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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO



No. 10-025622- -00007-0000

Fecha: 2013-02-15 15:20:52 Dep. 2020 DIR.NUEVASCR
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 523 RESPUESTA Folios: 76

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

DIVISION DE NUEVAS CREACIONES

E.

S.

D.

Re: Expediente No. 10.025.622 
Solicitud de patente a nombre de **ENDORECHERCHE, INC.**

Yo, DILIA MARIA RODRIGUEZ D'ALEMAN identificada como aparece al pie de mi firma, domiciliada en la Carrera 11 No. 86-53, piso 6, obrando como apoderada de la sociedad **ENDORECHERCHE, INC.**, domiciliada en Quebec, Canadá, solicitante de la patente de la referencia, en respuesta al oficio No. 17864 notificado por fijación en lista el 9 de octubre de 2012, me permito manifestar:

1. Con fundamento en la facultad que confiere el artículo 34 de la Decisión 486 al solicitante para modificar la solicitud de patente en cualquier momento del trámite, presento junto con este memorial nuevo capítulo reivindicatorio que consta de 24 reivindicaciones dirigidas a la composición farmacéutica y al supositorio intravaginal que comprende cantidades específicas de precursor esteroide sexual

En cuanto a estas modificaciones, me permito decir que las mismas no amplían de ninguna manera la materia objeto de la presente solicitud y se realizaron con el ánimo de superar las objeciones de claridad y novedad planteadas por el examinador en su concepto técnico.

Se adjunta recibo por pago de 13 reivindicaciones adicionales.

2. Excepciones a la patentabilidad

Esta objeción queda cabalmente superada dado que el nuevo capítulo reivindicatorio no contiene reivindicaciones de método o de uso.

3. Claridad de la solicitud

Respecto a este punto, el solicitante manifiesta que mediante las modificaciones realizadas tanto en el capítulo reivindicatorio queda subsanada la mayoría de objeciones, de modo tal que la nueva reivindicación proporciona claridad y

P-2461

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concisión a la invención descrita, siguiendo las recomendaciones realizadas por el examinador en su concepto técnico.

En cuanto a la antigua reivindicación 32 donde se establecían los precursores y los excipientes, atentamente nos permitimos manifestar que ahora las reivindicaciones son más claras. Adicionalmente, los excipientes enunciados en las reivindicaciones son los usualmente empleados para esta clase de composiciones.

Con respecto al soporte de los precursores esteroides sexuales diferentes de dehidroepiandrosterona y los excipientes diferentes de Witepsol H-15, el solicitante desea manifestar que las composiciones farmacéuticas que contienen sulfato de dehidroepiandrosterona, androst-5-eno-3 β , 17 β -diol o 4-androsteno-3, 17-diona se discuten en las páginas 4 a 5 y en la página 130 de la descripción en adición a la reivindicación original 32. En dichas páginas se describe un gran número de excipientes adicionales a los de la antigua reivindicación 32, con lo cual es claro que en la reivindicación 32 antigua, se está haciendo una limitación expresa de dichos excipientes y existe sustento adecuado para dichas composiciones dentro de la descripción.

En cuanto a la objeción de Witepsol H-15 como una marca, es deseo del solicitante indicar que el término Witepsol H-15 debe mantenerse, dado que se es específico. En este sentido, el solicitante desea indicar que el término Witepsol solo, podría causar una falta de claridad, pero en las reivindicaciones modificadas, el nombre Witepsol se combina con la descripción H-15. Esta información específica da al término una composición química bien definida, que sería evidente para una persona versada en el arte, particularmente en vista del uso bien conocido de este producto en forma de preparados farmacéuticos disponibles en el mercado. En otras palabras, la objeción de marca debe ser aplicada a Witepsol solo y no al Witepsol H-15 específico.

En aras de sustentar lo anterior, el solicitante respetuosamente se permite adjuntar copias del artículo De Muynck y colaboradores (Ver renglón 7 de la segunda columna de la página 945 y la Tabla 1) y de Van Lingen y colaboradores (Ver renglón final de la primera columna en la página 510) para apoyar esta afirmación con respecto a Witepsol H-15. (Ver anexo 1)

Aunado a lo anterior, también se ha incluido información de productos de Sasol Germany GmbH en (a) "Witepsol®" y Massa Estarinum®, y (b) los excipientes para la industria farmacéutica (ver página 6), ambos muestran las

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diferentes propiedades específicas de los diversos productos Witepsol®, incluyendo Witepsol H-15. (Anexo 2)

En vista de toda la información anterior en la literatura, una persona versada en el arte entenderá el significado y el alcance de las marcas comerciales utilizadas en la presente memoria incluso si la composición de estos productos fuera cambiada, lo cual es muy poco probable. Es mucho más probable, teniendo en cuenta el uso generalizado de Witepsol H-15 en la industria farmacéutica en todo el mundo, que tales composiciones, si se reformulan, fueran renombradas reemplazando el "H-15" con números diferentes. En vista del interés potencial comercial en la composición reivindicada, sería muy importante conservar esta marca.

No obstante lo anterior, es deseo del solicitante incluir en la nueva reivindicación 24 en donde el Witepsol H-15 podría ser sustituido por la mezcla de monoglicéridos, diglicéridos, y triglicéridos del ácido graso saturado C10 a C18, en el que el componente principal de la mezcla, en peso, es triglicérido láurico, teniendo en cuenta los componentes específicos del witepsol H-15.

Esta reivindicación se apoya en el anexo 1 de la referencia de De Muynck, que indica "Las cinco bases de triglicérido "puro" fueron tres glicéridos láuricos ... y Witepsol H15..." (ver columna de la derecha en la página 945), y por la referencia adjunta de Van Lingen que indica que Witepsol H 15 es una "mezcla sintética de monoglicéridos, diglicéridos, y triglicéridos de ácidos grasos saturados C10 a C18" (ver página 510).

4. Novedad

El examinador ha rechazado las antiguas reivindicaciones porque estarían anticipadas por, las referencias D1 a D4. En este sentido, nos permitimos manifestar que no nos encontramos de acuerdo.

En efecto, el artículo 16 de la Decisión 486 establece que: "*Una invención se considera nueva cuando no está comprendida en el estado de la técnica*."

El estado de la técnica comprenderá todo lo que haya sido accesible al público por una descripción escrita u oral, utilización, comercialización o cualquier otro medio antes de la fecha de presentación de la solicitud de patente o, en su caso, de la prioridad reconocida...."

(El resaltado es nuestro) y de acuerdo con el espíritu de la norma legal indicada, la invención no se considera como nueva si está "**comprendida**" en el estado de la técnica, dicha norma se está refiriendo, naturalmente, a la **totalidad del invento** y no a alguno o a algunos de los componentes que hacen parte de la nueva solicitud.

En efecto, para que la invención pierda su calidad de novedosa, es claro que ella, TODA ELLA, ha de estar contenida en el “*conjunto de conocimientos existentes que son aplicables al proceso industrial*” como define el estado de la técnica el Tribunal de justicia del Acuerdo de Cartagena (Proceso No. 6-IP-89, sentencia de 31 de octubre de 1989, publicada en Jurisprudencia del Tribunal de Justicia del Acuerdo de Cartagena, Tomo 11, BID-INTAL, Buenos Aires, 1994, página 60).

Esto se conoce como **NOVEDAD ABSOLUTA**, de conformidad con lo cual para desvirtuar el requisito de novedad de una invención será necesario que lo reivindicado por la invención bajo estudio corresponda de **forma total** con lo revelado en un documento o anterioridad señalada, conclusión a la que se llega después de un análisis exhaustivo mediante una comparación entre el capítulo reivindicatorio de la invención en estudio y la divulgación citada por ese despacho como anterioridad.

Ahora bien, si un determinado elemento, condición o circunstancia de la nueva creación ya hace parte del estado de la técnica no quiere ello decir, desde luego, que la invención deba ser rechazada por carecer del requisito de novedad. No sin razón ha dicho el mencionado Tribunal: “*hay que advertir que toda creación es en cierta forma, producto del estado de la técnica existente, en el sentido de que el inventor ha de tener en cuenta un conjunto de conocimientos técnicos que le permitan crear nuevos procedimientos aplicables a la industria*” (Ibidem, páginas 53 y 54) (El subrayado es nuestro).

Por lo tanto y teniendo en cuenta lo anteriormente expuesto, como mínimo la novedad debe contemplar los siguientes aspectos:

-Que la invención en estudio esté contenida de forma **TOTAL** y **ABSOLUTA** en cada uno de los documentos que se mencionan como anterioridades; y

-Que la invención en cuestión puede partir de conocimientos conocidos, caso en el cual habrá que hacer la comparación no de lo conocido sino de lo inédito y novedoso, teniendo que analizarse el **valor agregado** que aporta la invención con sus nuevas características al campo técnico al cual pertenece la solicitud en estudio.

Aplicando lo anterior al caso concreto, para que las razones que aduce ese despacho para desvirtuar la novedad fuesen ciertas, sería forzoso que se

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demostrara que la invención de mi mandante está comprendida en el estado de la técnica de forma **TOTAL**, lo cual no sucede toda vez que las características mostradas en el capítulo reivindicatorio modificado son totalmente nuevas en vista de los documentos citados como de muestra a continuación.

D1 CA 2154161 (Endorecherche)

Esta patente se refiere a la utilización de una composición que comprende DHEA o DHEA-S (o pro-medicamentos) en la prevención o tratamiento de la atrofia vaginal, incontinencia urinaria, así como otras condiciones no citadas en las reivindicaciones de la presente solicitud.

Las reivindicaciones originales de la referencia D1 y las reivindicaciones originales de la presente solicitud sólo comparten la atrofia vaginal y la incontinencia urinaria como enfermedades vaginales. Por otra parte, la reivindicación modificada es específica para dosificaciones bajas de precursores de esteroides sexuales (13 mg por día o menos). La base de la presente invención es una dosis baja de precursor de esteroides sexuales por administración intra-vaginal (local) sin absorción sistémica lo cual es diferente de la referencia D1 en donde se espera un aumento de los niveles en sangre de DHEA. La eficacia del tratamiento con la composición de la presente invención fue demostrada sin aumentar el nivel en sangre de estradiol y evitando el riesgo de cáncer de seno y cáncer uterino (ver Labrie F. *et al* Menopause 2009, 16(5), 897, 907 y 923) (Anexo 3).

Esta patente indica que "el nivel en sangre de DHEA es el criterio final de la dosis adecuada el cual tiene en cuenta la variación individual en la absorción y el metabolismo" (página 30, renglón 32) que es contrario a lo que se propone en la presente solicitud. Las reivindicaciones actuales pronostican una administración local (vaginal) sin absorción sistémica. En un ejemplo mostrado en la solicitud actual, algo de DHEA va en la circulación pero menos que en el grupo de mujeres pre-menopáusicas (3,18 ng/mL para DHEA 1,0% (13 mg) versus 4,47 ng/mL; ver tabla 2 de la presente solicitud).

De otro lado, un alto nivel en sangre de DHEA, el cual es sugerido por la referencia D1, aumenta el nivel de circulación del estradiol con lo cual se aumenta el riesgo de cáncer de seno y cáncer uterino en mujeres pos-menopáusicas. Adicionalmente, la patente citada sugiere la combinación del precursor de esteroides sexuales con estrógeno (página 11, último párrafo) lo cual es conocido por aumentar el riesgo de cáncer de seno y cáncer uterino.

Los ejemplos que se trabajaron se realizaron sobre un modelo animal (rata y no en vagina) y no en humanos (mujeres pos-menopáusicas) como se describe en la presente solicitud. Adicionalmente, la presente solicitud ha estudiado los

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parámetros en mujeres pos-menopáusicas (la mayoría de los molestos síntomas y los efectos sobre las células parabasales vaginales y superficiales y el pH) que están directamente relacionados con las enfermedades vaginales.

Esta referencia menciona la expresión "mucosa vaginal" (p. 32, renglón 22), pero esta referencia no sugiere una composición farmacéutica para aplicación vaginal. Además, esta referencia no se pronuncia sobre un supositorio vaginal.

D2 CA 2334577 (Endorecherche)

Esta solicitud de patente se refiere al uso de una combinación de precursor de esteroides sexuales incluyendo DHEA con Modulador Selectivo del Receptor de Estrógeno en la prevención o tratamiento de la sequedad vaginal, así como otras condiciones no reivindicadas en la presente solicitud. Aunque la aplicación se refiere a la administración transmucosal, y no se refiere a la administración intra-vaginal. Las reivindicaciones modificadas de la composición farmacéutica de la presente solicitud se limitan ahora a la administración intra-vaginal. Por consiguiente, la divulgación de las formulaciones tópicas en la referencia D2 no es relevante para destruir la novedad de las reivindicaciones de la composición farmacéutica. Esta referencia se relaciona a composiciones farmacéuticas de una combinación de SERM con precursores de esteroides sexuales, y no a formulaciones intra-vaginales de precursores de esteroides sexuales.

Esta patente indica que "el nivel en sangre de DHEA es el criterio final de la dosis adecuada el cual tiene en cuenta la variación individual en la absorción y el metabolismo" (página 31, primer párrafo) que es contrario a lo que se propone en la presente solicitud. Las reivindicaciones actuales pronostican una administración local (vaginal) sin absorción sistémica. En un ejemplo descrito en la solicitud actual, algo de DHEA va en la circulación pero menos que en el grupo de mujeres pre-menopáusicas (3,18 ng/mL para DHEA 1,0% (13 mg) versus 4,47 ng/mL; ver la tabla 2 en la descripción de la presente solicitud). El alto nivel en sangre de DHEA, aumenta el nivel de circulación del estradiol lo cual podría aumentar el riesgo de cáncer de seno y cáncer uterino en mujeres pos-menopáusicas.

Los ejemplos que se trabajaron se realizaron sobre modelos de animal (ratón y rata, y no en vagina) y no en humanos (mujeres pos-menopáusicas) como se describe en la presente solicitud. Adicionalmente, la presente solicitud ha estudiado los parámetros en mujeres pos-menopáusicas (la mayoría de los molestos síntomas y los efectos sobre las células parabasales vaginales y superficiales y el pH) que están directamente relacionados con las enfermedades vaginales.

Además, la referencia D2 no enseña o sugiere un supositorio vaginal con una

dosis baja (e.g., 13 mg o menos) ni siquiera sugiere cualquier supositorio vaginal (que es la formulación farmacéutica preferida de la presente invención).

D3 CA 2584524 (Endorecherche)

Esta solicitud de patente se refiere al uso de una composición de precursor de esteroides sexuales incluyendo DHEA solo o en combinación con el Modulador Selectivo del Receptor de Estrógeno en la prevención o tratamiento de la sequedad vaginal, la dispareunia y la disfunción sexual causada por la lámina de capa propia y/o capa muscular de la vagina incluyendo la disminución de las fibras de colágeno. La solicitud se refiere a las mujeres pos-menopáusicas y la administración intra-vaginal (para el precursor de esteroides sexuales solamente) pero no hay ninguna descripción de un precursor de esteroides sexuales en una composición farmacéutica con un peso de 13 mg o menos.

De hecho, D3 describe un supositorio vaginal de 0,66% a 2,0% en peso de la composición total (página 61, Ejemplo L) y 0,33, 0,66 y 1,00 mg/rata en el Ejemplo 3. La dosis más baja en rata de 0,33 mg de DHEA equivale a una dosis humana de 60 mg, que es casi cinco veces mayor que la dosis más alta reivindicada en la presente solicitud de patente (13 mg).

Así las cosas, la presente solicitud de patente demuestra la eficacia de tratar enfermedades vaginales con una dosis baja de aplicación local de precursor de esteroides sexuales. El dietilenglicol monometil éter es un ingrediente en la formulación del precursor de esteroides sexuales para administración tópica (gel) (Ejemplo A del párrafo [0112] de la referencia D6) pero no se menciona en la presente solicitud.

Además, la solicitud de patente citada (párrafos 0013, 0047, 0048 y 0111-0113, Ejemplo 1, y las reivindicaciones originales 3 y 43) sugieren la combinación de precursor de esteroides sexuales con estrógenos, que se sabe que aumenta el riesgo de cáncer de seno y cáncer uterino. Los ejemplos de trabajo se llevaron a cabo en modelos de animal (rata, incluyendo vagina) y no en humanos (mujeres pos-menopáusicas) como se describe en la presente solicitud con parámetros objetivos de las enfermedades vaginales reivindicadas.

La sugerencia de estrógeno en esta referencia demuestra la importancia de la contribución de la presente solicitud en evitar el uso de estrógenos y en la identificación de la importancia de la terapia de baja dosis de precursor de esteroides sexuales.

D4 U.S. 4.789.669 y D5 U.S. 2002/0013304

El examinador ha rechazado las reivindicaciones sobre las referencias citadas en

la Publicación de Patente U.S. 2002/0013304 (referencia '304) y la Patente U.S. 4.789.669 (referencia '669). Se argumenta, sin embargo, que estas referencias ni solas ni en combinación divulgan o sugieren que el Solicitante críticamente enseña la limitación del agente activo en las composiciones reivindicadas para que no sea mayor que 13 mg por forma de dosificación. Cada reivindicación (directa o indirectamente) limita la cantidad máxima permitida de precursor de esteroides sexuales en el producto reivindicado para que no sea más de 13 miligramos (mg) - un nivel crítico para evitar el riesgo de cáncer y otros problemas asociados con estrógeno elevado en sangre.

En este sentido, es importante hacer énfasis sobre el hecho que todas las reivindicaciones modificadas excluyen una cantidad más alta. El límite superior de 13 mg en las reivindicaciones del solicitante define un intervalo crítico de agente activo tal como se evidencia por la discusión más detallada a continuación.

En efecto, cuando, como en este caso, críticamente se establece un estrecho rango numérico, las reivindicaciones limitadas al rango crítico deben ser patentables sobre cualquier arte que refiera rangos más amplios. Aquí, como se explica en más detalle, las reivindicaciones de patente del solicitante son permisibles porque el solicitante ha demostrado que se consiguen ventajas inesperadas dentro de la gama reivindicada del solicitante que no se alcanzan de otra manera dentro de los rangos descritos por el arte citado.

Los solicitantes creen que los problemas vaginales tratados por la presente invención responden favorablemente tanto a andrógenos como a estrógenos. Sin embargo, la administración de andrógenos o estrógenos por vía intra-vaginal puede resultar en niveles elevados en sangre de aquellos esteroides sexuales. El estrógeno elevado en sangre presenta numerosos riesgos indeseables para la salud, incluyendo aumento en el riesgo de cáncer de seno y de cáncer uterino e hiperplasia del endometrio, como así se puede corroborar entre las páginas 38 a 58 de la memoria descriptiva de la presente solicitud, y las referencias allí citadas.

Sobre este particular, es necesario señalar que el solicitante ha descubierto ahora que los dos efectos deseados (efectos locales vaginales androgénicos y estrogénicos) se pueden lograr evitando al mismo tiempo una elevación significativa de los estrógenos y los andrógenos en sangre mediante la administración de una cantidad crítica de un precursor de esteroides sexuales, y no la administración de esteroides sexuales *per se*. El tejido vaginal en sí tiene las enzimas necesarias para catalizar la conversión de un precursor de esteroides sexuales, tal como la dehidroepiandrosterona (DHEA) para andrógenos y estrógenos, cada uno de los cuales proporciona efecto terapéutico separado y complementario en el tejido vaginal, y se cree que cuando el precursor del esteroide sexual se administra en una cantidad que no exceda el límite superior crítico citado en las reivindicaciones (13 mg), los andrógenos y los estrógenos son

Clarke, Modet & Cº

COLOMBIA

deseablemente producidos intracelularmente, realizan funciones terapéuticas y, posteriormente, se metabolizan a metabolitos de esteroides sexuales antes que los esteroides sexuales sean liberados en la sangre del tejido vaginal local como metabolitos inactivos, evitando así los efectos sistémicos.

Los estudios clínicos realizados en seres humanos de acuerdo con el Ejemplo 1 de la especificación muestran que no hay un aumento significativo en los resultados de concentración en sangre de estrógeno o andrógenos cuando se suministran menos de 13 mg de DHEA en la forma de dosificación (supositorios vaginales en el Ejemplo 1). La Figura 8 muestra el comienzo del estrógeno elevado en 13 mg y claramente la elevación es significativa cuando se usan cantidades más altas. En el tamaño de supositorio vaginal que se utiliza en los ensayos clínicos humanos cuyos datos se presentan en las Figuras 7 y 8, el supositorio 0,5% proporciona 6,5mg de DHEA, el supositorio 1,0% proporciona 13 mg de DHEA y el supositorio de 1,8% proporciona 23,4 mg de DHEA. **Esta conversión se establece en el párrafo [0060] de la especificación en la página 45 del mismo.** Las Figuras 7e y 7f muestran el efecto de cantidades crecientes de DHEA sobre los niveles en sangre de los andrógenos testosterona (TESTO) y dihidrotestosterona (DHT), respectivamente. Las figuras 8d y 8e muestran el efecto de cantidades crecientes de DHEA sobre los niveles en sangre de los estrógenos estrona (E1) y estradiol (E2), respectivamente. Las figuras 7 y 8 ilustran que los estrógenos y los andrógenos elevados en la sangre empiezan a aparecer cuando las cantidades precursoras alcanzan el nivel crítico de 13 mg. El arte previo no cita o sugiere de manera crítica el límite superior de 13 mg citado en las reivindicaciones del solicitante.

A modo de contraste con el límite superior de 13 mg reivindicado por el Solicitante, el Examinador cita la Referencia '669 que administra su agente activo en una dosis de 100 a 1500 mg con una frecuencia de una a tres veces al día. Ver la columna 2, renglones 22-26. Incluso en la dosis más baja recomendada de 100 mg, la dosis sería más de siete veces el límite superior crítico (13 mg) en las reivindicaciones del solicitante.

Asimismo, la citada referencia '304 recomienda un rango de dosis tan amplio como para ser casi sin sentido - - un insignificante 2 microgramos por gramo de supositorio en el extremo inferior y 500 miligramos por gramo de supositorio en el extremo superior. (Ver párrafo 92). Por otra parte, la referencia '304 no especifica un tamaño diferente de supositorio dando dos posibles -y muy diferentes - Ejemplos de tamaño en el párrafo 92 anterior. Sin que el tamaño se especifique, la conversión de la concentración a la cantidad de agente activo es indeterminada. Mucho más importante, sin embargo, se insiste en que los versados en el arte serian conducidos a utilizar el extremo alto de la recomendación de dosis de la referencia '304 - - el cual está muy por encima del límite crítico superior de 13 mg del Solicitante - - si se usa la DHEA en los

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supositorios de la referencia '304 en lugar de un compuesto andrógeno más potente, como los derivados de la testosterona preferidas en los ejemplos en las páginas 15 -16 de la referencia '304. La referencia '304 intenta entregar los andrógenos. En todos los ejemplos de su especificación (ver páginas 15-16), la referencia '304 utiliza el potente andrógeno testosterona en forma de propionato de testosterona, enantato o cipionato. Aunque que la DHEA se muestra como un "andrógeno" por la referencia '304, aquellos versados en el arte reconocerán que la DHEA *per se* no es inherentemente androgénica. Es más bien un precursor de los andrógenos que se pueden sintetizar en algunos tejidos, que tienen las enzimas adecuadas para catalizar la conversión. Ver el párrafo 19 de la especificación del solicitante, y las publicaciones citadas en el mismo. Basado en esto, y siendo el objetivo de la referencia '304 la actividad androgénica, el versado en el arte sería llevado al extremo alto del rango de dosificación recomendado de la referencia '304 cuando el agente utilizado sea la DHEA, un precursor, en lugar de testosterona, un andrógeno activo.

La conclusión anterior de que el versado en el arte sería llevado a utilizar el extremo alto del rango de dosis de la referencia '304 se refuerza por la referencia '669, que también ha sido citada por el examinador. Como se discutió anteriormente, la referencia '669 recomienda una dosis de 100 a 1500 mg (Ver columna 2, renglones 22-26) para un producto que, como en el producto reivindicado por el Solicitante, tiene un agente activo limitado a un precursor de esteroides sexuales - no andrógenos fuertes como la testosterona u otros andrógenos fuertes permitidos por la referencia '304. Incluso si se combinan las Referencias '669 y '304, un versado en el arte vería la referencia '669 sobre la referencia '304 para una dosificación apropiada de un precursor. Esto llevaría a un versado en el arte muy por encima de la cantidad crítica del precursor del solicitante que tiene un límite superior de 13 miligramos. Incluso si se utiliza la amplia gama de la referencia '304, la superposición parcial de rango no hace evidente la cantidad crítica estrecha reivindicada del agente activo descrito en las reivindicaciones del Solicitante.

Antes de los estudios del solicitante, no se conocía si cualquier cantidad de precursor de esteroides sexuales, tal como DHEA sería simultáneamente (1) adecuada para producir los efectos androgénicos y estrogénicos locales deseados en la vagina, aun más, (2) lo suficientemente baja como para evitar efectos estrogénicos o androgénicos sistémicos indeseables. En el ensayo clínico establecido en el Ejemplo 3, comenzando en la página 108 de la memoria descriptiva, se logra beneficio terapéutico sustancial por el tratamiento de acuerdo con la invención (ver página 125 desde la línea 8 hasta la página 126 línea 14 de la descripción), a la vez que los estrógenos séricos no se ven sustancialmente afectados (Ver página , 126 y 127).

Por todas las razones anteriores, se insta a que las referencias citadas, tomadas

Clarke, Modet & C^o

COLOMBIA

11

solas o en combinación, no describen o sugieren los productos farmacéuticos reivindicados en los que los precursores de esteroides sexuales están presentes en una cantidad crítica que tiene un límite superior de 13 mg como lo requieren las reivindicaciones del Solicitante con el fin de evitar los riesgos sistémicos de estrógenos y andrógenos.

Habiendo atendido cabalmente las objeciones presentadas por ese despacho, atentamente solicitamos se conceda el privilegio de patente de invención a la solicitud en estudio o si el señor examinador considera que subsisten razones para objetar la presente invención, rogamos expedir otro examen de fondo con los debidos motivos y razones que sustenten su objeción con el fin de que nuestro mandante tenga la posibilidad de exponer sus argumentos con base en la interpretación de los artículos 45 de la Decisión 486 -el cual otorga la facultad al examinador para emitir los exámenes de fondo-, 35 y 59 del Código Contencioso Administrativo que señala que: *"habiéndose dado oportunidad a los interesados para expresar sus opiniones, y con base en las pruebas e informes disponibles, se tomará la decisión que será motivada al menos en forma sumaria si afecta a particulares"* y *"(..) deberá proferirse la decisión definitiva. Esta se motivará en sus aspectos de hecho y de derecho, y en los de conveniencia si es el caso"*, respectivamente (El subrayado es nuestro).

En consecuencia, solicitamos a ese despacho pronunciarse en relación con todos los argumentos presentados en este memorial dando estricta aplicación al principio del debido proceso consagrado en el artículo 29 de la Constitución Nacional.

Señor Superintendente,



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000011

REIVINDICACIONES

1. Una composición farmacéutica para aplicación
5 intravaginal caracterizada porque comprende 13 miligramos o
menos por dosis de un precursor esteroide sexual seleccionado
del grupo que consiste de dehidroepiandrosterona, sulfato de
dehidroepiandrosterona, androst-5-ene-3 β ,17 β -diol y 4-
androst-3,17-diona y además comprende un excipiente,
10 diluyente o vehículo farmacéuticamente aceptable.

2. La composición farmacéutica de acuerdo con la
reivindicación 1 caracterizada porque comprende 0.5 a 13
15 miligramos por dosis de dicho precursor esteroide sexual.

3. Un supositorio vaginal que comprende un precursor
esteroide sexual seleccionado del grupo que consiste de
dehidroepiandrosterona, sulfato de dehidroepiandrosterona,
androst-5-ene-3 β ,17 β -diol y 4-androst-3,17-diona y un
20 excipiente, diluyente o vehículo farmacéuticamente aceptable
caracterizado porque dicho precursor esteroide sexual está
presente en un peso menor que 13 miligramos.

4. El supositorio vaginal de acuerdo con la
25 reivindicación 3, caracterizado porque comprende 6.5
miligramos del precursor esteroide sexual.

5. El supositorio vaginal de acuerdo con la
reivindicación 3, caracterizado porque comprende 3.25
30 miligramos del precursor esteroide sexual.

6. El supositorio vaginal de acuerdo con la
reivindicación 3, caracterizado porque comprende 3 a 13
miligramos del precursor esteroide sexual.

7. El supositorio vaginal de acuerdo con la
reivindicación 3, caracterizado porque el excipiente se
encuentra seleccionado del grupo que consiste de tri-, di- o
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000012

monoglicéridos semi sintéticos o naturales puros o mezclados de ácidos grasos saturados, no saturados o hidrogenados, mantequilla, palma, núcleo de palma, semilla de algodón parcialmente hidrogenada, y aceites de coco y sus derivados triglicéridos, ésteres y alcoholes grasos hidrogenados, estearato de polioxilo, aceites vegetales hidrogenados reconfigurados, mezcla eutéctica de mono-, di- triglicéridos derivada de aceites vegetales naturales, ésteres de triglicérido; Tween 61, aceite de teobroma y una combinación de cualquiera de los mismos.

8. Un supositorio vaginal que comprende dehidroepiandrosterona y un excipiente farmacéuticamente aceptable adecuado para la administración vaginal, caracterizado porque dicha dehidroepiandrosterona se encuentra presente en un peso menor de 13 miligramos.

9. El supositorio vaginal de acuerdo con la reivindicación 8, caracterizado porque comprende 6.5 miligramos de dehidroepiandrosterona.

10. El supositorio vaginal de acuerdo con la reivindicación 8, caracterizado porque comprende 3.25 miligramos de dehidroepiandrosterona.

11. El supositorio vaginal de acuerdo con la reivindicación 8, caracterizado porque comprende 3 a 13 miligramos de dehidroepiandrosterona.

12. El supositorio vaginal de acuerdo con la reivindicación 8, caracterizado porque el excipiente se encuentra seleccionado del grupo que consiste de tri-, di- o monoglicéridos semi sintéticos o naturales puros o mezclados de ácidos grasos saturados, no saturados o hidrogenados, mantequilla, palma, núcleo de palma, semilla de algodón parcialmente hidrogenada, y aceites de coco y sus derivados triglicéridos, ésteres y alcoholes grasos hidrogenados, estearato de polioxilo, aceites vegetales hidrogenados

reconfigurados, mezcla eutéctica de mono-, di- triglicéridos derivada de aceites vegetales naturales, ésteres de triglicérido; Tween 61, aceite de teobroma y una combinación de cualquiera de los mismos.

5

13. Un supositorio vaginal de 1.3 mililitros caracterizado porque comprende 6.5 miligramos de dehidroepiandrosterona y Witepsol H-15.

10

14. Un supositorio vaginal caracterizado porque comprende 3.25 miligramos de dehidroepiandrosterona y Witepsol H-15.

15

15. Un supositorio vaginal de 1.3 mililitros caracterizado porque comprende 3.25 miligramos de dehidroepiandrosterona y Witepsol H-15.

