Title: N-ACETYL - L - CYSTEINE FOR TREATMENT OF POLYCYSTIC OVARY SYNDROME

Abstract: The invention relates to a new prescription of NAC in the treatment of polycystic ovary syndrome (PCOS) and of symptoms associated with PCOS, in a human or mammalian animal patient. In addition an effective dose range of NAC in the treatment of PCOS is proposed, wherein the patient is treated for at least two months in a pulsed or intermittent fashion. The prescribed treatment regimen may be used e.g. in order to reduce ovary volume and number of follicles, achieve regular menstrual cycle, promote a desired pregnancy, ameliorate acne and/or hirsutism and reduce body mass index (BMI) in a subject with PCOS. Side effects of this treatment are virtually absent and, in particular, this treatment does not hinder pregnancy.
FIELD OF THE INVENTION

The present invention relates to a pharmaceutical composition comprising N-acetyl-L-cysteine useful for the treatment of polycystic ovary syndrome and indications associated with polycystic ovary syndrome.

BACKGROUND

Polycystic ovary syndrome (PCOS) is a relatively common disease, affecting 5-10% of women of reproductive age and is a leading cause of infertility. It is a complex endocrine disorder whose first symptoms include multiple ovary cysts and increased ovary volume. The cysts are actually immature ovarian follicles that have developed from primordial follicles but have been arrested at an early stage due to disturbed ovarian function. PCOS is properly characterized as a hormonal disorder, associated with excessive amounts or effects of androgenic hormones, leading to a variety of symptoms: lack of regular ovulation and therefore lack of regular menstrual cycle, acne, hirsutism and hypertrichosis (increased hair growth and hair density) and infertility. PCOS is often associated with metabolic syndrome, i.e. type 2 diabetes, with resulting elevated body mass index, obesity and increased risk for cardiovascular diseases.

Women with PCOS also are at risk of developing endometrial hyperplasia (benign proliferation of endometrial cells) and endometrial cancer.

The molecular mechanisms underlying PCOS is not fully understood. The ovaries of affected patients are stimulated to produce male (androgenic) hormones, in particular testosterone. This stimulation may be caused by e.g. excessive release of luteinizing hormone (LH) by the anterior pituitary gland or by the ovaries responding to high levels of insulin in the blood (hyperinsulinemia). Insulin resistance could be a leading cause. Hyperinsulinemia affects the pulsing of gonadotropin-releasing hormone (GnRH) and thus the balance between LH and follicle-stimulating hormone (FSH), the ovarian androgen production, follicular maturation and binding of sex hormones to sex hormone-binding globulin (SHBG). SHBG, in turn, regulates the bioavailability of sex
hormones. PCOS is thus associated with a complex hormonal imbalance, which leads to
cyst formation and the other symptoms associated with PCOS.

There is currently no cure for PCOS. Treatments have been developed to alleviate
symptoms, e.g. to lower insulin levels, normalize menstruation, improve fertility,
promote weight loss, reduce the symptoms of hirsutism and acne and prevent
endometrial hyperplasia and cancer. These therapies include hormonal therapies with
suppression of ovulation, insulin-sensitizing drugs, diet and exercise, and, in the most
severe cyst cases, surgery. Recurrence after surgery is quite common. The hormonal
treatments commonly used for PCOS related symptoms are often associated with
unwanted side effects and may also further aggravate the situation for infertile women
wanting to become pregnant.

N-acetyl-L-cysteine (hereinafter referred to as NAC) is a well-known drug, which has
been used mainly as a mucolytic agent and in the treatment of paracetamol poisoning. In
recent years it has also been acknowledged as having other beneficial properties, such
as being anti-inflammatory and anti-proliferative, and has been suggested for the
treatment of a variety of different disorders and symptoms such as diabetes and cancer.

NAC has been proposed for the treatment of insulin resistance and insulin imbalances in
women with PCOS. The use of NAC for the treatment of or symptoms associated with
PCOS has been proposed. No efficient long term dosage regimen has been proposed.

PRIOR ART

Following some earlier publications showing that NAC improves insulin secretion in
response to glucose and affects the regulation of the insulin receptor in human
erythrocytes Anna Maria Fulghesu et. al. set out to evaluate the effect of NAC on
insulin secretion and peripheral insulin resistance in patients with PCOS (Fertility and
Sterility 2002, Vol. 77, No. 6, pages 1128-1135). It was shown that, by administering
NAC to women with PCOS, at a dose of 1.8 g/day (normal weight persons) or 3 g/day
(obese persons) for 5-6 weeks, peripheral insulin sensitivity as well as insulin
circulating levels improved in hyperinsulinemic patients. The decrease in circulating
insulin levels was also followed by a reduction in testosterone and androgen levels. No
effect was seen in normoinsulinemic patients.

