated Postmenopausal Subjects

Subject No.	FCR (pools/d)	Pool Size of VLDL Apo B (mg/kg) ^a	Production Rate (mg/kg/d)
1			
Control	13	1.5	20
Treated	17	2.0	34
2			
Control	8.0	2.8	13
Treated	8.8	2.6	15
3			
Control	2.7	4.8	12
Treated	2.4	5.4	12
4			
Control	5.9	4.1	25
Treated	6.1	2.4	15
6			
Control	9.7	2.3	22
Treated	6.1	1.4	12
8			
Control	12	1.7	20
Treated	8.6	1.2	10
Mean ± SE			
Control	8.1 ± 1.7	2.8 ± 0.5	19 ± 2
Treated	8.0 ± 2.0	2.5 ± 0.6	18 ± 4

^aBased on kinetic analysis of ¹²⁵I-labeled VLDL apo B.

^bBased on product of FCR and radiolabelled determination of VLDL apo B pool size.

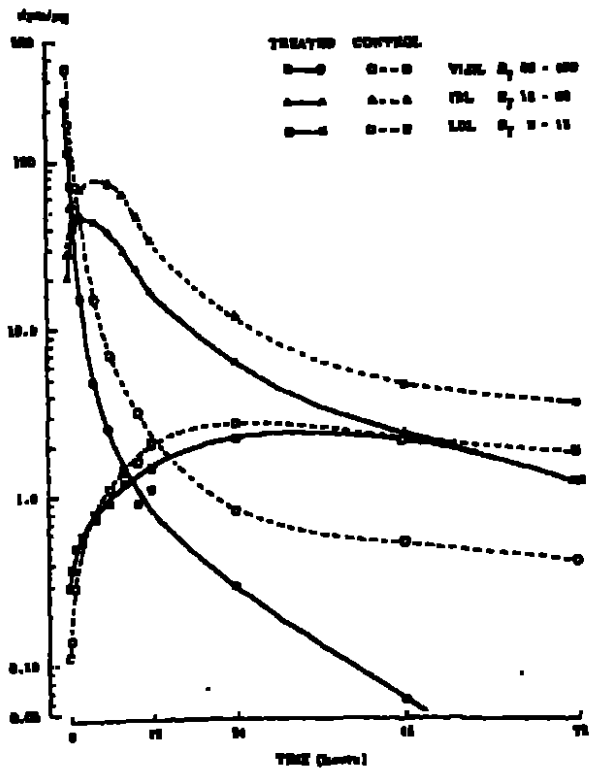


Fig 1. Specific activities of apo B of VLDL, IDL, and LDL after intravenous injection of autologous ¹²⁵I-labeled VLDL during control period and HRT for a representative subject.

consistent with a substantial portion of LDL entering the circulation directly⁴⁷ and/or via small, rapid-turnover, hepato-genous pool(s) of VLDL apo B,³⁴ which is not traceable with exogenous iodine labeling.⁴⁷ The present experiments could not distinguish relative contributions of the latter pathways.

DISCUSSION

The present studies are the first to investigate effects on composition and turnover of VLDL and LDL that result from administration of this recently recommended regimen of HRT.³² The 10% ± 2% mean reduction in the mean plasma concentration of lipoproteins of S₁ 0-20 during HRT in the present short-term study is similar to that observed during a 1-year treatment with a similar HRT regimen in a much larger group of subjects.³² Short-term studies using CEE 0.625 mg/d alone have also shown similar reductions in LDL cholesterol.³³⁻³⁵ The present study shows that HRT alters the composition of LDL (S₁ 0-12) by significantly reducing its cholesterol content. The trend toward an enrichment of triglyceride content of LDL (S₁ 0-12) at the expense of cholesterol with the present HRT is consistent with changes observed during daily administration of CEE 0.625 mg alone³³⁻³⁵; however, the effect on LDL particle size was not determined in the present study. Treatment with estrogen alone has also been associated with a decrease in the proportion of large LDL particles and an increase in the proportion of smaller LDL particles in postmenopausal women.^{36,37} This has been more pronounced in subjects with a preponderance of large LDL particles at baseline.³⁸ Low intakes of saturated fat and cholesterol, widely used to

Table 5. Kinetic Parameters of Human ^{125} I-LDL Apo B Turnover in HRT-Treated Postmenopausal Subjects

Subject No	PCR (pools/d)	LDL Apo B (mg/kg)	Production Rate (mg/kg/d)			Fraction of LDL Apo B Production Derived From LDL Apo B (%) ^a
			Total	Direct	Indirect	
1						
Control	0.23	42	9.6	6.4	3.2	33
Treated	0.27	80	13	7.7	5.4	41
2						
Control	0.26	30	11	8.5	2.4	22
Treated	0.43	28	12	9.1	2.9	24
3						
Control	0.25	48	12	10	2.2	18
Treated	0.29	48	14	11	2.2	18
4						
Control	0.34	45	16	9.7	6.7	37
Treated	0.43	36	15	12	3.5	23
5						
Control	0.31	49	16	9.9	5.4	36
Treated	0.29	32	15	12	3.6	24
6						
Control	0.31	42	13	9.2	3.8	30
Treated	0.36	36	13	7.0	5.7	45
Mean $\pm$ SE						
Control	0.30 $\pm$ 0.02	43 $\pm$ 3	13 $\pm$ 0.5	9.0 $\pm$ 0.5	3.9 $\pm$ 0.5	29 $\pm$ 3
Treated	0.34 $\pm$ 0.03 ^b	42 $\pm$ 4	14 $\pm$ 0.5	8.8 $\pm$ 0.9	3.9 $\pm$ 0.5	29 $\pm$ 5

^a Calculated according to method reported by Goldberg et al.⁴⁷^b Different from control, $P < .05$.

decrease the risk for CHD, have also been associated with reductions in LDL particle size. Hence, a small LDL particle size appears not to be a good indicator of atherothrombotic risk, at least in population studies.⁴²

Despite the change in lipoprotein composition with the present HRT, there was no change in apo B pool size of LDL (S_1 0-12; Table 4). HRT appeared to induce a change in the compositional pattern of lipoprotein particles of S_1 0-12; however, the present experimental design did not allow for analysis of LDL subfractions, and heterogeneity within LDL subfractions could obscure metabolic changes occurring in response to treatment. Although an increase in direct synthesis of LDL could conceivably result in changes in LDL composition, we found no statistically significant increase in LDL production via this pathway, although there were increases in five of six subjects (Table 5). HRT, like obesity,⁴³ increased PCR of LDL (Table 5); however, this has not been related to alteration of particle size in humans. While we acknowledge the uncertainty about the effect of gender on LDL kinetics, it is noteworthy that the mean control value for total production rate of LDL apo B obtained in women by the present kinetic analysis was similar to that obtained in non-obese men by multicompartmental analysis.⁴⁴

HRT induced increases in mean PCR of LDL apo B and total LDL apo B production (Table 4) that were less than half those reported with estrogen alone.⁵⁰ Some differences between these results may be accounted for by differences between subjects studied and estrogen type and dosage (CEE 0.625 mg v estradiol-17 β 2 mg) or by the present addition of low-dosage MPA, a C-21 progestin. The proges-

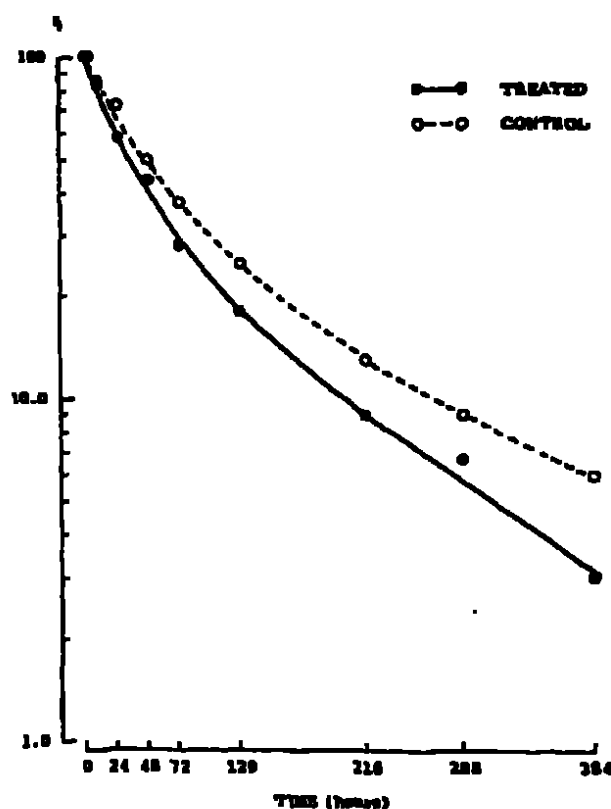


Fig 2. Specific radioactivity of LDL apo B expressed as % of peak specific activity after intravenous injection of autologous ^{125} I-labeled LDL for a representative subject.

tin effect on lipids has been related to the type of progestin (C-21 v C-19).⁴⁴ The present HRT decreased neither the pool size nor the production rate of LDL apo B, whereas continuous low-dosage DL-norgestrel (0.075 mg/d), a C-19 progestin, combined with estradiol-17 β 1 mg (25 of 28 days) decreased both LDL production and LDL pool size.³⁴

Although continuous administration of high-dose MPA (10 mg/d) abolishes the increment in HDL cholesterol induced by a daily dose of CEE 0.625 mg,²⁴ continuous administration of daily low-dose MPA (2.5 mg) combined with CEE 0.625 mg preserves the beneficial effects of CEE on plasma lipoproteins. The findings of a significant (16%) increase in HDL cholesterol and a significant (10%) decrease in LDL cholesterol during the present short-term HRT with MPA + CEE are consistent with results obtained in studies of 1 year's duration.^{24,45} The reported mean increments in HDL cholesterol with MPA + CEE range from 6% to 16%, versus 7% to 19% with CEE 0.625 mg alone.^{27,29,47} Mean reductions in LDL cholesterol with MPA + CEE range from 10% to 29%, versus 15% to 16% with CEE alone.^{27,29,47}

The addition of progestin to estrogen may have a beneficial effect on VLDL metabolism by modulating the action of estrogen thereon. Whereas administration of estradiol alone in another study of postmenopausal women increased VLDL apo B production, VLDL pool size, and plasma triglyceride concentration,³⁰ the present combined low-dose CEE + MPA (C-21 progestin) had no apparent adverse effects on VLDL metabolism (Table 4). The nature of the progestin (C-19 v C-21) coadministered with the estrogen also appears to influence the effect of the estrogen on VLDL metabolism. Whereas MPA + CEE had no significant effect on metabolic parameters of VLDL metabolism (Table 4), combined administration of low-dosage DL-norgestrel (C-19 progestin) and estradiol-17 β to postmenopausal women decreases VLDL pool size and plasma total triglyceride concentration by increasing FCR of VLDL.³⁰

Both the increase in HDL cholesterol and the decrease in cholesterol content of LDL underlie significant reductions

in mean values for the ratios plasma total cholesterol to HDL cholesterol and LDL (5- to 20) cholesterol to HDL cholesterol from ≥ 75 th percentile (ie, 5.2 and 3.5, respectively) during the control period to near the 50th percentile (ie, 4.2 and 2.7, respectively⁴⁸) during HRT. Estrogen-induced increases in HDL cholesterol concentration have been attributed to increased synthesis of apo A1⁴⁹ and/or reduced catabolism.⁵⁰

Thyroid hormone influences lipid metabolism, but long-term use in a physiologic dose for thyroid replacement in subject no. 2 would not be expected to perturb lipid metabolism. Hydrochlorothiazide can increase serum cholesterol⁵¹; however, any potential effect on lipid metabolism in subjects no. 4 and 6, who were treated with hydrochlorothiazide, was minimized by maintaining the same dosage during both control and treatment periods of each experiment. The sample size of the present study was too small to investigate a possible effect of hydrochlorothiazide; nevertheless, there was no indication of any such effect. To eliminate the contribution of intestinal lipoproteins to plasma triglyceride-rich lipoproteins during the period of frequent sampling on day 1, fat intake was reduced to less than 5 g/d.³⁵ The same dietary protocol was used during both the control and experimental periods of the study to minimize any potential effect on lipoprotein metabolism. In addition, lipoprotein concentrations remained steady throughout the turnover study.

In summary, the present combined low-dose MPA + CEE regimen significantly altered the composition and fractional catabolism of LDL and favorably influenced the overall plasma lipoprotein lipid vascular risk profile.⁵²

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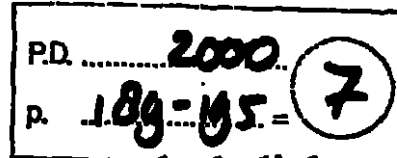


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Serum level of vascular endothelial growth factor is decreased by hormone replacement therapy in postmenopausal women without hypercholesterolemia

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Abstract

The administration of hormone replacement therapy (HRT) to postmenopausal women (PMW) reportedly has beneficial effects on their levels of lipid and lipoproteins. Estrogen retards the development of atherosclerosis induced by a high-fat diet in animals. Although vascular endothelial growth factor (VEGF) may be involved in the development of atherosclerosis in humans, there is no information on effect of estrogen administration on VEGF level and lipid metabolism. We evaluated 64 healthy normotensive or hypertensive PMW before and during the administration of HRT (0.625 mg conjugated equine estrogen combined with 2.5 mg medroxyprogesterone acetate orally) daily for 6 months. All hypertensive PMW were well-controlled on antihypertensive drug therapy. According to their total cholesterol level at baseline, we divided the PMW with HRT ($n = 54$) into a normocholesterolemic group (NC, total cholesterol < 220 mg/dl, $n = 35$) and a hypercholesterolemic group (HC, total cholesterol ≥ 220 mg/dl, $n = 19$). We evaluated the serum levels of VEGF at baseline, and again at 3 and 6 months after starting HRT. HRT significantly ($P < 0.01$) reduced the mean VEGF level from 31.5 ± 4.3 pg/ml at baseline to 18.2 ± 2.3 pg/ml after 6 months in the NC group. However, the VEGF levels in the HC group and the control group exhibited no significant change at either 3 or 6 months after starting HRT. In summary, HRT, using a combination of conjugated equine estrogen and medroxyprogesterone acetate, reduced the level of VEGF in normocholesterolemic PMW more effectively than in those with hypercholesterolemia.

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Keywords: Vascular endothelial growth factor; Estrogen; Hormone replacement therapy; High-density lipoprotein; Low-density lipoprotein; Postmenopausal women

1. Introduction

It is well established that the incidence of cardiovascular diseases increases in women after menopause [1]. Most epidemiologic studies have demonstrated that estrogen replacement therapy (ERT) markedly reduces the risk of cardiovascular disease in postmenopausal women (PMW) [2,3]. Although the cardioprotective benefits of estrogens are well established, the mechanism remains unclear and is currently the subject of

intense investigation. It was recently suggested that this cardioprotective effect may result from the beneficial effect of estrogen on the plasma concentrations of lipid, lipoproteins [4], hemostatic factors [4,5], glucose [5], nitric oxide (NO) [6], endothelin-1 [6], and insulin [5] and by reducing the arterial blood pressure [5]. However, these factors alone fail to explain the beneficial effects of estrogens on the cardiovascular system.

Vascular endothelial growth factor (VEGF) [7] is a 45-kD heparin-binding growth factor that is also known as the vascular permeability factor [8,9]. Studies performed in vitro [10] and in vivo [11] established that VEGF is mitogenic for endothelial cells. VEGF is

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extensively expressed in arteries that have been narrowed by atherosclerotic plaque [12]. Inoue et al. [13], using the reverse transcription-polymerase chain reaction, demonstrated that VEGF mRNA is present in atherosclerotic, but not in normal coronary arteries. This evidence suggests that VEGF may be involved in the pathogenesis of atherosclerosis in humans.

Oxidized-low density lipoprotein (LDL) is abundant in the walls of atherosclerotic arteries, and appears to augment the expression of VEGF mRNA in a dose-dependent manner [13]. Dulak et al. [14] demonstrated that modified LDL inhibits inducible NO synthase and NO production. Those authors also demonstrated that oxidized-LDL inhibits interleukin-1 and induces VEGF gene expression in the vascular smooth muscle cells of rats.

Since estrogen retards the development of atherosclerosis induced by a high-fat diet in ovariectomized monkeys [15], a decrease in estrogen levels may stimulate the development of atherosclerosis in PMW [16]. The progression of atherosclerosis after menopause also showed a close correlation with levels of total cholesterol and of low-density lipoprotein (LDL) cholesterol [17]. We therefore considered that the VEGF level might be influenced by the presence of lipid disorders in subjects receiving hormone replacement therapy (HRT). Because VEGF may be involved in the atherosclerotic process in humans, we hypothesized that the estrogen-induced cardioprotection might be related to the changes in VEGF expression by the vascular endothelium induced by estrogen.

This study was designed to investigate the effects of administering HRT to PMW on the serum VEGF, as a factor involved in the atherosclerotic process in humans.

2. Materials and methods

2.1. Subjects

A total of 64 Japanese PMW with the climacteric syndrome (aged 46–66, mean 56.7 ± 0.7 years), and without any evidence of gynecologic disorders, volunteered to participate in this study (Table 1).

Each woman was a normotensive or hypertensive individual, attending the Cardiovascular Hospital of Central Japan. Each subject had been amenorrheic for 1 year or longer, and had a plasma level of estrone (E1) and of estradiol (E2) of < 40 pg/ml, and a plasma level of follicle-stimulating hormone (FSH) of > 30 mIU/ml. None had received HRT for at least 3 months before enrollment, or had contraindication to such treatment. The hypertensive PMW continued to receive antihypertensive medications during the study. All were non-smokers.

None of the subjects had a history of diseases such as diabetes mellitus, chronic liver disease, atherosclerosis, cardiovascular or cerebrovascular disease, or thrombosis. Subjects were also excluded if they had experienced any clinically relevant illness during the 3 weeks preceding the 1st day of study, or when an abnormality considered to be clinically relevant by the investigator was found in the screening examination. Drop-out criteria were occurrence of any disease, genital bleeding of undiagnosed cause, oncotic colpocytology, any findings that would suggest the presence of malignant disease of the breast, presence of known or suspected estrogen-dependent tumors or fibroids, alcoholism, the Rotor or Dubin-Johnson syndrome, severe disorders of the liver or kidney, endometrial hyperplasia, and severe hypertension. Non-compliance with the study protocol was a final criterion for exclusion.

Table 1
Characteristics of control postmenopausal women (PMW) and PMW administered hormone replacement therapy^a

Characteristics	Control (range)	PMW with NC (range)	PMW with HC (range)
Number (n)	10	35	19
Age (years)	57.2 ± 2.1 (49–66)	57.0 ± 0.9 (46–65)	55.8 ± 1.3 (48–65)
Body height (cm)	151.2 ± 1.6 (145–159)	153.0 ± 0.8 (143–163)	151.9 ± 1.1 (142–160)
Body weight (kg)	55.1 ± 3.0 (32.4–62.8)	55.3 ± 1.3 (43.9–73.6)	54.8 ± 2.0 (38.1–76.6)
Complication (n)			
Hypertension	6	23	14
Antihypertensive drugs (n)			
ACE inhibitor	2	9	5
Calcium antagonist	3	11	8
β -Blocker	1	5	3
Diuretic	0	2	0

^a HC, hypercholesterolemia; NC, normocholesterolemia. Values are mean $\pm$ S.E.M.

The women enrolled in the study had similar lifestyles and dietary habits. They were instructed to avoid making any change in dietary habits during the investigation. The study protocol was approved by the Institutional Committee on Human Research of the Cardiovascular Hospital of Central Japan. Written informed consent was obtained from each subject. The participants consisted of 43 hypertensive and 21 normotensive PMW. Blood pressure was well controlled by antihypertensive medications in all hypertensive subjects. The following drugs were administered: calcium antagonist (22), angiotensin-converting enzyme inhibitor (16), β -blocker (nine), and furosemide (two). None of the normotensive subjects were receiving any medication. The PMW were divided into 54 with HRT and ten without HRT. The PMW with HRT were subdivided into two groups according to the level of total cholesterol at baseline: normocholesterolemic (NC) (total cholesterol < 220 mg/dl) group ($n = 35$) and hypercholesterolemic (HC) (total cholesterol $\geq$ 220 mg/dl) group ($n = 19$).

2.2. Protocol

Except for the controls, each subject received a daily dose of HRT (0.625 mg conjugated equine estrogen (Asahikasei, Osaka, Japan) combined with 2.5 mg medroxyprogesterone acetate (Sumitomo, Tokyo, Japan)) orally for 6 months. Subjects attended the HRT clinic at the Cardiovascular Hospital of Central Japan for physical check-ups, and to provide blood samples at baseline and then again at 3 and 6 months after starting HRT. The control subjects similarly attended the Cardiovascular Hospital of Central Japan.

Blood samples were taken in the morning after 12-h fast. Blood was drawn from an antecubital vein with an 18-gauge needle. Serum was separated by centrifuging the samples at 4°C for 10 min at 800 $\times$ g. The samples were stored at -80°C until assayed.

2.3. Physical examination

Resting blood pressure was measured after the subject had been seated for at least 30 min. Blood pressure in the patient's right arm was read three times by two investigators using a mercury sphygmomanometer. The mean of three measurements was used in this study.

2.4. Laboratory tests

The concentrations of total cholesterol and of triglyceride were determined by enzymatic methods, with the use of an automatic analyzer (Beckmann Synchron CX 7, Brea, CA). Concentrations of high-density lipoprotein (HDL) cholesterol were determined by an electrophoretic method, using the HDL Cholesterol Supply

Kit (Helena Laboratory, Beaumont, TX). The concentration of low-density lipoprotein (LDL) cholesterol was calculated using the Friedwald formula. The plasma levels of FSH, E1, and E2 were analyzed by radioimmunoassay using a commercially available kit (Boehringer Mannheim, Germany).

2.5. Measurement of VEGF

A VEGF assay was performed using a Sandwich Enzyme Immunoassay kit for human VEGF (IBL, Fujioka, Japan) according to the manufacturer's instructions. Briefly, 50- μl samples were diluted in 100 μl of buffer solution and serially diluted standard solution; recombinant human VEGF 165 (R&D Systems, Minneapolis, MN) was added to each well of the 96-well microtitre plate coated with mouse anti-human VEGF IgG 16F1 monoclonal antibody (IBL) and incubated for overnight at 4°C. For dilution, PBS containing 1% bovine serum albumin and 0.05% Tween was used. After washing the wells seven times with PBS, 100 μl of horseradish peroxidase (TOYOBO, Osaka, Japan)-conjugated Fab' of the affinity-purified rabbit polyclonal anti-human VEGF IgG (IBL) diluted with the buffer was added to each well and was incubated for 30 min at 37°C. Wells were washed nine times with PBS, then the enzyme reaction was carried out at room temperature for 30 min with Tetra Methyl Benzidine (Sigma, St. Louis, MO) with 0.01% hydrogen peroxide. The chemiluminescence of the wells was measured at 450 nm of absorbance by a plate luminometer (Immunoreader, Molecular Devices, Sunnyvale, CA), and the VEGF contents of the samples were estimated from the standard curve determined from the serially diluted standard VEGF solution. Linear range of this enzyme linked immunosorbent assay (ELISA) is from 7.8 to 500 pg/ml. There was no cross-reaction with human PDGF, human ET-1, human Big ET-1, human IL-8, human HGF, or human ET-3. The interassay coefficient of variation (CV) was 3.8%, and the interassay CV was 5.1%. For data processing, we allocated the minimum value detected by ELISA (< 0.009 ng/ml) to all samples with concentrations below the threshold of detection.

