

Use of gestagens in combination with (6S)-5-methyltetrahydrofolate for therapy of endometriosis with simultaneous reduction of therapy side effects and
5 reduction of the risk of congenital abnormalities at the onset of a pregnancy

Technical field

The invention relates to the use of gestagens in
10 combination with (6S)-5-methyltetrahydrofolate, wherein the daily dose of the gestagen corresponds to a maximum of twice the ovulation-inhibiting dose, for the preparation of pharmaceutical preparations for therapy of endometriosis with simultaneous reduction of therapy
15 side effects, such as the negative influence on bone density/bone metabolism and on the risk of osteoporosis, and, at the onset of a pregnancy, reduction in the risk of congenital abnormalities, such as neural canal defects and cleft lip, jaw and palate,
20 and pregnancy complications, such as abruptio placentae and premature birth. The invention is suitable for long-term use.

Prior art

25 Endometriosis is a chronic, gynaecological disease which primarily occurs in 5-20 % of women of child-bearing age. In the technical literature, endometriosis is defined as the occurrence of endometrium or endometrium-like tissue outside the
30 cavum uteri. Typical symptoms of endometriosis are dysmenorrhoea, dyspareunia and pain during defecation. Endometriosis patients frequently complain of pain in the pelvic region. Abdominal pain which occurs in the 2nd half of the cycle, followed by a painful period and
35 subsequent freedom from symptoms until the middle of the following cycle frequently suggest endometriosis, but permanent pain is also not infrequent. Nevertheless, approx. 30 - 40 % of endometriosis

sufferers have no symptoms. The disease is then detected only incidentally in connection with other diagnostic measures. In approx. 50-60 %, the diagnosis "endometriosis" is made as an incidental diagnosis when
5 clarifying sterility.

It is known from the technical and patent literature to treat endometriosis with medicaments using danazol, a derivative of 17 α -ethynyltestosterone, GnRH agonists,
10 gestagen/oestrogen combinations or gestagen monopreparations.

US 6,569,845 discloses treatment of angiogenic diseases with dienogest in a daily dose of from 0.5 to 10 mg.
15 Appropriate pharmaceutical compositions identified as examples which could also be employed in the long-term for treatment of endometriosis have a dienogest content of from 400 mg to 2 g.

20 Moore, C. et al., The treatment of endometriosis, Drugs of Today 1999, 35 (suppl. C): 41-52 investigated the efficacy of dienogest in the treatment of endometriosis in comparison with the treatment regime with danazol or GnRH agonists in clinical studies. Those affected by
25 endometriosis were given 2 mg of dienogest per day for 24 weeks. The result of the treatment is comparable to the result of a treatment with danazol or GnRH agonists. Up to 90 % of those affected reported irregular periods, but none reported intolerable
30 periods. The efficacy of standard treatment with danazol is diminished by significant androgenic effects, while GnRH agonists are connected with "menopausal symptoms".

35 Schweppe, K.-W, Stellenwert der Gestagene [Value of Gestagens], Zentralbl. Gynakol. 2003, 125: 276-280 states that during continuous oral gestagen treatment (for example with medroxyprogesterone acetate, dienogest, dydrogesterone or lynestrenol in a daily

dosage of from 5 mg to 20 mg, called low-dose and classified as an active treatment principle for endometriosis-related symptoms) low oestrogen levels are to be recorded. Spotting and intracyclic bleeding frequently result. This necessitates an increase in the dose and/or administration of oestrogen. Rates of recurrence in the long term are more than 50 %.

Safety information of 2005 on a medroxyprogesterone acetate product demonstrates that gestagen monopreparations can have a negative effect on bone density, especially during long-term treatment.

In combination with oestrogens, on the other hand, some gestagens have a positive influence on bone metabolism.

Further safety information, NDA 21-584, FDA 22.03.2005, on depo-subQ provera 104™ (medroxyprogesterone acetate i.m. - 104 mg/0.65 ml) points out that women who use this preparation suffer a loss of bone mineral density which increases with the duration of use of the preparation and is no longer completely reversible.

Knauth, R. and Habenicht U.F., Levonorgestrel has beneficial effects, Exp. Clin. Endocrinol. Diabetes 106 (1998) suppl. 1: 37 already demonstrate in 1998 that the partial androgenic action of a gestagen (levonorgestrel) and not the gestagenic activity is decisive here for this positive influence on bone metabolism.

