

SECRETARÍA DE INDUSTRIA Y COMERCIO



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As. 98.167

Señora

**JEFE DE DIVISIÓN DE NUEVAS CREACIONES
SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO**

E. S. D.

REF: Expediente No. 05 075 357 – solicitud de patente de invención a favor
de ASTRAZENECA AB

Respuesta al oficio No. 9909 del 28 de agosto de 2009

JUAN PABLO CADENA SARMIENTO, portador de la tarjeta profesional No. 57.492 del C.S.J., obrando como apoderado de la sociedad ASTRAZENECA AB, según lo acredita el documento de poder presentado ante esa División y me permito dar respuesta a las observaciones de carácter técnico y jurídico, relacionadas con la patentabilidad, para la concesión de la patente de acuerdo con la Decisión 486 según el concepto técnico No. 2399 notificado por fijación en lista de fecha 28 de agosto de 2009 cuyo término fue prorrogado el 19 de noviembre de 2009.

En primer lugar, adjuntamos un nuevo capítulo reivindicatorio de 1 reivindicación que subsana las objeciones realizadas por el Señor Examinador. Este capítulo no amplía la materia inicialmente divulgada y encuentra entero sustento en la descripción.

Asímismo, respetuosamente discrepamos de la opinión del Señor Examinador acerca de la carencia de novedad para lo cual presentamos los siguientes argumentos. De acuerdo al nuevo capítulo reivindicatorio, la combinación específica de anastrozol, levonorgestrel y etinil estradiol no se encuentra divulgada en D1. Aunque este documento enseña varias posibilidades de inhibidores de aromatasa, progestinas y estrógenos, no se

sugiere o indica en ningún momento la combinación específica de los tres componentes de la presente solicitud, por lo que consideramos que la presente invención es nueva.

Lo que los inventores encontraron de manera inesperada que la combinación específica del inhibidor de aromatasa anastrozol, la progestina levonorgestrel y el estrógeno etinil estradiol no solo es efectiva en el tratamiento de esta enfermedad sino que además reduce sustancialmente la pérdida ósea como efecto secundario no deseado. Además, de acuerdo a la descripción de la presente solicitud, se esperaría que la progestina ayude a prevenir las pérdidas óseas causadas por el inhibidor de aromatasa, sin embargo estos compuestos causan por lo general un exceso de sangrado como efecto secundario no deseado.

De acuerdo con lo anterior, no es esperado que al realizar una combinación de los tres compuestos específicos de la presente solicitud se logre una composición farmacéutica efectiva contra la endometriosis y que se evita al mismo tiempo la pérdida ósea característica de los inhibidores de aromatasa, el sangrado de las progestinas, y el aumento de las lesiones característico de los estrógenos. Por esta razón, consideramos que la presente solicitud concede un efecto técnico no esperado y constituye un avance en el estado de la técnica por lo que tiene nivel inventivo.

Con el fin de dar un mayor soporte al efecto técnico inesperado de la presente invención, se adjunta el artículo de referencia *Fang et al (2002) Journal of Clinical Endocrinology & Metabolism 87(7), 3460-3466*, donde se habla de los efectos esperados de realizar una combinación de un estrógeno y un inhibidor de aromatasa en una composición farmacéutica. Según este artículo, al realizar experimentos en ratones la adición de estrógenos en los inhibidores de aromatasa resulta en un incremento de los tamaños de lesión causada por endometriosis y por ende en la intensificación de esta condición médica.

En vista de lo anteriormente expuesto, respetuosamente solicitamos el retiro de las objeciones a la presente solicitud y la concesión del privilegio solicitado, ya que en opinión del solicitante cumple con todos los requisitos establecidos en la decisión 486.

De la señora Jefe,



JUAN PABLO CADENA SARMIENTO

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T.P. No. 57492 del C.S.J.

Calle 70A No. 4-41

Bogotá, 5 de enero de 2010

MXB/NFA

Anexo

REIVINDICACIONES

1. Una composición farmacéutica que comprende anastrozol, levonorgestrel y etinil estradiol.

Genetic or Enzymatic Disruption of Aromatase Inhibits the Growth of Ectopic Uterine Tissue

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Aromatase P450 (P450arom) is the key enzyme for the biosynthesis of estrogen that is essential for the growth of human endometriosis, a pathology characterized by endometrium-like tissue on the peritoneal surfaces of abdominal organs manifest by pelvic pain and infertility. Surgically transplanted autologous uterine tissue to ectopic sites on the peritoneum in mice has been used as an animal model to study endometriosis. Using this mouse model, we evaluated the roles of the P450arom gene and aromatase enzyme activity in the growth of endometriosis represented by ectopic uterine tissues in mice. Endometriosis was induced surgically in the following groups of mice: 1) untreated transgenic mice with disrupted P450arom gene (ArKO); 2) ArKO mice treated with systemic estrogen; 3) untreated wild-type (WT) mice; 4) WT mice treated with estrogen; 5) WT mice treated with the aromatase inhibitor, letrozole; and 6) WT mice treated with letrozole and estrogen. Each group contained eight mice; +/- littermates of ArKO mice were used as WT controls. Treatment with estrogen increased the size of ectopic uterine tissues in

ArKO and WT mice significantly. The ectopic uterine lesions in untreated and estrogen-treated ArKO mice were strikingly smaller than those in untreated and estrogen-treated WT controls, respectively. Systemic treatment of WT mice with letrozole significantly decreased the lesion size in a dose-dependent manner. The addition of estrogen to letrozole treatment increased the ectopic lesion size, although these lesions were significantly smaller than those in mice treated with estrogen only. As tissue controls, the effects of these conditions on normally located (eutopic) uterine tissue were evaluated. The effects of disruption of the P450arom gene and treatments with letrozole and estrogen seemed to be more profound on ectopic tissues, suggesting that ectopic tissues might be more sensitive to estrogen for growth. We conclude that both an intact P450arom gene and the presence of aromatase enzyme activity are essential for the growth of ectopic uterine tissue in a mouse model of endometriosis. (*J Clin Endocrinol Metab* 87: 3460-3466, 2002)