2.6. Statistical analyses

Data are reported as the mean $\pm$ S.E.M. One-way analysis of variance (ANOVA) was used to analyze the differences between the values of age, body height and body weight in the control, the PMW with NC and the PMW with HC (Table 1). The chi-square test and Fisher's probability test were also used to compare the incidence of the complication of hypertension or the combination of antihypertensive drugs (Table 1). Two-way analysis of variance (ANOVA) and Scheffe's *F*-test were used to analyze the differences between values

Table 2

Changes in blood pressure and heart rate in control postmenopausal women (PMW) and PMW with normocholesterolemia (NC) and hypercholesterolemia (HC) before and after starting the protocol^a

Characteristic	Control (n = 10)	PMW with NC (n = 35)	PMW with HC (n = 19)
SBP (mmHg)			
Baseline	129 ± 6	127 ± 2	131 ± 4
After 3 months	128 ± 6	127 ± 2	131 ± 5
After 6 months	127 ± 5	129 ± 3	130 ± 4
DBP (mmHg)			
Baseline	81 ± 4	80 ± 2	81 ± 3
After 3 months	82 ± 3	80 ± 1	82 ± 3
After 6 months	83 ± 3	80 ± 1	80 ± 4
Heart rate (beats/min)			
Baseline	64 ± 3	65 ± 2	63 ± 2
After 3 months	67 ± 2	64 ± 1	64 ± 2
After 6 months	65 ± 2	66 ± 1	63 ± 1

^a DBP, diastolic blood pressure; SBP, systolic blood pressure. Values are mean ± S.E.M.

recorded at baseline and again at 3 and 6 months (Tables 2–4; Fig. 1). A level of $P < 0.05$ was accepted as statistically significant.

3. Results

3.1. Study population

The characteristics of the study population appear in Table 1. Before treatment, there were no significant differences in age, body height, body weight, blood pressure, heart rate, existence of hypertension, and drug regimen between the control, NC and HC groups. Furthermore, no significant differences were observed in the blood pressure and heart rate between the three groups at 3 or 6 months after starting HRT (Table 2).

The baseline plasma levels of FSH, E1, and E2 were consistent with a postmenopausal state (Table 3). There were no significant differences in baseline FSH, E1 and E2 values between the three groups.

The plasma levels of FSH were significantly decreased compared with the baseline levels at 3 and 6 months after starting HRT in all but the control group. In contrast, the plasma levels of E1 and E2 were significantly increased compared with baseline levels 3 and 6 months after starting HRT in all but the control group. These findings indicate the good compliance of the participants to the HRT regimen.

3.2. VEGF profile

Serum VEGF levels at baseline, 3 and 6 months after starting HRT in the three groups are presented in Fig. 1. The baseline serum VEGF did not differ significantly among the three groups. The control group exhibited no significant change in serum VEGF during the study. However, the serum VEGF in the NC group was

significantly reduced by HRT from 31.5 ± 4.3 pg/ml at baseline to 24.4 ± 3.3 pg/ml 3 months after starting HRT and to 18.2 ± 2.3 pg/ml 6 months after starting HRT. However, the administration of HRT had no significant effect on serum VEGF in the HC group.

3.3. Lipid profile

The control group showed no significant change in the level of HDL or LDL cholesterol during the study (Table 4). The values for total and LDL cholesterol in the HC group at baseline significantly exceeded those in the NC group. The HRT increased the HDL cholesterol level and reduced the level of LDL cholesterol significantly in the NC group after 3 and 6 months as compared with baseline. Administration of HRT to the HC group also significantly increased the HDL cholesterol level after 3 and 6 months, and significantly reduced the LDL cholesterol level after 3 months, as compared with the baseline. The LDL cholesterol level in the HC group returned to baseline 6 months after the start of HRT.

4. Discussion

Our study showed that in PMW with normocholesterolemia, HRT reduced the serum levels of VEGF and LDL cholesterol, and increased those of HDL cholesterol. On the other hand, in PMW with hypercholesterolemia, HRT increased the serum level of HDL cholesterol, but did not reduce the serum levels of VEGF or LDL cholesterol.

Favorable changes in lipoprotein have been demonstrated in observational studies as well as clinical trials that evaluated the effects of exogenous estrogen on cardiovascular risk factors in women [3]. These favorable changes include an increase in HDL cholesterol and a decrease in LDL cholesterol. In the PHPI Trial [18], the

women randomized to receive conjugated equine estrogen and continuous or cyclical medroxyprogesterone acetate had smaller increases in HDL cholesterol levels from baseline than the women who received unopposed estrogen. Walsh et al. [3] have demonstrated an increase in HDL cholesterol and triglyceride levels and a decrease in LDL cholesterol and total cholesterol levels in PMW following treatment with equine estrogen.

In the present study, similar effects on lipoproteins were found in PMW with normocholesterolemia. In the NC group, the level of HDL cholesterol increased and the level of LDL cholesterol decreased. In the HC groups, the LDL cholesterol level also decreased 3 months after starting HRT, although it returned to the baseline pretreatment level 6 months after starting HRT. However, the levels of total cholesterol and triglyceride did not change significantly in either NC or HC groups after the start of HRT. From its effects on lipoprotein metabolism, HRT may exert a greater cardioprotective effect in PMW with normal, rather than with high, cholesterol levels.

Pathological analyses of specimens of human tissue have shown that postnatal VEGF expression is observed in arteries narrowed by atherosclerotic plaque [12]. A recent study [13] demonstrated the expression of VEGF in the atherosclerotic, but not in normal coronary arteries of humans. Activated endothelial cells in early atherosclerotic lesions with hypercellularity and in advanced atherosclerotic lesions with atheromatous plaques showed VEGF expression. Accumulated macrophages and de-differentiated smooth muscle cells also showed VEGF expression. This evidence suggests that VEGF could be involved in the atherosclerotic process in humans.

Angiographic study [19] has demonstrated a positive relationship between the presence of coronary artery disease and the serum levels of lipoprotein cholesterol.

Therefore, many researchers have speculated that treatment to reduce cholesterol levels may slow the progression of the coronary atherosclerotic lesion [20,21]. Oxidized-LDL is abundant in the atherosclerotic arterial wall and augments the expression of VEGF mRNA in a dose-dependent manner [13]. Such oxidized LDL inhibits IL-1 and induces VEGF gene expression in rat vascular smooth muscle cells [13] and in macrophage [22]. Thus, we believe that the levels of VEGF are influenced by concomitant lipid disorders. Hence, we divided our subjects with HRT into two groups, NC and HC, according to the cholesterol level at baseline. We found that the levels of VEGF and LDL cholesterol in the NC group were decreased by HRT.

It was recently shown that VEGF expression is regulated by estrogenic hormones [23,24]. This regulation could affect such physiological and pathological processes as the regeneration of the endometrium following menstruation, and the growth of hormone-responsive tumors, and could exert an even more generalized effect on the endothelium throughout the vascular system. This is an increasingly important area because of the widespread clinical use of estrogenic and antiestrogenic drugs. However, it is not clear how HRT affects VEGF in the vascular endothelium. We suggest that HRT with a combination of conjugated equine estrogen and medroxyprogesterone acetate reduces the level of VEGF, which could be involved in the atherosclerotic process in PMW. Hyder et al. [25] have reported that uterine expression of VEGF is increased by estradiol and tamoxifen. Therefore, HRT, using a combination of estrogen and progesterone in the present study, may have increased the VEGF in uterus. However, serum levels of VEGF were reduced by HRT in NC group. Thus, a source of VEGF other than in uterus should be more important in a decrease in serum levels of VEGF. Moreover, in PMW with normocholesterolemia, such combined HRT may

Table 3

Hormonal changes in control postmenopausal women (PMW) and in PMW with normocholesterolemia (NC) and hypercholesterolemia (HC) before and after starting the protocol^a

Characteristic	Control (n = 10)	PMW with NC (n = 35)	PMW with HC (n = 19)
FSH (mIU/ml)			
Baseline	65 ± 5	63 ± 5	61 ± 5
After 3 months	68 ± 7	21 ± 3**	24 ± 4**
After 6 months	72 ± 5	23 ± 16**	29 ± 4**
E1 (pg/ml)			
Baseline	14 ± 1	12 ± 1	13 ± 1
After 3 months	12 ± 1	79 ± 7**	98 ± 10**
After 6 months	14 ± 2	97 ± 8**	112 ± 17**
E2 (pg/ml)			
Baseline	12 ± 1	13 ± 2	12 ± 1
After 3 months	13 ± 2	76 ± 6**	87 ± 9**
After 6 months	12 ± 2	77 ± 6**	77 ± 11**

^a E1, estrone; E2, estradiol; FSH, follicle-stimulating hormone. Values are mean ± S.E.M.

** $P < 0.001$ compared with baseline.

Table 4

Changes in lipid for control postmenopausal women (PMW) and PMW with normocholesterolemia (NC) and hypercholesterolemia (HC) before and after starting the protocol^a

Characteristic	Control (n = 10)	PMW with NC (n = 35)	PMW with HC (n = 19)
Total cholesterol (mg/dl)			
Baseline	212 ± 10	193 ± 4	231 ± 6 [*]
After 3 months	212 ± 9	189 ± 4	218 ± 7
After 6 months	207 ± 6	187 ± 3	237 ± 7
Triglyceride (mg/dl)			
Baseline	126 ± 12	116 ± 14	131 ± 21
After 3 months	121 ± 22	128 ± 12	134 ± 17
After 6 months	123 ± 14	119 ± 13	135 ± 20
HDL-cholesterol (mg/dl)			
Baseline	46 ± 4	44 ± 2	51 ± 3
After 3 months	42 ± 3	49 ± 2 ^{***}	56 ± 3 [*]
After 6 months	44 ± 5	53 ± 3 ^{**}	60 ± 3 ^{**}
LDL-cholesterol (mg/dl)			
Baseline	141 ± 11	125 ± 4	155 ± 6 [*]
After 3 months	145 ± 11	114 ± 4 ^{**}	136 ± 6 [*]
After 6 months	138 ± 7	110 ± 3 ^{***}	151 ± 7

^a HDL, high density lipoprotein; LDL, low density lipoprotein. Values are mean ± S.E.M.

^{*} $P < 0.05$;

^{**} $P < 0.01$;

^{***} $P < 0.001$ compared with baseline.

^{*} $P < 0.05$ compared with NC at baseline.

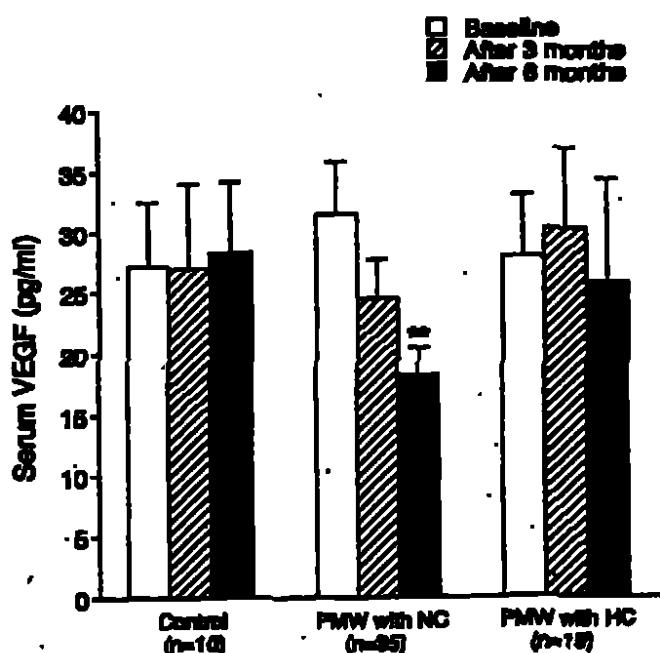


Fig. 1. Changes in serum vascular endothelial growth factor (VEGF) levels in control postmenopausal women (PMW) and in PMW with normocholesterolemia (NC) and hypercholesterolemia (HC) at baseline and at 3 and 6 months after the initiation of study. Open, oblique, and closed squares show the serum level of VEGF at baseline and at 3 and 6 months after starting the study, respectively. Data are expressed as means ± S.E.M. ** $P < 0.01$ versus baseline (ANOVA).

reduce the level of VEGF more effectively than it does in PMW with hypercholesterolemia.

In conclusion, HRT, using a combination of conjugated equine estrogen and medroxyprogesterone acetate reduced the serum level of VEGF more effectively in PMW who are normocholesterolemic than in PMW who are hypercholesterolemic.

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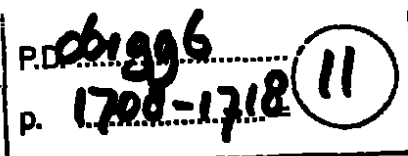
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Effects of estrogen and progestin on aortic size and compliance in postmenopausal women

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OBJECTIVE: Our purpose was to determine whether sex steroids alter aortic size and compliance in postmenopausal women.

STUDY DESIGN: Twenty-six postmenopausal women were randomized to receive either conjugated estrogens 0.625 mg per day (group 1) or conjugated estrogens 0.625 mg per day and medroxyprogesterone 2.5 mg per day (group 2). Aortic cross-sectional area was measured by magnetic resonance imaging before and after 3 months of hormone therapy.

RESULTS: Estradiol levels increased in both group 1 and group 2 ($p < 0.0001$). Ascending aortic cross-sectional area increased from $439 \pm 7 \text{ mm}^2$ to $466 \pm 7 \text{ mm}^2$ in group 1 ($p < 0.006$) but was unchanged in group 2. Within the range of aortic pressures studied, no change in aortic compliance could be detected.

CONCLUSION: Estrogen therapy in postmenopausal women was associated with an increase in aortic size; but this effect was not detectable with the addition of progestin. The potential antagonistic effect of progestin on estrogen-induced aortic enlargement suggests that the favorable cardiovascular effects of postmenopausal estrogen therapy cannot be automatically extended to the combination estrogen-progestin. (Am J Obstet Gynecol 1998;174:1708-18.)

Key words: Estrogen, progestin, aorta, menopause

Several studies have demonstrated that estrogen and progesterone have important effects on the female cardiovascular system during the reproductive years.¹⁻⁴ Estrogen and progesterone increase blood volume⁵ and red blood cell mass.^{6,7} Estrogen increases vascular capacitance and vascular compliance.⁸ In addition, progesterone but not estrogen increases left ventricular mass⁹ and estrogen increases left ventricular chamber size.^{3,4} Menotropin-induced increase in estrogen has been associated with increased left ventricular size in infertile women undergoing ovulation induction.¹⁰ Through these actions estrogen and progesterone are thought to play an important role in the cardiovascular adaptations to pregnancy.

Less is known about the effects of the sex steroids, estrogens or progestins, on the cardiovascular system of older women. Postmenopausal women have a substantial increase in the incidence of myocardial infarction,¹¹ and there is a decrease in the risk of coronary artery disease in

postmenopausal women receiving estrogen therapy.^{12,13} Although important, the favorable change in the lipid profile alone does not account for all the beneficial cardiovascular effects of estrogen therapy.^{14,15} Other mechanisms contribute to this reduction in risk of coronary heart disease. The aorta and its branches become less compliant or stiffer with age.^{16,17} After menopause this stiffening process may progress more rapidly. Acceleration of atherosclerosis¹⁸ and decreased aortic compliance after menopause may contribute to the development of hypertension. Furthermore, the reduction in aortic compliance coupled with increasing systemic vascular resistance with advancing age increases the systolic load of the heart.

The aorta is a potential target for an estrogen effect because estrogen receptors have been demonstrated in aortic tissue of several species.^{19,20} During human²¹ and guinea pig pregnancy,²² when estrogen and progesterone levels are elevated, the aorta is larger and more compliant. The high levels of these sex steroids seen during pregnancy could alter collagen synthesis, allowing remodeling of the aorta resulting in aortic enlargement. Conceivably the converse may be true (i.e., estrogen and progesterone withdrawal at menopause may result in a decrease in aortic size and aortic compliance). This study therefore asks if sex steroid administration in postmenopausal women could alter aortic size or compliance. The specific goal of this study was to determine whether estro-

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gen treatment or the combination of estrogen and progesterin treatment could increase thoracic aortic size and aortic compliance in postmenopausal women.

Material and methods

Postmenopausal women ($n = 28$) not receiving estrogen therapy were recruited from the Portland, Oregon, community. The study protocol was approved by the Institutional Human Research Committee at Oregon Health Sciences University. Women with historic evidence of coronary artery disease or valvular heart disease were not included. All women were enrolled at least 1 year after their last menstrual period. For the purpose of this study the postmenopausal state was defined as having had a bilateral salpingo-oophorectomy or natural menopause and a 17β -estradiol level of <25 pg/ml. After informed consent was obtained, women were randomly allocated to group 1, which received conjugated estrogens alone (Premarin, 0.625 mg daily) or conjugated estrogens plus progesterin (Premarin, 0.625 mg daily, and Provera, 2.5 mg daily). Two women in group 1 found to have 17β -estradiol levels >25 pg/ml in spite of no history of estrogen therapy were excluded from the study. As a result, group 1 consisted of 12 women who received conjugated estrogens, 0.625 mg per day, and group 2 consisted of 14 women who received conjugated estrogens, 0.625 mg per day, and medroxyprogesterone acetate, 2.5 mg per day (group 2). Subjects were studied before initiation of hormone therapy and after 3 months, while they were still receiving hormone therapy. Previous studies of the human aorta during pregnancy have shown a detectable change in aortic size between the end of gestation and approximately 15 weeks post partum.¹¹ The investigators were blinded to the treatment until all measurements had been made.

Aortic size was assessed by measuring cross-sectional area of the ascending and descending aorta by magnetic resonance imaging (MRI). Aortic cross-sectional area-aortic pressure relationships were determined over the range of the pulse pressure with the method of Stefanowicz et al.¹² Aortic pressure was estimated at 12 intervals (corresponding to the MRI images) from diastole to systole. A carotid pulse tracing, an electrocardiogram, and brachial artery pressures were taken simultaneously, immediately before MRI image acquisition. Brachial artery pressure was measured by the standard cuff technique with the fourth Korotkoff sound taken as diastole. The fractional rise in aortic pressure is equal to the fractional rise of the amplitude of the carotid pulse tracing at the same time point relative to a reference point, the R wave on the electrocardiogram. To compensate for the delay in the carotid pulse tracing, the MRI scan with the smallest cross-sectional aortic area was aligned with the end-diastole of the pulse tracing. This technique allowed estimation of distending aortic pressures from end-dia-

stole through systole, which correspond to measurements of aortic cross-sectional area made at the same times.

After the carotid artery pulse, electrocardiographic, and brachial artery cuff pressure measurements were made, the patient was positioned supine in a General Electric (Milwaukee) MRI unit. The R wave of the electrocardiogram was used to gate the image acquisition sequence. Brachial artery pressure was measured during the scan with a cuff free of ferromagnetic material and an automated pressure measuring device (Dinamapp; Critikon, Tampa). Blood pressure was monitored every 5 minutes during the MRI scan to ensure the stability of the blood pressure during scanning and to compare with the blood pressure obtained during the carotid pulse tracing. Blood pressure was obtained with an automated brachial artery cuff blood pressure device (Dinamapp) that had been previously standardized against simultaneously measured aortic blood pressures measured by intraaortic fluid-filled catheters in the cardiac catheterization laboratory at our institution.

The MRI magnet was operated at 0.35 T field strength. The initial tomographic scan series was in the coronal plane at the level of the midthorax. This allowed identification of the ascending aorta, aortic arch, descending aorta, and diaphragm. The center of the main pulmonary artery in the coronal plane, which showed a circular shape of the main pulmonary artery, served as a reference point to coordinate, that is, match, the scan level in each subject before and after 3 months, while she was still receiving hormone therapy. A series of 12 transaxial-plane tomographic images (perpendicular to the aorta) were obtained at the level of the center of the pulmonary artery with the first echo of the spin-echo pulse imaging sequence. The 12 MRI images were taken in rapid succession for serial imaging of the systolic expansion and diastolic contraction of the ascending and descending aorta in the transaxial plane. The aorta appeared as a circle of different sizes at different phases of the cardiac cycle in these cross-sectional images. The pulse sequences for this series of images began at the R wave of the electrocardiogram. The repetition time was set at approximately 550 msec to allow the image sequence to recycle for each cardiac cycle (heart rates of 60 to 100 beats/min have an R-R interval of 1000 to 600 msec, respectively). A slice thickness of 10 mm was used with either a 14 or 16 cm field of view with a 256×128 matrix interpolated to a 256×256 matrix. Total imaging time was 20 to 30 minutes, depending on heart rate. To allow for frequent sampling of aortic area, three scans with four phases of the cardiac cycle were interspersed so that 12 images were obtained beginning at end-diastole and ending at end-systole. An additional scan using a 256×256 matrix with a 16 cm field of view was obtained at end-diastole with the flow compensation technique to enhance aortic wall resolution at end-diastole. All subjects were in normal

sinus rhythm when imaged and beats of no more than 10% variation in R-R intervals were used for image accumulation. The transaxial tomograms of the thoracic aorta were displayed with the MRI computer in a 128 x 256 pixel image matrix. The area in square centimeters was calculated with calibration constants in centimeters per pixel, which were determined with a calibration phantom. Aortic area was digitized with the National Institutes of Health "Image" imaging processing program on an Apple (Cupertino, Calif.) Macintosh IIfx computer. Aortic cross-sectional area was divided by body surface area to reduce interindividual variability. Compliance was defined as the slope of the area-pressure relationship. Technically, compliance is dV/dP , where V is volume and P = pressure. This study assumes that aortic length can be ignored as others²⁴ have because the thoracic aorta is retropleural and does not change in length with the cardiac cycle.

The differences in compliance and aortic cross-sectional area, before and during estrogen treatment and progesterone and progestin treatment, were determined by analysis of covariance to test for differences in the slope and y -adjusted means.²⁵ Analysis of covariance was also used to determine whether there was a difference in compliance or aortic cross-sectional area between group 1 and group 2 before hormone therapy. A paired t test was used to determine differences in weight, heart rate, blood pressure, and blood levels of estrogen and estrone before and at 3 months of treatment.²⁶ Serum estrogen measurements were made with a standard radioimmunoassay (Coulter-Count Estradiol, Diagnostic Products, Los Angeles). This technique has an intraassay variability of 6% and an interassay variability of 9%. A t test for paired data was used to assess significant changes in serum estrogen levels.²⁶ A t test for nonpaired data was used to assess significant changes in the demographic parameters between group 1 and group 2.²⁶ Data were expressed as mean $\pm$ SD. A p value <0.05 was considered significant.

To determine the reproducibility of the MRI technique used in this study, the ascending aortic cross-sectional area-aortic pressure relationship was determined in five healthy women on the second or third day of their menstrual periods. These five women had no historic evidence of coronary artery disease or valvular heart disease. A second ascending aortic cross-sectional area-aortic pressure relationship was determined in these women approximately 1 month later on the second or third day of their menstrual periods. Ascending aortic cross-sectional area relationships for the two studies were not different. The adjusted y mean of ascending aortic cross-sectional area-aortic pressure relationship was $393 \pm 13 \text{ mm}^2/\text{m}^2$ for the first study and $386 \pm 13 \text{ mm}^2/\text{m}^2$ for the second study. With an α of 0.05 a minimum detectable difference of 20 mm^2 (5% change in aortic cross-sectional area) and a power of 0.80, a sample size of eight would be necessary. Previous studies of the human aorta during pregnancy have shown a detectable change in aortic size between the end of gestation and approximately 15 weeks post partum.²¹ On the basis of these observations, at least 12 women were randomized to each group and studied before and at the end of 3 months of hormone therapy.

Results

Twelve women received conjugated estrogens, 0.625 mg/day (group 1), and 14 women received a combination of conjugated estrogens, 0.625 mg/day, and medroxyprogesterone acetate, 2.5 mg/day (group 2). All women were compliant with treatments, as judged by pill count and appropriate increases in estrogen serum levels. Subject age, height, body weight, pregnancy history, and the time since the last menstrual period or estrogen use are shown in Table I. Five women in group 1 and six in group 2 had had a total abdominal hysterectomy and bilateral salpingo-oophorectomy. There was no difference in age, height, weight, gravidity, parity, or months since the last menstrual cycle or estrogen therapy between the two groups. Women in group 2 tended to be somewhat taller and heavier, although this was not statistically significant.