OBJECTS OF THE INVENTION

The clinical outcome, other than the effect on insulin sensitivity and androgen levels, of NAC treatment in PCOS has, to the knowledge of the inventors, not been determined in the prior art, nor has an efficient long term dosage regimen for the treatment of PCOS or the use of NAC for the treatment of indications associated with PCOS been proposed.

It is therefore a general object of the present invention to provide a solution to the problem of providing a pharmaceutical composition of N-acetyl-L-cysteine (NAC) for the treatment of PCOS and symptoms associated with PCOS. An aspect of the object is to provide a pharmaceutical composition of N-acetyl-L-cysteine (NAC) with an effective dosage regimen for a long-term treatment of PCOS and of symptoms related to PCOS.

SUMMARY OF THE INVENTION

It has previously been proposed that NAC could be used in new therapeutic strategies for improving insulin sensitivity and insulin circulating levels in patients with PCOS. The inventors of the present invention show that NAC has a much wider effect in the patients diagnosed with PCOS. For example, in patients treated with NAC ovary size is reduced, the number of follicles is reduced and menstrual cycle is normalized. The
inventors of the present disclosure have previously shown that NAC induces complex molecular and cellular changes mainly related to inhibition of proliferation and induction of differentiation in cancerous epithelial tissue towards normal tissue.

These findings have led the inventors of the present invention to propose a new prescription of NAC in the treatment of PCOS and of indications associated with PCOS, in a human or mammalian animal patient. In addition an effective dose range of NAC in the treatment of PCOS is proposed. Given a reported decreased plasma level of NAC after prolonged periods of treatment, the inventors propose a pulsed or intermittent treatment. The clinical outcome of pulsed or intermittent treatment with NAC includes a restoration of sex hormones balance, amelioration of ultrasonographic aspects of the ovaries, with reduction in the number of follicles, more regular periods, ovulation restoration, improvement of fertility and amelioration of skin and hair problems.

In one embodiment of the present invention the prescribed treatment regimen may be used e.g. in order to achieve a normalization of the ovary cortex functions, through a more regular proliferation-differentiation cycling, with a consequent normalization of estrogen-secretory functions of ovarian cells and normalization of menstrual cycle. In aspects of the present invention the prescribed treatment regimen may be used e.g. to reduce ovary volume, to reduce the number of follicles, to obtain regular menstrual cycle, to reduce hirsutism, to reduce acne and/or to reduce body weight and body mass index. In still other aspects of the present invention the prescribed treatment regimen may be used e.g. to improve fertility and allow a desired pregnancy, to reduce insulinemia and to reduce the risk of cardiovascular diseases, endometrial hyperplasia and endometrial cancer. As opposed to the current hormone treatments, side effects of the treatment with the composition according to the invention are virtually absent, highly improving the quality of life for the treated patients. In particular, this treatment does not hinder pregnancy.

The present invention provides a pharmaceutical composition comprising N-acetyl-L-cysteine for use in the treatment of a mammal, including a human, having polycystic ovary syndrome (PCOS), where the composition is for pulsed or intermittent, oral
administration, for a time period of two months or more, at a dose of N-acetyl-L-cysteine that is between 20 and 90 mg/kg/day on days when administered.

In one embodiment the invention provides a pharmaceutical composition comprising N-acetyl-L-cysteine for the above mentioned use where the composition is for administration for 3-5 consecutive days followed by 2-4 days of interruption. In another embodiment the pharmaceutical composition comprising N-acetyl-L-cysteine is for administration for 1-3 consecutive days, followed by 1-2 days of interruption.

In one embodiment the invention provides a pharmaceutical composition comprising N-acetyl-L-cysteine for the above mentioned use where the composition is for administration at a dose of N-acetyl-L-cysteine that is between 30 and 60 mg/kg/day on days when administered. In another embodiment the pharmaceutical composition is for administration at a dose of N-acetyl-L-cysteine that is between 30 and 45 mg/kg/day on days when administered.

In one embodiment the invention provides a pharmaceutical composition comprising N-acetyl-L-cysteine for the use described above where the pharmaceutical composition is protected from light. In another embodiment the pharmaceutical composition is a water soluble tablet. In still another embodiment the pharmaceutical composition contains sodium hydrogen carbonate. In one embodiment the pharmaceutical composition is a slow-release formulation and/or a gastric protected formulation.