20

16. Un supositorio vaginal caracterizado porque comprende 3 a 13 miligramos de dehidroepiandrosterona y Witepsol H-15.

25

17. Un supositorio vaginal que comprende un excipiente farmacéuticamente aceptable para la administración intravaginal y que además comprende una cantidad de dehidroepiandrosterona efectiva para proporcionar respuesta androgénica local por el tejido vaginal, caracterizado porque el peso total de la dehidroepiandrosterona en dicho supositorio es menor a 13 miligramos.

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18. El supositorio vaginal de la reivindicación 17 caracterizado porque el peso de la dehidroepiandrosterona en dicho supositorio es de 3 a 13 miligramos.

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19. El supositorio vaginal de acuerdo con la reivindicación 18, caracterizado porque el excipiente se encuentra seleccionado del grupo que consiste de tri-, di- o monoglicéridos semi sintéticos o naturales puros o mezclados de ácidos grasos saturados, no saturados o hidrogenados,

mantequilla, palma, núcleo de palma, semilla de algodón parcialmente hidrogenada, y aceites de coco y sus derivados triglicéridos, ésteres y alcoholes grasos hidrogenados, estearato de polioxilo, aceites vegetales hidrogenados reconfigurados, mezcla eutéctica de mono-, di- triglicéridos derivada de aceites vegetales naturales, ésteres de triglicérido; Tween 61, aceite de teobroma y una combinación de cualquiera de los mismos.

10 **20.** El supositorio vaginal de acuerdo con la reivindicación 16 caracterizado porque comprende 6.5 miligramos de dehidroepiandrosterona y Witepsol H-15.

15 **21.** El supositorio vaginal de acuerdo con la reivindicación 3 caracterizado porque dicho excipiente es lipofílico.

20 **22.** El supositorio vaginal de acuerdo con la reivindicación 8 caracterizado porque dicho excipiente es lipofílico.

25 **23.** El supositorio vaginal de acuerdo con la reivindicación 17 caracterizado porque dicho excipiente es lipofílico.

30 **24.** El supositorio vaginal de acuerdo con cualquiera de las reivindicaciones 13 a 16 y 20, en el que el Witepsol H-15 se sustituye por una mezcla de monoglicéridos, diglicéridos, y triglicéridos del ácido graso saturado C10 a C18, en el que el componente principal de la mezcla, en peso, es triglicérido láurico.

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ANEXO 1

Rectal Mucosa Damage in Rabbits After Subchronical Application of Suppository Bases

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Received November 1, 1990, accepted January 19, 1991

The effect of suppository bases on rabbit rectal mucosa was investigated using six triglyceride bases, polyethylene glycol, and a triglyceride base combined with monoglycerides or fatty acids and methyl esters of those acids. Rectal irritation was evaluated and scored according to defined pathological features. "Pure" triglycerides and a triglyceride to which a nonionic surfactant was added caused severe mucosal damage with ulceration and inflammation. Hyperemia was characteristic for irritation by polyethylene glycol suppositories. Mucosal damage by a pure triglyceride combined with monoglycerides or fatty acids and methyl esters of those acids was similar but statistically less pronounced than with all other bases.

KEY WORDS: suppositories; rectum; polyethylene glycol; triglyceride; monoglyceride; fatty acids; ulcer; hyperemia.

INTRODUCTION

Suppositories remain the most widely used dosage form in rectal therapy, although different other delivery systems such as microenemas, hydrogels, rectal capsules, and osmotic pumps are being used for therapeutic or research purposes.

Suppository bases can be divided into fatty bases (mixtures of mono-, di-, and triglycerides) and the water-soluble or water-dispersible bases (e.g., polyethylene glycol). Except for polyethylene glycol, suppository bases are considered to cause no major damage to the rectal mucosa. We emphasize that reports on the irritative effect of suppository bases without medication always concerned short-term experiments (1-3).

Therefore we investigated the effects of different suppository bases on the rectal mucosa of rabbits in subchronical experiments.

MATERIALS AND METHODS

Suppository Bases

Nine suppository bases were tested: a hydrophilic com-

position, five "pure" triglyceride bases, and three triglyceride formulations with adjuvants. The hydrophilic base was polyethylene glycol (Flandria, Zwijnaarde, Belgium), 1500:4000 (w/w, 3:7). The five "pure" triglyceride bases were three lauric glycerides [Novata B (Henkel & Cie GmbH, Düsseldorf, Germany), Suppocire AM (Gattefossé, St. Priest, France), and Witepsol H15 (Hüls AG, Witten, Germany)], a nonlauric glyceride [Mesuro PS (Vandemoortele N.V., Izegem, Belgium)], and cocoa butter (Flandria, Zwijnaarde, Belgium). The lauric glycerides contained mainly C12-C14 saturated fatty acids, while the nonlauric glyceride and cocoa butter contained mainly C16-C18 saturated and unsaturated fatty acids. All triglyceride bases were mono-, di-, and triglycerides mixtures containing more than 80% triglycerides and less than 2.5% monoglycerides.

We also tested Suppocire AP (Gattefossé, St. Priest, France), a lauric triglyceride supplemented with a nonionic surfactant (polyethylene glycol fatty acid esters), and two formulations of Mesuro PS to which 5% monoglycerides (Dimodan LS, Grindsted, Antwerpen, Belgium) or a mixture of 9% fatty acids of Mesuro PS origin and 1% methyl esters of those fatty acids (Vandemoortele, Izegem, Belgium) was added. The mono-, di-, and triglyceride content of the pure triglyceride bases and Suppocire AP as well as the composition of the monoglyceride, the fatty acids, and the fatty acid methyl esters is shown in Table I. From these bases 1-g suppositories were prepared.

Suppository Administration

To male New Zealand albino rabbits weighing ± 2.5 kg, provided with water and food ad libitum, three suppositories were administered daily (8, 16, and 24 hr) during 14 days. To guarantee a 30-min contact time between the base and the rectal mucosa, the anus was kept shut over this period with a clothes-peg. Each suppository base was tested on 20 rabbits. For the Novata B base 33 rabbits were used.

Controls and Placebo

Two control groups were used: rabbits receiving neither a suppository nor a clothes-peg and rabbits where the clothes-pegs were applied on a time schedule similar to the suppository administration in order to evaluate mucosal changes due to their application.

A nonmelting paraffin suppository (Flandria, Zwijnaarde, Belgium) was used as a placebo to study the influence of mechanical trauma on the rectal mucosa.

Each test was performed on 20 rabbits.

Tissue Processing

Eight hours after the last suppository administration the rabbits were exsanguinated. Rectum and anus were removed, opened lengthwise, and cleaned with a 0.9% NaCl solution before fixation in a 4% buffered formaldehyde solution. They were examined macroscopically before being cut longitudinally in three or four sections, covering the entire width of the rectum. The tissue specimens were then routinely embedded in paraffin, sectioned at 5 μ m, stained

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Table I. Composition of "Pure" Triglyceride Suppository Bases and of the Additives Used

Composition of "pure" triglyceride bases and of the Suppocire AP base Percentage of mono-, di-, and triglycerides and of PEG fatty acid esters						
	Mesuro PS	Cocoa butter	Suppocire AM	Suppocire AP	Witepsol H15	Novata B
Monoglycerides	—	±1	0.5	2.5	0.3	1.9
Diglycerides	2.9	±2	3.0	28.0	10.7	19.5
Triglycerides	97.1	±97	96.5	55-60	89.0	78.6
PEG-fatty acid esters	—	—	—	10-15	—	—
Lauric/nonlauric base	Nonlauric	Nonlauric	Lauric	Lauric	Lauric	Lauric

Composition of the additives Percentage of free fatty acids, fatty acid methyl esters, and mono-, di-, and triglycerides			
	Monoglyceride	Fatty acid	Fatty acid methyl ester
Free fatty acids	3.3	100	—
Fatty acid methyl ester	—	—	100
Monoglycerides	91.0	—	—
Diglycerides	5.7	—	—
Triglyceride	—	—	—

Percentage of (glyceride/methyl ester) fatty acid according to its chain length			
C 8:0-C 14:0	<1.0	<1.0	<1.0
C 16:0	10.2	7.5	17.5
C 18:0	11.5	6.5	6.5
C 18:1	15.0	67.5	67.5
C 18:2	59.2	6.2	6.2
C 20:0-C 22:0	<1.0	<1.0	<1.0

with hematoxylin-eosin, and examined with a light microscope.

Macroscopic evaluation was used only as a confirmation for the light-microscopic scoring of hyperemia or surface injuries (ulceration or erosion, regeneration, and polyp formation).

Evaluation of Rectal Irritation

The mucosa, the surface of the intestine, exists of crypts formed by epithelial cells extending vertically into the lamina propria. The lamina propria occupies only a small volume of the mucosa. Under the mucosa, the muscularis mucosa and the submucosa can be found.

Mucosal capillary congestion with red blood cells in the lamina propria was defined as hyperemia. A red-stained rectum, being macroscopically visible, was an indication of the diffuse presence of erythrocytes (Fig. 4d). Edema was seen when the mucosa appeared thicker than normal, with the crypts failing to reach the muscularis mucosa and the space between the crypts being increased. Inflammation in the scored preparations was always acute. This was confirmed by the increase in inflammatory cells (polymorphonuclear cells or neutrophilic cells) in the lamina propria (Fig. 1a). This in contrast to chronical inflammation, where plasma-cell infiltrates are predominant in the lamina propria. Erosion was defined as the detachment of surface epithelium and the presence of fibrin and inflammatory cells in the lamina propria. When necrotic material and fibrin exudates were observed, the erosion evoluted to an ulceration. Regeneration occurred when a new epithelial layer was present on

the ulceration (arrow in Fig. 1b). Irregular healing of ulcerations can induce polyp formation (pseudopolyps or "inflammatory polyps"). These are localized lesions projecting from the mucosa (Figs. 1c and d and Fig. 4c) (4).

To allow a statistical interpretation of the results, a scoring table was used (Table II) based on the severity of the inflammation. Each rectum was scored microscopically only according to the type and degree of mucosal damage. The



Fig. 1. Rectal mucosa featuring (a) hyperemia and inflammation after polyethylene glycol application, (b) ulceration with epithelial regeneration at the edge (arrow) after Suppocire AM treatment, (c) inflammatory polyp and underlying ulcer after using Novata B suppositories, and (d) inflammatory polyp at the anorectal junctional zone in Suppocire AP-treated rabbits. (a, b) ×75, (c) ×32, and (d) ×28; reduced 50% for reproduction.

000018

Table II. Scoring Table for the Evaluation of Rectal Irritation

Pathological feature	Degree of irritation		Score
Hyperemia	0	Negative	0
	+	Focal	1
	++	Diffuse	2
Edema	0	Negative	0
	+	Focal	1
	++	Diffuse	2
Inflammatory cells	0	Normal numbers	0
	+	Focal	3
	++	Diffuse	4
	+++	Heavy diffuse	5
Erosion, ulceration, regeneration, and/or polyps	Neg.	Absent	0
	Pos.	Present	15

scorings were performed in a blind fashion by three observers simultaneously.

Histological sections showing severe hyperemia, diffuse inflammation, ulceration, and inflammatory polyps are shown in Figs. 1a-d. When erosion, ulceration, regeneration, and/or inflammatory polyps were present, a score of 15 was attributed. As these types of lesions are the consequence of epithelial damage, we assumed that they had a major impact compared to hyperemia, edema, or inflammation. The maximal score per rectum was 24. For the different suppository bases, controls, and placebo, a mean total score was calculated by grouping the individual data. For each pathological characteristic the number of positive scoring rabbits was then expressed as percentages. This was also done for the degree (focal, diffuse, or heavy diffuse) to which hyperemia, edema, and inflammation occurred.

Statistical Evaluation

Statistical analysis was performed on the total scores and on the values obtained for hyperemia, edema, and granulocytes. The presence of erosion, ulceration, polyps, and regeneration was evaluated separately.

Two nonparametric tests were used: the Kruskal-Wallis one-way analysis of variance by ranks when more than two groups were compared and the median test when two groups were compared. A probability value of $P < 0.05$ was considered to be statistically significant (5).

RESULTS

The mean total scores for each suppository base, control group, or placebo are shown in Fig. 2.

For some representative groups Figs. 3a-d show the number of positive scoring rabbits for each pathological characteristic and for the degree in which hyperemia, edema and inflammation occurred.

Because a high variability (important interanimal variation) was observed for the response of the rabbits to the suppository bases, it was necessary to use groups of 20 rabbits.

Hyperemia or surface injuries such as erosions, ulcers, regeneration, and polyps were seen macroscopically (Figs.

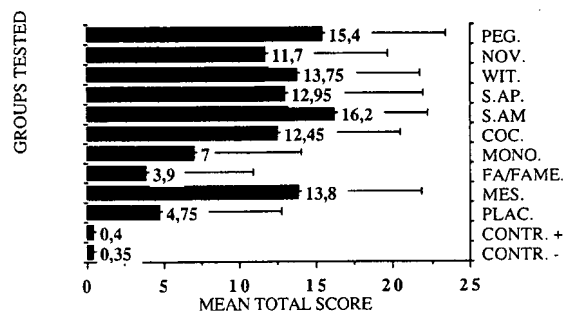


Fig. 2. The mean total scores ($\pm$ SD) for each group tested. CONTR. -, control without clothes-peg; CONTR. +, control with clothes-peg; PLAC., placebo; MES., Mesuro PS; FA/FAME, Mesuro PS + 9% fatty acids + 1% fatty acid methyl esters; MONO., 5% monoglycerides; COC., cocoa butter; S.AM, Suppocire AM; S.AP, Suppocire AP; WIT., Witpsol H15; NOV., Novata B; PEG., polyethylene glycol. $n = 20$ except for NOV., where $n = 33$.

4a-d). Light microscopy showed that the mucosal damage was always limited to the epithelium and the lamina propria. In the submucosa we did not find any abnormalities (such as edema, inflammation, or misplaced glands).

The mucosal irritation of recta treated with "pure" triglycerides was similar for all bases. Hyperemia occurred only focally. There was active inflammation with increased polymorphonuclear cells and crypt abscesses (polymorphonuclear cells present in the lumen of the crypt). A significant difference for the occurrence of edema between the five "pure" triglycerides was found (Kruskal-Wallis test, $P < 0.05$). Erosion evolving in ulceration with replacement of surface epithelium by fibrin, mucus, and inflammatory cells was a frequent finding, as well as the regeneration of the mucosal damage and inflammatory polyps.

The effects of Suppocire AP on the rabbit rectal mucosa were similar to these induced by "pure" triglycerides (Kruskal-Wallis test, $P = 0.26$).

The sections of recta treated with polyethylene glycol revealed the same abnormalities as in the pure triglyceride and the Suppocire AP groups, but hyperemia was more pronounced than with polyethylene glycol (Kruskal-Wallis test, $P < 0.05$). For the presence of erosion, ulceration, regeneration, and polyps and for the total scores, no significant difference was found among the pure triglycerides, the Suppocire AP, and the polyethylene glycol.

The addition of fatty acids and fatty acid methyl esters or the addition of monoglycerides to Mesuro PS decreased the mucosal damage dramatically. Scores for each type of abnormality and total scores for these bases differed significantly from those for pure triglycerides, Suppocire AP, or polyethylene glycol (Kruskal-Wallis or median test, $P < 0.05$).

Both control groups showed no differences. They had very low total scores and were significantly different from the triglycerides, the Suppocire AP, polyethylene glycol, and all formulations with Mesuro PS.

The scores of the placebo suppositories were not significantly different from those of both control groups (median test on total scores, $P = 0.27$ for control group without clothes-peg and $P = 0.12$ for control group with clothes-

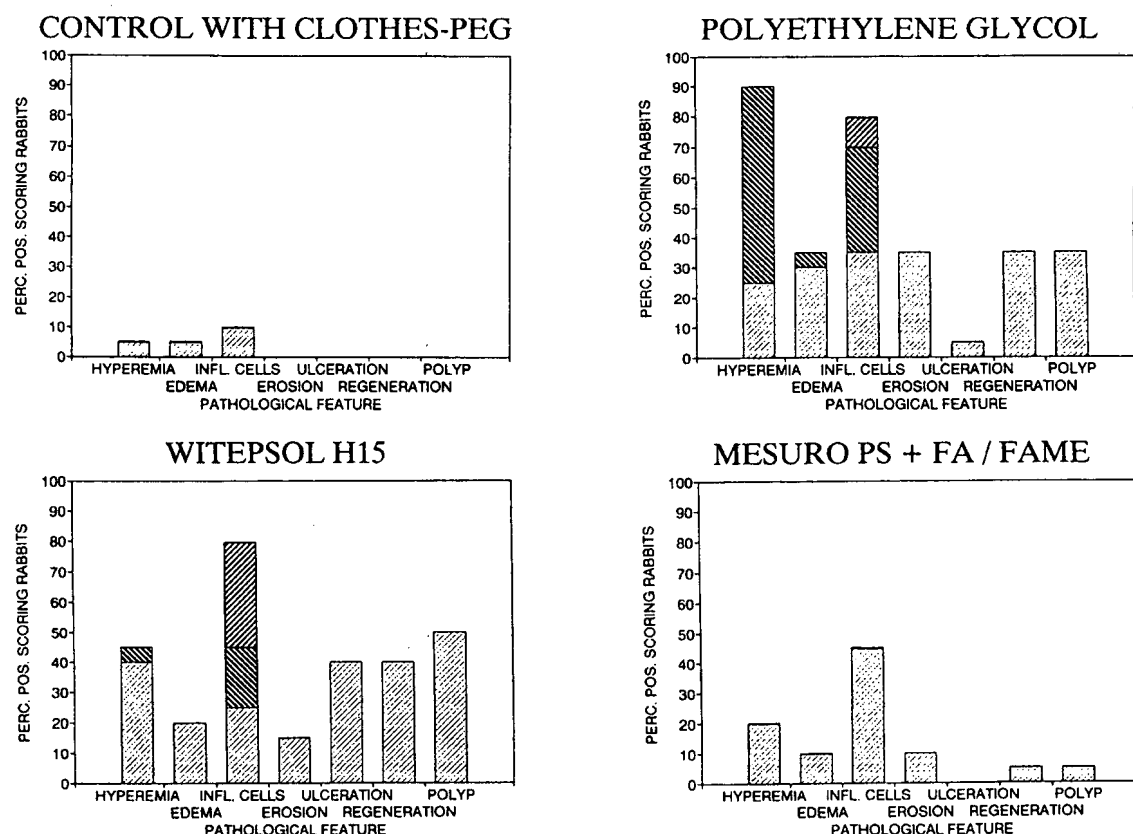


Fig. 3. Number of positive-scoring rabbits for each pathological characteristic and degree (focal, diffuse, or heavy diffuse) of hyperemia, edema, and inflammation (all data are expressed as percentages). (+) Focal; (++) diffuse; (+++) heavy diffuse. Infl cells, inflammatory cells; FA/FAME, Mesuro PS + 9% fatty acids + 1% fatty acid methyl esters.

peg). The Mesuro PS formulations with additives did not differ significantly from the placebo group [median test on total scores, $P = 0.20$ for Mesuro PS suppositories with monoglycerides (5% Dimodan LS) and $P = 0.19$ for Mesuro PS suppositories with 9% fatty acids and 1% methyl esters].

DISCUSSION

Rectal ulceration with therapeutic use of suppositories has been reported but this effect was always attributed to the drug, and never to the suppository base. Case reports mentioned either long-term application (e.g., indomethacin) (4,7) or drug abuse (analgesics or drugs used for the treatment of migraine) (8–10).

The rabbit was chosen as a model because its rectum is histologically comparable to the human rectum. The size of a rabbit rectum is similar to the size of the rectum of a 6-month-old baby.

Differences between the total scores of both control groups were not observed, indicating that rectal irritation was not due to increased strain of the rectoanal muscles induced by the clothes-peg technique.

Administration of the placebo suppositories caused focal inflammation. Because of the nonmelting nature of these suppositories at the animal's body temperature, the injuries may be due to suppository administration or to their pres-

ence in the rectal cavity. However, those data exclude the possibility that mucosal damage induced by polyethylene glycol and triglyceride bases was due to their insertion only.

Polyethylene glycol caused severe mucosal hyperemia in 65% of the rabbits. This was the main characteristic of polyethylene glycol-induced rectal irritation, although diffuse infiltration of granulocytes, erosion, regeneration, and inflammatory polyps were also observed. Recta treated with Suppocire AP base (containing a polyethylene glycol derivative) also showed serious mucosal damage not significantly different from triglyceride bases. The irritative effect of polyethylene glycol and its derivatives after a single exposure to the gastrointestinal tissue has been reported (11). Due to this effect the absorption of poorly absorbed polar molecules (antibiotics, peptides) was increased (12,13). The use of polyethylene glycol lauryl ether was described as a method to produce rectal ulcers after single application (14).

An unexpected result was the severe mucosal damage observed after frequent application of the triglycerides. Most of the literature on this subject describes fatty bases as being nonirritant. A report where rectal irritation induced by semi-synthetic triglycerides was compared to cocoa butter after chronic application to dogs concluded that these bases did not induce inflammation (15). In other studies, several semi-synthetic triglycerides were considered to be nonirritant be-

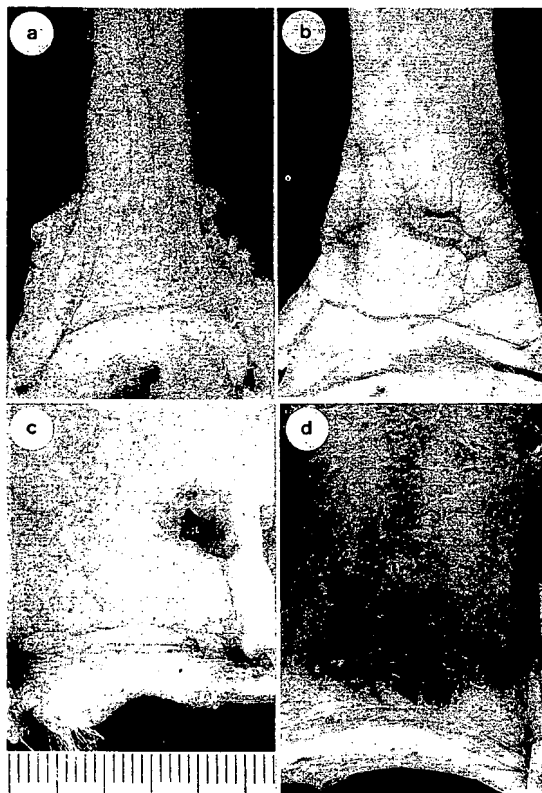


Fig. 4. Rabbit rectoanal preparations: (a) normal rectum after placebo treatment; (b) ulcerated rectoanal area after Witepsol H 15 suppositories; (c) inflammatory polyp developed after application of Suppocire AP suppositories; (d) hyperemic rectal mucosa typical for PEG administration.

cause the mucosal damage after single daily application did not exceed the natural capacity of the epithelium to prevent its disruption (1,2,16). In a recent study by Van Hoogdalem *et al.* (17) where, after single application, hyperemia, edema, and localized epithelial cell detachment were observed, these findings were considered to be reversible damaging effects, not necessarily excluding clinical applicability. In our study all triglycerides caused severe lesions similar to the solitary ulcer syndrome in man (8–10). The composition of the triglycerides regarding diglyceride content, glyceride fatty acid chain length (C12–C14 or C16–C18), and their degree of saturation did not influence the results. Considering the fact that polyethylene glycol and triglyceride bases induced rectal ulcers in rabbits, one might assume that rectal ulcerations in humans observed after chronic use of suppositories (6,7,8–10) were induced not only by the drug but also by the suppository base.

From these results it should be clear that in the early stages of suppository formulation, irritation studies in rabbits should be performed to determine if local irritation occurs after subchronic application of suppository bases with and without the active substance.

Adding 5% monoglycerides or a combination of 9% fatty acid and 1% methyl ester of those fatty acids decreased the mucosal damage. These results are in agreement with the literature data on the use of MGK (glyceride mixture with

$\pm 60\%$ monocaprylate) (3), fatty acids, or fatty acid derivatives in single-dose experiments (18). However, these results are in contrast with the results obtained by Van Hoogdalem *et al.* (17) using glycerylmonooctanoate and glycerolmonodecanoate and fatty acids. In this study cefoxitin solutions were administered with and without the different adjuvants. The cefoxitin solution in saline already caused fairly high scores. This finding and the fact that (i) these monoglycerides had other fatty acid chain lengths, (ii) the amounts used were different, and (iii) PEG 400 was added to the monoglyceride formulations may contribute to the discrepancy in the observed results (PEG suppositories can induce complete desquamation of epithelial cells in the rat rectum after single application) (1).

From our experiments and because monoglycerides, fatty acids, and fatty acid derivatives have been tested successfully as absorption enhancing products for poorly absorbed drugs such as cephalosporins (18,19), the use of monoglycerides and fatty acids and derivatives could be very promising as absorption promoting agents. However, the influence of the fatty acid chain length, the amount of additives used, and the type of ester on the reduction of the mucosal damage remains unclear, as only one monoglyceride and one mixture of fatty acids and fatty acid methyl esters were added to Mesuro PS.

Summarizing, this study demonstrated that frequent application of polyethylene glycol or pure triglycerides induced damage resulting in ulceration and inflammation of the rectal mucosa. This effect was similar for triglycerides supplemented with polyethylene glycol fatty acid esters. However, rectal mucosa damage was significantly reduced with triglyceride suppositories where monoglycerides or fatty acid and fatty acid methyl esters were added.

ACKNOWLEDGMENTS

The authors wish to thank Mrs. N. Baeck, of the Department of Probability and Mathematical Statistics, for technical assistance with the statistical evaluation of the results and Mr. D. Tensy for excellent assistance with the animal experiments.

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Multiple-dose pharmacokinetics of rectally administered acetaminophen in term infants

Objective: To investigate pharmacokinetics and pharmacodynamics of rectally administered acetaminophen (INN, paracetamol) in term neonates directly after birth.

Methods: In this prospective clinical trial, term neonates with painful conditions or who were undergoing painful procedures received multiple-dose acetaminophen. Serum concentrations were determined serially with an HPLC method, and pharmacokinetic analysis was performed. Pain assessment was performed by means of a validated pain score.

Results: Ten consecutive term neonates received four rectal doses of acetaminophen, 20 mg/kg body weight, every 6 hours. Mean peak serum concentrations ($\pm$ SD) during multiple-dose administration were 10.79 ± 6.39 mg/L, 15.34 ± 5.21 mg/L, and 6.24 ± 3.64 mg/L for the entire group, boys, and girls, respectively. There was a significant difference between the boys and the girls ($P = .01$). No serum concentrations associated with toxicity (>120 mg/L) were found. Median time to peak serum concentration was 1.5 hours after the first dose and 15 hours for multiple doses. Mean ($\pm$ SD) half-life was 2.7 ± 1.4 hours in eight patients. There was no correlation between dose and serum concentration or between pain score and serum concentration. There was a significant inverse relationship between the preceding pain score and peak serum concentrations.

Conclusions: In term neonates, multiple rectal doses of acetaminophen, 20 mg/kg body weight, led to widely varying serum concentrations but did not result in therapeutic concentrations in all infants. Boys had higher peak concentrations. Because accumulation was not found, a dose of 30 mg/kg followed by doses of 20 mg/kg at 6- to 8-hour administration intervals are appropriate to reach therapeutic concentrations. A concentration-effect relationship could not be determined. (Clin Pharmacol Ther 1999;66:509-15.)

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Although little is known about its pharmacokinetics and pharmacodynamics, acetaminophen (INN, paracetamol) is frequently used by both doctors and parents to treat minor and moderate pain in children. Fear of toxicity caused by accumulation in cases of multiple

dosing has been the main reason that acetaminophen has been refused to infants in the first days after birth.¹⁻³ Its effectiveness in treating neonates and both older and younger infants has not been clearly evaluated. In a recent study in preterm infants, we showed that a single rectal dose of acetaminophen can be given safely.⁴ Moreover, it has been proven that metabolism of acetaminophen in newborn infants is comparable to that documented in older children and adults, not as a result of glucuronidation but because acetaminophen is predominantly conjugated to sulfate.⁴⁻⁶

The appropriate analgesic dose for term infants is not known, but single doses between 10 mg/kg (oral) and 20 mg/kg body weight (rectal) that result in serum concentrations between 4 and 20 mg/L are reported to be sufficient to have an antipyretic effect.⁷⁻⁹ By taking this range and by combining other data based on validated pain assessment instruments and oral dose, the thera-

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Presented in part at the Fourth International Symposium on Pediatric Pain, Helsinki, Finland, July 2-4, 1997.

Dr Quak was supported in part by a grant from the Sophia Hospital Affiliation Fund.

Received for publication April 5, 1999; accepted Aug 17, 1999.

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0009-9236/99/\$8.00 + 0 13/1/102337

peutic range for analgesia in adults and children (10 to 20 mg/L) has been extrapolated.^{3,10,11} In older infants and children, the advised rectal dose is 20 mg/kg every 4 hours.^{12,13} This study was therefore designed to investigate the serum concentrations resulting from multiple rectal doses of 20 mg/kg acetaminophen in term neonates and to determine the relationship between dose and effect and between concentration and effect.

METHODS

Patients. The study included 11 consecutive neonates who were admitted to the neonatal intensive care unit. Entry criteria were traumatic delivery that resulted in hematoma, fractures, or scalp birth injuries or that resulted in the need for insertion of arterial and venous catheters or other potential painful procedures, such as insertion of a chest drain and subsequent drainage. The study protocol was approved by the ethical review committee of the Isala Clinics/ Sophia Hospital (Zwolle, The Netherlands). Written informed parental consent was obtained for each patient before enrollment. The gestational age of the neonates was estimated from maternal menstrual history, by routine ultrasound examination during pregnancy, and by postnatal physical characteristics (Farr score).¹⁴ Patients were excluded if they had major congenital anomalies, severe asphyxia (APGAR score ≤ 3 after 5 minutes), if their mother had received tocolysis with indomethacin (INN, indometacin), or if she had been given analgesics within 24 hours before delivery.

The infants were studied during the first 2 days after birth. Nasal intubation, if necessary, was performed in the delivery room in a standardized fashion without administration of medication, and the patient was transported to the neonatal intensive care unit. The heart rate, respiratory rate, arterial blood pressure, and oxygen saturation of each patient were monitored continuously, together with rectal and peripheral temperatures. Patients were nursed under a radiant heater. In each patient, umbilical venous and arterial access was obtained, and parenteral or enteral nutrition was started within 24 hours after birth according to standard procedures in the neonatal intensive care unit.

Within 1 hour of birth, patients were given acetaminophen rectally. The dose used was as close to 20 mg/kg as the available strengths of suppository would allow (50 mg for birth weights 2500 to 2749 g, 60 mg for 2750 to 3249 g, 70 mg for 3250 to 3749 g, and 80 mg for ≥ 3750 g). Subsequent doses were given after 6, 12, and 18 hours. Care was taken by the nurses to ensure that the suppository was retained. Suppositories contained 50, 60, 70, or 80 mg acetaminophen (particle size $<45 \mu\text{m}$) and hard fat (Witepsol H 15), a syn-

thetic mixture of monoglycerides, diglycerides, and triglycerides of the saturated fatty acids C10 to C18. The suppositories were prepared and analyzed for acetaminophen content and content uniformity by the quality assurance laboratory of the hospital pharmacy.

Blood samples (0.2 mL) were taken at 30, 60, and 90 minutes and at 3, 9, 15, 21, 24, and 27 hours after the first dose had been administered. After blood collection, serum was separated and frozen at -20°C , until analysis.