The interval between MRI imaging acquisitions was 97.8 ± 9.9 days (range 84 to 126 days) for the estrogen-treated women (group 1) and 92.0 ± 6.4 days (range 91 to 132 days) for the estrogen- and progestin-treated women (group 2). Estradiol and estrone levels, heart rate, systolic and diastolic blood pressures before and during hormone therapy for groups 1 and 2 are shown in Table II. Administration of conjugated estrogen was associated with elevations of both estradiol and estrone levels in both groups 1 and 2. There was no difference in heart rate before and during hormone therapy in either group 1 or 2. Systolic and diastolic blood pressure decreased during hormone therapy in group 1. Diastolic blood pressure decreased during hormone therapy in group 2. Systolic pressure before and during hormone therapy in group 2 was not significantly different. There was no difference in ascending or descending aortic size or in aortic compliance between group 1 and group 2 before hormone therapy.

The upper panel of Fig. 1 shows a plot of the ascending aortic cross-sectional area and blood pressure data for the 12 women in group 1 receiving estrogen therapy. On average, the data points obtained during hormone therapy (open circles) lie above the data points obtained before hormone therapy (solid circles). The lower panel of Fig. 1 shows the line of best fit for the data of ascending aortic cross-sectional area-body surface area plotted against aortic pressure for group 1. During hormone therapy the position of this ascending aortic cross-sectional area

Table I. Demographic variables for women receiving estrogen treatment (group 1) and women receiving estrogen and progestin treatment (group 2)

	Group 1	Group 2	Significance
Age (yr)	55.8 ± 4.2 (50-65)	57.4 ± 3.8 (51-68)	NS
Height (in)	64.1 ± 1.9 (60.0-69.5)	65.0 ± 2.3 (63-71)	NS
Weight (lb)	148.1 ± 24.0 (122-195)	160.4 ± 32.6 (125-250)	NS
Gravidity	3.4 ± 2.5 (1-9)	3.5 ± 2.2 (1-8)	NS
Parity	2.9 ± 2.1 (1-9)	3.4 ± 2.2 (1-8)	NS
Time since last menstrual period or estrogen therapy (mn)	104.9 ± 84.5 (7-232)	121.0 ± 103 (6-312)	NS

Values are ± SD of the mean; numbers in parentheses are range for values shown. NS, Not significant.

Table II. Estrogen and estrone levels, heart rate, systolic and diastolic blood pressures for women receiving estrogen treatment (group 1) or estrogen and progestin treatment (group 2)

	Before hormone therapy	During hormone therapy	Significance
Group 1			
Estrodiol (pg/ml)	<20	103.8 ± 63.0 (25-201)	p < 0.0001
Estrone (pg/ml)	25.3 ± 6.6 (16-38)	174.0 ± 112.0 (34-379)	p < 0.001
Heart rate (beats/min)	68.2 ± 0.3 (56-87)	62.8 ± 7.7 (48-78)	NS
Systolic blood pressure (mm Hg)	131.4 ± 13.4 (116-153)	122.6 ± 14.8 (105-148)	p < 0.005
Diastolic blood pressure (mm Hg)	78.4 ± 8.8 (63-90)	69.8 ± 10.0 (53-90)	p < 0.01
Group 2			
Estrodiol (pg/ml)	<20	106.5 ± 52.5 (34-235)	p < 0.0001
Estrone (pg/ml)	25.7 ± 10.1 (7-43)	144.1 ± 78.3 (34-380)	p < 0.0001
Heart rate (beats/min)	70.9 ± 7.8 (54-86)	68.3 ± 7.6 (53-80)	NS
Systolic blood pressure (mm Hg)	126.0 ± 13.1 (103-150)	125.1 ± 11.6 (110-141)	NS
Diastolic blood pressure (mm Hg)	72.7 ± 10.0 (54-90)	69.0 ± 9.0 (53-80)	p < 0.02

Values are ± SD of the mean; numbers in parentheses are range for values shown. NS, Not significant.

tional area-aortic pressure relationship is shifted upward, indicating that during estrogen therapy the aorta is larger at a given aortic pressure. Ascending aortic cross-sectional area normalized for body surface area increased from an adjusted $\bar{y}$ mean of $439 \pm 7 \text{ mm}^2/\text{m}^2$ to $468 \pm 7 \text{ mm}^2/\text{m}^2$ in group 1 ($p < 0.008$). The slope of the line before estrogen administration was $1.18 \text{ mm}^2/\text{m}^2/\text{mm Hg}$ and was not significantly changed during estrogen administration ($1.39 \text{ mm}^2/\text{m}^2/\text{mm Hg}$). The slope of this line, that is, the relationship between change in area and change in pressure, represents aortic compliance. The slope of this relationship (or the compliance of the aorta) was not significantly altered within the range of blood pressures seen in this study.

In Fig. 2 the upper panel illustrates the descending aortic cross-sectional area-aortic pressure relationship for group 1. Although not as marked as with the ascending aorta, the data points obtained during hormone therapy for the descending aorta (*open circles*) lie above the data points obtained before hormone therapy (*solid circles*). The lower panel of Fig. 2 shows line of best fit for the data of descending aortic cross-sectional area-body surface area plotted against aortic pressure for group 1. During hormone therapy the position of this descending

aortic cross-sectional area-aortic pressure relationship is shifted upward, indicating that during estrogen therapy the aorta is larger for a given aortic pressure. Although the increase in cross-sectional area was not as large as with the ascending aorta, the adjusted $\bar{y}$ mean for the descending aortic cross-sectional area increased from $220 \pm 5 \text{ mm}^2/\text{m}^2$ to $236 \pm 5 \text{ mm}^2/\text{m}^2$ ($p < 0.02$). The slope of the relationship for the descending aorta before estrogen administration was $0.86 \text{ mm}^2/\text{m}^2/\text{mm Hg}$ and was $0.76 \text{ mm}^2/\text{m}^2/\text{mm Hg}$ during estrogen administration. The slope of this relationship (or the compliance of the aorta) was not significantly different within the range of blood pressures seen in this study. There was no difference in compliance or in aortic cross-sectional area between group 1 and group 2 before hormone therapy.

Figs. 3 and 4 illustrate the aortic cross-sectional area-aortic pressure relationships for the 14 women receiving the combination estrogen-progestin therapy, group 2. The *solid circles* represent data before hormone therapy and the *open circles* represent data during hormone therapy. There is no difference in the distribution of the data points before or during hormone therapy for either the ascending or the descending aorta for the women in group 2. During estrogen-progestin therapy there was no

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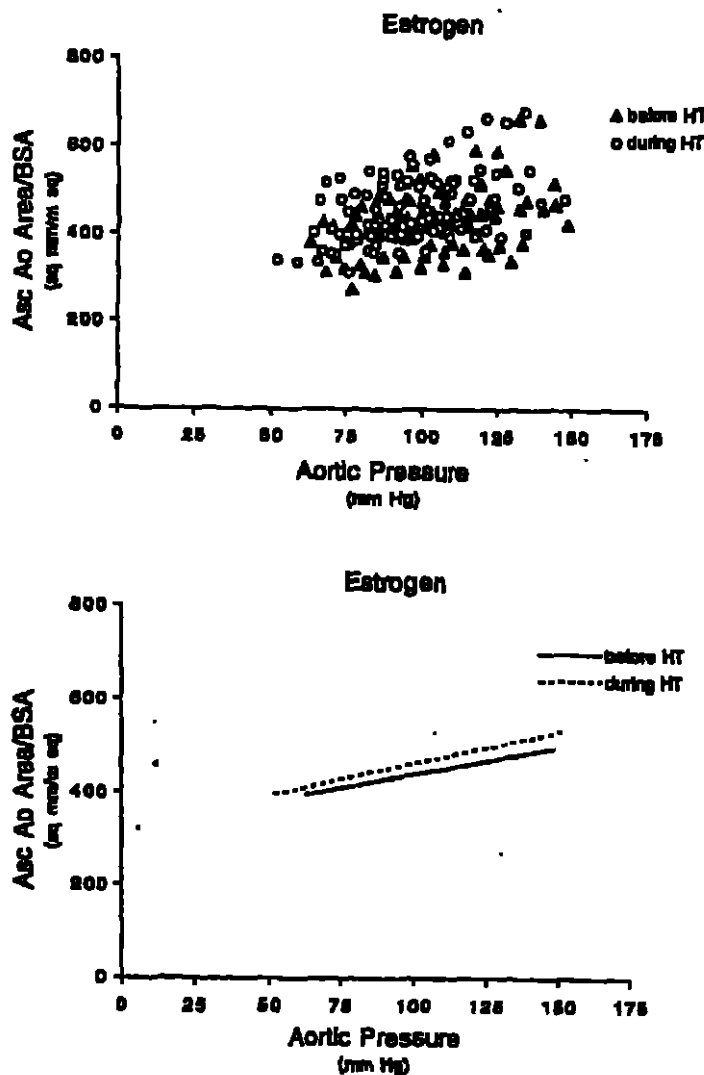


Fig. 1. Upper panel, Ascending aortic cross-sectional area-aortic pressure relationship for estrogen-treated group (group 1). Lower panel, Composite ascending aortic cross-sectional area-aortic pressure relationship for group 1. Aa Area/BSA, Ascending aortic cross-sectional area divided by body surface area in square millimeters per meter squared; HT, hormone therapy.

difference in the position of the two relationships for either the ascending ($455 \pm 8 \text{ mm}^2/\text{m}^2$ compared with $461 \pm 8 \text{ mm}^2/\text{m}^2$) or descending thoracic aortic ($217 \pm 4 \text{ mm}^2/\text{m}^2$ compared with $221 \pm 4 \text{ mm}^2/\text{m}^2$). The slope of the relationship for the ascending aorta was $1.01 \text{ mm}^2/\text{m}^2/\text{mm Hg}$ before estrogen-progestin administration and $0.97 \text{ mm}^2/\text{m}^2/\text{mm Hg}$ during administration. The slope of the relationship for the descending aorta was $0.51 \text{ mm}^2/\text{m}^2/\text{mm Hg}$ before estrogen-progestin administration and $0.50 \text{ mm}^2/\text{m}^2/\text{mm Hg}$ during administration. The combination of estrogen-progestin therapy had no effect on the compliance of the thoracic aorta in these postmenopausal women.

Comment

The goal of this study was to determine the effects of estrogen and the combination of estrogen and progestin on aortic size and aortic compliance in postmenopausal women. Studying aortic size and compliance under conditions where the changes are small in healthy humans presents some special problems. Such a study requires an accurate, precise technique that is reproducible between studies and that is noninvasive with no harmful effects. The MRI technique fulfills these requirements. MRI is, however, associated with some trade-offs. It requires that the imaging be done in a ferromagnetic-free environment. Blood pressure cannot be easily increased or re-

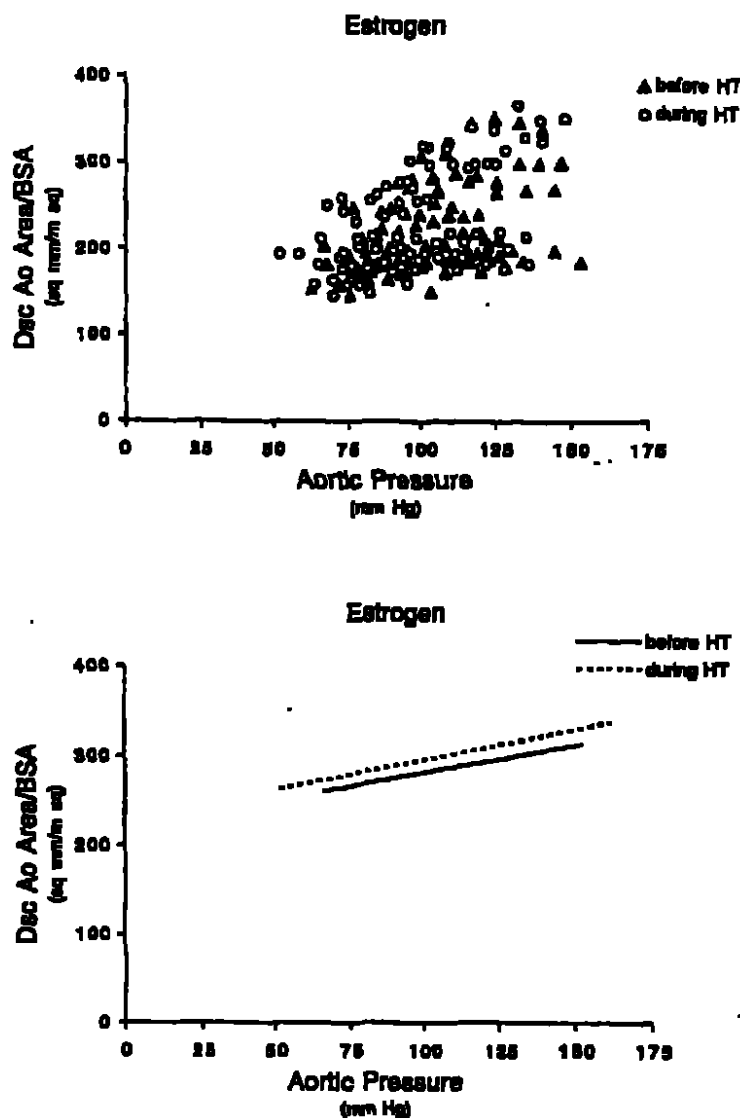


Fig. 2. Upper panel, Descending aortic cross-sectional area–aortic pressure relationship for estrogen-treated group (group 1). Lower panel, Composite descending aortic cross-sectional area–aortic pressure relationship for group 1. Dec Ao Area/BSA, Descending aortic cross-sectional area divided by body surface area in square millimeters per meter squared; HT, hormone therapy.

duced to expand the range over which the aortic cross-sectional area–aortic pressure relationship is studied. As a result, the aortic cross-sectional area–aortic pressure relationship is studied within the range of each individual's pulse pressure, that is, over a fairly narrow range. Therefore this study did not demonstrate changes in aortic compliance; but such changes have been excluded by this small study. Nevertheless this study did demonstrate that estrogen therapy alone increases both ascending and descending thoracic aortic size in postmenopausal women.

Estrogen therapy resulted in an increase in serum estradiol and estrone levels in both group 1 and group 2. Neither estrogen nor the combination of estrogen and

progestin affected heart rate. Estrogen therapy was associated with a decrease in both systolic and diastolic pressure in group 1. The combination of estrogen and progestin therapy was associated with a decrease in diastolic blood pressure but not systolic pressure group 2. Although there was no statistical significant difference in systolic or diastolic blood pressures before hormone therapy between group 1 and group 2, group 2 had a slightly lower blood pressure at the beginning of the study. Other investigators

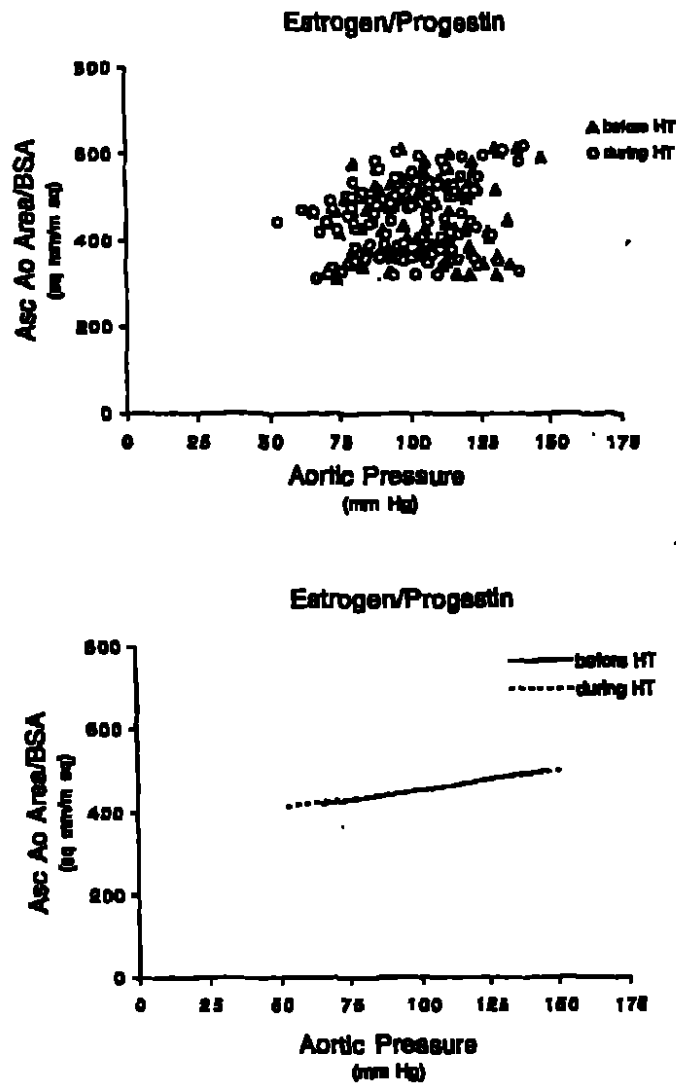


Fig. 3. Upper panel, Ascending aortic cross-sectional area-aortic pressure relationship for estrogen-progestin-treated group (group 2). Lower panel, Composite ascending aortic cross-sectional area-aortic pressure relationship for group 2. Aa/Aa/BSA, Ascending aortic cross-sectional area divided by body surface area in square millimeters per meter squared; HT, hormone therapy.

have demonstrated a decrease in blood pressure in postmenopausal women.¹⁷ A more recent study¹⁸ found no significant effect of estrogen therapy on systolic or diastolic pressure in a large number of women over the 3 years of the study.

After 3 months of estrogen therapy alone, both the ascending and descending aortic cross-sectional area was larger in the estrogen-treated group (group 1). This enlargement was manifest within the physiologic pressure range, that is, the usual arterial pressure of the individual. In contrast, the combination of estrogen and progestin therapy did not alter aortic size in postmenopausal women in this 3-month study. The reason for this finding is not clear. However, it is recognized that progestins exert and-

estrogenic actions and deplete target tissues of estrogen receptors. In the presence of progestins a longer exposure to estrogen (>3 months) may be necessary for combination therapy to produce an increase in aortic size.

The slopes of the aortic cross-sectional area-aortic pressure relationships in Fig. 1 and 2 are a measure of the compliance of the aorta within the range of arterial pressures for the women in this study. Although there was a trend for an increase in slope of the ascending aortic cross-sectional area-aortic pressure relationship, the slope of the relationship for both the ascending and descending aorta was not significantly different in group 1 or 2. It might be expected that the aorta would be larger and more compliant after estrogen and progestin administra-

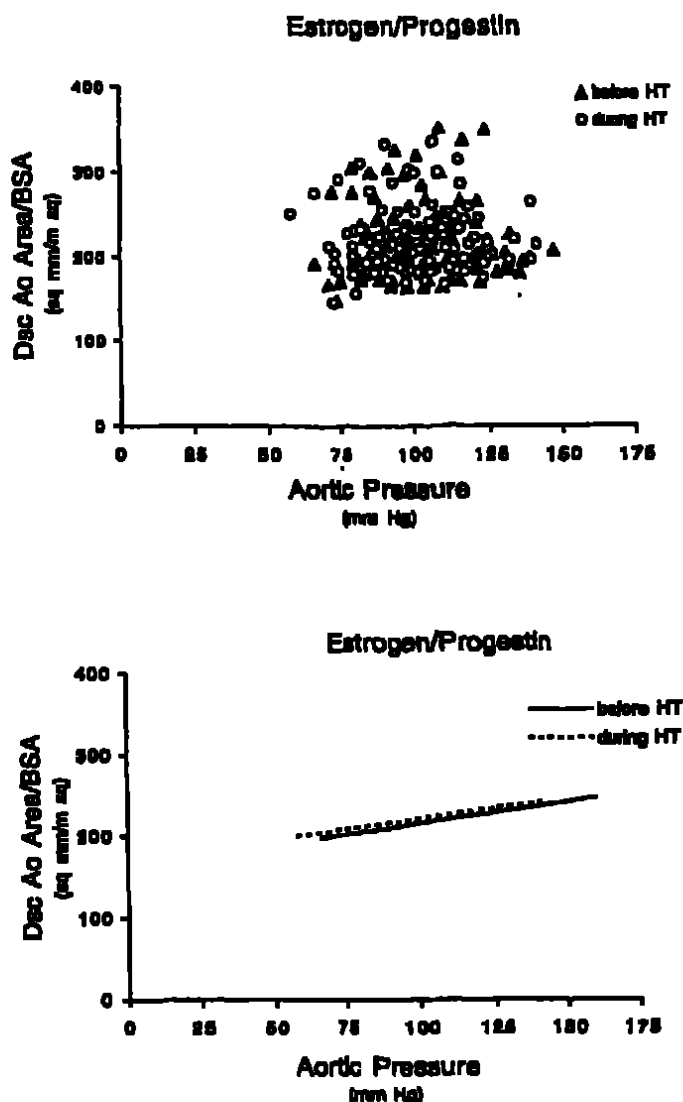


Fig. 4. Upper panel, Descending aortic cross-sectional area–aortic pressure relationship for estrogen-progestin-treated group (group 2). Lower panel, Composite descending aortic cross-sectional area–aortic pressure relationship for group 2. Dec Ao Area/BSA, Descending aortic cross-sectional area divided by body surface area in square millimeters per meter squared; HT, hormone therapy.

tion. For example, during human²⁸ and guinea pig pregnancy²⁹ when estrogen and progestin levels are elevated, the aorta is larger and more compliant. The aorta could be more compliant after estrogen and after combination estrogen and progestin administration, but the current experimental design could not demonstrate an increase in compliance in this relatively small group of subjects. It is also possible that a longer duration of therapy with estrogen and the combination of estrogen and progestin is necessary for the change in compliance to be seen.

Because MRI requires, even with the fastest acquisition routines, a relatively long period of time to acquire the cross-sectional images, arterial pressure cannot easily be increased or reduced to expand the range over which the

aortic cross-sectional area–aortic pressure relationship is studied. As a result, the aortic cross-sectional area–aortic pressure relationship was studied over a relatively narrow range. The slopes of the aortic cross-sectional area–aortic pressure relationship in the lower panels of Figs. 1 and 2 are not identical. Given the curvilinear nature of the aortic cross-sectional area–aortic pressure relationship and greater difference seen at higher arterial pressures, this study may not have been able to detect subtle differences in aortic compliance within the range of blood pressures studied. The fact that compliance was measured over the normal operating range of blood pressure is still important because aortic impedance is determined by systemic vascular resistance and aortic compliance in

the range of pressures present during normal left ventricular ejection. Increasing the pressures to show statistical differences would have been less meaningful except during exercise, when blood pressure might be much higher.

Postmenopausal women receiving estrogen therapy have a small but significant increase in thoracic aortic size observed within 3 months, whereas women receiving the combination of estrogen and progestin in this short-term study did not demonstrate a change. The clinical significance of this finding is unknown. The potential antagonistic effect of progestin on estrogen-induced aortic enlargement suggests that the favorable cardiovascular effects of postmenopausal estrogen therapy cannot be automatically extended to the combination of estrogen and progestin. Longer-term therapy needs to be similarly evaluated to see whether this difference persists.

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Editors' note: This manuscript was revised after these discussions were presented.

Discussion

Dr. William H. Fowler, Los Angeles, California. This meticulous MRI study adds to the published data on the site-specific effects of estrogen in the human female. Several hundred other site-specific effects of estrogen on tissue, cells, and cellular nuclei have been identified. The actions of estrogen, and contrarily estrogen deficiency, are complex, extensive, and physiologically significant throughout the female body.

