



No. 04-001222- -00012-0000

Fecha: 2009-10-28 12:38:22 Dep. 2020 NUECREACIONE
Tra. 11 PATENTEIYII Eve: 379 FASENACIONALII
Act. 329 CTOINFORMACION Folios: 70

Señor Jefe de la
DIVISION DE NUEVAS CREACIONES
Superintendencia de Industria y Comercio
E. S. D.

Trámite 11 - Evento 379 – Actuación

Re: No. Solicitud: 04 - 001222
Fase Nacional: Solicitud de Patente Internacional No.
PCT/US02/21524
Titulo: Composiciones para el Tratamiento de
Deficiencias de Testosterona en Varones
Solicitante: Zonagen, Inc.
The Woodlands, Texas, E.U.A.

ADICIÓN A RESPUESTA AL OFICIO NO. 8176 DEL 31 DE JULIO DEL
2009

Obrando en mi condición de apoderado del solicitante en el proceso de la referencia, dentro del término legal, respetuosamente procedo a adicionar la respuesta presentada en Octubre 9, 2009 al Oficio No. 8716 de Julio 31, 2009 de ese despacho, en la forma que aparece a continuación:

1. Concesión del invento de la referencia en otras jurisdicciones

Siendo concientes de la independencia en las decisiones de esa esa oficina de patentes con respecto a otras oficinas de patentes, consideramos importante informar que contrapartes del invento de la referencia ha sido concedido en las siguientes jurisdicciones:

Nueva Zelandia

No. 551013

Australia	No.2002318225
China	No. ZL02817441.0
Hong Kong	No. HK 1071521
Corea	No. 10-0851648
Nueva Zelandia	No. 530491
Singapur	No. 101818
Sudáfrica	No. 2004/0241

2. Copia de la Figura 3

Para mayor claridad, anexamos a la presente una mejor copia de la Figura 3.

ANEXOS

1. Copia de la Figura 3.
2. Copia del Certificado de Patente No. 530491 de Nueva Zelandia.
3. Copia del Certificado de Patente No. 2002318225 de Australia.
4. Copia del Certificado de patente No. ZL02817441.0 de China.
5. Copia del Certificado de patente No. HK 1071521 de Hong Kong.

De usted atentamente,


CAMILO H. ALVAREZ BERNATE
T.P.A. No. 20,343 del CSJ
C.C. 19,219,009 de Bogotá
Codigo 5352

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+

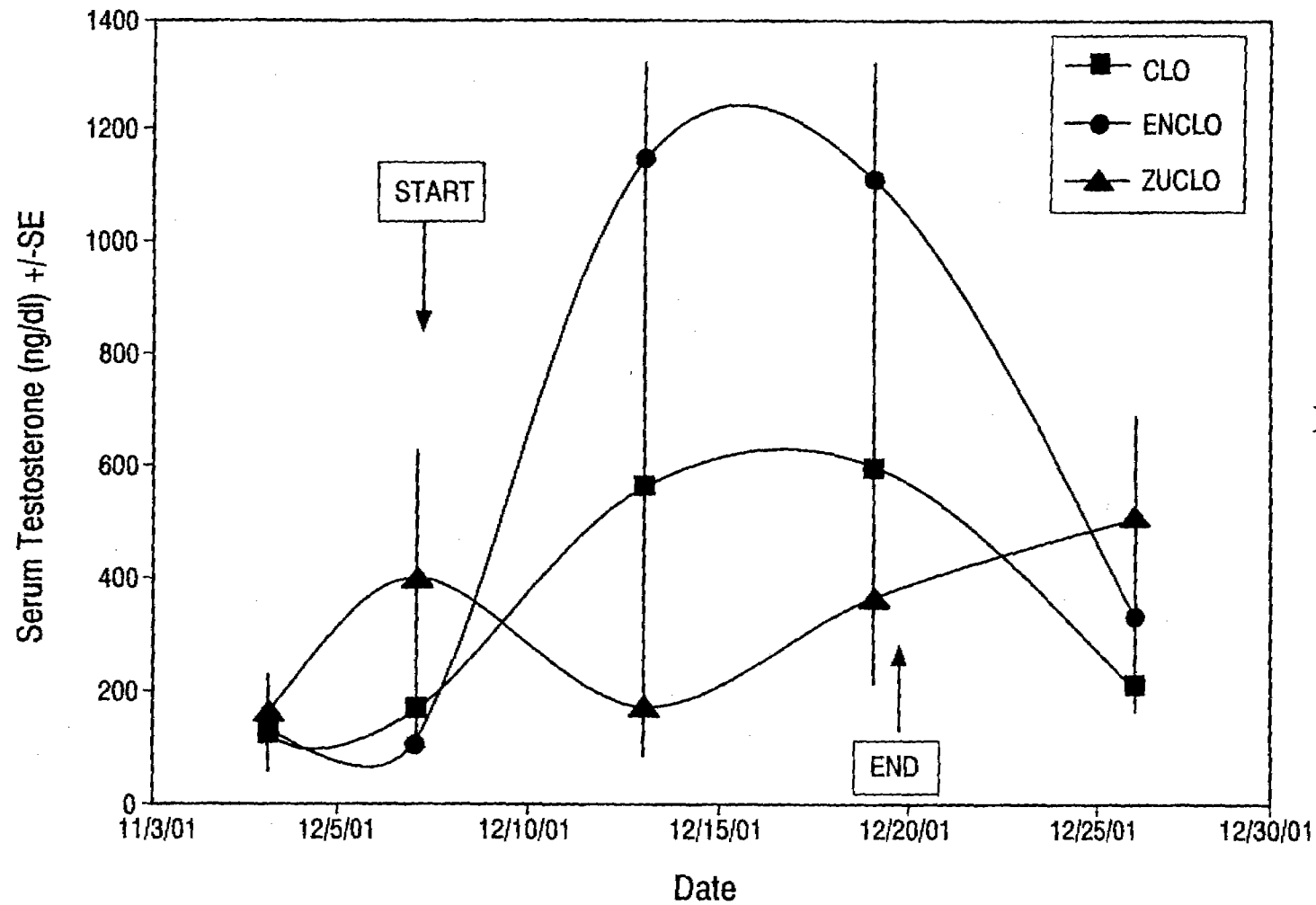


FIG. 3

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LETTERS PATENT

Number 551013

ELIZABETH THE SECOND, by the Grace of God Queen of New Zealand and Her Other Realms and Territories, Head of the Commonwealth, Defender of the Faith; To all to whom these presents shall come, Greeting:

WHEREAS pursuant to the Patents Act 1953 an application has been made for a patent of an invention for

Methods and materials for the treatment of testosterone deficiency in men

(more particularly described in the complete specification relating to the application).

AND WHEREAS

Repros Therapeutics Inc., 2408 Timberloch Place, B-7, The Woodlands, Texas 77380, United States of America

(hereinafter together with his or their successors and assigns or any of them called "the patentee") is entitled to be registered as the proprietor of the patent hereinafter granted:

Address for service: A J PARK, 6th Floor, Huddart Parker Building, 1 Post Office Square, Wellington 6011, New Zealand

NOW, THEREFORE, We by these letters patent give and grant to the patentee our special licence, full power, sole privilege, and authority, that the patentee by himself, his agents, or licensees and no others, may subject to the provisions of any statute or regulation for the time being in force make, use, exercise and vend the said invention within New Zealand and its dependencies during a term of twenty years from 9 July 2002 and that the patentee shall have and enjoy the whole profit and advantage from time to time accruing by reason of the said invention during the said term:

AND WE strictly command all our subjects whomsoever within New Zealand and its dependencies that they do not at any time during said term either directly or indirectly make use of or put into practice the said invention, nor in any way imitate the said invention without the consent, licence, or agreement of the patentee in writing under his hand, on pain of incurring such penalties as are prescribed by law and of being answerable to the patentee according to law for his damages thereby occasioned:

PROVIDED ALWAYS:

- (1) That these letters patent shall determine and become void if the patentee does not from time to time pay the renewal fees prescribed by law in respect of the patent;
- (2) That these letters patent are revocable on any of the grounds prescribed by the Patents Act 1953 as grounds for revoking letters patent;
- (3) That nothing in these letters patent shall prevent the granting of licences in the manner in which and for the considerations on which they may by law be granted;
- (4) That these letters patent shall be construed in the most beneficial sense for the advantage of the patentee.

IN WITNESS whereof We have caused these letters patent to be signed and sealed on 14 August 2008 with effect from 9 July 2002.



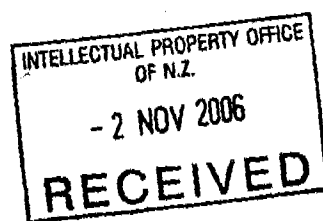
Neville Harris

Neville Harris
Commissioner of Patents, Trade Marks and Designs

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10052750002



NEW ZEALAND
PATENTS ACT, 1953

No:
Date:

Divided out of No. 530491
Dated 9 July 2002

COMPLETE SPECIFICATION

METHODS AND MATERIALS FOR THE TREATMENT OF TESTOSTERONE
DEFICIENCY IN MEN

We, ZONAGEN, INC., of 2408 Timberloch Place, B-4, The Woodlands, Texas 77380, United States of America, do hereby declare the invention for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:

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secretion of testosterone in the Leydig cells of the testes, while FSH assists in inducing spermatogenesis.

[0005] Testosterone is most often measured as "total testosterone." This measurement includes testosterone that is bound to sex hormone-binding globulin (SHBG) (~44%) and is therefore not bioavailable and testosterone which either is free (~2%) or loosely bound to other proteins (non-SHBG-bound) (~54%).

[0006] Results from a WHO study indicate that testosterone is normally secreted in a circadian rhythm, with higher levels in the morning and nadir levels occurring around 8 to 10 p.m. See FIG. 1. This variation in testosterone secretion throughout the day becomes much less pronounced in older men (mean age equals 71 years). The importance of this rhythm is not known at this time.

[0007] Samples were obtained from both young and elderly patients every 10 minutes for 24 hours via an indwelling cannula. According to Tenover (1987) the mean 24 hr total serum testosterone levels in healthy young men (age range 22 yrs.-35 yrs. mean 27.3 yrs) was 4.9 ± 0.3 ($\pm$ SEM) mg/ml (17.0 nmol/L) while older men (age range 65yrs -- 84 yrs. mean 70.7 yrs.) had a significantly lower mean 24 hrs. total serum testosterone level of 4.1 ± 0.4 mg/ml. ($P < 0.5$; 14.2 nmol/L).

[0008] Total serum testosterone levels obtained from single random samples were also significantly lower in older men (4.0 ± 0.2 mg/ml [13.9 n nmol/L]) as compared to 4.8 ± 0.2 mg/ml [16.6 nmol/L] in healthy young men.

[0009] Testosterone deficiency can result from underlying disease or genetic disorders and is also frequently a complication of aging. For example, primary hypogonadism results from primary testicular failure. In this situation, testosterone levels are low and levels of pituitary gonadotropins (LH and FSH) are elevated. Secondary hypogonadism is due to inadequate secretion of the pituitary gonadotropins. In addition to a low testosterone level, LH and FSH

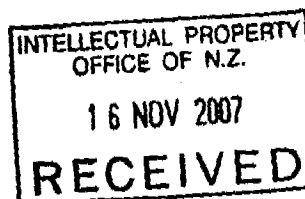
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levels are low or low-normal. Some of the sequelae of adult testosterone deficiency include a wide variety of symptoms including: loss of libido, erectile dysfunction, oligospermia or azoospermia, absence or regression of secondary sexual characteristics, progressive decrease in muscle mass, fatigue, depressed mood and increased risk of osteoporosis.

[0010] Several forms of testosterone therapy exists in the United States today. Recently, transdermal preparations have gained favor in the market. However, a scrotal testosterone patch results in supraphysiologic levels of 5 α -dihydrotestosterone (DHT) due to the high concentration of 5 α -reductase in scrotal skin. It is not known whether these elevated DHT levels have any long-term health consequences. Nonscrotal systems are considered more convenient and most patients achieve average serum concentrations within the normal range and have normal levels of DHT. Oral testosterone therapy is not recommended because doses required for replacement therapy are associated with significant risk of hepatotoxicity.

SUMMARY

[0011] The present invention is directed to compositions useful for increasing testosterone levels in male mammals and for ameliorating or preventing the sequelae of low testosterone levels. In one of its aspects the invention is directed to compositions having active ingredients comprising 0% to 29% weight/weight of (*cis*-, *-Z*-, *trans*-clomiphene) (hereinafter "*cis*-clomiphene") and 100% to 71% w/w (*trans*-, *E*-, *cis*-clomiphene) (hereinafter "*trans*-clomiphene") or pharmaceutically acceptable salts thereof or solvates thereof and optionally one or more pharmaceutically acceptable diluents, adjuvants, carriers or excipients, with the proviso that said composition does not comprise 11% *cis*-clomiphene and 89% *trans*-clomiphene. Among the preferred compositions of the present invention which contain both *cis*-clomiphene and *trans*-clomiphene invention are compositions wherein the ratio of *trans*-clomiphene and *cis*-clomiphene is greater than 1. A more preferred composition according to the present invention comprises about 100% w/w of active ingredients of *trans*-clomiphene or a pharmaceutically acceptable salt thereof. All compositions of the present invention may further comprise suitable pharmaceutical excipients diluents, carriers, and the like. Analogs



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of the *cis*-clomiphene and *trans*-clomiphene are also contemplated for use in all aspects of the present invention.

[0011a] The present invention is also directed to a composition comprising *cis*-clomiphene and *trans*-clomiphene or analogs thereof or pharmaceutically effective salts or solvates thereof and optionally one or more pharmaceutically acceptable diluents, adjuvants, carriers or excipients and wherein the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 71/29, with the proviso that said ratio is not 8/1.

[0012] Described are methods for increasing serum testosterone levels in hypogonadal male mammals (and for ameliorating or preventing the sequelae of low testosterone levels), the method comprising administering to a subject male an effective amount of a composition according to the present invention, the compositions having active ingredients comprising 0% to 29% weight/weight of *cis*-clomiphene and 100% to 71% w/w *trans*-clomiphene including any of their pharmaceutically acceptable salts thereof. Among the preferred methods are those in which the administered compositions contain both isomers wherein the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 1. A more preferred method comprises administering to the male a composition comprising about 100% w/w of *trans*-clomiphene.

BRIEF DESCRIPTION OF THE DRAWING

[0013] FIG. 1 is a graphic representative of the normal secretory total serum testosterone profiles in healthy men (young and old).

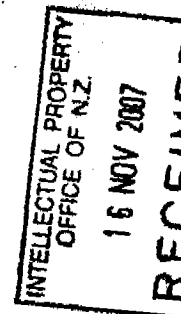
[0014] FIG. 2 shows the chemical structure of clomiphene citrate.

[0015] FIG. 3 is a graphic demonstration of the time course of serum testosterone levels with Clomid, Enclomid and Zuclomid.

DETAILED DESCRIPTION

[0016] The present invention describes methods and compositions useful for increasing testosterone levels in male mammals and for ameliorating or preventing the sequelae of low testosterone levels including but not limited to those described above.

[0017] Clomiphene (FIG. 2) is an antiestrogen related to tamoxifen that blocks the normal estrogen feedback on the hypothalamus and subsequent negative feedback on the pituitary. This leads to increases in luteinizing hormone (LH) and follicle stimulating hormone (FSH).



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In men, these increased levels of gonadotropins stimulate the Leydig cells of the testes and result in the production of higher testosterone levels. Clomiphene citrate has the following structure:

[0018] Ernst *et al.*, J. Pharmaceut. Sci. 65:148 (1976), have shown that clomiphene is a mixture of two geometric isomers which they refer to as *cis*-, *Z*-, clomiphene (*cis*-clomiphene or zuclomiphene) and *trans*-, *E*-, clomiphene, (*trans*-clomiphene or enclomiphene).

According to Ernst, *et al.* *trans*-clomiphene HCl has a melting point of 149°C-150.5°C, while *cis*-clomiphene HCl has a melting point of 156.5°C-158°C.