Analytical procedures. The assay was performed within a month of sample collection by a modified HPLC method as described previously.^{4,15} In short, serum samples were extracted with perchloric acid and, after centrifugation, the supernatant was injected into the HPLC column. Standards for serum acetaminophen were injected at the start and end of each run. HPLC conditions, retention times, and ultraviolet detection have been described previously.^{4,15} The limit of detection for serum acetaminophen was 0.2 mg/L, the recovery rate was 96%, and the precision of the assay was 2%. The calibration curves were linear across the range of 0.5 to 40 mg/L.

Pharmacokinetics and pharmacodynamics. Pharmacokinetic data and parameters were calculated from standard equations with use of the Multifit program (J.H. Proost, University Centre for Pharmacy, Groningen, The Netherlands). Concentration-time curves were constructed to determine peak serum concentration (C_{max}), time to reach C_{max} (t_{max}), time to reach therapeutic levels (T_1), and serum half-life of the drug ($t_{1/2}$). The mean C_{max} was determined by averaging the maximum serum concentrations, and the mean t_{max} was determined by averaging the times of peak serum concentration. Area under the concentration-time curve (AUC) was calculated with the trapezoidal rule. For calculations of the quotient of clearance and bioavailability (CL/F), a bioavailability of 90% was assumed. Serum concentrations ranging from 10 to 20 mg/L were considered to be therapeutic.

Because all infants were nursed under radiant heaters with temperature settings between 36.8°C and 37.2°C to achieve a neutral temperature, any antipyretic effects could not be measured. All infants had repeated painful procedures, including suctioning, skin puncture, dressing change or tape removal, and discontinuation of intravenous lines. Pain was assessed at standard times 1 hour after each dose was given by nurse pairs or nurse-doctor pairs, with a modified 5 faces pain score that showed increasing levels of discomfort from 0 (no pain) to 4 (clearly/obviously in pain), as described previously.^{4,12} Observers were trained by video instruction for 3 hours, resulting in a high inter-rater reliability (Cohen's $\kappa = 0.74$) as published previously.⁴

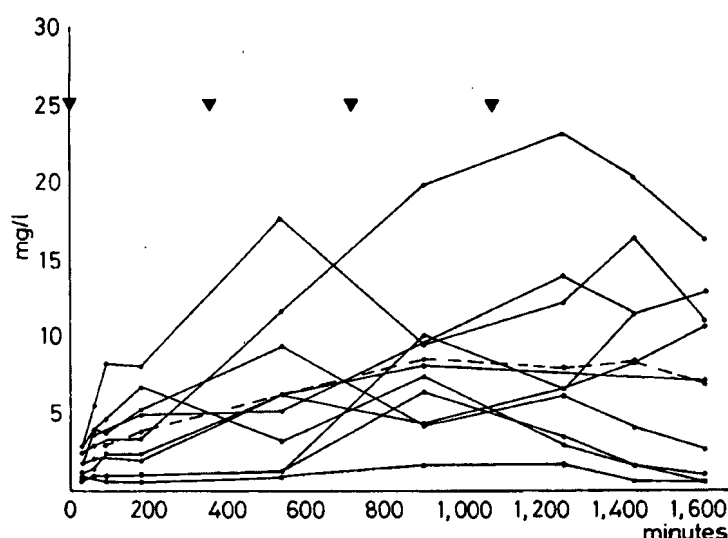


Fig 1. Acetaminophen serum concentrations following multiple doses (at 0, 6, 12, and 18 hours) after 30 minutes to 27 hours ($n = 10$). Triangles, Time of drug administration; broken line, average concentration.

Statistical analysis. Data were analyzed with SPSS-5 (SPSS Inc, Chicago, Ill). Student t tests were used for normally distributed data and Mann-Whitney U tests for nonparametric data to compare the two groups. Least-squares regression was used to evaluate linear correlation between variables, and P values $\leq .05$ (two-tailed) were considered to be significant.

RESULTS

Eleven infants were included in the study; serum samples from one infant were lost. Of the remaining 10 infants, the lungs of nine were ventilated. Diagnosis at admittance was pneumonia in two infants and pneumothorax in two infants. The clinical details of these infants are shown in Table I.

Acetaminophen was detected in the serum of all infants studied. Individual serum concentration-time curves are shown in Fig 1.

Absorption of acetaminophen after the first dose was slow. C_{max} was reached between 30 and 180 minutes (median, 90). The C_{max} after the first dose reached a mean ($\pm$ SD) value of 3.36 ± 2.11 mg/L. Even after multiple doses, it took a considerable time to reach a C_{max} within the therapeutic range (10 to 20 mg/L), with only the five boys reaching a C_{max} that exceeded 10 mg/L during the total study period.

There was a significant difference in the mean ($\pm$ SD) C_{max} between the boys ($n = 5$; 15.34 ± 5.21 mg/L) and the girls ($n = 5$; 6.24 ± 3.64 mg/L; $P = .01$). The C_{max}

Table I. Clinical characteristics of study participants

Sex	
Male	5
Female	5
Birth weight (g)	3542 ± 777
Gestational age (wk)	39.34 ± 2.35
Artificial ventilation	9
Mode of delivery	
Vaginal	5
Vaginal vacuum extraction	1
Cesarian section	4
Diagnosis at admittance	
Pneumonia	2
Pneumothorax	2
PPHN	1
Wet lung (TTN)	5

for all infants throughout the entire study period was 10.79 ± 6.39 mg/L (range, 1.7 to 23.2 mg/L; Fig 2). The coefficient of variation for all infants was 37.2%. The median t_{max} values after the first dose and during multiple dosing were 1.5 hours and 15 hours, respectively.

The serum $t_{1/2}$ after the first dose could be calculated for eight patients only (elimination of acetaminophen was not yet apparent in two patients) and was 2.7 ± 1.4 hours. The T_1 for the five boys was between 9 and 27 hours; this was reached after two, three, or even four doses ($n = 2, 2$, and 1). After the last dose, elimination seemed to be slower in four infants, with serum con-

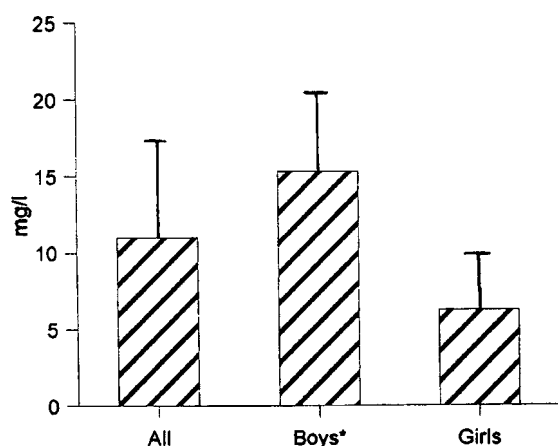


Fig 2. Peak serum concentration (C_{max} ; hatched bars) and SD (error bars) during the study period for all infants ($n = 10$), boys ($n = 5$), and girls ($n = 5$). * $P = .01$, boys versus girls.

centrations remaining in the therapeutic range even 9 hours after the last dose.

Because of the fixed strengths of the suppositories, the actual doses administered ranged from 16.6 to 21.2 mg/kg. A correlation between dose and C_{max} was not found. Almost all infants with a short t_{max} after the first dose had a low C_{max} , but this correlation ($r = 0.57$) did not reach a significant level. After multiple doses, no correlation between t_{max} and C_{max} was observed. The $t_{1/2}$, area under the serum concentration-time curve (AUC), clearance (CL) for individual patients after all doses, and quotient of clearance and bioavailability (CL/F) are shown in Table II.

In all infants, pain assessment by means of the facies score, performed 1 hour after the dose was given before the infant's C_{max} , showed a significant inverse linear regression in relation to the C_{max} ($r = -0.39$; $P = .023$). There was no correlation between the pain scores and the serum concentrations: pain scores from 0 to 1 were obtained in the infants when serum concentrations were >2.61 mg/L.

DISCUSSION

As far as we are aware, this is the first study to investigate both dose-concentration and concentration-effect relationships of acetaminophen multiple-dose pharmacokinetics in term neonates. The pharmacokinetic effect of a single dose has been studied after rectal and intravenous administration in infants <14 days of age,^{16,17} but there are still few data regarding multiple doses and analgesia.¹⁸

The antipyretic effect of acetaminophen has been extensively studied in infants,^{10,19,20} children,^{7,10,19-21} and adults.^{9,22} Plasma and serum values between 4 and 20 mg/L are considered to be therapeutic,⁹ and by combining data from orally administered acetaminophen that results in concentrations of 10 to 20 mg/L²³ with data from a dose-effect study,¹⁰ a therapeutic range for analgesia has been proposed but has not been validated.¹¹ Using tonsillectomy as a pain model, Anderson et al²⁴ found that acetaminophen (40 mg/kg orally) provided satisfactory analgesia for most children when plasma concentrations were within the range known to reduce fever. In the literature, data on the effect of acetaminophen on fever or analgesia in children are either dose-concentration^{16,20,25} or dose-effect studies.²⁶

The values for C_{max} in our study are comparable with results from previous single-dose studies with a 20 mg/kg rectal dose of acetaminophen in both preterm and term neonates^{4,16,27} and with high-dose rectal acetaminophen (30 to 45 mg/kg) in children from 3 to 13 years old.^{25,28} In a recent study by Sanderson et al,²⁹ plasma values at 24 hours in children from 3.9 to 9.8 years old ranged from 6.8 to 20.9 mg/L after a scheduled dosing regimen of 20 mg/kg orally with a 6-hour dosing interval. In comparison, we found values from 0.59 to 20.4 mg/L at 24 hours. Our data with a dosing schedule of 20 mg/kg four times a day do not support the estimates made by Autret et al¹⁷ for infants under 10 days of age. With computer simulations of 15 mg/kg propacetamol four times a day (ie, 30 mg/kg/day acetaminophen) Autret et al¹⁷ estimated that C_{max} would reach concentrations from 10.1 to 22.9 mg/L (mean $\pm$ SD, 17.46 ± 4.97 mg/L), whereas these values were reached in only half of our study infants with 80 mg/kg/day. We agree with Anderson et al¹¹ that with the large variability of results shown by the coefficient of variation of 40%, which compares with our value of 37.2%, we should be careful not to overstate the worth of mean values when considering individual patients, and that this coefficient of variation implies that some patients may not achieve a therapeutic C_{max} .

In all studies in infants, individual values vary widely, and when acetaminophen is given rectally the t_{max} also varies widely and is seldom reached before 3 hours. This may be a result of the variability of venous drainage from the rectum, resulting in bypassing of the liver by drugs delivered distally, whereas drugs delivered in the proximal rectum will be subjected to the hepatic first-pass effect.³⁰

Even after four doses, accumulation was not observed in our patients, as was the case in one study of children from 6 months to 6 years old to whom oral

Table II. Pharmacokinetic data of study participants

Patient	Serum elimination $t_{1/2}$ (h)	AUC (mg · h/L)	CL (L/h)	CL/F
G1	4.9	28.2	9.4	8.46
B1	8.6	461.8	0.8	0.72
G2	3.8	151.8	1.4	1.26
G3	1.2	115.2	3.9	3.51
G4	5.3	73.3	5.3	4.77
B2	—	397.2	—	—
G5	52.4	164.5	0.3	0.27
B3	36	229.8	0.4	0.36
B4	—	118.6	—	—
B5	11.8	160.4	1.2	1.08

$t_{1/2}$, Half-life; AUC, area under the serum concentration–time curve; CL, serum clearance; F, fraction of drug absorbed; G, girl; B, boy.

doses between 10 and 15 mg were administered.²³ In adults, orally administered acetaminophen exhibits linear pharmacokinetics for doses of 18 mg/kg or less when given five times at 6-hour intervals.³¹ In a computer model that simulated rectal dosing in children, a loading dose of 50 mg/kg followed by 30 mg/kg at 6-hour intervals achieved plasma concentrations of 9 to 18 mg/L, without accumulation.³² However, the effect of acetaminophen given for more than 24 hours in this age group is not known. Nahata et al³ found accumulation after 2 to 3 days in an older age group (2 to 8 years) when given 22 to 27 mg/kg orally, whereas doses of 13.3 mg/kg or more may also accumulate after this period. The highest serum concentration recorded in our study was 23.2 mg/L, which is still well below the concentration associated with toxicity in adults (>120 mg/L).¹ However, elimination appeared to be slower after the last dose in our study.

In some studies with older children, a starting dose of 30 to 40 mg/kg has been proposed, but we refrained from following this schedule in this study because the metabolism in neonates is somewhat slower.^{4,11,28,32} The $t_{1/2}$ varies widely between individuals, and in our study on the first day of life it ranged between 1.2 and 52.4 hours but decreased with postnatal age. In term infants it is reported to be between 2.7 to 4.9 hours in the first month of life and ranges from 1.0 to 2.4 hours up to the age of 1 year.^{5,8}

On the basis of our study, a starting dose of 30 mg/kg is proposed, followed by 20 mg/kg rectally at dosing intervals that increase from 6 to 8 hours. In this way, the maximum daily dose of 90 mg/kg is not exceeded.

Surprisingly, we found a significant difference in C_{max} between the girls and the boys; the mean value for girls was lower than that for boys. Clearance was no higher in girls than in boys. Although we have tried to collect urine by means of an adhesive urinary bag in

five infants, too many samples were lost to give a reliable impression of the excretion and recovery in the urine. Nothing is known about a gender-related difference in pharmacokinetics for any drug in this age group in humans. Data from our recent study in preterm infants do not show any difference.⁴ Because our group is relatively small, this difference may become negligible when a larger sample group is taken.

Serum concentrations between 10 and 25 mg/L are suggested as the appropriate analgesic range.³² However, no studies have been performed to compare serum or plasma concentration with effect in the age group under study. Except for the study by Anderson et al,²⁴ until now, therapeutic acetaminophen concentration ranges have not been determined in infants or in older children and adults. Studies in adults on the central analgesic effect propose that the time course of acetaminophen in cerebral spinal fluid may parallel that of analgesic effect because a time delay exists between serum concentrations and the analgesic effect of acetaminophen measured by means of the R-III nociceptive flexion reflex.³³⁻³⁵ Painful procedures such as those experienced by the infants in our study are reported to generate painful responses 75% to 100% of the time and therefore seem to be equivalent to each other.³⁶ A dose–effect study showed that 20 mg/kg oral acetaminophen was ineffective for decreasing the pain from heel prick in term neonates.³⁷ Recently Anderson³⁸ stated that neonates may have altered pharmacodynamics compared with older children.

Although low serum concentrations were found, low scores of pain assessment were determined, suggesting appropriate analgesia in all infants. This may be the result of a greater permeability of the blood-brain barrier in neonates, leading to greater central analgesia. Because there was no correlation between the pain scores and the serum concentrations and because val-

000027

ues that exceeded 2.6 mg/L were associated with facies pain scores from 0 to 1, we are not able to indicate a therapeutic acetaminophen concentration range for this age group.

In conclusion, we found that acetaminophen can be administered safely to neonates on the first day of life in rectal multiple doses of 20 mg/kg. A higher starting dose of 30 mg/kg, as recommended for older children, is proposed because (1) therapeutic concentrations were not reached in all infants, (2) the time to reach therapeutic concentrations was relatively long, and (3) accumulation was not found. Further research with a longer sampling time is needed to determine whether acetaminophen given to neonates for periods exceeding 24 hours is effective and safe.

We gratefully acknowledge Willem Grol for his statistical advice and John van den Anker and Kumar Jamdagni for their help in preparing the manuscript.

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ANEXO 2

000030

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Excipients for pharmaceuticals

Sasol brochure - Excipients (1)

000731

Contents

	Page
<i>Foreword</i>	3
<i>Liquid lipids</i>	4
<i>Solid triglycerides</i>	6
<i>Partial glycerides</i>	10
<i>Emulsifiers</i>	12
<i>Special fatty acid esters</i>	14
<i>Fatty alcohols</i>	16
<i>Polyethylene glycols (PEGs)</i>	18
<i>Register of chemical/INCI names or synonyms</i>	20
<i>Register of trademarks and product names</i>	24

Abbreviations:

BP	= British Pharmacopoeia
CFR	= Code of Federal Regulations
DAB	= Deutsches Arzneibuch
DMF	= Drug Master File
GRAS	= Generally Recognized As Safe
JCIC	= Japanese Cosmetic Ingredients Codex
JPE	= Japanese Pharmaceutical Excipients
Ph. Eur.	= European Pharmacopoeia
USP(-NF)	= United States Pharmacopoeia(-National Formulary)

000032

Solid triglycerides

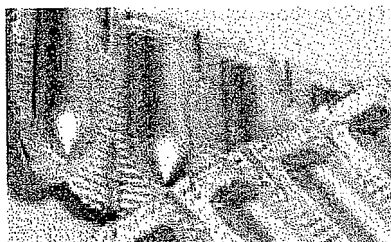
Apart from suppository manufacturing, solid triglycerides (hard fats) are used as carriers in capsule fillings, inlets, ointments and creams and in dental products. They also act as surface treatment and binder in tablets.

Solid triglycerides are manufactured through direct esterification of glycerol with defined fatty acid blends and have therefore precise properties regarding melting point, polarity (Hydroxyl Value) and consistency.

Being glycerol esters of edible fatty acids, they are classified as food additives.

Product	Chemical description/INCI name	Listed in
Hard fat suppository bases		
WITEPSOL H 5	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL H 12	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL H 15	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL H 19	Hydrogenated Coco-Glycerides + Glyceryl Ricinoleate**	—
WITEPSOL H 185	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL H 32	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL H 35	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL H 37	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL W 25	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL W 31	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL W 32	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL W 35	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL W 45	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE, DMF
WITEPSOL S 51	Hydrogenated Coco-Glycerides + Ceteareth-25 + Glyceryl Ricinoleate**	—
WITEPSOL S 55*	Hydrogenated Coco-Glycerides + Ceteareth-25 + Bees Wax	USP-NF, Ph. Eur. *, DMF
WITEPSOL S 58	Hydrogenated Coco-Glycerides + Ceteareth-25 + Glyceryl Ricinoleate**	—

* Compound, single components Ph. Eur. + USP-NF.
** Compounds contain unsaturated components, Interactions with oxygen must be considered.



All suppository hard fats have a low content of short chain fatty acids (C_8/C_{10}), convenient for improved application. The solid fat index curve is very stable. Hence WITEPSOLs are still solid and applicable, even a few degrees below melting point. EXCEPTION: the more soft adjusted WITEPSOL H 35.

Important selection criteria are melting point and hydroxyl value. During formulation developments the tendency of the post-harden effect should be observed. Our technical application department in Witten will be glad to advise you.

Main characteristics

Melting point °C	Hydroxyl value mg KOH/g	Properties and applications
34.0 to 36.0	max. 5	WITEPSOL H grades are hard fats with a low hydroxyl value of max. 15. They comprise mainly triglycerides with proportions of max. 15 % of diglycerides and max. 1 % of monoglycerides.
32.0 to 33.5	5 to 15	
33.5 to 35.5	5 to 15	
33.5 to 35.5	20 to 30	
38.0 to 39.0	5 to 15	They are characterized by a short gap between melting and solidification temperatures, showing a low tendency of post-hardening.
31.0 to 33.0	max. 3	
33.5 to 35.5	max. 3	Shock cooling should be avoided because of their brittleness.
36.0 to 38.0	max. 3	
33.5 to 35.5	20 to 30	WITEPSOL W grades are hard fats with hydroxyl values between 20–50. They comprise a mixture of 65–80 % of triglycerides, 10–35 % of diglycerides and 1–5 % of monoglycerides. (Correspondingly they have a wider gap between melting and solidification temperatures and are therefore more insensitive (elastic) to shock cooling.) They solidify more slowly. The amount of partial glycerides delays the sedimentation of solid active ingredients and promotes wetting of mucous membranes.
35.5 to 37.0	25 to 35	
32.0 to 33.5	40 to 50	
33.5 to 35.5	40 to 50	
33.5 to 35.5	40 to 50	
30.0 to 32.0	55 to 70	WITEPSOL S grades contain special additives. They are suitable for manufacturing vaginal and rectal preparations, requiring a more intensive wetting of mucosae, a greater dispersability or an enhanced absorption. They all contain an ethoxylated emulsifier (Ph. Eur.) which makes them suitable for use in aqueous or ethanolic/aqueous active ingredients (emulsion suppositories).
ca. 35***	50 to 65	
31.5 to 33.0	60 to 70	

*** Due to additive bees wax, determination of solidification point is recommended.

Solid triglycerides (continued)

Product	Chemical description/INCI name	Listed in
Hard fat suppository bases		
WITEPSOL E 75*	Hydrogenated Coco-Glycerides + Bees Wax	USP-NF, Ph. Eur. *
WITEPSOL E 76	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE
WITEPSOL E 85	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur., JPE
MASSA ESTARINUM 299	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur.
MASSA ESTARINUM B	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur.
MASSA ESTARINUM BC*	Hydrogenated Coco-Glycerides + Glyceryl Ricinoleate**	—
MASSA ESTARINUM C	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur.*
MASSA ESTARINUM E*	Hydrogenated Coco-Glycerides + Ceteareth-25 + Bees Wax	—
SOFTISAN 378	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur. JCIC, DMF
Solid Wax Esters ¹⁾		
DYNACERIN CP	Cetyl Palmitate	USP-NF, Ph. Eur. JCIC
Triglycerides with Melting Point above Body Temperature		
DYNASAN 114	Trimyristin	—
DYNASAN 118	Tristearin	21 CFR §172.811 JCIC
DYNASAN P 60	Hydrogenated Palm Oil	USP-NF
SOFTISAN 154	Hydrogenated Palm Oil	—
WITOCAN 42/44	Hydrogenated Coco-Glycerides	USP-NF, Ph. Eur.

* Compound, Single Products Ph. Eur. + USP-NF.
** Compounds contain unsaturated components, interactions with oxygen must be considered.
¹⁾ Other wax esters on request, please also see "solid alcohols".



Main characteristics

Melting point °C	Hydroxyl value mg KOH/g	Properties and applications
ca. 38***	max. 15	WITEPSOL E grades are hard fats with a melting range above body temperature. They can be used to raise the melting point in case of active ingredients that decrease the melting point.
37.0 to 39.0	30 to 40	
42.0 to 44.0	5 to 15	
33.5 to 35.5	max. 2	Suppositories, see WITEPSOLs.
33.5 to 35.5	20 to 30	
33.5 to 35.5	30 to 40	
36.0 to 38.0	20 to 30	
ca. 34 to 36***	45 to 60	
~ 30	7 to 17	Hard fat with softer consistency, similar to natural lard, but stable, odourless, emollient in creams, capsule filling mass.
~ 50	max. 6	Stable waxy ester, replacement for natural spermaceti, consistency agent in creams, for coating, sustained release.
55 to 58	DYNASAN grades are suitable for tablet manufacturing as a lubricant.	
70 to 74	The stable β-modification of these fats shows a sharp melting point. Suitable in suppositories and vaginal ovules as crystalization accelerators and seeding agents.	
58 to 62	max. 10	Palmitic/stearic triglycerides, similar to hydrogenated vegetable oil, USP – type I.
53 to 58	max. 10	
42 to 44	max. 15	Consistency agent, coating, sustained release.

*** Due to additive bees wax determination of solidification point is recommended.

Product Information

01.06.2007

WITEPSOL® and MASSA ESTARINUM®

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1. Description

Suppository bases according to the current monographs Hard Fat, Adeps solidus Ph.Eur. and Hard Fat USP. They are white, odorless hard fats in pastill shape which comprises of glycerides of plant origin.

2. Characteristic values

Each of the following products is characterized by 10 parameters. The sorting is oriented at the meaning for the suppository formulation.

Method →	Ascending melting point °C	Hydroxyl value mg KOH/g	Acid value mg KOH/g	Iodine value g I ₂ /100 g	Peroxide value mequi O/kg
	Ph.Eur. 2.2.15	Ph.Eur. 2.5.3	Ph.Eur. 2.5.1	Ph.Eur. 2.5.4	Ph.Eur. 2.5.5
WITEPSOL H 5	34,0 – 36,0	max. 5	max. 0,2	max. 2	max. 1
WITEPSOL H 12	32,0 – 33,5	5 – 15	max. 0,2	max. 3	max. 1
WITEPSOL H 15	33,5 – 35,5	5 – 15	max. 0,2	max. 3	max. 1
WITEPSOL H 19*	33,5 – 35,5	20 – 30	max. 0,2	max. 7	max. 3
WITEPSOL H 175*	34,5 – 36,5	5 – 15	max. 0,7	max. 3	max. 3
WITEPSOL H 32	31,0 – 33,0	max. 3	max. 0,2	max. 3	max. 1
WITEPSOL H 35	33,5 – 35,5	max. 3	max. 0,2	max. 3	max. 1
WITEPSOL H 37	36,0 – 38,0	max. 3	max. 0,2	max. 3	max. 1
WITEPSOL W 25	33,5 – 35,5	20 – 30	max. 0,3	max. 3	max. 1
WITEPSOL W 31	35,5 – 37,0	25 – 35	max. 0,3	max. 3	max. 1
WITEPSOL W 32	32,0 – 33,5	40 – 50	max. 0,5	max. 3	max. 1
WITEPSOL W 35	33,5 – 35,5	40 – 50	max. 0,3	max. 3	max. 1
WITEPSOL W 45	33,5 – 35,5	40 – 50	max. 0,3	max. 3	max. 1
WITEPSOL S 51*	30,0 – 32,0	55 – 70	max. 0,5	max. 8	max. 4
WITEPSOL S 52*	32,0 – 33,5	50 – 65	max. 1,0	max. 3	max. 3
WITEPSOL S 55*	ca. 35**	50 – 65	max. 1,0	max. 3	max. 3
WITEPSOL S 58*	31,5 – 33,0	60 – 70	max. 1,0	max. 7	max. 4
WITEPSOL E 75*	ca. 38**	max. 15	max. 1,3	max. 3	max. 3
WITEPSOL E 76	37,0 – 39,0	30 – 40	max. 0,3	max. 3	max. 1
WITEPSOL E 85	42,0 – 44,0	5 – 15	max. 0,3	max. 3	max. 1
MASSA ESTARINUM 299	33,5 – 35,5	max. 2	max. 0,2	max. 3	max. 1
MASSA ESTARINUM B	33,5 – 35,5	20 – 30	max. 0,3	max. 3	max. 1
MASSA ESTARINUM BC*	33,5 – 35,5	30 – 40	max. 0,3	max. 3	max. 1
MASSA ESTARINUM C	36,0 – 38,0	20 – 30	max. 0,3	max. 3	max. 1
MASSA ESTARINUM E*	ca. 34 – 36**	45 – 60	max. 1	max. 3	max. 3
Monographs					
Ph.Eur.	30 – 45	max. 50	max. 0,5	max. 3,0	max. 3
USP	27 – 44	max. 70	max. 1,0	max. 7,0	-
Jap. Pharm. Excipients	30 – 45	max. 70	max. 2,0	max. 3,0	-

* WITEPSOL grades H 19, H 175, S 51, S 52, S 55, S 58, E 75 and MASSA ESTARINUM BC, E respectively are mixtures of hard fats (Ph.Eur.) and additives. These mixtures do not comply with Ph.Eur. More specific descriptions are given in our brochure „Fatty bases for suppositories“.

** Due to additive bees wax the ascending melting points are hard to reproduce (adhesion at the glas capillary). The solidification points are more precisely. They are for WITEPSOL E 75 = 34 – 36,5 °C, for WITEPSOL S 55 = 28 – 33 °C and for MASSA ESTARINUM E = 29 – 31 °C.

Sasol - Product information - Witepsol

000037

Product Information

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WITEPSOL® and MASSA ESTARINUM®

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Characteristic values

Method →	Saponification value mg KOH/g	Alkaline impurities ml HCl/2g	Heavy metals ppm	Ash %	Unsaponifiable matter %
	Ph.Eur. 2.5.6	Ph.Eur. 2.4.19	Ph.Eur. 2.4.8	Ph.Eur. 2.4.16	Ph.Eur. 2.5.7
WITEPSOL H 5	235 – 245	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL H 12	240 – 255	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL H 15	230 – 245	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL H 19*	230 – 240	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL H 175*	225 – 245	max. 0,15	max. 10	max. 0,05	max. 1,0
WITEPSOL H 32	240 – 250	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL H 35	240 – 250	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL H 37	225 – 245	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL W 25	225 – 240	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL W 31	225 – 240	max. 0,15	max. 10	max. 0,05	max. 0,5
WITEPSOL W 32	225 – 245	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL W 35	225 – 235	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL W 45	225 – 240	max. 0,15	max. 10	max. 0,05	max. 0,3
WITEPSOL S 51*	215 – 230	max. 0,15	max. 10	max. 0,05	max. 0,5
WITEPSOL S 52*	220 – 230	max. 0,15	max. 10	max. 0,05	max. 2,0
WITEPSOL S 55*	215 – 230	max. 0,15	max. 10	max. 0,05	max. 2,0
WITEPSOL S 58*	215 – 225	max. 0,15	max. 10	max. 0,05	max. 2,0
WITEPSOL E 75*	220 – 230	max. 0,15	max. 10	max. 0,05	max. 3,0
WITEPSOL E 76	220 – 230	max. 0,15	max. 10	max. 0,05	max. 0,5
WITEPSOL E 85	220 – 240	max. 0,15	max. 10	max. 0,05	max. 0,5
MASSA ESTARINUM 299	240 – 255	max. 0,15	max. 10	max. 0,05	max. 0,5
MASSA ESTARINUM B	225 – 240	max. 0,15	max. 10	max. 0,05	max. 0,5
MASSA ESTARINUM BC*	225 – 240	max. 0,15	max. 10	max. 0,05	max. 0,5
MASSA ESTARINUM C	225 – 235	max. 0,15	max. 10	max. 0,05	max. 0,5
MASSA ESTARINUM E*	215 – 230	max. 0,15	max. 10	max. 0,05	max. 2,0
Monographs					
Ph.Eur.	210 – 260	max. 0,15	max. 10	max. 0,05	max. 0,6
USP	215 – 255	max. 0,15	-	max. 0,05	max. 3,0
Jap. Pharm. Excipients	210 – 255	-	-	-	max. 3,0

3. Handling and Storage

Dry, protected from light, in original containers and at temperatures below 25°C, shelf life is at least three years.

We reserve the right to make any changes according to technological progress or further developments. The customer is not released from the obligations to conduct careful inspection and testing of incoming goods. Reference to trade names used by other companies is neither a recommendation, nor is it intended or suggested that similar products could not be used. All our business transactions shall be governed exclusively by our General Sales Conditions of SASOL Germany GmbH.