The effects of estrogen on the cardiovascular system are clinically the most important, particularly the effect on mortality. The statistical and public health significance of the effects of estrogen (or lack thereof) on the female cardiovascular system increases with our aging population and swelling numbers of postmenopausal women.

All cardiovascular studies should be adjusted for the known risk factors of age, smoking, diabetes, hypertension, and obesity.

Autopsy studies reveal that, adjusted for age, the incidence of aortic lesions is the same for women as for men,

whereas with coronary lesions there is a 10-year lag in the curve for women compared with that for men.

The incidence of atherosclerosis, coronary artery contraction, coronary artery occlusion, myocardial infarction, and cardiovascular death are significantly decreased in estrogen-deficient women who are treated with estrogen. However, women have an increased mortality from myocardial infarction compared with men.

Estrogen receptors have been demonstrated in the female cardiovascular arterial walls, both in the endothelium and the muscle layers. Estrogen affects all layers of the walls of coronary arteries. In effect, the cardiovascular endothelium is an estrogen endocrine end-organ. Several human female coronary angiography studies substantiate the direct arterial dilation effect of estrogen. Estrogen increases left ventricular diastolic filling and stroke volume, probably by direct action on the musculature with increased contractile strength.

Measuring the percentage change in coronary artery diameter response to acetylcholine in monkeys, Williams et al.¹ demonstrated that the increase in diameter obtained on conjugated equine estrogen therapy was decreased by the addition of cyclic medroxyprogesterone acetate and was decreased further by continuous medroxyprogesterone acetate therapy. Estrogen or progesterone therapy did not affect the response in atherosclerotic arteries. However, in other studies of female monkeys a direct coronary artery atherosclerosis effect of estrogen has been demonstrated.

It is of keen clinical interest that in this short-term therapy study, progesterone negated what is presumed to be a positive effect of estrogen on the thoracic aorta. This is consistent with the findings by Lieberman et al.² in double-blind, randomized, crossover, placebo-controlled trials, that the estrogen-induced improvements in endothelium-dependent, flow-mediated vasodilation in postmenopausal women is negated by the addition of progesterone.

I have the following questions. (1) What insight into hormone therapy can clinicians gain from this study? (2) What mechanism, and at what site of action, might explain the differences in MRI aortic measurement between groups 1 and 2 when the serum estradiol measurements are essentially the same in both groups? (3) How were the investigators "blinded to the treatment until all measurements had been made?" (4) Were placebo studies or crossover studies (preferably with placebo) considered? (5) Are longer-term studies on these same MRI aortic measurements and treatment groups being undertaken?

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Dr. Charles March, Los Angeles, California. Have you had an opportunity to study two other groups of women,

even if the numbers are few? One is the postmenopausal women on chronic estrogen replacement, either estrogen alone or in combination with varying regimens of progestins. The second is women who are markedly obese and may have high levels of estrone, the ones who some suggest don't need estrogen replacement?

Dr. Glynis Von Drin Ann, Los Angeles, California. Once a woman is placed on estrogen therapy, whether you use estrogen and progesterone, how long do you keep her on hormone replacement therapy?

Dr. Row Umehara, Santa Rosa, California. Have you studied any women in whom you added micronized progesterone instead of a progestin?

Dr. Brame (Closing). Dr. Hindle raised several important questions. The first was what insights into hormone replacement therapy can clinicians gain from this study. As would be expected after review of the current literature, estrogen has effects on the cardiovascular system independent of its effects on the lipid profile. These cardiovascular effects may be advantageous after menopause, just as they are during pregnancy. However, caution should be applied in extending the results of this study. This study first looked at the effects of sex steroids on the aorta in postmenopausal women. These effects are likely complex, especially given that the combination of estrogen and progestin over the short term was not associated with an enlargement of the aorta.

The second question was what mechanism and what site of action might explain the differences in MRI aortic measurement between groups 1 and 2, when the serum estradiol measurements are essentially the same in both groups. Although a considerable body of information is available regarding the effects of estrogen on the uterine and coronary arteries, less is known about the effects of estrogen on the aorta. Because there is not any significant vasoactivity in the mature aorta, it is most likely that the aortic enlargement is associated with remodeling of the aorta. The aorta is made of collagen and elastin and doesn't have the smooth muscle fibers that would be vasoactive. This remodeling would involve a rearrangement of the matrix to allow an increase in size during an increase in pressure. The mechanisms for this are not known. The reasons why progestin in the short term counters this effect is not known and is definitely an area for future investigation.

The third question was how were the investigators blinded to treatment until all the measurements had been made. The individuals collecting and analyzing the hemodynamic and MRI data were blinded to all other data. Dr. Speroff and I screened all of these women for acceptance into the study. The nurses in our unit did a standard randomization protocol with mixing envelopes, and there was a drawing for what patient was on what protocol and nothing was unblinded until all data points were in.

In response to the fourth question, were placebo studies or crossover studies preferably with placebo considered, placebo arm or crossover studies are definitely appropriate designs and they were considered. However, this is a pilot study and the magnet is an expensive tool so we were limited as to how many arms of this study we could have. We weren't sure that there would be a

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change, so adding multiple arms to an already expensive study didn't seem to be affordable. We don't think it negates the results we have reported.

The last question was are longer-term studies in the undertaking? Yes, we are hopeful of getting more funding to do a study at least at 1 year, possibly longer.

Dr. March asked about women who had been treated with hormone replacement therapy over the long term. We have no data on that. We only have the data with the young reproductive-aged women. Additionally, we have not studied anyone who is markedly obese. The magnet does put some constraints on that, but we have not specifically studied those two areas.

How long do we keep people on hormone replacement therapy? Many clinicians are keeping women on hormone replacement therapy indefinitely. We do see that

patients will stop taking it on their own after a period of time. If patients have ever taken estrogen, they have a lower mortality rate than those who have never taken it. How long is long enough? That is another question to be answered.

We have looked at micronized progesterone, not in this study, but in others, and see similar negative effects on estrogen action on the heart. We have recently done a study on postmenopausal rhesus monkeys using natural progesterone. We measured oxidized low-density lipoprotein and within 2 weeks of estradiol therapy saw a decrease in oxidized low-density lipoprotein. At 4 weeks it is about maximum. Within 4 weeks of natural progesterone administration, the oxidized low-density lipoprotein returned to preestradiol levels.

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(12) SOLICITUD INTERNACIONAL PUBLICADA SEGÚN EL TRATADO DE COOPERACION DE PATENTES (PCT)

(19) Organización Mundial de Propiedad Intelectual
Oficina Internacional



(43) Fecha de Publicación

Septiembre 27 de 2001 (27.09.2001)

PCT

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WO 01/70208 A2

(51) Clasificación Internacional de Patentes⁷: A61K 31/00,

(21) Número de Solicitud Internacional: PCT/US01/40302

(22) Fecha de Registro Internacional: Marzo 16 de 2001
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(30) Información de Prioridad:

60/190,630 Marzo 20 de 2000 (20.03.2000) US
60/268,607 Febrero 14 de 2001 (14.02.2001) US

(71) Solicitante: AMERICAN HOME PRODUCTS
CORPORATION (US/US); Five Giralda Farms, Madison, NJ 07940
(US).

(72) Inventores: PICKAR, James, H.; 816 Crum Creek Road,
Springfield, PA 19064 (US).

(74) Agentes: MILOWSKY, Arnold, S.; American Home Products
Corporation, Patent Law Department, Five Giralda Farms, Madison, NJ
07940 et al. (US).

(81) Estados Designados (nacionales): AE, AG, AL, AM, AT, AU, AZ,
BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ,
EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP,
KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ,
UA, UG, UZ, VN, YU, ZA, ZW.

(84) Estados Designados (regionales): Patente ARIPO (GH, GM, KE, LS,
MW, MZ, SD, SL, SZ, TZ, UG, ZW), patente Eurasiática (AM, AZ, BY, KG,
KZ, MD, RU, TJ, TM) Patente Europea (AT, BE, CH, CY, DE, DK, ES, FI,
FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), Patente OAPI (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TO).

Publicado:

- sin reporte de búsqueda internacional y para ser republicado en el
momento de recibir este reporte

Para los códigos de dos letras y otras abreviaturas, véase a
"Las Notas Guía sobre Códigos y Abreviaturas" que aparecen
al comienzo de cada emisión regular de la Gaceta PCT.

(54) Título: "TERAPIA DE REEMPLAZO DE HORMONAS "

(57) Resumen: Esta invención se relaciona con métodos y composiciones farmacéuticas para proporcionar una
terapia de reemplazo de hormonas en mujeres perimenopáusicas, menopáusicas y post-menopáusicas a través de la
administración de combinaciones de estrógenos conjugados y acetato de medroxiprogesterona.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
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PCT

(10) International Publication Number
WO 01/70208 A2

(51) International Patent Classification⁷: A61K 31/00

(21) International Application Number: PCT/US01/40302

(22) International Filing Date: 16 March 2001 (16.03.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/190,630 20 March 2000 (20.03.2000) US
60/268,607 14 February 2001 (14.02.2001) US

(71) Applicant: AMERICAN HOME PRODUCTS CORPORATION [US/US]; Five Giralda Farms, Madison, NJ 07940 (US).

(72) Inventor: PICKAR, James, H.; 816 Crum Creek Road, Springfield, PA 19064 (US).

(74) Agents: MILOWSKY, Arnold, S.; American Home Products Corporation, Patent Law Department, Five Giralda Farms, Madison, NJ 07940 et al. (US).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 01/70208 A2

(54) Title: HORMONE REPLACEMENT THERAPY

(57) Abstract: This invention relates to methods and pharmaceutical compositions for providing hormone replacement therapy in perimenopausal, menopausal, and postmenopausal women through the continuous administration of combinations of conjugated estrogens and medroxyprogesterone acetate.

(12) SOLICITUD INTERNACIONAL PUBLICADA SEGÚN EL TRATADO DE COOPERACION DE PATENTES (PCT)

(19) Organización Mundial de Propiedad Intelectual
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(51) Clasificación Internacional de Patentes¹: A61K 31/57,
A61P 5/30 // (A61K 31/57, 31:565)

(74) Agentes: MILOWSKY, Arnold, S.; American Home Products Corporation, Patent Law Department, Five Giralda Farms, Madison, NJ 07940 et al. (US).

(21) Número de Solicitud Internacional: PCT/US01/40302

(81) Estados Designados (nacionales): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, GR, GU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(25) Idioma de registro: Inglés

(84) Estados Designados (regionales): Patente ARIPO (GH, GM, KE, LS, MW, MZ, SD, SI, SZ, TZ, UG, ZW), patente Eurasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM) Patente Europea (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), Patente OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

(26) Idioma de Publicación: Inglés

(30) Información de Prioridad:

60/190,630 Marzo 20 de 2000 (20.03.2000) US
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Publicando:

- con reporte de búsqueda internacional
- antes de la expiración del límite de tiempo para modificar las reivindicaciones y ser publicada de nuevo en el caso de recibo de modificaciones

(71) Solicitante: AMERICAN HOME PRODUCTS CORPORATION (US/US); Five Giralda Farms, Madison, NJ 07940 (US).

(88) Fecha de publicación del reporte de búsqueda internacional:
Abril 25 de 2002

(72) Inventores: PICKAR, James, H.; 816 Crum Creek Road, Springfield, PA 19064 (US).

Para los códigos de dos letras y otras abreviaturas, refiérase a "Las Notas Guías sobre Códigos y Abreviaturas" que aparecen al comienzo de cada emisión regular de la Gaceta PCT.

(54) Título:

"TERAPIA DE REEMPLAZO DE HORMONAS EMPLEANDO UNA COMBINACION DE ESTROGEN S CONJUGADOS Y ACETATO DE MEDROXIPROGESTERONA "

(57) Resumen: Esta invención se relaciona con métodos y composiciones farmacéuticas para proporcionar una terapia de reemplazo de hormonas en mujeres perimenopáusicas, menopáusicas y post-menopáusicas a través de la administración de combinaciones de estrógenos conjugados y acetato de medroxiprogesterona.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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WO 01/70208 A3(51) International Patent Classification⁷: A61K 31/57,
A61P 5/30 // (A61K 31/57, 31:565)

(21) International Application Number: PCT/US01/40302

(22) International Filing Date: 16 March 2001 (16.03.2001)

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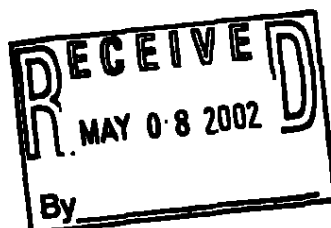
(30) Priority Data:
60/190,630 20 March 2000 (20.03.2000) US
60/268,607 14 February 2001 (14.02.2001) US(71) Applicant: AMERICAN HOME PRODUCTS COR-
PORATION [US/US]; Five Giralda Farms, Madison, NJ
07940 (US).(72) Inventor: PICKAR, James, H.; 816 Crum Creek Road,
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AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,
CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM,
HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,
LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX,
MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL,
TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.(84) Designated States (*regional*): ARIPO patent (GH, GM,
KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian
patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European
patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE,
IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF,
CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

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amendments

(88) Date of publication of the international search report:
25 April 2002For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

WO 01/70208 A3

(54) Title: HORMONE REPLACEMENT THERAPY USING A COMBINATION OF CONJUGATED ESTROGENS AND
MEDROXYPROGESTERONE ACETATE(57) Abstract: This invention relates to methods and pharmaceutical compositions for providing hormone replacement therapy in
perimenopausal, menopausal, and postmenopausal women through the continuous administration of combinations of conjugated
estrogens and medroxyprogesterone acetate.

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TRATADO DE COOPERACION DE PATENTES
PCT
REPORTE DE EXAMEN PRELIMINAR INTERNACIONAL

(PCT Artículo 36 y Regla 70)

Registro de referencia del solicitante o agente AM100226	PARA FUTURAS ACCIONES Ver Notificación o Transmisión del Reporte de Examen Preliminar Internacional (Formato PCT/PEA/416)	
Solicitud Internacional No. PCT/US 01/40302	Fecha de registro internacional (día/mes/año) 16/03/2001	Fecha de prioridad (día/mes/año) 20/03/2000
Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC 7 A61K31/00		
Solicitante AMERICAN HOME PRODUCTS CORPORATION		

1.	Este informe de examen preliminar internacional ha sido preparado por esta Autoridad Examinadora Preliminar Internacional y se transmite al solicitante de conformidad con el Artículo 36.
2.	Este REPORTE consta de un total de <u> 2 </u> páginas, incluyendo la carátula. ☐ Este reporte también está acompañado de ANEXOS, es decir, páginas de descripción, reivindicaciones y/o dibujos que han sido modificados y son la base de este informe y/o páginas que contienen rectificaciones hechas ante esta Autoridad (ver Regla 70.16 y la Sección 607 de las Instrucciones Administrativas de conformidad con el PCT). Estos anexos constan de un total de <u> </u> páginas.
3.	Este informe contiene indicaciones relacionadas con los siguientes ítems: - I ☒ Bases del Reporte - II ☐ Prioridad - III ☐ No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad Industrial. - IV ☐ Falta de unidad de invención - V ☒ Afirmación razonada de conformidad con el Artículo 35(2) con respecto a novedad, nivel inventivo o aplicabilidad industrial; citaciones y explicaciones que sustentan dicha afirmación. - VI ☐ Ciertos documentos citados - VII ☐ Ciertos defectos en la solicitud internacional - VIII ☐ Ciertas observaciones sobre la solicitud internacional

Fecha de presentación de la demanda 29/09/2001	Fecha de terminación de este reporte 15/06/2002
Nombre y dirección de la autoridad internacional de examen preliminar: European Patent Office D-80298 Munich Tel. (+49-89) 2399 - 0, Tx: 523656 epmu d Fax: (+49- 89) 2399- 4465	Funcionario autorizado MEYLAERTS H Teléfono No. (+49-89) 2399 2828

42
43
44

REPORTE DE EXAMEN PRELIMINAR INTERNACIONAL Solicitud internacional No. PCT/US 01/40302

I. Bases del Reporte

La base de este examen preliminar internacional es la solicitud como se presentó originalmente.

V. Afirmación razonada bajo la Regla 66.2(a)(ii) con respecto a la novedad, nivel inventivo o la aplicación industrial.

A la luz de los documentos citados en el reporte de búsqueda internacional, se considera que la invención como se define en al menos algunas de las reivindicaciones no parece cumplir con los criterios mencionados en el Artículo 33(1) de la PCT, esto es, no parece ser novedoso y/o involucrar una etapa inventiva (ver el reporte de búsqueda internacional, en particular los documentos citados X y/o Y y las referencias a las reivindicaciones correspondientes)

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference AM100226	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/16)	
International application No. PCT/US 01/ 40302	International filing date (day/month/year) 16/03/2001	Priority date (day/month/year) 20/03/2000
International Patent Classification (IPC) or national classification and IPC A61K31/00		
Applicant AMERICAN HOME PRODUCTS CORPORATION		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 2 sheets, including this cover sheet.
- ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of _____ sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 29/09/2001	Date of completion of this report 15/06/2002
Name and mailing address of the IPEA/  European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 epmu d Fax: (+49-89) 2399-4465	Authorized officer MEYLAERTS H Tel. (+49-89) 2399 2828

Form PCT/IPEA/409 (cover sheet) (July 1998)



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No PCT/US 01/40302

I. Basis of the report

The basis of this international preliminary examination is the application as originally filed.

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability

In light of the documents cited in the international search report, it is considered that the invention as defined in at least some of the claims does not appear to meet the criteria mentioned in Article 33(1) PCT, i.e. does not appear to be novel and/or to involve an inventive step (see international search report, in particular the documents cited X and/or Y and corresponding claim references).

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TRATADO DE COOPERACION DE PATENTES

De la
AUTORIDAD EXAMINADORA PRELIMINAR INTERNACIONAL

To: WYETH LABORATORIES Patents & Trademarks Department Huntercombe Lane South Taplow Maidenhead Berkshire SL6 OPH GRABDE BRETAGNE		OPINION ESCRITA (PCT Regla 66)	
Registro de referencia del solicitante o agente AM100226		Fecha de envío (día/mes/año) 27/03/2002	
		RESPUESTA VENCE Dentro de 2/00 meses/días desde la fecha anterior de envío	
Solicitud Internacional No. PCT/US 01/40302	Fecha de registro internacional (día/mes/año) 16/03/2001	Fecha de prioridad (día/mes/año) 20/03/2000	
Clasificación Internacional de Patentes (IPC) o clasificación nacional e IPC 7 A61K31/00			
Solicitante AMERICAN HOME PRODUCTS CORPORATION			

1. Esta opinión escrita la redacta primero la Autoridad Examinadora Preliminar Internacional.
2. Esta opinión contiene indicaciones relacionadas con los siguientes ítems: I ☒ Bases de opinión II ☐ Prioridad III ☐ No establecimiento de opinión respecto a novedad, nivel inventivo y aplicabilidad Industrial. IV ☐ Falta de unidad de invención V ☒ declaración razonada de según la regla 66.2(a)(ii) respecto a novedad, nivel inventivo o aplicabilidad industrial; citaciones y explicaciones que sustentan dicha afirmación. VI ☐ Ciertos documentos citados VII ☐ Ciertos defectos en la solicitud internacional VIII ☐ Ciertas observaciones sobre la solicitud internacional
3. El solicitante por el presente está invitado a contestar a esta opinión. ¿Cuándo? Ver el límite de tiempo indicado anteriormente. El solicitante puede, antes de la expiración del límite de tiempo, solicitar a esta Autoridad que otorgue una extensión, ver Regla 66.2(d). ¿Cómo? Presentando una respuesta escrita, acompañada de, cuando sea apropiado, de enmiendas, según Regla 66.3. Para el formulario y el idioma de las enmiendas, ver las Reglas 66.8 y 66.9

También Para una oportunidad adicional de presentar enmiendas, ver Regla 66.4. Para la obligación del examinador de considerar las enmiendas y/o argumentos, ver Norma 66.4bis. Para una comunicación informal con el examinador, ver Regla 66.6. Si no se registra una respuesta, el informe del examen preliminar internacional se establecerá según esta opinión. 4. La fecha final por medio de la cual se debe establecer el informe del examen preliminar internacional según la Regla 69.2 es: 20/07/2002	
Nombre y dirección de correo de IPEA/ Oficina de Patentes Europea D-80298 Munich Tel. (+49-89) 2399 - 0, Tx: 523656 epmu d Fax: (+49- 89) 2399- 4465	Funcionario autorizado Examinador
	Funcionario para Formalidades (incl. Extensión de límites de tiempo) Tel. (++49-89) 2399 2828

OPINION ESCRITA

Solicitud internacional No. PCT/US 01/40302

I. Bases de la Opinión

La base de esta opinión escrita es la solicitud como se presentó originalmente.

V. Afirmación razonada bajo la Regla 66.2(a)(ii) con respecto a la novedad, nivel inventivo o la aplicación industrial.

1. A la luz de los documentos citados en el reporte de búsqueda internacional, se considera que la invención como se define en al menos algunas de las reivindicaciones no parece cumplir con los criterios mencionados en el Artículo 33(1) de la PCT, esto es, no parece ser novedoso y/o involucrar una etapa inventiva (ver el reporte de búsqueda internacional, en particular los documentos citados X y/o Y y las referencias a las reivindicaciones correspondientes).
2. Si se presentan modificaciones, el solicitante deberá cumplir con los requerimientos de la Regla 66.8 PCT e indicar la base de las modificaciones en los documentos de la solicitud como fue presentada originalmente (Artículo 34(2) b) PCT o de lo contrario estas modificaciones pueden no ser tomadas en consideración para el establecimiento del reporte de examen preliminar internacional.
La atención del solicitante se dirige al hecho de que si la solicitud contiene una pluralidad innecesaria de reivindicaciones independientes, ningún examen de cualquiera de las reivindicaciones será realizada.

Docketed

02 APR 2002

PATENT COOPERATION TREATY.

C.O. PADNOR

53
80

AY100246 52

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

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PCT

To:

02 APR 2002

WYETH LABORATORIES
 Patents & Trademarks Department
 Huntercombe Lane South
 Taplow
 Maidenhead
 Berkshire SL6 0PH
 GRANDE BRETAGNE

PATENTS DEPT.

WRITTEN OPINION

(PCT Rule 66)

Date of mailing
(day/month/year)

27/03/2002

Applicant's or agent's file reference

AM100226

REPLY DUE

within 2 / 00 months/days
from the above date of mailing

International application No.

PCT/US 01/40302

International filing date (day/month/year)

16/03/2001

Priority date (day/month/year)

20/03/2000

International Patent Classification (IPC) or both national classification and IPC

A61K31/00

Applicant

AMERICAN HOME PRODUCTS CORPORATION

1. This written opinion is the first drawn up by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

- I ☒ Basis of the opinion
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

APR 04 2002

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. The applicant may, before the expiration of that time limit, request this Authority to grant an extension, see Rule 66.2(d).

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4. For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4bis. For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is 20/07/2002.

Name and mailing address of the IPBA/

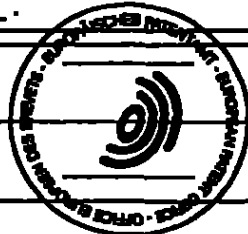


European Patent Office
 D-80298 Munich
 Tel. (+49-89) 2399-0, Tx: 523656 epro d
 Fax: (+49-89) 2399-4465

Authorized officer

Examiner

Formalities officer
 (incl. extension of time limits)
 Tel. (+49-89) 2399 2828



WRITTEN OPINION

International application No. PCT/US 01/40302

I. Basis of the opinion

1. The basis of this written opinion is the application as originally filed.

V. Reasoned statement under Rule 66.2(a)(II) with regard to novelty, inventive step or industrial applicability

1. In light of the documents cited in the international search report, it is considered that the invention as defined in at least some of the claims does not appear to meet the criteria mentioned in Article 33(1) PCT, i.e. does not appear to be novel and/or to involve an inventive step (see international search report, in particular the documents cited X and/or Y and corresponding claims references).
2. If amendments are filed, the applicant should comply with the requirements of Rule 66.8 PCT and indicate the basis of the amendments in the documents of the application as originally filed (Article 34 (2) (b) PCT) otherwise these amendments may not be taken into consideration for the establishment of the international preliminary examination report. The attention of the applicant is drawn to the fact that if the application contains an unnecessary plurality of independent claims, no examination of any of the claims will be carried out.