Kuhl, H., Klimakterium, Postmenopause and Hormonsubstitution [Menopause, Postmenopause and Hormone Replacement], 3rd ed., Bremen, UNI-MED, 2006, 117 also emphasizes that certain gestagens become active via their partial androgenic action. Kuhl furthermore states that androgens considerably intensify the positive action of oestrogens on bone density.

Osteoporosis is described in the technical literature as non-reversible bone degradation with increased bone fragility. More than 5 million people in Germany suffer from osteoporosis. Women are more frequently affected than men. Osteoporosis is a painless, slowly progressing process during which not only does the amount of bone substance decrease, but the "architecture" of the bone is also modified such that this no longer withstands normal loads. A frequent consequence of osteoporosis is fracture of the neck of the femur or extremely painful fractures of the vertebrae. In spite of treatment, the risk of further bone fractures is very high. The base medication for osteoporosis is combination of calcium with vitamin D. Agents of first choice for osteoporosis therapy are bisphosphonate alendronate and risedronate and the selective oestrogen receptor modulator raloxifen.

Bröll, H. et al. states in "Konsensus-Statement: Therapie der postmenopausalen Osteoporosis [Consensus Statement: Therapy of Postmenopausal Osteoporosis]", J. Miner. Stoffwechs. 1/2007. 45-48 that the side effects are the prime reasons for discontinuation of therapy with oral bisphosphonates. The side effects of the bisphosphonates described include abdominal pain, influenza-like syndrome, constipation, peripheral oedema, tinnitus and bronchitis. It likewise proves to be a disadvantage that bisphosphonates must be taken strictly according to instructions because of the risk of inflammations of the mucous membranes; in the morning on an empty stomach with a large glass of water while standing. After intake, the patient should not lie down for at least half an hour.

The disadvantages of raloxifen are described in the literature as possible hot flushes, calf cramps and oedemas.

Oestrogen-containing preparations are used in hormone replacement therapy to maintain bone mass. An oestrogen-containing therapy is likewise associated with certain risks. They should be used to avoid
5 endometrial cancer only in women with an intact womb.

It is also known that endometriosis is associated with subfertility and is frequently diagnosed during clarification of an unfulfilled desire for children.
10 One aim of therapy of endometriosis is therefore also frequently to increase the probability of a pregnancy.

It is furthermore known that an inadequate folate status during pregnancy can lead to congenital
15 abnormalities, such as, for example, congenital heart defects, congenital abnormalities of the urinary tract, acute lymphoblastic leukaemia, cleft lip, jaw and palate or abnormalities of the central nervous system, such as neural canal defects (spina bifida or
20 anencephaly).

The Deutsche Gesellschaft für Ernährung e.V. therefore recommends a daily dose of in principle 400 µg of folic acid, for pregnant women 600 µg and for breast-feeding
25 mothers 600 µg. This is a global statement. A deficiency of vitamin B₁₂ and folate deficiency show identical changes in the blood count. By administration of folate/folic acid, folate deficiency can be compensated, but deficiency of vitamin B₁₂ is not
30 indicated. There is therefore the risk of a masked vitamin B₁₂ deficiency.

Folic acid, also called pteroyl-monoglutamic acid, N-(4-(((2-amino-1,4-dihydro-4-oxo-6-pteridinyl)methyl)-
35 amino)benzoyl)glutamic acid (empirical formula: C₁₀H₁₉N₇O₆), folinic acid, is a heat- and light-sensitive, water-soluble vitamin from the vitamin B complex (vitamin B₉).

It is known that folates are predominantly present in food as pteroylpolyglutamates. After food intake, these are first hydrolyzed in the mucosa cells to pteroylmonoglutamates and then chiefly absorbed in the intestine by active transport.

In the liver, the predominantly non-methylated folates are converted into methylated folates, and are chiefly transported further to the cells as 5-methyltetrahydrofolate (5-MTHF) bonded to albumin and α -macroglobulin, absorbed there, demethylated and converted into the polyglutamate form.

The amino acid homocysteine and an enzyme which requires vitamin B₁₂ as a coenzyme are involved in the demethylation.

It is furthermore known that losses in the folate content of foodstuffs can arise due to preparation (cooking) and storage. It is moreover known that intensive UV radiation acting on human skin reduces the folic acid in the body. Pale-skinned humans are particularly affected in this context.