In one embodiment the invention provides a pharmaceutical composition comprising N-acetyl-L-cysteine for reducing ovary volume and/or follicles number in a subject with PCOS. In another embodiment the pharmaceutical composition is for achieving regular menstrual cycle in a subject with PCOS. In still another embodiment the pharmaceutical composition is for reducing body weight and body mass index in a subject with PCOS. In one embodiment the pharmaceutical composition is for treating infertility or promoting a desired pregnancy in a subject with PCOS. In another embodiment the pharmaceutical composition is for reducing acne and/or hirsutism in a subject with PCOS. In still another embodiment the pharmaceutical composition is for treating insulinemia in a subject with PCOS. In one embodiment the pharmaceutical
composition is for reducing the risk for cardiovascular diseases in a subject with PCOS. In another embodiment the pharmaceutical composition is for reducing the risk for endometrial hyperplasia and endometrial cancer in a subject with PCOS.

In one aspect the invention provides a method for the treatment of a mammal having PCOS, comprising orally administering a pharmaceutical composition comprising N-acetyl-L-cysteine to said mammal in a pulsed or intermittent dosage regimen, for a time period of two months or more, at a dose of N-acetyl-L-cysteine that is between 20 and 90 mg/kg/day on days when administered. In one embodiment of the method the pharmaceutical composition is administered for 3-5 consecutive days followed by 2-4 days of interruption. In another embodiment the pharmaceutical composition is administered for 1-3 consecutive days, followed by 1-2 days of interruption.

In one embodiment of the method the dose of N-acetyl-L-cysteine is between 30 and 60 mg/kg/day on administration days. In another embodiment of the method the dose of N-acetyl-L-cysteine is between 30 and 45 mg/kg/day on administration days.

In one embodiment the method is for reducing ovary volume in a subject with PCOS. In other embodiments the method is for achieving regular menstrual cycle in a subject with PCOS, for reducing body weight and body mass index in a subject with PCOS, for treating infertility in a subject with PCOS, for reducing acne and/or hirsutism in a subject with PCOS, for reducing insulinemia in a subject with PCOS, for reducing the risk for cardiovascular diseases in a subject with PCOS or for reducing the risk for endometrial hyperplasia and endometrial cancer in a subject with PCOS.

BRIEF DESCRIPTION OF THE FIGURE

The invention will be explained in more detail in the following description, referring to the enclosed figure, where:

Fig. 1 shows a diagram of the effect of NAC treatment on ovary diameter after three months of treatment. The ratio between final (D3) and initial (DO) average ovary diameter is reported for the right (■) and left (●) ovary, and for their average (♦).
DETAILED DESCRIPTION OF THE INVENTION

NAC in general

N-acetyl-L-cysteine (NAC) is a well known low molecular weight pharmaceutical drug, with the chemical formula

\[
\text{NAC} \quad \text{O} \quad \text{SH} \\
\text{HO} \quad \text{HN} \quad \text{CH}_3 \\
\text{O}
\]

The features of NAC are mainly related to its thiol group, which makes it effective in most biochemical pathways where the tripeptide glutathione (GSH), present in all human tissues at relatively high concentrations, even above 10 mM, acts. Cysteine is indeed among the three aminoacids composing GSH, so NAC is considered a precursor of GSH with its de-acetylated cysteine. NAC has been and still is largely used as a mucolytic agent, where the mode of action is generally attributed to the redox breakage of sensitive cysteine disulfur bridges in the mucus proteins. In fact NAC participates to the complex redox cycling of thiol groups, where several enzymes acts. Indeed, of extreme physiological importance is the disulfide formation and breakage cycle, a common mechanism by which protein activity and cellular signaling is regulated. Enzymes such as protein tyrosine phosphatases and tyrosine kinases, for example, play pivotal roles in the control of the cell cycle, cell proliferation and differentiation, and many of them are regulated by the redox state of their cysteines.

Overall, although detailed mechanisms of action have not been finally elucidated, NAC appears to act in all biochemical pathways where GSH does. Enzymes and proteins whose activity is modulated by GSH, or by the redox enzymes utilizing GSH, operate in several processes either directly or through a net of signals transduction pathways. In this picture, NAC may either parallel GSH action, or may be even more effective than GSH.
GSH is e.g. normally conjugated to reactive metabolites formed by paracetamol and helps detoxify them. When paracetamol is overdosed GSH is however depleted and the paracetamol metabolites start reacting with cellular proteins, eventually leading to cell death. In the treatment of fulminant hepatic failure after paracetamol poisoning NAC acts instead of GSH in the detoxification of paracetamol metabolites. NAC is believed to be virtually absent of undesired side effect, which is also indicated by the high NAC doses that are used in the treatment of paracetamol poisoning, estimated, for a 70 kg individual, of about 40 g/day.