[0019] Ernst *et al.* have also noted that (the *trans*-isomer) is antiestrogenic (AE) while the *cis*-isomer is the more potent and more estrogenic form and has also been reported to have anti-estrogenic activity. The authors attribute the effect of the drug on ovulatory activity to both forms stating that the mixture is more effective than *trans*-clomiphene alone. The *trans*-isomer aids ovulation at the level of the hypothalamus. The estrogenic isomer *cis*-clomiphene contributes to enhanced ovulation elsewhere in the physiologic pathway leading to ovulation. The isomers are also reported to have different *in vivo* half-life. Furthermore the *cis* form has been reported to leave residual blood levels for in excess of one month following a single dose.

[0020] Vandekerckhove, *et al.* (Cochrane Database Syst Rev 2000;(2):CD000151 (2000)) noted that ten studies involving 738 men have suggested that anti-estrogens appear to have a beneficial effect on endocrinal outcomes, *i.e.* testosterone, but there is not enough evidence to evaluate fertility effects. Nevertheless should clomiphene administration enhance testosterone levels then one could easily conclude that the drug should positively impact the side effects of testosterone deprivation as long as the testes still retain the ability to respond to gonadotropin stimulation.

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[0021] Clomiphene is currently approved as a mixture of both cis- and trans-isomers, the cis-isomer being present as about 30% to 50% (Merck Manual) for fertility enhancement in the anovulatory patient. Clomiphene improves ovulation by initiating a series of endocrine events culminating in a preovulatory gonadotropin surge and subsequent follicular rupture. The drug is recommended to be administered for 5 days at a dose of up to 100 mg daily. Clomiphene has also been associated with numerous side effects including: blurred vision, abdominal discomfort, gynaecomastia, testicular tumors, vasomotor flushes, nausea, and headaches. Furthermore, other studies suggest that clomiphene possesses both genotoxic and tumor enhancement effects. The net outcome of these observations is that clomiphene in its current format, having between 30% and 50% of the cis isomer, would be unacceptable for chronic therapy in men for the treatment of testosterone deficiency.

[0022] Clomiphene has also been used for therapeutic intervention in men with low testosterone levels. Tenover *et al.*, *J. Clin. Endocrinol. Metab.* 64:1103, (1987) and Tenover *et al.*, *J. Clin. Endocrinol. Metab.* 64:1118 (1987) found increased in FSH, LH in both young and old men after treatment with clomiphene. They also found increases in free and total testosterone in men with young men showing significant increases

[0023] Studies were also conducted to determine whether or not clomiphene could be used to improve fertility in men by improving semen quality. Homonnai *et al.* *Fertil. and Steril* 50:801 (1988) saw increases in sperm concentration and count but others have not. (See e.g., Sokel, *et al.*, *Fertil. and Steril.* 49:865 (1988); Check, *et al.*, *Int. J. Fertil.* 34:120 (1989); Purvis, *et al.*, *Int. J. Androl* 21:109 (1989); and Breznik, *Arch. Androl.* 21:109 (1993).) One group saw a deterioration in the percentage of normal sperm with long-term treatment. Shamis, *et al.*, *Arch. Androl* 21:109 (1991). A WHO study showed no changes in semen quality or fertility after 6 months of treatment. (Anonymous *Androl.* 15:299 (1992).) A meta-analysis seems to confirm that testosterone levels go up in men with poor quality sperm

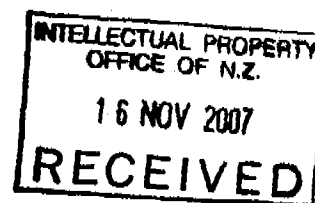
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but not fertility. (Vanderkerckhove, *et al.*, 2000). Studies have also suggested that long term treatment with clomiphene does not seem to have a drastic deleterious effect on health, although it did show that treatment resulted in poorer sperm quality after 4 months. Studies have kept men on clomiphene for as long as 18 months and at levels of 25 mg per day or 100 mg every other day.

[0024] In 1991, Guay *et al* (Urology 38:377 (1991)) suggested that clomiphene could treat sexual dysfunction in men. Their hypothesis seems to be that sexual function follows testosterone levels. This was supported by early studies showing positive influence of androgens and sexual function, Davidson, *et al.*, J. Clin. Endocrinol. Metab. 48:955 (1979), and studies that rated sleep-related erections as a strong response to T, Cunningham, *et al.*, J. Clin. Endocrinol. Metab. 70:792 (1990). However, in 1995, Guay *et al.* (Gray, *et al.*, J. Clin. Endocrinol. Metab. 80:3546 (1995)) published a study in which they saw increase in I.H, FSH, and testosterone after 2 months of clomiphene but no effects on erectile dysfunction. There might be some advantage for young men and specific groups of older men, but it seems that just raising the testosterone level is not enough. Effects of testosterone on sleep-related erections may have been taken too seriously (Herskowitz, *et al.*, J. Psychosomat. Res. 42:541 (1997)).

[0025] Described is a composition comprising of one isomer preferably *trans*-clomiphene or a predefined blend of the isomers of clomiphene as described below differing from the normally produced mixture are used to enhance testosterone levels while reducing the side effects of the drug. Thus, also described is an oral therapy for increasing testosterone levels, which lacks or has diminished side effects connected with the existing clomiphene formulations.

[0026] In one described embodiment a patient who has a need or desire to increase their serum testosterone levels are administered one or more dosages of an effective



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amount of composition comprising *trans*-clomiphene at a dosage between one mg to about 200 mg (although the determination of optimal dosages is with the level of ordinary skill in the art). *Cis*-clomiphene may also be present in the composition so long as the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 1. Analogs of the *trans*- and *cis*- isomers of clomiphene such as those described in Ernst, *et al. supra* are also useful in the practice of the present invention.

[0027] Dosages are preferably (but not necessarily) administered as part of a dosage regimen designed to give rise to serum testosterone levels that mimic or correspond to the normal secretory total serum testosterone profile described in FIG. 1. For example, according to FIG. 1 a dosage of the preferred composition may be administered in a pharmaceutical formulation that would give rise to peak serum testosterone levels at around 8 a.m. Such pharmaceutical formulations may be in the form of sustained release formulations prepared as described for example in U.S. Patent No. 6,221,399, Japanese patent 4-312522, Meshali *et al*, *Int. J. Phar.* 89:177-181 (1993), Kharenko *et al*, *Intern. Symp. Control Rel. Bioact. Mater.* 22:232-233 (1995), WO 95/35093, Dangprasit *et al.*, *Drug. Devel. and Incl. Pharm.* 21 (20):2323-2337 (1995); U.S. Patent Nos. 6,143,353, 6,190,591, 6,096,338, 6,129,933, 6,126,969, 6,248,363 and other sustained release formulations well known in the art.

[0028] Suitable pharmaceutical compositions or unit dosage form may be in the form of solids, such as tablets or filled capsules or liquids such as solutions suspensions, emulsions, elixirs or capsules filled with the same, all for oral use. The compositions may also be in the form of sterile injectable solutions or emulsions for parenteral (including subcutaneous) use. Such pharmaceutical compositions and unit dosage forms thereof may comprise ingredients in conventional proportions.

[0029] Compositions according to the present invention may also be administered by the intravenous, subcutaneous, buccal, transmucosal, intrathecal, intradermal, intracisternal or

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other routes of administration. After administration of the composition serum testosterone levels may be measured as described above and dosages may be altered to achieve a sufficient increase in the serum testosterone levels to achieve the desired physiological results associated with normal testosterone described above.

[0030] All of the references discussed herein are incorporated by reference in their entirety.

[0030a] The term "comprising" as used in this specification means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present.

Related terms such as "comprise" and "comprises" are to be interpreted in the same manner.

[0030b] The term "consisting essentially of" as used in this specification and claims means the claimed composition is limited to presence of the specified materials but can also include materials that do not materially affect the basic characteristics of the composition.

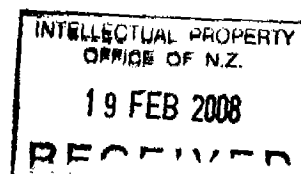
[0030c] In the description in this specification reference may be made to subject matter which is not within the scope of the claims of the current application. That subject matter should be readily identifiable by a person skilled in the art and may assist in putting into practice the invention as defined in the claims of this application.

[0031] The following Example is meant to be illustrative of the invention and is not intended to limit the scope of the invention as set out in the appended claims.

EXAMPLE 1

Effects of Clomids on Serum Testosterone in Male Baboons

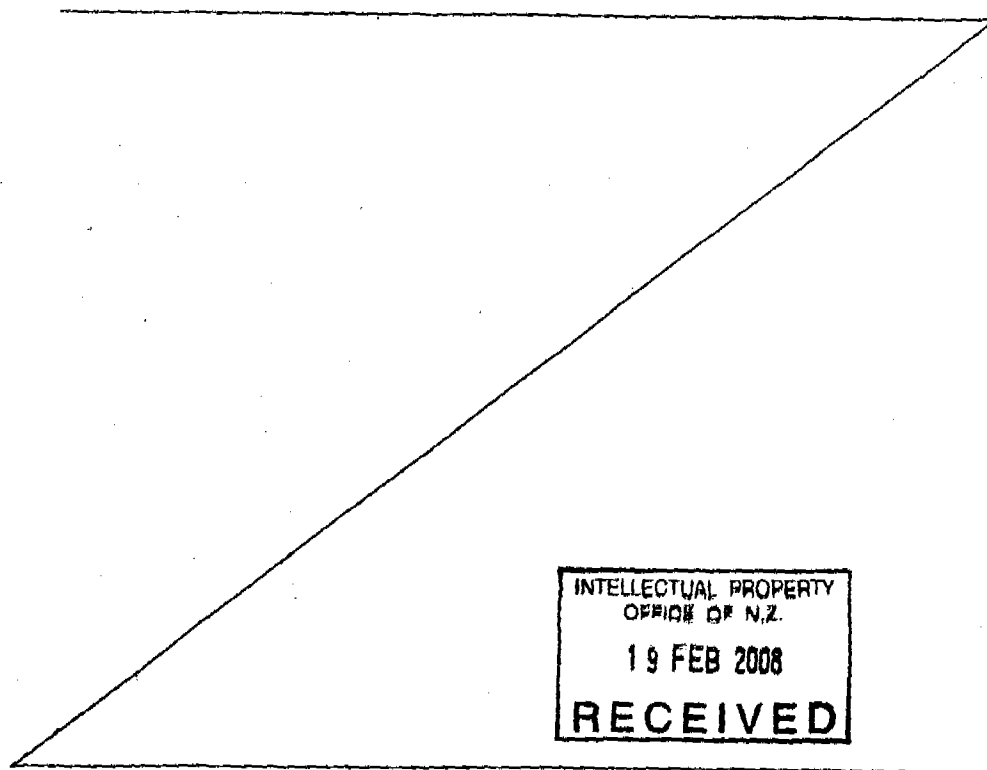
[0032] Adult, male, Baboons were given 1.5 mg/kg of Clomid, Enclomid (*trans*-Clomid) or Zuclomid (*cis*-Clomid) for 12 consecutive days. The samples analyzed were sera taken on the day of first treatment before being given test article (day 0), after 12 days of treatment (day 12) and 7 days after the last treatment (end or wash-out).



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1. Effects on Body Weight and Serum LH, FSH, PRL and Testosterone

[0033] There were significant increases in total serum testosterone in the group receiving Enclomid. See Table 1. There were no differences among groups in the baseline period or at day 0. There were also no differences among the three groups 7 days after treatment (the washout period). However, Enclomid produced higher levels of testosterone compared to Clomid and Zuclomid on day 6 ($p = 0.03$ and $p = 0.00002$ respectively) and compared to Zuclomid on day 12 ($p = 0.047$). Zuclomid clearly did not raise total serum testosterone to any extent. Compared to the animals receiving Enclomid, the animals receiving Clomid exhibited more variable total testosterone levels on day 6 and later as judged by their coefficients of variations. When we looked at the time course of the effects (FIG. 3), we determined that only Enclomid significantly and statistically raised total serum testosterone



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on days 6 and 12 compared with either baseline or day 0 values. Moreover, cessation of Enclomid treatment, resulted in a significant drop in the level of total serum testosterone between day 12 and day 18 (washout). This indicates that Enclomid is readily cleared from the circulation consistent with the metabolic clearance seen for Enclomid in humans. Enclomid was clearly better and more consistent than Clomid itself and Zucloamid was ineffective.

Table 1 - Serum Testosterone Levels (ng/dl)

Group	ID	baseline 12/3/01	0 day 12/7/01	6 days 12/13/01	12 days 12/20/01	wash-out 12/26/01
CLO	7500	79.01	76.15	940.97	891.5	150.9
	9012	97.55	305.24	585.92	555.6	316.3
	9097	158.06	102.94	151.12	318.9	143.6
	mean	111.5	161.4	559.3	588.7	203.6
	SD	41.3	125.2	395.6	287.7	97.7
ENCLO	7223	64.57	74.96	1223.8	633.6	307.2
	8021	166.86	133.59	1128.2	1466	399.2
	8369	170.45	106.47	1081.1	1166	271
	mean	134.0	105.0	1144.4	1088.5	325.8
	SD	60.1	29.3	72.7	421.6	66.1
ZUCLO	7438	124.84	210.4	137.51	314.5	359.7
	8292	104.66	67.37	169.98	406.1	860.5
	10098	282.29	904.82	227.95	353.0	274.1
	mean	170.6	394.2	178.5	357.9	498.1
	SD	97.3	448.0	45.8	46.0	316.8
ANOVA		p = 0.61	p = 0.43	p = 0.007	p = 0.57	p = 0.256
K-W		p = 0.56	p = 0.84	p = 0.051	p = 0.079	p = 0.252

[0034] There were no changes in serum LH or FSH. The ratio of total serum testosterone to LH followed the same pattern as total serum testosterone, suggesting a lack of dependence (data not shown). There was also no change in body weight during the 12 day study. There was a decrease in serum prolactin (PRL) during the study in the group receiving Enclomid, suggesting an effect of antiestrogen that has been described in part (Ben-Jonathan and

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Hnasko, 2001) and expected on the basis of the fact that as men age, testosterone declines and Prolactin increase (Feldman *et al.*, 2002).

2. Effects on Clinical Chemistry Parameters

[0035] The mean values for each parameter did not differ among the three groups for any test parameter at the beginning of the study as determined by ANOVA or by the Kruskal-Wallis test. All groups exhibited normal values at each parameter except for (1) serum sodium; a related calculated parameter, anionic gap, which were low for all nine baboons throughout the trial; (2) serum glucose; and (3) BUN which were high on day 0 for the group which would be treated with Enclomid. On day 12 of treatment and 7 days after treatment (washout), there were no differences among groups for any parameter except anionic gap that showed that the Clomid and Zuclomid groups had lower values than the Enclomid group. The values of serum sodium and anionic gap appear to be anomalies associated with this group of baboons.

[0036] There were substantive effects on the red blood cell population with Enclomid and Zuclomid and on hematocrit with Zuclomid. All the compounds lower the mean cell hemoglobin concentration (MCHC) either at day 0 or at the endpoint. With no change in mean cell hemoglobin (MCH) and an increase in the mean cell volume (MCV), the lowering of MCHC is predictable. Although testosterone might be expected to raise hematocrit, only Zuclomid treatment, which did not increase total serum testosterone, demonstrated a statistical difference. Clearly, men in a clinical trial that uses Zuclomid should be monitored for the characteristics of their red blood cell population. Enclomid would be predicted to have less of an effect.

[0037] There appears to be a clear effect of 12-day Enclomid treatment on platelets although the values found stayed within the normal range. One thing to consider here is the sexual dimorphism in platelet counts between male and female baboons (279 for males vs. 348 for

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females). This is likely to be due to hormones. Since the Enclomid group demonstrated increased testosterone, the lowering of the platelet count could be secondary to the change in testosterone in this group. Moreover, treatment with Enclomid pushed the platelet count to its normal male level from a day 0 level that was the high end of the normal range for this group. Enclomid would not necessarily predict a deleterious effect on platelets.