If intake of folate and/or vitamin B₁₂ is inadequate, homocysteine metabolism is impeded, and as a consequence the concentration of homocysteine in the blood can rise. The concentration of homocysteine in the blood can accordingly be used as an indicator of the folate content.

The state of hyperhomocysteinaemia is defined by Malinow, M.R. et al., Homocyst(e)ine, diet, and cardiovascular disease, Statement for healthcare professionals from the Nutrition Committee, American Heart Association Circulation 99, 178-182, 1999, by the following concentration in the plasma: 16 - 30 $\mu\text{mol/l}$ (moderate); 31 - 100 $\mu\text{mol/l}$ (average); > 100 $\mu\text{mol/l}$ (severe). A concentration above 10 $\mu\text{mol/l}$ is regarded

as critical, and from 12 $\mu\text{mol/l}$ there is a need for action.

In addition to deficiency of folate and vitamin B₁₂,
5 however, enzyme defects can also cause the increase in the homocysteine concentration. The relationship between increased homocysteine concentrations in the blood and vascular diseases, for example, has also been discussed for some time as a risk factor of
10 cardiovascular diseases.

It has similarly been discussed whether folic acid/folate can protect against malignant diseases because of its importance for DNA methylation and DNA
15 strand stability.

The patent specification EP 0 898 965 claims the use of 5-methyl-(6S)-tetrahydrofolic acid or pharmaceutically acceptable salts thereof for prevention of neural canal
20 defects.

The patent specification EP 1 044 975 discloses crystalline salts of 5-methyl-(6R,S)-, -(6S)- and -(6R)-tetrahydrofolic acid and the use as a constituent
25 of a foodstuff supplement.

Description of the invention

The object of the invention is to discover a possibility for protecting endometriosis patients from
30 a risk of osteoporosis and reducing the endometriosis, and achievement of an aim of reducing endometriosis, namely of realizing the onset of a pregnancy, without side effects.

35 It has now been found that the object is achieved according to the invention by the use of gestagens in combination with (6S)-5-methyltetrahydrofolate (metafolin), wherein the daily dose of the gestagen corresponds to a maximum of two times and at least the

ovulation-inhibiting dose, together or separately with one or more pharmaceutically acceptable auxiliary substances/carriers for the preparation of pharmaceutical preparations.

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It has been found that, in addition to the reduction in endometriosis, surprisingly no negative influencing of bone metabolism takes place, so that no decrease/reduction in bone density is to be recorded, there is no risk of osteoporosis, and the risk of congenital abnormalities, such as neural canal defects and cleft lip, jaw and palate, and pregnancy complications, such as abruptio placentae and premature birth, at the onset of a pregnancy can likewise be reduced.

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Nevertheless, with the use according to the invention of the pharmaceutical preparation, the side effects known of the conventional medicaments for treatment of endometriosis, e.g. hot flushes, change in the lipid profile, are surprisingly successfully kept at a tolerable level.

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According to the invention, the gestagens having an antiandrogenic activity which are used are dienogest, cyproterone acetate or chlormadinone acetate.

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The daily dose of the gestagens can be up to a maximum of twice the ovulation-inhibiting dose.

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The daily dose of dienogest is 1 mg up to a maximum of 2 mg. Cyproterone acetate or chlormadinone acetate are employed according to the invention in a daily dosage of up to twice the ovulation-inhibiting dose. The ovulation-inhibiting dose of cyproterone acetate is 1 mg, and that of chlormadinone acetate is 1.7 mg.

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The object is also according to the invention by the use of from 0.1 to 10 mg of (6S)-5-

methylnetetrahydrofolate as a daily dose unit, preferably 0.4 to 1 mg of (6S)-5-methylnetetrahydrofolate, particularly preferably 451 µg of (6S)-5-methylnetetrahydrofolate.

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The use according to the invention also realizes the preparation of pharmaceutical preparations with a continuous administration of the dosage form for a duration of from at least 169 days or 25 weeks to
10 several years, preferably more than 2 years, and is therefore surprisingly suitable for long-term administration.

Tablets, capsules, coated tablets, wafers, transdermal
15 therapy systems, ampoules, suppositories, gels, ointments, implants, vaginal rings or nasal sprays can be employed for the use according to the invention.