Contrary to the tripeptide GSH, which can be degraded already in the stomach, the simple NAC molecule freely diffuses in almost all tissues and cells. NAC pharmacokinetic studies determined a peak concentration in plasma reached in about one hour, with a half-life of about three hours. Total clearance occurs between six and twelve hours.

**NAC as an antiproliferative, differentiating agent**

The inventors have recently found that N-acetyl-L-cysteine (NAC) possesses a marked antiproliferative effect on cancer cells of epithelial origin - the same origin as of ovarian and endometrial cells (Cell Death and Differentiation 2005, 12(10): 1285-1296). This antiproliferative effect of NAC was due to the activation of a physiological differentiation pathway, associated with a normalization of cell function towards the tissue of origin. Although cancer and PCOS are principally unrelated diseases the observed effects of NAC eventually led the inventors to the idea that NAC might also be useful in the treatment of PCOS. In particular, in PCOS the abnormal secretory functions are possibly related to defects in the differentiation of ovarian cells. Therefore NAC treatment could lead to a normalization of the ovary cortex functions, through a more regular proliferation-differentiation cycling, and thereby also to a normalization of estrogen-secretory functions and consequent normalization of menstrual cycle. The observations made in cancer will here be presented as a background to the effects of NAC and as a likely mode of action also in PCOS.

NAC was used to arrest proliferation and induce differentiation in two adenocarcinoma cell lines and in primary normal keratinocyte cells, all of epithelial origin. In these
systems, the differentiation was characterized morphologically, biochemically and through gene expression analysis (the gene expression analysis extensively reported in BMC Cancer 2005, 5:75).

As stated above, the antiproliferative effect of NAC, in the study of cancer, was not related to cell death or to toxicity but, instead, was due to the activation of a physiological differentiation pathway, which can be regarded as a normalization of cell functions towards the tissue of origin.

In addition to the decreased proliferation, the morphology of NAC-treated cancer cells was also altered. In vitro, epithelial cells under active proliferation display an irregular morphology - a mesenchymal morphology - and often form several multiple cell layers. On the contrary, when cells undergo a differentiation process, toward the structure and function of their final target tissue, they stop proliferation, their morphology becomes regularly polygonal, each cell sometimes thicker, and they form a single layer of adjacent cells. This process is accompanied by increased cell-cell and cell-substratum junctions, consistent with a shift from a proliferating mesenchymal to an adhesive, less motile and differentiated phenotype.

On a whole, a complex series of metabolic changes were detected after NAC supplementation to cancer cells, all converging in arresting the uncontrolled proliferation and in inducing their terminal differentiation.

As an example of the ability of NAC to modulate signal transduction in cells through the redox status of sensitive cysteines, the enzyme non-receptor tyrosine kinase c-Src was studied in an in vitro model of colon carcinoma (CaCo-2) cells and ovary carcinoma (OVCAR-3) cells (Free Radicals Biology and Medicine 2008, 45(11):1566-72). c-Src activity is crucial in the proliferation/differentiation switch, and c-Src is indeed activated and over expressed in a number of human cancers, particularly in colon and ovary carcinoma. Conspicuous international drug design efforts are presently devoted to the search of specific c-Src inhibitors. Instead, it was found that a simple treatment of adenocarcinoma cells with NAC can reach the objective of c-Src inhibition, with a mechanism related to redox transitions in sensitive cysteine residues in c-Src, able to switch off this kinase and to deliver it to endo-lysosomes, where it is stored or
degraded. Thus, the NAC induced terminal differentiation in adenocarcinoma cells was related to the inhibition of c-Src.

The affinity of receptors for their ligand hormones is often modulated through the redox status of crucial cysteine residues, with the formation/breakage of disulfur bridges. The observation that NAC could alter the redox status of sensitive cysteines in c-Src lead to the idea that the effect of NAC in PCOS can be related to a restored proper affinity of receptors for their hormone ligands, including insulin, rather than or in addition to appropriate hormone levels for a normalized secretion.

In this respect, while NAC affects the modulation of the cysteine redox status of hormone receptors relatively fast, a re-normalization of the proliferation/differentiation pathway requires longer periods of time, which highlights the need of prolonged treatments with an effective dosage regimen.

