Title: USE OF BIOGENIC ESTRIOL DIESTER PRODRUGS FOR THE TREATMENT OF AUTOIMMUNE DISEASES

Abstract: The invention relates to the use of esters of estriol, for example, an estriol 3,17-dipropionate or an estriol 3,17-dihexanoate, for the treatment of autoimmune diseases, such as multiple sclerosis (MS).
Use of biogenic estriol diester prodrugs for the treatment of
autoimmune diseases

Field of the invention
The present invention relates to the use of esters of estriol, for example, an
estriol 3,17-dipropionate or an estriol 3,17-dihexanoate, for the treatment of
autoimmune diseases, such as multiple sclerosis (MS).

Background of the invention
Autoimmune diseases are a type of immune pathologies that result from an
uncontrolled immune response against autoantigens. The susceptibility to
autoimmune diseases is affected by gender. During reproductive ages, there
exists a prevalence among females to suffer from an autoimmune disease such
as multiple sclerosis (MS) or rheumatoid arthritis (RA). For example, the female-
to-male ratio to develop MS is 2:1 (Voskuhl et al., 2001; Neuroscientist 7: 258-
270; Whitacre et al., 1999; Science 283: 1277-1278).

MS is an autoimmune disorder of the central nervous system affecting the
myelin sheath of neurons and leading to demyelination and subsequent
neuronal cell death. The disease is mediated by CD4+ T lymphocytes, which
are specific for proteins in the myelin sheath like myelin basic protein (MBP),
proteolipid protein (PLP), and myelin oligodendrocyte protein. One hypothesis is
that on the basis of a genetically determined predisposition, environmental
factors such as viral infections trigger the outbreak of the disease which results
in an imbalance in the Th1 and Th2 population of lymphocytes, thereby
promoting the accumulation of activated Th1 cells that are able to penetrate the
blood-brain barrier and exert proinflammatory actions in the CNS. Demyelination
of axons is in part caused by myelin-specific CD4+ lymphocytes secreting Th1
cytokines like interleukin(II)-12, interferon gamma (IFNγ) and tumour necrosis
factor alpha (TNFα). This pro-inflammatory cytokine pattern is characteristic for
the cell-mediated immune response. In healthy individuals the cell-mediated
Th1 immune response is in balance with the humoral Th2 immune response.
The humoral immune response is mediated by the anti-inflammatory Th2 cytokines IL-4, IL-5 and IL-10.

The treatment strategies of MS are currently based on immunomodulatory treatment using interferons or glatiramer acetate. However, these treatments delay progress of disease only in some patients. Corticosteroids are used for acute treatment of relapses due to their antiinflammatory effects. Treatment with corticosteroids alleviates some acute symptoms of MS but fails to affect long-term prognosis. In addition to the known, numerous side-effects of corticosteroids, they also inhibit endogenous immunosuppressive mechanisms, rendering them unsuitable for long-term therapy. For therapy of highly active disease or of patients not responding to standard treatments including patients suffering from secondary progressive MS, immunosuppressive agents like methotrexate or cyclosporine are used. These substances are often poorly tolerated.

Therefore, there exists a need for an additional treatment strategy of MS and other Th1-mediated immune diseases.

In many autoimmune diseases, such as in MS, the Th1/Th2 balance is disturbed. Female sex hormones seem to have an influence on the regulation of this balance. During pregnancy, a shift toward a Th2 cytokine pattern has been demonstrated. An improvement of the clinical symptoms of Th1-mediated immune diseases (like MS) during pregnancy has also been observed. Especially in the third trimester of pregnancy, the rate of relapse declines in women with MS (Confavreux et al., 1998; N Engl J Med 339(5): 285-291). The decrease in disease activity appears to be due at least in part to high levels of estrogens such as estradiol and estriol, which are observed during the last trimester of pregnancy. In an animal model of MS, the Th1-mediated experimental autoimmune encephalomyelitis (EAE), it has been shown that administration of estriol at levels equal to those found in pregnancy were capable of ameliorating disease (Kim et al., 1999; Neurology 52: 1230-1238; Jansson et al., 1994; J Neuroimmunol 53: 203-207). Furthermore, it has been
shown by Correale et al. (1998; J Immunol 161: 3365-3374) that the secretion of the anti-inflammatory cytokine IL-10 by CD4+ lymphocytes of MS patients is stimulated by estradiol, estrone and estriol at concentrations at a similar level as in pregnancy.