In this context, the daily dose of gestagen released by
20 the non-oral forms of the pharmaceutical preparation, such as transdermal therapy system, ampoule, suppository, gel, ointment, implant, vaginal ring or nasal spray, is equivalent to once to twice the daily dose unit amounting to the ovulation-inhibiting dose in
25 the oral forms, or a maximum of equivalent to the activity of 2 mg of dienogest.

The object is furthermore by a process for the preparation of a pharmaceutical preparation for
30 endometriosis therapy with simultaneous reduction of therapy side effects, such as the negative influence on bone density/bone metabolism and on the risk of osteoporosis, and, at the onset of a pregnancy, reduction in the risk of congenital abnormalities and
35 pregnancy complications, such as neural canal defects, cleft lip, jaw and palate, abruptio placentae and premature birth, containing a combination of gestagens with (6S)-5-methylnetetrahydrofolate and, together or separately, one or more pharmaceutically acceptable

auxiliary substances/carriers, wherein the daily dose of the gestagens corresponds to at least the ovulation-inhibiting dose to a maximum of twice the ovulation-inhibiting dose.

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Further embodiments of the pharmaceutical preparation prepared by the process according to the invention correspond to claims 11, 12, 13, 14, 15, 16, 17 and 18.

10 **Embodiment examples**

Example 1

Tablets having the following composition are prepared:

Core:

	Dienogest	2.000 mg
15	Metafolin	0.451 mg
	Lactose monohydrate	46.349 mg
	Microcrystalline cellulose	24.800 mg
	Hydroxypropylcellulose	1.600 mg
	Croscarmellose	3.200 mg
20	Magnesium stearate	1.600 mg

All the substances are mixed in a suitable manner, the mixture is formed into tablets and the tablets are optionally film-coated.

25 **Example 2**

Tablets having the following composition are prepared:

	Dienogest, micronized	2.000 mg
	min. 99 % $\leq$ 20 μ m, 100 % $<$ 30 μ m	
30	Lactose monohydrate	62.800 mg
	Microcrystalline cellulose	18.000 mg
	Potato starch	36.000 mg
	Povidone K 25	8.100 mg
	Magnesium stearate	1.350 mg
35	Talc	4.050 mg
	Crospovidone	2.700 mg

Dienogest is employed in micronized form having an average particle size of 20 μ m and is mixed with

lactose monohydrate, microcrystalline cellulose and potato starch. The povidone K 25 is sprayed in during the granulation. After drying and admixing of talc, crospovidone and magnesium stearate, the mixture of the substances is pressed to tablets having a diameter of 7 mm and a weight of 135 mg.

451 µg of (6S)-5-methyltetrahydrofolate are processed separately with one or more pharmaceutically acceptable auxiliary substances/carriers by known methods and administered simultaneously with the dienogest formulation.

Example 3

In a clinical study, 252 women with endometriosis diagnosed by laparoscopy were treated over a period of 6 months either with the GnRH agonist leuprorelin acetate (LA) 3.75 mg s.c. every 4 weeks or with 2 mg/d orally of the gestagen dienogest (DNG). 128 patients were randomized into the LA group and 124 patients into the DNG group. The efficacy of the particular therapy was investigated, inter alia, by means of a pain scale to be completed by the patient (visual analogue scale, VAS). At the end of the treatment, a similar reduction of the pain compared with the start of the study was found in the two comparison groups (-47.5 mm for DNG; -46.0 mm for LA). Statistical analysis showed the non-inferiority of DNG with respect to LA. At the same time, it was demonstrated in a sub-population that no decrease in bone density took place in the dienogest group, whereas the bone density decreased by 4 % in the LA group.

Patent claims

1. The use of gestagens in combination with (6S)-5-methyltetrahydrofolate, wherein the daily dose of the
5 gestagen corresponds to at least the ovulation-inhibiting dose to a maximum of twice the ovulation-inhibiting dose, for the preparation of pharmaceutical preparations for therapy of endometriosis with simultaneous reduction of therapy side effects, such as
10 the negative influence on bone density/bone metabolism and the risk of osteoporosis, and reduction in the risk of congenital abnormalities and pregnancy complications at the onset of a pregnancy.
- 15 2. The use of gestagens and (6S)-5-methyltetrahydrofolate as claimed in claim 1, wherein the congenital abnormalities and pregnancy complications at the onset of a pregnancy are neural canal defects, cleft lip, jaw and palate, abruptio
20 placentae and premature birth.
3. The use of gestagens and (6S)-5-methyltetrahydrofolate as claimed in claim 1 or 2, characterized in that the gestagen component is 17 α -
25 cyanomethyl-17 β -hydroxyestra-4,9-dien-3-one (dienogest), cyproterone acetate or chlormadinone acetate.
4. The use of gestagens and (6S)-5-methyltetrahydrofolate as claimed in claim 1, 2 or 3,
30 characterized in that the daily gestagen dose is 1 to 2 mg of dienogest or an equivalent amount of cyproterone acetate or chlormadinone acetate.
5. The use of gestagens and (6S)-5-methyltetrahydrofolate as claimed in claim 1, 2, 3 or
35 4, characterized in that the daily dose of (6S)-5-methyltetrahydrofolate is 0.1 to 10 mg.