**NAC and PCOS**

From the epithelial cancer studies described above the inventors of the present invention noticed that NAC had some advantageous effects on epithelial cancer cells and that those effects could also be useful in the treatment of ovary cortex cells and endometrial cells in PCOS, if such cells should respond similarly. In particular, it was hoped that the anti-proliferative and differentiating effects of NAC seen in cancer would also occur when NAC was used in the treatment of PCOS.

It was believed that if NAC had similar molecular effects on ovary cortex as those which had been observed in cancer, they would also be beneficial for the treatment of PCOS. It was hypothesized that NAC might have the following physiological effects on PCOS:

i) a normalization of the ovary cortex and of endometrial tissue functions, through a more regular proliferation-differentiation cycling;

ii) a normalization of estrogen secretory functions of ovarian cells;

iii) a normalization of hormone balance;

iv) consequences of above points include: ovulation restoration, regularization of menstrual cycle, improvement of fertility, weight loss, decreased
hirsutism and hypertrichosis, amelioration of acne, finally an overall amelioration of the patients' quality of life.

On a whole, the hypothesized outcomes were verified either directly or indirectly through the patients' response in a pilot clinical study. In particular, the inventors have found that NAC indeed reduces ovary size and follicles number, normalizes menstrual cycle, reduced BMI and ameliorates acne.

Dosage regimen

From the study of NAC treatment on adenocarcinoma cell lines and primary normal keratinocyte cells (Cell Death and Differentiation 2005, 12(10): 1285-1296), it was concluded that the effective dose of NAC for induction of the antiproliferative-differentiating effect varied and was cell type dependent. The tissue of origin thus dictates the effective NAC concentration required to observe a complete block of proliferation, and has to be determined for each tissue. In addition, the NAC dose was also related to the cell malignancy. In detail, while normal cells required a low dose to stop proliferating and start differentiating, carcinoma cells with characteristic poorer prognosis required a higher NAC concentration.

For the purpose of the present invention a dosage regimen of NAC for the treatment of PCOS was developed based on the following criteria:

1) a dosage of NAC per day which is in agreement with other current clinical treatments and is considered without undesirable side effects;

2) a dose which is considered high enough to fulfill the requirement of abnormally proliferating cells, to switch them into the differentiation pathway;

3) a dose which is daily fractionated as to warrant an almost constant plasma level, in consideration of NAC pharmacokinetics and pharmacodynamics;

4) given a reported decrease in NAC plasma level after prolonged treatments (Pendyala L, Creaven PJ. Cancer Epidemiol Biomarkers Prev. 1995; 4:245-51), the suspension of the treatment for about half of each week was considered for an optimal biological response in a two months or longer treatment.
The composition of the present invention, comprising NAC for the treatment of PCOS or indications associated with PCOS is according to one embodiment to be administered at a dose between approximately 20 and 90 mg/kg/day. The lower limit is based on twice the dose used for mucolytic action, i.e. it is per se known to have a physiological effect. The upper limit is based on the consideration that higher doses have been found to cause gastric problems in many patients. In another embodiment of the present invention the composition comprises NAC to be administered at a dose of approximately 30-60 mg/kg/day. The lower limit has been shown to be effective in PCOS and the higher limit is known to have virtually no side effects. In still another embodiment of the present invention the composition comprises NAC to be administered at a dose of approximately 30-45 mg/kg/day. This low dosage has surprisingly been shown to be effective in PCOS.

In one embodiment the composition is to be administered for a period of time which is two months or more, or preferably three months or more. To counteract a decrease in NAC plasma level after prolonged treatment NAC may be administered at the prescribed dosage in an intermittent fashion, i.e. intermittent dosage regimen/treatment. By intermittent administration or treatment is meant that the treatment is interrupted in periods, i.e. that the pharmaceutical composition is administered for a period of time, e.g. a few days, followed by an interruption in administration, where no pharmaceutical composition is administered for a period of time, e.g. for a few days. Intermittent treatment can be regular, e.g. treatment for a fixed number of days or weeks, followed by interruption for a fixed number of days or weeks. Examples include repeated schemes with treatment for 4 days followed by interruption for 3 days each week or treatment for 2 weeks followed by interruption 1 week. A special case of regular intermittent treatment is pulsed treatment, i.e. with regular treatment and interruption duration, e.g. administration every other day or administration for two days followed by two days of interruption etc. Irregular intermittent treatment schemes that are not regularly repeated or have a more complex scheme that is repeated is also conceivable, e.g. dependent on response to treatment. In different exemplifying embodiments of the present invention the prescribed dose of NAC is administered for 3-5 consecutive days followed by 2-4 days of interruption, or administered for 1-3 consecutive days followed by 1-2 days of interruption.
In one embodiment, by referring to a body weight of approximately 60 kg, the NAC dose is in the range between 1.2 and 5.4 g/day, preferably between 1.8 and 3.6 g/day, on days when administered. The dose may be divided in two or more, preferably three or four, daily administrations of either one or two doses (e.g. pills) each, where each dose may comprise e.g. 0.15-2.7 g of NAC or preferably 0.6-1.2 g of NAC. For patients with other weights, e.g. over- or underweight persons the daily dose needs to be adjusted accordingly.