TRADUCCION No. 2002-08-29-492
DE UN DOCUMENTO PRESENTADO EN INGLES

12 de abril de 2.002

Via Facsímile

(41-22) 740 14 35

The International Bureau of WIPO

34, chemin des Colombettes

1211 Geneva 20, Switzerland

Re: Solicitud de Patente Internacional No. PCT/US01/40302

Referencia del Archivo del Solicitante: AM100226

Señores:

Adjunto encontrará copia de The International Bureau of WIPO de una solicitud de cambio de nombre del Solicitante bajo la Norma 92 bis PCT.

Una copia de confirmación vía courier seguirá.

Atentamente,

(firma: ilegible)

Margaret Doran

Anexo

EN LA OFICINA DE PATENTES Y MARCAS DE LOS ESTADOS UNIDOS
TRATADO DE COOPERACION DE PATENTES

Solicitante: Wyeth (antiguamente American Home Products Corporation)

Solicitud Internacional No. PCT/US01/14302

Fecha de Registro Internacional: 16 de marzo de 2.001

Para TERAPIA DE REMPLAZO DE HORMONAS

Commissioner of Patents and Trademarks

Box PCT

Washington, D.C. 20231

El Solicitante Solicita Cambio de Nombre bajo la Norma 92 bis PCT

En la Solicitud Internacional

Desde el 11 de marzo de 2.002, American Home Products Corporation cambió su nombre a Wyeth. El solicitante solicita que el Solicitante sea cambiado en la solicitud internacional anterior a Wyeth para reflejar este cambio de nombre.

El solicitante solicita que la Dirección del Agente sea también actualizada para reflejar el nombre nuevo de la compañía. Nuestros números de teléfono y facsímil permanecerán igual.

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Con el presente se encuentra un documento certificado del Secretario del Estado de Delaware que confirma este cambio de nombre.

Respetuosamente,

(firma: ilegible)

Egon E. Berg

Vice-Presidente, Wyeth

Patent Law Department

Five Giralda Farms

Madison, NJ 07940

United States of America

CERTIFICACION DE ENVIO POR CORREO SEGÚN 37 CFR §1.10

Etiqueta de Correo Expreso No. EV080130656US

Fecha de Depósito 12 de abril de 2.002

Mediante el presente certifico que este documento se deposita en el Servicio de Correo Postal de los Estados Unidos "Correo Expreso" Oficina de Correo Postal al Destinatario según 37 cfr §1.10 en un sobre dirigido al Sub-Comisionado de Patentes, Box PCT, Washington, DC 20231.

(firma: ilegible)

Delaware

El Primer Estado

Página 1

YO, HARRIET SMITH WINDSOR, SECRETARIO DE ESTADO DEL ESTADO DE DELAWARE, MEDIANTE EL PRESENTE CERTIFICO EL CERTIFICADO DE PROPIEDAD, QUE FUSIONA A:

"WYETH COMPANY", UNA CORPORACION DE DELAWARE,

CON Y EN "AMERICAN HOME PRODUCTS CORPORATION" BAJO EL NOMBRE DE "WYETH", UNA CORPORACION ORGANIZADA Y EXISTENTE SEGÚN LAS LEYES DEL ESTADO DE DELAWARE, FUE RECIBIDO Y REGISTRADO EN ESTA OFICINA EL DIA VEINTIDOS DE ENERO, A.D. DE 2.002, A LAS 10:00 A.M.

Y ADEMAS CERTIFICO QUE LA CORPORACION ANTERIORMENTE MENCIONADA ESTARA REGIDA POR LAS LEYES DEL ESTADO DE DELAWARE.

Y ADEMAS CERTIFICO QUE LA FECHA EFECTIVA DEL CERTIFICADO DE PROPIEDAD ANTERIORMENTE MENCIONADO ES EL DIA ONCE DE MARZO, A.D. DE 2.002.

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(firma: ilegible)

HARRIET SMITH WINDSOR, Secretario de Estado

0196024 8330

AUTENTICACION:

1667538

020171263

FECHA:

03-14-02

Five Girdle Farms
Madison, NJ 07940

Margaret Doran
Administrative Assistant
Law Department - Patent Section
Tel (973) 683-2108
Fax (973) 683-2112
doranm2@wyeth.com

C.C. RADNOR

A7100226 1

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ASM 57

Wyeth

April 12, 2002

Via Facsimile
(41-22) 740 14 35

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

WYETH
APR 15 2002
PATENT DEPT.

Re: International Patent Application No. PCT/US01/40302
Applicants File Reference: AM100226

Gentlemen:

Attached please find The International Bureau of WIPO's copy of a request to change the name of the Applicant under PCT Rule 92 *bis*.

A confirmation copy will follow via courier.

Sincerely,



Margaret Doran

Enclosure

L:\PATENTS\Bmd\AFCT FORMS\PCT Name Change\AM100226WIPO.doc

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT COOPERATION TREATY

Applicant: Wyeth (formerly American Home Products Corporation)

International Application No. PCT/US01/40302

International Filing Date: 16 March 2001

For: HORMONE REPLACEMENT THERAPY

Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

REQUEST TO CHANGE THE NAME of the APPLICANT UNDER PCT Rule 92b/s
IN THE INTERNATIONAL APPLICATION

Effective March 11, 2002, American Home Products Corporation changed its name to Wyeth. Applicant requests that the Applicant in the above international application be changed to Wyeth to reflect this name change.

Applicant requests that the Address for Agent also be updated to reflect the new name of the company. Our telephone and facsimile numbers will remain the same.

Submitted herewith is a certified document from the Secretary of the State of Delaware confirming this name change

Respectfully submitted,

Egon E Berg

Egon E. Berg
Vice-President, Wyeth
Patent Law Department
Five Giralda Farms
Madison, New Jersey 07940
Telephone: (973) 883-2188
Fax: (973) 883-2112

Certificate of Mailing 37 CFR 1.10
Express Mail Label No. EY080130856US
Date of Deposit April 12, 2002
I hereby certify that this paper is being deposited
with the United States Postal Service "Express Mail"
Post Office to Addressee" service under 37 CFR 1.10
in an envelope addressed to The Assistant Commissioner
of Patents, Box PCT, Washington, D.C. 20231

Margaret Loran

cc: The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Delaware

PAGE 1

AM100226 362
59

The First State

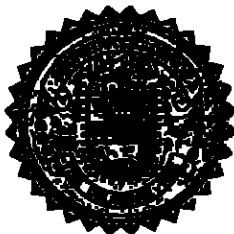
I, HARRIET SMITH WINDSOR, SECRETARY OF STATE OF THE STATE OF DELAWARE, DO HEREBY CERTIFY THE CERTIFICATE OF OWNERSHIP, WHICH MERGES:

"WYETH COMPANY", A DELAWARE CORPORATION,

WITH AND INTO "AMERICAN HOME PRODUCTS CORPORATION" UNDER THE NAME OF "WYETH", A CORPORATION ORGANIZED AND EXISTING UNDER THE LAWS OF THE STATE OF DELAWARE, WAS RECEIVED AND FILED IN THIS OFFICE THE TWENTY-SECOND DAY OF JANUARY, A.D. 2002, AT 10 O'CLOCK A.M.

AND I DO HEREBY FURTHER CERTIFY THAT THE AFORESAID CORPORATION SHALL BE GOVERNED BY THE LAWS OF THE STATE OF DELAWARE.

AND I DO HEREBY FURTHER CERTIFY THAT THE EFFECTIVE DATE OF THE AFORESAID CERTIFICATE OF OWNERSHIP IS THE ELEVENTH DAY OF MARCH, A.D. 2002.



0196024 8330

020171263

Harriet Smith Windsor

Harriet Smith Windsor, Secretary of State

AUTHENTICATION: 1667538

DATE: 03-14-02

wyeth
AM 100226
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TRADUCCION OFICIAL No. 2002-08-29-491
DE UN DOCUMENTO PRESENTADO EN INGLES

TRATADO DE COOPERACION DE PATENTES

PCT

NOTIFICACION DEL REGISTRO DE UN CAMBIO

(PCT Norma 92bis. 1 e

Instrucciones Administrativas, Sección 422)

De la OFICINA INTERNACIONAL

Para: MILOWSKY, Arnold S.
American Home Products Corporation
Patent Law Department
Five Giralda Farms
Madison, NJ 07940
United States of America

Fecha de envío (día/mes/año) 22 de abril de 2.002 (22.04.02)	NOTIFICACION IMPORTANTE
Referencia del archivo del solicitante o agente AM 100226	Fecha de registro internacional 16 de marzo de 2.001 (16.03.01)

Solicitud internacional No. PCT / US 01 / 40302		
1. Las siguientes indicaciones aparecieron en el registro en relación con:		
☒ El solicitante ☐ el inventor ☐ el agente ☐ el representante común		
Nombre y Dirección	Nacionalidad	Estado de residencia
American Home Products Corporation	EE.UU.	EE.UU.
Five Giralda Farms		
Madison, NJ 07940	Teléfono No.	Facsímil No.
United States of America		
2. La Oficina Internacional por el presente le notifica al solicitante que se ha registrado el siguiente cambio en relación con:		
☐ la persona ☒ el nombre ☐ la dirección ☐ la nacionalidad ☐ la residencia		
Nombre y Dirección	Nacionalidad	Estado de residencia
WYETH		
Five Giralda Farms		
Madison, NJ 07940	Teléfono No.	Facsímil No.
United States of America		
Más observaciones, si es necesario:		

3. Una copia de esta notificación se ha enviado a:	
☒	la oficina que recibe
☐	la Autoridad de Búsqueda Internacional
☐	la Autoridad de Examen Preliminar Internacional
☐	las Oficinas designadas
☐	las Oficinas elegidas
☐	otro:
The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20 Switzerland	Funcionario autorizado David LOPEZ-RAMIREZ Teléfono No.: (41-22) 338.83.38

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

MILOWSKY, Arnold S.
American Home Products Corporation
Patent Law Department
Five Giralda Farms
Madison, NJ 07940
ETATS-UNIS D'AMERIQUE

MAY 07 2002

Date of mailing (day/month/year) 22 April 2002 (22.04.02)	
Applicant's or agent's file reference AM100226	IMPORTANT NOTIFICATION
International application No. PCT/US01/40302	International filing date (day/month/year) 16 March 2001 (16.03.01)

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address

AMERICAN HOME PRODUCTS CORPORATION
Five Giralda Farms
Madison, NJ 07940
United States of America

State of Nationality

US

State of Residence

US

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☒ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address

WYETH
Five Giralda Farms
Madison, NJ 07940
United States of America

State of Nationality

State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☐ the elected Offices concerned
☐ the International Preliminary Examining Authority ☐ other:The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

David LOPEZ-RAMIREZ

Telephone No.: (41-22) 338.83.38

TRADUCCION No. 2002-08-29-493
DE UN DOCUMENTO PRESENTADO EN INGLES

No. SERIAL **60/190.630**
REGISTRADO: **20 de marzo de 2.000**

CESION

POR CUANTO, Yo, James H. Pickar de Springfield en el Condado de Delaware y Estado de Pennsylvania, he inventado ciertas Mejoras nuevas y útiles en

TERAPIA DE REMPLAZO DE HORMONAS

para lo cual he, respectivamente, el día 27 de marzo de 2.000, ejecutado una solicitud de Patente de Invención de los Estados Unidos, la fecha de registro y número serial de dicha solicitud, cuando sean conocidos deben insertarse arriba; y

POR CUANTO, AMERICAN HOME PRODUCTS CORPORATION, una corporación debidamente organizada y existente según las leyes del Estado de Delaware, que tiene su lugar de negocios en Five Giralda Farms, en el Condado de Morris y Estado de New Jersey, en adelante llamada

CESIONARIO, desea adquirir todos los derechos, títulos e intereses en y de dicha invención, en los Estados Unidos de América, sus territorios, dependencias y posesiones y en todos los países, y en y de cualquier Patente de Invención de los Estados Unidos o extranjera que se otorgue para esto, y en y para dichas otras formas de protección de propiedad industrial según se otorguen por esto según las leyes de cualquier país:

AHORA, POR LO TANTO, en consideración de la suma de UN DÓLAR (\$1.00) en mano pagada a cada uno de nosotros, y de causas contractuales adecuadas, el recibo de lo cual por el presente se reconoce, yo, por el presente, he vendido, cedido y transferido y mediante el presente vendo, cedo y transfiero al CESIONARIO, sus sucesores, representantes legales y cesionarios, todos los derechos, títulos e intereses en y para dicho invento, en los Estados Unidos de América, sus territorios, dependencias y posesiones, y en todos los países, y en y para cualquier Patente de Invención de los Estados Unidos y otros países (incluyendo todas las divisiones, reemisiones, continuaciones y extensiones de la misma) que se otorguen por ello, y en y para dichas otras formas de protección de propiedad industrial según se otorguen por ello según las leyes de cualquier país, y el derecho de solicitar Patente de Invención y dichas otras formas de protección de propiedad industrial según se proporcionen en cualquier país, con pleno beneficio de dichas prioridades según se otorguen ahora o después a nosotros por las leyes locales o por tratado o por convención internacional, junto con el derecho de

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extender la protección de dichas Patentes de Invención de los Estados Unidos a posesiones territoriales ahora en posesión o que en un futuro se puedan adquirir por los Estados Unidos de América, por todo el término para el cual dichas Patentes de Invención o dichas otras formas de protección se puedan otorgar;

Y yo acuerdo y acepto con el CESIONARIO que tengo un título total y libre de gravamen sobre la invención por el presente cedida, título que garantizo al CESIONARIO;

Y además acuerdo que yo, a solicitud del CESIONARIO, sin exigir más consideraciones por ello pero enteramente a expensas del CESIONARIO, haré todos los actos legales y justos, incluyendo la ejecución y reconocimiento de instrumentos, que puedan ser o llegar a ser necesarios para obtener, sostener o reemitir la Patente de Invención de los Estados Unidos y extranjera o dichas otras formas de protección que cubran la invención por el presente cedida, y para mantener y perfeccionar el derecho del CESIONARIO sobre dicha invención, Patente de Invención y otras formas de protección, particularmente en casos de interferencia y litigio.

EN TESTIMONIO DE LO CUAL, he colocado mi firma y sello este día 27 de marzo de 2.000.

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(1) (firma: ilegible) (L.S.)

James H. Pickar

TESTIGO RESPECTO A (1).

(firma: ilegible)

Estado de Pennsylvania)
) ss.
Condado de Delaware)

En este día 27 de marzo de 2.000, ante mí compareció personalmente James H. Pickar, a quien conozco y quien sé que es la persona descrita y quien ejecutó el documento anterior y quien reconoció ante mí que él firmó el mismo.

(firma: ilegible)
Notario Público

Aparece el sello del Notario.
ROXANNE L. KELLY, Notario Público
Condado de Radnor Twp Delaware
Mi Comisión Expira el 23 de noviembre de 2.002

SERIAL NO. 60/190,630FILED: March 20, 2000**ASSIGNMENT**

WHEREAS, I, James H. Pickar of Springfield in the County of Delaware and State of Pennsylvania, have invented certain new and useful Improvements in

HORMONE REPLACEMENT THERAPY

for which I have, respectively, on the 27 day of March, 2000 executed an application for Letters Patent of the United States, the date of filing and serial number of said application, when known to be inserted above; and

WHEREAS, AMERICAN HOME PRODUCTS CORPORATION, a corporation duly organized and existing under the laws of the State of Delaware, and having a place of business at Five Giralda Farms, in the County of Morris, and State of New Jersey, hereinafter called ASSIGNEE, is desirous of acquiring the entire right, title and interest in and to said invention, in the United States of America, its territories, dependencies and possessions, and in all foreign countries, and in and to any United States or foreign Letters Patent that may be granted therefor, and in and to such other forms of protection of industrial property as may be granted therefor pursuant to the laws of any country:

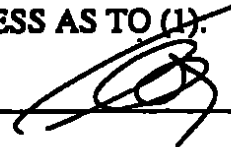
NOW, THEREFORE, in consideration of ONE DOLLAR (\$1.00) in hand paid to each of us, and other good and valuable considerations, the receipt of which is hereby acknowledged, I by these presents, have sold, assigned and transferred, and do hereby sell, assign and transfer, to ASSIGNEE, its successors, legal representatives and assigns, the entire right, title and interest in and to the said invention, in the United States of America, its territories, dependencies and possessions, and in all foreign countries, and in and to any Letters Patent of the United States and foreign countries (including all divisions, reissues, continuations and extensions thereof) that may be granted therefor, and in and to such other forms of protection of industrial property as may be granted therefor pursuant to the laws of any country, and the right to apply for Letters Patent and such other forms of protection of industrial property as may be provided by any country, with full benefit of such priorities as may now or hereafter be granted to us by local laws or by treaty or by international convention, together with the right to extend the protection of said United States Letters Patent to territorial possessions now owned or which may hereafter be acquired by the United States of America, for the full term for which said Letters Patent or said other forms of protection may be granted;

And I covenant and agree with ASSIGNEE that I have a full and unencumbered title to the invention hereby assigned, which title I warrant to ASSIGNEE;

And I further agree that I will, at ASSIGNEE'S request, without demanding further consideration therefor but entirely at ASSIGNEE'S expense, do all lawful and just acts, including the execution and acknowledgement of instruments, that may be or become necessary for obtaining, sustaining or reissuing United States and foreign Letters Patent or said other forms of protection covering the invention hereby assigned, and for maintaining and perfecting ASSIGNEE's right to said invention, Letters Patent and other forms of protection, particularly in cases of interference and litigation.

IN WITNESS WHEREOF, I have hereunto set my hand and seal this
27 day of March, 2000.

(1)  (L.S.)
James H. Pickar

WITNESS AS TO (1).

Jane Burton

STATE OF PENNSYLVANIA)
 : SS:
COUNTY OF DELAWARE)

On this 27th day of March, 2000, before me personally appeared James H. Pickar, to me known and known to me to be the individual described in and who executed the foregoing instrument, and he acknowledged to me that he executed the same.


Notary Public
NOTARIAL SEAL
ROXANNE L. KELLY Notary Public
Radnor Twp. Delaware County
My Commission Expires Nov. 23, 2002

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TRADUCCION No. 2002-08-29-494
DE UN DOCUMENTO PRESENTADO EN INGLES

No. SERIAL **60/268.607**
REGISTRADO: **14 de febrero de 2.001**

CESION

POR CUANTO, Yo, James H. Pickar de Springfield en el Condado de Delaware y Estado de Pennsylvania, he inventado ciertas Mejoras nuevas y útiles en

TERAPIA DE REMPLAZO DE HORMONAS

para lo cual he, presentado como Solicitud Provisional de Patente de Invención de los Estados Unidos, la fecha de registro y número de solicitud de dicha Solicitud Provisional, cuando sean conocidos deben insertarse arriba; y

POR CUANTO, AMERICAN HOME PRODUCTS CORPORATION, una corporación debidamente organizada y existente según las leyes del Estado de Delaware, que tiene su lugar de negocios en Five Giralda Farms, en el Condado de Morris y Estado de New Jersey, en adelante llamada CESIONARIO, desea adquirir todos los derechos, títulos e intereses en y de

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dicha invención, en los Estados Unidos de América, sus territorios, dependencias y posesiones y en todos los países, y en y de cualquier Patente de Invención de los Estados Unidos o extranjera que se otorgue para esto, y en y para dichas otras formas de protección de propiedad industrial según se otorguen por esto según las leyes de cualquier país:

AHORA, POR LO TANTO, en consideración de la suma de UN DÓLAR (\$1.00) en mano pagada a cada uno de nosotros, y de causas contractuales adecuadas, el recibo de lo cual por el presente se reconoce, yo, por el presente, he vendido, cedido y transferido y mediante el presente vendo, cedo y transfiero al CESIONARIO, sus sucesores, representantes legales y cesionarios, todos los derechos, títulos e intereses en y para dicho invento, en los Estados Unidos de América, sus territorios, dependencias y posesiones, y en todos los países, y en y para cualquier Patente de Invención de los Estados Unidos y otros países (incluyendo todas las divisiones, reemisiones, continuaciones y extensiones de la misma) que se otorguen por ello, y en y para dichas otras formas de protección de propiedad industrial según se otorguen por ello según las leyes de cualquier país, y el derecho de solicitar Patente de Invención y dichas otras formas de protección de propiedad industrial según se proporcionen en cualquier país, con pleno beneficio de dichas prioridades según se otorguen ahora o después a nosotros por las leyes locales o por tratado o por convención internacional, junto con el derecho de extender la protección de dichas Patentes de Invención de los Estados Unidos

a posesiones territoriales ahora en posesión o que en un futuro se puedan adquirir por los Estados Unidos de América, por todo el término para el cual dichas Patentes de Invención o dichas otras formas de protección se puedan otorgar;

Y yo acuerdo y acepto con el CESIONARIO que tengo un título total y libre de gravamen sobre la invención por el presente cedida, título que garantizo al CESIONARIO;

Y además acuerdo que yo, a solicitud del CESIONARIO, sin exigir más consideraciones por ello pero enteramente a expensas del CESIONARIO, haré todos los actos legales y justos, incluyendo la ejecución y reconocimiento de instrumentos, que puedan ser o llegar a ser necesarios para obtener, sostener o reemitir la Patente de Invención de los Estados Unidos y extranjera o dichas otras formas de protección que cubran la invención por el presente cedida, y para mantener y perfeccionar el derecho del CESIONARIO sobre dicha invención, Patente de Invención y otras formas de protección, particularmente en casos de interferencia y litigio.

EN TESTIMONIO DE LO CUAL, he colocado mi firma y sello este día 14 de marzo de 2.001.

(1) (firma: ilegible) (L.S.)

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(firma: ilegible)

)
) **ss.**
)

Notario Público

Mi Comisión Expira el 23 de noviembre de 2.002

APPLICATION NO. 60/268,607

FILED: February 14, 2001

ASSIGNMENT

WHEREAS, I, James H. Pickar of Springfield in the County of Delaware and State of Pennsylvania, have invented certain new and useful Improvements in

HORMONE REPLACEMENT THERAPY

which will be filed as a Provisional Application for Letters Patent of the United States, the date of filing and application number of said Provisional Application, when known to be inserted above; and

WHEREAS, AMERICAN HOME PRODUCTS CORPORATION, a corporation duly organized and existing under the laws of the State of Delaware, and having a place of business at Five Giralda Farms, in the County of Morris, and State of New Jersey, hereinafter called ASSIGNEE, is desirous of acquiring the entire right, title and interest in and to said invention, in the United States of America, its territories, dependencies and possessions, and in all foreign countries, and in and to any United States or foreign Letters Patent that may be granted therefor, and in and to such other forms of protection of industrial property as may be granted therefor pursuant to the laws of any country:

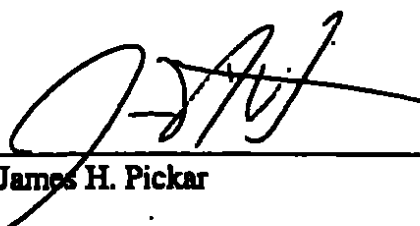
NOW, THEREFORE, in consideration of ONE DOLLAR (\$1.00) in hand paid to each of us, and other good and valuable considerations, the receipt of which is hereby acknowledged, I by these presents, have sold, assigned and transferred, and do hereby sell, assign and transfer, to ASSIGNEE, its successors, legal representatives and assigns, the entire right, title and interest in and to the said invention, in the United States of America, its territories, dependencies and possessions, and in all foreign countries, and in and to any Letters Patent of the United States and foreign countries (including all divisions, reissues, continuations and extensions thereof) that may be granted therefor, and in and to such other forms of protection of industrial property as may be granted therefor pursuant to the laws of any country, and the right to apply for Letters Patent and such other forms of protection of industrial property as may be provided by any country, with full benefit of such priorities as may now or hereafter be granted to us by local laws or by treaty or by international convention, together with the right to extend the protection of said United States Letters Patent to territorial possessions now owned or which may hereafter be acquired by the United States of America, for the full term for which said Letters Patent or said other forms of protection may be granted;

And I covenant and agree with ASSIGNEE that I have a full and unencumbered title to the invention hereby assigned, which title I warrant to ASSIGNEE;

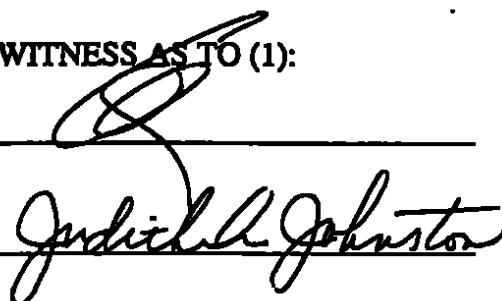
-12 7B
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And I further agree that I will, at ASSIGNEE'S request, without demanding further consideration therefor but entirely at ASSIGNEE'S expense, do all lawful and just acts, including the execution and acknowledgement of instruments, that may be or become necessary for obtaining, sustaining or reissuing United States and foreign Letters Patent or said other forms of protection covering the invention hereby assigned, and for maintaining and perfecting ASSIGNEE's right to said invention, Letters Patent and other forms of protection, particularly in cases of interference and litigation.