6. The use of gestagens and (6S)-5-methyltetrahydrofolate as claimed in claim 1, 2, 3, 4 or 5, characterized in that the daily dose of (6S)-5-methyltetrahydrofolate is 0.4 to 1 mg.

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7. The use of gestagens and (6S)-5-methyltetrahydrofolate as claimed in claim 1, 2, 3, 4, 5 or 6, characterized in that the daily dose of (6S)-5-methyltetrahydrofolate is 451 µg of the calcium salt of (6S)-5-methyltetrahydrofolic acid.

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8. The use of gestagens and (6S)-5-methyltetrahydrofolate as claimed in claim 1, 2, 3, 4, 5, 6 or 7 for the preparation of pharmaceutical preparations with continuous administration for a duration of from at least 169 days or 25 weeks to several years, preferably more than 2 years, for avoiding side effects, such as the negative influence on bone density/bone metabolism and the risk of osteoporosis, and for reducing the risk of congenital abnormalities at the onset of a pregnancy, during or after endometriosis therapy.

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9. The use of gestagens and (6S)-5-methyltetrahydrofolate as claimed in claim 1, 2, 3, 4, 5, 6, 7 or 8, characterized in that the pharmaceutical preparation is present in the form of tablets, capsules, coated tablets, wafers, transdermal therapy systems, ampoules, suppositories, gels, ointments, implants, vaginal rings or nasal sprays.

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10. A process for the preparation of a pharmaceutical preparation for endometriosis therapy with simultaneous reduction of therapy side effects, such as the negative influence on bone density/bone metabolism and the risk of osteoporosis, and reduction in the risk of congenital abnormalities and pregnancy complications at the onset of a pregnancy, characterized in that a combination of gestagens with

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(6S)-5-methyltetrahydrofolate, wherein the daily dose of the gestagen corresponds to at least the ovulation-inhibiting dose to a maximum of twice the ovulation-inhibiting dose, is used.

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11. The process as claimed in claim 10, characterized in that the congenital abnormalities and pregnancy complications at the onset of a pregnancy are neural canal defects, cleft lip, jaw and palate,
10 abruptio placentae and premature birth.

12. The process as claimed in claim 10 or 11, characterized in that the gestagen component is 17 α -cyanomethyl-17 β -hydroxyestra-4,9-dien-3-one
15 (dienogest), cyproterone acetate or chlormadinone acetate.

13. The process as claimed in claim 10, 11 or 12, characterized in that the daily dose is 1 to 2 mg of
20 dienogest or an equivalent amount of cyproterone acetate or chlormadinone acetate and (6S)-5-methyltetrahydrofolate.

14. The process as claimed in claim 10, 11, 12 or
25 13, characterized in that the daily dose is 0.1 to 10 mg of (6S)-5-methyltetrahydrofolate.

15. The process as claimed in claim 10, 11, 12, 13 or 14, characterized in that the daily dose is 0.4 to 1
30 mg of (6S)-5-methyltetrahydrofolate.

16. The process as claimed in claim 10, 11, 12, 13, 14 or 15, characterized in that the daily dose is 451 μ g of the calcium salt of (6S)-5-methyltetrahydrofolic
35 acid.

17. The process as claimed in claim 10, 11, 12, 13, 14, 15 or 16, with a continuous administration for a

duration of from at least 169 days or 25 weeks to several years, preferably more than 2 years.

18. The process as claimed in claim 10, 11, 12, 13,
5 14, 15, 16 or 17, characterized in that the pharmaceutical preparation is present in the form of tablets, capsules, coated tablets, wafers, transdermal therapy systems, ampoules, suppositories, gels ointments, implants, vaginal rings or nasal sprays.