Naltrexone treatment in clomiphene resistant women with polycystic ovary syndrome

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BACKGROUND: Endogenous opiates may affect various aspects of reproductive and metabolic function in patients with polycystic ovary syndrome (PCOS). This study evaluated long-term inhibition of the opioid system using naltrexone in clomiphene citrate (CC)-resistant women with PCOS. METHODS: A group of 30 infertile females with PCOS were evaluated; all subjects were obese, hyperandrogenic and hyperinsulinemic; 16 patients were amenorrheic and 14 were oligomenorrheic. All subjects received naltrexone (50 mg p.o. daily) for 6 months. Patients who did not ovulate after 12 weeks of naltrexone monotherapy, also received CC (starting at 50 mg/day for 5 days and, for non-responders, increasing it up to 150 mg/day). RESULTS: Of the 30 women, 3 ovulated during naltrexone monotherapy and 19 of the remaining 27 ovulated during naltrexone + CC therapy. There were no conceptions during naltrexone monotherapy, but 9 of 27 women (33.3%) conceived during naltrexone + CC; there was one missed abortion at 9 weeks, one preterm delivery at 34 weeks and seven term live births. Naltrexone therapy was also followed by significant reductions in BMI, fasting serum insulin, luteinizing hormone (LH), LH/follicle-stimulating hormone ratio and testosterone. CONCLUSIONS: In this preliminary trial, naltrexone improved endocrine and metabolic function in women with CC-resistant PCOS. Furthermore, naltrexone restored CC sensitivity in the majority of subjects, resulting in a significant number of pregnancies.

Keywords: naltrexone; PCOS; infertility; clomiphene resistance

Introduction

Polycystic ovary syndrome (PCOS) is the most common cause of anovulatory infertility (Homburg, 2003). It is a complex disorder involving the CNS-hypothalamus, the pituitary gland, the adrenal glands and the ovaries (Talbott et al., 2000; Doi et al., 2005). Although multiple alterations in the function of these organs have been documented, it is uncertain which, if any, of these organs initiates the development of PCOS (Chang et al., 2000; Lobo and Carmina, 2000). Prevalence of PCOS has been studied in several populations and it appears that it affects as many as 5–10% of women of reproductive age (Diamanti-Kandarakis et al., 1999; Azziz et al., 2004). In the view of a diversity of clinical presentations and a wide range of endocrine and metabolic profiles, it is probable that PCOS does not represent a single disorder, but a group of related pathologic conditions.

Salient features of PCOS include hyperandrogenism and chronic anovulation, often in association with insulin resistance, as well as alterations in GnRH pulse frequency and gonadotrophin release (Barnes and Rosenfield, 1989; Morales et al., 1996; Blank et al., 2007). There is also evidence that PCOS is associated with hyperactivity of the sympathetic nervous system, stress and altered β-endorphin release. In a study directly evaluating sympathetic nerve activity, Sverrisdottir et al. (2008) found that women with PCOS have significantly higher muscle vascular bed sympathetic nerve activity than matched controls. The increased sympathetic outflow was related to testosterone and to a lesser degree to cholesterol. They concluded that the augmented sympathetic activity contributes to the vascular risk factors associated with PCOS and recommended studies of therapies aimed at reducing sympathetic activity.

Several other studies have indirectly measured the nerve activity in women with PCOS by comparing the heart autonomic innervation characteristics of women with PCOS and control subjects (Yildirim et al., 2006; Tekin et al., 2007; Giallauria et al., 2008). These studies consistently have shown that autonomic innervation of the heart in PCOS is altered, with increased sympathetic activity.