000038

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ANEXO 3

Serum steroid levels during 12-week intravaginal dehydroepiandrosterone administration

Fernand Labrie, MD, PhD,^{1,2} David Archer, MD,³ Céline Bouchard, MD,⁴ Michel Fortier, MD,⁴ Leonello Cusan, MD, PhD,¹ José-Luis Gomez, MD, PhD,¹ Ginette Girard, MD,⁵ Mira Baron, MD,⁶ Normand Ayotte, MD,⁷ Michèle Moreau, MD,⁸ Robert Dubé, MD,⁹ Isabelle Côté, BSc, CCRP,² Claude Labrie, MD, PhD,^{1,2} Lyne Lavoie, MSc,² René Bérubé, MSc,¹ Patrick Bélanger, MSc,¹ Louise Berger, PhD,² Lucy Gilbert, MD,¹⁰ Céline Martel, PhD,² and John Balser, PhD¹¹

Abstract

Objective: Because a previous 1-week study has shown no or minimal changes in the serum levels of dehydroepiandrosterone (DHEA) and its metabolites after up to daily 1.8% (23.4 mg) intravaginal DHEA, the objective of the present study was to investigate the serum steroid levels during a 12-week daily intravaginal administration of 0%, 0.25%, 0.5%, and 1.0% DHEA (Prasterone) 1.3 mL ovules.

Methods: In a double-blind, placebo-controlled phase III study, 218 postmenopausal women (age range, 42-74 y) were randomized to receive daily one of four DHEA concentrations intravaginally. Serum steroids were measured by a Good Laboratory Practice-validated mass spectrometry technology in samples obtained at time of visit.

Results: The serum levels of DHEA and 11 of its metabolites measured at screening, day 1, and weeks 2, 4, 8, and 12 in women showed no or minimal changes during the whole observation period, with all values remaining well within the limits of normal postmenopausal women. No accumulation of the steroid metabolites nor change in DHEA bioavailability was detected.

Conclusions: The present data show that local daily intravaginal DHEA administration at DHEA doses of 3.25-13 mg was able to rapidly and efficiently achieve correction of all the signs and symptoms of vaginal atrophy and improve sexual function and caused no or minimal changes in serum sex steroid levels, which all remain within the normal postmenopausal range, thus avoiding the risks of all estrogen formulations.

Key Words: Dehydroepiandrosterone – Prasterone – Intracrinology – Estrogens – Androgens – Vaginal atrophy.

Because all previous measurements of serum estradiol (E₂) after intravaginal administration of estrogens used radioimmunoassays, a technology frequently lacking specificity, accuracy, and sensitivity for low serum E₂ and testosterone levels found in postmenopausal women,¹

we have recently, for the first time, measured serum estrogens using Good Laboratory Practice-validated mass spectrometry assays after intravaginal administration of the two most commonly used estrogen formulations.² That study definitively showed that both the E₂ pill (25 µg E₂/d) and conjugated estrogen cream (1 g of 0.625 mg conjugated equine estrogens/d), after 1 week of daily intravaginal administration caused an approximately fivefold increase in serum E₂ in postmenopausal women.

In addition to the safety issue of estrogens administered systemically or locally for the treatment of vaginal atrophy, recent data have clearly demonstrated that postmenopausal women are not only deficient in estrogens at the time of menopause but also deficient in androgens.³ This new knowledge of the endocrinology of sex steroids in women opens novel possibilities for true physiological hormone (or pre-hormone) therapy in postmenopausal women. These new data essentially pertain to the observation that sex steroids in postmenopausal women originate completely from the tissue-specific local conversion of the inactive precursor dehydroepiandrosterone (DHEA) of adrenal origin into androgens and/or estrogens.^{4,5} It should also be considered

Received November 10, 2008; revised and accepted January 26, 2009.

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Funding/support: This study was sponsored by Endoceutics.

Financial disclosure/conflicts of interest: Dr. Labrie is President and CEO of Endoceutics. The other authors report no conflicts of interest, although the study was financed by Endoceutics. Patents for intravaginal Prasterone are the property of Endoceutics, Inc.

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that relatively high levels of androgens are synthesized in peripheral tissues of women independently from a minor contribution of the ovaries.³ Most interestingly, healthy postmenopausal women synthesize approximately 50% as much androgens as intact men of comparable age.^{6,7}

The androgens testosterone and dihydrotestosterone (DHT) as well as E₂ made in peripheral tissues from DHEA exert their action locally in the same cells where their synthesis takes place with only minimal release into the circulation.^{4,5} This sophisticated mechanism called intracrinology^{4,8} permits the maintenance of biologically active levels of intracellular estrogens and/or androgens only in specific tissues having a physiological need for these sex steroids. Moreover, these steroids made in peripheral tissues diffuse at very low levels as active steroids in the blood, thus preventing adverse reactions in other tissues. Most importantly, after their local formation and immediate availability for local intracellular action, testosterone and DHT (the most active natural androgen) are inactivated and transformed in the same cells into water-soluble glucuronide derivatives, which then diffuse quantitatively into the general circulation and are eliminated.⁵ Serum DHEA and DHEA-sulfate (DHEA-S), however, progressively decrease from the peak values seen at the age of 30 years⁹ to reach, on average, 60% lower values at time of menopause.³

In previous studies in which the serum levels of DHEA and 11 of its metabolites were measured during a 24-hour period on the 1st and 7th day of daily intravaginal administration of 0%, 0.5%, 1.0%, and 1.8% DHEA,^{10,11} the serum concentrations of E₂ and testosterone remained within the values found in normal postmenopausal women at all DHEA doses. Similar observations were made for serum androstenedione (4-dione), estrone (E₁), estrone-sulfate (E₁-S), and DHEA-S. Even at the highest 1.8% DHEA dose, serum DHEA was increased at the levels observed in normal premenopausal women. Those data have shown that the intravaginal administration of DHEA permits rapid achievement of the local beneficial effects against vaginal atrophy¹⁰ without significant changes in serum estrogens,^{10,11} thus avoiding the increase in serum estrogens associated with the current intravaginal or systemic estrogenic formulations. A similar observation was made on serum androgens, although androgens exert an inhibitory effect on proliferation of the normal mammary gland and breast cancer.^{6,12,13}

The present study investigates the changes in the serum levels of DHEA and 11 of its metabolites during a 12-week treatment period with the daily 0%, 0.25%, 0.5%, and 1.0% intravaginal DHEA doses to determine the possible effect of duration of treatment on DHEA bioavailability and metabolism.

METHODS

This study was a phase III, prospective, multicenter, randomized, placebo-controlled, and double-blind trial planned for 50 participants per arm (for a total of 200 participants to complete the study). Two hundred eighteen

postmenopausal women (216 women in the intent-to-treat analysis) enrolled at eight sites in Canada and in the United States were thus randomized to receive a daily intravaginal ovule of the following DHEA concentrations: 0.0% (53 women), 0.25% (3.25 mg DHEA; 53 women), 0.5% (6.5 mg DHEA; 56 women), or 1.0% (13 mg DHEA; 54 women), applied intravaginally with an applicator at bedtime. The ovules or suppositories containing DHEA in a lipophilic base were manufactured by Recipharm (Karlskoga, Sweden). The median (range) age of women was 58 (49-70), 57 (42-72), 58 (50-74), and 59 (46-69) years in the four groups. The study was divided into two phases, namely, screening followed by a treatment period of 12 weeks. The protocol was approved by the institutional review board of the Centre Hospitalier de l'Université Laval, Quebec City, QC, Canada; McGill University, Montreal, QC, Canada; Ethica, Montreal, QC, Canada; Eastern Virginia Medical School, Norfolk, VA; and the Western Institutional Review Board, Los Angeles, CA.

Inclusion/exclusion criteria were described in detail in an accompanying article.¹⁷ The main inclusion criteria were the following:

- Postmenopausal women
- Women having self-identified at least one moderate to severe of the following symptoms:
 - Vaginal dryness (none, mild, moderate, or severe)
 - Vaginal and/or vulvar irritation/itching (none, mild, moderate, or severe)
 - Vaginal pain associated with sexual activity (none, mild, moderate, or severe)
- Women between 40 and 75 years of age
- Women having a low maturation index (no greater part of guidance than 5% of superficial cells on vaginal smear)
- Women having a vaginal pH above 5

The main exclusion criteria were the following:

- Undiagnosed abnormal genital bleeding
- Previous diagnosis of cancer, except skin cancer (non-melanoma)
- Endometrial hyperplasia at biopsy performed at screening or endometrial cancer
- Use of estrogen-alone injectable drug therapy or progestin implant within 3 months before study entry (screening visit)
- Use of estrogen pellet or progestin injectable drug within 6 months before study entry (screening visit)
- Use of oral estrogen, progestin, or DHEA exposure or intrauterine progestin therapy in the 8 weeks before baseline assessments (screening visit)
- Use of vaginal hormonal products (rings, creams, or gels) or transdermal estrogen alone or estrogen/progestin products in the 4 weeks before baseline assessments (screening visit)

Informed consent

Written informed consent was obtained from all participants before the performance of any study-related procedure. The participants had a medical history, a medical examination, and a complete gynecological examination (including an

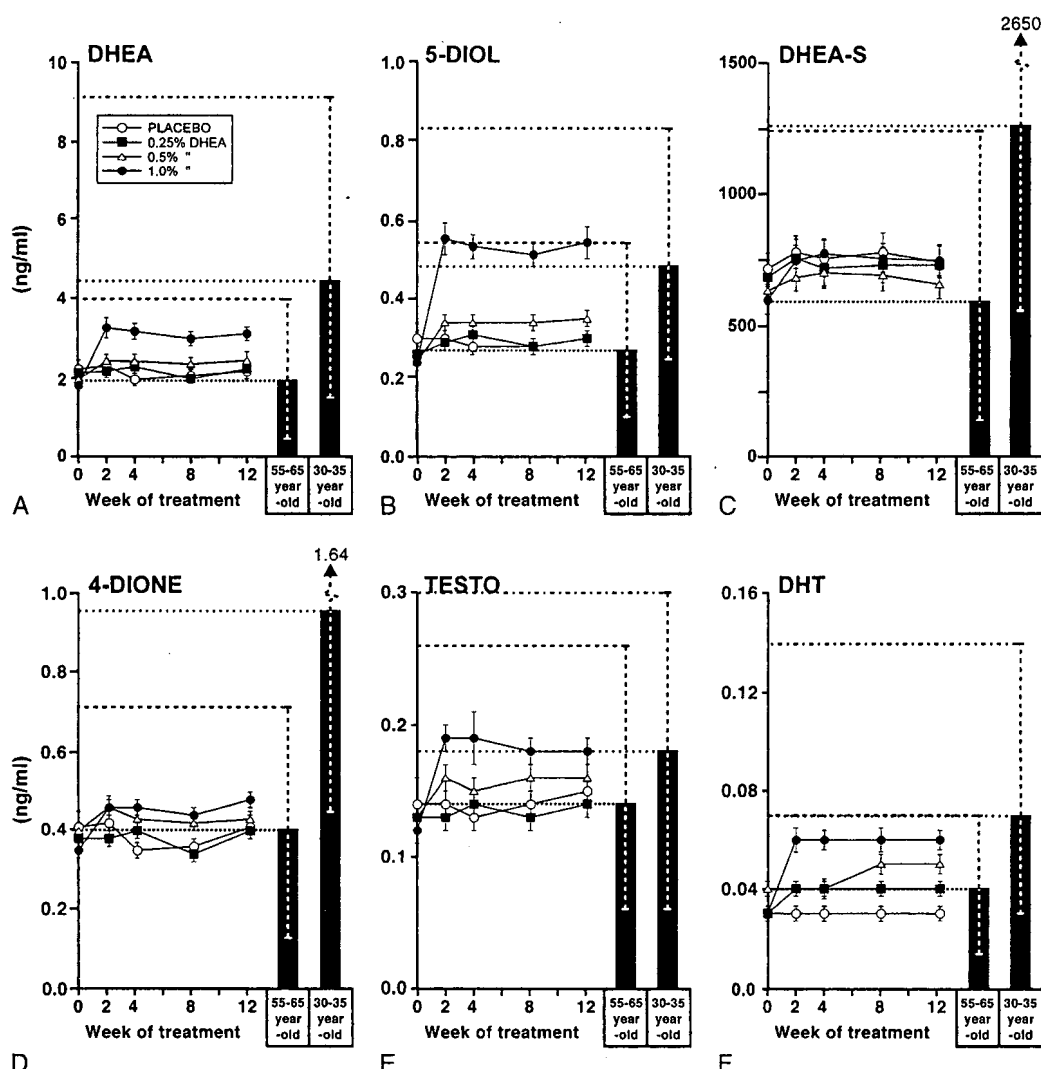


FIG. 1. Serum levels of dehydroepiandrosterone (DHEA), androst-5-ene-3 β ,17 β -diol (5-diol), DHEA-sulfate (DHEA-S), androstenedione (4-dione), testosterone (Testo), and dihydrotestosterone (DHT) measured on day 1 and at weeks 2, 4, 8, and 12 during once-daily intravaginal administration of ovules containing 0.0% (n = 53), 0.25% (n = 53), 0.5% (n = 56), and 1.0% (n = 54) DHEA. Data are expressed as means $\pm$ SEM (n = 53-56). Serum steroid concentrations measured in 30- to 35-year-old premenopausal (n = 47) and 55- to 65-year-old postmenopausal (n = 377) women are added as reference data, which are expressed as means and 5th and 95th centiles (dashed lines).

endometrial biopsy) at screening. A partial gynecological examination was performed to evaluate the aspect of the mucosa and tolerance to the medication on day 1 and at weeks 2, 4, 8, and 12.

Laboratory tests

The standard laboratory tests, namely, hematology (including complete blood count and coagulation), blood chemistry, and urinalysis were performed at screening and at week 12.¹⁷ Serum follicle-stimulating hormone was measured only in women who had no menses for more than 6 months but less than 12 months or who were premenopausal at the time of hysterectomy. Serum steroid levels of DHEA, DHEA-S, androst-5-ene-3 β ,17 β -diol (5-diol), DHT, testosterone, 4-dione, E₁, E₂, E₁-S, androsterone glucuronide (ADT-G), androstane-3 α ,17 β -diol-3 glucuronide (3 α -diol-3 G), and 3 α -

diol-17 G were measured at the Laboratory of Molecular Endocrinology, Laval University Hospital Research Center, by mass spectrometry according to Good Laboratory Practice-validated methodology as described.^{3,10,14} Intra-assay and interassay coefficients of variation were reported.

Statistics

Summary tabulations were prepared, displaying the number of observations, mean, SD, SEM, 95% two-sided CI, median, and minimum and maximum for continuous variables. Statistical analyses were performed at the two-sided significance level of 0.05 using SAS software. The analysis comparing each dose of DHEA to placebo was performed by analysis of covariance, with the baseline value used as the covariate. The changes from baseline within each treatment group were assessed for statistical significance

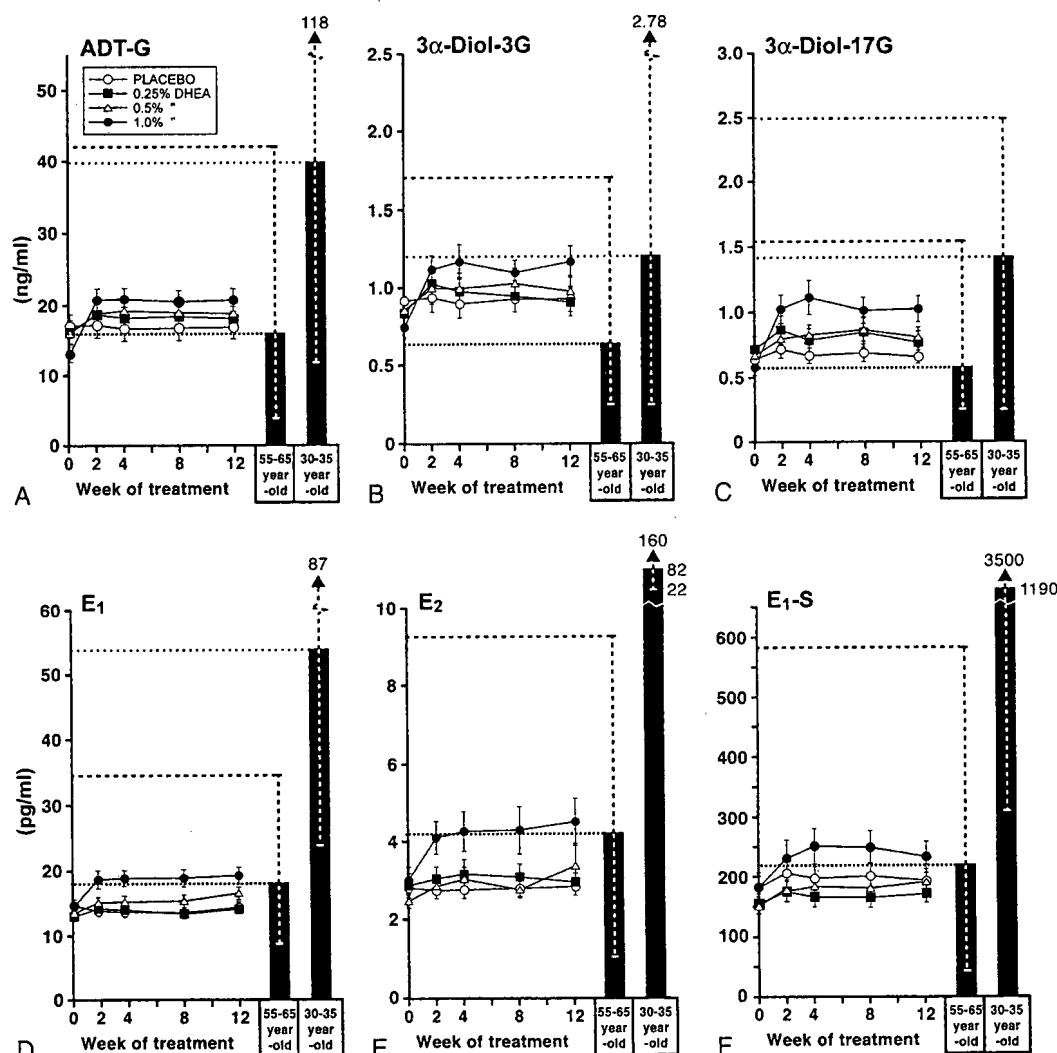


FIG. 2. Serum levels of androsterone glucuronide (ADT-G), androstane-3 α ,17 β -diol-3 glucuronide (3 α -diol-3 G), 3 α -diol-17 G, estrone (E₁), estradiol (E₂), and estrone-sulfate (E₁-S) measured on day 1 and at weeks 2, 4, 8, and 12 during once-daily administration of vaginal ovules containing 0% (n = 53), 0.25% (n = 53), 0.5% (n = 56), and 1.0% (n = 54) dehydroepiandrosterone (DHEA). Data are expressed as means $\pm$ SEM (n = 53-56). Serum steroid concentrations measured in 30- to 35-year-old premenopausal (n = 47) and 55- to 65-year-old postmenopausal (n = 377) women are added as reference data, which are expressed as means and 5th and 95th centiles (dashed lines).

using paired *t* tests. Baseline was defined as the value on day 1, obtained before first use of study treatment, and change from baseline to week 12 was calculated for each participant for each steroid. Missing values for any endpoint for a participant were replaced using a last value carried forward approach.

For Figures 5 and 6, statistical analysis of the days 1 and 7 average serum levels compared with baseline was performed using the multiple-range test of Duncan-Kramer.¹⁵

RESULTS

As illustrated in Figures 1 and 2 and shown in Table 1, all the 12 steroids measured by mass spectrometry from samples obtained between 7:20 AM and 7:40 PM remained within the limits of normal postmenopausal women after daily administration of DHEA ovules, except the levels of 5-diol at the

1.0% DHEA dose, which were measured at the upper limit of postmenopausal values but were well below premenopausal concentrations. Figure 1A shows that serum DHEA increases at 12 weeks by 4.7% (*P* = 0.449), 24.7% (*P* = 0.0036), and 70.7% (*P* < 0.0001) at the 0.25%, 0.5%, and 1.0% DHEA doses, respectively, with all these values remaining well within the normal postmenopausal range.³ The same figure and the following figures show, for comparison, the means as well as the 5th and 95th centile values in 55- to 65- and 30- to 35-year-old women.^{3,10} Figures 1 and 2 and Table 1 also show that there was no accumulation of any steroid or change in DHEA bioavailability during the 12-week period as illustrated by the same concentrations measured at 2, 4, 8, and 12 weeks for all steroids.

Serum 5-diol was increased by 15.4%, 45.8%, and 125% at the three DHEA doses (Fig. 1B), this last value remaining

INTRAVAGINAL DHEA AND SEX STEROIDS

TABLE 1. Serum concentrations of DHEA and 11 of its metabolites measured at baseline (day 1) and at 12 weeks of treatment with placebo (0%) and 0.25%, 0.5%, and 1.0% DHEA

Steroid	Placebo (n = 53)		0.25% DHEA (n = 53)		0.5% DHEA (n = 56)		1.0% DHEA (n = 54)	
	Baseline (day 1)	12 wk	Baseline (day 1)	12 wk	Baseline (day 1)	12 wk	Baseline (day 1)	12 wk
DHEA, ng/mL	2.25 ± 0.23	2.19 ± 0.18 <i>P</i> = 0.6733	2.15 ± 0.21	2.25 ± 0.15 <i>P</i> = 0.4491	1.98 ± 0.18	2.47 ± 0.22 <i>P</i> = 0.0036	1.84 ± 0.15	3.14 ± 0.17 <i>P</i> < 0.0001
5-Diol, ng/mL	0.30 ± 0.02	0.30 ± 0.02 <i>P</i> = 0.6291	0.26 ± 0.02	0.30 ± 0.02 <i>P</i> = 0.0021	0.24 ± 0.02	0.35 ± 0.02 <i>P</i> < 0.0001	0.24 ± 0.02	0.54 ± 0.04 <i>P</i> < 0.0001
DHEA-S, ng/mL	716 ± 59	742 ± 60 <i>P</i> = 0.2609	683 ± 68	730 ± 69 <i>P</i> = 0.0150	633 ± 54	657 ± 53 <i>P</i> = 0.1179	597 ± 52	748 ± 58 <i>P</i> < 0.0001
4-Dione, ng/mL	0.41 ± 0.04	0.41 ± 0.03 <i>P</i> = 0.8955	0.38 ± 0.03	0.40 ± 0.02 <i>P</i> = 0.5396	0.40 ± 0.02	0.43 ± 0.02 <i>P</i> = 0.2160	0.35 ± 0.02	0.48 ± 0.02 <i>P</i> < 0.0001
Testo, ng/mL	0.14 ± 0.02	0.15 ± 0.02 <i>P</i> = 0.5088	0.13 ± 0.01	0.14 ± 0.01 <i>P</i> = 0.0076	0.13 ± 0.01	0.16 ± 0.01 <i>P</i> = 0.0002	0.12 ± 0.01	0.18 ± 0.01 <i>P</i> < 0.0001
DHT, ng/mL	0.03 ± 0.003	0.03 ± 0.003 <i>P</i> = 0.0907	0.03 ± 0.002	0.04 ± 0.003 <i>P</i> = 0.0005	0.04 ± 0.003	0.05 ± 0.004 <i>P</i> < 0.0001	0.03 ± 0.003	0.06 ± 0.004 <i>P</i> < 0.0001
ADT-G, ng/mL	16.95 ± 1.66	16.77 ± 1.68 <i>P</i> = 0.7008	16.14 ± 1.64	17.95 ± 1.72 <i>P</i> = 0.0010	15.89 ± 1.54	18.70 ± 1.71 <i>P</i> < 0.0001	12.96 ± 1.13	20.58 ± 1.61 <i>P</i> < 0.0001
3α-Diol-3 G, ng/mL	0.91 ± 0.09	0.92 ± 0.08 <i>P</i> = 0.8280	0.83 ± 0.08	0.90 ± 0.09 <i>P</i> = 0.0561	0.85 ± 0.09	0.97 ± 0.10 <i>P</i> = 0.0093	0.74 ± 0.07	1.16 ± 0.10 <i>P</i> < 0.0001
3α-Diol-17 G, ng/mL	0.63 ± 0.05	0.65 ± 0.05 <i>P</i> = 0.7404	0.71 ± 0.10	0.76 ± 0.09 <i>P</i> = 0.1796	0.66 ± 0.06	0.80 ± 0.08 <i>P</i> = 0.0066	0.57 ± 0.06	1.02 ± 0.10 <i>P</i> < 0.0001
E ₁ , pg/mL	14.58 ± 0.99	14.29 ± 0.88 <i>P</i> = 0.6562	12.96 ± 0.74	14.00 ± 0.74 <i>P</i> = 0.0546	13.47 ± 0.80	16.46 ± 0.99 <i>P</i> = 0.0001	14.47 ± 1.06	19.17 ± 1.33 <i>P</i> < 0.0001
E ₂ , pg/mL	2.81 ± 0.25	2.84 ± 0.23 <i>P</i> = 0.8838	2.87 ± 0.31	2.95 ± 0.23 <i>P</i> = 0.7364	2.47 ± 0.17	3.37 ± 0.56 <i>P</i> = 0.0867	3.01 ± 0.34	4.50 ± 0.61 <i>P</i> = 0.0003
E ₁ -S, pg/mL	182 ± 19	192 ± 20 <i>P</i> = 0.3132	154 ± 16	171 ± 15 <i>P</i> = 0.0854	151 ± 14	190 ± 22 <i>P</i> = 0.0043	181 ± 25	232 ± 26 <i>P</i> = 0.0002

Data are expressed as means ± SEM. DHEA, dehydroepiandrosterone; 5-diol, androst-5-ene-3β,17β-diol; DHEA-S, DHEA-sulfate; 4-Dione, androstenedione; Testo, testosterone; DHT, dihydrotestosterone; ADT-G, androsterone glucuronide; 3α-Diol-3 G, androstane-3α,17β-diol-3 glucuronide; 3α-Diol-17 G, androstane-3α,17β-diol-17 glucuronide; E₁, estrone; E₂, estradiol; E₁-S, estrone-sulfate.

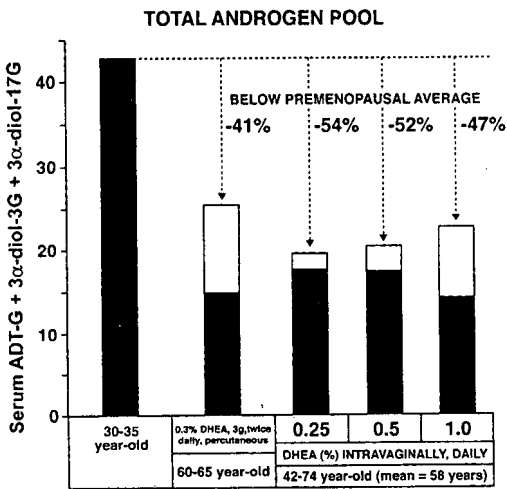


FIG. 3. Changes in the sum of the androgen metabolites androsterone glucuronide (ADT-G), androstane-3α,17β-diol-3 G (3α-diol-3 glucuronide), and 3α-diol-17 G in postmenopausal women (42-74 years old) who received daily intravaginal administration of 0.25% (n = 53), 0.5% (n = 56), and 1.0% (n = 54) dehydroepiandrosterone (DHEA) ovules for 12 weeks. Comparison is made with 60- to 65-year-old women who received twice-daily application on the skin of 3.0 g of a 0.3% DHEA emulsion.¹⁶ The average 61.6% loss in the androgen pool in the women treated for vaginal atrophy in the present study was only replaced by 4.4%, 7.2%, and 19.8% compared with that of premenopausal women, thus remaining 54%, 52%, and 47% below the values of the androgen pool in normal 30- to 35-year-old women.³ The total androgen pool in women who received percutaneous DHEA for 12 months remained 41% below the value in normal premenopausal women.¹⁶

at the upper limit of the values observed in normal postmenopausal women but, as mentioned above, well within the premenopausal value.

It should be mentioned at this stage that the changes in serum DHEA and 5-diol after percutaneous administration of DHEA have been found to be an 286% overestimation of the

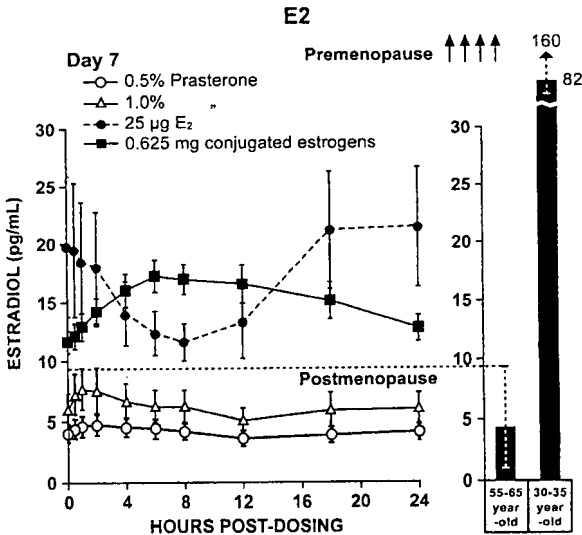


FIG. 4. Changes in serum estradiol (E₂) during the 24-hour period after the seventh daily intravaginal administration of 0.5% dehydroepiandrosterone (DHEA; n = 10), 1.0% DHEA (n = 10), 25 µg E₂ (n = 10; Vagifem), and 0.625 µg conjugated estrogens (Premarin cream; n = 10).^{2,10,11}

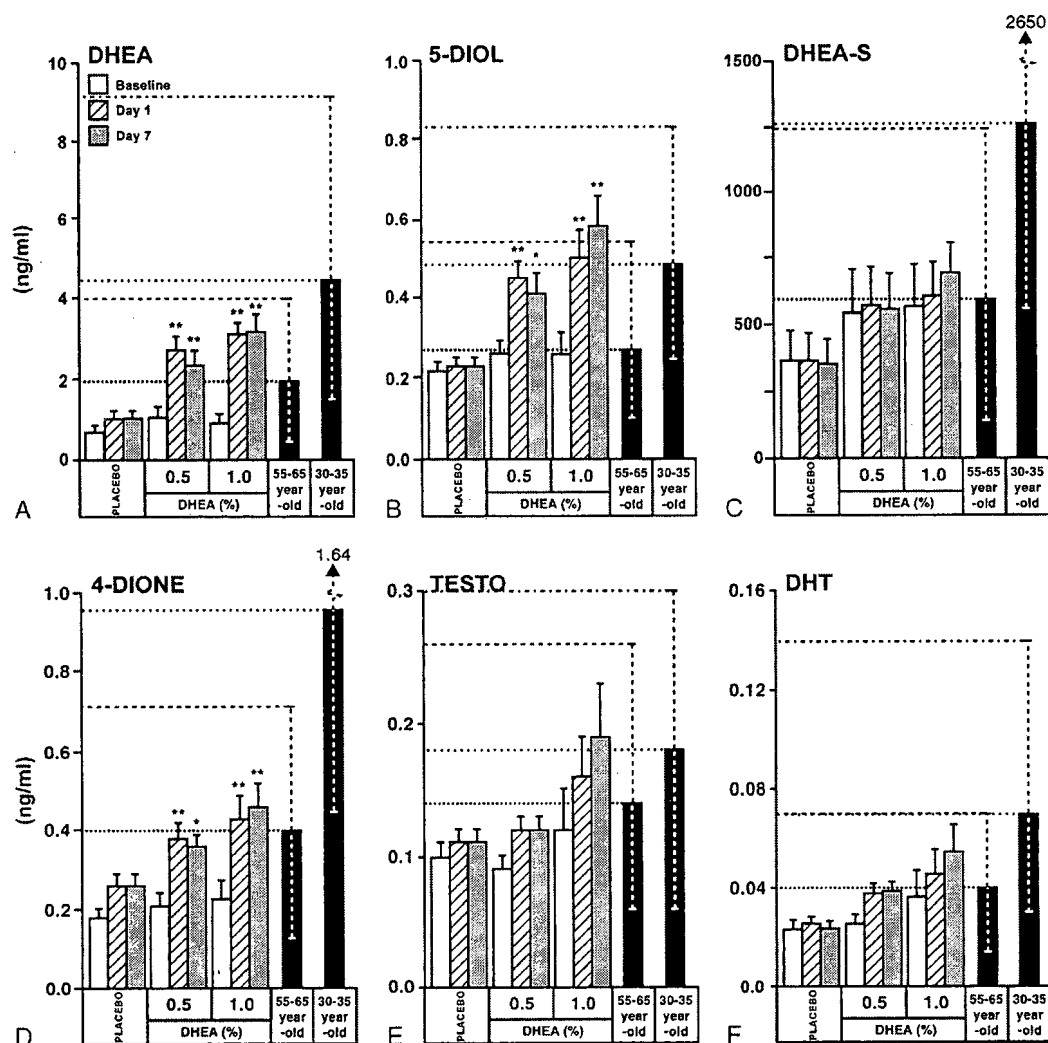


FIG. 5. Average 24-hour serum concentrations (area under the curve [0-24 h]/24) of dehydroepiandrosterone (DHEA), androst-5-ene-3 β ,17 β -diol (5-diol), DHEA-sulfate (DHEA-S), androstenedione (4-dione), testosterone (Testo), and dihydrotestosterone (DHT) measured at baseline and on days 1 and 7 during once-daily administration of vaginal ovules containing 0.5% and 1.0% DHEA.^{10,11} Data are expressed as means $\pm$ SEM ($n = 10$). Serum steroid concentrations measured in 30- to 35-year-old premenopausal ($n = 47$) and 55- to 65-year-old postmenopausal ($n = 377$) women³ are added as reference data, which are expressed as means and 5th and 95th centiles (dashed lines). * $P < 0.05$, ** $P < 0.01$, experimental versus baseline.

true changes in androgen formation, whereas the over-estimation of estrogen production is even higher¹⁶ (Fig. 3). In fact, changes of much smaller amplitude are seen for the steroids derived from DHEA and 5-diol. Serum DHEA-S, for example, increased by 6.9% ($P = 0.015$), 3.8% ($P = 0.118$), and 8.5% ($P < 0.0001$) at the 0.25%, 0.5%, and 1.0% DHEA doses (Fig. 1C).