[0038] All the Clomids tested had effects on the white blood cell (WBC) population, the most striking was that of Enclomid on raising the counts of lymphocytes and eosinophiles. The effects are not as straightforward as they would seem to be. There appears to be a strong effect of Enclonud on lowering the per cent of granulocytes in the blood. The effects are very strong after the 7-day washout period when the values are decreased below the normal range. (This time course could reflect the relatively long time required to affect change the WBC population.) There is little sexual dimorphism in baboons with respect to the white blood cell populations, so the effects are more likely to be due to the compound itself than changes in testosterone. However, when we look at the calculated count of granulocytes using the WBC count, we find no differences in granulocyte count due to any compound. Concomitantly, it is the lymphocyte story that is the most interesting. Both the count and per cent lymphocytes in the population increase with Enclomid treatment. Whereas the mean values of per cent lymphocytes remain in the normal range, given the trend for an increase in WBC count, the net effect is an increase in lymphocyte count with Enclomid. This eosinophil result is analogous. There is a clear implication for treating men who have low lymphocytes, such as men who are HIV-positive. Since Enclomid is unlikely to lower lymphocytes based on this result, a case could be made for its use in the population of men with AIDS. These individuals are often treated with agents that are intended to raise testosterone due to the wasting effects of disease. Low liver and kidney toxicity and favorable effects on cholesterol

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and lipids are also highly favored attributes for any medication intended for use HIV-positive men who are already compromised by their disease.

[0039] The increase in serum glucose with Clomid or Zucloimid was within the normal range. In the case of Enclomid where the mean serum glucose values were high on day 0, there were no increases with treatment. There was no evidence that Enclomid would have a deleterious effect on blood glucose.

[0040] No clearly adverse effects on liver function are apparent as judged by the enzymes AST and ALT. The trend in these values was a decrease with treatment. An increase in the level of enzymes in the serum would indicate liver damage. ALT/SGPT was out of range low at the end of the study for the Clomid group although the differences over the treatment period were not statistically significant. The changes with Enclomid and Zucloimid were within the normal range. AST is depressed in pregnancy; thus the action of an estrogen agonist such as Zucloimid in lowering the marginal AST level could be rationalized. Alkaline phosphatase (ALP) is also found in the liver and is elevated various disease states. The lowering of ALP argues further against hepatic damage. There were no changes in serum albumin, also a liver product. A strong suppression of serum albumin over an extended time period could contribute to free serum steroid hormone levels in humans although a more important role is played by sex hormone binding globulin. As a bottom line, none of the compounds could be linked to liver damage on the basis of the parameters assayed.

[0041] Osteoblastic activity and diseases of the bone are accompanied by high serum ALP values. ALP was not elevated following Zucloimid treatment and was decreased in value following Enclomid treatment. The trends would predict a more benign result for the use of Enclomid compared to Zucloimid.

[0042] Although BUN and BUN/creatinine were altered during the study in the Clomid and Enclomid groups, the lack of a definitive change in creatinine argues against renal

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dysfunction. A loss of glomerular filtration capacity would result in an increase in BUN. Decreased BUN occurs in humans due to poor nutrition (not likely in a controlled setting), or high fluid intake (presumably accompanied by edema). Also, despite an increase in total serum testosterone between day 0 and Day 12 with Enclomid, there were no differences between serum creatinine values, arguing against an increase in muscle mass over this short time interval.

[0043] Serum sodium levels were lower than reference values for all animals throughout the study. Serum carbon dioxide was higher than reference values on day 12 for the Clomid and Zuclomid groups. Serum anion gap was lower for all animals throughout the study, paralleling the sodium results. Enclomid raised this parameter towards normal values. The electrolyte imbalances detected in the test animals throughout all treatment periods remains elusive but might be part of the same fluid derangement phenomenon suggested by the BUN results.

[0044] Treatment with Enclomid tended to decrease serum cholesterol and Zuclomid tended to increase the same parameter although neither change reached statistical significance. Those changes were within the normal range although the trend for the two isomers to demonstrate opposite effects over a short period of time merits the further monitoring and might not be unexpected given that the isomers have, alternatively, estrogen agonist or antagonist activity. Enclomid might be expected to be more benign than Zuclomid with respect to serum cholesterol if used chronically.

[0045] The foregoing results indicate that Enclomid is more effective than Clomid or Zuclomid at enhancing total serum testosterone. Zuclomid is clearly not effective and that deficiency limits any use of Clomid for hypogonadism, particularly since the Zuclomid component of Clomid would predominate in the circulation over time given its longer half-life.

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[0046] Enclomid appeared to be relatively benign in all aspects when compared to Zuclomid and, often, even Clomid. This is particularly true when consideration is given to the trend of Enclomid to lower cholesterol, and liver enzymes as opposed to Zuclomid's trend to raise the same parameters. The surprising trend for Enclomid to raise the lymphocyte count may be useful for men with AIDS if it can be shown the CD4+ subpopulation of lymphocytes is not lowered or is enhanced.

EXAMPLE 2

Method for Increasing Testosterone Level in Men Using *Trans*-clomiphene and Mixtures of *Trans*-clomiphene and *Cis*-clomiphene at Ratios Greater Than 1

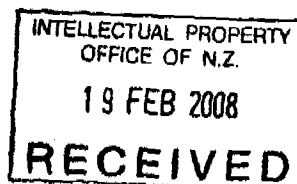
[0047] Prior to administration of *trans*-clomiphene, blood samples are taken from subject males and testosterone levels are measured using methodologies described for example in Matsumoto, *et al.* Clin. Endocrinol. Metab. 56; 720 (1983) (incorporated herein by reference). Sex hormone binding globulin (SHBG), both free and bound to testosterone, may also be measured as described for example in Tenover *et al.* J. Clin. Endocrinol. Metab. 65:1118 (1987) which describe measurement of SHBG by both a [³H] dihydrotestosterone saturation analysis and by radioimmunoassay. Non-SHBG-bound testosterone levels (bioavailable testosterone) are also measured for example according to Tenover *et al.* J. Clin. Endocrinol and Metab. 65:1118 (1987). See also Soderguard *et al.* J. Steroid Biochem 16:801 (1982) incorporated herein by reference.

[0048] Patients are given daily dosages of 1.5 mg/kg clomiphene; wherein the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 1. Patients are monitored for testosterone levels such that the dosage amount and dosage frequency may be adjusted to achieve therapeutic levels of testosterone in the patient.

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WHAT WE CLAIM IS:

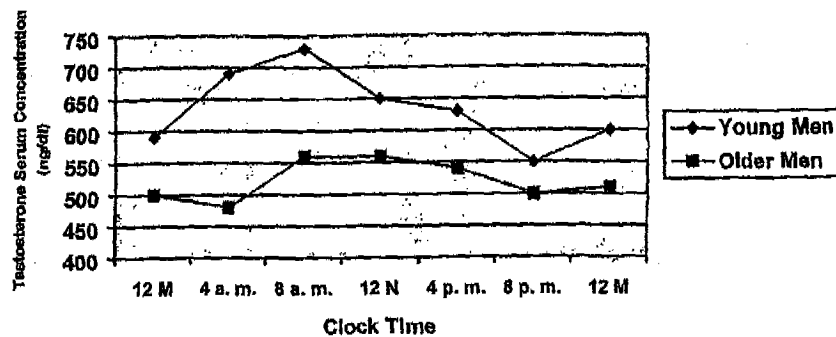
1. A composition comprising 0% to about 29% w/w of *cis*-clomiphene and about 100% to about 71% *trans*-clomiphene or analogs thereof or pharmaceutically acceptable salts or solvates thereof and optionally one or more pharmaceutically acceptable diluents, adjuvants, carriers or excipients, with the proviso that said composition does not comprise 11% *cis*-clomiphene and 89% *trans*-clomiphene.
2. A composition comprising *cis*-clomiphene and *trans*-clomiphene or analogs thereof or pharmaceutically effective salts or solvates thereof and optionally one or more pharmaceutically acceptable diluents, adjuvants, carriers or excipients and wherein the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 71/29, with the proviso that said ratio is not 8/1.
3. The composition of claim 1 or 2, wherein the composition consists essentially of an effective amount of *trans*-clomiphene or an analog thereof or a pharmaceutically effective salt or solvate thereof and optionally one or more pharmaceutically acceptable diluents, adjuvants, carriers or excipients.
4. The composition of claim 3, wherein the composition comprises 1-200 mg of *trans*-clomiphene or an analog thereof or a pharmaceutically effective salt or solvate thereof and optionally one or more pharmaceutically acceptable diluents, adjuvants, carriers or excipients.
5. The composition of claim 3, wherein the composition consists essentially of 12.5 mg of *trans*-clomiphene or an analog thereof or a pharmaceutically effective salt or solvate thereof and optionally one or more pharmaceutically acceptable diluents, adjuvants, carriers or excipients.
6. The composition of any one of claims 1, 2, 4 or 5, wherein the composition is provided in a capsule form.
7. The composition of claim 3, wherein the composition is provided in a capsule form.
8. A composition of claim 1 or 2, substantially as herein described with reference to any example thereof.



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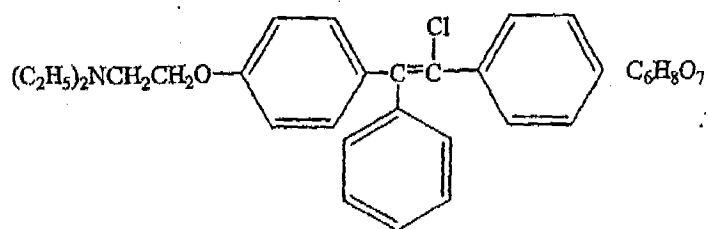
FIG. 1

Normal Secretory Total Serum Testosterone Profiles In Healthy Young and Older Men



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FIG. 2



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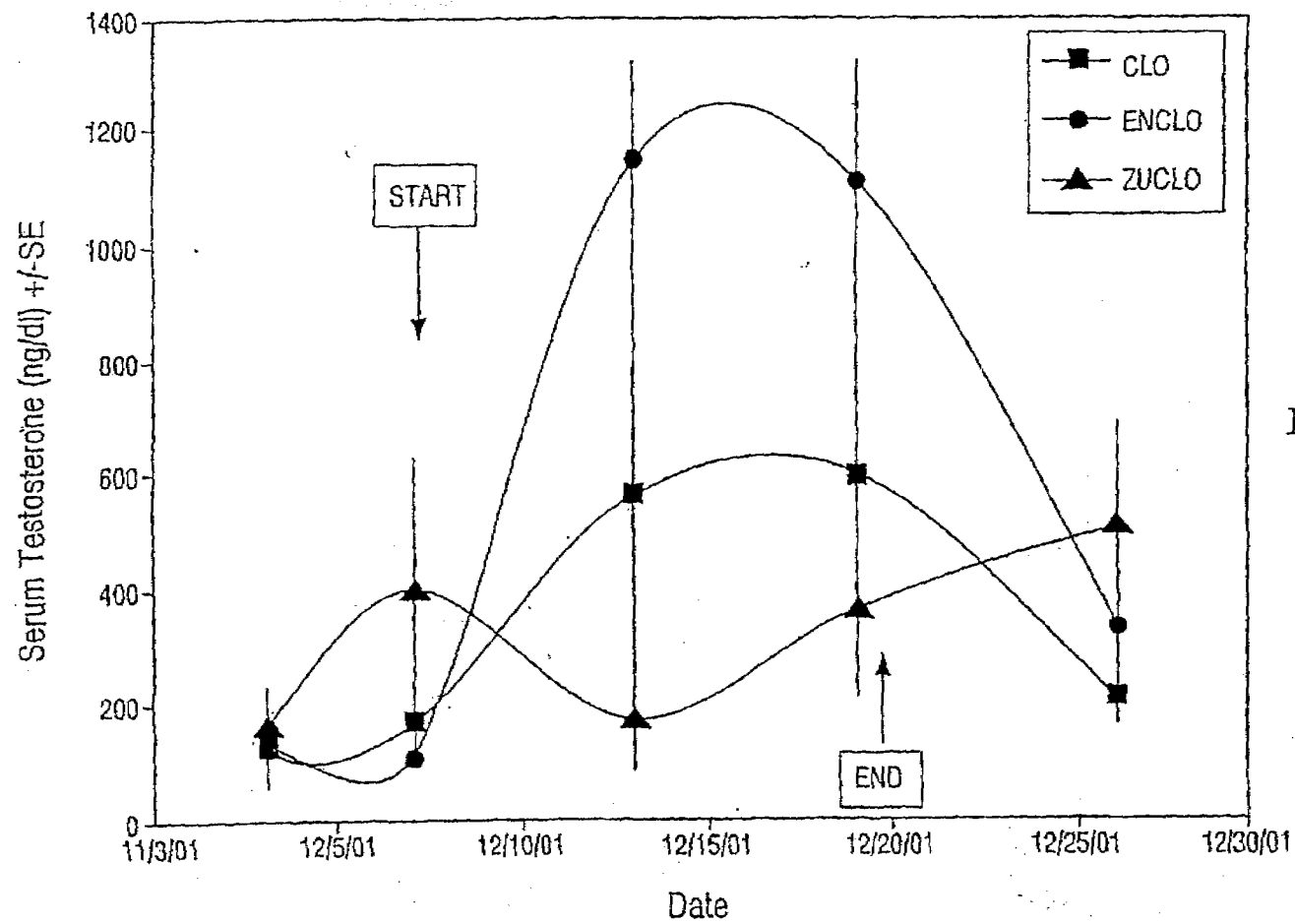


FIG. 3

END

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Australian Government

IP Australia

LETTERS PATENT

STANDARD PATENT

2002318225

I, Fatima Beattie, the Commissioner of Patents, grant a Standard Patent with the following particulars:

Name and Address of Patentee(s):

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Name of Actual Inventor(s):

Podolski, Joseph S..

Title of Invention:

Methods and materials for the treatment of testosterone deficiency in men

Term of Letters Patent:

Twenty years from 9 July 2002

Priority Details :

Number
60/304,313

Date
9 July 2001

Filed with
US



Dated this 27th day of March 2008

PATENTS ACT 1990

Fatima Beattie
Commissioner of Patents

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26
164

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. AU 2002318225 B2

- (54) Title
Methods and materials for the treatment of testosterone deficiency in men
- (51) International Patent Classification(s)
A61K 31/225 (2006.01) A61K 31/138 (2006.01)
A61K 31/135 (2006.01) A61P 15/08 (2006.01)
- (21) Application No: **2002318225** (22) Date of Filing: **2002.07.09**
- (87) WIPO No: **WO03/005954**
- (30) Priority Data
- (31) Number **60/304,313** (32) Date **2001.07.09** (33) Country **US**
- (43) Publication Date: **2003.01.29**
(43) Publication Journal Date: **2003.05.22**
(44) Accepted Journal Date: **2007.12.13**
- (71) Applicant(s)
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- (56) Related Art
EP 0430388 A2
AU 2001261684

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
23 January 2003 (23.01.2003)

PCT

(10) International Publication Number
WO 03/005954 A3

(51) International Patent Classification: A61K 31/225,
31/135

(21) International Application Number: PCT/US02/21524

(22) International Filing Date: 9 July 2002 (09.07.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/304,313 9 July 2001 (09.07.2001) US

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(81) Designated States (national): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU,

CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH,
GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC,
LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW,
MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG,
SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ,
VN, YU, ZA, ZM, ZW.

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

Published:

with international search report

— with amended claims and statement

(88) Date of publication of the international search report:
23 October 2003

Date of publication of the amended claims and statement:
4 December 2003

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

WO 03/005954 A3

(54) Title: METHODS AND MATERIALS FOR THE TREATMENT OF TESTOSTERONE DEFICIENCY IN MEN

(57) Abstract: The present invention relates to the use of compositions comprising *trans*-clomiphene for treating men with hypogonadism. The invention is also directed to methods for treating males with hypogonadism.

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**METHODS AND MATERIALS FOR THE TREATMENT OF TESTOSTERONE
DEFICIENCY IN MEN**

FIELD OF THE INVENTION

5 [0002] The present invention relates to the compositions and methods for increasing testosterone levels. More specifically, the present invention relates to a composition comprising clomiphene enriched for trans-clomiphene. The present invention also relates to the use of a composition comprising clomiphene enriched for trans-clomiphene reagents for increasing testosterone levels.