In one embodiment of the present invention the pharmaceutical composition for treatment of PCOS or PCOS related symptoms comprises NAC in a dose of 150-5400 mg to be administered in two or more administrations per day, for a period of at least 2 months, such as at least 3 months. In a preferred embodiment of the present invention the pharmaceutical composition comprises NAC in a dose of 230-3600 mg to be administered in two or more administrations per day, for a period of at least 2 months, such as at least 3 months. In another embodiment of the present invention the pharmaceutical composition for treatment of PCOS or PCOS related symptoms comprises NAC to be administered in a single or more administrations per day, 3-5 days per week, for a period of at least 2 months, such as at least 3 months.

**Pharmaceutical formulations**

A pharmaceutical composition according to the present invention may be prepared in a manner per se known by a person skilled in the pharmaceutical art. The composition may comprise an effective amount of NAC, in accordance with the invention, as well as a suitable carrier or excipient that serves as a vehicle or medium for the active ingredient. Such carriers or excipients are known in the art and include solid materials such as citric acid, natrium citrate, natrium (acid) carbonate plus flavoring. The pharmaceutical composition is preferably adapted for oral administration. Such compositions could be administered in different forms, at present preferably as tablets. Other forms, such as capsules, suppositories, solutions, suspensions, syrups or the like are also conceivable.

The invention requires a strict assessment of the pharmaceutical quality of NAC preparation for obtaining the effective dose. Therefore, brand or certified generic
preparations have to be used. NAC is not a stable molecule, its active thiol residue can be easily oxidized by oxygen, light and other radiations, so that the effective dose would not be reached. The preparation is thus preferably protected from light, e.g. by a light protecting package such as a blister foil package. Soluble tablets preferably comprise sodium hydrogen carbonate, which helps in a partial removal of oxygen from water during dissolution.

It has been observed that high doses of NAC may cause abdominal pain. To overcome this, an option is to provide NAC in a gastric protected formulation, suitable for preventing NAC release/solubility in the stomach. Such formulations are well known in the art and may be used with the present invention. For example, tablet coatings that are resistant to gastric fluids and allow release of the drug only in the intestine, after its transit through the stomach, may be used. Commonly used formulations include polymers such as cellulose derivatives, methacrylate amino ester copolymers. The coating protects the tablet core from disintegration in the acidic environment of the stomach by employing a pH sensitive polymer, which swells or solubilises after having passed through the stomach, in response to the increase in pH, whereafter the drug is released.

Another option is to lower the dose of NAC entering the blood stream at any one time. Administration of NAC three or more times daily can be difficult to accomplish for the patient. Nevertheless, a repeated administration can be desirable to achieve a nearly constant serum concentration of NAC. To overcome these problems, a once or twice-a-day administration could be easier to handle for the patient, for instance morning and night. One option is to provide NAC in a slow-release formulation (also denoted sustained-release or controlled-release). By being able to reduce the rate of diffusion and uptake of NAC into the blood stream such a formulation enables administration of a larger dose at longer intervals. The dose is then distributed in the blood over a long time in small quantities, e.g. over 12+12 hours in the case of a twice-a-day regimen scheme. Many different technologies and formulations for slow-release are since long known in the art and may be applied with the present invention. In such technologies the active substance is for example encapsulated in a coating or matrix that is insoluble or less soluble in the body fluid where it is administered.
Formulations having a combined effect of slow-release and gastric protection is also possible and may be used within the present invention.

Use/medical indications of the present invention

The present invention has been shown to have beneficial properties in many aspects related to PCOS and may be used both for the treatment of PCOS and for treatment of various indications associated with PCOS. By treatment of various indications associated with PCOS is meant e.g. treatment to reduce the symptoms of the disease such as irregular menstruation, overweight, hirsutism and acne. Such treatment of various indications associated with PCOS also includes e.g. treatment to reduce ovary volume and number of follicles, treatment to increase the likelihood of getting pregnant, treatment to reduce insulinemia and treatment to reduce the risk of cardiovascular diseases, endometrial hyperplasia and endometrial cancer in subjects diagnosed with PCOS.