ENDOMETRIOSIS IS CHARACTERIZED by the presence of endometrial glands and stroma within the pelvic peritoneum and other extrauterine sites and is linked to pelvic pain and infertility. It is a common condition, affecting 1 in 10 women in the reproductive age group (1-3). Although the etiology and exact mechanism for the development of endometriosis are not well understood, both circumstantial and laboratory evidence are indicative of a critical role of estrogen in the establishment and maintenance of this disease (4). The usefulness of GnRH agonists, which suppress ovarian steroidogenesis in the management of endometriosis, is well recognized. There are, however, two important caveats, which are not addressed by the GnRH agonist treatment. First, large quantities of estrogen can be produced locally within the endometriotic cells; this represents an intracrine mechanism in addition to ovarian secretion, which is the classical mechanism for estrogen production (5). Local estrogen biosynthesis is not blocked by any of the currently used treatments for endometriosis. Second, estrogen produced in peripheral tissue sites (e.g. adipose tissue and skin fibroblasts) may give rise to significant circulating levels of estrogen (6). Additionally, defective inactivation of estrogen in endometriosis in contrast to eutopic endometrium may

further increase local concentrations of the potent estrogen (*i.e.* estradiol) (7). Thus, the origin of estrogen in target tissues can arise from three likely sources: 1) ovarian secretion via an endocrine mechanism (endocrine), 2) peripheral formation that is sufficient to increase circulating estrogen (endocrine), and 3) local formation in the target tissue (intracrine or paracrine).

Aromatase is the key enzyme for estrogen formation in human tissues. The conversion of C₁₉ steroids to estrogens by aromatase P450 (P450arom) takes place in a number of human tissues, e.g. ovary, placenta, adipose tissue, skin, and brain (8). We previously demonstrated significant levels of P450arom mRNA and activity in the stromal cell component of endometriotic tissues, whereas aromatase expression is either only barely detectable or most commonly absent in the eutopic endometrium (9, 10). Moreover, aromatase activity and P450arom mRNA levels in cultured endometriotic stromal cells could be induced strikingly by cAMP analogs or PGE₂ to extremely high levels comparable to those found in ovarian granulosa cells or the placental syncytiotrophoblast (10). The clinical significance of local aromatase activity in endometriotic tissue was exemplified recently by the successful use of an aromatase inhibitor to treat an unusually aggressive case of recurrent postmenopausal endometriosis that was resistant to progestin (11). Collectively, these data are suggestive that aromatase expression in one or more human tissues is essential for the growth and persistence of

Abbreviations: ArKO, Aromatase knockout; CE, conjugated estrogens; E, estrogen; P450arom, aromatase P450; PCNA, proliferating cell nuclear antigen; WT, wild type.

77
77

ectopic endometrium-like lesions, *i.e.* endometriosis, as this pathological tissue is dependent on biologically active estradiol.

Studies in rodents in which endometriosis was surgically induced have demonstrated a critical effect of surgically transplanted uterine tissues on fertility (12–14). Estrogen gave rise to growth, whereas pregnancy and lactation caused atrophy of these ectopic uterine lesions (14). These findings have demonstrated that the rodent model of endometriosis is useful for the study of responsiveness of ectopic lesions to steroid hormones (12–14).

The aromatase knockout (ArKO) mouse has been developed as a mouse model of estrogen insufficiency by targeted disruption of the *P450arom* (*Cyp19*) gene (15). We have used ArKO mice by surgically transplanted uterine tissue to peritoneal surfaces to directly determine the role of aromatase in the development and growth of ectopic uterine lesions. We demonstrated for the first time that in mice genetically deprived of estrogen production, ectopic uterine tissues remain atrophic. Adding back systemic estrogen causes growth of this ectopic tissue in ArKO mice. By analogy, treatment of wild-type mice systemically with an aromatase inhibitor significantly reduces the size of ectopic uterine implants. Using this *in vivo* model, we studied the growth properties of aberrantly located (*i.e.* ectopic) *vs.* normally located (*i.e.* eutopic) endometrial tissue in response to genetically and pharmacologically induced estrogen deficiency and thus the role of aromatase in endometriosis.

Materials and Methods

Animals

All mice had C57BL/6 backgrounds. All mice were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals. Disrupting the *Cyp19* gene as described previously (15) generated ArKO mice. Mice were genotyped by PCR as described previously (16). We maintain an ArKO mouse colony in this laboratory and genotype them routinely. Wild-type (WT) littermates were used as controls. Animals were maintained under specific pathogen-free conditions and had unlimited access to drinking water.

Surgery

At the time of surgery, all mice were 6 months of age. We induce endometriosis-like lesions through transplantation of one of the uterine horns to the bowel mesentery as described previously (12). Briefly, the animal was placed under general anesthesia, and a midventral incision was made to expose the uterus and intestines. The left uterine horn was ligated at both ends and removed to warm medium (Ham's F-12). The uterine horn was split longitudinally and then cut into three square pieces measuring approximately 2 mm on each dimension. Three equal pieces of left uterine tissues were sutured with serosal layer in direct apposition to the peritoneum close to a vessel, as is the endometrial layer of the uterine square facing the abdominal cavity. A single suture (4-0 VP557) was used for each piece of tissue. The abdomen was then closed using the same suture material. The mice were killed within 8 wk to assess the size, histology, and proliferating cell nuclear antigen (PCNA) content of the endometriotic lesion.

Experimental design

Initially female mice were divided into six groups. WT littermates of ArKO mice were used to assess the effects of the aromatase inhibitor letrozole. WT littermates also served as control groups for ArKO mice. Endometriosis was induced surgically in all of the following groups of mice: 1) ArKO mice, 2) untreated WT mice, 3) ArKO mice treated with

conjugated estrogens (CE), 4) WT mice treated with CE, 5) WT mice treated with letrozole, and 6) WT mice treated with letrozole added to the CE regimen. The transplanted (ectopic) and eutopic uterine tissues were evaluated within 8 wk after surgery. Mice were killed with CO₂ immediately before the tissues were harvested.

Estrogen supplementation

CE were purchased from Ayerst Laboratories, Inc. (Philadelphia, PA). Ten micrograms of CE were dissolved in 100 μ l saline and administered sc. Various quantities of the aromatase inhibitor letrozole [4,4'-(1H-1,2,4-triazol-1-ylmethylene) dibenzonitrile; Novartis, Basel, Switzerland] were first dissolved in ethanol and then diluted with saline and administered sc every day in a volume of 100 μ l.