WO 01/85154 discloses a method of treating immune pathologies with low dose estrogen raising the serum concentration above basal level, but below pregnancy levels.

Because of the involvement of estrogens in the regulation of the balance between pro-inflammatory and anti-inflammatory conditions, a potential therapy for patients suffering from a Th1-mediated immune disease is to administer estrogens, in particular estriols, preferably to achieve continuous serum concentrations typically found in pregnancy.

However, the therapeutic use of estrogens is afflicted with several problems. One disadvantage of the use of estrogens in therapy is their potential ability to cause uterine cancer (endometrium carcinoma) or breast cancer. For example, the use of estradiol could lead to the metabolite 16alpha-hydroxyestrone, a metabolite with known tumor-promoting activity (Bradlow et al., 1985; Proc Natl Sci USA 82: 6295-6299; Kabat et al., 1997; Cancer Epidemiol Biomarkers Prev 6: 505-509).

Estriol as an active principle circumvents this problem. It is believed that estriol therapy is associated with small risks of cancer development in the human. Because of the much faster dissociation of estriol-estrogen receptor (ER) complexes than the dissociation of estradiol-ER complexes, estriol acts as a weaker and only short lasting estrogen. Therefore, estriol causes minimal endometrial proliferation. In addition, estriol displays antagonistic activity on the binding of estradiol to the receptor (Clark et al, 1984; J Steroid Biochem 20: 1005-1013) and therefore estriol seems to have a protective role opposing carcinogenic effects of estradiol. The antagonistic effects of estriol are only observed if the ratio of estriol to estradiol and estrone is 10:1, below this ratio
estradiol is only partially or minimally antagonized and acts as a potent estrogen (Melamed et al., 1997; Mol Endocrinol 11: 1868-1878). This ratio is achieved in late pregnancy.

One problem encountered in the prior art is the inability to achieve continuous pregnancy blood levels of estriol with a form of administration that is comfortable for the patient. When administered orally, the bioavailability of estriol is low. To achieve comparable serum levels of estriol as after intravaginal application, ten times more estriol had to be administered orally (Head et al., 1998; Altern Med Rev 3: 101-113). Thus, estriol had to be orally administered in high doses, giving rise to possible side effects. Oral application of estriol leads to high estrogenicity in the liver. Hepatic effects include, for example, the increased synthesis of factors of the blood clotting system and angiotensinogen.

Another problem known from the prior art for oral therapy with estriol is that blood levels of estriol vary widely from patient to patient, so that general recommendations of the doses are not possible.

Problematic is also the very short half-life of estriol of about 1.5-5.3 h (Heithecker et al., 1991; Horm Res 35: 234-238). Thus, to achieve well-defined and sustained blood levels of estriol similar to those found in pregnancy, high doses of oral estriol would have to be administered at short time intervals which is not convenient for the patient, and several side effects have to be taken into account.

Increase of the orally administered dose of estriol is not the way to increase the desired systemic estrogenicity. Osteoprotective properties of estriol are a quite good marker for the systemic estrogenicity of estriol. EP 0 630 248 teaches that if estriol is administered transdermally in a system which continuously releases estriol for at least 24 h and thereby a constant blood level of estriol is achieved, estriol exhibits anti-osteoporotical effects. The decisive factor for these effects is the constant estriol blood level. Although Lindsay et al. (1979; Maturitas 1: 279-
administered orally estriol in very high doses (12 mg/day), they were not able to show the osteoprotective effects.

EP 0163 596 discloses estra-1,3,5(10)-triene ester derivatives, methods of preparing such compounds and pharmaceutical compositions containing them.

In view of the problems encountered by the prior art, a new therapeutic approach for the treatment of autoimmune diseases by achieving a well-defined and sustained blood level of estriol without the described disadvantages would be desirable.

Object of the present invention
It is the object of the present invention to prevent or reduce the disadvantages of the prior art, i.e. to provide a new strategy for the treatment of autoimmune diseases, especially MS. In an aspect of the invention, the autoimmune disease is Th1-mediated.