As can be seen in Figure 1D and Table 1, serum 4-dione increased by 37% ($P < 0.0001$) at the 1% DHEA dose, whereas the 5.3% and 7.5% increases observed at the two lower doses were not statistically significant despite the large population size. Serum testosterone, on the other hand, increased by 7.7% ($P = 0.0076$), 23.1% ($P = 0.0002$), and 50% ($P < 0.0001$) at the 0.25%, 0.5%, and 1.0% DHEA doses (Fig. 1E). Serum DHT, on the other hand, increased by 33.3% ($P = 0.0005$), 25% ($P < 0.0001$), and 100% ($P <$

0.0001) at the same DHEA doses (Fig. 1F), with all these values and those of testosterone remaining within the normal postmenopausal range.

As can be seen in Figure 2D, serum E_1 increased by 8% ($P = 0.0546$), 22.2% ($P = 0.0001$), and 32.5% ($P < 0.0001$) at the 0.25%, 0.5%, and 1.0% DHEA doses, whereas serum E_2 increased from 3.01 ± 0.34 to 4.50 ± 0.61 pg/mL ($P = 0.0003$) at the 1.0% DHEA dose, and no statistically significant change was observed at the two lower DHEA doses (from 2.87 ± 0.31 to 2.95 ± 0.23 pg/mL and from 2.47 ± 0.17 to 3.37 ± 0.56 pg/mL at the 0.25% and 0.5% doses with P values of 0.7364 and 0.0867, respectively; Fig. 2E). Serum E_1 -S increased by 11.0% ($P = 0.0854$), 25.8% ($P = 0.0043$), and 17.1% ($P = 0.0002$) at the same three DHEA doses (Fig. 2F). These values of serum estrogens observed with the highest dose of DHEA are comparable with the mean values

INTRAVAGINAL DHEA AND SEX STEROIDS

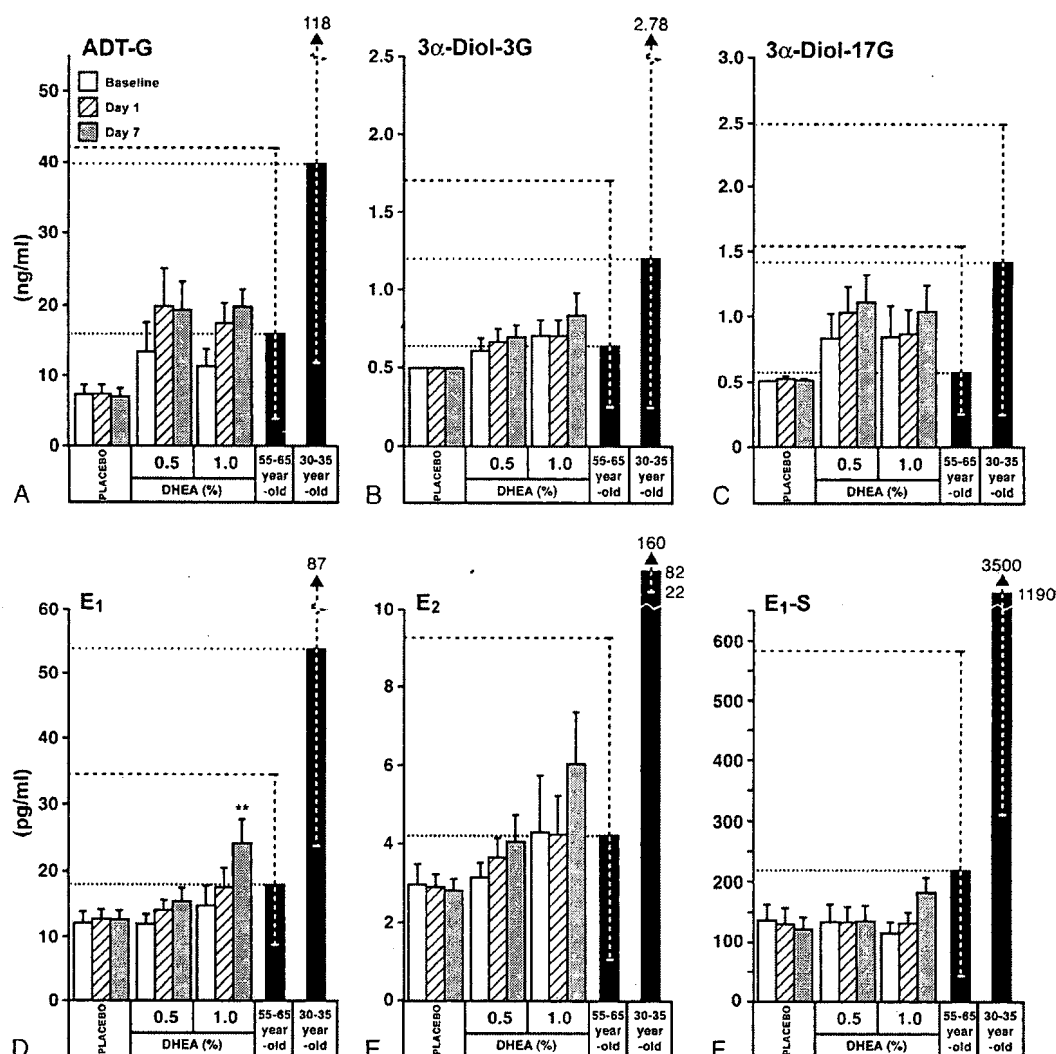


FIG. 6. Average 24-hour serum concentrations (area under the curve [0-24 h]/24) of androsterone glucuronide (ADT-G), androstane-3 α ,17 β -diol-3 glucuronide (3 α -diol-3 G), 3 α -diol-17 G, estrone (E₁), estradiol (E₂), and estrone-sulfate (E₁-S) measured at baseline and on days 1 and 7 after once-daily administration of vaginal ovules containing 0.5% and 1.0% dehydroepiandrosterone (DHEA).^{10,11} Data are expressed as means $\pm$ SEM (n = 10). Serum steroid concentrations measured in 30- to 35-year-old premenopausal (n = 47) and 55- to 65-year-old postmenopausal (n = 377) women³ are added as reference data, which are expressed as means and 5th and 95th centiles (dashed lines). ***P* < 0.01, experimental versus baseline.

measured in 55- to 65-year-old postmenopausal women (Fig. 2D-F),^{7,10} whereas the values observed with 0.25% and 0.5% DHEA are below the mean value of normal postmenopausal women.

As can be seen in Figures 2A, B and C and Table 1, the 1.0% DHEA dose led to 58.8% (*P* < 0.0001), 56.8% (*P* < 0.0001), and 78.9% (*P* < 0.0001) increases at 12 weeks compared with baseline in the serum levels of ADT-G, 3 α -diol-3 G, and 3 α -diol-17 G, respectively. The changes observed with the 0.25% dose were 5.0% (*P* = 0.001), 8.4% (*P* = 0.0561), and 7.0% (*P* = 0.1796) for ADT-G, 3 α -diol-3 G, and 3 α -diol-17 G, respectively, whereas the corresponding increases with the 0.5% DHEA dose were 17.7% (*P* < 0.0001), 16.5% (*P* = 0.0093), and 21.2% (*P* = 0.0066). All these values remain well within those observed

in normal postmenopausal women and very much below those of premenopausal women.

The sums of the androgen metabolites in the placebo, 0.25% DHEA, 0.5% DHEA, and 1.0% DHEA groups at 12 weeks are 18.34, 19.61, 20.47, and 22.76 ng/mL, respectively, for differences of 6.9%, 11.6%, and 24.1% of the DHEA-treated groups compared with placebo. It is of interest to see in Figure 3 that the increase (8.49 ng/mL) in total serum androgen metabolites seen at the highest DHEA dose (from 14.27 to 22.76 ng/mL) reaches only 53.1% of the mean value of 42.85 ng/mL found in normal 30- to 35-year-old women.³ In fact, the sum of total androgen metabolites reached with daily 1.0% DHEA ovule administration is still 46.9% below the value found in normal premenopausal women, whereas, for comparison, our previous 12-month

study gave a value of 41.0% below the value found in premenopausal women¹⁶ (Fig. 3). At the 0.25% and 0.5% DHEA doses, the sum of the androgen glucuronidated metabolites remained 54% and 52%, respectively, below the values found in 30- to 35-year-old women and well within the values found in normal postmenopausal women.

DISCUSSION

Our previous studies and the new knowledge recently gained on the endocrinology of sex steroids in women^{4,6} indicate that a relative lack of endogenous DHEA is responsible for the symptoms and signs of vaginal atrophy in postmenopausal women. It is important to mention that because estrogen secretion from the ovaries into the systemic circulation ceases at menopause in all women, the administration of estrogens to postmenopausal women is not a physiological solution. In agreement with these new findings, the data recently obtained in the two accompanying studies^{17,18} clearly demonstrate that local application of DHEA can rapidly and very efficiently correct all the symptoms and signs of vaginal atrophy.

Although 75% of postmenopausal women experience vaginal atrophy,^{19,20} thus affecting their quality of life during a major part of their lifetime, only 20% of these women seek treatment.²¹ The fear of breast cancer associated with the well-recognized increase in the serum levels of estrogens after intravaginal or systemic estrogen therapy is the main cause of the lack of acceptance of estrogen therapy by most women and their physicians. In the aftermath of the Women's Health Initiative trial, the scientific challenge is to explore alternative hormonal therapy types and formulations that could provide all the menopausal advantages of estrogens (and more) while improving women's quality of life, minimizing risks, and maximizing benefits.²² On the other hand, because the nonestrogen-based treatments have not shown convincing efficacy,^{23,24} women and their physicians are thus left with no safe treatment for vaginal atrophy.

The recent data on serum E_2 obtained with the most accurate technology² indicate that the effects of estrogens applied locally in the vagina are unlikely to be limited to the vagina and that systemic action is expected, as previously suggested.²⁵⁻⁴³ The absence of significant or minimal changes in serum E_2 after intravaginal administration of DHEA ovules (Figs. 2 and 4) is in marked contrast with the fivefold increase in serum E_2 measured by mass spectrometry during treatment with intravaginal E_2 or conjugated estrogens (Fig. 4).²

Most importantly, it does not seem reasonable or acceptable to increase the serum E_2 levels during breast cancer therapy when the objective of treatment with aromatase inhibitors is precisely to inhibit E_2 biosynthesis.⁴¹

It is important to remember that the specific hormonal difference between the postmenopausal women who do not have vaginal atrophy (estimated at 25% of the postmenopausal population) and most (75%) postmenopausal women who have vaginal atrophy^{19,20} is not related to differences in the secretion of estrogens in the systemic circulation. In fact,

estrogen secretion by the ovaries ceases in all women at time of menopause; consequently, a deficit in estrogen secretion into the systemic circulation is not a valid explanation for the occurrence of symptoms of vaginal atrophy in 75% of postmenopausal women, whereas the remaining and more fortunate 25% of women live through all their postmenopausal years without significant bothersome or evident vaginal atrophy symptoms. Therefore, other factors may be at work. Consequently, estrogens are not a physiological solution to menopausal symptoms.

Because blood sampling was performed at a variable time during the day at each visit (from 7:20 AM to 7:40 PM), it is likely, as previously observed,^{10,11} that higher serum levels of DHEA and 5-diol occur at earlier time intervals but not for the other steroids, which remain practically on a plateau during the 24-hour period on the 7th day of treatment.^{10,11} The objective of the present study was to study, as mentioned in the Introduction, "the possible effect of duration of treatment on DHEA bioavailability and metabolism," the study being of a 3-month duration.

It is important to see in Figures 5 and 6 the average 24-hour (calculated from area under the curve [0-24 h]/24 measured on days 1 and 7 of treatment) serum steroid levels of DHEA, 11 of its metabolites taken from a recent study, in which serum steroid levels were measured at multiple time intervals during a 24-hour period to obtain maximal precision and information about the 24-hour exposure.^{10,11} These data show that after daily treatment with 0.5% or 1.0% DHEA ovules, only serum DHEA, 5-diol and 4-dione (and E_1 on day 7 at 1.0% DHEA) are increased significantly from baseline but remain well within the limits of values found in normal postmenopausal women.^{3,10,11} Serum E_2 and E_1S as well as serum androgens (testosterone and DHT) are not significantly affected compared with baseline. Most interestingly, the metabolites of androgens (ADT-G, 3 α -diol-3 G, and 3 α -diol-17 G) and estrogens (E_1S) are unchanged from baseline or minimally changed after intravaginal DHEA administration, thus clearly demonstrating the absence of significant systemic androgenic and estrogenic formation and, therefore, potential systemic effects of sex steroids at the intravaginal DHEA doses used.

The present study shows that DHEA and its 11 metabolites remain within the range of values found in normal postmenopausal women.^{3,10,11} For example, although statistically significant with a 70.7% increase observed with the 1.0% DHEA dose, the average serum DHEA concentration of 3.14 ± 0.17 ng/mL is well within the normal postmenopausal range (5th-95th centiles of 0.56 and 3.99 ng/mL, respectively) and below the mean value of 4.47 ± 2.19 ng/mL (5th-95th centiles of 1.53 and 9.14 ng/mL) observed in normal premenopausal women. In a recent study performed in 150 women randomized to receive placebo or 3 g of 0.3% DHEA cream applied on the skin twice daily, serum DHEA increased to 4.53 ± 0.26 ng/mL in the DHEA group¹⁶ with an excellent tolerance to treatment and no DHEA-related significant adverse event during the 12-month clinical trial.

In agreement with our 12-month study with percutaneous DHEA,¹⁶ the changes in serum DHEA-S and 4-dione were minor or nonsignificant. The serum testosterone levels in our previous 12-month study with percutaneous DHEA were measured at 0.20 ± 0.01 ng/mL compared with 0.18 ± 0.01 ng/mL in the present study. These values are almost superimposable to the serum testosterone concentration of 0.18 ± 0.07 ng/mL observed in normal premenopausal women as well as within the range of serum testosterone observed in 55- to 65-year-old postmenopausal women (0.14 ± 0.07 ng/mL, 5th-95th centiles of 0.06-0.26 ng/mL).^{3,16} All the small changes of the other steroids (when statistically significant due to a large population) remain well within the range of normal postmenopausal women and correspond to the average values found in 55- to 65-year-old postmenopausal women.³ Because serum E₁S is likely to reflect total estrogen formation, the present and previous data^{3,16,44} confirm that very little estrogen formation follows DHEA administration.

Concerning androgens, it is of interest to mention that in our previous 12-month percutaneous DHEA study, the sum of the androgen metabolites was measured at 25.46 ± 1.78 ng/mL,¹⁶ whereas the present study shows the sum of the androgen metabolites at 18.34, 19.61, 20.47, and 25.46 ng/mL at the 0%, 0.25%, 0.5%, and 1.0% DHEA doses, respectively (Fig. 3). An important observation made in our previous study is that an increase in serum DHEA of 203% over control led to only a 71% increase of the serum androgen metabolites, thus showing that the changes in serum DHEA are a 2.8-fold overestimation of the true changes in androgen formation.¹⁶ That the changes in serum DHEA seen after daily intravaginal administration of the DHEA ovules induce minimal changes in the total androgen pool correctly estimated by the changes in serum ADT-G, 3 α -diol-3 G, and 3 α -diol-17 G³ is illustrated by the observation that treatment with the 0.25%, 0.5%, and 1.0% DHEA doses leaves the total androgen pool 54%, 52%, and 47% below the average values observed in normal premenopausal women, respectively (Fig. 3). Moreover, all these values are well within the range observed in normal postmenopausal women.

Although measurements of serum DHEA may suggest that the low postmenopausal serum levels of DHEA can be corrected relatively easily by oral or percutaneous DHEA administration, our previous clinical trials^{10,11,14,16} have consistently shown that correction of the androgen deficit is extremely difficult or most likely impossible to achieve due to the limited efficacy of the enzymatic machinery responsible for DHEA transformation. This is even truer for estrogen formation, which seems to be extremely low after DHEA administration.^{10,11,14,16} Such findings are not surprising when looking at the extremely low serum E₂ levels observed in women after menopause, whereas serum DHEA and the serum levels of androgens metabolites are only reduced by 60%.³ There is, in fact, no reason to believe that exogenous DHEA would be metabolized differently from endogenous DHEA of adrenal origin.

The beneficial morphological changes observed concomitantly with the strong modulation of rat vaginal androgen receptor levels^{45,46} by androgens suggest that the DHEA-derived androgens are decreased in postmenopausal women, thus contributing to the decreased vaginal health and eventually to the loss of libido and sexual enjoyment observed in this age group. Decreased serum total testosterone, free testosterone, and DHEA-S were indeed found in women who consulted their physicians for decreased sexual desire.⁴⁷

Concerning safety, no drug-related significant adverse event was observed either in the present study or in our previous 1-week pharmacokinetics study with doses of 0.5%, 1.0%, and 1.8% DHEA ovules.^{10,11} Moreover, no significant adverse event related to DHEA was observed in a study performed in 75 women randomized to receive 3 g of 0.3% DHEA percutaneously twice daily for 12 months¹⁶ or in 15 women who received daily 3 to 5 g of 10% DHEA cream for 1 year.⁴⁸

CONCLUSIONS

Although all symptoms and signs of vaginal atrophy are rapidly and markedly improved with intravaginal DHEA,^{17,18} all serum steroids measured by Good Laboratory Practice-validated mass spectrometry remain unchanged or within the reference range observed in postmenopausal women, thus indicating a strictly local action that eliminates systemic exposure to sex steroids and the related risks.

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Intravaginal dehydroepiandrosterone (Prasterone), a physiological and highly efficient treatment of vaginal atrophy

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Abstract

Objective: Because the secretion of dehydroepiandrosterone (DHEA), the exclusive source of sex steroids in postmenopausal women, is already decreased by 60% and continues to decline at the time of menopause, the objective of this study was to examine the effect of intravaginal DHEA on the symptoms and signs of vaginal atrophy.

Methods: This prospective, randomized, double-blind and placebo-controlled phase III clinical trial studied the effect of Prasterone (DHEA) applied locally in the vagina on the signs and symptoms of vaginal atrophy in 216 postmenopausal women.

Results: All three doses (0.25%, 0.5%, and 1.0%) of DHEA ovules applied daily intravaginally induced a highly significant beneficial change in the percentage of vaginal parabasal and superficial cells and pH as well as in the most bothersome symptom at 2 weeks. At the standard 12-week time interval, 0.5% DHEA caused a 45.9 ± 5.31 ($P < 0.0001$ vs placebo) decrease in the percentage of parabasal cells, a $6.8 \pm 1.29\%$ ($P < 0.0001$) increase in superficial cells, a 1.3 ± 0.13 unit ($P < 0.0001$) decrease in vaginal pH, and a 1.5 ± 0.14 score unit ($P < 0.0001$) decrease in the severity of the most bothersome symptom. Similar changes were seen on vaginal secretions, color, epithelial surface thickness, and epithelial integrity. Comparable effects were observed at the 0.25% and 1.0% DHEA doses.

Conclusions: Local Prasterone, through local androgen and estrogen formation, causes a rapid and efficient reversal of all the symptoms and signs of vaginal atrophy with no or minimal changes in serum steroids, which remain well within the normal postmenopausal range. This approach avoids the fear of systemic effects common to all presently available estrogen formulations and adds a novel physiological androgenic component to therapy.

Key Words: Vaginal atrophy – Dehydroepiandrosterone – Prasterone – Intracrinology – Androgens – Tissue-specific prehormone replacement therapy.

Vaginal dryness is found in 75% of postmenopausal women.^{1,2} For various reasons, especially the fear of adverse effects caused by estrogens, only 20% to

25% of symptomatic women who have vaginal atrophy seek medical treatment.^{2,3} There is thus a clear medical need and a major opportunity to remove the fear of breast cancer associated with therapy while improving the quality of life of most women who are left with the problem of vaginal atrophy for a large proportion of their lifetime. It can be mentioned that although hot flashes abate spontaneously with time without treatment, vaginal atrophy symptoms, namely, vaginal dryness, vulvovaginal irritation/itching, and pain at sexual activity, usually increase in severity with time in the absence of treatment.

Based on the knowledge that estrogen secretion by the ovaries ceases at menopause in all women, systemic and local estrogens have so far been the traditional and exclusive treatment for vaginal atrophy. A series of reports have indicated, however, that systemic estrogens + progestin (hormone therapy) and estrogens alone (estrogen therapy) increase the risk of breast,⁴⁻¹⁰ ovarian,^{5,6,11,12} and endometrial (estrogens alone)^{13,14} cancer. The publicity that followed knowledge by

Received November 10, 2008; revised and accepted January 26, 2009.

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Funding/support: This study was sponsored by Endoceutics.

Financial disclosure/conflicts of interest: Dr. Labrie is President and CEO of Endoceutics. The other authors report no conflicts of interest, although the study was financed by Endoceutics. Patents for intravaginal Prasterone are the property of Endoceutics, Inc.

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TABLE 1. Laboratory tests evaluating the hematology, coagulation, blood chemistry, and urinalysis parameters

Laboratory tests	Parameters
Hematology	WBC
	RBC
	Hemoglobin
	Hematocrit
	Platelets
	Neutrophils
	Lymphocytes
	Monocytes
	Eosinophils
	Basophils
	Bands
	Fibrogen
	Prothrombin
Coagulation	Partial thromboplastine time
Blood chemistry	Albumin
	Alkaline phosphatase
	AST (SGOT)
	ALT (SGPT)
	Total bilirubin
	Blood urea nitrogen (BUN)
	Calcium
	Chloride
	Creatinine
	Glucose
	Inorganic phosphorus
	LDH
	Magnesium
	Potassium
	Total protein
Urinalysis	Sodium
	Uric acid
	Color
	Aspect
	Specific gravity
	pH
	Glucose
	Ketones
	Blood
	Protein
	Nitrites
	Leukocytes
	Microscopic RBC
	Microscopic WBC
	Bacteria
	Epithelial cells
	Casts
	Crystals

WBC, white blood cells; RBC, red blood cells; AST, aspartate amino-transferase; SGOT, serum glutamic-oxaloacetic transaminase; ALT, alanine aminotransferase; SGPT, serum glutamic-pyruvic transaminase; LDH, lactate dehydrogenase.

the public of the data of the Women’s Health Initiative study⁵ had the greatest impact, thus adding serious doubts about the safety of the available estrogen-based treatments of menopaual symptoms.¹⁵

Although intravaginal formulations were developed to avoid systemic exposure to estrogens, a series of studies have unanimously demonstrated that all intravaginal estrogen formulations lead to significant increases in serum estrogen levels measured directly by radioimmunoassay or through their systemic effects.¹⁶⁻²⁵ Recent data obtained with the most accurate technology²⁶ indicate that the effects of estrogens applied locally in the vagina are unlikely to be limited to the vagina and that systemic action is expected, as previously suggested.¹⁶⁻²⁴

Because serum dehydroepiandrosterone (DHEA) is the almost exclusive source of androgens, which play important physiological roles in women,^{27,28} the 60% decrease in circulating DHEA already found at time of menopause is accompanied by a similar 60% decrease in the total androgen pool.²⁹ Such a marked decrease in androgens can potentially lead to signs and symptoms of hypoandrogenicity in the bone, muscle, skin, mammary gland, vagina, and brain as well as on glucose, insulin, and lipid metabolism.^{27,28}

Among the androgen target tissues, recent preclinical data obtained in experimental animals have clearly shown beneficial effects of the androgens made locally from DHEA in the vagina, not only in the superficial epithelial layer but most importantly on collagen fibers of the lamina propria and on the muscularis.³⁰ These data clearly indicate the importance of androgens for normal vaginal physiology, a role that cannot be achieved with estrogens.

Based on the data of both our preclinical^{30,31} and clinical³²⁻³⁴ studies showing beneficial effects on the vagina of DHEA administered percutaneously or locally, the present clinical trial is a prospective, randomized, and placebo-controlled study of the effect of four doses of intravaginal DHEA administered daily for 12 weeks on the changes in vaginal superficial and parabasal cells as well as on vaginal pH and on the most bothersome symptoms of vaginal atrophy as coprimary objectives. The data clearly show that locally administered DHEA is very efficient and rapid in correcting the signs and symptoms of vaginal atrophy, a near maximal effect being already achieved at 2 weeks.

METHODS

This study was a phase III, prospective, multicenter, randomized, placebo-controlled, and double-blind trial planned for 50 participants per arm (for a total of 200 participants to complete the study). Two hundred eighteen postmenopausal women (216 women in the intent-to-treat [ITT] analysis) were thus randomized to receive a daily ovule of the following DHEA concentrations: 0.0% (53 women), 0.25% (3.25 mg DHEA; 53 women), 0.5% (6.5 mg DHEA; 56 women), or 1.0% (13 mg DHEA; 54 women) applied intravaginally with an applicator at bedtime. Two hundred sixteen women were included in the ITT population, which served as the basis of the analysis. The median age of women was 58 years (range, 49-70 y), 57 years (range, 42-72 y), 58 years (range, 50-74 y), and 59 years (46-69 y) in the four groups. The DHEA ovules or suppositories (Vaginorm) containing Prasterone in a lipophilic base were manufactured by Recipharm (Karlskoga, Sweden). The study was divided into two phases, namely, screening followed by a treatment period of 12 weeks. The protocol was approved by the institutional review board of the Centre Hospitalier de l’Université Laval, Quebec City, QC, Canada; McGill University, Montreal, QC, Canada; Ethica, Montreal, QC, Canada; Eastern Virginia Medical School, Norfolk, VA; and the Western Institutional Review Board, Los Angeles, CA.

000051

HIGH EFFICACY OF LOCAL DHEA FOR VAGINAL ATROPHY

The inclusion criteria were the following:

- Postmenopausal women who satisfy a or b or c:
 - a. No menses for at least 1 year; or
 - b. Follicle-stimulating hormone levels of 40 mIU/mL or more (within 60 d before day 1) in women with no menses for 6 months or more but less than 12 months, or hysterectomized women who were premenopausal at the time of hysterectomy; or
 - c. 6 weeks or more (of screening visit) after bilateral oophorectomy.
 - Women having self-identified at least one moderate to severe of the following symptoms:
 - Vaginal dryness (none, mild, moderate, or severe);
 - Vaginal and/or vulvar irritation/itching (none, mild, moderate, or severe);
 - Vaginal pain associated with sexual activity (none, mild, moderate, or severe).
- Women were asked to identify which symptom is the most bothersome to her at the start of treatment. The change in this symptom was followed and served to evaluate the effect of treatment.
- Women between 40 and 75 years of age
 - Women willing to participate in the study and sign an informed consent form
 - Women having a low maturation index (no greater part of guidance than 5% of superficial cells on vaginal smear)
 - Women having a vaginal pH above 5
 - Women having a normal mammogram within 9 months of study start
 - Women having a normal breast examination
 - Women having normal Papanicolaou (Pap) smear (which in-

cludes inflammatory changes) within the previous 12 months of day 1 (for hysterectomized women, the Pap smear was done with at least one slide)

- Women having no former or present narcotic addiction or alcoholism
- Women having body weight within the range of 18.5 to 35 kg/m² of ideal body weight according to body mass index (World Health Organization)
- Women having no hepatic or renal impairment or condition known to affect drug or steroid metabolism
- Women having normal baseline hematology, clinical chemistry, and urinalysis results
- Women having negative serology results for HIV-1/HIV-2 and hepatitis B and C.

The exclusion criteria were as follows:

- Undiagnosed abnormal genital bleeding
- Previous diagnosis of cancer, except skin cancer (non-melanoma)
- Endometrial hyperplasia at biopsy performed at screening or endometrial cancer
- Active or history of thromboembolic disease
- Significant metabolic or endocrine disease
- Clinically significant gastrointestinal, liver, or gallbladder disease
- Recurrent migraine headache not controlled by conventional therapy
- Diabetes mellitus not controlled by conventional therapy
- Significant complication on previous hormone therapy
- Use of estrogen-alone injectable drug therapy or progestin implant within 3 months before study entry (screening visit)

PARABASAL CELLS

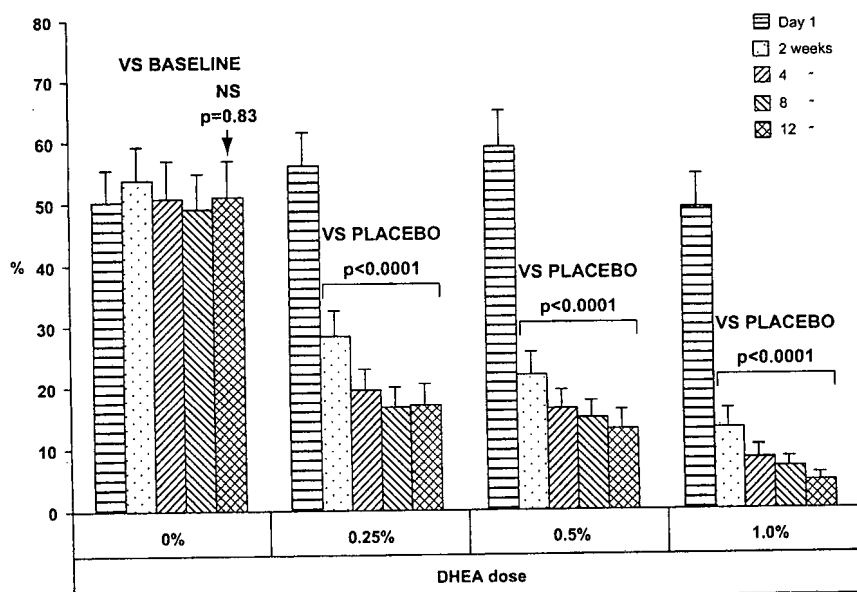


FIG. 1. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the percentage of vaginal parabasal cells in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values for the three DHEA doses are comparisons with placebo at all time intervals, whereas for the placebo group at 12 weeks, comparison is with baseline.