Evaluation of ectopic and eutopic uterine tissues

Eight weeks after peritoneal transplantation of uterine tissues in treated or untreated mice, animals were killed with CO₂. Mice were immediately fixed in the supine position on a platform, the abdomen was opened, and all three dimensions of ectopic and eutopic uterine tissues were measured using a caliper. The volume of each ectopic uterine tissue was calculated by following formula: $V_{\text{ectopic}} = 0.52 \times A \times B \times C$, where A, B, and C denote width, length, and height, respectively. The volume of the cylinder-shaped eutopic uterine horn was calculated by the following formula: $V_{\text{eutopic}} = \pi r^2 h$, where $r = (D_1 + D_2)/4$, D_1 and D_2 are diameters at each end of the uterine horn, and h is length of the uterine horn. Tissues were also photographed using a digital camera. All images of transplanted tissues and remaining eutopic uterine horns were taken using the DP10 microscope digital camera system (Olympus Corp., Melville, NY) at $\times 2.5$ magnification. We used the remaining eutopic uterine horn as a control tissue in each mouse. These normally located eutopic uterine tissues were evaluated with respect to size, weight, and proliferation. For histological assessment, sections of ectopic tissues together with adherent organs (bowel and its mesentery) and eutopic uterine tissues were fixed in 10% neutral buffered formalin. After appropriate fixing for 2 d before dehydration, tissues were trimmed, dehydrated through ascending grades of ethyl alcohol, cleared in xylene, and infiltrated with paraffin wax. Tissues were embedded in paraffin and 5- μ m sections were prepared. The sections were heat-dried and stained with hematoxylin and eosin.

Western blot

Western blotting was used to determine the levels of PCNA in ectopic uterine tissues. Briefly, tissue were homogenized in lysis buffer and transferred to nylon membrane. Nonspecific binding was blocked by incubation overnight at 4 $^{\circ}$ C with Tris-buffered saline containing 0.05% Triton X-100 and 5% nonfat dry milk. Filters were exposed to antibody raised against PCNA (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 1 h at room temperature, and antimouse IgG-horseradish peroxidase for 40 min at room temperature. Chemiluminescent Super Signal West Fento Stable Peroxide Buffer (Santa Cruz Biotechnology, Inc.) was used to detect the signals.

Statistics

We initially used two-tailed ANOVA to determine the statistical significance of the differences between multiple groups. This was followed by Newman-Keuls multiple comparisons test to calculate differences between individual groups. The P value for the difference between the means of two groups was calculated, where α was equal to 0.05 and power ($1 - \beta$) was 0.80.

Results

Effects of the disruption of the *P450arom* (*Cyp19*) gene on the growth of ectopic uterine tissue

To assess the *in vivo* role of the *P450arom* gene in the growth of ectopic uterine tissue, uterine tissues were surgically transplanted to the peritoneum of the bowel mesentery in adult female ArKO and WT mice. We preserved ovaries

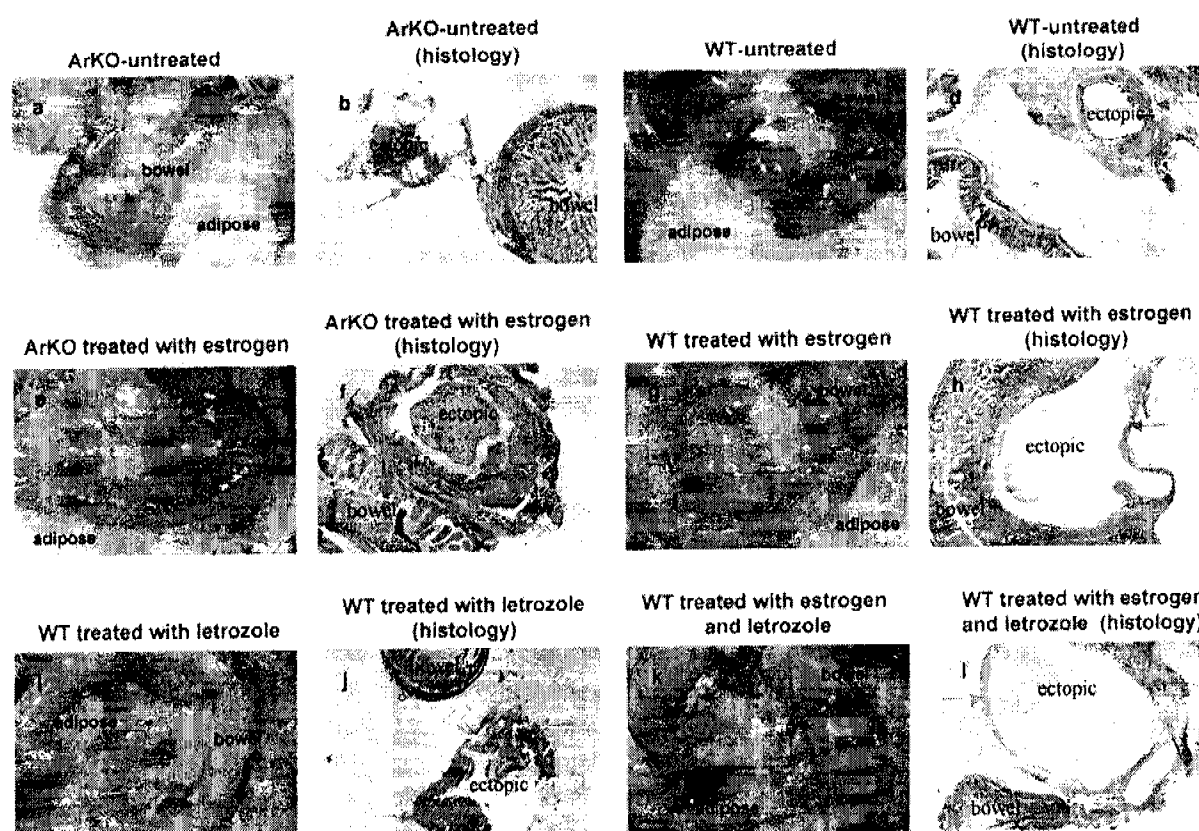


FIG. 1. Ectopic uterine tissues of six treatment groups. Images were taken immediately after the mice were killed. *Top panels* (A and C) show ectopic uterine tissue (*between arrows*) of untreated ArKO and WT mice. Ectopic uterine tissues are barely visible in untreated ArKO mice (A), whereas untreated WT mice (C) had lesions larger than those in ArKO mice. *Middle panels* show the growth of ectopic tissues in both ArKO (E) and WT (G) mice treated with conjugated estrogens. The *bottom panel* (I) shows diminished tissue size in WT mice treated with the aromatase inhibitor letrozole (relative to C). K, The addition of estrogen to letrozole treatment increases the size of ectopic uterine tissue (magnification, $\times 2.5$). Histological sections confirm the development and extent of growth of ectopic tissues transplanted to the bowel mesentery. Histological examination is consistent with the gross anatomy of corresponding ectopic tissues (hematoxylin and eosin stain; magnification, $\times 4$).