The object is achieved by prodrugs of estriol, specifically estriol diesters, preferably, estriol 3,17-dipropionate or 3,17-dihexanoate. Most preferred is the estriol ester 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol. The compound is a preferably parenterally administered prodrug and thereby liver-estriol interactions should be prevented. Estriol diester prodrugs such as 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol achieve a well-defined and sustained blood level of estriol. Administration of the estriol diester prodrugs according to the invention, such as 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol, causes a shift toward an anti-inflammatory Th2-type of immune response, and therefore, provides a promising method and use for ameliorating autoimmune diseases like MS.
Summary of the invention

The present invention relates to the use of estriol diester prodrugs for the preparation of a medicament for the treatment of an autoimmune disease such as MS in a mammal. Preferably, such estriol diester is an estriol 3,17-dipropionate or an estriol 3,17-dihexanoate. The most preferred estriol diester is 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol. In particular, the administration of low doses of the invention estriol esters, such as 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol, results in such high and sustained estriol blood levels as observed in late pregnancy, such as the second or third trimester of pregnancy, preferably during the last trimester, without any effects on hepatic functions.

In another aspect, the present invention provides a method for the treatment of autoimmune diseases such as MS in a mammal in need of such treatment, said method comprising administering a pharmaceutically effective amount of an estriol diester to a mammal in need thereof. For the invention methods and uses, 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol is the most preferred estriol diester prodrug.

Figure 1 represents the summary of the clinical scores (rat acute EAE model) for vehicle and 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol-treated animals.

Detailed description of the invention

The invention relates to the methods and uses of estriol diester prodrugs, such as 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol, for the treatment of autoimmune diseases, such as MS.

Estriol diesters are represented by the general formula below:
wherein R is a C₁⁻¹₀ straight or branched alkyl group or phenyl.

The estriol diester for use in the invention is preferably an estriol 3,17-dipropionate or an estriol 3,17 dihexanoate. The most preferred compound for use in the invention is 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol. Although 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol is the preferred estriol diester for the purpose of the present invention, this does not exclude the possibility to use other suitable estriol diesters as well.

The term “prodrug” in the context of the present invention means a biologically inactive substance, which is metabolised to the active form in the organism.

Estriol diester prodrugs suitable for use in the invention and methods for their manufacture are described in EP 0 163 596. In particular, the method for preparing 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol is described in Example 6 of EP 0 163 596.

The present invention provides a new therapy strategy for the treatment of autoimmune diseases, such as MS. It provides the possibility to achieve and to sustain blood levels of estriol as high as in the second or third trimester of pregnancy. This blood level is high enough to cause an immune shift in Th1-mediated immune diseases.
The superiority of the present invention over the prior art results from the high bioavailability of the estriol released from the diester prodrug compared to the conventionally used estriol (E3). The estriol diester prodrugs of the invention are particularly suitable for use in large dosage intervals. Due to the slow release of estriol from the diester prodrug, high, well-defined and sustained blood levels of estriol may be reached after administration of relatively low dosages. Preliminary studies by Heithecker et al. 1991 (Horm Res 35: 234-238) have shown that estriol diester derivatives increase estriol blood levels for much longer periods than estriol.

A favourable consequence of the administration of estriol esters as a prodrug for the purpose of the present invention is that much reduced interactions with liver functions during the first-pass can be observed. This has the advantage that the released estriol is well tolerated.

In a preferred aspect, the present invention relates to the use of estriol esters (e.g., estriol 3,17-propionate or estriol 3,17-dihexanoate) for the manufacture of a medicament for the treatment of an autoimmune disease. Preferably, the disease is a Th1-mediated autoimmune disease, most preferably, MS. The most preferred estriol diester prodrug is 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol. Preferably, the medicament is for the treatment in a human.

In a second preferred aspect, the present invention relates to a method for the treatment of an autoimmune disease. Preferably, the disease is a Th1-mediated autoimmune disease, most preferably, MS. The method comprises administering estriol diester to a mammal, preferably a human, in need of such treatment. The estriol diester is preferably an estriol 3,17- dipropionate or an estriol 3,17-dihexanoate, most preferably the diester 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol.