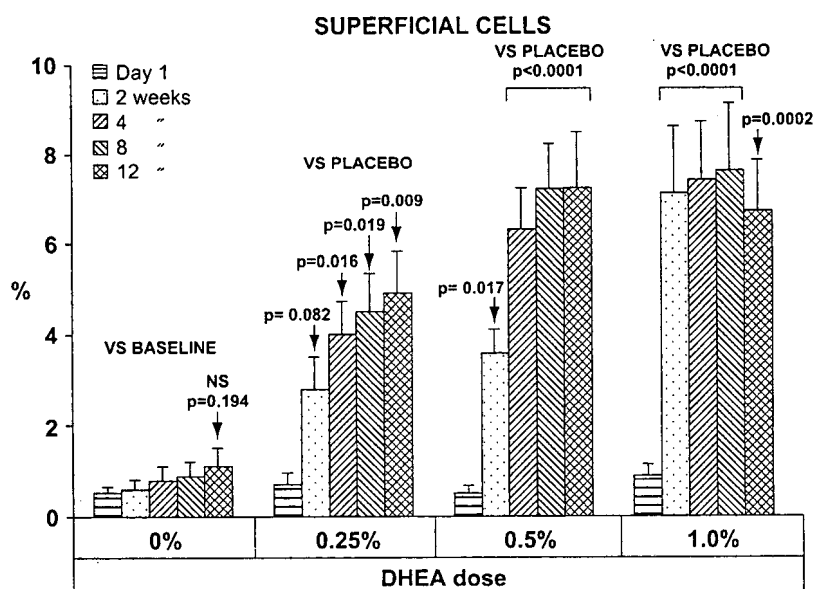


FIG. 2. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the percentage of vaginal superficial cells in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values for the three DHEA doses are comparisons with placebo at all time intervals, whereas for the placebo group at 12 weeks, comparison is with baseline.

- Use of estrogen pellet or progestin injectable drug within 6 months before study entry (screening visit)
- Use of oral estrogen, progestin, or DHEA exposure or intrauterine progestin therapy in the 8 weeks before baseline assessments (screening visit)
- Use of vaginal hormonal products (rings, creams, or gels) or transdermal estrogen alone or estrogen/progestin products in the 4 weeks before baseline assessments (screening visit)

Participants could washout as follows, but the questionnaire on vaginal atrophy was answered after the required washout period.

- At least an 8-week washout period for prior oral estrogen, DHEA, and/or progestin therapy
- At least a 4-week washout period for prior transdermal hormone therapy
- At least a 4-week washout period for locally delivered hormone therapy for vaginal dryness (rings, creams, gels, or tablets)
- At least 6 months for prior estrogen pellet therapy or progestin injectable drug therapy
- Eight weeks or longer for prior intrauterine progestin therapy
- Six months or longer for prior progestin implants and estrogen-alone injectable drug therapy
- Previous treatment with androgens or anabolic steroids within 3 months before screening visit
- Oral corticosteroid treatment within 6 weeks of study start (day 1)
- No chronic use of corticosteroid allowed (intermittent nasal spray or topical on skin, eyes, or ears is permitted)
- Cardiac failure or manifest coronary heart disease
- Hypertension equal to or above 160/95 mm Hg or not controlled by standard therapy

- Confirmed clinically significant depression or confirmed history of severe psychiatric disturbance
- The administration of any investigational drug within 30 days of screening visit
- Clinically relevant abnormal serum biochemistry, hematology, or urinalysis
- Baseline cervical cytology showing low-grade squamous intraepithelial lesion or worse
- Smoking more than 10 cigarettes a day
- Drugs that interfere with the metabolism of estrogens (eg, ketoconazole), steroid formation, or action inhibitors
- Selective estrogen receptor modulators or drug interacting with steroid receptors
- Known presence of uterine fibroma or fibroma palpable at gynecological examination
- Coagulation disorders or receiving anticoagulant drug therapy

Informed consent

Written informed consent was obtained from all participants before the performance of any study-related procedure. The participants had a medical history, a medical examination, and a complete gynecological examination (including an endometrial biopsy) at screening. A partial gynecological examination was performed to evaluate the aspect of the mucosa and tolerance to the medication on day 1 and at weeks 2, 4, 8, and 12.

Laboratory tests

The standard laboratory tests, namely, hematology (including complete blood count and coagulation), blood chemistry, and urinalysis were performed at screening and at week 12 (Table 1). Serum follicle-stimulating hormone was measured only in women who had no menses for 6 months or more but less than 12 months or who were premenopausal at the time

HIGH EFFICACY OF LOCAL DHEA FOR VAGINAL ATROPHY

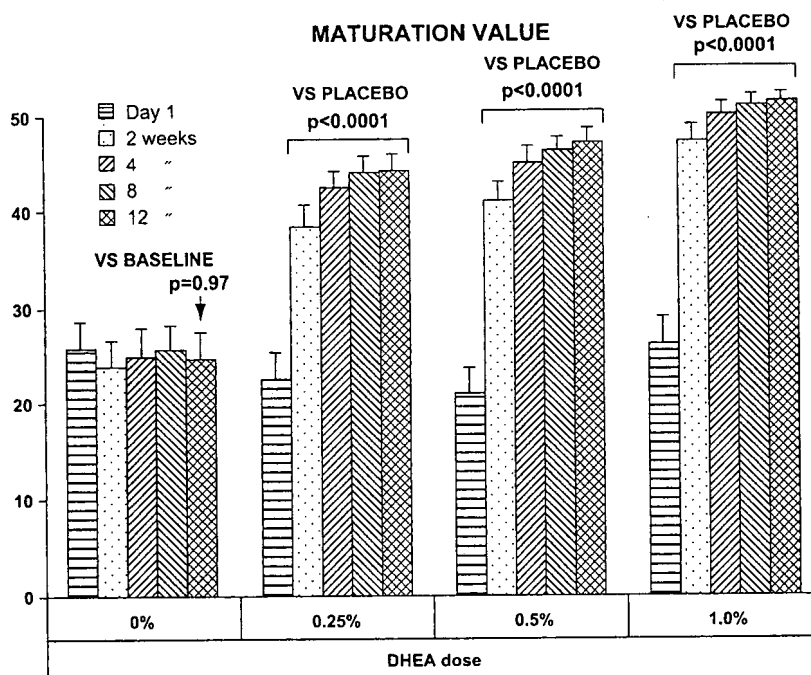


FIG. 3. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the maturation value of vaginal epithelial cells in postmenopausal women. Values are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals for the three DHEA doses, whereas for the placebo group at 12 weeks, comparison is with baseline.

of hysterectomy. Serum steroid levels of DHEA; DHEA-sulfate; androst-5-ene-3 β ,17 β -diol; dihydrotestosterone; testosterone; androstenedione; estrone (E_1); estradiol (E_2); E_1 sulfate (E_1 -S); androsterone glucuronide; androstane-3 α 17 β -diol-3G; and 3 α -diol-17G were measured at the Laboratory of Molecular Endocrinology, Laval University Hospital Centre Research Center, by mass spectrometry as described^{26,29,32,33,35,36} (data are presented in the accompanying article).³⁷

Vaginal pH and cytology and endometrial histology

Endometrial histology was examined by Dr. Robert Dubé and Dr. Valérie Dubé (Department of Cytology-Pathology, Centre Hospitalier Affilié Universitaire de Québec, Quebec City, QC, Canada) and they were blinded to the treatment regimens. For the vaginal cell maturation evaluation, samples were examined by Lucie Genest and Claudette Girard, under the supervision of Dr. Robert Dubé while Pap tests were evaluated to the site's local laboratories. A 100-cell count was performed to classify cells as superficial, intermediate, and parabasal squamous cell types.^{38,39} Vaginal smears were obtained by scraping the second third of the side wall of the vagina with the rounded end of an Ayre spatula. The material was then applied to a glass slide and immediately fixed with Spray-Cyte. These samples were sent to the central laboratory for determination of the maturation index. Vaginal pH was measured by applying a pH indicator strip directly to the lateral wall of the vagina with a forceps. For the Pap smear—if not done in the last 12 months—specimens were obtained from the endocervix, exocervix, and vaginal vault

and immediately fixed with cytospray. The specimens were collected with an Ayre spatula.

Mammography

Mammography was performed if not done during the past 9 months.

Endometrial biopsy

Endometrial biopsy was performed at screening and at the end of the study (12 wk). All biopsies were examined by the same pathologist at the central laboratory (Dr. Robert Dubé).

Vaginal examination

Vaginal examination was performed at screening and then at day 1 and weeks 2, 4, 8, and 12. Vaginal secretions, vaginal color, vaginal epithelial integrity, and vaginal epithelial surface thickness were evaluated according to the following degrees of severity: none, mild, moderate, or severe. The definitions of severity were as follows:

- Vaginal secretions**
 - No atrophy: normal clear secretions noted on vaginal walls
 - Mild: superficial coating of secretions, difficulty with speculum insertion
 - Moderate: scant not covering the entire vaginal vault, may need lubrication with speculum insertion to prevent pain
 - Severe: none, inflamed, ulceration noted, need lubrication with speculum insertion to prevent pain
- Vaginal epithelial integrity**
 - No atrophy: normal
 - Mild: vaginal surface bleeds with scraping

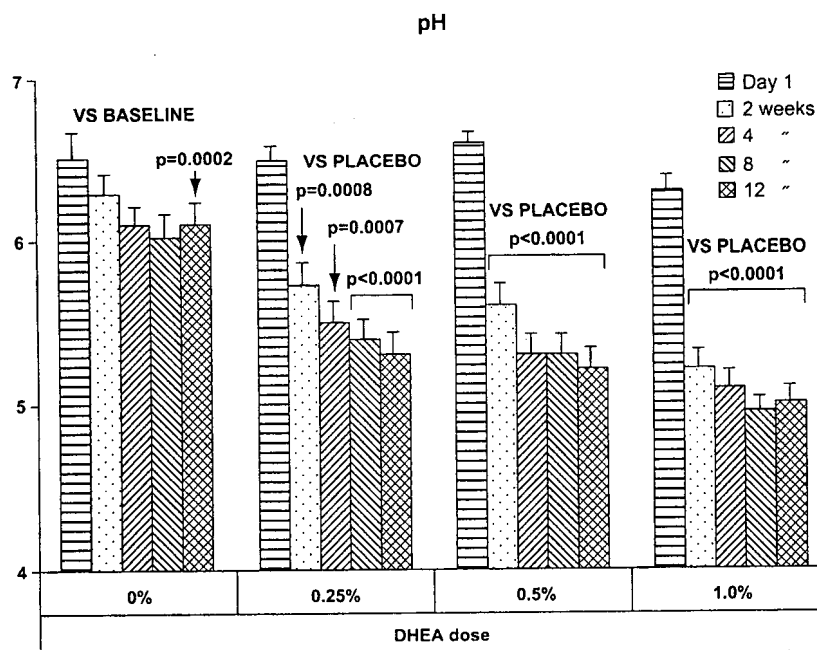


FIG. 4. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on vaginal pH in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals for the three DHEA doses, whereas for the placebo group at 12 weeks, comparison is with baseline.

Moderate: vaginal surface bleeds with light contact

Severe: vaginal surface has petechiae before contact and bleeds with light contact

c) Vaginal epithelial surface thickness

No atrophy: rognation and elasticity of vault

Mild: poor rognation with some elasticity noted of vaginal vault

Moderate: smooth, some elasticity of vaginal vault

Severe: smooth, no elasticity, constriction of the upper one third of vagina or loss of vaginal tone (cystocele and rectocele)

d) Vaginal color

No atrophy: pink

Mild: lighter in color

Moderate: pale in color

Severe: transparent, either no color or inflamed

Statistics

Summary tabulations were prepared that display the number of observations, mean or geometric mean as appropriate, SD, SEM, 95% two-sided CI, median, minimum, and maximum for continuous variables, and the number and percent per category for categorical data. Statistical analyses were performed at the two-sided significance level of 0.05, unless otherwise stated. The categories for summarization generally consisted of the dose levels of the DHEA treatments: 0% (placebo), 0.25%, 0.5%, and 1.0% DHEA. The analysis comparing each dose of DHEA to placebo was performed by analysis of covariance, with the baseline value used as the covariate. The changes from baseline within each treatment group were assessed for statistical significance using paired

t tests. Baseline was defined as the value on day 1, obtained before the first use of study treatment, and change from baseline to weeks 2, 4, 8, and 12 was calculated for each participant for each domain endpoint. Missing values for any endpoint for a participant were replaced using a last value carried forward approach.

The primary endpoints for analysis consisted of the following:

- Statistically significant decrease in parabasal cells and a statistically significant increase in superficial cells. The data were measured as a percentage of a total of 100 cells analyzed per smear. The maturation value was also calculated.
- Statistically significant lowering of vaginal pH
- Statistically significant improvement in the moderate to severe symptom identified by the participant as most bothersome to her. The symptom severity was based on symptoms of increasing severity: none, mild, moderate, or severe. These ratings were analyzed using the values 0, 1, 2, and 3, respectively; all participants needed to have at least one baseline symptom graded as 2 or 3. The symptoms of interest were vaginal dryness, vaginal and/or vulvar irritation/itching, and vaginal pain associated with sexual activity.

Analysis populations

The ITT population consisted of the treated participants with a baseline and at least one postbaseline efficacy assessment. Participants who may have received the wrong treatment were to be analyzed as randomized. This analysis population was considered the primary analysis population. Participants in this

HIGH EFFICACY OF LOCAL DHEA FOR VAGINAL ATROPHY

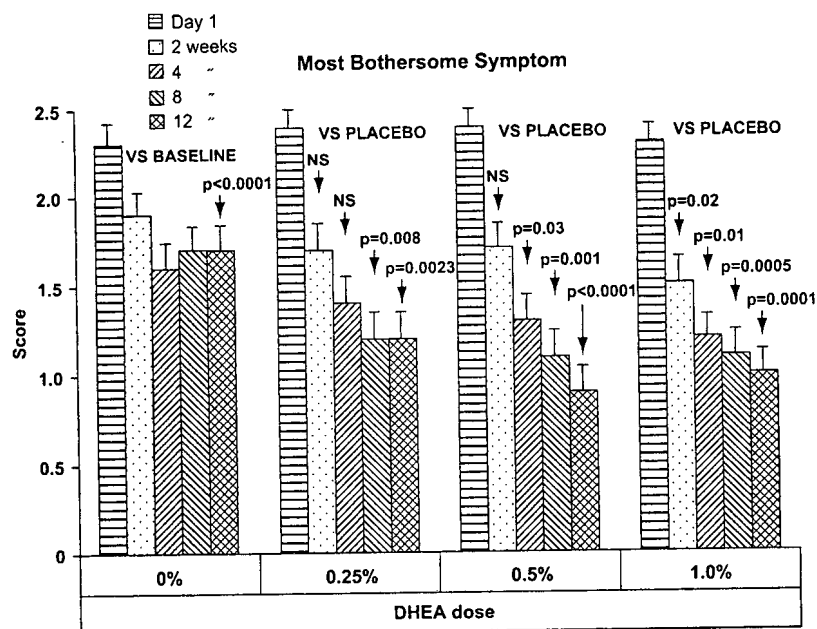


FIG. 5. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the severity score of the symptom of vaginal atrophy judged by women at baseline as being the most bothersome. Values are expressed as means $\pm$ SEM; the *P* values at 12 weeks are comparisons with placebo for the three DHEA doses, whereas for the placebo group, comparison is with baseline.

population who had missing observations postbaseline had the last value carried forward for efficacy analyses.

The safety population was defined as all participants who received one administration of either test article (DHEA at any dose) or placebo and who had any safety information available. All safety data analyses were based on this population. Analysis was based on the treatment actually received.

Efficacy evaluation

Efficacy analyses were performed on the ITT population. The primary study objective was to evaluate the dose response of vaginal mucosal parameters to the local action of DHEA in postmenopausal women experiencing vaginal atrophy, specifically by determination of the minimal dose of DHEA that produces maximal effect on the vaginal mucosa. The coprimary efficacy endpoints to address this objective were decrease in parabasal cells, decrease in vaginal pH, increase in superficial cells, and improvement of participant self-reported most bothersome symptom among vaginal dryness, vaginal and/or vulvar itching/irritation, and vaginal pain associated with sexual activity. The self-reported symptom scores had the values, none, mild, moderate, or

severe, and were analyzed using values of 0, 1, 2, or 3, respectively. All endpoints had to demonstrate statistically significant effects relative to placebo. The primary time point for analysis was the 12-week assessment, with additional presentations of the data for 2, 4, and 8 weeks.

RESULTS

Because parabasal cells are usually the predominant category in the vaginal smear of postmenopausal women with at least one moderate to severe symptom of vaginal atrophy, it can be seen in Fig. 1 that already at 2 weeks of treatment, the lowest dose of DHEA (0.25%) decreased the percentage of parabasal cells by 28.6 ± 4.95 from 56.9 ± 5.54 to 28.3 ± 4.34 , whereas decreases of 37.1 ± 4.55 (from 58.8 ± 5.37 to 21.6 ± 3.47) and 36.0 ± 4.91 (from 48.9 ± 5.46 to 12.9 ± 2.81) were observed, respectively, with the 0.5% and 1.0% DHEA doses at the same early time interval of 2 weeks. At the standard duration of 12 weeks of treatment, decreases of 39.8 ± 5.33 , 45.9 ± 5.31 , and 44.5 ± 5.19 were observed in the percentage of parabasal cells with the 0.25%, 0.5%, and 1.0% DHEA doses, respectively, whereas no significant effect was observed in the placebo group at any time interval. It can also be seen in Fig. 1 that the effect of DHEA is rapid, with 72%, 81%, and 81% of the effect measured at 12 weeks being already observed at 2 weeks at

TABLE 2. List of number of women reporting vaginal atrophy symptoms of various degrees of severity at baseline

Severity	Dryness	Irritation/itching	Pain at sexual activity
None = 0	15	61	2
Mild = 1	35	85	27
Moderate = 2	118	57	49
Severe = 3	48	13	110
Total	216	216	188 ^a

^aTwenty-eight women reported no sexual activity at baseline.

TABLE 3. Most bothersome symptom identified by participants at baseline

	Dryness	Irritation/itching	Pain at sexual activity
n	66	16	129

TABLE 4. Change from day 1 (baseline) in the categories of severity scores of the most bothersome symptoms at 12 weeks of treatment with 0% (placebo), 0.25%, 0.50%, and 1.0% DHEA

Doses	% Women with category change				
	-3	-2	-1	0	+1
0%	3.77	15.09	20.75	54.72	5.66
0.25%	7.84	35.29	25.49	29.41	1.96
0.5%	21.82	29.09	27.27	21.82	0.0
1.0%	11.54	34.62	30.77	21.15	1.92

Change from one category (severe → moderate → mild → none) was taken as -1, whereas a change of two categories was -2, etc. Data are expressed as a percentage of the total of the three symptoms for each DHEA dose. DHEA, dehydroepiandrosterone.

the above-indicated increasing DHEA doses. The effect of all doses of DHEA was highly significant from placebo at all time intervals ($P < 0.0001$ for all).

Although no significant change was seen at 12 weeks in the placebo group in the percentage of superficial cells (Fig. 2), increases of 4.3 ± 0.96 (from 0.7 ± 0.26 to 5.0 ± 0.90 , $P = 0.009$ vs placebo), 6.8 ± 1.29 (from 0.5 ± 0.16 to 7.2 ± 1.29 , $P < 0.0001$), and 5.8 ± 1.13 (from 0.9 ± 0.24 to 6.7 ± 1.16 , $P = 0.0002$) were measured in the 0.25%, 0.5%, and 1.0% DHEA groups, respectively. It can also be seen that with the 0.5% DHEA dose, 52% of the maximal effect was achieved at 2 weeks, whereas at 4 and 8 weeks, 85% and 99% of the maximal effect were observed. At the 1.0% DHEA dose, 93% of the maximal effect seen at 8 weeks was already reached at 2 weeks. All doses of DHEA had a highly significant effect above placebo ($P = 0.009$, $P = 0.0002$, and $P < 0.0001$) at 12 weeks, whereas at 4 and 8 weeks, the P values were both less than 0.0001 for the 0.5% and 1.0% doses and 0.016 and 0.019, respectively, for the 0.25% dose. At 2 weeks, the P values were 0.082, 0.017, and less than 0.0001 compared with placebo for the 0.25%, 0.5%, and 1.0% DHEA doses, respectively.

Analysis was also made of the maturation value of the vaginal smear at the same time intervals. As can be seen in Fig. 3, the maturation value obtained by multiplying the percentage of parabasal cells by 0, the percentage of intermediate cells by 0.5, and the number of superficial cells by 1.0^{38} went from 21.0 ± 2.72 on day 1 to 41.2 ± 1.84 , 45.2 ± 1.67 , 46.5 ± 1.51 , and 47.3 ± 1.58 at 2, 4, 8, and 12 weeks, respectively, with the 0.5% DHEA dose. Comparable values were obtained with the 1.0% dose, whereas slightly smaller changes were observed with the 0.25% dose. It can be seen in Figure 3 that the difference between treatment and placebo was highly significant ($P < 0.0001$) at all doses and at all time intervals.

Vaginal pH was decreased at 12 weeks by 1.1 ± 0.12 units ($P < 0.0001$) from 6.5 ± 0.09 units, by 1.3 ± 0.13 units ($P < 0.0001$) from 6.6 ± 0.07 units, and by 1.4 ± 0.13 units ($P < 0.0001$) from 6.3 ± 0.09 units in the 0.25%, 0.50%, and 1.0% DHEA-treated groups, respectively (Fig. 4). In the placebo group, pH decreased by 0.4 ± 0.11 unit ($P = 0.0002$) at 12 weeks from 6.5 ± 0.09 units. At the 0.5% DHEA dose,

77%, 92%, and 100% of the maximal effect on pH (reduction of 1.3 pH units) was achieved at 2, 4, and 8 weeks of treatment, respectively. As indicated in Fig. 4, the difference between treatment and placebo is highly significant at all doses and at all time intervals ($P = 0.0008$ to < 0.0001).

At screening, all women needed to have one or more of the following symptoms of vaginal atrophy evaluated by herself as moderate to severe: vaginal dryness, vaginal or vulvar irritation/itching, or vaginal pain at sexual activity. The self-identified symptoms reported during the course of the study as none, mild, moderate, or severe were analyzed using values of 0, 1, 2, and 3, respectively. The women also needed to identify one of the three symptoms as being the most bothersome, which was a coprimary objective along with the changes in vaginal parabasal cells, superficial cells, and pH. Figure 5 illustrates the changes in the severity score of the most bothersome symptoms at the different DHEA doses and time intervals. As indicated in this figure, at the 12-week interval, the severity of the most bothersome symptom was reduced by 0.6 ± 0.13 in the placebo group, 1.2 ± 0.14 in the 0.25% DHEA group ($P = 0.002$ vs placebo), 1.5 ± 0.14 in the group receiving 0.5% DHEA ($P < 0.0001$ vs placebo), and 1.3 ± 0.14 in the group receiving the 1.0% DHEA dose ($P = 0.0001$ vs placebo).

Starting at 8 weeks for the 0.25% DHEA dose ($P = 0.008$), 4 weeks for the 0.5% dose ($P = 0.03$), and 2 weeks for the 1.0% dose ($P = 0.02$), the difference with placebo was significant to highly significant at all time intervals and doses ($P = 0.03$ to < 0.0001). In the 0.25%, 0.5%, and 1.0% DHEA groups, the effect was clearly time dependent because only 50%, 53%, and 62% of the effect, respectively, observed at 12 weeks were achieved at 2 weeks. At 4 weeks, however, 75%, 73%, and 92% of the maximal change of the most bothersome symptom were observed at the three increasing DHEA doses.

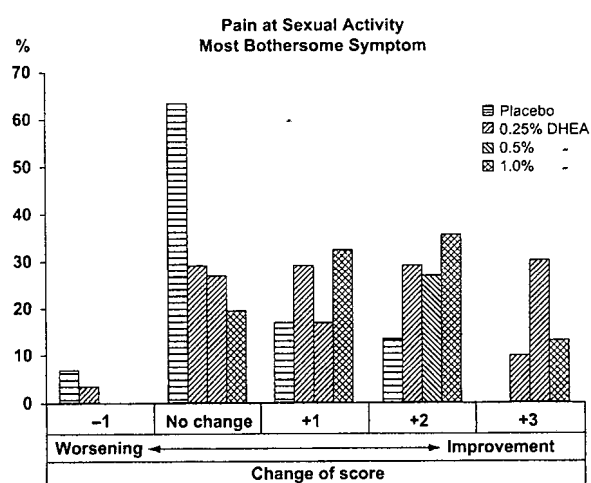


FIG. 6. Changes from day 1 (baseline) in the severity of vaginal pain at sexual activity. Change from one category (severe → moderate → mild → none) was taken as -1, whereas a change of two categories (severe to mild or moderate to none) was taken as -2, etc. Data are expressed as a percentage of all participants having identified vaginal pain as most bothersome symptom. DHEA, dehydroepiandrosterone.

HIGH EFFICACY OF LOCAL DHEA FOR VAGINAL ATROPHY

TABLE 5. Change from day 1 (baseline) in the categories of severity scores of vaginal dryness, irritation/itching, and vaginal pain at sexual activity (identified as most bothersome symptom) at 12 weeks of treatment with 0% (placebo), 0.25%, 0.5%, and 1.0% DHEA

DHEA doses	Symptoms	Category change				
		-3	-2	-1	0	+1
0%	Vaginal dryness	12.50	18.75	31.25	37.50	0
	Irritation/itching	0	50.00	0	50.00	0
	Vaginal pain	0	13.33	16.67	63.33	6.67
0.25%	Vaginal dryness	0	42.86	21.43	35.71	0
	Irritation/itching	0	100.00	0	0	0
	Vaginal pain	9.68	29.03	29.03	29.03	3.23
0.5%	Vaginal dryness	10.53	36.84	31.58	21.05	0
	Irritation/itching	25.00	25.00	50.00	0	0
	Vaginal pain	30.00	26.67	16.67	26.67	0
1.0%	Vaginal dryness	13.33	40.00	26.67	20.00	0
	Irritation/itching	0	20.00	40.00	20.00	20
	Vaginal pain	12.90	35.48	32.26	19.35	0

Change from one category (severe → moderate → mild → none) was taken as -1, whereas a change of two categories was taken as -2, etc. Data are expressed in percentage for each symptom. DHEA, dehydroepiandrosterone.

As indicated in Table 2, the presence of vaginal dryness, irritation/itching, and pain at sexual activity of moderate to severe intensity was reported at baseline by 166, 70, and 159 women, respectively. Twenty-eight women did not report sexual activity at baseline evaluation (day 1). Only 16 women identified irritation/itching as the most bothersome symptom, whereas 66 and 129 women, respectively, identified dryness and pain at sexual activity as the most bothersome symptom (Table 3).

The percentage of women with no change or a worsening by a score of 1 of the most bothersome symptom at 12 weeks decreased from 60.4% in the placebo group to 31.4%, 21.8%, and 23.1% in the 0.25%, 0.5%, and 1.0% DHEA groups,

respectively (Table 4). Improvement by two or three categories of the severity score was observed in 18.9% of women treated with placebo, whereas 43.1%, 50.9%, and 46.2% of women who received the 0.25%, 0.5%, and 1.0% DHEA formulations reported such an improvement. Only 3.8% of women indicated a decrease from severe to none in the placebo group compared with 7.8%, 21.8%, and 11.5% in the DHEA-treated groups.

As illustrated in Fig. 6, vaginal pain at sexual activity, the symptom identified as being the most bothersome by most women, remained unchanged in 63.3% of women in the placebo group, whereas it decreased to 29.0%, 26.7%, and 19.3% in the groups of women receiving the 0.25%, 0.5%,

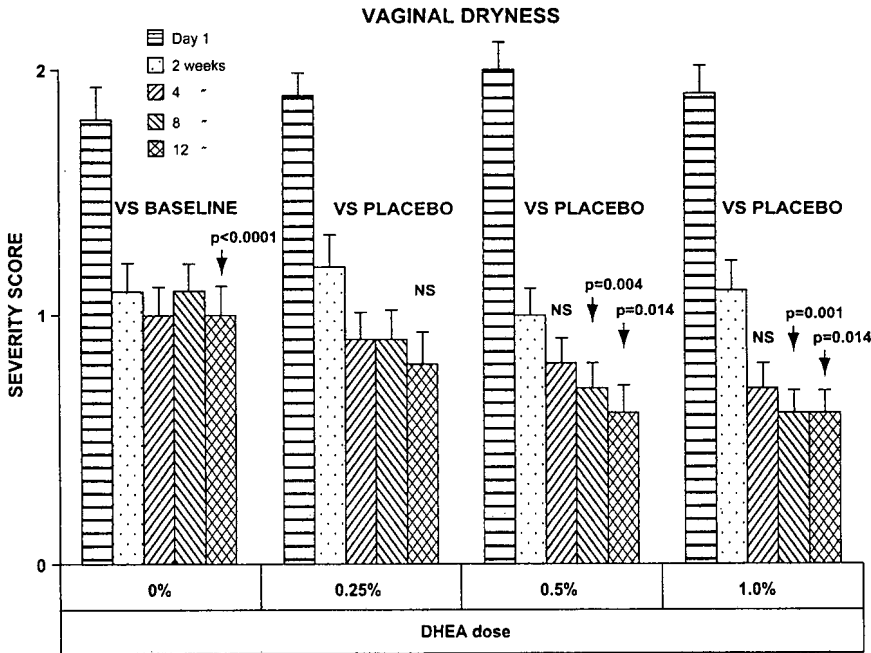


FIG. 7. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the severity of vaginal dryness. Data are expressed as means ± SEM; the *P* values are comparisons with placebo at all time intervals for the three DHEA doses, whereas for the placebo group at 12 weeks, comparison is with baseline.