IN WITNESS WHEREOF, I have hereunto set my hand and seal this
14th day of March, 2001.

(1)  (L.S.)
James H. Pickar

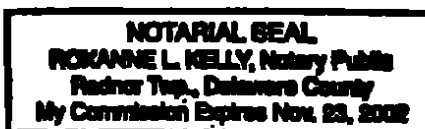
WITNESS AS TO (1):



STATE OF PENNSYLVANIA)
: SS:
COUNTY OF DELAWARE)

On this 14th day of March, 2001, before me personally appeared James H. Pickar, to me known and known to me to be the individual described in and who executed the foregoing instrument, and he acknowledged to me that he executed the same.


Notary Public



RESUMEN, OBJETO Y FINALIDAD DE LA INVENCION

Esta invención se relaciona con métodos y composiciones farmacéuticas para proporcionar una terapia de reemplazo de hormonas en mujeres perimenopáusicas, menopáusicas y post-menopáusicas a través de la administración de combinaciones de estrógenos conjugados y acetato de medroxiprogesterona.

TERAPIA DE REEMPLAZO DE HORMONAS EMPLEANDO UNA COMBINACION DE ESTROGENOS CONJUGADOS Y ACETATO DE MEDROXIPROGESTERONA

ANTECEDENTES DE LA INVENCION

La presente invención se refiere a métodos y composiciones farmacéuticas para proporcionar terapia de reemplazo de hormonas en mujeres perimenopáusicas, menopáusicas y postmenopáusicas a través de la administración continua de combinaciones de estrógenos conjugados y acetato de medroxiprogesterona.

La menopausia se define, en términos generales, como el último período menstrual natural, caracterizado por el cese de las funciones ováricas que conduce a la disminución sustancial de estrógeno circulante en la corriente sanguínea. La menopausia es usualmente identificada, en retrospectiva, después de 12 meses de amenorrea. No es un evento súbito, pero con frecuencia va precedido por un tiempo de ciclos menstruales irregulares previos al eventual cese de las menstruaciones. Luego del cese de la menstruación, la declinación en las concentraciones de estrógenos endógenos normalmente es rápida. Se observa un decrecimiento en los estrógenos séricos desde niveles circulantes que varían desde 40-250 pg/mL de estradiol y 40-170 pg/mL de estrona durante los ciclos ovulatorios hasta menos de 15 pg/mL de estradiol y 30 pg/mL de estrona en mujeres postmenopáusicas.

A medida que dichos estrógenos declinan durante la época precedente (perimenopausia) y posterior a la menopausia (postmenopausia), pueden presentarse diversos cambios fisiológicos, entre los que se incluye la atrofia vulvar y vaginal causante de resequedad vaginal, prurito y dispareunia, e inestabilidad vasomotora manifestada a manera de sensaciones pseudofebriles o bochornos. También pueden presentarse otros trastornos menopáusicos tales como depresión, insomnio, y nerviosismo. Los efectos fisiológicos de largo plazo de la disminución postmenopáusica de los estrógenos pueden dar como resultado una significativa morbilidad y mortalidad debido al incremento en los factores de riesgo de desarrollo de enfermedad cardiovascular y

osteoporosis. Los cambios menopaúsicos en los niveles de lípidos sanguíneos, uno de los componentes principales de la patogénesis de la enfermedad arterial coronaria (EAC), pueden ser precursores respecto de la incidencia incrementada de enfermedad isquémica cardíaca, de aterosclerosis, y de otras enfermedades cardiovasculares. Una rápida disminución en la masa ósea tanto del tejido óseo cortical (columna vertebral) como del trabecular (caderas) puede ser observada inmediatamente después de la menopausia, con una pérdida total de masa ósea situada entre 1% y 5% por año, que puede continuar durante 10 - 15 años.

La terapia de reemplazo de estrógenos (TRE) resulta beneficiosa para el alivio sintomático de los bochornos y la atrofia genital y para la prevención de la osteoporosis postmenopáusica.

La TRE ha sido reconocida como un tratamiento ventajoso para aliviar los síntomas vasomotores. No existe ninguna alternativa aceptable frente al tratamiento con estrógenos de los cambios atróficos en la vagina: la terapia con estrógenos incrementa la mucosa vaginal, reduce la resequedad vaginal. La TRE de largo plazo constituye la clave para la prevención de la osteoporosis debido a que reduce la pérdida de tejido óseo, reduce las fracturas de columna y cadera, y previene la pérdida de estatura. Por otra parte, se ha demostrado que la TRE es eficaz para incrementar el colesterol del tipo de lipoproteínas de alta densidad (HDL-C) y para reducir el colesterol del tipo de lipoproteínas de baja densidad (LDL-C), lo cual hace posible la protección contra la EAC. La TRE también puede suministrar protección antioxidante contra los desórdenes o estados patológicos mediados por la acción de los radicales libres. Se ha reportado también que los estrógenos confieren neuroprotección, e inhiben los desórdenes neurodegenerativos, tales como la enfermedad de Alzheimer (véase Patente de los EE.UU. No. 5,554,601, citada aquí a manera de solo referencia). La siguiente Tabla contiene una lista de preparaciones de estrógenos disponibles hoy en día en el mercado.

Terapias de reemplazo de estrógenos disponibles en los Estados Unidos y/o Europa

Nombre Genérico	Nombre Comercial	Potencias
Estrógenos orales		
Estrógenos equinos conjugados (naturales)	Premarin	0.3, 0.625, 0.9, 1.25, 2.5 mg
Estrógenos conjugados (sintéticos)	Cenestin	0.625, 0.9 mg
Estrógenos esterificados (75-80% de sulfato de estrona + 6-15% de sulfato de equilina derivado de esteroides de plantas)	Estratab	0.3, 0.625, 1.25, 2.5 mg
Estropipato (Piperazina estrona sulfato)	Ogen Ortho-Est	0.625, 1.25, 2.5 mg
Estradiol micronizado	Estrace	0.5, 1.0, 2.0 mg
Raloxifeno (modulador selectivo de los receptores de los estrógenos)	Evista	60 mg
Estrógenos esterificados y metiltestosterona	Estratest	1.25 mg de estrógeno esterificado + 2.5 mg de metiltestosterona
	Estratest HS	0.625 mg esterificado estrógeno + 1.25 mg metiltestosterona
Valerato de estradiol	Climaval	1 mg, 2 mg
Estradiol	Elleste Solo	1 mg, 2 mg
Estradiol	Estrofem	2 mg
Estradiol	Estrofem Forte	4 mg
Piperazine esterona sulfato	Harmogen	1.5 mg
Combinación: Estrona Estradiol Estriol	Hormonin	1.4 mg 0.6 mg 0.27 mg
Valerato de estradiol	Progynova	1 mg, 2 mg
Estradiol	Zumenon	1 mg, 2 mg
Estrógenos transdérmicos		
Estradiol	Alora (dos veces a la semana)	0.025, 0.0375, 0.05, 0.075, 0.1 mg de estradiol liberados cada día (opciones de dosificación para los diversos productos)
	Climara (semanal)	
	Estraderm (2 veces a la semana)	
	Fem Patch (semanal)	
	Vivelle (dos veces a la semana)	
Estradiol	Dermestril	25, 50, 100 µg
Estradiol	Estraderm	25, 50, 100 µg

Estradiol	Evorel (System)	25, 50, 75, 100 µg
Estradiol	Fematrix	40, 80 µg
Estradiol	Menorest	25, 37.5, 50, 75 µg
Estradiol	Progynova TS And TS Forte (Climara)	50, 100 µg
Estrógenos vaginales		
Estrógenos de equino conjugados	Premarin, crema vaginal	0.625 mg/g
Dienestrol	Ortho, crema de dienestrol	0.1 mg/g
Estradiol	Estring	7.5 µg
Estropipato	Ogen, crema vaginal	1.5 mg/g
Estradiol micronizado	Estrace, crema vaginal	1.0 mg/g

A fin de minimizar la ocurrencia de efectos secundarios asociados con los estrógenos y maximizar la relación riesgo/beneficio, deberá usarse la dosis efectiva más baja para el alivio de los síntomas y la prevención de la osteoporosis. Aunque la TRE reduce el riesgo relativo (RR) de la enfermedad isquémica cardíaca (RR, 0.50) y la osteoporosis (RR, 0.40), puede incrementarse el riesgo relativo de cáncer endometrial en mujeres postmenopáusicas con útero.

Se encuentra a disposición una extensa literatura clínica la cual revela que puede reducirse el riesgo relativo de cáncer endometrial mediante la adición de una progestina (nombre antiguo de progestágeno), ya sea de manera secuencial o continua. La adición de un progestágeno a la terapia con estrógenos, previene la proliferación endometrial estrógeno-inducida. La terapia de reemplazo de hormonas (TRH) combinada y continua, usando las dosis diarias apropiadas de estrógeno y progestágeno, ha demostrado ser eficaz en el alivio de la atrofia vaginal y de los síntomas vasomotores, así como también en la prevención de la osteoporosis postmenopáusica, y en la reducción del riesgo de cáncer endometrial por cuanto previene la hiperplasia endometrial. La siguiente Tabla contiene una lista de algunos productos TRH orales actualmente disponibles del tipo de combinación.

Productos TRH Orales del Tipo de Combinación

Nombre Comercial	Estrógeno/Progestágeno	Potencias
Activelle	Estradiol Noretisterona, acetato (NETA)	1 mg 0.5 mg
Climagest	Valerato de estradiol (Climaval) Noretisterona (NET)	1 ó 2 mg 1 mg, días 17-28
Cyclo Progynova	Valerato de estradiol Levonorgestrel	1 ó 2 mg, días 1-21 250 ó 500 µg, días 2-21
Elleste Duet	Estradiol Noretisterona, acetato	1 ó 2 mg 1 mg, días 17-28
Femoston	Estradiol Dihidrogesterona	1 ó 2 mg 10 ó 20 mg
Kliogest	Estradiol Noretisterona, acetato	2 mg 1 mg
Improvera	Piperazina estrona, sulfato Acetato de medroxiprogesterona (MPA)	1.5 mg 10 mg, días 17-28
Nuvelle	Valerato de estradiol (Progynova) Levonorgestrel	2 mg 75 µg, días 17-28
Premphase	Estrógenos conjugados MPA	0.625 mg 5.0 mg
Prempro	Estrógenos conjugados MPA	0.625 mg 2.5 ó 5.0 mg
Trisequens And Trisequens Forte	Estradiol Noretisterona	2 ó 4 mg, días 1-22 1 mg, días 23-28 1 mg, días 13-22
Ortho-Prefest	Estradiol Nogestimate	1.0mg, días 1-6 0.09 mg, días 4-6
Femhrt 1/5	Etinilestradiol Noretindrona, acetato	1.0mg 5 µg

Debido a la posibilidad de que los progestágenos mengüen los efectos favorables de los estrenos sobre los lípidos y puedan potencialmente deteriorar la tolerancia a la glucosa, resulta deseable, y constituirá un objetivo, encontrar el producto TRH con la dosis más baja de estrógeno + progestágeno, el cual también minimice o elimine la hiperplasia endometrial. Por otra parte, un factor importante que afecta la decisión de la mujer para iniciar y para continuar tomando la TRH es el sangrado vaginal, y por supuesto, muchas mujeres preferirían un producto que no produjera sangrado. Por lo tanto, otro objetivo es el de suministrar la dosis efectiva más baja que proporcione un patrón de sangrado aceptable. En el pasado se han usado dosis tan bajas como lo son las de 0.5 mg de NETA, 0.35 mg de NET, 2.5 mg de acetato de medroxiprogesterona (MPA), 0.25 mg de levonorgestrel, y 5 mg de dihidrogesterona, en regímenes de TRH continuos e ininterrumpidos.

En consecuencia, y en concordancia con la presente invención, se proporciona aquí un paquete farmacéutico que contiene unidades de dosificación diaria conformadas por una combinación de estrógenos conjugados y acetato de medroxiprogesterona, suministrada está última a una dosificación diaria de aprox. 1.5 mg, y adicionalmente se proporciona el uso de estrógenos conjugados y acetato de medroxiprogesterona en la manufactura de tales paquetes farmacéuticos para el tratamiento o inhibición de los desórdenes menopáusicos o postmenopáusicos en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de los mismos.

BREVE DESCRIPCION DE LAS FIGURAS

La FIG. 1 ilustra el número promedio de bochornos (acaloramientos) por día en pacientes que reciben combinaciones de PREMARIN + MPA, ó placebo.

La FIG. 2 ilustra la severidad promedio de los bochornos en pacientes que reciben combinaciones de PREMARIN + MPA, o placebo.

La FIG. 3 ilustra el porcentaje de pacientes con amenorrea en pacientes que reciben combinaciones de PREMARIN + MPA, o placebo.

DESCRIPCION DE LA INVENCION

El propósito de la presente invención es proporcionar los beneficios significativos de un producto TRH comercialmente exitoso, tal como el PREMPRO (0.625 mg de estrógenos de equino conjugados, USP más 2.5 mg de MPA), pero con la característica de que se ha disminuido la dosificación del MPA a un nivel por debajo de aquel que previamente ha demostrado ser eficaz, y preferiblemente también disminuir la dosificación de los estrógenos conjugados. La presente invención proporciona un método de tratamiento o inhibición de desórdenes menopáusicos o postmenopáusicos en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho tratamiento o inhibición, método que comprende la administración a dicha mujer, de manera continua e ininterrumpida en el curso de un período de tratamiento, de una combinación de estrógenos conjugados (naturales o sintéticos) y una dosificación diaria de aprox. 1.5 mg de MPA. La dosificación preferiblemente se suministra a manera de una composición farmacéutica para uso en el tratamiento de desórdenes menopáusicos o postmenopáusicos, composición farmacéutica la cual comprende una combinación de estrógenos conjugados y una dosificación de aprox. 1.5 mg de MPA. La presente invención proporciona además un empaque farmacéutico que contiene las unidades de dosificación diaria de estrógeno conjugados y MPA para administración diaria y continua.

El término "estrógenos conjugados" se refiere a sustancias esteroidales estrogénicas en las cuales uno ó más grupos funcionales (típicamente, grupos hidroxilo) en el esteroide existen a modo de un conjugado (típicamente, un sulfato o un glucurónido). Los estrógenos conjugados pueden tratarse de un solo estrógeno conjugado, o puede tratarse de mezclas de diversos estrógenos conjugados. En la literatura se encuentran descritos numerosos estrógenos conjugados y muchos están a disposición en el comercio, los cuales pueden ser formulados para uso en la presente invención ya sea a manera de un estrógeno unitario, o pueden ir en mezclas junto con otros estrógenos sintéticos y/o naturales.

Los estrógenos conjugados pueden también contener otros compuestos esteroidales o no esteroidales, los cuales pueden o no contribuir con el efecto biológico total. Entre tales compuestos se incluye, pero sin limitarse a,

estrógenos no conjugados, androstanos, y pregnanos. Los estrógenos conjugados preferidos para uso en la presente invención son PREMARIN (estrógenos de equino conjugados, USP) y CENESTIN (estrógenos conjugados sintéticos, A).

El producto PREMARIN (tabletas de estrógenos conjugados, USP) para administración oral, contiene una mezcla de estrógenos obtenidos exclusivamente de fuentes naturales, los cuales se presentan a manera de las sales sódicas de sulfatos de estrógenos solubles en agua mezclados de modo que representen la composición promedio del material derivado de la orina de yeguas embarazadas. Este producto es una mezcla de sulfato sódico de estrona y sulfato sódico de equilina, y por lo menos los siguientes 8 componentes concomitantes, también en la forma de sulfatos sódicos conjugados: 17α -dihidroequilina, 17α -estradiol, $\Delta 8,9$ -deshidroestrona, 7β -dihidroequilina, 17β -estradiol, equilenina, 17α -dihidroequilenina, y 17β -dihidroequilenina. La composición y formulación del PREMARIN continúa aún siendo analizada, y otros componentes se encuentran en el proceso de ser identificados y caracterizados. El PREMARIN está indicado en el tratamiento de síntomas vasomotores moderados a severos asociados con la menopausia; en el tratamiento de la atrofia vulvar y vaginal; y en la prevención de la osteoporosis, así como también para otras indicaciones aprobadas para los productos estrogénicos.

Las tabletas de CENESTIN (estrógenos conjugados sintéticos, A) para administración oral, contienen una mezcla de 9 sustancias estrogénicas sintéticas: sulfato sódico de estrona, sulfato sódico de 17α -dihidroequilina, sulfato sódico de 17α -estradiol, sulfato sódico de equilenina, sulfato sódico de 17β -dihidroequilenina, sulfato sódico de equilina, sulfato sódico de 17α -dihidroequilina, sulfato sódico de 17β -estradiol, sulfato sódico de 17α -dihidroequilenina. CENESTIN está indicado en el tratamiento de síntomas vasomotores moderados a severos asociados con la menopausia.

Los productos PREMARIN, CENESTIN, y el acetato de medroxiprogesterona se encuentran todos disponibles en el comercio (Wyeth-Ayerst - PREMARIN y medroxiprogesterona acetato; Duramed - CENESTIN). La dosificación del MPA es de aprox. 1.5 mg por día. Se prefiere que el constituyente estrógeno

conjugado sea el PREMARIN. Se prefiere también que la dosificación del PREMARIN sea de aprox. 0.625 mg por día o menos, y mejor aún, que la dosificación del PREMARIN sea de aprox. 0.45 mg por día ó de aprox. 0.30 mg por día.

Tal como se usa en concordancia con la presente invención, el término "desorden menopáusico o postmenopáusico" se refiere a aquellas condiciones, trastornos, o estados de enfermedad que sean por lo menos parcialmente causados por la producción disminuida de estrógenos ocurrida durante las etapas perimenopáusica, menopáusica, o post-menopáusica de la vida de una mujer. Entre tales desórdenes típicamente se incluyen, sin hacer una lista exhaustiva de éstos, uno o más de los siguientes:

- inestabilidad vasomotora, síntomas vasomotores tales como las sensaciones de acaloramiento o bochorno;
- desmineralización ósea, causante de una reducida densidad mineral del tejido óseo, y de un riesgo incrementado de desarrollar osteoporosis;
- atrofia vaginal o vulvar; vaginitis atrófica; resequedad vaginal; prurito; dispareunia; disuria;
- micción frecuente; incontinencia urinaria; infecciones del tracto urinario, niveles elevados de colesterol, triglicéridos, Lp(a), o de LDL; hipercolesterolemia; hiperlipidemia;
- enfermedad cardiovascular; aterosclerosis; enfermedad vascular periférica; restenosis, vasoespasmo; daños de las paredes vasculares provocados por eventos celulares conducentes a daños vasculares mediados por el sistema inmunológico;
- involucramiento de radicales libres en el desarrollo de cánceres, desórdenes del sistema nervioso central, enfermedad de Alzheimer, enfermedad de los huesos, envejecimiento, desórdenes inflamatorios, enfermedad vascular periférica, artritis reumatoidea, enfermedades autoinmunes, insuficiencia respiratoria, enfisema, prevención de lesiones por reperfusión, hepatitis viral, hepatitis crónica activa, tuberculosis, psoriasis, lupus eritematoso sistémico, esclerosis lateral amiotrófica, efectos del envejecimiento, síndrome de dificultad respiratoria del adulto, traumas y ataques del sistema nervioso central, o de lesiones durante los procedimientos de reperfusión;
- demencias, desórdenes neurodegenerativos, y enfermedad de Alzheimer.

Tal como se usa en el presente documento, el término "desorden menopaúsico" también abarca aquellas condiciones de producción disminuida de estrógenos que puedan ser causados quirúrgica o químicamente, o que puedan ser causados por un estado patológico que conduzca a la disminución o cesación prematura de la función ovárica.

El término "diaria" significa que la dosificación debe administrarse por lo menos una sola vez al día. Se prefiere que la frecuencia de administración sea de una sola vez al día, aunque puede ser más de una vez al día, con la condición de que toda dosificación diaria especificada no sea excedida.

El término "combinación" de estrógenos conjugados y MPA significa que la dosificación diaria de cada uno de los componentes de la combinación se debe administrar día tras día del tratamiento. Los componentes de la combinación se administran preferiblemente al mismo tiempo; ya sea a manera de una forma de dosificación unitaria que contenga ambos componentes, o a manera de unidades de dosificación separadas. Los componentes de la combinación pueden administrarse a diferentes horas durante el día, con la condición de que se logre la dosificación diaria deseada.

El término "continua e ininterrumpida" significa que no hay descanso en el régimen de tratamiento, durante el período de tratamiento. Así, "administración continua, no interrumpida" de una combinación, significa que la combinación se administra por lo menos una sola vez al día y durante todo el período de tratamiento. Se espera que el período de tratamiento con la combinación de estrógenos conjugados y MPA se realice durante por lo menos 28 días, preferiblemente 30 días y preferiblemente por un período de tratamiento más prolongado, posiblemente un período indefinido, en la medida en que una de las razones fundamentales para efectuar la administración de las combinaciones de estrógenos conjugados y MPA es la de tratar o inhibir los desórdenes menopaúsicos o postmenopaúsicos. Se prefiere que el tratamiento se suministre durante por lo menos 120 días, preferiblemente 180 días, y mejor aún a manera de un tratamiento por término indefinido. Los empaques farmacéuticos contienen suficientes unidades de dosificación diaria apropiadas para el período de tratamiento o en cantidades que faciliten el cumplimiento del paciente con el régimen de tratamiento. Por ejemplo, el empaque farmacéutico puede contener de 28 a 180 unidades de dosificación diaria, o por ejemplo de

30 a 120 unidades de dosificación diaria. Los empaques farmacéuticos que contienen un número grande unidades de dosificación pueden encontrarse a su vez sub-empacados, por ejemplo, para períodos de 28 días, o de 30 días. Los períodos de tratamiento también pueden variar dependiendo de los síntomas a ser tratados. Por ejemplo, para el tratamiento de síntomas vasomotores, se prefiere que el tratamiento dure desde un mes a varios años, dependiendo de la severidad y duración de los síntomas. La evaluación por parte de un médico junto con la interacción de la paciente puede ayudar a la determinación de la duración del tratamiento. Para el tratamiento o inhibición de la osteoporosis, se prefiere que el período de tratamiento pueda durar desde seis meses hasta cierto número de años, o indefinidamente.