Autoimmune diseases are caused in part by T cells, which recognize a host component (autoantigen) in a specific tissue (organ specific) or in various tissues as foreign and attack that tissue. Autoimmune diseases in the context of the
present invention include, but are not limited to, e.g. multiple sclerosis (MS), experimental autoimmune encephalomyelitis (EAE), rheumatoid arthritis, juvenile oligoarthritis, collagen-induced arthritis, type I diabetes mellitus, inflammatory bowel disease, Hashimoto's thyroiditis, Crohn's disease, Graft-versus-host-disease, lupus disorders, Addison's disease, and the like.

It is desirable that the prodrugs of the invention are administered in an amount sufficient to raise the serum concentration of estriol equivalent to pregnancy levels. For example, it has been observed that estriol is secreted in the order of 40 mg/24 h and circulates at a concentration of 1 - 100 ng/ml during late pregnancy in the blood (see: Katagiri et al, 1976, Am J Obstet Gynecol 272 – 280; Klopper et al, 1977, Obstet Gynecol, 459 – 461; Fischer-Rasmussen et al, 1981, Acta Obstet Gynecol Scand 417 – 420).

According to the invention, a diester prodrug of estriol of the general formula (see page 6) permits the uptake and/or binding of estriol in a metabolically stabilized form. Accordingly, a high systemic estriol level may be achieved without hepatic estrogenicity side effects. Furthermore, because of the generated depot of estriol, a well-defined and sustained estriol blood level is obtained. The half-life of estriol in the organism is extended. Accordingly, the administration in large intervals is possible. Due to improved bioavailability, this can be achieved with relatively low dosages.

The advantages of the invention estriol diester prodrugs, such as the most preferred 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol, are numerous, namely:

1. reduction of side effects in the liver;
2. extension of the half-life of estriol in the organism; and
3. enhancement of the bioavailability.

As a consequence, the prodrugs according to the invention may be administered at relatively low dosages with longer intervals between the doses. Furthermore, the individual variability among patients is decreased.
Due to the depot effect of the diesters, the frequency of the application for the purposes of the invention could be reduced in comparison to the application of estriol. The duration of the depot effect depends on the chain length of the esterified mono carbonic acid.

The active agent suitable for the purposes of the present invention as defined above, e.g., estriol diesters such as 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol, may be incorporated into pharmaceutical compositions according to known methods of preparing galenics.

The manufacture of the medicaments and pharmaceutical compositions for use in the invention may be performed according to methods known in the art. Commonly known and used adjuvants as well as further suitable carriers or diluents may be used. Suitable carriers and adjuvants may be such as recommended for pharmacy, cosmetics and related fields in: Ullmann's Encyclopedia of Technical Chemistry, Vol. 4, (1953), pp. 1-39; Journal of Pharmaceutical Sciences, Vol. 52 (1963), p. 918ff; H.v.Czetsch-Lindenwald, "Hilfsstoffe für Pharmazie und angrenzende Gebiete"; Pharm. Ind. 2, 1961, p.72ff; Dr. H.P. Fiedler, Lexikon der Hilfsstoffe für Pharmazie, Kosmetik und angrenzende Gebiete, Cantor KG, Aulendorf in Württemberg, 1971.

The administration of the estriol diester prodrugs of the invention, such as 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol, as a medicament may be oral, rectal, intrauterine, intravaginal, local, transdermal or parenteral. The oral application could be in the form of powder, granules, tablets, pills, pastilles, dragees, capsules, fluid extracts, tinctures and syrups. Rectal or intravaginal application could be in the form of suppositories or intrauterine devices. Local application could be in the form of suspensions or emulsions, ointments, creams or gels. Transdermal could be in form of a patch. The parenteral application of injectible sterile aqueous or oily solutions or suspensions could be subcutaneous or intramuscular as well as percutaneous. The medicament according to the present invention may be administered via a depot injection or
an implant preparation, optionally for sustained delivery of the active agent. The preferred mode of application is the administration via an injection.

Implants can comprise as inert materials e.g. biologically degradable polymers or synthetic silicones such as e.g. silicone rubber.

Suitable diluents for preparing a pharmaceutical composition are defined in EP 0 163 596.