NAC, according to the present invention, has been shown to improve the quality of life of the patient in the absence of undesired side effects; to reduce ovary size and number of follicles, to ameliorate acne, hirsutism to produce more regular menstrual cycles and to decrease body mass index. Thus, in one embodiment of the present invention the pharmaceutical composition comprises NAC for the treatment of PCOS in order to convert ovary cortex function, including estrogen secretory function, towards normal.

In one embodiment of the present invention the pharmaceutical composition comprises NAC for the treatment of PCOS in order to reduce symptoms associated with PCOS.

In another embodiment of the present invention the pharmaceutical composition comprises NAC for treating infertility or promoting a desired pregnancy in a subject with PCOS.

In another embodiment of the present invention the pharmaceutical composition comprises NAC for reducing ovary volume in a subject with PCOS.

In still another embodiment of the present invention the pharmaceutical composition comprises NAC for achieving a regular menstrual cycle in a subject with PCOS.
In another embodiment of the present invention the pharmaceutical composition comprises NAC for reducing hirsutism in a subject with PCOS.

In another embodiment of the present invention the pharmaceutical composition comprises NAC for reducing acne in a subject with PCOS.

In still another embodiment of the present invention the pharmaceutical composition comprises NAC for reducing body weight and body mass index in a subject with PCOS.

In another embodiment of the present invention the pharmaceutical composition comprises NAC for reducing insulinemia in a subject with PCOS.

In another embodiment of the present invention the pharmaceutical composition comprises NAC for reducing the risk for cardiovascular diseases in a subject with PCOS.

In still another embodiment of the present invention the pharmaceutical composition comprises NAC for reducing the risk for endometrial hyperplasia and endometrial cancer in a subject with PCOS.

In other embodiments the present invention provides a method for treating a subject having PCOS by oral administration of a pharmaceutical composition comprising N-acetyl-L-cysteine, using the dosage regimen of the present invention.

In one embodiment of the present invention such method is used for reducing ovary volume in a subject with PCOS.

In another embodiment of the present invention the method is for achieving regular menstrual cycle in a subject with PCOS.

In still another embodiment of the present invention the method is for reducing body weight and body mass index in a subject with PCOS.

In a further embodiment of the present invention the method is for treating infertility or promoting a desired pregnancy in a subject with PCOS.

In another embodiment of the present invention the method is for reducing acne and/or hirsutism in a subject with PCOS.

In another embodiment of the present invention the method is for reducing insulinemia in a subject with PCOS.
In another embodiment of the present invention the method is for reducing the risk for cardiovascular diseases in a subject with PCOS.

In still another embodiment of the present invention the method is for reducing the risk for endometrial hyperplasia and endometrial cancer in a subject with PCOS.

EXAMPLE

The invention is further described and illustrated by reference to the following example. It should be noted, however, that this example should not be considered as limiting the invention in any way.

Example 1. Pilot clinical study on the effect of NAC to treat PCOS.

Aim and set up of the study. This pilot clinical study was designed to treat women with polycystic ovary syndrome (PCOS) with NAC as an alternative to other treatments, particularly those involving hormones. Five women, average age 25±5 years, diagnosed with PCOS through sonographic and clinical diagnosis, and having more than 10 ovary follicles, were enrolled.

Treatment. The prescribed NAC dose was 30 mg/kg/day, corresponding for an average body weight of 60 kg, to three oral doses of 600 mg NAC, three times a day, for three consecutive days each week, with four days of interruption. This resulted in 1.8 g of NAC per day, 5.4 g per week, 21.6 g per month. These precise modalities of treatment were based on the following considerations: 1) the dose of 1.8 g of NAC per day is in agreement with other current clinical treatments and is considered without undesirable side effects; 2) given NAC pharmacokinetics, the three daily doses of 0.6 mg approach a nearly constant plasma level, without discomfort of patients for a complex treatment; 3) given a reported decrease in NAC plasma level after prolonged treatments (Pendyala L, Creaven PJ. Cancer Epidemiol Biomarkers Prev. 1995; 4:245-51), the suspension of the treatment for about half of each week warrants an optimal biological response in a six-months or longer treatment.

Follow-up. Clinical outcome was evaluated after the first three months of treatment, with a planned follow-up evaluation at the end of the study after six months.
Results.