Growing evidence points to a prominent and complex relationship between endogenous opiates, insulin and GnRH output (Piva et al., 1986). Endogenous opioids (β-endorphins) have been identified in the pancreas and gastrointestinal tract. These may influence pancreatic β-cell function and glycemic
control (Hadziomerovic et al., 2006), and thus may play a role in the pathogenesis of conditions such as obesity and PCOS (Lanzone et al., 1995). Among many proposed actions, β-endorphins can modulate appetite and food-intake (Guido et al., 2006). Lanzone et al. (1993) have shown that both acute and chronic inhibition of the opioid system significantly decreases the insulin response to an oral glucose tolerance test (OGTT) in a group of hyperinsulinemic PCOS patients without significant change in plasma androgen levels.

Narcotic antagonists are defined as substances which block the actions of opioids. Thus, narcotic antagonists, such as naltrexone, block such phenomena as analgesia, euphoria, and other physiologic changes produced by opiates. By blocking the effects of opiates, narcotic antagonists also prevent the development of physical dependence and tolerance to opiate drugs (Li et al., 2003). Fruzzetti et al. (2002) studied 10 obese women with PCOS and found that the long-term naltrexone treatment resulted in a decrease in BMI, improvement of menstrual cyclicity, reduction of androgen levels and improvement of some parameters of insulin sensitivity.

This study was designed to assess endocrine and metabolic effects of naltrexone in clomiphene-resistant, obese, hyperinsulinemic patients with PCOS. The end-points studied also included clinical parameters such as ovulatory function, hirsutism and acne, and, most importantly, pregnancy.

Materials and Methods

Patients

This study evaluated 30 infertile females with PCOS, 18–35 years of age, who were attending the fertility clinic of Benha University Hospital (Benha, Egypt) between March 2003 and April 2005. The protocol of the study was approved by the ethics committee of Benha Medical School and written consent was obtained from each patient.

Diagnosis of PCOS was based on the evidence of: (i) oligo- or amenorrhea responsive with withdrawal bleeding to progestin and (ii) hyperandrogenism and/or hyperandrogenemia. Oligomenorrhea was defined as irregular menstrual bleeding at intervals varying between 5 weeks and 6 months, and amenorrhea as absence of bleedings for at least 6 months. Hyperandrogenism was defined as hirsutism with a Ferriman–Gallwey score ≥ 7 (Ferriman and Gallwey, 1961) and/or acne (Kołodziejczyk et al., 2000). Hyperandrogenemia was defined as total testosterone > 0.8 ng/ml. Ultrasound evaluations of ovaries documented polycystic ovarian appearance.

All subjects had primary infertility for over 1 year, chronic anovulation with a progesterone level < 4 ng/ml, and a body mass index (BMI) > 25 kg/m² (Legro et al., 2005), android obesity with waist/hip ratio (WHR) > 0.80 (Ovesen et al., 1993) and a fasting glucose to fasting insulin ratio < 4.5 mg/unit.

All subjects were resistant to clomiphene citrate (CC) therapy, which was defined as failure to ovulate in response to CC administered at a dose of up to 200 mg/day (Mitwally et al., 1999). Hence, all subjects did not ovulate during four consecutive cycles while receiving CC, starting at a dose of 50 mg/day during cycle days 4–8 and increasing the dose by 50 mg during each consecutive cycle. In all subjects, follicle-stimulating hormone (FSH) was < 10 mIU/ml and luteinizing hormone (LH)/FSH ratio was > 2. All patients had normal levels of thyroid stimulating hormone (TSH), prolactin and 17-hydroxy progesterone (17-OHP) as well as normal renal and liver functions. None of the subjects had any other identifiable factors which might contribute to infertility; they all had normal hysterosalpingogram, no evidence of uterine leiomyomas, normal post-coital test and their partners had normal semen analysis.

Women with diabetes mellitus or other endocrinopathies and those who had received sex hormones within 2 months before the start of the study were excluded.