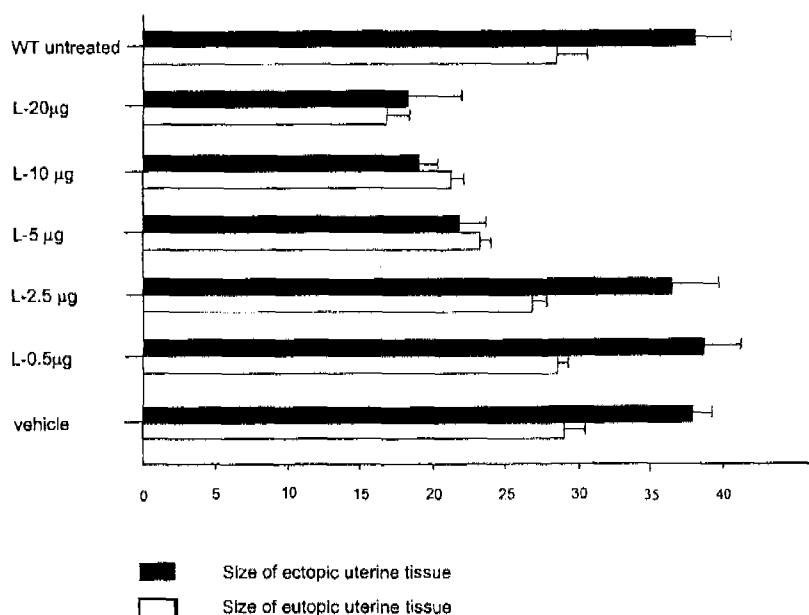
TABLE 1. Volumes of ectopic and eutopic uterine tissues

Group	No.	Ectopic uterine tissues		Eutopic uterine tissues	
		Volume (mm ³)	P	Volume (mm ³)	P
1. ArKO-untreated	8	0.83 $\pm$ 0.08	P ¹⁻² < 0.0001	4.49 $\pm$ 0.24	P ¹⁻² < 0.0001
2. WT-untreated	8	36.06 $\pm$ 1.73		29.60 $\pm$ 1.06	
3. ArKO-estrogen	8	30.53 $\pm$ 1.74	P ¹⁻³ < 0.0001	27.65 $\pm$ 1.15	P ¹⁻³ < 0.0001
			P ³⁻⁴ < 0.0001		
4. WT-estrogen	8	81.05 $\pm$ 3.88	P ²⁻⁴ < 0.0001	60.68 $\pm$ 1.85	P ²⁻⁴ < 0.001
5. WT-letrazole	8	18.62 $\pm$ 1.16	P ²⁻⁵ < 0.001	22.03 $\pm$ 0.79	P ²⁻⁵ < 0.001
6. WT-estrogen-letrazole	8	40.92 $\pm$ 2.30	P ⁴⁻⁶ < 0.001	37.40 $\pm$ 2.30	P ⁴⁻⁶ < 0.001
			P ⁵⁻⁶ < 0.001		P ⁵⁻⁶ < 0.001

in all groups of mice to mimic the intact function of ovary as in premenopausal women with endometriosis. First, we compared the size of ectopic lesions in untreated ArKO and WT mice (Fig. 1, A and C). Eight weeks from the time of transplantation of uterine tissues to the peritoneum, transplants remained as small scars in untreated ArKO mice at the end of the 8-wk period (0.83 $\pm$ 0.08 mm³), whereas transplanted uterine tissues in untreated WT mice grew as round

lesions (36.06 $\pm$ 1.73 mm³; Fig. 1, A and C, and Table 1). When exogenous estrogen was administered to these mice (10 μ g CE, sc, every day) for 10 wk (starting from 2 wk before surgery), the size of ectopic uterine tissues increased significantly in both groups (Fig. 1, E and G, and Table 1). These gross anatomical findings were accompanied by similar change in histological sections (Fig. 1, B, D, F, and H). These findings are suggestive that a functional P450arom gene is

FIG. 2. Volume of ectopic and eutopic uterine tissues in response to increasing doses of letrozole in WT mice. Six sc doses of letrozole (0, 0.5, 2.5, 5, 10, or 20 μ g) were used to treat WT animals for 4 wk. The minimum critical dose for size reduction was 5 μ g/d. The overall size decrease in eutopic uterine tissue was less than that seen in ectopic uterine tissue using the same dose.



essential for the growth of ectopic uterine implants. Exogenous estrogen partially substitutes for the effects of an intact P450arom gene, but does not fully restore the WT phenotype with respect to lesion size (see statistically significant difference between groups 3 and 4 in Table 1).

Effects of the aromatase inhibitor, letrozole on the growth of ectopic uterine tissue

Thus, we concluded that the growth of ectopic uterine implants is dependent on the product of the P450arom gene or estrogen. To demonstrate that aromatase enzyme activity was essential for the growth of these implants, WT animals were treated with letrozole, a potent medication that inhibits the function of the P450arom protein. Letrozole was administered sc for 4 wk with or without treatment of animals with CE before and during letrozole treatment. Letrozole treatment (10 μ g/d, sc) significantly decreased the implant size (Fig. 1I and groups 2 and 5 in Table 1), whereas adding CE (10 μ g/d, sc) back to letrozole treatment partially restored the lesion size (Fig. 1K and Table 1). Add-back estrogen to letrozole-treated mice, however, did not fully restore the ectopic lesion size to that observed in controls treated with estrogen only (see statistically significant difference between groups 4 and 6 in Table 1).

The suppressive effect of letrozole on the growth of ectopic uterine tissue was dose dependent (Fig. 2). We found that the minimum dose of letrozole that gave rise to a significant decrease in the lesion size was 5 μ g/d, sc continued for 4 wk. Increases in the dose up to 20 μ g/d did not result in further reduction in the ectopic lesion size (Fig. 2). Three mice were included in each group for this dose-response study (total $n = 21$ in Fig. 2).

In summary, P450arom protein with an intact enzyme activity and an intact P450arom (*Cyp19*) gene are required for

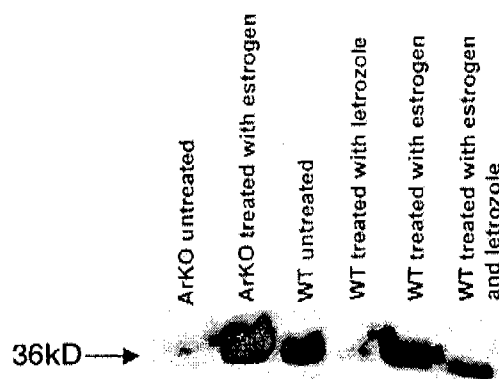


FIG. 3. Levels of PCNA in ectopic uterine tissues of mice under various treatments. Western analysis showed minimal proliferative activity (PCNA levels) in ectopic uterine tissues in ArKO mice and WT mice treated with the aromatase inhibitor letrozole. Treatment of these mice with estrogen increased levels of PCNA protein (35KD) significantly.

the growth of ectopic uterine tissues in mice. Disruption of either the gene or enzyme activity of its product gives rise to significant reductions in the lesion size.