The dose of the estriol diester prodrug of the invention such as 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol, which has to be administered, can raise the serum concentration of estriol to 0,1 – 100 ng/ml. A serum concentration of estriol in the range of 0,1 - 100 ng/ml is desirable, more preferably in the range of 0,1 – 10 ng/ml, most preferably in the range of 1 – 10 ng/ml. According to the invention, this is achieved by the application of the estriol diester prodrugs of the invention in a cumulative dose of 1 – 1000 mg, preferably 10 – 500 mg per month. The interval between applications may be between 1 to 60 days, preferably 5 – 50, most preferably 20 – 40 days. Methods of measuring the estriol serum concentration are known in the art, for example, a suitable radioimmunassay is disclosed in Heithecker et al 1991 (Horm Res 35: 234-238).

Optionally, the pharmaceutical uses and methods according to the present invention further comprise other pharmaceutically active agents. For example, the pharmaceutically active agent may be a hormone, e.g., progesterone (gestagen), or a progesterone precursor, analog, progesterone receptor agonist or mesoprogestin. The combination of the compounds of the invention with, e.g., progesterone (gestagen) may have an additional protective effect against endometrial proliferation and certain other risks, associated with the long term use of estriol. A combination with testosterone or other androgens may be needed to avoid the loss of libido due to a loss of testosterone secretion.
The continuous administration of 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol can lead to proliferation of the endometrium. This undesired effect in conjunction with estrogen treatment is withdrawn by the accompanying treatment with a progestin, e.g. „Kontrazeption mit Hormonen“, H.-D. Taubert und H. Kuhl, Georg Thieme Verlag, Stuttgart, New York, 1995, or mesoprogesterin (= progesterone antagonist with significant partial agonistic activity), e.g. compounds mentioned in EP 0648778 B1, EP 0648779 B1, EP 1157996 A1, WO 01/34126 and WO 99/45023. The progestin or the mesoprogesterin can be administered in usual forms of administration and dosages and the administration may be e.g. oral, parenteral or intrauterine.

This treatment does not influence the course of the disease of multiple sclerosis. This accompanying treatment can be omitted in hysterectomized women.

The treatment of autoimmune diseases, for example Th1-mediated diseases, such as MS, with the invention estriol diester prodrug may further comprise the administration of a conventional immunotherapeutic agent. The term "immunotherapeutic agent" in the context of the present invention includes, but is not limited to, immunomodulatory or immunosuppressive agents such as corticosteroids, cyclosporine, FK 506, methotrexate, azathioprine, Mitoxantrone, cyclophosphamid, glatiramer acetate copolymer-1, anti-inflammatory cytokines such as IL-4, IL-5, IL-10, IFNβ, e.g. betaferon®, cytokine-antagonists such as against IL-1, IL-2 and IL-12, TNFα, antiinflammatory PDE IV receptor antagonists e.g. mesopram, integrin α 4 antagonists and antiinflammatory chemokine antagonists such as CCR1 receptor antagonists, including antibodies, antisense oligonucleotides and soluble receptors.

The estriol diester prodrug according to the invention such as 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol, and the pharmaceutically active immunotherapeutic agent may be administered either together or separately, at the same time and/or sequentially. The mode of administration may differ
between the prodrugs of the invention and the second pharmaceutically active agent.
Efficacy of 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol
in rat acute EAE

Experimental Design:

In order to investigate the efficacy of 3,17β-di-n-hexanoyloxy-1,3,5(10)-
estratriene-16α-ol in animal model of MS, this compound has been tested in the
Lewis rat EAE model.

1. Disease Induction

Female Lewis rats were immunized at 8 weeks of age with antigen emulsion.
On day 1, rats were immunized with a 0.05 ml subcutaneous injection into each
hind footpad with the following mixture: whole guinea pig spinal cord,
homogenized and mixed 1 g: 1ml saline. This homogenate is then mixed 1:1
with Freund's incomplete adjuvant containing 1 mg/ml Mycobacterium
tuberculosis. 0.05ml of spinal cord homogenate (SCH) was injected by single
bolus injections on day 1 into each hind limb footpad for a total of 0.1 ml per rat.