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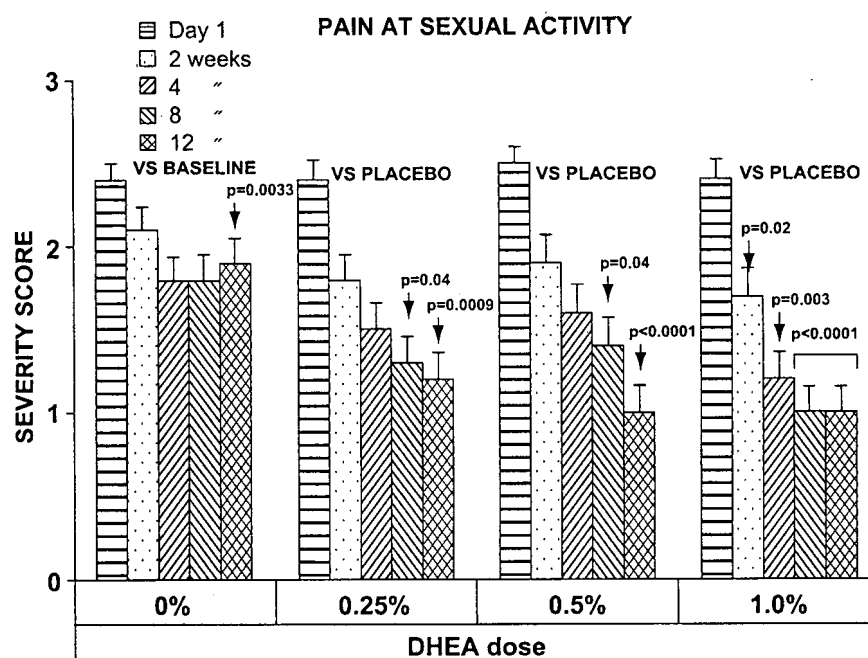


FIG. 8. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the severity of pain at sexual activity. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals for the three DHEA doses, whereas for the placebo group at 12 weeks, comparison is with baseline.

and 1.0% DHEA doses, respectively. The effect of DHEA treatment was particularly well illustrated by a decrease of three scores (severe [3] to none) in 0.0%, 9.7%, 30.0%, and 12.9% of women treated with the 0.0%, 0.25%, 0.5%, and 1.0% DHEA doses, respectively.

It is of interest that although the beneficial effect of placebo on vaginal pain was observed in 30% of women who had this symptom identified as the most bothersome at the start of study, a much greater effect of placebo was found on vaginal dryness, namely, in 62.5% of women who had this symptom as most bothersome (Table 5). Despite this large placebo effect on such a highly subjective parameter, vaginal dryness improved in 78% and 80% of women who received the 0.5% and 1.0% DHEA doses, respectively.

At 12 weeks, the standard time interval, the severity score of vaginal dryness identified at screening at any level of severity was decreased by 47%, 53% (NS vs placebo), 63% ($P = 0.014$ vs placebo), and 68% ($P = 0.014$ vs placebo) at the 0%, 0.25%, 0.5%, and 1.0% DHEA doses (Fig. 7). For irritation/itching identified as the most bothersome symptom by only 16 women, a statistically significant difference from placebo was observed with the 0.5% dose at 8 weeks ($P = 0.05$ vs placebo) and 12 weeks ($P = 0.002$ vs placebo) (data not shown).

The most important effect of DHEA among the vaginal atrophy symptoms was observed on pain at sexual activity, the most bothersome symptom identified by most women (129/216) (Table 3). As can be seen in Fig. 8, at 12 weeks, the 0.25%, 0.5%, and 1.0% doses of DHEA caused 50% ($P = 0.0009$ vs placebo), 60% ($P < 0.0001$ vs placebo), and 58% ($P < 0.0001$ vs placebo) decreases in the severity of this

symptom. The effect was already significant at 8, 8, and 2 weeks for the 0.25%, 0.5%, and 1.0% DHEA doses, respectively.

At the same time intervals of 2, 4, 8, and 12 weeks, the gynecologist or physician in charge of the clinical trial at each study site performed a vaginal examination to evaluate the severity (none, mild, moderate, or severe analyzed using values of 0, 1, 2, and 3, respectively) for the main signs of vaginal atrophy, namely, vaginal secretions, vaginal color, vaginal epithelial integrity, and vaginal epithelial surface thickness. As can be seen in Figs. 9 to 12, a time-dependent and dose-related as well as highly statistically significant improvement of all four signs of vaginal atrophy was seen. In fact, the beneficial effects observed by the gynecologist or physician in charge are almost superimposable to those self-reported by women on their most bothersome symptoms as well as to the effects on vaginal parabasal and superficial cells and pH, the coprimary objectives of DHEA action described above.

As shown in Fig. 9, a rapid effect of treatment is observed on vaginal secretions as early as 2 weeks with the three doses of DHEA administered intravaginally, the difference with placebo being highly significant versus placebo at all doses of DHEA and durations of treatment, the effect increasing with the duration of treatment and with dose. At 12 weeks, the severity score decreased by 39%, 44%, and 47% in the 0.25%, 0.5%, and 1.0% DHEA groups (all $P < 0.0001$ vs placebo) (Fig. 9). For vaginal color (Fig. 10), similar findings were observed with highly significant differences with placebo ($P = 0.008$ to $P < 0.0001$) at 12 weeks at all doses. At

HIGH EFFICACY OF LOCAL DHEA FOR VAGINAL ATROPHY

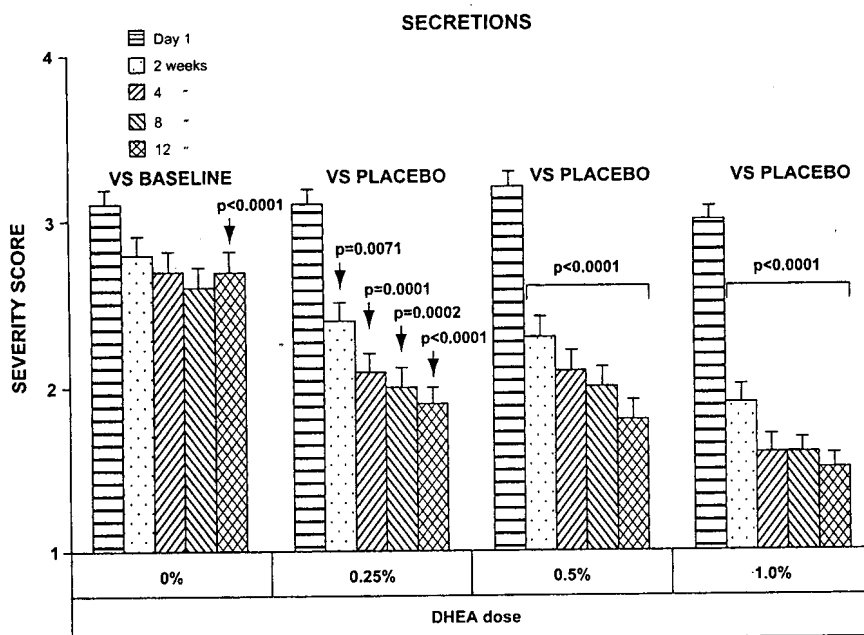


FIG. 9. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the change in vaginal secretions evaluated at vaginal examination. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals for the three DHEA doses, whereas for the placebo group at 12 weeks, comparison is with baseline.

12 weeks, 33%, 42%, and 48% improvements in the color score were observed ($P = 0.0002$ vs placebo [0.25% DHEA]) or $P < 0.0001$ vs placebo [0.5% and 1% DHEA]).

When the epithelial integrity score was considered, 37%, 46%, and 48% improvements were observed at 12 weeks for the 0.25%, 0.5%, and 1.0% DHEA doses (all $P < 0.0001$ vs

placebo; Fig. 11). For the epithelial surface thickness, on the other hand (Fig. 12), similar improvements of 34%, 42%, and 47% were observed at 12 weeks for the three increasing doses of DHEA (all $P < 0.0001$ vs placebo).

Concerning safety, no drug-related significant adverse event was observed in the present study nor in our previous

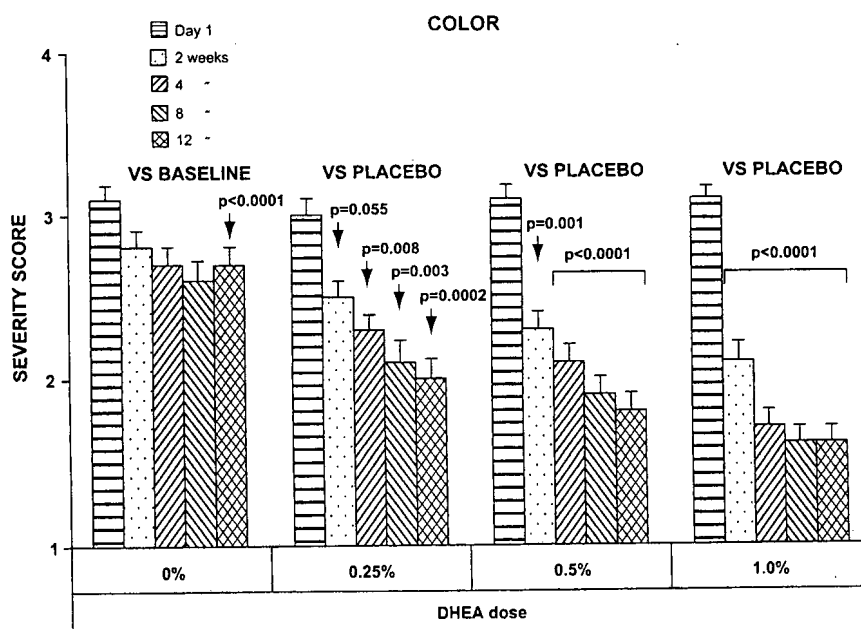


FIG. 10. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the change in vaginal color evaluated at vaginal examination. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals for the three DHEA doses, whereas for the placebo group at 12 weeks, comparison is with baseline.

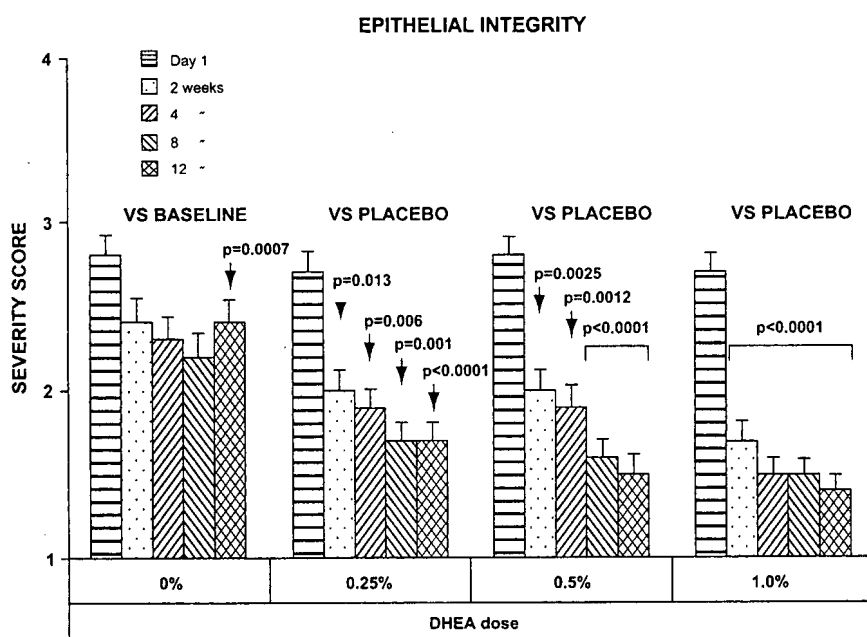


FIG. 11. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the change in vaginal epithelial integrity evaluated at vaginal examination. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals for the three DHEA doses, whereas for the placebo group at 12 weeks, comparison is with baseline.

1-week pharmacokinetics study with doses of 0.5%, 1.0%, and 1.8% DHEA ovules.^{32,33} No adverse effect was observed on hepatic tests, hematocrit, or any hematological or biochemical parameter. Moreover, no significant adverse event related to DHEA was observed in a study performed in 75 women randomized to receive 3 g of 0.3% DHEA

percutaneously twice daily for 12 months³⁵ or 15 women who received daily 3 to 5 g of 10% DHEA cream for 1 year.³⁴

DISCUSSION

The present data show for the first time that the local administration of DHEA, the essential precursor of all sex

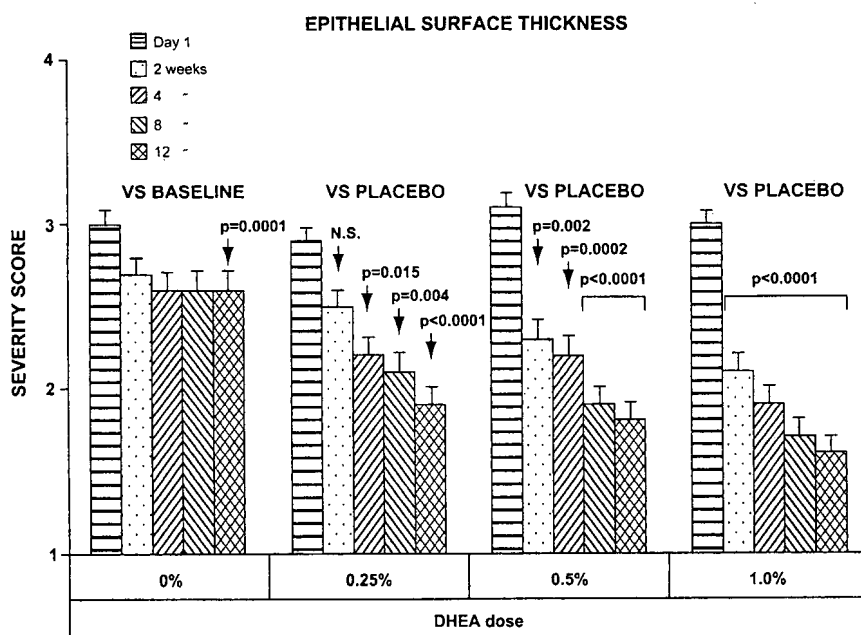


FIG. 12. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 2, 4, 8, and 12 weeks on the change in vaginal epithelial thickness evaluated at vaginal examination. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals for the three DHEA doses, whereas for the placebo group at 12 weeks, comparison is with baseline.

HIGH EFFICACY OF LOCAL DHEA FOR VAGINAL ATROPHY

steroids but an inactive compound by itself, is highly efficient and rapid in correcting all the symptoms and signs of vaginal atrophy in postmenopausal women. Most importantly, this is achieved at DHEA doses, which have no or minimal effects on serum estrogens and androgens and their metabolites, which all remain well within the range of values found in normal postmenopausal women.^{32,33,37}

Despite their well-recognized safety issues, various forms of estrogens are an efficient treatment for vulvovaginal atrophy.^{3,40} In fact, the vaginal E₂ tablet has shown an efficacy similar to that of the E₂ ring²⁰ and the conjugated estrogen cream. It is estimated, however, that approximately 40% of women taking oral hormone therapy have persistent vaginal dryness.⁴¹

As mentioned above, although 75% of postmenopausal women suffer from vaginal atrophy,^{1,2} thus affecting their quality of life during a major part of their lifetime, only 20% seek treatment.³ The fear of breast cancer associated with the prescription of estrogens which increases the serum levels of estrogens is the main reason for the lack of acceptance of estrogen therapy by most women and their physicians. In the aftermath of Women's Health Initiative study, the scientific challenge is to explore alternative hormone therapy types and formulations that would provide all the menopausal advantages of estrogens while improving women's quality of life, minimizing risks, and maximizing benefits.¹⁵

It is pertinent to mention that because estrogen secretion into the systemic circulation in women ceases at menopause, administration of estrogens to postmenopausal women is not a physiological solution. On the other hand, because the nonestrogen-based treatments have not shown convincing efficacy,^{42,43} women and their physicians are left with no safe treatment for vaginal atrophy.

A recent study in which serum steroid levels were measured at multiple time intervals to obtain maximal precision and information during the 24-hour period^{32,33} has shown that after daily treatment with 0.5% or 1.0% DHEA ovules, only serum DHEA and androst-5-ene-3 β ,17 β -diol (and androstenedione at day 1 and E₁ on day 7) are increased significantly from baseline but well within the limits of values found in normal postmenopausal women.²⁹ Serum estrogens (E₂ and E₁-S) and serum androgens (testosterone and dihydrotestosterone) are not significantly affected. Although DHEA can be transformed into both androgens and estrogens in the mammary gland, the global effect of DHEA on the human mammary gland is inhibitory.^{27,28} Most importantly, the metabolites of androgens (androsterone glucuronide; androstane-3 α ,17 β -diol-3G, and 3 α -diol-17G) and estrogens (E₁-S) are unchanged or minimally changed after intravaginal DHEA administration, thus clearly demonstrating the absence of significant systemic androgenic and estrogenic formation and, therefore, potential systemic effects of sex steroids at the doses used.

In addition to the increased breast cancer risk associated with the administration of estrogens, it is important to recognize that the specific hormonal difference between the post-

menopausal women who do not have vaginal atrophy (estimated at 25% of the postmenopausal population) and the majority (75%) of postmenopausal women who have vaginal atrophy^{1,2} is absolutely not related to the secretion of estrogens in the systemic circulation because estrogen secretion by the ovaries has ceased in all women at time of menopause. Consequently, a deficit in estrogen secretion into the systemic circulation is not a valid explanation for the occurrence of symptoms of vaginal atrophy in 75% of postmenopausal women, whereas the remaining and more fortunate 25% of women live throughout all their postmenopausal years without significant bothersome or evident vaginal atrophy symptoms. Therefore, estrogens may not be a physiological replacement therapy for the 75% of postmenopausal women who have vaginal atrophy, as other factors may be at play.

Contrary to the estrogens of ovarian origin, which are secreted into the general circulation where they are exposed to all tissues, DHEA is an inactive precursor that is transformed only in the peripheral tissues that possess the required steroidogenic enzymes. The process of intracrinology thus permits local intratissular formation of active sex steroids with no significant release of the active steroids into the circulation,⁴⁴⁻⁴⁷ thus avoiding exposure of the other tissues not physiologically in need of these active sex steroids.

As mentioned above, however, the secretion of DHEA, the only source of sex steroids after menopause, markedly and variably decreases with age, with an average 60% loss being already observed at time of menopause.^{27-29,47-49} Consequently, the only difference between the symptomatic (75%) and the asymptomatic (25%) postmenopausal women is the amount of DHEA secreted by the adrenal glands, the ability of each tissue to transform DHEA into active sex steroids, and/or the sensitivity of the vaginal tissue to DHEA. The difference in sensitivity of different women is likely to be related, up to an unknown extent, to the level of activity of the enzymatic machinery specific to each cell type in each tissue.^{44,49} With this knowledge, it is clear that DHEA and not estrogens should be the physiological hormone therapy at menopause.

Change in pH is now recognized as a valid parameter that reflects the beneficial effect of therapy on vaginal atrophy. After 12 weeks of intravaginal treatment with 25 μ g E₂, the percentage of participants having a pH less than 5.0 was 51% compared with 21% in the placebo group.²⁴ At baseline, however, 11.2% and 13% of women in that study already had a pH below 5.0 in the two corresponding groups. In the present study, no participant had a pH value below 5.0 at screening, whereas 29%, 53%, 61%, and 71% had pH values at or below 5.0 at 12 weeks in the 0%, 0.25%, 0.5%, and 1.0% DHEA groups, respectively.

As demonstrated in the present study, the effect of DHEA on the maturation of the vaginal epithelial cells is particularly rapid: with the 0.5% DHEA ovule, 81% of the maximal effect on parabasal cells was already observed at 2 weeks, whereas 52% of the maximal stimulatory effect exerted on

superficial cells was observed at the same time interval. On the other hand, 85% of the maximal effect of 0.5% DHEA on the percentage of superficial cells was achieved at 4 weeks. Similarly, 53% of the maximal effect of 0.5% DHEA on the most bothersome symptom was observed at 2 weeks, and 73% was reached at 4 weeks. Moreover, only 17.8% of women reported no improvement in their most bothersome symptom at 12 weeks in the 0.5% DHEA group compared with 48.8% in the placebo group.

The effect of DHEA on parabasal cells is especially rapid because the percentage of parabasal cells was decreased to less than 20% at 1 month with the three DHEA doses used. The effect on the percentage of superficial cells is also very rapid, with 96% of the effect being seen at 2 weeks with the high (1%) DHEA dose. In a study with vaginal estrogen cream or tablet, approximately 50% of the effect measured at 12 weeks was observed at 2 weeks.¹⁶ Such data indicate that the rapidity of the effect of DHEA is not inferior and is possibly superior to the effect of the vaginal E₂ and conjugated estrogen formulations.

In a recent study, the vaginal maturation value increased from 27.4 at baseline to 56.8 ($P < 0.0001$) in the estrogen-treated group.⁵⁰ The percentage of superficial cells increased by 17.1 from baseline, whereas the percentage of parabasal cells decreased by 41.7% in the estrogen-treated group. In the same study, the vaginal pH decreased from 6.74 at baseline to 5.05 (decrease of 1.69% or 24% in the estrogen group). The severity of the most bothersome symptoms decreased from 2.58 to 1.04 (-1.54) in the estrogen group compared with a decrease from 2.59 to 1.84 (-0.75) in the placebo group. Such data observed with estrogens are comparable to the 1.56 decrease in severity of the most bothersome symptoms at 12 weeks in the 0.5% DHEA group and the 0.67 decrease in the placebo group observed in our study.

At week 12, 11% of Estring participants and 24% of Vagifem participants had persistent atrophic epithelium. At week 48, the respective values were 8% and 14%.²⁰ At 48 weeks of treatment with Vagifem or Estring, vaginal dryness was still present in 33% of women.²⁰ Pruritus vulvae, on the other hand, remained present in 15% and 20% of women after treatment with Estring and Vagifem, respectively, whereas 33% and 28% of women still had dyspareunia after treatment with Estring and Vagifem, respectively. Bleeding after the progestogen test was 7% in the Vagifem group and 0% in the Estring group. In the present study, after 12 weeks of treatment with the 0.25%, 0.5%, and 1.0% DHEA doses, improvement of the most bothersome symptom was observed in 72.5%, 82.4%, and 81.4% of women, respectively, thus leaving only 23.5%, 17.8%, and 19.6% of women with no reported improvement of their most bothersome symptoms.

After 3 months of daily administration of 0.625 mg Premarin orally or intravaginally (cream), respective 70.6% and 75% improvements of dyspareunia were observed.²³ It was concluded in that study that 1 g of 0.625 mg Premarin was the minimal dose for the treatment of sexual dysfunction. In women who received 25 µg E₂ intravaginally, dyspareunia

persisted in 12.4% after 12 months of treatment.⁵¹ The success rate of therapy of local E₂ tablets was 84.5% as judged by participants and 86.1% as judged by physicians, but 40% to 50% of women receiving oral estrogen therapy had persistent complaints of vaginal dryness.^{51,52}

As reported previously after 12 months of treatment with DHEA,³⁴ the present study shows no effect on endometrial histology after 3 months of intravaginal administration of the hormone precursor DHEA as shown by histopathological examination of the endometrial biopsies obtained before and after 12 weeks of treatment. These findings are in agreement with the absence of aromatase activity in the human endometrium.^{53,54} This observation is also strongly supported by the well-recognized clinical observation that endometrial atrophy is characteristic of postmenopause despite the continuous secretion of DHEA.^{29,47} The absence in the human endometrium of the steroidogenic enzymes necessary to transform DHEA into estrogens is in agreement with the physiological role of the endometrium, which is active exclusively during the reproductive years when its function is essentially controlled by hormones of ovarian and placental origins. In fact, there is no physiological role of the endometrium after menopause which would justify any need for estrogens. Moreover, exposure of the postmenopausal endometrium to estrogens is associated with a well-known increased risk of endometrial cancer. Accordingly, the enzymes required for the synthesis of estrogens from DHEA are not expressed in the endometrium, a tissue fully dependent on estrogens of ovarian origin and limited in its function to premenopause and pregnancy.

We believe that the androgenic component of DHEA action plays a so-far unrecognized role in general vaginal health. In fact, as mentioned above, our recent preclinical data have shown an important effect of DHEA on all three layers of the vaginal wall, including the collagen fibers of the lamina propria and the muscularis.³⁰ It is expected that such a global action of DHEA exerts benefits on the severity of the signs of vaginal atrophy, especially those related to a reduction in the length and diameter of the vagina, the loss of vaginal rugal folds, and the disappearance of vaginal fornices. All these atrophic changes are accompanied by decreased vascularization, decreased vaginal secretions, and more susceptibility to trauma and pain. The specific androgenic effect on collagen formation could play a major role in the particularly positive results obtained in this study.

CONCLUSIONS

The Food and Drug Administration Guidance to Industry encourages sponsors to develop the lowest doses and exposures for both estrogens and progestins. We must recognize that although estrogens are efficient in correcting the symptoms of vaginal atrophy and vasomotor symptoms, systemic estrogens are not the physiological hormones that permit reduction of symptoms of vaginal atrophy in approximately 25% of postmenopausal women who remain

relatively asymptomatic throughout their postmenopausal years.² Because the only variable source of sex steroids available in postmenopausal women is DHEA secreted by the adrenals and locally transformed in specific cells and tissues into estrogens and/or androgens by the mechanisms of intracrinology,^{44,47} replacement with DHEA seems to be the only physiological replacement therapy for postmenopausal women experiencing menopausal symptoms. With the novel approach that we have called tissue-specific prehormone replacement therapy, we theorize vaginal atrophy symptoms should be corrected with no more risk than those present in the 25% of postmenopausal women who have no symptoms of vaginal atrophy because of the presence in these women of a higher exposure or sensitivity to DHEA.

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LABRIE ET AL

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Effect of intravaginal dehydroepiandrosterone (Prasterone) on libido and sexual dysfunction in postmenopausal women

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Abstract

Objective: The objective of this study was to provide evidence that the transformation of DHEA into both androgens and/or estrogens locally in cells of the three layers of the vagina (epithelium, lamina propria, and muscularis) would have effects of greater impact, including effects on sexual function, than only effects on superficial epithelial cells as achieved with estrogens.

Methods: This prospective, randomized, double-blind, and placebo-controlled phase III clinical trial has evaluated the effect of daily local intravaginal application of Prasterone (dehydroepiandrosterone; DHEA) for 12 weeks on the domains of sexual dysfunction, namely, desire/interest, arousal, orgasm, and pain at sexual activity, in 216 postmenopausal women with moderate to severe symptoms of vaginal atrophy.

Results: A time- and dose-dependent improvement of the four domains of sexual function was observed. At the 12-week time interval, the 1.0% DHEA dose led, compared with placebo, to 49% ($P = 0.0061$) and 23% ($P = 0.0257$) improvements of the desire domains in the Menopause Specific Quality of Life and Abbreviated Sex Function questionnaires, respectively. Compared with placebo, the Abbreviated Sex Function arousal/sensation domain was improved by 68% ($P = 0.006$), the arousal/lubrication domain by 39% ($P = 0.0014$), orgasm by 75% ($P = 0.047$), and dryness during intercourse by 57% ($P = 0.0001$).

Conclusions: By a local action in the vagina, DHEA applied daily at doses at which serum steroids remain well within normal postmenopausal values exerts relatively potent beneficial effects on all four aspects of sexual dysfunction. Such data indicate that combined androgenic/estrogenic stimulation in the three layers of the vagina exerts important beneficial effects on sexual function in women without systemic action on the brain and other extravaginal tissues.

Key Words: Dehydroepiandrosterone – Prasterone – Libido – Sexual dysfunction – Postmenopause.

Sexual dysfunction, especially low libido, is a common problem with rates of up to 50% self-reported among women in community studies using questionnaires.¹⁻⁴

Received November 10, 2008; revised and accepted January 26, 2009.

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Funding/support: This study was sponsored by Endoceutics.

Financial disclosure/conflicts of interest: Dr. Labrie is President and CEO of Endoceutics. The other authors report no conflicts of interest, although the study was financed by Endoceutics. Patents for intravaginal Prasterone are the property of Endoceutics, Inc.

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In the United States, it has been observed that 43% of women have sexual dysfunction of one type or another.⁵ More recently, it has been found that more than 40% of women reported sexual problems, with 25% having sexual dysfunction.⁶ Overall, in another study, approximately 42% of women complained of one or more sexual problems compared with 30% of men.⁷ The prevalence increases after oophorectomy and with age.^{5,8}

As indicated in the *Diagnostic and Statistical Manual of Mental Disorders, 4th ed.* (1994) of the American Psychiatric Association,⁹ the general categories of female sexual dysfunction (FSD) include desire disorders (hypoactive sexual desire disorder), arousal disorders, orgasmic disorders, and sexual pain disorders. These disorders can occur individually or in any combination. The common problem of sexual dysfunction is believed to be a composite of biological, psychological, and interpersonal components.¹⁰

Although psychological factors are believed to play an important role in the loss of sexual desire/interest and arousal, many studies have reported a beneficial effect of androgens

on sexual function in women.¹¹⁻²⁰ These observations have resulted in an increased use of testosterone for this indication,^{21,22} although some controversy about the efficacy of androgens on sexual dysfunction still persists.^{23,24}

Persistent controversial data relate to the search for a potential correlation between desire and serum testosterone in healthy women.²⁵⁻²⁷ In fact, most studies have found no correlation between serum testosterone and arousal.²⁶ It is important to indicate that this lack of correlation can be fully explained by the finding that serum testosterone is clearly not a valid parameter of androgenic activity in women.²⁸

The finding that women make approximately 50% as much androgens as men has important implications for the design of the most appropriate hormone therapy for women at menopause and postmenopause. This issue is particularly important because approximately 60% of the androgens present at the age of 30 years in women are already lost at time of menopause in parallel with the decrease in serum dehydroepiandrosterone (DHEA; Prasterone).^{28,29}

The important clinical health issues facing women at menopause pertain to vaginal atrophy, hot flashes, bone loss, loss of muscle mass and strength, fat accumulation, and type 2 diabetes. It is of particular interest that all these medical problems have been found to respond positively to androgens and, in most cases, to the administration of DHEA when used at the proper dose and appropriate experimental conditions^{14,30-32} (see review of Labrie³³).

Considering the clinical evidence mentioned above strongly suggesting a role of androgens in sexual dysfunction¹¹⁻²⁰ and our recent preclinical findings of beneficial effects of DHEA in the three layers of the vagina,³⁴ it seemed particularly important to study the effect of DHEA applied locally in the vagina on the various domains of sexual dysfunction. Such a study would also provide evidence for the relative role played by the brain and the genital organs in the four domains of defective sexual function, namely, desire/interest, arousal, orgasm, and pain at sexual activity. It is important to indicate that intravaginal or systemic administration of estrogens has not been found to have significant effects on libido and coital frequency.^{15,35,36}

The changes in the sexual dysfunction parameters (desire/interest, arousal, orgasm, and pain at sexual activity) were analyzed at screening, at day 1, and at weeks 4, 8, and 12 in 216 postmenopausal women who have vaginal trophy and are randomly treated daily with 0% (placebo), 0.25%, 0.5%, and 1.0% DHEA intravaginal ovules. The validated questionnaires used were the Abbreviated Sexual Function (ASF)^{37,38} and the Menopause-Specific Quality of Life (MENQOL)³⁹ questionnaires. Quality of life was evaluated with the Psychological General Well Being (PGWB) questionnaire.⁴⁰

METHODS

This study was a phase III, prospective, multicenter, randomized, placebo-controlled, and double-blind trial planned for 50 participants per arm (for a total of 200 participants to complete the study). Two hundred eighteen postmenopausal women (216 women in the intent-to-treat [ITT] analysis) were

thus randomized to receive a daily ovule of the following DHEA concentrations: 0.0% (53 women), 0.25% (3.25 mg DHEA; 53 women), 0.5% (6.5 mg DHEA; 56 women), or 1.0% (13 mg DHEA; 54 women) applied intravaginally with an applicator at bedtime and were part of the ITT analysis. The median range age of women was 58 (49-70), 57 (42-72), 58 (50-74), and 59 (46-69) years in the four groups. The DHEA ovules or suppositories containing Prasterone in a lipophilic base were manufactured by Recipharm (Karlskoga, Sweden). The study was divided into two phases, namely, the screening phase where all the required tests were done to make sure that all inclusion and exclusion criteria were met, followed by the treatment phase of 12 weeks. The protocol was approved by the institutional review board of the Centre Hospitalier de l'Université Laval, Quebec City, QC, Canada; McGill University, Montreal, QC, Canada; Ethica, Montreal, QC, Canada; Eastern Virginia Medical School, Norfolk, VA; and the Western Institutional Review Board, Los Angeles, CA.