La presente invención también cubre tanto los tratamientos de corto plazo como los tratamientos a término definido, que puedan tener una duración inferior a los períodos de tratamiento de 28 ó 30 días preferidos. Se prevee que una paciente pueda perder, u olvidar tomar, una o varias dosis durante el curso de un régimen de tratamiento, sin embargo, se puede considerar aún que tales pacientes siguen aún recibiendo la administración de manera continua e ininterrumpida.

El término "dosificación diaria fija" se refiere a que se suministra una misma cantidad de dosificación todos los días durante el período de tratamiento. Se prefiere que el MPA se suministre en una dosificación diaria fija de aprox. 1.5 mg, junto con la dosis de estrógenos conjugados apropiada, preferiblemente una equivalente a aprox. 0.45 mg ó aprox. 0.30 mg de PREMARIN. Un aspecto de la presente invención también cubre situaciones en las cuales una dosificación diaria fija de la combinación estrógenos conjugados más MPA no sea suministrada todos los días durante el período de tratamiento. Por ejemplo, la dosificación para una paciente en particular puede requerir que sea ajustada (ya sea hacia arriba o hacia abajo), a fin de lograr el efecto deseado hacia la mitad de un período de tratamiento.

El término "suministro" con respecto a la administración de una dosificación de uno ó de ambos componentes de la presente invención, se refiere ya sea a la administración directa de tal componente de la presente invención, o a la administración de un precursor de droga, derivado, o compuesto análogo el cual forme la cantidad equivalente del componente en cuestión dentro del

cuerpo.

La unidad de dosificación diaria preferiblemente se suministra en una forma para administración oral que contiene tanto los estrógenos conjugados como el MPA.

La capacidad de las combinaciones estrógenos conjugados + MPA de la presente invención para tratar o inhibir los desórdenes menopaúsicos o postmenopaúsicos se confirmó a través de un estudio clínico doble ciego realizado en mujeres postmenopáusicas usando combinaciones de PREMARIN + MPA, o placebo. En este estudio, las pacientes recibieron un tratamiento continuo e ininterrumpido durante 13 ciclos (1 año). Se midió el alivio de los síntomas vasomotores, la prevención de la hiperplasia endometrial, los efectos sobre los lípidos, y el sangrado vaginal a través de todo el período del estudio. Adicionalmente, se evaluó el efecto sobre la densidad mineral ósea en las pacientes que recibieron tratamiento continuo e ininterrumpido hasta por 26 ciclos (2 años).

La inestabilidad vasomotora es un desorden menopaúsico o postmenopaúsico el cual suele manifestarse a manera de episodios de bochorno (acaloramiento, o sofocos). En el estudio clínico antes descrito, se analizó el alivio de los síntomas vasomotores en un subconjunto de pacientes quienes experimentaban por lo menos un promedio de 7-8 episodios diarios de bochorno moderados a severos durante los 7 días previos a la iniciación del tratamiento del estudio. Los resultados obtenidos se resumen en las Tablas presentadas más adelante. La primera Tabla ilustra el número promedio de bochornos, y la segunda Tabla ilustra la severidad diaria promedio de los bochornos. Estos resultados se ilustran también en las FIGS. 1 y 2.

Número Promedio (+ S.E.) de Bochornos Por día

Semana	Grupo de Tratamiento			
	Placebo	0.625 P + 2.5 M*	0.45 P + 1.5 M	0.3 P + 1.5 M
1	9.41 ± 0.81	9.50 ± 0.73	9.99 ± 0.79	10.60 ± 0.76
2	8.55 ± 0.80	6.38 ± 0.72	6.98 ± 0.80	6.88 ± 0.74
3	8.51 ± 0.76	4.47 ± 0.69	4.47 ± 0.76	4.62 ± 0.71
4	8.09 ± 0.72	3.38 ± 0.66	3.23 ± 0.72	3.84 ± 0.67
8	7.10 ± 0.65	1.55 ± 0.61	1.49 ± 0.65	2.41 ± 0.60
12	5.36 ± 0.55	1.16 ± 0.49	0.94 ± 0.53	1.13 ± 0.50

*Dosificación diarias de P (PREMARIN) y M (MPA).

Severidad Promedio (+ S.E.) de los Bochomos

Semana	Grupo de Tratamiento			
	Placebo	0.625 P + 2.5 M*	0.45 P + 1.5 M	0.3 P + 1.5 M
1	2.10 ± 0.10	2.08 ± 0.09	2.14 ± 0.10	2.07 ± 0.10
2	2.06 ± 0.13	1.75 ± 0.12	1.78 ± 0.13	1.73 ± 0.12
3	1.97 ± 0.14	1.36 ± 0.13	1.42 ± 0.15	1.48 ± 0.14
4	2.03 ± 0.15	1.07 ± 0.14	1.21 ± 0.15	1.45 ± 0.14
8	1.75 ± 0.16	0.54 ± 0.14	0.70 ± 0.16	0.87 ± 0.14
12	1.57 ± 0.16	0.51 ± 0.14	0.51 ± 0.16	0.56 ± 0.14

*Dosificación diarias de P (PREMARIN) y M (MPA).

Tal como se ilustra tanto en las Tablas como en las Figuras, todas las dosificaciones de PREMARIN + MPA redujeron el número y severidad de los episodios de bochomo experimentados por las mujeres durante el estudio clínico, en comparación con las mujeres que tomaron el placebo. Todas las diferencias respecto del placebo fueron significativas ($p < 0.05$) hacia las semanas 3-4. Fue inesperado, sin embargo, cómo las dosificaciones más bajas de PREMARIN (0.45 y 0.3 mg) y de MPA (1.5 mg), redujeron de manera más rápida el número y severidad de los bochomos en el mismo grado en que lo hicieron las dosis más alta de la combinación que contenía 0.625 mg de PREMARIN + 2.5 mg de MPA.

La atrofia vaginal es un desorden menopaúsico o postmenopaúsico de frecuente ocurrencia y que conduce a la resequedad vaginal, prurito, y la dispareunia.

La atrofia vaginal es el resultado de una degradación de las células epiteliales vaginales que no son reemplazadas, lo cual conduce a un adelgazamiento del revestimiento vaginal. Los efectos de las dosis más bajas de los regímenes de estrógeno conjugado + MPA sobre la atrofia vaginal se evaluaron por comparación del índice de maduración vaginal de las células superficiales medido al inicio del tratamiento y después de los ciclos 6 y 13 del tratamiento. El índice de maduración vaginal es una medida del número de células epiteliales vaginales superficiales. Un incremento (número positivo) en el índice de maduración vaginal indica una reversación (tratamiento exitoso) de la atrofia vaginal. La siguiente Tabla resume los resultados obtenidos.

Indice de Maduración Vaginal para Células Superficiales
Mediana del Cambio versus los valores iniciales de referencia

Grupo de Tratamiento	Ciclo 6	Ciclo 13
0.625 P + 2.5 M*	10	10
0.45P + 1.5M	10	10
0.3P + 1.5M	10	10
Placebo	0	0

***Dosificaciones diarias de P (PREMARIN) y M (MPA).**

Estos datos sugieren que todas las dosificaciones evaluadas de estrógenos conjugados + MPA suministraron un mejoramiento significativo ($p < 0.001$) en el índice de maduración vaginal versus el placebo, demostrando así su capacidad para exitosamente tratar o inhibir la atrofia vaginal. Es notable cómo las dosificaciones más bajas de estrógenos conjugados + MPA fueron igualmente eficaces que la dosificación de 0.625 mg de PREMARIN + 2.5 mg de MPA en cuanto a facilitar el crecimiento de las células vaginales superficiales.

Puesto que se ha demostrado que la TRH usando estrógenos solos incrementa el riesgo relativo de hiperplasia endometrial en las mujeres postmenopáusicas con útero, se evaluó la incidencia de hiperplasia endometrial en el estudio clínico en grupos de pacientes tratados con PREMARIN + MPA, y placebo. Dos patólogos independientes evaluaron las biopsias endometriales en un modelo de estudio ciego. Se consideró que una paciente presentaba hiperplasia endometrial si los primeros dos patólogos concordaban con el diagnóstico. Si no concordaban, se consultaba a un tercer patólogo, y el diagnóstico de la hiperplasia se basaba entonces en el diagnóstico de la mayoría. La siguiente Tabla resume los resultados obtenidos después de 13 ciclos de tratamiento.

Porcentaje de Pacientes que Desarrollaron Hiperplasia Endometrial

Grupo de Tratamiento (Dosificación)*	No. de Hiperplasias	No. de Pacientes/Grupo	Calificación de la Hiperplasia (%)
Placebo	0	261	0.00
PREMARIN/MPA (0.625/2.5)	0	278	0.00

PREMARIN/MPA (0.45/1.5)	1	272	0.37
PREMARIN/MPA (0.3/1.5)	1	272	0.37

* Todas las dosificaciones son en mg/día

Estos resultados demostraron que el uso de estrógenos de equino conjugados + MPA a una dosificación de 0.625/2.5 mg/día, previno eficazmente el desarrollo de hiperplasia endometrial. Los resultados también revelan el inesperado resultado de que al bajar la dosificación del MPA a 1.5 mg/día en las combinaciones PREMARIN + MPA, también se observó una eficaz inhibición en el desarrollo de hiperplasia endometrial. La diferencia entre los resultados obtenidos en el grupo de tratamiento que recibió 1.5 mg de MPA versus el que recibió 2.5 mg MPA no fue estadísticamente significativa.

Al proporcionar un régimen de TRH que sea aceptable para las mujeres menopaúsicas, y particularmente para las postmenopaúsicas, resulta altamente deseable que el régimen produzca una alta tasa de amenorrea, en la medida en que las mayoría de las mujeres preferirán un producto que no cause manchado o un excesivo sangrado. La siguiente Tabla ilustra el porcentaje de mujeres que experimentaron ciclos amenorreicos durante los ciclos 1, 3, 6, 9, y 13.

Porcentaje de Ciclos de Amenorrea

Ciclo	Grupo de Tratamiento			
	Placebo	0.625 P + 2.5 M*	0.45 P + 1.5 M	0.3 P + 1.5 M
1	91.1	51.3	71.1	75.2
3	96.4	54.9	70.3	80.4
6	91.8	68.4	72.8	85.1
9	93.6	70.8	80.5	86.6
13	95.2	77.4	79.8	88.4

*Dosificación diarias de P (PREMARIN) y M (MPA).

Los resultados demuestran que más del 90 por ciento de las mujeres que recibieron placebo fueron amenorreicas durante todo el estudio. Si bien el porcentaje de mujeres amenorreicas que recibieron las dosificaciones diarias de 0.625 mg de PREMARIN + 2.5 mg de MPA es satisfactorio, según se determina también por el éxito comercial del producto PREMPRO (0.625 mg de estrógenos de equino conjugados + 2.5 mg de MPA), la disminución de las

dosificaciones de PREMARIN a ya sea 0.45 mg ó a 0.3 mg y del MPA a 1.5 mg, dio lugar a un porcentaje igual, si no significativamente mayor (0.3 mg PREMARIN + 1.5 mg de MPA) de mujeres que lograron amenorrea, en tanto que se mantienen aún los demás beneficios de la TRH. Adicionalmente, tal como se ilustra en la tabla anterior, y en la FIG. 3, se logró un mayor porcentaje de amenorrea de manera más rápida con las combinaciones al usar las dosis menores.

Es bien sabido que la adición de progestágenos a los regímenes TRE puede menguar algunos de los efectos cardioprotectores beneficiosos conferidos por los estrógenos, ó incluso producir efectos nocivos sobre los niveles de los lípidos. En el presente estudio, se midieron los niveles de colesterol total (TC), HDL, HDL₂, y LDL. Se observó una tendencia dosis-respuesta general entre los grupos de tratamiento, la cual reveló unos perfiles de lípidos más favorables con el uso de las dosis más altas de PREMARIN y las dosis más bajas de MPA. Los pacientes que recibieron 0.625 mg de PREMARIN + 2.5 mg de MPA presentaron ligeras reducciones en el nivel de TC, incrementos significativos en los niveles de HDL y HDL₂, y significativas disminuciones en los niveles de LDL. La dosificación de 0.45 mg de PREMARIN + 1.5 mg de MPA produjeron en las mujeres tratadas un perfil favorable similar (pero de menor magnitud) a la dosificación de 0.625 mg de PREMARIN + 2.5 mg de MPA. Las mujeres tratadas con 0.3 mg de PREMARIN + 1.5 mg de MPA exhibieron un perfil de lípidos menos favorable (TC, HDL, HDL₂ y LDL), que las mujeres tratadas con 0.625 mg de PREMARIN + 2.5 mg de MPA; sin embargo, este perfil fue incluso mejor que el observado en las que tomaron placebo.

Durante el estudio, se registraron y analizaron los eventos adversos. Los eventos adversos emergentes con el tratamiento fueron consistentes con aquellos observados con la terapia hormonal. Con la excepción del dolor en los senos, el perfil de efectos secundarios fue comparable entre los grupos de tratamiento con PREMARIN + MPA. Las mujeres que recibieron la dosificación diaria de 0.3 mg de PREMARIN + 1.5 mg de MPA experimentaron significativamente menor dolor en los senos que las mujeres que tomaron 0.625 mg de PREMARIN + 2.5 mg de MPA.

En resumen, los resultados del estudio clínico demostraron que los regímenes TRH de estrógenos conjugados que contienen dosificaciones de 1.5 mg/día de

MPA fueron igualmente eficaces en el tratamiento de los desórdenes menopáusicos o postmenopáusicos, en comparación con los regímenes que contenían la dosis más alta de 2.5 mg de MPA (específicamente, 0.625 mg de PREMARIN + 2.5 mg de MPA). Se lograron además tasas más altas de amenorrea, y de manera más rápida.

Adicionalmente, se observó menos sensibilidad al tacto en los senos en las mujeres que tomaron 0.3 mg de PREMARIN + 1.5 mg de MPA, que en las mujeres que recibieron la combinación de 0.625 mg de PREMARIN + 2.5 mg de MPA comercialmente disponible.

Es bien sabido que puede ocurrir un rápido decrecimiento en la masa ósea tanto del hueso cortical (columna vertebral) como del hueso trabecular (caderas) inmediatamente después de la menopausia, con una pérdida total de masa ósea de 1% a 5% por año, la cual continúa por 10 - 15 años. En el estudio clínico antes descrito, se determinó la densidad mineral ósea (BMD, sigla en Inglés) usando mediciones de absorciometría de rayos x de energía dual (DEXA, sigla en Inglés) de la porción lumbar de la columna vertebral (L2-L4), cuello femoral, trocánter, y del cuerpo total. Se efectuaron mediciones de la BMD por lo menos dos veces antes del estudio (con un intervalo de 7-14 días pero no mayor a 3 semanas), durante los ciclos 6, 13, 19, y dos veces durante el ciclo 26 (a intervalos de 7-14 días pero sin exceder de 3 semanas). Los resultados observados en el momento de la cita médica final (ciclo 26) se resumen en la siguiente Tabla.

Porcentaje de Cambio en la BMD (± S.E.) - Inicio del Tratamiento Versus Cita Médica Final

Grupo de Tratamiento	Vértabras Lumbares	Cuello Femoral	Trocánter	Cuerpo Total
Placebo	-2.63 ± 0.37 ⁺	-1.97 ± 0.46 ⁺	0.82 ± 0.58	-1.56 ± 0.17 ⁺
0.625 P + 2.5 M [*]	3.77 ± 0.37 ^{+, #}	1.67 ± 0.46 ^{+, #}	4.05 ± 0.59 ^{+, #}	0.96 ± 0.18 ^{+, #}
0.45 P + 1.5 M	2.45 ± 0.37 ^{+, #}	1.43 ± 0.47 ^{+, #}	3.60 ± 0.59 ^{+, #}	0.56 ± 0.18 ^{+, #}
0.3 P+ 1.5 M	1.77 ± 0.36 ^{+, #}	1.51 ± 0.44 ^{+, #}	4.66 ± 0.56 ^{+, #}	0.55 ± 0.17 ^{+, #}

* Dosificación diarias de P (PREMARIN) y M (MPA).
 + Cambio estadísticamente significativo (p <0.001) respecto de los valores iniciales.
 ++ Cambio estadísticamente significativo (p = 0.004) respecto de los valores

iniciales.

Diferencia estadísticamente significativa ($p < 0.001$) respecto del placebo.

Los resultados revelaron que todas las dosificaciones de PREMARIN + MPA significativamente incrementaron la BMD versus los valores iniciales de referencia y el placebo en la porción lumbar de la columna vertebral, cuello femoral, trocánter, y cuerpo total demostrando así que las combinaciones de estrógenos conjugados + MPA inhiben o retardan la desmineralización ósea. Estos datos también indican que las combinaciones de estrógenos conjugados + MPA efectivamente incrementaron la densidad mineral ósea respecto de los niveles observados antes del estudio, y también respecto de los pacientes que recibieron el placebo.

Con base en los resultados observados en el estudio clínico antes descrito, se ha encontrado que la administración continua e ininterrumpida de una combinación de estrógenos conjugados más una dosificación de aprox. mg de acetato de medroxiprogesterona, es útil en el tratamiento o inhibición de los desórdenes menopáusicos o postmenopáusicos en mujeres perimenopáusicas, menopáusicas, o postmenopáusicas (principalmente en estas últimas). Más específicamente, las combinaciones descritas en el presente documento son útiles en el tratamiento o inhibición de la atrofia vaginal o vulvar; vaginitis atrófica; resequedad vaginal; prurito; dispareunia; disuria; micción frecuente; incontinencia urinaria; infecciones del tracto urinario; síntomas vasomotores, incluyendo bochornos, mialgia, artralgia, insomnio, irritabilidad, y similares; como también para inhibir o retardar la desmineralización ósea; incrementar la densidad mineral ósea; y útiles en el tratamiento o inhibición de la osteoporosis.

Las combinaciones de la presente invención también ejercen un efecto cardioprotector en mujeres perimenopáusicas, menopáusicas y postmenopáusicas, y son por lo tanto útiles para disminuir los niveles de colesterol, triglicéridos, Lp(a), y LDL; para inhibir o tratar la hipercolesterolemia; hiperlipidemia; enfermedad cardiovascular; aterosclerosis; enfermedad vascular periférica; restenosis, y vasoespasmo; y para inhibir los daños de las paredes vasculares provocados por eventos celulares conducentes a daños vasculares mediados por el sistema inmunológico.

Las combinaciones de la presente invención son antioxidantes, y son por lo

tanto útiles para inhibir desórdenes o estados patológicos que involucren radicales libres. De manera más específica, las combinaciones de la presente invención son útiles en el tratamiento o inhibición del involucramiento de los radicales libres en el desarrollo de cánceres, desórdenes del sistema nervioso central, enfermedad de Alzheimer, enfermedad de los huesos, envejecimiento, desórdenes inflamatorios, enfermedad vascular periférica, artritis reumatoidea, enfermedades autoinmunes, insuficiencia respiratoria, enfisema, prevención de lesiones por reperfusión, hepatitis viral, hepatitis crónica activa, tuberculosis, psoriasis, lupus eritematoso sistémico, esclerosis lateral amiotrófica, efectos del envejecimiento, síndrome de dificultad respiratoria del adulto, traumas y ataques del sistema nervioso central, o lesiones durante los procedimientos de reperfusión.

Las combinaciones de la presente invención son útiles en el tratamiento o inhibición de demencias, desórdenes neurodegenerativos, y enfermedad de Alzheimer; y suministran neuroprotección o mejoramiento de la cognición.

Los estrógenos conjugados y el acetato de medroxiprogesterona descritos en la presente invención pueden ir formulados ya sea a manera de tabletas por separado o a manera de una tableta de combinación unitaria.

Cualquiera de los componentes o la combinación pueden formularse puros o pueden combinarse con uno o más portadores farmacéuticamente aceptables para su administración. Por ejemplo, se incluyen dentro de los portadores sólidos el almidón, lactosa, fosfato dicálcico, celulosa microcristalina, sacarosa y el caolín, en tanto que entre los portadores líquidos se mencionan el agua estéril, polietilenglicoles, tensioactivos no iónicos y aceites comestibles tales como los aceites de maíz, maní y ajonjolí, según resulten apropiados frente a la naturaleza del ingrediente activo y la forma de administración que en particular se desee. Pueden incluirse ventajosamente auxiliares de formulación usualmente empleados en la preparación de composiciones farmacéuticas, tales como agentes saborizantes, agentes colorantes, agentes preservantes, y antioxidantes, por ejemplo, vitamina E, ácido ascórbico, BHT y BHA.

Las composiciones farmacéuticas preferidas desde el punto de vista de la facilidad de su preparación y administración son las composiciones sólidas,

particularmente las tabletas y las cápsulas de relleno duro o de relleno líquido. Se prefiere la administración oral de los compuestos.

Los estrógenos conjugados y el MPA puede formularse en cierto número de maneras que proporcionen una sola forma de dosificación para la combinación. Los estrógenos conjugados puede ir incorporados dentro del núcleo de una tableta comprimida y el progestágeno puede ir colocado en un revestimiento consistente en un revestimiento comprimido, pelicular o de azúcar, tal como se describe en la Patente de los EE.UU. No. 5,547,948, citada aquí a manera de solo referencia. Las tabletas descritas en la Patente de los EE.UU. No. 5,547,948 son apropiadas para la formulación de los estrógenos conjugados y el MPA descrito en la presente invención a manera de una tableta unitaria. La Patente de los EE.UU. No. 5,908,638, citada aquí a manera de solo referencia, también describe tabletas de combinación las cuales son apropiadas para la formulación de los estrógenos conjugados y el MPA descritos en la presente invención a manera de un tableta unitaria.

Los estrógenos conjugados puede formularse en un núcleo que contenga los estrógenos conjugados, junto con diversos componentes entre las que se incluyen el alcohol, al hidroxipropilmetil celulosa, la lactosa monohidrato, el estearato de magnesio, y el almidón. El núcleo puede ir recubierto con un revestimiento elaborado de componentes tales como etilcelulosa, y trietil citrato. Ambos componentes de la combinación pueden incorporarse en el núcleo comprimido de la tableta o en un revestimiento de la tableta formulado para mantener la estabilidad de la droga y proporcionar la adecuada biodisponibilidad oral. Por ejemplo, el progestágeno puede ir micronizado.

Los estrógenos conjugados pueden incorporarse en gránulos, en esferúlas o en otras formas multiparticulares (en múltiples partículas), y, si es necesario, pueden revestirse para proporcionar la adecuada estabilidad. Estas formas multiparticulares pueden combinarse, en las apropiadas proporciones, con una mezcla de polvos, por granulación o en forma de multipartículas que contenga el progestágeno, para luego llenarse en cápsulas de gelatina dura.

La presente invención también proporciona un sistema de paquete farmacéutico, el cual contiene cierto número de unidades de dosificación farmacéutica diaria. Preferiblemente, y tradicionalmente, el paquete contiene 28

tabletas o múltiples series de éstas. El paquete deberá indicar que las unidades de dosificación deben ser tomadas consecutivamente y de manera diaria hasta que finalice el período de tratamiento, o hasta que se haya terminado el contenido del paquete. El siguiente paquete deberá comenzarse a usar en el siguiente consecutivo día. En el caso de las combinaciones que contengan una tableta de dosificación unitaria que contiene los estrógenos conjugados + el MPA, resulta preferible que el paquete contenga una tableta que corresponda a cada día de administración. Para el caso de las combinaciones conformadas por unidades de dosificación separadas de estrógenos conjugados y de MPA, se prefiere que cada tableta de cada una de las unidades corresponda a cada día de administración, según se indique en el paquete de las tabletas.