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BACKGROUND

[0003] Testosterone is the primary male androgen, playing a vital role in overall male health. Testosterone is essential to the development and maintenance of specific reproductive tissues (testes, prostate, epididymis, seminal vesicle, and penis) and male
15 secondary sex characteristics. It plays a key role in libido and erectile function and is necessary for the initiation and maintenance of spermatogenesis. Testosterone also has important functions not related to reproductive tissues. For example, it positively affects body composition by increasing nitrogen retention, which supports lean body mass, muscle size and strength. It also acts on bone to stimulate bone formation.

20 [0004] Testosterone secretion is the end product of a series of hormonal processes. Gonadotropin-releasing hormone (GnRH), which is secreted in the hypothalamus, controls the pulsatile secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH), which are secreted by the anterior pituitary. LH, in turn, regulates the production and

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secretion of testosterone in the Leydig cells of the testes, while FSH assists in inducing spermatogenesis.

[0005] Testosterone is most often measured as "total testosterone." This measurement includes testosterone that is bound to sex hormone-binding globulin (SHBG) (~44%) and is therefore not bioavailable and testosterone which either is free (~2%) or loosely bound to other proteins (non-SHBG-bound) (~54%).

[0006] Results from a WHO study indicate that testosterone is normally secreted in a circadian rhythm, with higher levels in the morning and nadir levels occurring around 8 to 10 p.m. *See* FIG. 1. This variation in testosterone secretion throughout the day becomes much less pronounced in older men (mean age equals 71 years). The importance of this rhythm is not known at this time.

[0007] Samples were obtained from both young and elderly patients every 10 minutes for 24 hours via an indwelling cannula. According to Tenover (1987) the mean 24 hr total serum testosterone levels in healthy young men (age range 22 yrs.-35 yrs. mean 27.3 yrs) was 4.9 ± 0.3 ($\pm$ SEM) mg/ml (17.0 nmol/L) while older men (age range 65yrs - 84 yrs. mean 70.7 yrs.) had a significantly lower mean 24 hrs. total serum testosterone level of 4.1 ± 0.4 mg/ml. ($P < 0.5$; 14.2 nmol/L).

[0008] Total serum testosterone levels obtained from single random samples were also significantly lower in older men (4.0 ± 0.2 mg/ml [13.9 n nmol/L]) as compared to 4.8 ± 0.2 mg/ml [16.6 nmol/L] in healthy young men.

[0009] Testosterone deficiency can result from underlying disease or genetic disorders and is also frequently a complication of aging. For example, primary hypogonadism results from primary testicular failure. In this situation, testosterone levels are low and levels of pituitary gonadotropins (LH and FSH) are elevated. Secondary hypogonadism is due to inadequate secretion of the pituitary gonadotropins. In addition to a low testosterone level, LH and FSH

levels are low or low-normal. Some of the sequelae of adult testosterone deficiency include a wide variety of symptoms including: loss of libido, erectile dysfunction, oligospermia or azospermia, absence or regression of secondary sexual characteristics, progressive decrease in muscle mass, fatigue, depressed mood and increased risk of osteoporosis.

[0010] Several forms of testosterone therapy exists in the United States today. Recently, transdermal preparations have gained favor in the market. However, a scrotal testosterone patch results in supraphysiologic levels of 5 α -dihydrotestosterone (DHT) due to the high concentration of 5 α -reductase in scrotal skin. It is not known whether these elevated DHT levels have any long-term health consequences. Nonscrotal systems are considered more convenient and most patients achieve average serum concentrations within the normal range and have normal levels of DHT. Oral testosterone therapy is not recommended because doses required for replacement therapy are associated with significant risk of hepatotoxicity.

SUMMARY

In a first aspect, the invention provides a method for increasing serum levels of testosterone in a male mammal, the method comprising administering to the mammal an effective amount of a composition comprising *cis*-clomiphene and *trans*-clomiphene or analogs thereof or pharmaceutically acceptable salts or solvates thereof and optionally one or more pharmaceutically acceptable diluents, adjuvants, carriers or excipients, wherein the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 71/29 w/w.

In a second aspect, the invention provides use of a composition comprising *cis*-clomiphene and *trans*-clomiphene or analogs, pharmaceutically acceptable salts or solvents thereof wherein the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 71/29 w/w for the preparation of a medicament for increasing serum levels of testosterone in a male mammal.

[0011] The present invention is directed to compositions useful for increasing testosterone levels in male mammals and for ameliorating or preventing the sequelae of low testosterone levels. Described herein are compositions having active ingredients comprising 0% to 29% weight/weight of (*cis*,-Z- *trans*-clomiphene) (hereinafter "*cis*-clomiphene") and 100% to 71% w/w (*trans*-, E-, *cis*-clomiphene) (hereinafter "*trans*-clomiphene") or pharmaceutically acceptable salts

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thereof. Among the preferred compositions of the present invention which contain both *cis*-clomiphene and *trans*-clomiphene are compositions wherein the ratio of *trans*-clomiphene and *cis*-clomiphene is greater than 1. A more preferred composition according to the present invention comprises about 100% w/w of active ingredients of *trans*-clomiphene or a pharmaceutically acceptable salt thereof. All compositions of the present invention may further comprise suitable pharmaceutical excipients, diluents, carriers and the like. Analogs

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of the *cis*-clomiphene and *trans*-clomiphene are also contemplated for use in all aspects of the present invention.

[0012] The present invention is also directed to methods for increasing serum testosterone levels in hypogonadal male mammals (and for ameliorating or preventing the sequelae of low testosterone levels), the method comprising administering to a subject male an effective amount of a composition according to the present invention, the compositions having active ingredients comprising 0% to 29% weight/weight of *cis*-clomiphene and 100% to 71% w/w *trans*-clomiphene including any of their pharmaceutically acceptable salts thereof. Among the preferred methods are those in which the administered compositions contain both isomers wherein the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 1. A more preferred method comprises administering to the male a composition comprising about 100% w/w of *trans*-clomiphene.

BRIEF DESCRIPTION OF THE DRAWING

[0013] FIG. 1 is a graphic representative of the normal secretory total serum testosterone profiles in healthy men (young and old).

[0014] FIG. 2 shows the chemical structure of clomiphene citrate.

[0015] FIG. 3 is a graphic demonstration of the time course of serum testosterone levels with Clomid, Enclomid and Zuclomid.

DETAILED DESCRIPTION

[0016] The present invention provides methods and compositions useful for increasing testosterone levels in male mammals and for ameliorating or preventing the sequelae of low testosterone levels including but not limited to those described above.

[0017] Clomiphene (FIG. 2) is an antiestrogen related to tamoxifen that blocks the normal estrogen feedback on the hypothalamus and subsequent negative feedback on the pituitary. This leads to increases in luteinizing hormone (LH) and follicle stimulating hormone (FSH).

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In men, these increased levels of gonadotropins stimulate the Leydig cells of the testes and result in the production of higher testosterone levels. Clomiphene citrate has the following structure:

[0018] Ernst *et al.*, J. Pharmaceut. Sci. 65:148 (1976), have shown that clomiphene is a mixture of two geometric isomers which they refer to as *cis*-, *Z*-, clomiphene (*cis*-clomiphene or zuclophene) and *trans*-, *E*-, clomiphene, (*trans*-clomiphene or enclomiphene).

According to Ernst, *et al.* *trans*-clomiphene HCl has a melting point of 149°C-150.5°C, while *cis*-clomiphene HCl has a melting point of 156.5°C-158°C.

[0019] Ernst *et al.* have also noted that (the *trans*-isomer) is antiestrogenic (AE) while the *cis*-isomer is the more potent and more estrogenic form and has also been reported to have anti-estrogenic activity. The authors attribute the effect of the drug on ovulatory activity to both forms stating that the mixture is more effective than *trans*-clomiphene alone. The *trans*-isomer aids ovulation at the level of the hypothalamus. The estrogenic isomer *cis*-clomiphene contributes to enhanced ovulation elsewhere in the physiologic pathway leading to ovulation. The isomers are also reported to have different *in vivo* half-life. Furthermore the *cis* form has been reported to leave residual blood levels for in excess of one month following a single dose.

[0020] Vandekerckhove, *et al.* (Cochrane Database Syst Rev 2000;(2):CD000151 (2000)) noted that ten studies involving 738 men have suggested that anti-estrogens appear to have a beneficial effect on endocrinal outcomes, *i.e.* testosterone, but there is not enough evidence to evaluate fertility effects. Nevertheless should clomiphene administration enhance testosterone levels then one could easily conclude that the drug should positively impact the side effects of testosterone deprivation as long as the testes still retain the ability to respond to gonadotropin stimulation.

[0021] Clomiphene is currently approved as a mixture of both cis- and trans-isomers, the cis-isomer being present as about 30% to 50% (Merck Manual) for fertility enhancement in the anovulatory patient. Clomiphene improves ovulation by initiating a series of endocrine events culminating in a preovulatory gonadotropin surge and subsequent follicular rupture. The drug is recommended to be administered for 5 days at a dose of up to 100 mg daily. Clomiphene has also been associated with numerous side effects including: blurred vision, abdominal discomfort, gynecomastia, testicular tumors, vasomotor flushes, nausea, and headaches. Furthermore, other studies suggest that clomiphene possesses both genotoxic and tumor enhancement effects. The net outcome of these observations is that clomiphene in its current format, having between 30% and 50% of the cis isomer, would be unacceptable for chronic therapy in men for the treatment of testosterone deficiency.

[0022] Clomiphene has also been used for therapeutic intervention in men with low testosterone levels. Tenover *et al.*, *J. Clin. Endocrinol. Metab.* 64:1103, (1987) and Tenover *et al.*, *J. Clin. Endocrinol. Metab.* 64:1118 (1987) found increased in FSH, LH in both young and old men after treatment with clomiphene. They also found increases in free and total testosterone in men with young men showing significant increases

[0023] Studies were also conducted to determine whether or not clomiphene could be used to improve fertility in men by improving semen quality. Homonnai *et al.* *Fertil. and Steril* 50:801 (1988) saw increases in sperm concentration and count but others have not. (See e.g., Sokel, *et al.*, *Fertil. and Steril.* 49:865 (1988); Check, *et al.*, *Int. J. Fertil.* 34:120 (1989); Purvis, *et al.*, *Int. J. Androl* 21:109 (1989); and Breznik, *Arch. Androl.* 21:109 (1993).) One group saw a deterioration in the percentage of normal sperm with long-term treatment. Shamis, *et al.*, *Arch. Androl* 21:109 (1991). A WHO study showed no changes in semen quality or fertility after 6 months of treatment. (Anonymous *Androl.* 15:299 (1992).) A meta-analysis seems to confirm that testosterone levels go up in men with poor quality sperm

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but not fertility. (Vanderkerckhove, *et al.*, 2000). Studies have also suggested that long term treatment with clomiphene does not seem to have a drastic deleterious effect on health, although it did show that treatment resulted in poorer sperm quality after 4 months. Studies have kept men on clomiphene for as long as 18 months and at levels of 25 mg per day or 100 mg every other day.

[0024] In 1991, Guay *et al.* (Urology 38:377 (1991)) suggested that clomiphene could treat sexual dysfunction in men. Their hypothesis seems to be that sexual function follows testosterone levels. This was supported by early studies showing positive influence of androgens and sexual function, Davidson, *et al.*, J. Clin. Endocrinol. Metab. 48:955 (1979), and studies that rated sleep-related erections as a strong response to T, Cunningham, *et al.*, J. Clin. Endocrinol. Metab. 70:792 (1990). However, in 1995, Guay *et al.* (Gray, *et al.*, J. Clin. Endocrinol. Metab. 80:3546 (1995)) published a study in which they saw increase in LH, FSH, and testosterone after 2 months of clomiphene but no effects on erectile dysfunction. There might be some advantage for young men and specific groups of older men, but it seems that just raising the testosterone level is not enough. Effects of testosterone on sleep-related erections may have been taken too seriously (Herskowitz, *et al.*, J. Psychosomat. Res. 42:541 (1997)).

[0025] According to the present invention, a composition comprising of one isomer preferably *trans*-clomiphene or a predefined blend of the isomers of clomiphene as described below differing from the normally produced mixture are used to enhance testosterone levels while reducing the side effects of the drug. Thus, the present invention provides an oral therapy for increasing testosterone levels, which lacks or has diminished side effects connected with the existing clomiphene formulations.

[0026] In one embodiment of the present invention, a patient who has a need or desire to increase their serum testosterone levels are administered one or more dosages of an effective

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amount of composition comprising *trans*-clomiphene at a dosage between one mg to about 200 mg (although the determination of optimal dosages is with the level of ordinary skill in the art). *Cis*-clomiphene may also be present in the composition so long as the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 1. Analogs of the *trans*- and *cis*- isomers of clomiphene such as those described in Ernst, *et al. supra* are also useful in the practice of the present invention.

[0027] Dosages are preferably (but not necessarily) administered as part of a dosage regimen designed to give rise to serum testosterone levels that mimic or correspond to the normal secretory total serum testosterone profile described in FIG. 1. For example, according to FIG. 1 a dosage of the preferred composition may be administered in a pharmaceutical formulation that would give rise to peak serum testosterone levels at around 8 a.m. Such pharmaceutical formulations may be in the form of sustained release formulations prepared as described for example in U.S. Patent No. 6,221,399, Japanese patent 4-312522, Meshali et al, Int. J. Phar. 89:177-181 (1993), Kharenko et al, Intern. Symp. Control Rel. Bioact. Mater. 22:232-233 (1995), WO 95/35093, Dangprasit *et al.*, Drug. Devel. and Incl. Pharm. 21 (20):2323-2337 (1995); U.S. Patent Nos. 6,143,353, 6,190,591, 6,096,338, 6,129,933, 6,126,969, 6,248,363 and other sustained release formulations well known in the art.

[0028] Suitable pharmaceutical compositions or unit dosage form may be in the form of solids, such as tablets or filled capsules or liquids such as solutions suspensions, emulsions, elixirs or capsules filled with the same, all for oral use. The compositions may also be in the form of sterile injectable solutions or emulsions for parenteral (including subcutaneous) use. Such pharmaceutical compositions and unit dosage forms thereof may comprise ingredients in conventional proportions.

[0029] Compositions according to the present invention may also be administered by the intravenous, subcutaneous, buccal, transmucosal, intrathecal, intradermal, intracisternal or

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other routes of administration. After administration of the composition serum testosterone levels may be measured as described above and dosages may be altered to achieve a sufficient increase in the serum testosterone levels to achieve the desired physiological results associated with normal testosterone described above.

[0030] All of the references discussed herein are incorporated by reference in their entirety.

[0031] The following Example is meant to be illustrative of the invention and is not intended to limit the scope of the invention as set out in the appended claims.

EXAMPLE 1

Effects of Clomids on Serum Testosterone in Male Baboons

[0032] Adult, male, Baboons were given 1.5 mg/kg of Clomid, Enclomid (*trans*-Clomid) or Zucloimid (*cis*-Clomid) for 12 consecutive days. The samples analyzed were sera taken on the day of first treatment before being given test article (day 0), after 12 days of treatment (day 12) and 7 days after the last treatment (end or wash-out).

1. Effects on Body Weight and Serum LH, FSH, PRL and Testosterone

[0033] There were significant increases in total serum testosterone in the group receiving Enclomid. See Table 1. There were no differences among groups in the baseline period or at day 0. There were also no differences among the three groups 7 days after treatment (the washout period). However, Enclomid produced higher levels of testosterone compared to Clomid and Zucloimid on day 6 ($p = 0.03$ and $p = 0.00002$ respectively) and compared to Zucloimid on day 12 ($p = 0.047$). Zucloimid clearly did not raise total serum testosterone to any extent. Compared to the animals receiving Enclomid, the animals receiving Clomid exhibited more variable total testosterone levels on day 6 and later as judged by their coefficients of variations. When we looked at the time course of the effects (FIG. 3), we determined that only Enclomid significantly and statistically raised total serum testosterone

on days 6 and 12 compared with either baseline or day 0 values. Moreover, cessation of Enclomid treatment, resulted in a significant drop in the level of total serum testosterone between day 12 and day 18 (washout). This indicates that Enclomid is readily cleared from the circulation consistent with the metabolic clearance seen for Enclomid in humans. Enclomid was clearly better and more consistent than Clomid itself and Zucloamid was ineffective.