2. Treatment

3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol was prepared in a vehicle
benzyl benzoate:castor oil (3:2 g/g). 3,17β-di-n-hexanoyloxy-1,3,5(10)-
estratriene-16α-ol was dosed subcutaneously (s.c.) at 1000 ug/rat in 0.5
ml/injection. 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol was
administered twice during this study (on day 1 and day 15). Solution was
prepared fresh before each dose 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-
16α-ol first dissolved in benzyl benzoate and gently heated before addition of
caster oil. There were 10 animals per each group (vehicle-benzyl
benzoate/castor oil and treated-1000 ug/rat 3,17β-di-n-hexanoyloxy-1,3,5(10)-
estratriene-16α-ol).
3. Clinical Evaluation
Clinical Evaluation was performed according to the well established protocol. EAE score of zero means that animal had no neurological symptoms and was classified as normal. Clinical score of 1 means that animal had a limp tail and 2 represents incomplete paralysis of one or both hind limbs. Animals with the complete paralysis of one hind limb or both hind limbs can move but do not help in movement of the body are scored as 3. EAE score of 4 represents complete paralysis of both hind limbs and 5 is complete paralysis of hind limbs and weakness of one or both forelimbs or moribund, or death.

Rats were weighed and scored every few days up to day 4, then weighed and scored daily up to day 20. Plasma and serum samples were collected for blood chemistry analysis.

Rats which are borderline in scores are given a one half score, such as 3.5.

Results and Conclusions:
3,17β-di-n-hexanoyloxy-1,3,5(10)-estraatriene-16α-ol was efficacious in lowering the clinical score in Lewis rat EAE model during the chronic stage of a disease.

The clinical scores for the vehicle and 3,17β-di-n-hexanoyloxy-1,3,5(10)-estraatriene-16α-ol treated animals are represented in Fig.1. Analyzing the scores conducting an ANOVA there is a significant difference between the vehicle and 3,17β-di-n-hexanoyloxy-1,3,5(10)-estraatriene-16α-ol treated animals in the chronic stage of the disease (p≤ 0.028).

These results suggest that 3,17β-di-n-hexanoyloxy-1,3,5(10)-estraatriene-16α-ol might be a potential therapeutic for the treatment of autoimmune demyelination that could be administrated twice a month or less and still exhibit its protective effect.
Claims

1. Use of an estriol ester of the general formula

![Chemical Structure](image)

wherein R is a C₁₋₁₀ straight or branched alkyl group or phenyl, for the manufacture of a medicament for the treatment of an autoimmune disease in a mammal.

2. Use according to claim 1, wherein the estriol ester is estriol 3,17-propionate or estriol 3,17-dihexanoate.

3. Use according to claim 1, wherein the estriol ester is 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol.

4. Use according to any one of claims 1 to 3, wherein the mammal is a human.

5. Use according to any one of claims 1 to 4, wherein the autoimmune disease involves a Th1-mediated immune response.

6. Use according to any one of claims 1 to 5, wherein the disease is selected from the group consisting of multiple sclerosis, rheumatoid arthritis, juvenile oligoarthritis, type I diabetes mellitus, inflammatory bowel disease, Hashimoto’s thyroiditis, Addison’s disease, lupus disorders, acute graft-versus-host disease, Crohn’s disease.
7. Use according to any one of claims 1 to 6, wherein the disease is multiple sclerosis.

8. Use according to any one of claims 1 to 7, wherein the estriol ester is administered in a cumulative dose of 1 – 1000 mg per month.

9. Use according to any one of claims 1 to 8, wherein treatment further comprises the administration of a second therapeutic agent.

10. Use according to any one of claims 1 to 9, wherein the medicament is to be administered subcutaneously or intramuscularly by injection.

11. Use of an estriol ester of the general formula

![Chemical Structure Image]

, wherein R is a C_{1-10} straight or branched alkyl group or phenyl, for the manufacture of a medicament for providing a blood level of estriol as observed during late pregnancy in a woman.

12. Use according to claim 11, wherein the estriol ester is estriol 3,17-propionate or estriol 3,17-dihexanoate.

13. Use according to claim 11, wherein the estriol ester is 3,17β-di-n-hexanoyloxy-1,3,5(10)-estratriene-16α-ol.

14. Use according to any one of claims 11 to 13, wherein the estriol blood level is between 0,1 – 100 ng/ml.
15. Use according to any one of claims 11 to 14, wherein the medicament is to be administered subcutaneously or intramuscularly by injection.
Fig. 1
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

<table>
<thead>
<tr>
<th>IPC 7</th>
<th>A61K31/565</th>
<th>A61P37/02</th>
<th>A61P19/02</th>
<th>A61P21/00</th>
<th>A61P3/10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A61P1/04</td>
<td>A61P37/06</td>
<td>A61P5/00</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