- Four cases presented a decrease in the average ovary diameter, with an average decrease of 20%, see table 1 and figure 1.

- Three cases having more than 10 follicles at the start of the study presented a decrease in follicle number, two of them achieving a completely normal ovary sonographic appearance.

- Three cases with irregular menstrual cycles, reported regularization.

- One case reported a decrease in body mass index from 26.5 to 25.2. The remaining patients already had a low BMI.

- One patient of the five suffered from acne before the start of the treatment. After treatment amelioration of acne and improvement of hair quality was reported by both patient and clinician.

Table I.

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<td>40</td>
<td>23</td>
<td>30</td>
<td>&gt;10</td>
<td>&lt;10</td>
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In Table I results are summarized for each patient 1-5. Shown are: the average ovary diameter (D), in mm, for the right (dx) and left (sn) ovary, at the first sonographic evaluation at enrolment (0), and after three months (3). Also reported are cases starting with high follicle number and irregular menstrual cycle at enrolment (0) and the corresponding results after three months (3).
CLAIMS

1. A pharmaceutical composition comprising N-acetyl-L-cysteine for use in the treatment of a mammal having polycystic ovary syndrome (PCOS), characterized in that the composition is for pulsed or intermittent, oral administration, for a time period of two months or more and wherein the administration takes place for 3-5 consecutive days followed by 2-4 days of interruption or alternatively for 1-3 consecutive days followed by 1-2 days of interruption at a dose of N-acetyl-L-cysteine that is between 20 and 90 mg/kg/day on administration days.

2. A pharmaceutical composition comprising N-acetyl-L-Cysteine for use according to claim 1 characterized in that it is administered for 3-5 consecutive days followed by 2-4 days of interruption.

3. A pharmaceutical composition comprising N-acetyl-L-Cysteine for use according to claim 1 characterized in that it is administered for 1-3 consecutive days followed by 1-2 days of interruption.

4. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claim 1, characterized in that it is for administration at a dose of N-acetyl-L-cysteine that is between 30 and 60 mg/kg/day on administration days.

5. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claim 1, characterized in that it is for administration at a dose of N-acetyl-L-cysteine that is between 30 and 45 mg/kg/day on administration days.

6. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to any of the previous claims, characterized in that it is protected from light.

7. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to any of the previous claims, characterized in that it is a water soluble tablet.

8. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to any of the previous claims, characterized in that it contains sodium hydrogen carbonate.
9. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claims 1-6, characterized in that it comprises a formulation for slow-release and/or gastric protected formulation.

10. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claims 1-9 for reducing ovary volume and/or follicles number in a subject with PCOS.

11. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claims 1-9 for achieving regular menstrual cycle in a subject with PCOS.

12. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claims 1-9 for reducing body weight and body mass index in a subject with PCOS.

13. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claims 1-9 for treating infertility or promoting a desired pregnancy in a subject with PCOS.

14. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claims 1-9 for reducing acne and/or hirsutism in a subject with PCOS.

15. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claims 1-9 for treating insulinemia in a subject with PCOS.

16. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claims 1-9 for reducing the risk for cardiovascular diseases in a subject with PCOS.

17. A pharmaceutical composition comprising N-acetyl-L-cysteine for use according to claims 1-9 for reducing the risk for endometrial hyperplasia and endometrial cancer in a subject with PCOS.
### A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K31/198 A61P15/00 A61P43/00

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)
A61K A61P

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)
EPO-Internal, BIOSIS, EMBASE, FSTA, WPI Data, CHEM ABS Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

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<td>WO 2007/056851 AI (MOUNT SINAI HOSPITAL CORP [CA]; BEDAIWY MOHAMED [CA]; RIZK AHMED Y [EG]) 24 May 2007 (2007-05-24) page 10, lines 14-31; claims 1, 2, 5, 6, 9 ------</td>
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Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search: 31 January 2012

Date of mailing of the international search report: 07/02/2012

Name and mailing address of the ISA:
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer: Baumgartner, Heike
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<td>FULGHESU A M ET AL: &quot;N-acetyl-cysteine treatment improves insulin sensitivity in women with polycystic ovary syndrome&quot;, FERTILITY AND STERILITY, ELSEVIER SCIENCE INC, NEW YORK, NY, USA, vol. 77, no. 6, 1 June 2002 (2002-06-01), pages 1128-1135, XP003013234, ISSN: 0015-0282, DOI: 10.1016/S0015-0282(02)03133-3 abstract --------</td>
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