Table 1 - Serum Testosterone Levels (ng/dl)

Group	ID	baseline 12/3/01	0 day 12/7/01	6 days 12/13/01	12 days 12/20/01	wash-out 12/26/01
CLO	7500	79.01	76.15	940.97	891.5	150.9
	9012	97.55	305.24	585.92	555.6	316.3
	9097	158.06	102.94	151.12	318.9	143.6
	mean	111.5	161.4	559.3	588.7	203.6
	SD	41.3	125.2	395.6	287.7	97.7
ENCLO	7223	64.57	74.96	1223.8	633.6	307.2
	8021	166.86	133.59	1128.2	1466	399.2
	8369	170.45	106.47	1081.1	1166	271
	mean	134.0	105.0	1144.4	1088.5	325.8
	SD	60.1	29.3	72.7	421.6	66.1
ZUCLO	7438	124.84	210.4	137.51	314.5	359.7
	8292	104.66	67.37	169.98	406.1	860.5
	10098	282.29	904.82	227.95	353.0	274.1
	mean	170.6	394.2	178.5	357.9	498.1
	SD	97.3	448.0	45.8	46.0	316.8
ANOVA		p = 0.61	p = 0.43	p = 0.007	p = 0.57	p = 0.256
K-W		p = 0.56	p = 0.84	p = 0.051	p = 0.079	p = 0.252

[0034] There were no changes in serum LH or FSH. The ratio of total serum testosterone to LH followed the same pattern as total serum testosterone, suggesting a lack of dependence (data not shown). There was also no change in body weight during the 12 day study. There was a decrease in serum prolactin (PRL) during the study in the group receiving Enclomid, suggesting an effect of antiestrogen that has been described in part (Ben-Jonathan and

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Hnasko, 2001) and expected on the basis of the fact that as men age, testosterone declines and Prolactin increase (Feldman *et al.*, 2002).

2. Effects on Clinical Chemistry Parameters

[0035] The mean values for each parameter did not differ among the three groups for any test parameter at the beginning of the study as determined by ANOVA or by the Kruskal-Wallis test. All groups exhibited normal values at each parameter except for (1) serum sodium; a related calculated parameter, anionic gap, which were low for all nine baboons throughout the trial; (2) serum glucose; and (3) BUN which were high on day 0 for the group which would be treated with Enclomid. On day 12 of treatment and 7 days after treatment (washout), there were no differences among groups for any parameter except anionic gap that showed that the Clomid and Zucloimid groups had lower values than the Enclomid group. The values of serum sodium and anionic gap appear to be anomalies associated with this group of baboons.

[0036] There were substantive effects on the red blood cell population with Enclomid and Zucloimid and on hematocrit with Zucloimid. All the compounds lower the mean cell hemoglobin concentration (MCHC) either at day 0 or at the endpoint. With no change in mean cell hemoglobin (MCH) and an increase in the mean cell volume (MCV), the lowering of MCHC is predictable. Although testosterone might be expected to raise hematocrit, only Zucloimid treatment, which did not increase total serum testosterone, demonstrated a statistical difference. Clearly, men in a clinical trial that uses Zucloimid should be monitored for the characteristics of their red blood cell population. Enclomid would be predicted to have less of an effect.

[0037] There appears to be a clear effect of 12-day Enclomid treatment on platelets although the values found stayed within the normal range. One thing to consider here is the sexual dimorphism in platelet counts between male and female baboons (279 for males vs. 348 for

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females). This is likely to be due to hormones. Since the Enclomid group demonstrated increased testosterone, the lowering of the platelet count could be secondary to the change in testosterone in this group. Moreover, treatment with Enclomid pushed the platelet count to its normal male level from a day 0 level that was the high end of the normal range for this group. Enclomid would not necessarily predict a deleterious effect on platelets.

[0038] All the Clomids tested had effects on the white blood cell (WBC) population, the most striking was that of Enclomid on raising the counts of lymphocytes and eosinophiles. The effects are not as straightforward as they would seem to be. There appears to be a strong effect of Enclonud on lowering the per cent of granulocytes in the blood. The effects are very strong after the 7-day washout period when the values are decreased below the normal range. (This time course could reflect the relatively long time required to affect change the WBC population.) There is little sexual dimorphism in baboons with respect to the white blood cell populations, so the effects are more likely to be due to the compound itself than changes in testosterone. However, when we look at the calculated count of granulocytes using the WBC count, we find no differences in granulocyte count due to any compound. Concomitantly, it is the lymphocyte story that is the most interesting. Both the count and per cent lymphocytes in the population increase with Enclomid treatment. Whereas the mean values of per cent lymphocytes remain in the normal range, given the trend for an increase in WBC count, the net effect is an increase in lymphocyte count with Enclomid. This eosinophil result is analogous. There is a clear implication for treating men who have low lymphocytes, such as men who are HIV-positive. Since Enclomid is unlikely to lower lymphocytes based on this result, a case could be made for its use in the population of men with AIDS. These individuals are often treated with agents that are intended to raise testosterone due to the wasting effects of disease. Low liver and kidney toxicity and favorable effects on cholesterol

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and lipids are also highly favored attributes for any medication intended for use HIV-positive men who are already compromised by their disease.

[0039] The increase in serum glucose with Clomid or Zucloimid was within the normal range. In the case of Enclomid where the mean serum glucose values were high on day 0, there were no increases with treatment. There was no evidence that Enclomid would have a deleterious effect on blood glucose.

[0040] No clearly adverse effects on liver function are apparent as judged by the enzymes AST and ALT. The trend in these values was a decrease with treatment. An increase in the level of enzymes in the serum would indicate liver damage. ALT/SGPT was out of range low at the end of the study for the Clomid group although the differences over the treatment period were not statistically significant. The changes with Enclomid and Zucloimid were within the normal range. AST is depressed in pregnancy; thus the action of an estrogen agonist such as Zucloimid in lowering the marginal AST level could be rationalized. Alkaline phosphatase (ALP) is also found in the liver and is elevated various disease states. The lowering of ALP argues further against hepatic damage. There were no changes in serum albumin, also a liver product. A strong suppression of serum albumin over an extended time period could contribute to free serum steroid hormone levels in humans although a more important role is played by sex hormone binding globulin. As a bottom line, none of the compounds could be linked to liver damage on the basis of the parameters assayed.

[0041] Osteoblastic activity and diseases of the bone are accompanied by high serum ALP values. ALP was not elevated following Zucloimid treatment and was decreased in value following Enclomid treatment. The trends would predict a more benign result for the use of Enclomid compared to Zucloimid.

[0042] Although BUN and BUN/creatinine were altered during the study in the Clomid and Enclomid groups, the lack of a definitive change in creatinine argues against renal

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dysfunction. A loss of glomerular filtration capacity would result in an increase in BUN. Decreased BUN occurs in humans due to poor nutrition (not likely in a controlled setting), or high fluid intake (presumably accompanied by edema). Also, despite an increase in total serum testosterone between day 0 and Day 12 with Enclomid, there were no differences between serum creatinine values, arguing against an increase in muscle mass over this short time interval.

[0043] Serum sodium levels were lower than reference values for all animals throughout the study. Serum carbon dioxide was higher than reference values on day 12 for the Clomid and Zuclomid groups. Serum anion gap was lower for all animals throughout the study, paralleling the sodium results. Enclomid raised this parameter towards normal values. The electrolyte imbalances detected in the test animals throughout all treatment periods remains elusive but might be part of the same fluid derangement phenomenon suggested by the BUN results.

[0044] Treatment with Enclomid tended to decrease serum cholesterol and Zuclomid tended to increase the same parameter although neither change reached statistical significance. Those changes were within the normal range although the trend for the two isomers to demonstrate opposite effects over a short period of time merits the further monitoring and might not be unexpected given that the isomers have, alternatively, estrogen agonist or antagonist activity. Enclomid might be expected to be more benign than Zuclomid with respect to serum cholesterol if used chronically.

[0045] The foregoing results indicate that Enclomid is more effective than Clomid or Zuclomid at enhancing total serum testosterone. Zuclomid is clearly not effective and that deficiency limits any use of Clomid for hypogonadism, particularly since the Zuclomid component of Clomid would predominate in the circulation over time given its longer half-life.

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[0046] Enclomid appeared to be relatively benign in all aspects when compared to Zuclomid and, often, even Clomid. This is particularly true when consideration is given to the trend of Enclomid to lower cholesterol, and liver enzymes as opposed to Zuclomid's trend to raise the same parameters. The surprising trend for Enclomid to raise the lymphocyte count may be useful for men with AIDS if it can be shown the CD4+ subpopulation of lymphocytes is not lowered or is enhanced.

EXAMPLE 2

Method for Increasing Testosterone Level in Men Using *Trans*-clomiphene and Mixtures of *Trans*-clomiphene and *Cis*-clomiphene at Ratios Greater Than 1

[0047] Prior to administration of *trans*-clomiphene, blood samples are taken from subject males and testosterone levels are measured using methodologies described for example in Matsumoto, *et al.* Clin. Endocrinol. Metab. 56; 720 (1983) (incorporated herein by reference). Sex hormone binding globulin (SHBG), both free and bound to testosterone, may also be measured as described for example in Tenover *et al.* J. Clin. Endocrinol. Metab. 65:1118 (1987) which describe measurement of SHBG by both a [³H] dihydrotestosterone saturation analysis and by radioimmunoassay. Non-SHBG-bound testosterone levels (bioavailable testosterone) are also measured for example according to Tenover *et al.* J. Clin. Endocrinol. and Metab. 65:1118 (1987). See also Soderguard *et al.* J. Steroid Biochem. 16:801 (1982) incorporated herein by reference.

[0048] Patients are given daily dosages of 1.5 mg/kg clomiphene; wherein the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 1. Patients are monitored for testosterone levels such that the dosage amount and dosage frequency may be adjusted to achieve therapeutic levels of testosterone in the patient.

The claims defining the invention are as follows:

1. A method for increasing serum levels of testosterone in a male mammal, the method comprising administering to the mammal an effective amount of a composition comprising *cis*-clomiphene and *trans*-clomiphene or analogs thereof or pharmaceutically acceptable salts or solvates thereof and optionally one or more pharmaceutically acceptable diluents, adjuvants, carriers or excipients, wherein the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 71/29 w/w.
2. The method of claim 1, wherein the composition consists essentially of an effective amount of *trans*-clomiphene or an analog thereof or a pharmaceutically effective salt or solvate thereof and optionally one or more pharmaceutically acceptable diluents, adjuvants, carriers or excipients.
3. The method of claim 1 or claim 2, wherein the composition comprises a dosage of between 1 to 200 mg of *trans*-clomiphene.
4. The method of claim 1 or claim 2, wherein the composition is administered at a dosage of 1.5 mg/kg of *trans*-clomiphene per day.
5. The method of any one of claims 1 to 4, wherein the composition is in the form of a filled capsule for oral use.
6. A filled capsule for oral use, comprising a composition according to claim 1 or claim 2.
7. A method according to any one of the preceding claims, wherein the method is for treatment of secondary hypogonadism, erectile dysfunction, oligospermia, azoospermia, progressive decrease in muscle mass, fatigue, or depressed mood.
8. Use of a composition comprising *cis*-clomiphene and *trans*-clomiphene or analogs, pharmaceutically acceptable salts or solvents thereof wherein the ratio of *trans*-clomiphene to *cis*-clomiphene is greater than 71/29 w/w for the preparation of a medicament for increasing serum levels of testosterone in a male mammal.

9. A method for increasing serum levels of testosterone in a male mammal according to claim 1, substantially as hereinbefore described with reference to any one of the examples.

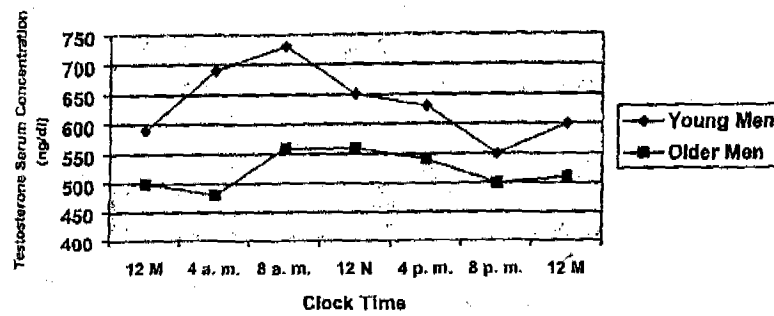
Dated 26 November, 2007
Repros Therapeutics Inc.

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

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FIG. 1

Normal Secretory Total Serum Testosterone Profiles in Healthy Young and Older Men



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FIG. 2

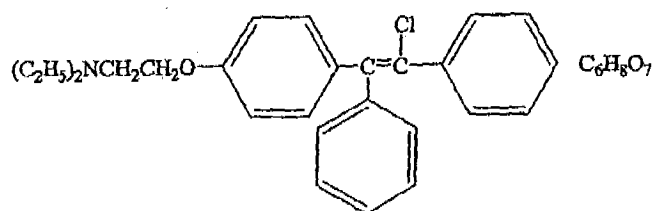
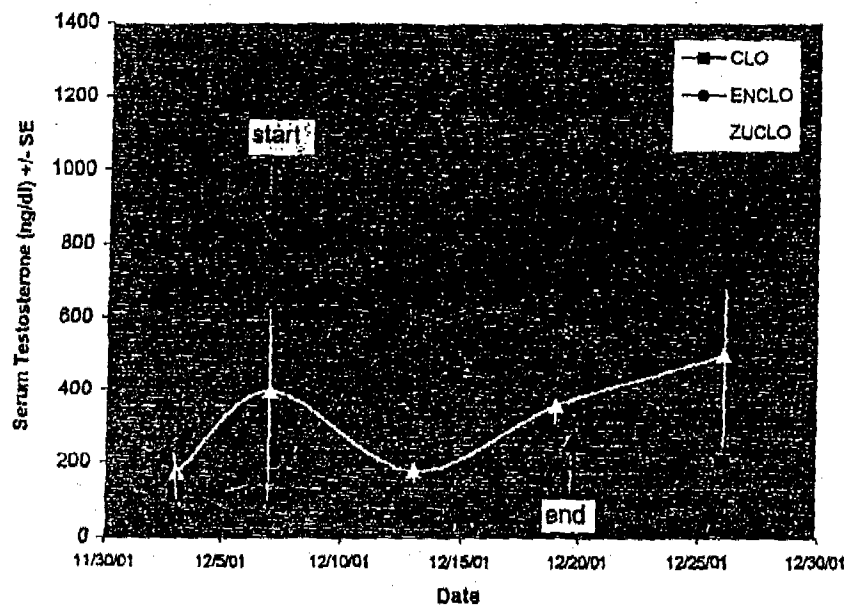


FIG. 3



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**CERTIFICATE OF PATENT
FOR INVENTION
Cert. No. 298237**

Title of the Invention:

USE OF A COMPOSITION COMPRISING CIS-CLOMIPHENE AND TRANS-
CLOMIPHENE IN THE PREPARATION OF A MEDICAMENT FOR INCREASING
SERUM LEVELS OF TESTOSTERONE

Inventor(s):

Patent No.: ZL02817441.0 Int'l Cl.: A61K 31/225

Date of Filing: July 9, 2002

Patentee(s):

ZONAGEN, INC.

Date of Issue: December 13, 2006

Cert. No. 298237

Patent No. ZL02817441.0

This is to certify that this Office examined the invention pursuant to the Patent Law, decided to grant the patent right, issue this certificate, and record the grant in the Patent Register on December 13, 2006. The patent right shall come into effect from the date of issue.

The duration of the patent right in respect of this patent is 20 years counted from the date of filing. The patentee shall pay the annual fee as prescribed in accordance with the Chinese Patent Law and its Implementing Regulations. The time limit for payment of the annual fee for this patent is within one month before July 9, each year. Where the prescribed annual fee is not paid, the patent right shall cease from the date of expiration of the time limit by which the annual fee is to be paid.