<table>
<thead>
<tr>
<th>IPC 7</th>
<th>A61K</th>
<th>A61P</th>
</tr>
</thead>
</table>

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

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

CHEMABS Data, SCISEARCH, EMBASE, MEDLINE, BIOSIS, EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>HEITHECKER, R. ET AL: &quot;Plasma estriol levels after intramuscular injection of estriol and two of its esters&quot; HORM. RES. (1992), 35(6), 234-8, XP008008290 the whole document</td>
<td>1-15</td>
</tr>
</tbody>
</table>

Date of the actual completion of the international search 26 May 2003

Date of mailing of the international search report 02/06/2003

Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk, Tel. (+31) 70 340-2500, Te. 31 051 epo nl, Fax. (+31-70) 340-3016

Authorized officer van der KooiJ, M
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>WO 01 85154 A (UNIV OREGON HEALTH SCIENCES; OFFNER HALINA (US); GOVERNMENT OF THE); 15 November 2001 (2001-11-15) cited in the application claims 1-7 —</td>
<td>1-15</td>
</tr>
<tr>
<td>A</td>
<td>US 4 681 875 A (LAURENT HENRY ET AL) 21 July 1987 (1987-07-21) column 1, line 19 – line 34 column 1, line 61 – line 65 column 5, line 29 – line 31; table 2 —</td>
<td>1-15</td>
</tr>
</tbody>
</table>
Continuation of Box I.2

Present claims 1-5 and 8-15 relate to the treatment of a disease which actually is not well defined. It is not fully possible to determine the diseases for which protection might legitimately be sought using the definition of "autoimmune disease" (claims 1-5 and 8-10) and "autoimmune disease involving a Th1-mediated immune response" (claim 5). In the present context, these definitions are considered to lead to a lack of clarity within the meaning of Article 6 PCT.

Furthermore, the use of the definition "providing a blood level of estriol as observed during late pregnancy in a woman" in the present context is also considered to lead to a lack of clarity within the meaning of Article 6 PCT. It is not possible to determine the diseases for which protection might legitimately be sought. The lack of clarity is such as to render a meaningful complete search impossible.

In addition, present claim 9 relates to a large number of undefined compounds in terms of "a second therapeutic agent". In fact, the claim contains so many options that a lack of clarity (and conciseness) within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible.

Consequently, the search has been restricted to the use of compounds according to the general formula of claim 1 in relation to the real and defined diseases mentioned in claim 6.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.
**INTERNATIONAL SEARCH REPORT**

### Box I  Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international Search Report has not been established in respect of certain claims under Article 17(3)(a) for the following reasons:

1. ☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. X Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
   - see FURTHER INFORMATION sheet PCT/ISA/210

3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II  Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the Invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.
**INTERNATIONAL SEARCH REPORT**

**Information on patent family members**

<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family member(s)</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CA 2408935 A1</td>
<td>15-11-2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 1286664 A2</td>
<td>05-03-2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 0185154 A2</td>
<td>15-11-2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 571090 B2</td>
<td>30-03-1988</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 4330185 A</td>
<td>09-01-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 1285274 A1</td>
<td>25-06-1991</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 85103839 A ,B</td>
<td>24-12-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DD 232920 A5</td>
<td>12-02-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DE 3579193 D1</td>
<td>20-09-1990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DK 213385 A ,B</td>
<td>17-11-1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0163596 A1</td>
<td>04-12-1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ES 8603193 A1</td>
<td>01-04-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FI 851826 A ,B</td>
<td>17-11-1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GR 851164 A1</td>
<td>25-11-1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HU 37943 A2</td>
<td>28-03-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IE 58116 B1</td>
<td>14-07-1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL 75206 A</td>
<td>31-03-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 1850931 C</td>
<td>21-06-1994</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 5064637 B</td>
<td>16-09-1993</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 6100094 A</td>
<td>06-01-1986</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO 851966 A ,B</td>
<td>18-11-1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO 860233 A</td>
<td>18-11-1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NO 161375 B</td>
<td>02-05-1989</td>
</tr>
<tr>
<td></td>
<td></td>
<td>PT 80472 A ,B</td>
<td>01-06-1985</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZA 8503715 A</td>
<td>29-01-1986</td>
</tr>
</tbody>
</table>