Effects of disruption of the P450arom gene or the function of its product on cell proliferation

We determined the effects of an intact P450arom gene or its product on cell proliferation in ectopic uterine lesions. We determined the protein levels of PCNA for this purpose. PCNA levels in ectopic uterine tissues were strikingly decreased in ArKO mice (Fig. 3, lane 1) and letrozole-treated WT mice (Fig. 3, lane 4) compared with untreated WT mice

(Fig. 3, lane 3). The addition of CE to ArKO or letrozole-treated WT mice (Fig. 3, lanes 2 and 6) restored cell proliferation (Fig. 3). This experiment was performed three times with reproducible results (Fig. 3).

Effects of disruption of the P450arom gene or the function of its product on eutopic uterine tissue

The volume and weight of the remaining left uterine horn after surgery and treatment were determined, and eutopic uterine tissue was evaluated as an additional control (Fig. 4, A and C, and Tables 1 and 2). Significant changes in the size and weight of eutopic uterine tissue accompanied disruption of the P450arom gene and inhibition of aromatase activity with letrozole (Tables 1 and 2). These changes were similar to those seen in ectopic uterine tissues and were reversible in part by treatment with CE. Histological examination confirmed the changes in volume and weight (Fig. 4).

The decreases in eutopic uterine tissue weight or volume in response to the disruption of the P450arom gene or inhibition of aromatase enzyme were significant and dose de-

pendent (Tables 1 and 2 and Figs. 4 and 5). These decreases, however, were not as pronounced as the corresponding conditions for ectopic uterine tissues (Table 1). In ArKO mice, the volume of the ectopic uterine tissues was 2% that in WT mice, whereas the volume of mean eutopic uterine tissues was 15% that in WT mice (Table 1). By the same token, ectopic uterine tissue volume decreased by 48% in response to letrozole, whereas letrozole-induced volume reduction was 26% for eutopic uterine tissue (Table 1). These observations suggest that ectopic uterine tissue is more dependent for growth on

TABLE 2. Weight of normally located (eutopic) uterine tissues

Group	Weight (mg)	No.	P
1. ArKO-untreated	1.9 ± 0.2	5	P ¹⁻² < 0.0001
2. WT-untreated	45.0 ± 0.8	5	
3. ArKO-estrogen	36.2 ± 0.9	5	P ¹⁻³ < 0.0001
4. WT-estrogen	53.2 ± 1.1	5	P ²⁻⁴ < 0.001
5. WT-letrazole	39.8 ± 0.4	5	P ²⁻⁵ < 0.001
6. WT-estrogen-letrazole	43.0 ± 0.9	5	P ⁵⁻⁶ < 0.05

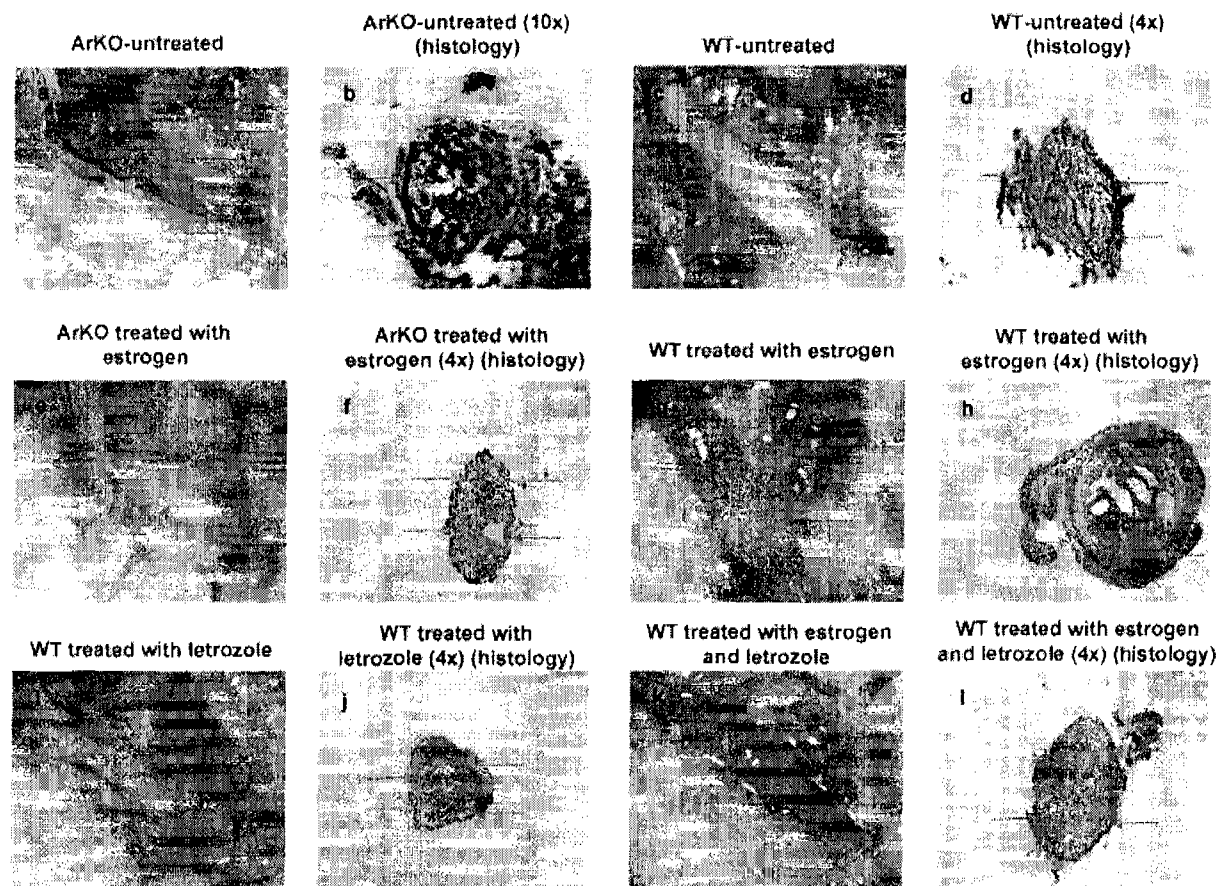
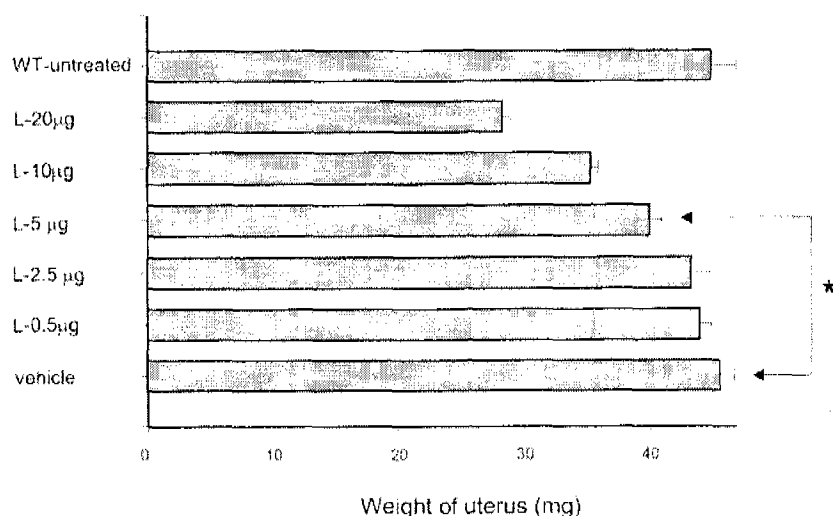