This patent certificate records the legal status of the patent right at the time of registration. Other matters, such as patent transfer, pledge, invalidation, cessation and restoration, and any change of the patentee's name, nationality and address, shall be recorded in the Patent Register.

Tian Lipu
Director General

The State Intellectual Property Office of
The People's Republic of China

证书号第298237号



发明专利证书

发明名称：富含反式克罗米酚的克罗米酚组合物的用途

发明人：J·S·波多尔斯基

专利号：ZL 02 8 17441.0

专利申请日：2002年7月9日

专利权人：佐纳根有限公司

授权公告日：2006年12月13日

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[19] 中华人民共和国国家知识产权局



[12] 发明专利说明书

专利号 ZL 02817441.0

[51] Int. Cl.

A61K 31/225 (2006.01)

A61K 31/135 (2006.01)

A61P 15/08 (2006.01)

[45] 授权公告日 2006 年 12 月 13 日

[11] 授权公告号 CN 1289075C

[22] 申请日 2002.7.9 [21] 申请号 02817441.0

[30] 优先权

[32] 2001. 7. 9 [33] US [31] 60/304,313

[86] 国际申请 PCT/US2002/021524 2002.7.9

[87] 国际公布 WO2003/005954 英 2003.1.23

[85] 进入国家阶段日期 2004.3.5

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权利要求书 1 页 说明书 10 页 附图 3 页

[54] 发明名称

富含反式克罗米酚的克罗米酚组合物的用途

[57] 摘要

本发明涉及使用包含反式克罗米酚的组合物治疗患性腺机能减退的男性。本发明还针对治疗性腺机能减退男性的方法。

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1. 一种组合物在制备用于提高雄性哺乳动物血清睾酮水平的药物中的用途，该组合物包括顺式克罗米酚和反式克罗米酚或其药学上可接受的盐或溶剂化物，以及任选一种或多种药学可接受的稀释剂、佐剂、载体或赋形剂，其中反式克罗米酚和顺式克罗米酚的比例大于 71/29 w/w。

2. 权利要求 1 的用途，其中组合物基本上由有效量的反式克罗米酚或其药学上有效的盐或溶剂化物以及任选一种或多种药学可接受的稀释剂、佐剂、载体或赋形剂组成。

3. 权利要求 1 或 2 的用途，其中该组合物以胶囊形式提供。

ST 192

富含反式克罗米酚的克罗米酚组合物的用途

根据 35 U. S. C. § 119 (e), 本申请要求 2001 年 7 月 9 日提交的美国临时专利申请 60/304, 313 的权益。

发明领域

本发明涉及用于提高睾酮水平的组合物和方法。更具体地, 本发明涉及包括富含反式克罗米酚的克罗米酚的组合物。本发明还涉及使用包括富含反式克罗米酚试剂的克罗米酚的组合物来提高睾酮水平。

背景

睾酮是主要的男性雄激素, 在整个男性健康中起重要作用。睾酮对特殊的生殖组织(睾丸、前列腺、附睾、精囊和阴茎)和男性第二性征的发育及维持是必要的。它在性欲和勃起功能中起关键作用, 并对精子发生的开始和维持是必须的。睾酮还具有与生殖组织无关的重要功能。例如, 它通过提高支持纤维体重、肌肉大小和力量的氮储备对身体组成具有有利影响。它还对骨发挥作用以刺激骨形成。

睾酮分泌是一系列激素过程的终产物。下丘脑分泌的促性腺激素释放激素(GnRH)控制垂体前叶脉冲性地分泌黄体生成激素(LH)和卵泡刺激素(FSH)。LH 依次调节睾丸 Leydig 细胞生产和分泌睾酮, 而 FSH 协助诱导精子发生。

睾酮最通常作为“总睾酮”测定。测定包括与性激素结合球蛋白(SHBG)(约 44%)结合并因此是生物不可利用的睾酮和游离(约 2%)或者与其他蛋白松散结合(非 SHBG 结合)(约 54%)的睾酮。

WHO 研究结果显示睾酮通常以昼夜节律分泌, 较高水平出现在早晨, 最低水平发生在约 8-10p.m.。见图 1。老年男性(平均年龄 71 岁)一天中睾酮分泌的这种差异变得不那么显著。目前还不知道这一节律的重要意义。

通过留置导管每 10 分钟对年青和老年患者采集样品, 进行 24 小时。根据 Tenover (1987), 健康年青男性(年龄 22 岁-35 岁, 平均 27.3 岁)的平均 24 小时总血清睾酮水平为 4.9 ± 0.3 ($\pm$ SEM) mg/ml (17.0 nmol/L), 而老年男性(年龄 65 岁-84 岁, 平均 70.7 岁)的平均 24 小时总血清睾酮水平显著偏低, 为 4.1 ± 0.4 mg/ml. ($P < 0.5$;

14.2nmol/L)。

来自单个随机样品的总血清睾酮水平在老年男性中 (4.0 ± 0.2 mg/ml [13.9 ± 0.2 nmol/L]) 也比健康年青男性的 4.8 ± 0.2 mg/ml [16.6 ± 0.2 nmol/L] 显著低。

睾酮缺乏可以是基础疾病或遗传病的结果，还通常是年龄老化的并发症。例如，原发性腺机能减退是原发睾丸衰竭的结果。在这种情况下，睾酮水平低下，垂体促性腺激素 (LH 和 FSH) 水平升高。继发性腺机能减退是因为垂体促性腺激素分泌不足。除了睾酮水平低下，LH 和 FSH 水平也低或是正常低值。成人睾酮缺乏的一些后遗症包括多种多样的症状，包括：性欲丧失，勃起障碍，精子减少或精子缺乏，第二性征消失或减退，肌肉质量进行性减少，疲乏，情绪抑郁和骨质疏松危险增加。

现在美国存在几种形式的睾酮治疗。最近经皮制剂在市场上取得优势。然而，因为阴囊皮肤高浓度的 5α -还原酶，阴囊睾酮贴剂导致生理水平以上的 5α -二氢睾酮 (DHT)。不知道这种 DHT 水平的升高是否具有任何长期的健康后果。非阴囊系统被认为更方便，并且大多数患者达到的平均血清浓度在正常范围以内，具有正常水平的 DHT。不推荐口服睾酮治疗，因为替代治疗需要的剂量与显著的肝毒性危险相关。

概述

本发明涉及用于提高雄性哺乳动物中睾酮水平和用于改善或预防低睾酮水平后遗症的组合物。其中一方面本发明涉及活性成分包括 0% - 29% w/w 的 (顺式, -Z-, 反式克罗米酚) (下文中为“顺式克罗米酚”) 和 100% - 71% w/w 的 (反式, E-, 顺式克罗米酚) (下文中为“反式克罗米酚”) 或其药理学可接受的盐的组合物。在同时含有顺式克罗米酚和反式克罗米酚的本发明中，优选组合物是反式克罗米酚和顺式克罗米酚比例大于 1 的组合物。本发明更优选的组合物包括约 100% w/w 的反式克罗米酚活性成分或其药理学可接受的盐。本发明所有的组合物可以进一步包括合适的药理学赋形剂稀释剂、载体等等。也考虑将顺式克罗米酚和反式克罗米酚的类似物应用在本发明的所有方面。

本发明还涉及提高性腺机能减退的雄性哺乳动物血清睾酮水平

(并改善和防止低睾酮水平的后遗症)的方法,该方法包括给予受试雄性有效剂量的本发明的组合物,该组合物的活性成分包括 0% - 29% w/w 的顺式克罗米酚和 100% - 71% w/w 的反式克罗米酚,包括其任何药理学可接受的盐。优选方法包括给予包含两种异构体,其中反式克罗米酚与顺式克罗米酚的比例大于 1 的组合物的方法。更优选的方法包括给予雄性包含约 100% w/w 反式克罗米酚的组合物。

附图简述

图 1 是健康男性(年青人和老年人)正常分泌总血清睾酮曲线的图示。

图 2 显示克罗米酚柠檬酸盐的化学结构。

图 3 是使用氯米芬(Clomid)、恩氯米芬(Enclomid)和珠氯米芬(Zuclomid)的血清睾酮水平的时程图示。

详述

本发明提供用于提高雄性哺乳动物睾酮水平并改善和防止包括但不限于上文所描述的那些低睾酮水平后遗症的方法和组合物。

克罗米酚(图 2)是与三苯氧胺相关的抗雌激素药物,它阻断作用于下丘脑的正常的雌激素反馈和随后作用于垂体的负反馈。这导致黄体生成激素(LH)和卵泡刺激素(FSH)增加。在男性中,促性腺激素水平的提高刺激睾丸 Leydig 细胞并导致生成更高水平的睾酮。克罗米酚柠檬酸盐具有以下结构:

Ernst 等, J. Pharmaceut. Sci. 65 : 148 (1976) 显示克罗米酚是两种几何异构体的混合物,它们叫做顺式, -Z-, 克罗米酚(顺式克罗米酚或 zuclomiphene) 和反式, E-, 克罗米酚(反式克罗米酚或 enclomiphene)。根据 Ernst 等所述,反式克罗米酚盐酸盐熔点为 149 °C - 150.5 °C, 而顺式克罗米酚盐酸盐熔点为 156.5 °C - 158 °C。

Ernst 等还注意到(反式异构体)是抗雌激素的(AE), 而顺式异构体是更强和更雌激素性的形式, 并且也被报道具有抗雌激素活性。作者将药物对排卵活性的作用归于同时具备两种形式, 称混合物比单独的反式克罗米酚更有效。反式异构体在下丘脑水平帮助排卵。雌激素性异构体顺式克罗米酚在导致排卵的生理途径的其他环节对促进排卵有帮助。据报道异构体还具有不同的体内半衰期。另外据报道单剂给药后顺式形式的血液残留水平持续超过 1 个月。

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Vandekerckhove 等 (Cochrane Database Syst Rev 2000; (2): CD000151 (2000)) 注意到包括 738 位男性的 10 项研究显示抗雌激素药对内分泌结果, 即睾酮, 表现出具有有益的作用, 但没有足够的证据来评价生育作用。然而如果克罗米酚给药提高睾酮水平, 人们就可以容易地得出结论, 药物应该对睾酮剥夺的副作用产生积极影响, 只要睾丸仍然保留对促性腺激素刺激的反应能力。

克罗米酚目前作为顺式和反式异构体的混合物获得批准, 其中顺式异构体占约 30% - 50% (Merck 手册), 用于提高不排卵患者的生育力。克罗米酚通过启动一系列在排卵前促性腺激素大量释放中和随后的卵泡破裂中达到高峰的内分泌事件来促进排卵。推荐药物以每天至多 100mg 的剂量给药 5 天。克罗米酚还和许多副作用相关, 包括: 视物模糊、腹部不适、男子女性型乳房、睾丸肿瘤、血管舒缩性潮热、恶心和头痛。另外, 其他研究显示克罗米酚具有基因毒性和促肿瘤作用。这些观察的净结果是不能接受的含有 30% - 50% 顺式异构体的目前形式克罗米酚作为男性睾酮缺乏治疗的长期疗法。

克罗米酚还被应用于低睾酮水平男性的治疗干预。Tenover 等, J. Clin. Endocrinol. Metab. 64 : 1103, (1987) 和 Tenover 等, J. Clin. Endocrinol. Metab. 64 : 1118 (1987) 发现用克罗米酚治疗后年青和老年男性的 FSH、LH 都升高。他们还发现男性游离和总睾酮升高, 其中年青男性显示了显著的升高。

还进行了研究以明确克罗米酚是否能通过提高精液质量而被用来提高男性生育力。Homonnai 等, Fertil. and Steril 50 : 801 (1988) 观察到精子浓度和计数的增加, 但其他人未观察到 (参见例如 Sokel 等, Fertil. and Steril. 49 : 865 (1988); Check 等, Int. J. Fertil. 34 : 120 (1989); Purvis 等, Int. J. Androl 21 : 109 (1989) 和 Breznik, Arch. Androl. 21 : 109 (1993))。一组发现长期治疗后正常精子百分比的衰减。Shamis 等, Arch. Androl 21 : 109 (1991)。一项 WHO 研究显示在 6 个月的治疗后精液质量或生育力没有变化。(Anonymous Androl. 15 : 299 (1992)。一项荟萃分析似乎证实男性睾酮水平升高, 伴随精子质量差, 并非不育。

(Vandekerckhove 等, 2000)。研究还提示长期使用克罗米酚治疗似乎对健康没有强烈的有害影响, 尽管它的确显示 4 个月后治疗导致

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精子质量更差。研究中男性服用克罗米酚长达 18 个月，每天 25mg 或隔日 100mg。

1991 年 Guay 等 (Urology 38 : 377 (1991)) 建议克罗米酚可以治疗男性性功能障碍。他们的假说看起来是性功能遵循睾酮水平。Davidson 等, J. Clin. Endocrinol. Metab. 48 : 955 (1979) 的显示雄激素和性功能积极影响的早期研究, 和 Cuxuilingham 等, J. Clin. Endocrinol. Metab. 70: 792 (1990) 认为睡眠相关勃起是对睾酮的强烈反应的研究支持这一假说。然而, 1995 年, Guay 等 J. Clin. Endocrinol. Metab. 80 : 3546 (1995) 发表了一项研究, 在研究中他们观察到克罗米酚治疗 2 个月后, LH、FSH 和睾酮增加, 但对勃起功能障碍无效。对于年青男性和老年男性中的某些特殊群体这可能有某些益处, 但看起来仅仅提高睾酮水平是不够的。睾酮对睡眠相关勃起的作用可能被看得过于重要了 (Herskowitz 等, J. Psychosomat. Res. 42 : 541 (1997))。

根据本发明, 使用一种组合物以提高睾酮水平同时减少药物的副作用, 该组合物包括优选是反式克罗米酚的一种异构体或如下所述的与通常生产的混合物不同的预先确定的克罗米酚异构体混和物。因此, 本发明提供一种提高睾酮水平的口服治疗, 它没有与现有的克罗米酚制剂相关的副作用或副作用减少。

在本发明的一项实施方案中, 给予有提高血清睾酮水平需要和愿望的患者一剂或多剂有效量的包括反式克罗米酚的组合物, 剂量为 1mg 到约 200mg (尽管最佳剂量的确定在本领域普通技术人员水平范围内)。只要反式克罗米酚和顺式克罗米酚的比例大于 1, 顺式克罗米酚也可以存在于组合物中。在本发明的实施中诸如前面 Ernst 等描述的那些克罗米酚反式和顺式异构体的类似物也有用。

优选 (但不必须) 作为设计成提高血清睾酮水平的剂量方案的一部分给予剂量, 该血清睾酮水平模拟或对应于图 1 描述的正常分泌总血清睾酮曲线。例如, 根据图 1 采用使大约 8 a. m. 时的血清睾酮水平峰值升高的药物学制剂, 可以给予一剂优选组合物。该药物学制剂可以采用按例如美国专利 6, 221, 399, 日本专利 4-312522, Meshali 等, Int. J. Phar. 89: 177-181 (1993), Kharenko 等, Intern. Symp. Control Rel. Bioact. Mater. 22: 232-233 (1995), WO 95/35093,

Dangprasit 等, Drug. Devel. And Incl. Pharm. 21 (20): 2323-2337 (1995), 美国专利 6,143,353, 6,190,591, 6,096,338, 6,129,933, 6,126,969, 6,248,363 描述制备的持续释放制剂和本领域熟知的其他持续释放制剂的形式。

合适的药物组合物或单位剂型可以采用诸如片剂或填充胶囊的固体形式,或诸如溶液悬浮液、乳液、酏剂的液体形式或用同样物质填充的胶囊,都用于口服。组合物还可以采用无菌注射溶液或肠胃外(包括皮下)应用的乳液形式。该药物组合物和其单位剂型可以包括常规比例的组成成分。

本发明的组合物还可以通过静脉、皮下、颊、经粘膜、鞘内、皮内、脑池内或其他给药途径给药。在给予组合物后可以如上所述测定血清睾酮水平,并且可以改变剂量使血清睾酮水平充分提高以获得与上面描述的正常睾酮相关的所需的生理学结果。