REIVINDICACIONES

- 1. El uso de estrógenos conjugados y acetato de medroxiprogesterona en la manufactura de paquetes farmacéuticos que contienen unidades de dosificación diaria para el tratamiento o inhibición de los desórdenes menopáusicos o postmenopáusicos en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho tratamiento o inhibición, en donde cada unidad de dosificación diaria comprende una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.**
- 2. El uso acorde con la Reivindicación 1, el cual comprende el tratamiento o inhibición de los síntomas vasomotores en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho tratamiento o inhibición.**
- 3. El uso acorde con la Reivindicación 2, en donde el síntoma vasomotor es el bochorno o sofocación.**
- 4. El uso acorde con la Reivindicación 1, el cual comprende la inhibición o retardo de la desmineralización ósea o el tratamiento o inhibición de la osteoporosis en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho tratamiento o inhibición.**
- 5. El uso acorde con la Reivindicación 1, el cual comprende el tratamiento o inhibición de la atrofia vaginal o vulvar; vaginitis atrófica; resequedad vaginal; prurito; dispareunia; disuria; micción frecuente; incontinencia urinaria; y de infecciones del tracto urinario en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho tratamiento o inhibición.**
- 6. El uso acorde con la Reivindicación 1, el cual comprende la disminución de los niveles de colesterol, triglicéridos, Lp(a), ó de LDL; la inhibición o tratamiento de la hipercolesterolemia; hiperlipidemia; enfermedad cardiovascular; aterosclerosis; enfermedad vascular periférica; restenosis, vasoespasmo; o la inhibición de daños de las paredes vasculares provocados por eventos celulares conducentes a daños vasculares mediados por el sistema inmunológico, en una mujer perimenopáusica, menopáusica, o**

postmenopáusica en necesidad de dicha disminución, tratamiento o inhibición.

7. El uso de estrógenos conjugados y acetato de medroxiprogesterona en la manufactura de paquetes farmacéuticos que contienen unidades de dosificación diaria para el tratamiento o inhibición del involucramiento de los radicales libres en el desarrollo de cánceres, desórdenes del sistema nervioso central, enfermedad de Alzheimer, enfermedad de los huesos, envejecimiento, desórdenes inflamatorios, enfermedad vascular periférica, artritis reumatoidea, enfermedades autoinmunes, insuficiencia respiratoria, enfisema, prevención de lesiones por reperfusión, hepatitis viral, hepatitis crónica activa, tuberculosis, psoriasis, lupus eritematoso sistémico, esclerosis lateral amiotrófica, efectos del envejecimiento, síndrome de dificultad respiratoria del adulto, traumas y ataques del sistema nervioso central, o lesiones durante los procedimientos de reperfusión, en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho tratamiento o inhibición, en donde cada unidad de dosificación diaria comprende una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

8. El uso de estrógenos conjugados y acetato de medroxiprogesterona en la manufactura de paquetes farmacéuticos que contienen unidades de dosificación diaria para el tratamiento o inhibición de demencias, desórdenes neurodegenerativos, y de la enfermedad de Alzheimer; y para suministrar neuroprotección o mejoramiento de la cognición en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho tratamiento o inhibición, en donde cada unidad de dosificación diaria comprende una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

9. El uso de estrógenos conjugados y acetato de medroxiprogesterona en la manufactura de paquetes farmacéuticos que contienen unidades de dosificación diaria para incrementar la densidad mineral ósea en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho incremento, en donde cada unidad de dosificación diaria comprende una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

10. El uso de estrógenos conjugados y acetato de medroxiprogesterona en

la manufactura de paquetes farmacéuticos que contienen unidades de dosificación diaria para minimizar o reducir los niveles de dolor en los senos en una mujer que reciba terapia hormonal de reemplazo, en donde cada unidad de dosificación diaria comprende una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

11. El uso de estrógenos conjugados y acetato de medroxiprogesterona en la manufactura de paquetes farmacéuticos que contienen unidades de dosificación diaria para minimizar el manchado o el excesivo sangrado, o para lograr la amenorrea en una mujer que reciba terapia de reemplazo de hormonas, en donde cada unidad de dosificación diaria comprende una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

12. El uso acorde con la Reivindicación 11, en donde el tiempo para minimizar el manchado o el excesivo sangrado o para lograr el inicio de la amenorrea es acelerado.

13. El uso acorde con cualquiera de las Reivindicaciones 1 a 12, en donde los estrógenos conjugados son los estrógenos conjugados sintéticos, A.

14. El uso acorde con cualquiera de las Reivindicaciones 1 a 12, en donde los estrógenos conjugados son los estrógenos de equino conjugados, USP.

15. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde la dosificación diaria de estrógenos conjugados se sitúa entre aprox. 0.625 mg y aprox. 0.3 mg.

16. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde la dosificación diaria de estrógenos conjugados, se sitúa entre aprox. 0.45 mg y aprox. 0.3 mg.

17. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde la dosificación diaria de estrógenos conjugados es de aprox. 0.45 mg.

18. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde la dosificación diaria de estrógenos conjugados es de aprox. 0.3 mg.

19. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde el paquete farmacéutico contiene unidades de dosificación diaria fijas.
20. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde el paquete farmacéutico contiene por lo menos 28 unidades de dosificación diaria.
21. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde el paquete farmacéutico comprende hasta 180 unidades de dosificación diaria.
22. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde el paquete farmacéutico contiene por lo menos 30 unidades de dosificación diaria.
23. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde el paquete farmacéutico comprende hasta 120 unidades de dosificación diaria.
24. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde el acetato de medroxiprogesterona es micronizado.
25. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde la unidad de dosificación diaria se suministra una sola vez al día.
26. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde la unidad de dosificación diaria se suministra para administración oral.
27. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde la unidad de dosificación diaria contiene 0.45 mg de estrógenos de equino conjugados y 1.5 mg de acetato de medroxiprogesterona.
28. El uso acorde con cualquiera de las Reivindicaciones precedentes, en donde la unidad de dosificación diaria contiene 0.3 mg de estrógenos de equino conjugados y 1.5 mg de acetato de medroxiprogesterona.

29. Una composición farmacéutica para uso en el tratamiento de desórdenes menopaúsicos o postmenopaúsicos en una mujer perimenopaúsica, menopaúsica, o postmenopaúsica en necesidad de dicho tratamiento, la cual comprende estrógenos conjugados, una dosificación de aprox. 1.5 mg acetato de medroxiprogesterona, y un portador farmacéutico.
30. Una unidad de dosificación farmacéutica la cual comprende estrógenos conjugados, una dosificación de aprox. 1.5 mg de acetato de medroxiprogesterona, y un portador farmacéutico.
31. Un paquete farmacéutico que contiene unidades de dosificación diaria las cuales contienen una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.
32. La composición, unidad o empaque acordes con las Reivindicaciones 29, 30 ó 31, en donde los estrógenos conjugados son estrógenos de equino conjugados, USP.
33. La composición, unidad o paquetes acordes con las Reivindicaciones 29, 30 ó 31, en donde los estrógenos conjugados son los estrógenos conjugados sintéticos, A.
34. La composición, unidad o paquetes acordes con las Reivindicaciones 32 ó 33, en donde la dosificación se sitúa entre aprox. 0.45 mg y aprox. 0.30 mg.
35. La composición, unidad o paquetes acordes con las Reivindicaciones 32 ó 33 en donde la dosificación es de aprox. 0.45 mg ó de aprox. 0.30 mg.
36. La composición, unidad o paquetes acordes con las Reivindicaciones 29, 30 ó 31, en donde el acetato de medroxiprogesterona es micronizado.
37. El paquete farmacéutico acorde con la Reivindicación 31, el cual comprende hasta 180 unidades de dosificación diaria fijas.
38. El paquete farmacéutico acorde con la Reivindicación 37, el cual contiene por lo menos 28 unidades de dosificación diaria fijas.

39. El paquete farmacéutico acorde con la Reivindicación 37, el cual contiene por lo menos 30 unidades de dosificación diaria fijas.

40. Un método de tratamiento o inhibición de desórdenes menopaúsicos o postmenopaúsicos en una mujer perimenopaúsica, menopaúsica, o postmenopaúsica en necesidad de dicho tratamiento o inhibición, método que comprende la administración a dicha mujer, de manera continua e ininterrumpida en el curso de un período de tratamiento, de una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

41. Un método acorde con la Reivindicación 40, el cual comprende el tratamiento o inhibición de los síntomas vasomotores en una mujer perimenopaúsica, menopaúsica, o postmenopaúsica en necesidad de dicho tratamiento o inhibición.

42. El método acorde con la Reivindicación 41, en donde el síntoma vasomotor es el bochorno o sofocación.

43. Un método acorde con la Reivindicación 40, el cual comprende la inhibición o retardo de la desmineralización ósea o el tratamiento o inhibición de la osteoporosis en una mujer perimenopaúsica, menopaúsica, o postmenopaúsica en necesidad de dicha inhibición, retardo o tratamiento.

44. Un método acorde con la Reivindicación 40, el cual comprende el tratamiento o inhibición de la atrofia vaginal o vulvar; vaginitis atrófica; resequedad vaginal; prurito; dispareunia; disuria; micción frecuente; incontinencia urinaria; infecciones del tracto urinario en una mujer perimenopaúsica, menopaúsica, o postmenopaúsica en necesidad de dicho tratamiento o inhibición.

45. Un método acorde con la Reivindicación 40, el cual comprende la disminución de los niveles de colesterol, triglicéridos, Lp(a), o de LDL; la inhibición o tratamiento de la hipercolesterolemia; hiperlipidemia; enfermedad cardiovascular; aterosclerosis; enfermedad vascular periférica; restenosis, vasoespasmo; o la inhibición de los daños de las paredes vasculares provocados por eventos celulares conducentes a daños vasculares mediados

por el sistema inmunológico, en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicha disminución, tratamiento o inhibición.

46. Un método de tratamiento o inhibición del involucramiento de los radicales libres en el desarrollo de cánceres, desórdenes del sistema nervioso central, enfermedad de Alzheimer, enfermedad de los huesos, envejecimiento, desórdenes inflamatorios, enfermedad vascular periférica, artritis reumatoidea, enfermedades autoinmunes, insuficiencia respiratoria, enfisema, prevención de lesiones por reperfusión, hepatitis viral, hepatitis crónica activa, tuberculosis, psoriasis, lupus eritematoso sistémico, esclerosis lateral amiotrófica, efectos del envejecimiento, síndrome de dificultad respiratoria del adulto, traumas y ataques del sistema nervioso central, o lesiones durante los procedimientos de reperfusión, en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho tratamiento o inhibición, método que comprende la administración a dicha mujer, de manera continua e ininterrumpida en el curso de un período de tratamiento, de una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

47. Un método de tratamiento o inhibición de demencias, desórdenes neurodegenerativos, y de la enfermedad de Alzheimer; y para proporcionar neuroprotección o mejoramiento de la cognición en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho tratamiento o inhibición, método que comprende la administración a dicha mujer, de manera continua e ininterrumpida en el curso de un período de tratamiento, de una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

48. Un método para incrementar la densidad mineral ósea en una mujer perimenopáusica, menopáusica, o postmenopáusica en necesidad de dicho incremento, método que comprende la administración a dicha mujer, de manera continua e ininterrumpida en el curso de un período de tratamiento, de una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

49. Un método para minimizar o reducir los niveles de dolor en los senos en una mujer que reciba terapia de reemplazo de hormonas, método que comprende la administración a dicha mujer, de manera continua e

ininterrumpida en el curso de un período de tratamiento, de una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

50. Un método para minimizar el manchado o el excesivo sangrado; o para lograr la amenorrea en una mujer que siga una terapia de reemplazo de hormonas, método que comprende la administración a dicha mujer, de manera continua e ininterrumpida en el curso de un período de tratamiento, de una combinación de estrógenos conjugados y una dosificación diaria de aprox. 1.5 mg de acetato de medroxiprogesterona.

51. El método acorde con la Reivindicación 50, en donde el tiempo para minimizar el manchado o el excesivo sangrado, o para lograr el inicio de la amenorrea es acelerado.

52. El método acorde con cualquiera de las Reivindicaciones 40 a 51, en donde los estrógenos conjugados son los estrógenos conjugados sintéticos, A.

53. El método acorde con cualquiera de las Reivindicaciones 40 a 51, en donde los estrógenos conjugados son los estrógenos de equino conjugados, USP.

54. El método acorde con cualquiera de las Reivindicaciones 40 a 51, en donde la dosificación diaria de estrógenos conjugados se sitúa entre aprox. 0.625 mg y aprox. 0.3 mg.

55. El método acorde con cualquiera de las Reivindicaciones 40 a 53, en donde la dosificación diaria de estrógenos conjugados se sitúa entre aprox. 0.45 mg y aprox. 0.3 mg.

56. El método acorde con cualquiera de las Reivindicaciones 40 a 53 en donde la dosificación diaria de estrógenos conjugados, es de aprox. 0.45 mg ó de aprox. 0.3 mg.

57. El método acorde con cualquiera de las Reivindicaciones 40 a 53, en donde la unidad de dosificación diaria contiene 0.45 mg de estrógenos de equino conjugados y 1.5 mg de acetato de medroxiprogesterona.

58. El método acorde con cualquiera de las Reivindicaciones 40 a 53, en donde la unidad de dosificación diaria contiene 0.30 mg de estrógenos de equino conjugados y 1.5 mg de acetato de medroxiprogesterona.

NUMERO PROMEDIO DE BOCHORNOS POR DIA

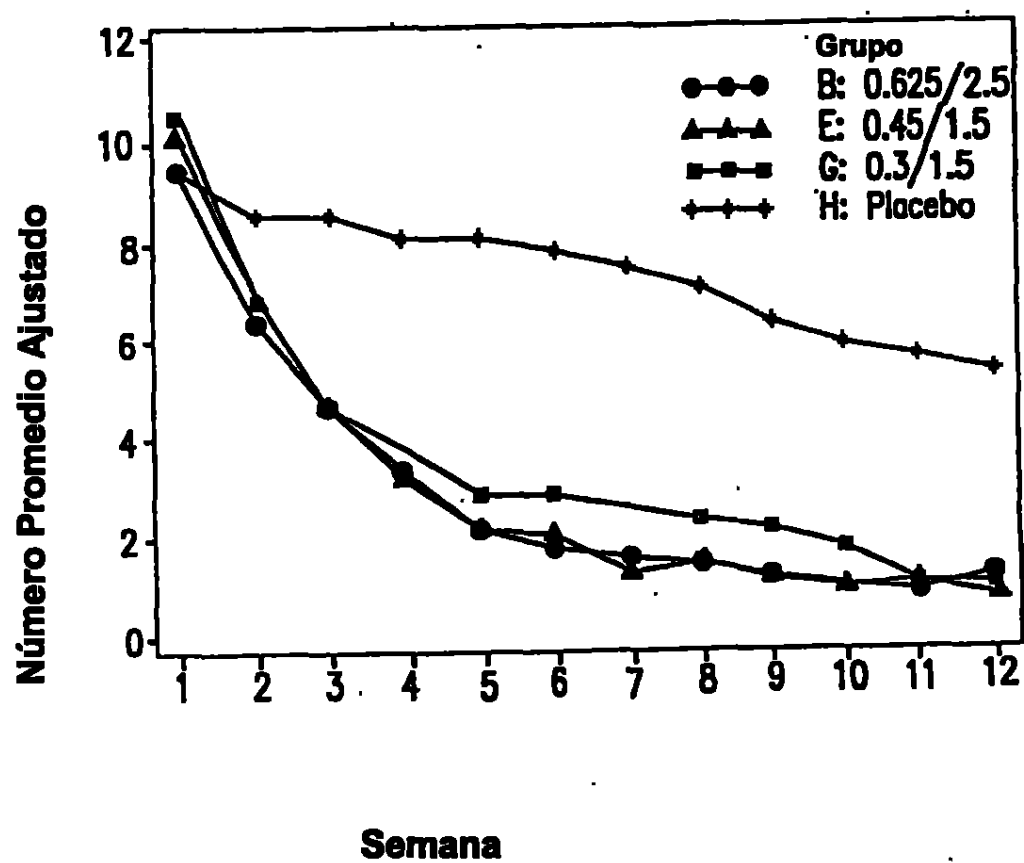


FIG. 1

SEVERIDAD PROMEDIO DE LOS EPISODIOS DE BOCHORNO

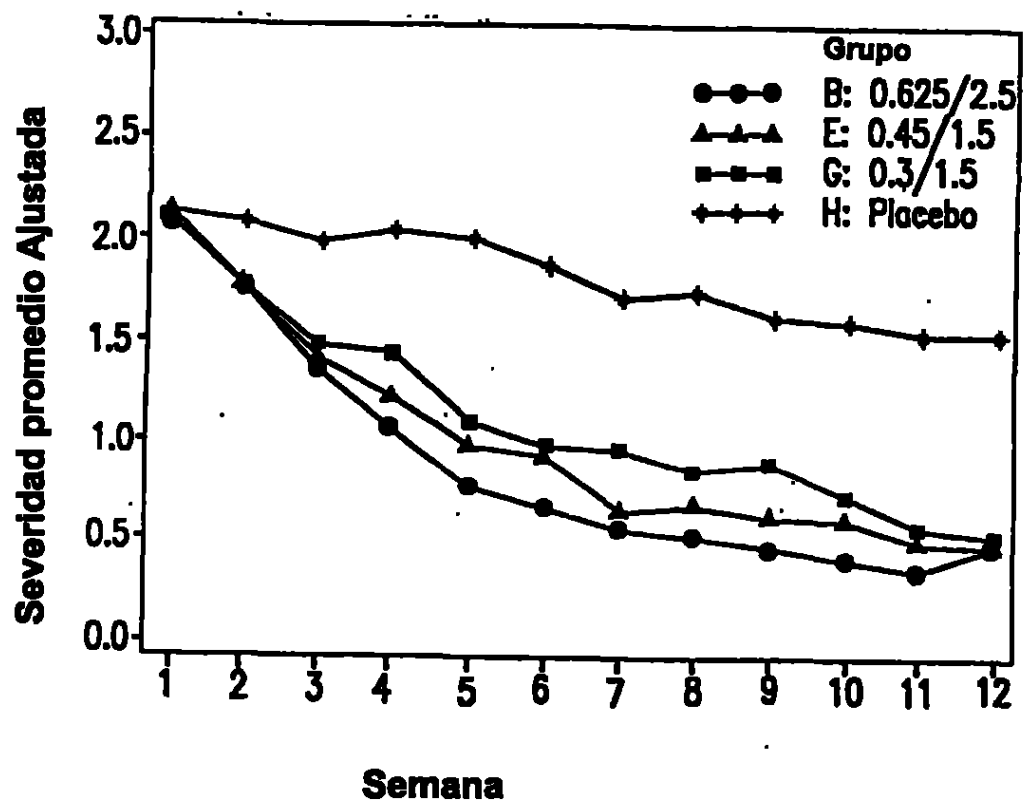


FIG. 2

PORCENTAJE DE PACIENTES CON AMENORREA

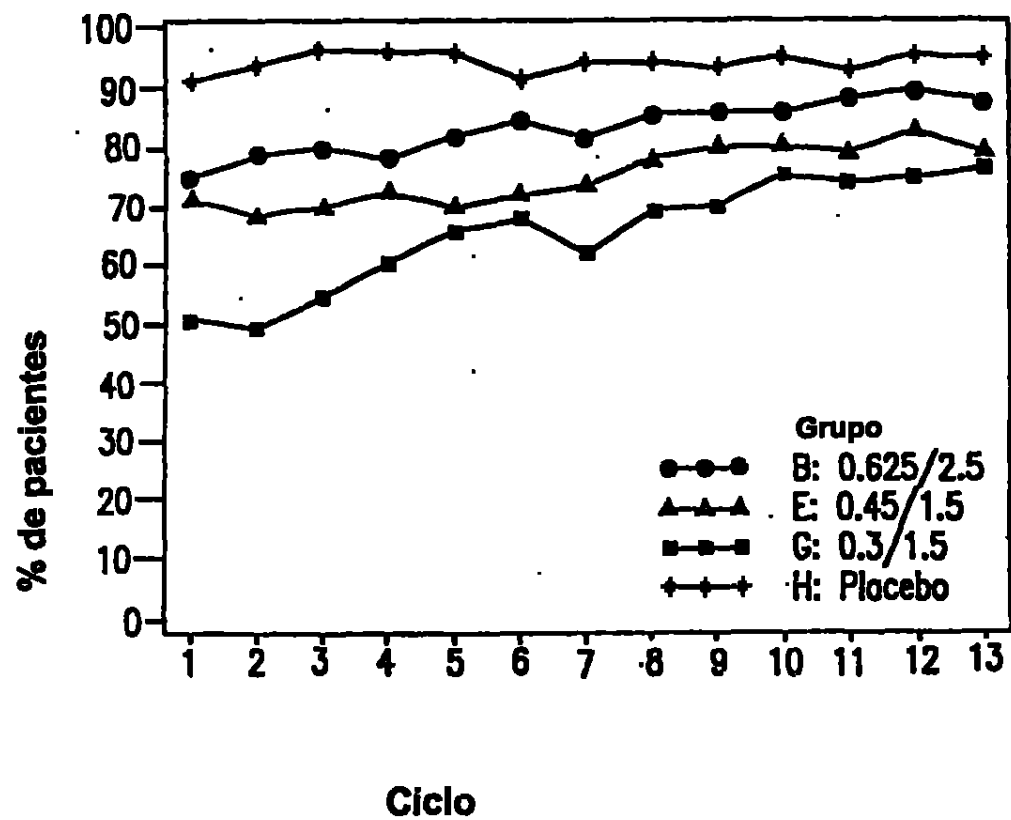


FIG. 3

REQUISITOS MINIMOS PARA ADMISION A TRAMITE

PCT

PATENTE DE INVENCION

MODELO DE UTILIDAD []

- | | |
|---|-------|
| • Indicación de que se solicita la concesión de una patente | 111 |
| • Datos de identificación del solicitante o de la persona que presenta la solicitud | 111 |
| • Descripción de la invención | 7911 |
| • Dibujos de ser estos pertinentes | 11111 |
| • Comprobante de Pago de las tasas establecidas | 21111 |

COMPLETA ☒

INCOMPLETA [1]

DISEÑO INDUSTRIAL [1]

- Indicación de que se solicita el registro de Diseño Industrial. []
- Datos de identificación del solicitante o de la persona que presenta la solicitud. []
- Representación gráfica y fotográfica del Diseño Industrial o muestra del material que incorpora el diseño . []
- Comprobante de pago de las tasas establecidas. []

COMPLETA ()

INCOMPLETA []

ESQUEMA DE TRAZADO []

- Indicación de que se solicita el registro de un Esquema de trazado. []
- Datos de identificación del solicitante o de la persona que presenta la solicitud. []
- Representación gráfica del esquema de trazado []
- Comprobante de pago de las tasas establecidas. []

COMPLETA []

INCOMPLETA []

~~Firma Responsable~~

SUPERINTENDENCIA Y COMERCIO

Folios: 113

Radicación : 020363 500000

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Trámite : 011 PCT-PATENT 379 FASE NUCI 411 PRESENTAC
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2020
Bogotá DC

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JOSE ANTONIO LLOREDA
Apoderado(a)

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	Fecha Inicio fase nacional	20/10/2002
	Trámite	011
	Evento	379
	Actuación	416
	Oficio No.	952
	Folios	001

Teniendo en cuenta que la solicitud de privilegio de patente que se tramita bajo el expediente indicado en la referencia, reúne los requisitos de forma establecidos en la Decisión 486 de la Comisión de la Comunidad Andina, el Tratado de Cooperación en Materia de Patentes (PCT), el Reglamento y la Circular Unica de la Superintendencia de Industria y Comercio, se envía el extracto de la solicitud a la Oficina de Comunicaciones para efecto de su publicación en la Gaceta de Propiedad Industrial.

Cúmplase
Dado en Bogotá DC,

09 JUL 2009


ALIX CARMENZA CÉSPEDES DE VERGEL
Jefe de la División de Nuevas Creaciones

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