FIG. 4. Eutopic (normally located) uterine tissues of mice under various treatments. The sizes of eutopic uterine tissues of untreated and treated ArKO and WT mice showed changes comparable to those observed in ectopic uterine tissues shown in Fig. 1. Magnification: A, C, E, G, I, and K, ×2.5). Histological sections confirmed gross anatomic changes in corresponding eutopic uterine tissues. Magnification: B, ×10; D, F, H, J, and L, ×4).

FIG. 5. Weight of eutopic uterine tissues in response to increasing doses of letrozole in WT mice. Weight of eutopic uterine tissues decreased progressively in response to letrozole (5, 10, or 20 $\mu\text{g}/\text{d}$ for 4 wk). Results were comparable to changes in volume.



a functional P450arom gene and protein compared with eutopic uterine tissue.

Discussion

In this study we used ArKO mice as an *in vivo* model to determine the role of the P450arom gene in the growth of ectopic uterine tissue. We also employed the aromatase inhibitor letrozole to determine the role of aromatase enzyme activity in the growth of ectopic uterine tissue in WT mice. This model clearly indicated that both P450arom gene and P450arom protein with an intact enzyme activity are essential for the growth of ectopic and eutopic uterine tissues.

The antiproliferative effects of aromatase inhibitors on the eutopic endometrium have been reported previously (17). Our findings were consistent with this report. Our results were also suggestive that ectopic tissue is more sensitive to the proliferative effects of the P450arom gene and aromatase enzyme. Exogenous estrogen could be used as a surrogate treatment to induce significant growth in these tissues of mice with genetically disrupted P450arom gene or abolished aromatase enzyme activity. Thus, we demonstrated clearly that estrogen is essential for the growth of ectopic uterine tissue.

We have not analyzed the role of local aromatase expression in endometriosis, because we did not use ovariectomy in this particular study for two reasons: 1) to mimic endometriosis in premenopausal women, and 2) to study the role of ovarian aromatase expression in this model. In mice, aromatase is expressed primarily in the brain and ovaries, and estradiol is secreted only from the ovary (18–20). In our model we do not know yet whether aromatase expression is present in ectopic uterine tissues of mice. Thus, negative effects of disrupted P450arom gene or enzyme activity in this model may be a result of the absence of ovarian aromatase activity, which would preclude ovarian estradiol secretion. In fact, it was reported that sc letrozole at a dose of 5 $\mu\text{g}/\text{d}$ abolished ovarian function in mice (21).

Studies are underway to determine the role of tissue-specific P450arom expression in the mouse endometriosis model. Nonetheless, regardless of site-specific P450arom expression, this model allowed us to demonstrate the essential roles of the P450arom gene and enzyme in the growth of endometriosis.

Acknowledgments

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References

1. Sampson JA 1927 Metastasis of embolic endometriosis due to menstrual dissemination of endometrial tissue into the venous circulation. *Am J Pathol* 3:93–109
2. Sampson JA 1927 Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am J Obstet Gynecol* 144:22–25
3. Ekenazi B, Warner ML 1997 Epidemiology of endometriosis. *Obstet Gynecol Clin North Am* 24:235–258
4. Dizerga GS, Barber DL, Hodgen GD 1980 Endometriosis: role of ovarian steroids in initiation, maintenance and suppression. *Fertil Steril* 33:649–653
5. Zeitoun KM, Bulun SE 1999 Aromatase: a key molecule in the pathophysiology of endometriosis and a therapeutic target. *Fertil Steril* 72:961–969
6. Bulun SE 1999 Aromatase in aging women. *Semin Reprod Endocrinol* 17:349–358
7. Zeitoun K, Takayama K, Sasano H, Suzuki T, Moghrabi N, Andersson S, Johns A, Meng L, Putman M, Carr B, Bulun S 1998 Deficient 17 β -hydroxysteroid dehydrogenase type 2 expression in endometriosis: failure to metabolize 17 β -estradiol. *J Clin Endocrinol Metab* 83:4474–4480
8. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD, Mendelson CR, Bulun SE 1994 Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. *Endocr Rev* 15:342–355
9. Noble LS, Simpson ER, Johns A, Bulun SE 1996 Aromatase expression in endometriosis. *J Clin Endocrinol Metab* 81:174–179
10. Noble LS, Takayama K, Putman JM, Johns DA, Hinshelwood MM, Agarwal VR, Zhao Y, Carr BR, Bulun SE 1997 Prostaglandin F₂ stimulates aromatase expression in endometriosis-derived stromal cells. *J Clin Endocrinol Metab* 82:600–606
11. Takayama K, Zeitoun K, Gunby RT, Sasano H, Carr BR, Bulun SE 1998