Inclusion/exclusion criteria were described in detail in an accompanying article.⁴¹ The main inclusion criteria were the following:

- Postmenopausal women
- Women having self-identified at least one moderate to severe of the following symptoms
 - Vaginal dryness (none, mild, moderate, or severe)
 - Vaginal and/or vulvar irritation/itching (none, mild, moderate, or severe)
 - Vaginal pain associated with sexual activity (none, mild, moderate, or severe)
- Women between 40 and 75 years of age
- Women having a low maturation index (no greater part of guidance than 5% of superficial cells on vaginal smear)
- Women having a vaginal pH above 5

The main exclusion criteria were the following:

- Undiagnosed abnormal genital bleeding
- Previous diagnosis of cancer, except skin cancer (non-melanoma)
- Endometrial hyperplasia at biopsy performed at screening or endometrial cancer
- Use of estrogen-alone injectable drug therapy or progestin implant within 3 months before study entry (screening visit)
- Use of estrogen pellet or progestin injectable drug within 6 months before study entry (screening visit)
- Use of oral estrogen, progestin, or DHEA exposure or intrauterine progestin therapy in the 8 weeks before baseline assessments (screening visit)
- Use of vaginal hormonal products (rings, creams, or gels) or transdermal estrogen alone or estrogen/progestin products in the 4 weeks before baseline assessments (screening visit)

Informed consent

Written informed consent was obtained from all participants before the performance of any study-related procedure. The participants had a medical history, a medical examination, and a complete gynecological examination (including

endometrical biopsy) at screening. A partial gynecological examination was performed to evaluate the aspect of the mucosa and tolerance to the medication on day 1 and at weeks 2, 4, 8, and 12.

Laboratory tests

The standard laboratory tests, namely, hematology (including complete blood count and coagulation), blood chemistry, and urinalysis, were performed at screening and week 12.⁴¹

Statistics

Summary tabulations were prepared, displaying the number of observations; mean or geometric mean as appropriate; SD; SEM, 95% two-sided CI; median, minimum, and maximum for continuous variables; and the number and percentage per category for categorical data. Statistical analyses were performed at the two-sided significance level of 0.05, unless otherwise stated. The categories for summarization in general consisted of the dose levels of the DHEA treatments, 0% (placebo), 0.25%, 0.5%, and 1.0% DHEA.

The endpoints for analysis consisted of the following. Desire/interest, arousal, orgasm, and vaginal pain at intercourse were self-rated by the women at screening, at day 1, and at weeks 4, 8, and 12 using the ASF and MENQOL questionnaires. The PGWB questionnaire was also completed by participants, whereas the Sexual Concern (SC) questionnaire was used to obtain an estimate of the interest for sexual activity by women, especially at entry in the protocol. For each type of questionnaire, the endpoints for analysis consisted of domains comprised of several individual questions. Baseline was defined as the value on day 1, obtained before first use of the study treatment, and change from baseline to weeks 4, 8, and 12 was calculated for each participant for each domain endpoint. The analysis comparing each dose of DHEA to placebo was performed by analysis of covariance, with the baseline value used as the covariate. Missing values for the domain were excluded from analysis.

Analysis populations

The ITT population consisted of the treated participants with a baseline and at least one postbaseline efficacy assessment. Participants who may have received the wrong treatment were analyzed as randomized. This analysis population was considered the primary analysis population. The percentage of participants who had questionnaire data through the final visit at week 12 was greater than 90% in each treatment group for each questionnaire; therefore, it was not considered necessary to conduct a completers or evaluable population analysis in addition to the ITT analysis.

The safety population was defined as all participants who received one administration of either test article (DHEA at any dose) or placebo and who had any safety information available. All safety data analyses were based on this population.

RESULTS

In addition to self-assessment of the most bothersome symptom (coprimary objective) and of the severity of three

symptoms of menopause,⁴¹ women were asked, as a secondary objective, to answer questionnaires related to libido, sexual function, and quality of life at postmenopause. At screening, at day 1, and at weeks 4, 8, and 12, the ASF, the MENQOL, the SC, and the PGWB questionnaires were completed.

Of 171 women who reported one of the three symptoms of vaginal atrophy, namely, vaginal dryness, irritation/itching, and vaginal pain at sexual activity, as being severe, most women (110; 64%) indicated vaginal pain at sexual activity, whereas 48 (28%) and 13 (8%) reported vaginal dryness and irritation/itching, respectively.

Because vaginal pain is most closely associated with loss of libido and sexual dysfunction, it is of major interest to see that at the standard 12-week interval, treatment with 0.25% DHEA decreased the score of the sexual domain of the MENQOL questionnaire by 2.85 ± 0.282 ($P = 0.0001$ vs placebo). On the other hand, decreases of 1.92 ± 0.289 ($P = 0.0036$ vs placebo) and 2.50 ± 0.267 ($P < 0.0001$ vs placebo) were achieved with the 0.5% and 1.0% DHEA doses, respectively (Fig. 1). In fact, the sexual domain score was improved at 12 weeks by 54%, 43%, and 53% with the three increasing doses of DHEA, whereas the placebo effect was 32%.

The three questions covered by the sexual domain of MENQOL are change in your sexual desire, vaginal dryness during intercourse, and avoiding intimacy. The question most directly related to libido, namely, sexual desire, was improved by 55% ($P < 0.0001$ vs baseline), 32% ($P = 0.0006$ vs baseline), and 49% ($P < 0.0001$ vs baseline) in the 0.25%, 0.5%, and 1.0% DHEA groups, respectively. The placebo effect was a reduction of 23% ($P = 0.0187$ vs baseline; Fig. 2).

As can be seen in Fig. 3, the score "avoiding intimacy" decreased by 46% ($P = 0.105$ vs placebo), 53% ($P = 0.043$ vs placebo), and 51% ($P = 0.0056$ vs placebo) for the 0.25%, 0.5%, and 1.0% DHEA doses, respectively, whereas a decrease of 26% from baseline was observed in the placebo group ($P = 0.0079$ vs baseline; Fig. 3).

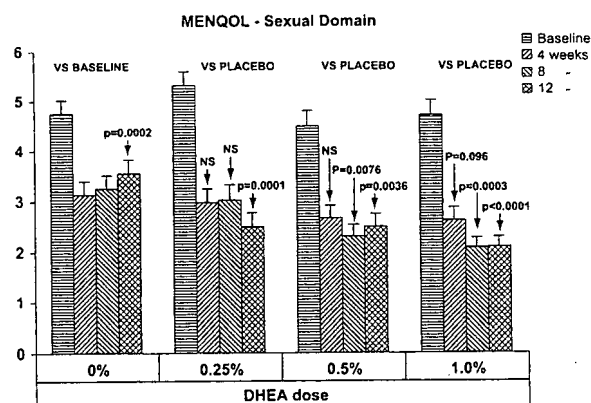


FIG. 1. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 4, 8, and 12 weeks on the score of the sexual domain of the Menopause-Specific Quality of Life (MENQOL) questionnaire in postmenopausal women. Data are expressed as means $\pm$ SEM; the P values are comparisons with placebo at all time intervals except for the placebo group at 12 weeks, which is compared with baseline.

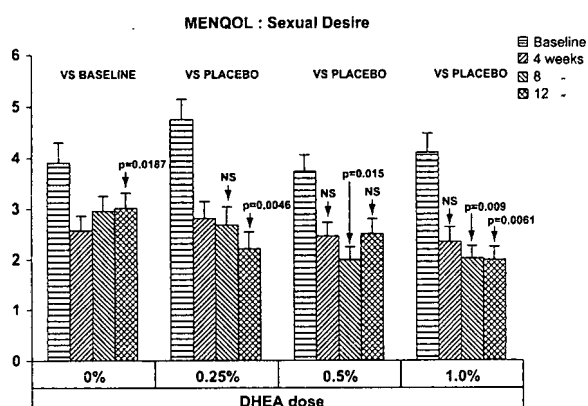


FIG. 2. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 4, 8, and 12 weeks on the score of the sexual desire of the Menopause-Specific Quality of Life (MENQOL) questionnaire in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals except for the placebo group at 12 weeks, which is compared with baseline.

The third question of the sexual domain of the MENQOL questionnaire is vaginal dryness during intercourse. As can be seen in Fig. 4, treatment with the 0.25%, 0.5%, and 1.0% DHEA doses led, at 12 weeks, to 58% ($P = 0.0001$ vs placebo), 53% ($P = 0.0042$ vs placebo), and 57% ($P = 0.0001$ vs placebo) decreases in the severity score of vaginal dryness at intercourse, whereas the placebo effect was 28% ($P = 0.0003$ vs baseline).

Although no significant effect was noticed on the psychological and physical domains of MENQOL, the particularly strong effect described above on the sexual domain (Fig. 1) led to a significant effect on the global MENQOL score (Fig. 5). The MENQOL summary (total) score decreased at 12 weeks by 0.28 ± 0.13 (9.5%; $P = 0.03$ vs baseline), 0.83 ± 0.131 (27%) in the 0.25% group ($P = 0.001$ vs placebo), 0.48 ± 0.096 (17.5%) in the 0.5% DHEA group ($P = 0.07$ vs

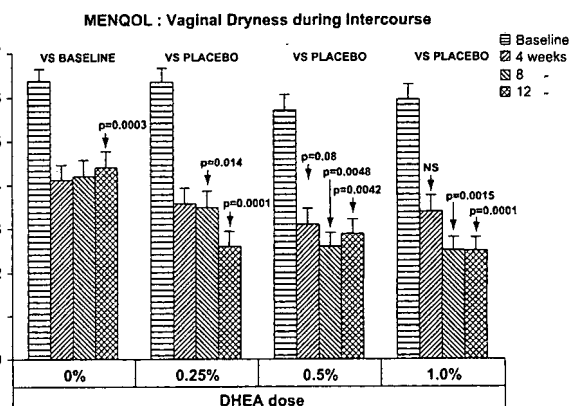


FIG. 4. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 4, 8 and 12 weeks on the score of vaginal dryness during intercourse of the Menopause-Specific Quality of Life (MENQOL) questionnaire in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals except for the placebo group at 12 weeks, which is compared with baseline.

placebo), and 0.64 ± 0.103 (21%) in the 1.0% DHEA group ($P = 0.03$ vs placebo; Fig. 5).

An important questionnaire related to libido and sexual dysfunction is the ASF questionnaire. When the data of this questionnaire were analyzed, it was found that although placebo had no significant effect on the domain desire, the 0.25% DHEA dose improved the score by 3.40 ± 0.786 ($P = 0.034$ vs placebo; Fig. 6), whereas the increase in the 0.5% DHEA group did not reach statistical significance, and treatment with the 1.0% DHEA ovules increased the desire score at 12 weeks by 23% ($P = 0.0257$ vs placebo).

When the arousal/sensation domain was examined at the standard 12-week time interval, a highly significant effect of the three DHEA doses was observed versus baseline with *P* values equal to 0.0004 and less than 0.0001, whereas placebo has no significant effect ($P = 0.56$; Fig. 7). At 12 weeks, a

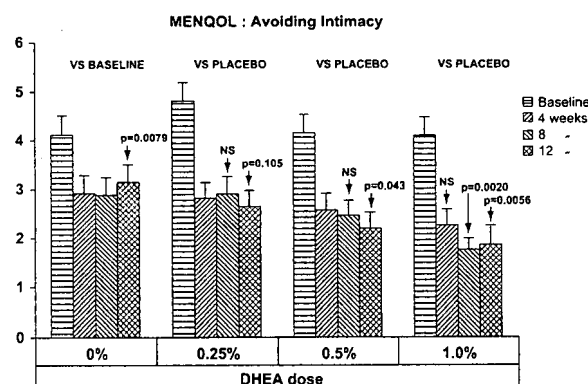


FIG. 3. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 4, 8, and 12 weeks on the score of avoiding intimacy of the Menopause-Specific Quality of Life (MENQOL) questionnaire in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals except for the placebo group at 12 weeks, which is compared with baseline.

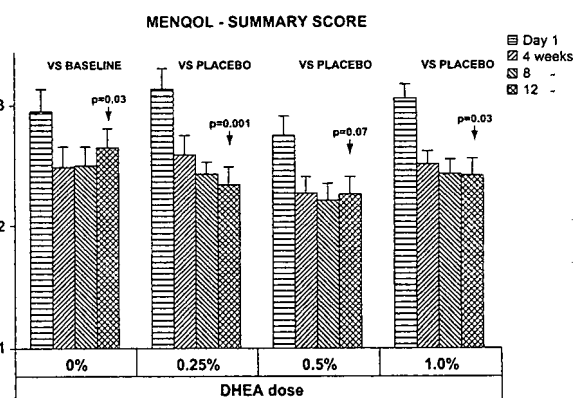


FIG. 5. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 4, 8, and 12 weeks on the summary score of the Menopause Specific Quality of Life (MENQOL) questionnaire in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals except for the placebo group at 12 weeks, which is compared with baseline.

INTRAVAGINAL DHEA AND SEXUAL FUNCTION

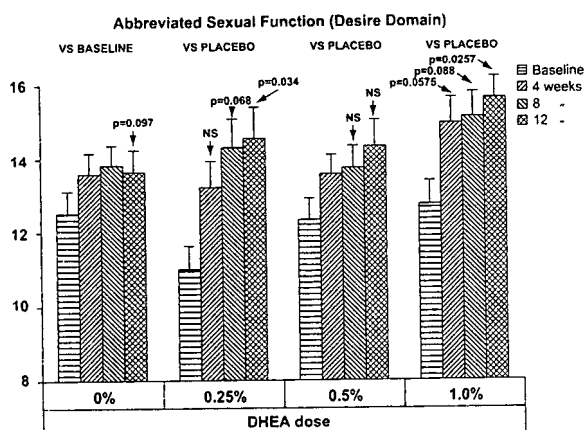


FIG. 6. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 4, 8, and 12 weeks on the score of the desire domain of the Abbreviated Sexual Function questionnaire in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals except for the placebo group at 12 weeks, which is compared with baseline.

statistically significant difference from placebo was observed with the 1.0% DHEA dose ($P = 0.006$ vs placebo; Fig. 7). Even greater beneficial effects were also observed at 12 weeks on the domain arousal-lubrication, with improvements of 169% ($P < 0.0001$ vs baseline), 162% ($P < 0.0001$ vs baseline), and 139% ($P < 0.0001$ vs baseline), whereas the placebo effect was 49% ($P = 0.02$; Fig. 8). At 12 weeks, all three doses have shown a statistically significant difference from placebo. The two questions of this domain were the following: "Over the last 4 weeks, in general, how often did you notice vaginal wetness/lubrication when you took part in sexual activity?" and "Over the last 4 weeks, in general, how much vaginal wetness/lubrication did you notice when you took part in sexual activity?"

With this test, at the standard 12-week interval, the score increased by 2.13 ± 0.352 ($P = 0.0325$ vs placebo) in the

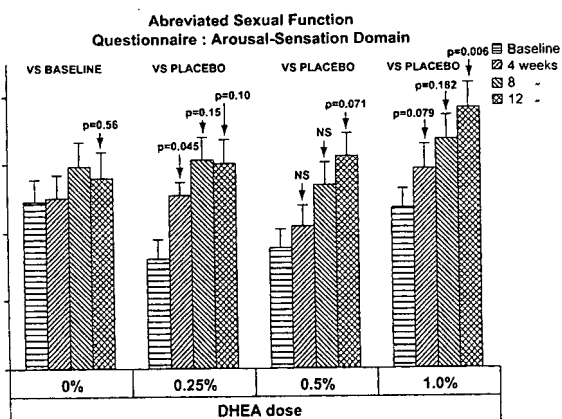


FIG. 7. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 4, 8, and 12 weeks on the score of the arousal-sensation domain of the Abbreviated Sexual Function questionnaire in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals except for the placebo group at 12 weeks, which is compared with baseline.

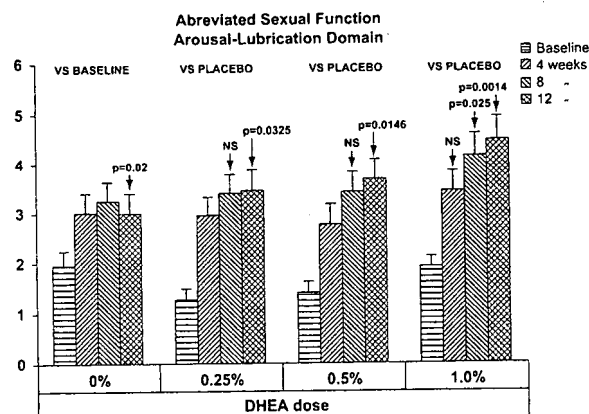


FIG. 8. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 4, 8, and 12 weeks on the score of the arousal-lubrication domain of the Abbreviated Sexual Function questionnaire in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals except for the placebo group at 12 weeks, which is compared with baseline.

women treated with 0.25% DHEA (Fig. 8). For the women treated with 0.5% DHEA, the score increased by 2.25 ± 0.31 ($P = 0.0146$ vs placebo), whereas an increase of 2.62 ± 0.371 ($P = 0.0014$ vs placebo) was observed in the group of women treated with 1.0% DHEA.

For the orgasm domain of the ASF questionnaire, at the 12-week time interval, as can be seen in Fig. 9, improvements of 1.88 ± 0.628 (NS vs placebo) from 2.87 ± 0.502 to 4.83 ± 0.69 , 2.00 ± 0.57 (NS vs placebo) from 3.54 ± 0.57 to 5.42 ± 0.62 , and 2.76 ± 0.546 ($P = 0.047$ vs placebo) from 3.70 ± 0.626 to 6.18 ± 0.696 were observed for the groups treated with the 0.25%, 0.5%, and 1.0% DHEA doses, respectively. The changes observed from baseline were 65% ($P = 0.004$), 56% ($P = 0.001$), and 75% ($P < 0.0001$) for the three increasing doses of DHEA, whereas the placebo effect was 31% ($P = 0.096$).

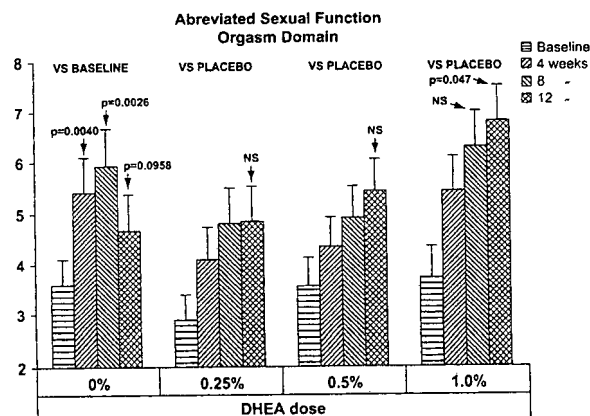


FIG. 9. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 4, 8, and 12 weeks on the score of the orgasm domain of the Abbreviated Sexual Function questionnaire in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals except for the placebo group at 12 weeks, which is compared with baseline.

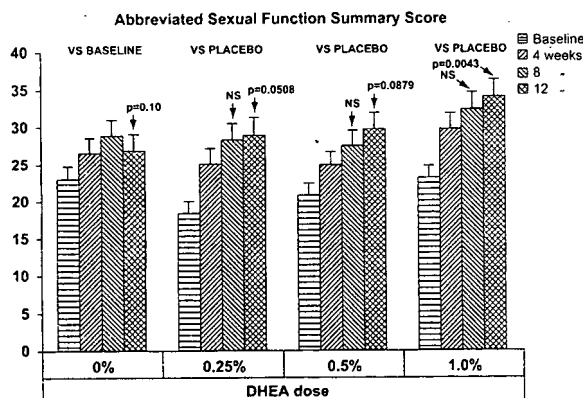


FIG. 10. Effect of daily intravaginal application of 0.0%, 0.25%, 0.5%, and 1.0% dehydroepiandrosterone (DHEA; Prasterone) for 4, 8, and 12 weeks on summary score of the Abbreviated Sexual Function questionnaire in postmenopausal women. Data are expressed as means $\pm$ SEM; the *P* values are comparisons with placebo at all time intervals except for the placebo group at 12 weeks, which is compared with baseline.

The three questions of the orgasm domain were the following: "Over the last 4 weeks, how often did you have an orgasm when you took part in sexual activity (may be with or without a partner)", "Over the last 4 weeks, in general, how pleasurable were the orgasms that you had" and "Over the last 4 weeks, in general, how easy was it for you to reach orgasm?"

As can be seen in Fig. 10, the 0.25%, 0.5%, and 1.0% doses of DHEA improved the summary score of the ASF questionnaire at 12 weeks compared with baseline by 55%, 42%, and 50% (all $P < 0.0001$ vs baseline and $P = 0.0508$, 0.0879, and 0.0043 vs placebo).

Concerning the more general PGWB, the summary score increased from day 1 to 12 weeks by 0.67 ± 1.936 (1%; $P = 0.73$ vs baseline) in the 0% DHEA (placebo) group, 7.40 ± 1.69 (9.3%; $P < 0.0001$ vs baseline) in the 0.25% DHEA group, 2.92 ± 1.54 (3.5%; $P = 0.064$ vs baseline) in the 0.5% group, and 3.06 ± 1.909 (3.9%; $P = 0.115$ vs baseline) in the 1.0% DHEA group.

When the six domains of the PGWB questionnaire are looked at individually, the positive well-being domain at 12 weeks improved by 1.56 ± 0.37 (12%; $P = 0.0001$ vs baseline) in the 0.25% DHEA group, 0.73 ± 0.341 (5.4%; $P = 0.04$ vs baseline) in the 0.5% DHEA group, and 0.61 ± 0.397 (4.6%; $P = 0.013$ vs baseline) in the 1.0% DHEA group. In the placebo group, the improvement of 0.15 ± 0.355 was not statistically significant.

Although some positive effects were observed in the more general PGWB questionnaire, the major effects revealed by the questionnaires were clearly those observed on libido and sexual function in the ASF and MENQOL questionnaires.

DISCUSSION

The present data show for the first time that local intravaginal treatment with DHEA, a compound inactive by itself that acts as precursor for the cell-specific local formation of all androgens and/or estrogens after menopause,

causes a marked improvement of all four aspects of women's sexual dysfunction, namely, desire/interest, arousal, orgasm, and pain at sexual activity.

Although the role of psychological, biological, and interpersonal factors in sexual function is a matter of debate,¹⁰ the data described above clearly show that local intravaginal changes induced by sex steroids can exert marked influences on all aspects of sexual function, including desire/interest, a characteristic component of brain function. It thus seems that increased favorable sensitive outputs from a more healthy vaginal area influence the brain to feel increased desire/interest without the need for a direct action of hormones on the brain. The present findings also indicate that there is a highly efficient bidirectional interplay between the genital area and the brain. Most importantly, these benefits are achieved by an exclusive peripheral action of Prasterone, with no or minimal changes in the serum levels of E_2 and testosterone which remain within the values observed in normal postmenopausal women.⁴²⁻⁴⁴

Several studies have reported diminished sexual desire or interest, decreased sexual receptivity, and decreased sexual responsiveness in postmenopausal women.⁴⁵⁻⁵¹ Low sexual desire is strongly associated with a decrease in the other aspects of the sexual response, namely, arousal, orgasm, and pleasure.⁵²

The definition of women's sexual dysfunction has been revised recently.¹⁰ The disorder of the sexual desire/interest domain is illustrated by absent or diminished feelings of sexual interest or desire. In this situation, the incentive/motivation to become sexually aroused is scarce or absent.

The disorders of the arousal domain require the presence of a normal vasocongestive response of the genitalia after erotic sexual stimulation. The disorders of the sexual arousal domain can be divided into (1) subjective sexual arousal disorder (lack of pleasure after sexual stimulation), whereas the vaginal lubrication and other signs of physical response still occur, and (2) genital sexual arousal disorder illustrated by minimal vaginal lubrication and sexual sensations, whereas the subjective (psychological) sexual excitement is present. Orgasm will not occur or occurs with low intensity with loss of sexual excitement and pleasure. A combination of the subjective and genital arousal disorders occurs. The women's orgasmic disorder, on the other hand, is either the lack of orgasm, markedly diminished orgasmic sensations, or a marked delay in orgasm.

With the present data showing a parallel effect on desire/interest, arousal, orgasm, and pain at sexual activity, it is unlikely that the linear model of desire leading to sexual arousal and orgasm is correct, as already mentioned.^{53,54} The present data clearly show that beneficial effects induced by DHEA at the level of the vagina have parallel effects on desire/interest and arousal and also similar beneficial effects on orgasm and decreased pain at sexual activity. In other words, the present study shows that all aspects of women's sexual dysfunction can be successfully treated by local intravaginal DHEA therapy.

INTRAVAGINAL DHEA AND SEXUAL FUNCTION

The current therapeutic recommendations for sexual dysfunction in women are diverse but are generally deceptive. For low sexual desire and arousal (combined and subjective), cognitive-behavioral approaches, sex therapy, and psychodynamic therapies are recommended.¹⁰ Systemic estrogens combined with progestins are also used with the known risks of such therapy for the breast and uterus.¹⁰

In general, androgens have been reported to increase desire in postmenopausal women,¹³ although some data have been controversial.²³ In fact, starting in the 1940s, testosterone was found not only to eliminate menopausal symptoms but also to restore libido.⁵⁵ Sexual function has been found to be improved in many studies by adding methyltestosterone to estrogens.¹⁵ Similar findings have been reported, and high doses of testosterone enanthate by intramuscular injection alone or in combination with estrogens have resulted in sexual desire, fantasies, and arousal increase more than placebo or estrogen alone in ovariectomized women.^{12,14,19,56} On the other hand, an improvement in sexual activity but not in desire was observed.⁵⁷ Sexual arousal was not found to be modified by estrogens plus methyltestosterone in another study.²⁴ Testosterone in association with repeated erotic stimuli led to increased arousal after a delay,¹⁶ whereas no subjective increase in arousal was observed after testosterone administration and one episode of erotic stimulus.¹⁷

A recent large-scale study of a population of 483 spontaneous postmenopausal women with hypoactive sexual desire who received 300 µg testosterone or placebo patches twice a week for 24 weeks in addition to oral estrogens or estrogens + progestin therapy.²⁰ The number of satisfying episodes and desire were significantly increased by 73% and 48%, respectively, in the testosterone group, compared with 19% and 20% in the placebo group. The same dose of testosterone has shown improvement of hypoactive sexual desire disorder in surgically induced menopausal women.⁵⁸⁻⁶¹

Few data are available for DHEA: systemic therapy (50 mg/d for 4 mo) with DHEA in women with adrenal insufficiency⁶² improved the frequency of sexual thoughts or fantasies and sexual interest, and measurable improvements were made in both mental and physical satisfaction. Increased sexual thoughts and fantasies were apparent within 1 month of starting DHEA. Three small randomized, placebo-controlled studies of DHEA replacement (20-50 mg/d) in women with hypopituitarism reported improvement in quality of life,⁶³⁻⁶⁵ whereas improved sexual function was reported by those who found improved mood.

In postmenopausal women with normal adrenal function, an increase in sexual excitation and libido was reported at 6 months of DHEA administration.⁶⁶ In other studies, DHEA has been reported to improve sexual and mood disorders.⁶⁷⁻⁶⁹ DHEA has also been observed to improve psychological and physical well-being in postmenopausal women.^{70,71}

Some other studies, however, have not reported a beneficial effect of DHEA on sexual function.^{72,73} Two studies in women with adrenal insufficiency found no improvement in well-being or sexual function^{73,74} after DHEA administra-

tion. In another study in Addison disease,⁷⁵ a significant improvement in sexuality was not observed after 12 weeks of treatment with the same DHEA dose. An improvement in mood and spatial cognition was observed in androgen-deficient women with anorexia nervosa who received DHEA, some of whom were depressed.⁷⁶

We believe that the androgenic component of DHEA action plays a so-far unrecognized role in the physiology of the vagina. In fact, our recent preclinical data have shown an important effect of DHEA on all three layers of the vaginal wall, including the collagen fibers of the lamina propria and the muscularis.³⁴ It is expected that such a previously unknown global action of DHEA on the vagina exerts benefits not previously observed on the signs of vaginal atrophy which can include a reduction in the length and diameter of the vagina, the loss of vaginal rugal folds, and the disappearance of vaginal fornices. All these atrophic changes are accompanied by decreased vascularization, decreased vaginal secretions, and more susceptibility to trauma and pain. The specific androgenic effect on collagen formation could play a major role in the particularly positive results obtained in the present study.

Most importantly, the present data clearly show that intravaginal DHEA is a very efficient treatment for FSD without significant systemic exposure to a drug because all steroids remain within the postmenopausal range.⁴²⁻⁴⁴ The other advantage is that the drug used is already present in women at a relatively high level, thus practically eliminating the risk of an unexpected secondary effect at any site.

It should be mentioned that no women were excluded from the trial on the basis of other medications potentially affecting FSD, except sex hormones which could have affected vaginal atrophy. Another important aspect of the present study is that women were not selected on the basis of personal distress or desire to see improved sexual function. In fact, approximately 50% of women indicated that they desired improved sexual life, thus suggesting that interest in sexual life is not a prerequisite to achieve an improvement in the four domains of sexual function in that population of women.

The Food and Drug Administration Guideline for Industry in FSD recommends that "the endpoints be based on the number of successful and satisfactory sexual events or encounters over time." The present data not only show a significantly increased number of successful and satisfactory events compared with placebo (Fig. 10) but also show that all four components of FSD are highly statistically ($P < 0.01$ or more) improved compared with placebo.

CONCLUSIONS

The new knowledge gained about intracrinology in women²⁹ clearly indicates that the appearance of symptoms of vaginal atrophy in postmenopausal women is due to a lack of DHEA and/or insufficient transformation of this precursor into active androgens and/or estrogens in the vagina. Most interestingly, the present data show that in addition to very

rapidly and efficiently correcting the symptoms and signs of vaginal atrophy,⁴¹ the local application of DHEA in the vagina has a marked influence on all aspects of sexual function, thus indicating the role of an androgenic component of DHEA transformation on vaginal health, which permits improvement in desire, arousal, orgasm, and pleasure.

The present beneficial effects of intravaginal DHEA on sexual dysfunction in women, including orgasm, can be compared to the success of oral therapy for erectile dysfunction in men. A marked difference, however, between the treatment of erectile dysfunction in men and the present treatment of sexual dysfunction in women is that local treatment with DHEA achieves its beneficial effects without significant systemic exposure to a drug, thus avoiding potential systemic risks.

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INTRAVAGINAL DHEA AND SEXUAL FUNCTION

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76