Protocol

All subjects underwent baseline evaluations on the 3rd day of spontaneous or progestin-induced menstruation and commenced treatment with naltrexone on the 4th day of menstruation (50 mg p.o. daily) for 6 months. Patients who did not ovulate on naltrexone alone after 12 weeks were given a combination of naltrexone + CC, starting at a dose of 50 mg/day orally on the 5th day of menstruation after withdrawal bleeding. If ovulation was not detected by ultrasound, the dose of CC was increased in 50 mg increments per cycle (in 12 patients) up to a maximum dose of 150 mg/day. Combined therapy of naltrexone + CC was continued until conception was diagnosed by a positive chemical pregnancy test or, in the absence of pregnancy, up to the end of the study (a total of 6 months), whichever occurred first.

Each cycle was monitored by ultrasound examinations on the 11th and 13th day and serum progesterone was measured on cycle day 21 to assess the ovulation. BMI and menstrual cyclicity were assessed. Fasting insulin, fasting glucose, total testosterone, sex hormone binding globulin (SHBG), DHEAS, 17-OH progesterone, LH and FSH measurements were performed at the start of this study, 3 months after naltrexone monotherapy and at the end of the study. Renal function (serum creatinine concentration < 1.4 mg/dl) and liver function tests included SGPT and SGOT, and were assessed before and at 3 month intervals during the study. Pregnancy was diagnosed by detection of the β-subunit of HCG > 20 mIU/ml performed 2 weeks after documented ovulation.

Ultrasonographic evaluation of the ovaries and the endometrium was performed on the 2nd day of menstruation in real time using a high-frequency 7.5 MHz Voluson transvaginal transducer attached to the Combison 580 system (Kretztechnik AG, Zipf, Austria). Women with ovarian follicles ≥ 10 mm or any other ovarian cysts or endometriomas were excluded from the study. Polycystic ovarian morphology was diagnosed as the presence of more than 12 follicles (2004) with diameter ≤ 8 mm arranged peripherally or scattered throughout an echodense core of stroma (Adams et al., 1986; Polson et al., 1988).

Assays

LH, FSH and SHBG were measured by chemiluminescent enzyme immunoassay (Immulite 2000; Diagnostic Products Corporation, Los Angeles, CA, USA) according to the method described by Winter et al. (1978). Total testosterone (T) was measured by a chemiluminescent immunometric method (Immulite 2000; Diagnostic Products Corporation), Serum 17-OH progesterone was measured by RIA with standard commercially available kits (Biermann Inc and Diagnostic Products Corp). DHEAS was measured by RIA (Diagnostic Products Corporation).

Plasma insulin levels were measured by chemiluminescent enzyme immunoassay (Immulite 2000; Diagnostic Products Corporation). Fasting glucose was measured by glucose oxidase technique using Beckman glucose analyzer 2 (Fullerton, CA, USA). Fasting glucose to fasting insulin ratios of < 4.5 mg/unit are consistent with insulin resistance (Legro et al., 1998). The OGTT was performed after an overnight fast, with a 100 g glucose load administered orally and blood sampled for blood glucose and insulin at 0 and 120 min (Acien et al., 1999). All tests were performed at baseline, repeated
after 12 weeks of naltrexone monotherapy and again at the end of naltrexone + CC therapy.

**Statistical analysis**
Data were analyzed using the Statistical Package for Social Science, release 11 (SPSS Inc., Chicago, IL, USA). All data are presented as arithmetic means ± standard error of the mean (SEM). The changes in hormone levels and BMI during naltrexone treatment and results of both OGTTs were compared using Student’s t-test for paired samples, with each patient serving as her own control. Values of \( P < 0.05 \), two-sided, were considered statistically significant. In this study, 30 subjects were enrolled in order to detect a statistically significant effect of treatment (at \( P < 0.05 \) with power of 0.8) assuming at least a 25% pregnancy rate.