X82
82

- Treatment of severe postmenopausal endometriosis with an aromatase inhibitor. *Fertil Steril* 69:709-713
12. Venon MW, Wilson EA 1985 Studies on the surgical induction of endometriosis in the rat. *Fertil Steril* 44:684-694
 13. Cummings AM, Metcalf J 1995 Induction of endometriosis in mice: a new model sensitive to estrogen. *Reprod Toxicol* 9:233-238
 14. Barragan JC, Brotons J, Ruiz JA, Acién P 1992 Experimentally induced endometriosis in rats: effect on fertility and the effects of pregnancy and lactation on the ectopic endometrial tissue. *Fertil Steril* 58:1215-1219
 15. Fisher CR, Graves KH, Parlow AF, Simpson ER 1998 Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the *cyp19* gene. *Proc Natl Acad Sci USA* 95:6965-6970
 16. Robertson K, O'Donnell L, Jones MEE, Meachem SJ, Boon WC, Fisher CR, Graves KH, McLachlan RI, Simpson ER 1999 Impairment of spermatogenesis in mice lacking a functional aromatase (*cyp19*) gene. *Proc Natl Acad Sci USA* 96:7986-7991
 17. Yue W, Wang J, Savinov A, Brodie A 1995 Effect of aromatase inhibitors on growth of mammary tumors in a nude mouse model. *Cancer Res* 55:3073-3077
 18. Hickey GJ, Richards JS 1988 Hormonal regulation, tissue distribution and content of aromatase cytochrome P450 messenger ribonucleic acid and enzyme in rat ovarian follicles and corpora lutea: relationship to estradiol biosynthesis. *Endocrinology* 122:1426-1436
 19. Harada N 1989 Gene structure and gene expression regulation of estrogen synthetase. *Nippon Rinsho* 47:2287-2292
 20. Britt KL, Simpson ER 2000 An age-related ovarian phenotype in mice with targeted disruption of the *CYP19* (aromatase) gene. *Endocrinology* 141:2614-2623
 21. Tekmal PR, Kirma, Gill K, Fowler K 1999 Aromatase overexpression and breast hyperplasia, an *in vivo* model-continued overexpression of aromatase is sufficient to maintain hyperplasia without circulating estrogens, and aromatase inhibitors abrogate these preneoplastic changes in mammary glands. *Endocr Relat Cancer* 6:307-314

[0035] The combination preparations of the invention are produced with the commonly used solid or liquid vehicles or diluents and the commonly used pharmaceutical-technical adjuvants corresponding to the desired type of administration with a suitable dosage in a known way. The preferred preparations consist in a dispensing form that is suitable for oral administration. Such dispensing forms are, for example, tablets, film tablets, coated tablets, capsules, pills, powder, solutions or suspensions or else depot forms.

[0036] Of course, parenteral preparations such as injection solutions can also be considered. In addition, for example, suppositories and agents for vaginal use can also be mentioned as preparations.

[0037] Corresponding tablets can be obtained by, for example, mixing the active ingredient with known adjuvants, for example inert diluents such as dextrose, sugar, sorbitol, mannitol, polyvinyl pyrrolidone, explosives such as corn starch or alginic acid, binders such as starch or gelatin, lubricating agents such as magnesium stearate or talc and/or agents for achieving a depot effect such as carboxylpolymethylene, carboxymethyl cellulose, cellulose acetate phthalate or polyvinyl acetate. The tablets can also consist of several layers.

[0038] Correspondingly, coated tablets can be produced by coating nuclei that are produced analogously to the tablets with agents that are commonly used in tablet coatings, for example polyvinyl pyrrolidone or shellac, gum arabic, talc, titanium oxide or sugar. In this case, the coated tablet shell can also consist of several layers, whereby the adjuvants that are mentioned above in the tablets can be used.

[0039] The solutions or suspensions can contain additional taste-improving agents such as saccharin, cyclamate or sugar, as well as, e.g., flavoring substances such as vanilla or orange extract. In addition, they can contain suspending adjuvants such as sodium carboxymethyl cellulose or preservatives such as p-hydroxybenzoates.

[0040] In addition, capsules can be produced, for example, by inert vehicles such as lactose or sorbitol being added and encapsulated in gelatin capsules.

[0041] Suitable suppositories can be produced by, for example, mixing with vehicles that are provided for this purpose, such as neutral fats or polyethylene glycol or derivatives thereof.

[0042] The subject of the invention is also the packaging unit, which comprises at least two components. It contains the active ingredients that are manufactured together or separated in space and as a further component directions on simultaneous administration and/or administration that is sequential in time of the dispensing forms.

[0043] Below, the invention is to be explained in more detail by examples without being limited thereto, however.

EXAMPLE 1

[0044] Combination preparation for women in menopause who do not require endometrium protection by gestagens (e.g., after a hysterectomy) for continuous daily administration:

[0045] a) Estrogen Preparation Daily dosage unit based on the preparation, e.g., 1-2 mg of estradiol-17 β or 0.625 mg of a conjugated estrogen in one dose and

[0046] b) Aromatase Inhibitors e.g., 200 mg of atamestane in one dose.

EXAMPLE 2

[0047] Combination preparation for perimenopausal women with intact uteri for a 28-day cycle:

[0048] a) Estrogen Preparation Daily dosage unit based on the preparation, e.g., 1-2 mg of estradiol-17 β or 0.625 mg of a conjugated estrogen in one dose,

[0049] b) Gestagen preparation for 10-14 days (sequential addition) Daily dosage unit based on the respective gestagen preparation, e.g., 5 mg of MPA, 1 mg of NETA, 75-150 μ g of LNG, 1 mg of CPA or 50 μ g of gestodene in one dose, and

[0050] c) Aromatase Inhibitor e.g., 200 mg of atamestane in one dose.

EXAMPLE 3

[0051] Combination preparation for postmenopausal women who have not undergone a hysterectomy for continuous daily administration:

[0052] a) Estrogen Preparation Daily dosage unit based on the preparation, e.g., 1-2 mg of estradiol-17 β or 0.625 mg of a conjugated estrogen in one dose,

[0053] b) Gestagen Preparation Daily dosage unit based on the respective gestagen preparation, e.g., 5 mg of MPA, 1 mg of NETA, 75-150 μ g of LNG, 1 mg of CPA or 50 μ g of gestodene or less in one dose, and

[0054] c) Aromatase Inhibitor e.g., 200 mg of atamestane in one dose.

[0055] List of Abbreviations

[0056] MPA=medroxyprogesterone acetate

[0057] NETA=norethisterone acetate

[0058] LNG=levonorgestrel

[0059] CPA=cyproterone acetate

1. Pharmaceutical combination preparation that comprises at least one aromatase inhibitor and at least one substance with estrogenic action.

2. Combination preparation according to claim 1, characterized in that the aromatase inhibitor is a selective aromatase inhibitor, preferably atamestane, formestane, pentrozole, arimidex, fadrozole, CGS 20267 or vorozole.