将这里讨论的所有参考文献整体引入作为参考。

以下实施例是为了说明本发明,并不意味着限制所附权利要求所限定的本发明的范围。

实施例 1

氯米芬对雄性狒狒血清睾酮的作用

连续十二天给予成年雄性狒狒 1.5 mg/kg 的氯米芬、恩氯米芬(反式氯米芬)或珠氯米芬(顺式氯米芬)。分析的样品是在初次治疗当天给予检验物品前(第 0 天)、治疗 12 天后(第 12 天)和末次治疗后 7 天时(终点或洗脱期)采集的血清。

1. 对体重和血清 LH、FSH、PRL 和睾酮的作用

在接受恩氯米芬的组中总血清睾酮显著增加。见表 1。在基线期或第 0 天各组间没有差别。治疗后 7 天时(洗脱期)三组间也没有差别。然而,在第 6 天与氯米芬和珠氯米芬相比(分别为 $p = 0.03$ 和 $p = 0.00002$),在第 12 天与珠氯米芬相比($p = 0.047$),恩氯米芬产生更高水平的睾酮。珠氯米芬显然不提高总血清睾酮。根据它们的变异系数判断,与接受恩氯米芬的动物相比,接受氯米芬的动物在第 6 天和之后显示出更多变化的总睾酮水平。当我们观察作用时程时(图 3),我们确定与基线或第 0 天值比较,只有恩氯米芬在第 6 天和第 12 天显著地和统计学上升高总血清睾酮。另外,终止恩氯米芬治疗导致

第 12 天和 18 天（洗脱期）之间总血清睾酮水平显著下降。这说明恩氯米芬易于从循环中清除，与人类中见到的恩氯米芬代谢清除一致。恩氯米芬显然比氯米芬本身更好和更一致，珠氯米芬无效。

表1-血清睾酮水平 (ng/dl)

组别	ID	基线 12/3/01	第0天 12/7/01	第6天 12/13/01	第12天 12/20/01	洗脱 12/26/01
氯米芬	7500	79.01	76.15	940.97	891.5	150.9
	9012	97.55	305.24	585.92	555.6	316.3
	9097	158.06	102.94	151.12	318.9	143.6
	均值	111.5	161.4	559.3	588.7	203.6
	标准差	41.3	125.2	395.6	287.7	97.7
恩氯米芬	7223	64.57	74.96	1223.8	633.6	307.2
	8021	166.86	133.59	1128.2	1466	399.2
	8369	170.45	106.47	1081.1	1166	271
	均值	134.0	105.0	1144.4	1088.5	325.8
	标准差	60.1	29.3	72.7	421.6	66.1
珠氯米芬	7438	124.84	210.4	137.51	314.5	359.7
	8292	104.66	67.37	169.98	406.1	860.5
	10098	282.29	904.82	227.95	353.0	274.1
	均值	170.6	394.2	178.5	357.9	498.1
	标准差	97.3	448.0	45.8	46.0	316.8
ANOVA		p=0.61	p=0.43	p=0.007	p=0.57	p=0.256
K-W		p=0.56	p=0.84	p=0.051	p=0.079	p=0.252

血清 LH 或 FSH 没有变化。总血清睾酮与 LH 的比例遵循与总血清睾酮一样的模式，提示缺乏依赖性（数据未显示）。在 12 天的研究中体重也没有变化。研究过程中接受恩氯米芬的组血清泌乳素（PRL）下降，提示已经部分描述（Ben-Jonathan 和 Hnasko, 2001）和根据随男性年龄增长睾酮下降和泌乳素增加的事实预期（Feldman 等, 2002）的抗雌激素作用。

2. 对临床化学参数的作用

通过方差分析（ANOVA）或 Kruskal-Wallis 检验测定，研究开始时对于任何检验参数，三组之间每个参数的平均值没有差异。所有组的每一参数呈现正常值，除了（1）血清钠；一项相关的计算参数，阴离子间隙在整个试验过程中所有 9 只狒狒的值均低；（2）血清葡萄糖；和（3）BUN，第 0 天它在将接受恩氯米芬治疗组中的值高。在治疗第 12 天和治疗后 7 天（洗脱期），除了氯米芬和珠氯米芬组比恩氯米芬组

的阴离子间隙值低外，各组间任何参数没有差异。此组狒狒的血清钠和阴离子间隙值表现出异常。

恩氯米芬和珠氯米芬对红细胞数量，珠氯米芬对血细胞比容有很大影响。在第 0 天或终点，所有的化合物都降低平均红细胞血红蛋白浓度 (MCHC)。因为平均红细胞血红蛋白 (MCH) 没有变化而平均红细胞体积 (MCV) 增加，MCHC 下降是可以预料的。尽管认为睾酮可能提高血细胞比容，只有不提高总血清睾酮的珠氯米芬治疗显示出统计学上的差异。显然，应当监测临床试验中使用珠氯米芬的男性的红细胞数量的特征。预计恩氯米芬会具有更小的作用。

尽管值在正常范围内，看起来 12 天恩氯米芬的治疗对血小板有明确作用。此处需要考虑的一件事是雄性和雌性狒狒之间血小板计数的性别双态性 (雄性 279 对雌性 348)。这可能是激素的原因。由于恩氯米芬组显示睾酮增加，血小板计数的降低可能继发于此组睾酮的变化。另外，恩氯米芬治疗使血小板计数从处于此组正常范围高值的第 0 天水平变成它的正常雄性水平。恩氯米芬并不必定对血小板产生有害作用。

所有检验的氯米芬对白细胞 (WBC) 数量有作用。最惊人的是恩氯米芬升高淋巴细胞和嗜酸细胞计数的作用。该作用并不象它们应该看起来的那样直接。看起来恩氯米芬对降低血液粒细胞百分比有强大作用。7 天洗脱期后当值降到正常范围以下时作用非常强大。(这一时程可以反映出影响 WBC 数量改变需要相对长的时间。) 狒狒白细胞数量的性别双态性很小，因此作用更可能是因为化合物本身而非睾酮的变化造成的。然而，当观察使用 WBC 计数器计算的粒细胞计数时，我们发现粒细胞计数没有因为任何化合物产生差异。同时，最有趣的是淋巴细胞。恩氯米芬治疗群体中淋巴细胞计数和百分比均上升。尽管淋巴细胞百分比的平均值仍在正常范围，因为 WBC 计数的升高趋势，净作用是使用恩氯米芬淋巴细胞计数增加。嗜酸细胞结果相似。这对治疗淋巴细胞低的男性，诸如 HIV 阳性的男性有明确的意义。既然根据此结果恩氯米芬可能不降低淋巴细胞，可以安排病例将其应用于患 AIDS 的男性人群。因为疾病的消耗作用这些个体经常使用用来提高睾酮的制剂进行治疗。低肝肾毒性以及对胆固醇和脂质有利的作用也是任何用于已经受到疾病损害的 HIV 阳性男性的药物高度有利的品质。

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氯米芬或珠氯米芬引起的血清葡萄糖的升高在正常范围内。

对于第0天平均血清葡萄糖值高的恩氯米芬，治疗后值没有升高。没有证据表明恩氯米芬会对血液葡萄糖产生有害作用。

根据酶AST和ALT判断，显然对肝功能没有明确的副作用。随治疗进行这些值呈下降趋势。血清酶水平升高将提示肝损害。在研究结束时氯米芬组的ALT/SGPT超出低限，尽管治疗期间的差异在统计学上不显著。恩氯米芬和珠氯米芬引起的变化在正常范围以内。妊娠时AST降低，因此诸如珠氯米芬的雌激素激动剂降低边缘AST水平的作用可以是合理的。碱性磷酸酶(ALP)也在肝中发现并在多种疾病状态下升高。ALP的降低进一步不支持肝损害。同样是肝脏产物的血清白蛋白没有变化。尽管性激素结合球蛋白发挥更重要的作用，血清白蛋白长时间的强抑制可以对人类游离血清类固醇激素水平有影响。作为底线，根据检测的参数，没有化合物与肝损害相关联。

成骨活性和骨疾病伴随血清ALP值高。ALP不随珠氯米芬治疗升高，并随恩氯米芬治疗降低。此趋势预计与珠氯米芬相比，使用恩氯米芬产生的结果更好。

尽管研究中氯米芬和恩氯米芬组的BUN和BUN/肌酐有变化，肌酐没有明确的变化不支持肾功能障碍。肾小球滤过功能丢失会导致BUN升高。人类BUN的下降是因为营养差（在控制情况下不可能发生），或大量液体摄入（假设伴发水肿）。同样，尽管使用恩氯米芬在第0天和第12天之间总血清睾酮升高，血清肌酐值没有差异，不支持在此短时间间隔内肌肉质量增加。

在研究过程中所有动物的血清钠水平比参考值低。在第12天氯米芬和珠氯米芬组的血清二氧化碳比参考值高。整个研究过程中所有动物的血清阴离子间隙更低，与钠结果平行。恩氯米芬使此参数向正常值方向增长。在所有治疗过程中受试动物中检测到的电解质失衡尚不明朗，但可能是BUN结果提示的同一液体紊乱现象的一部分。

尽管每种变化都没有达到统计学显著性，恩氯米芬治疗倾向于降低血清胆固醇，珠氯米芬倾向于提高同一参数。这些变化处于正常范围内，但是在短时间内两种异构体显示相反作用的趋势对进一步的监测有益，并且因为异构体选择性地具有雌激素激动剂或拮抗剂活性，趋势并不是不可预测的。可以预料如果长期应用，恩氯米芬比珠氯米

芬对血清胆固醇更有利。

前面的结果说明对于提高总血清睾酮恩氯米芬比氯米芬或珠氯米芬更有效。珠氯米芬显然无效并且该缺陷限制了氯米芬在性腺机能减退中的任何应用，特别是因为它半衰期较长，氯米芬的珠氯米芬成分会随时间在循环中占优势。

当与珠氯米芬，以及甚至通常与氯米芬比较时，恩氯米芬在所有方面表现得相对优良。当考虑恩氯米芬降低胆固醇和肝酶的趋势而相反珠氯米芬升高同样参数的趋势时，这特别正确。如果能显示淋巴细胞 CD4+ 亚群没有降低或升高，恩氯米芬升高淋巴细胞计数的令人吃惊的趋势可能对患 AIDS 的男性有用。

实施例 2

使用反式克罗米酚以及反式克罗米酚和顺式克罗米酚比例大于 1 的混合物提高男性睾酮水平的方法

在给予反式克罗米酚之前，收集受试雄性的血液样品并测定睾酮水平，其中使用例如 Matsumoto 等，Clin. Endocrinol. Metab. 56: 720 (1983) (引入此处作为参考) 中描述的方法学。也可以按照例如 Tenover 等，J. Clin. Endocrinol. Metab. 65: 1118 (1987) 中所描述的测定游离的和与睾酮结合的性激素结合球蛋白 (SHBG) (该文描述了通过 α [^3H] 二氢睾酮饱和度分析和放射免疫检测进行 SHBG 测定) 进行测量。例如根据 Tenover 等，J. Clin. Endocrinol. Metab. 65: 1118 (1987) 还测定了非 SHBG 结合的睾酮水平 (生物可利用睾酮)。还可以参见引入此处作为参考的 Soderguard 等，J. Steroid Biochem 16: 801 (1982)。

给予患者每日 1.5 mg/kg 剂量的克罗米酚，其中反式克罗米酚和顺式克罗米酚的比例大于 1。监测患者的睾酮水平以便可以调整剂量大小和剂量频率使睾酮在患者体内达到治疗水平。

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健康年青和老年男性的正常分泌总血清睾酮曲线

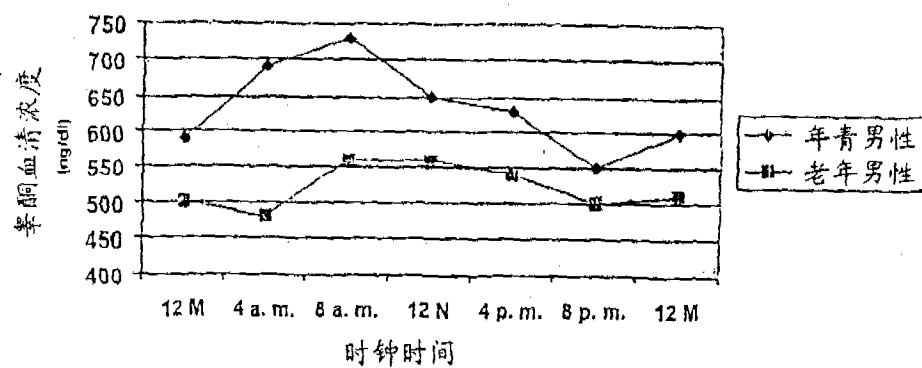


图 1

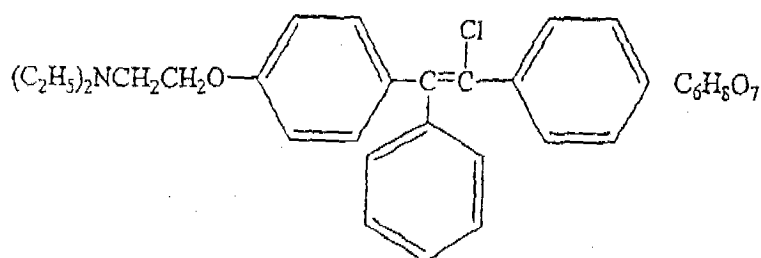


图 2

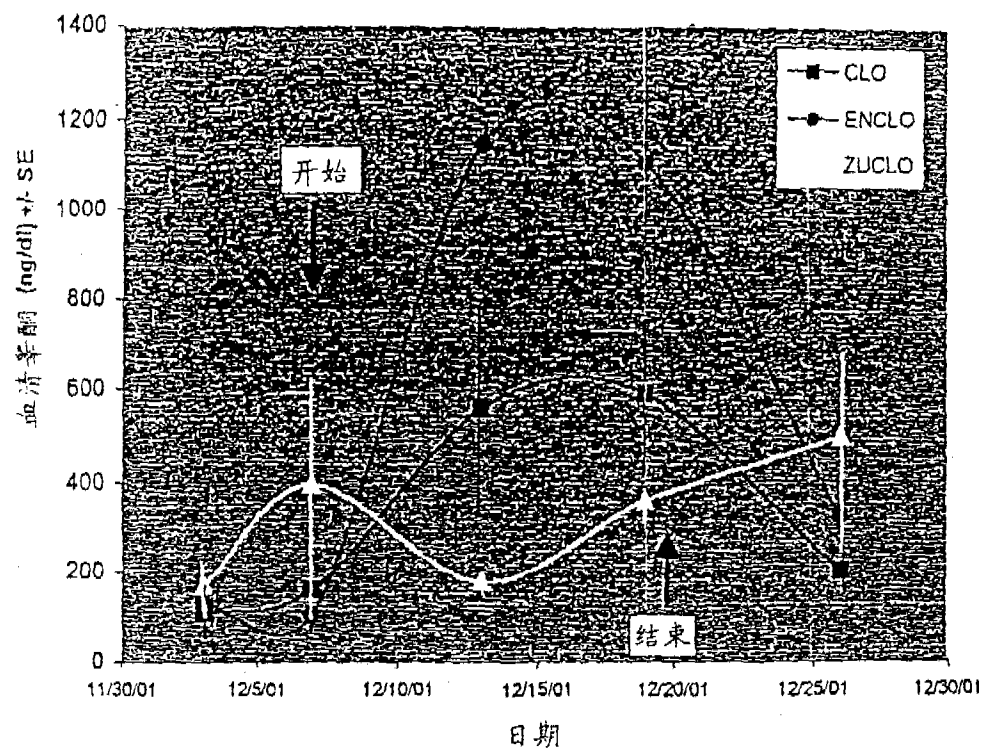
58
204
204

图 3

香港特別行政區政府知識產權署專利註冊處
Patents Registry, Intellectual Property Department
The Government of the Hong Kong Special Administrative Region

知識產權署
Intellectual Property Department

In reply please quote this ref.: 05104390.3

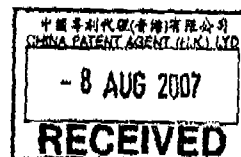
Your ref.: HKP0321986-CN-2:60003; HKP9821016-CN-2:62146

Tel.: 2961 6034

Fax.: 2838 6315

7 August, 2007

China Patent Agent (H.K.) Ltd.
22/F, Great Eagle Centre
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Request for Change of Name of Applicant / Proprietor

I refer to your request filed on 30 July, 2007. I am pleased to inform you that the following change has been effected:

Application No.