**Results**
Figure 1 shows the flowchart summarizing the study. The study included 30 obese patients with mean age of 28 ± 1.4 years (mean ± SEM). The clinical, endocrine and metabolic profiles of these patients are shown in Table I, presenting data at the baseline, after naltrexone monotherapy and at the end of combined naltrexone + CC. All 30 subjects were re-evaluated after the 12 week course of naltrexone alone. Since three subjects became ovulatory during naltrexone monotherapy, only 27 remaining women were re-evaluated at the end of the combined naltrexone + CC treatment. It is apparent, that naltrexone monotherapy resulted in a significant decline of BMI in the absence of dietary restrictions. There was no significant difference between the BMI of ovulating versus non-ovulating women (respectively, 31.4 ± 1.2 versus 31.7 ± 1.3). The decline of BMI in ovulating women was also comparable to that in non-ovulating women (1.37% versus 1.21%). Naltrexone therapy also caused a pronounced improvement in clinical end-points including hirsutism, acne and menstrual regularity. There were also improvements in biochemical markers including fasting insulin, glucose to insulin ratio, total testosterone and LH.

Notably, the pattern of changes in insulin secretion was different among responders (subjects who ovulated) and non-responders (those who remained anovulatory). The insulin AUC of responders significantly decreased from the baseline \((110.5 ± 2.3)\) to the end of treatment \((84.12 ± 1.3)\) \((P < 0.01)\). The insulin AUC of non-responders also decreased from the baseline \((107.9 ± 1.4)\) to the end of treatment \((89.7 ± 2.7)\) \((P < 0.05)\). However, the decline of the insulin AUC among responders \((26.4 ± 2.1)\) was significantly greater than that among non-responders \((18.2 ± 1.4)\) with \( P < 0.05 \). In contrast, there were no significant differences in the glucose AUC between responders and non-responders.

Sonographic evaluations revealed that all the ovulatory cycles during treatment with naltrexone alone were associated with monofollicular ovulations. During combined therapy with CC and naltrexone, 44.4% of cycles had monofollicular development and 25.9% of cycles had multifollicular development. Furthermore, naltrexone + CC treatment resulted in a significant decrease in the total ovarian volume \((13.8 ± 1.3 \text{ ml before treatment and } 9.2 ± 1.1 \text{ ml after})\); a decrease in the total antral follicle count \((12.6 ± 12.6 \text{ before and } 6.4 ± 1.2 \text{ after})\) and a decrease in the total follicle volume \((5.7 ± 1 \text{ ml before and } 3.4 ± 0.8 \text{ ml after})\).

Ovulatory and pregnancy rates in response to both naltrexone monotherapy and combined naltrexone + CC are shown in Table II. Naltrexone alone induced a modest rate of ovulatory response; however, the combination of naltrexone and CC restored ovulation in over 70% of subjects and resulted

**FLOWCHART**

![Flowchart](image_url)

Figure 1: Flowchart summarizing the study.
Glucose AUC (mg/dl) 30
Serum LH (mIU/ml) 27
Serum FSH (mIU/ml) 14
LH

*After 24 weeks of treatment or earlier, if pregnant.

All values represent means ± SEM.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Number of women who ovulated (%)</th>
<th>Number of women who conceived (%)</th>
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<tr>
<td>Naltrexone monotherapy (n = 30)</td>
<td>3/30 (10)</td>
<td>0/30 (0%)</td>
</tr>
<tr>
<td>Naltrexone + CC (n = 27)</td>
<td>19/27 (70.3)</td>
<td>9/27 (33.3)</td>
</tr>
<tr>
<td>Total (n = 30)</td>
<td>22/30 (73.3)</td>
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Above all, naltrexone treatment restored CC sensitivity and resulted in a high rate of ovulations and pregnancies in previously clomiphene-resistant patients.

Improvement of clinical and biochemical markers of PCOS may be related to a significant decline of BMI, which occurred in the absence of apparent change in diet and without overt dietary restrictions. These observations are in agreement with several previous studies (Givens and Kurtz, 1986; Apa et al., 1995; Villa et al., 1999; Fruzzetti et al., 2002). On the other hand, some researchers did not observe significant changes in BMI after naltrexone therapy (Couzinet et al., 1995). The discrepancy between different studies may reflect differences in patients’ characteristics; thus, e.g. in the present study, patients were more obese. Ultimately, the potential role of naltrexone in modulation of BMI should be addressed in a placebo-controlled randomized trial.