3. Combination preparation according to claim 1 or 2, wherein the substance with estrogenic action represents an estrogen or an estrogen in combination with a gestagen.

4. Combination preparation according to claim 3, wherein the estrogen is selected from the group of natural estrogens or the synthetic estrogens or derivatives thereof.

5. Combination preparation according to claim 3, wherein the gestagen is selected from the group of compounds gestodene, progesterone, desogestrel, 3-ketodesogestrel,

84

84

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES

Radicación No. 05075357

Clasificación Internacional (IPC): A61K31/5685, 31/519,31/4196, A61P15/00.

Concepto Técnico No. 263

Patente de Invención

☒

Patente de Modelo de Utilidad

☐

Vía Nacional

☐

Vía PCT (fase nacional)

☐

Cap.I

☐

Cap. II

☒

No. Sol. Internacional

PCT/GB/2004/000414

Fecha de Presentación Internal.

03/02/2004.

Como resultado del examen definitivo, realizado con fundamento en el artículo 48 de la Decisión 486 de la Comisión de la Comunidad Andina, se le comunica:

1. Documentos estudiados

1.1. Descripción: Folios: 14 a 25 como se radicó inicialmente

1.2. Reivindicaciones: Folios: 26 a 27 como se radicó inicialmente
Folio: 75 (1 reivindicación) Fecha 2009/11/19.

1.3. Rta. del Solicitante: Folios: 72 a 82.

1.4. Prioridad: 05/02/2003; GB; 0302572.3

2. Objeto de la invención

Título de la invención: **COMPOSICIÓN QUE COMPRENDE UNA COMBINACIÓN DE UN INHIBIDOR DE AROMATASA, UNA PROGESTINA Y UN ESTRÓGENO Y SU USO PARA EL TRATAMIENTO DE ENDOMETRIOSIS.**

Problema Técnico Planteado: Necesidad de una estrategia que inhiba los síntomas de endometriosis, estimulados por estrógenos, con efectos secundarios mínimos.

Solución: Una combinación de un inhibidor de Anastrozol, Levonorgestrel y Etinil estradiol.

IPC: A61K31/5685, 31/519,31/4196, A61P15/00.

3. De la solicitud de Patente

3.1 Reivindicaciones (artículo 30 D 486)

De la composición reclamada en la reivindicación 1, no existe soporte en la descripción, ya que lo mencionado en el folio 23 pagina 10, se refiere a un estudio clínico el cual se realizó administrando Anastrozol y al mismo tiempo el levonorgestrel y un estrógeno etinilestardiol; es decir que no se utilizó una composición que contenga los tres principios activos y que ni se presentó una única composición que muestre un efecto sorprendente, (por ejemplo , una sinergia) logrado por la administración de una única composición, en comparación con el efecto obtenido por la administración de cada uno de los principios, por separado.

4. Determinación del Estado de la Técnica

La fecha que se ha tenido en cuenta para determinar el Estado de la Técnica es:

Fecha de presentación de la solicitud prioritaria: 05/02/2003.

Teniendo en cuenta la fecha indicada se realizó la búsqueda en el estado de la técnica de documentos relacionados con la solicitud encontrándose los siguientes documentos más relevantes:

Nº	Documento	Título	Fecha de publicación*
D1	US2002156059	Pharmaceutical, combination preparations that contain aromatase inhibitors and substances with estrogenic action as well as their use	Octubre 24 de 2002

D1 (folios 64-66)

5. Respuesta a los argumentos del solicitante:

Con el fin de subsanar las objeciones expuestas en el concepto técnico No. 9909 del 28 de agosto del 2009; el solicitante presenta los siguientes argumentos técnicos y modificaciones:

-En folios 72 a 75, el solicitante indica que realiza modificaciones en el capítulo reivindicatorio, el cual no amplía materia, dejando como nuevo capítulo una única reivindicación, folio 75.

Por parte de esta oficina se acepta el nuevo capítulo y con base en él se realiza el nuevo análisis de patentabilidad.

-El solicitante afirma que D1, no afecta la novedad de la reivindicación 1, ya que no hay una combinación específica de anastrozol, levonorgestrel y etinilestradiol.

Se calara que en D1, se revela implícitamente la combinación, con los tres principios activos, en el ejemplo 2 (folio 83, pg 3, párrafo 47), se divulga una combinación de un:

-estrógeno (que según el párrafo 20 y 21) puede ser etinilestradiol,

-un gestágeno LNG, el levonorgestrel, ejemplo 2.

- y el inhibidor de aromatasa, (según el párrafo 13) que puede ser el anastrozol.

86

Así mismo se aclara al solicitante que todo el documento D1, se refiere a combinaciones farmacéuticas de: inhibidores de aromatasa con sustancias de acción estrogénica y preferible en combinación con gestágenos. Por lo tanto la composición reclamada no es nueva.

-El solicitante afirma que los inventores encontraron un efecto inesperado en la combinación reclamada (folio 73) y además allegan copia de un artículo científico (folios: 76-82), como soporte técnico inesperado de la presente invención.

Se aclara que dado que la composición no es nueva, no es necesario realizar análisis de nivel inventivo, para evaluar algún efecto inesperado. Respecto al artículo allegado, en caso de ser necesario evaluarlo, se observa que la materia es diferente a lo reclamado en la reivindicación 1, ya que dicho artículo habla de la administración de un estrógeno y un inhibidor de aromatasa, en donde no aparece el géstageno, que hace parte de las características esenciales de la reivindicación 1, que son: un estrógeno, un gestágeno y el inhibidor de aromatasa, en una composición.

6. Análisis de patentabilidad (artículo 14 D486)

6.1 Novedad (artículo 16 D486)

La reivindicación 1 reclama: Una composición farmacéutica que comprende Anastrozol, Levonorgestrel y Etinilestradiol.

D1: menciona combinaciones que contienen los tres componentes: Arimidex, cuyo nombre genérico es, anastrozol (folio 65, pg 1, párrafo 13); levonorgestrel (folio 66, pg 2, párrafo 24) y el etinilestradiol (folio 66, pg 2, párrafos 20 y 21).

Las características esenciales de la composición de la reivindicación 1, es decir: Anastrozol, Levonorgestrel y Etinilestradiol, ya se divulgan en D1.

Por lo tanto se observa, que el objeto reclamado en la reivindicación 1, no es nuevo.

7. Conclusión:

En consecuencia y por todo lo expuesto anteriormente, se recomienda **Negar** las reivindicación: 1, folio: 75

Bogotá, D.C.,

CONCEPTO TECNICO No.263	EXAMINADOR TECNICO Código No.202078
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