05104390.3

98111244.4

Publication No.

1071521

1017603

New Name : REPOS THERAPEUTICS INC.

This is a letter issued by Queenie Tai for Registrar of Patents.

(This is a computer-generated copy. No signature is required.)

C.C. (related file(s))

香港特別行政區政府知識產權署專利註冊處
Patents Registry, Intellectual Property Department
The Government of the Hong Kong Special Administrative Region

知識產權署
Intellectual Property Department

In reply please quote this ref.: 05104390.3

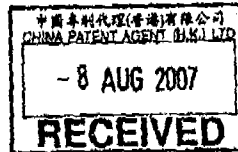
Your ref.: HKP0321986-CN-2:60003; HKP9821016-CN-2:62146

Tel.: 2961 6934

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7 August, 2007

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Request for Change of Address of Applicant / Proprietor

I refer to your request filed on 30 July, 2007. I am pleased to inform you that the following change has been effected:

Application No.
05104390.3
98111244.4

Publication No.
1071521
1017603

New Address : 2408 TIMBERLOCH PLACE
SUITE B-1, THE WOODLANDS
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美國/美利堅合眾國

This is a letter issued by Queenie Tai for Registrar of Patents.

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C.C. (related file(s))

(19) Patents Registry
The Hong Kong Special Administrative Region
香港特別行政區
專利註冊處

(11) 1071521 B
CN 1269075 C

(12)

STANDARD PATENT SPECIFICATION
標準專利說明書

(21) Application No. 申請編號
05104390.3

(51) Int.Cl.⁸ A61K A61P

(22) Date of filing 提交日期
23.05.2005

(54) USE OF A COMPOSITION COMPRISING CIS-CLOMIPHENE AND TRANS-CLOMIPHENE IN THE PREPARATION OF A MEDICAMENT FOR INCREASING SERUM
LEVELS OF TESTOSTERONE 含有反式克羅米酚的克羅米酚組合物的用途

(30) Priority 優先權

09.07.2001 US 60/304,313

(43) Date of publication of application 申請發表日期

22.07.2005

(45) Publication of the grant of the patent 授予專利的發表日期

27.07.2007

CN Application No. & Date 中國專利申請編號及日期

CN 02817441.0 09.07.2002

CN Publication No. & Date 中國專利申請發表編號及日期

CN 1551765 01.12.2004

Date of Grant in Designated Patent Office 指定專利當局授予專利日期

13.12.2006

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70 208
208

CERTIFICATE OF GRANT OF PATENT

批予專利證明書

Patents Ordinance (Chapter 514)

專利條例 (第 514 章)

STANDARD PATENT 標準專利

I hereby certify that a standard patent with the following particulars has this day been granted 茲證明下述標準專利在今日批予:

Name and Address of Proprietor 專利所有人姓名或名稱及地址:

ZONAGEN, INC.

2408 TIMBERLOCH PLACE, B-4

THE WOODLANDS, TEXAS 77380

UNITED STATES/UNITED STATES OF AMERICA

Patent No. 專利編號 : HK1071521

Application No. 申請編號 : 05104390.3

Title of Invention 發明名稱 :

USE OF A COMPOSITION COMPRISING CIS-CLOMIPHENE AND
TRANS-CLOMIPHENE IN THE PREPARATION OF A MEDICAMENT FOR
INCREASING SERUM LEVELS OF TESTOSTERONE
富含反式克羅米酚的克羅米酚組合物的用途

Term of Standard Patent 標準專利有效期 :

Twenty years commencing on 09.07.2002

由 09.07.2002 年起計 20 年

Dated this 27th July, 2007

二零零七年七月二十七日



Stephen Selby

Registrar of Patents

專利註冊處處長謝爾方

Patents Registry
Intellectual Property Department
The Hong Kong Special Administrative Region
香港特別行政區知識產權署專利註冊處

Please see the important notes overleaf 請細閱背頁的專利所有人須知

**SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIRECCION DE NUEVAS CREACIONES**

Radicación No. 04-1222

Clasificación Internacional (IPC): A61K 31/225

Concepto Técnico No. 578

Patente de Invención



Patente de Modelo de Utilidad



Vía Nacional



Vía PCT (fase nacional)



Cap. I



Cap. II



No. Sol. Internacional

PCT/US02/21524

Fecha de Presentación Internal.

2002/07/09

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

Descripción:

Folios: 29 a 56

Reivindicaciones:

Folios 57 a 58 como se radicó inicialmente

Folios 65 a 69 como se modificó posteriormente

Folio 116 modificadas como respuesta al primer examen de fondo

Folio 138 modificadas como respuesta al segundo examen de fondo

Rta. del Solicitante:

Folios: 109 a 113 y 130 a 135 y 139 a 141

Prioridad:

fecha: 9/07/2001, US, 60/304.313

2. Objeto de la invención

Título de la solicitud: Composiciones para el tratamiento de deficiencias de testosterona en varones. Número de folio: 110

El problema planteado en esta solicitud es la necesidad de compuestos útiles para aumentar las concentraciones de testosterona en mamíferos machos.

Para solucionar este problema técnico la solicitud en estudio proporciona métodos no escrotales para administración de testosterona en composiciones tales como una mezcla de los isómeros de clomifeno de 0-29% p/p (cis-clomifeno) y de 100-71% p/p (trans-clomifeno) en vez de una mezcla en donde el "cis" se encuentre entre el 30-50% con el fin de aumentar las concentraciones de testosterona en mamíferos machos proporcionando igualmente sus correspondientes métodos.

- Clasificación: A61K 31/225

3. 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 9/07/2001

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*
D ₁	US 4,061,733	Veterinary compositions for inducing estrus in animals and method	6/12/1977
D ₂	EP 0 888 775	Control of selective estrogen receptor modulators	1/07/1999

4. Respuesta a los argumentos del solicitante:

- Dice el solicitante que se han excluido las composiciones que comprenden una proporción de clomifeno trans / cis 8:1 y que por lo tanto con estas modificaciones se debe tener por superada la objeción por falta de novedad con respecto a D₂.

Se aclara al solicitante si bien con la excepción realizada en cuanto a la proporción 8:1 ahora la reivindicación 1 es nueva, no tendrá sin embargo, nivel inventivo puesto que la solicitud, resulta obvia, ya que el experto medio en la materia enfrentado al problema de necesitar compuestos útiles para aumentar las concentraciones de testosterona en mamíferos machos y conociendo el estado de la técnica haría lo que dice en D₂. Además si el experto medio se apoya también con lo que dice D₁, en donde se muestran otras posibilidades de proporciones adicionales para la mezcla de los isómeros cis/trans clomifeno, le sugerirá al técnico medio que las proporciones de estos isómeros se pueden variar fácilmente sin perder sus efectos farmacológicos o bien consiguiendo otros efectos técnicos.

- Dice el solicitante que D1 no contiene sugerencia alguna que motive al experto en la técnica a modificar esta proporción de isómeros con el objeto de obtener composiciones útiles para aumentar los niveles de testosterona y de hecho una persona versada que le a D1 entendería que dicha invención está limitada a una proporción específica 3:2

Se aclara al solicitante que D1 con la proporción que enseña por si solo tal vez no daría a entender al experto medio en la materia que podría llegar a realizar variaciones a las proporciones de los isómeros cis y trans, pero si el experto medio también tiene acceso también a D2, observa que variar las proporciones es una posibilidad válida.

- Dice el solicitante que a pesar de que D₂ revela el uso de composiciones que contienen citrato de clomifeno (fuera del alcance de las reivindicaciones) y revela una proporción trans a cis de 8:1 (también fuera del alcance de la presente invención), D₂ no proporciona ninguna guía que conduzca a la

persona versada en la materia a las composiciones modificadas que se presentan.

Se aclara al solicitante que las reivindicaciones deben ser claras por si mismas y en el capítulo reivindicatorio modificado se menciona el clomifeno mas no dice que no sea citrato. Se reivindica el clomifeno con sus isómeros, sales o solvatos. Por lo tanto no se puede asegurar que el citrato de clomifeno esta fuera del alcance y asumir que sea una diferencia más con respecto a la solicitud. De otro lado, ni la descripción ni la figura 3 anexa a la respuesta presentan datos contundentes que muestren un efecto técnico sorprendente de las proporciones reivindicadas contra la proporción 8:1 que presenta la anterioridad, ya que lo que se corroboró con dichos ensayos fue precisamente que cada isómero "por separado" tuviera características estrogénicas y antiestrogénicas más no se muestra el ensayo de las "proporciones mayores de 71/29" - tal como se presenta la reivindicación independiente, para mostrar superioridad o un efecto que marque la diferencia. Adicionalmente la proporción exceptuada del capítulo reivindicatorio está coincidentalmente justo en el medio del rango que se pretende proteger por lo tanto es un mero solapamiento de una característica técnica que sigue siendo básicamente la misma. además si el experto medio se apoya también con lo que dice D₁, en donde se muestran otras posibilidades de proporciones adicionales para la mezcla de los isómeros cis/trans clomifeno, le sugerirá al técnico medio que las proporciones de estos isómeros se pueden variar fácilmente sin perder sus efectos farmacológicos o bien consiguiendo otros efectos técnicos.

- Dice el solicitante que en la página 5 en D2 se contempla administrar (1) citrato de clomifeno (2) una mezcla de clomifeno de trans a cis en relación 8:1 (3) cis-clomifeno solo, sin embargo la administración de **trans- clomifeno solo** no está contemplada bajo los ejemplos. Por ello, un técnico en la materia que lea D2, aprende que el cis-clomifeno solo es útil para tratar las condiciones enlistadas o que las composiciones útiles para el tratamiento de dichas condiciones deben comprender clomifeno trans-cis 8:1. Esta enseñanza es opuesta a la presente invención que demuestra que al incrementar la proporción de clomifeno trans a cis por encima de las del arte anterior, se observan beneficios inesperados. Se pide verificar la figura 3 en donde el resultado es sorprendente en vista de las enseñanzas del arte anterior en las que cada isómero tiene ambas características estrogénicas y antiestrogénicas y que el efecto farmacéutico del citrato de clomifeno se atribuyó parcialmente al cis-clomifeno. La figura 3 compara los efectos de composiciones que comprenden ya sea trans al 100 %, cis al 100% o citrato de clomifeno y se observa que proporciona resultados inesperados al demostrar que el cis al 100% disminuye los niveles de testosterona, por otro lado el isómero trans incrementa los niveles de testosterona a más del doble sobre la misma dosis del citrato de clomifeno. Por lo tanto, la invención si tiene un efecto mejorado y sorprendente.

Se aclara al solicitante que un experto medio a partir de D2 no solo aprenderá que el cis-clomifeno solo es útil para tratamiento o con la proporción específica 8:1 sino también como ya que como bien lo dice el solicitante en su respuesta folio 131 página 2, en dicho documento se sugieren también claramente que las proporciones pueden variar en los rangos 30-50% (cis) y 50-70% (trans).

De otro lado la figura 3 no ha sido contundente prueba del efecto pretendido por la solicitud ya que se evalúan los isómeros por separado corroborando que son activos, pero no se evalúan las proporciones específicas reivindicadas.

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Un experto en la materia a partir de D2 no solamente va a entender que el cis-clomifeno solo es útil para tratar las condiciones enlistadas o que las composiciones útiles para el tratamiento de dichas condiciones deben comprender clomifeno trans-cis 8:1, sino que leyendo además D1 se va a dar cuenta que puede realizar variantes a las proporciones mucho mas amplias obteniendo efectos farmacológicos.

- Dice el solicitante que la patente ha sido concedida en otras jurisdicciones.

Se recuerda al solicitante que la concesión de patentes es territorial y no implica que por el hecho de haber sido concedida en otro lugar, en Colombia se tenga obligatoriamente que tomar la misma decisión.

5. Análisis de patentabilidad (artículo 14 D486)

Nivel inventivo (artículo 18 D486)

La presente Solicitud de Patente reivindica:

Reivindicaciones 1 – 3: composición de cis-clomifeno y trans clomifeno en proporción de trans-clomifeno con relación a cis-clomifeno mayor de 71/29 pero no 8:1:

Dicha composición está afectada por los siguientes documentos del Estado de la Técnica:

D₁ que enseña mezclas de uso veterinario compuestas por cis-clomifeno y trans-clomifeno en proporciones aproximadamente de 3:2 (60-40%). Estas composiciones pueden formularse como tabletas, tabletas cubiertas, gránulos, emulsiones, jarabes, soluciones o suspensiones con vehículos acuosos, no acuosos, almidón, azúcar, miel, etc. pueden administrarse oralmente o parenteralmente. (ver folios 97 y 98 columna 2 líneas 5 a 22 y 35 a 38).

D₂ que enseña (ver folios 99-100, 117 -121, documento completo) enseña que el clomifeno vía oral en forma de tabletas, grageas, cápsulas, píldoras, etc., con sus isómeros en relación 8:1 (89-11%) o de 30-50% (cis) y 50-70% (trans) puede ser usado solo para prevenir la osteoporosis, enfermedades cardiovasculares, para mantener perfiles lipídicos sanguíneos saludables, prevenir tumores mamarios o para la amenorrea.

TABLA 1	
CARACTERÍSTICAS REIVINDICADAS	D ₂
composición en cápsula	SI Pág 4, pág 121
0-29% cis-clomifeno	SIMILAR Abstract, pág 121 Solo se diferencia en que se exceptua la proporción 11% (8:1), la cual está contenida en el rango intermedio de lo reivindicado
100-71% trans clomifeno	SIMILAR Abstract, pág 121 Solo se diferencia en que se exceptua la proporción 88% (8:1) la cual está contenida en el rango intermedio de lo reivindicado

La única **diferencia con D₂** es que esta solicitud **exceptúa de su capítulo reivindicatorio la relación de isómeros de clomifeno 8:1** pero esta diferencia no parece conceder un efecto técnico inesperado.

No puede determinarse un efecto técnico que logre la diferencia con la anterioridad encontrada porque ni la descripción ni la figura 3 anexa a la respuesta presentan datos contundentes en tal sentido ya que lo que se corroboró con dichos ensayos fue precisamente que cada isómero "por separado" tuviera características estrogénicas y antiestrogénicas más no se muestra el ensayo de las "proporciones mayores de 71/29" - tal como se presenta la reivindicación independiente - en donde precisamente se verifiquen los efectos de las proporciones reivindicadas de los isómeros mezclados y se corrobore que la proporción exceptuada 88/11 no presenta el efecto que también soluciona el problema técnico. Adicionalmente la proporción exceptuada del capítulo reivindicatorio está coincidentalmente justo en el medio del rango que se pretende proteger.

El Problema Técnico Objetivo se puede definir en como lograr **composiciones de mezclas de isómeros cis/trans clomifeno alternativas a las previamente conocidas.**

Por tanto, la solicitud, tal como se reivindica, es obvia, porque el experto medio en la materia enfrentado al problema de necesitar compuestos útiles para aumentar las concentraciones de testosterona en mamíferos machos y conociendo el estado de la técnica haría lo que dice en D₂ apoyado también con lo que dice D₁, ya que tanto en D₁ como en D₂ se muestran proporciones diferentes para la mezcla de los isómeros cis/trans clomifeno, lo cual sugiere al experto medio en la materia que las proporciones de estos isómeros se pueden variar para llegar así a conseguir efectos técnicos diferentes.

En conclusión, no se considera que la presente solicitud merezca reconocimiento de nivel inventivo.

6. Conclusión:

En consecuencia y por todo lo expuesto anteriormente, se recomienda **Negar** las reivindicaciones: 1 a 3 (folio: 138)

Bogotá, D.C.,

CONCEPTO TECNICO No. 578	EXAMINADOR TECNICO Código No. 202087
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