It is known that weight loss is the most physiologic method of inducing ovulation and improvement of insulin sensitivity (Harwood et al., 2007; Nelson and Fleming, 2007). The exact mechanism of action of naltrexone on weight has not been established, but it may be related to the involvement of opioid peptides in the control of appetite and in the pathogenesis of obesity. Plasma levels of β-endorphin are increased in obese subjects, both in adults and adolescents and this increase is not corrected by weight loss (Malcolm et al., 1986). Exaggerated β-pancreatic cell responsiveness to β-endorphin stimulation in human obesity is well documented (Cozzolino et al., 1996). Moreover, the administration of naltrexone results in a reduced food intake in android obesity (Fruzzetti et al., 2002). β-Endorphins are detected in the pancreas and are believed to influence insulin and glucagon release. Hence, endogenous opioids may play a role in the regulation of glucose metabolism and in the pathogenesis of obesity beyond the effects on appetite. Metabolic abnormalities, such as hyperinsulinemia, insulin-resistance and obesity, are the common features of

Table II. Ovulation and pregnancy rates during naltrexone monotherapy and naltrexone + CC therapy.

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PCOS and are likely to be involved in the pathogenesis of this disorder. A link between opioids and PCOS-related hyperinsulinism is suggested by the finding of altered central opioid tone evidenced by elevated β-endorphins levels, which directly correlated with body weight (Guido et al., 2006). Furthermore, naloxone and naltrexone significantly reduce the insulin response to glucose load only in hyperinsulinemic PCOS patients.

In this study, the decline of insulin secretion was not accompanied by significant changes in glucose levels; these observations are consistent with improvement of insulin sensitivity. We also noted that the decline in insulin AUC in responders who ovulated was significantly greater than that of non-responders. Hence, these observations strengthen the argument that insulin sensitivity improvement is related to ovulation.

Since insulin and testosterone levels declined after naltrexone therapy, one would expect an increase in SHBG. However, in this study, no significant changes of SHBG were noted. This observation is not readily explainable; one may speculate that other factors not evaluated in this report may have been involved in the maintenance of SHBG levels.

In this study, naltrexone therapy was associated with a significant decrease in total testosterone. This finding correlates well with the observations of Fruzzetti et al. (2002) who reported a significant decrease in plasma androstenedione, plasma-free testosterone and DHEAS after naltrexone treatment, and a statistically insignificant trend toward lower total testosterone.

Importantly, we also observed improvements with regard to the clinical effects of androgens including hirsutism and acne. These findings agree with most, but not all, previous reports (Villa et al., 1999; Fulghesu et al., 2001; Fruzzetti et al., 2002; Hadziomerovic et al., 2006). These effects may be related to a decreased LH and improvement of hyperinsulinemia, resulting in lower ovarian androgen production (Nestler and Jakubowicz, 1996).

Beneficial effects of naltrexone also included restoration of menstrual cyclicity. This effect is also likely specific to individual patient populations, since comparable findings were observed by some (Fruzzetti et al., 2002), but not other investigators (Lanzone et al., 1993; Guido et al., 1998). In the present study, naltrexone monotherapy partly restored the menstrual cyclicity, and further improvement was observed during combined naltrexone + CC treatment indicating a restoration of CC sensitivity in these patients. The design of the study did not allow us to determine whether the effect of naltrexone on menstrual cycle was related to its central effect leading to changes in LH secretion or to indirect effects related to weight reduction and decrease in hyperinsulinemia. A decrease in fasting serum insulin level was not accompanied by a significant change in glucose levels. Hence, it appears that naltrexone therapy resulted in improved insulin sensitivity rather than a change in pancreatic β-cell function. The increase in insulin sensitivity may be related to decreased BMI and/or to a direct effect of naltrexone on insulin sensitivity (Fruzzetti et al., 2002).

In this study, the baseline levels of both LH and FSH were in a typical range for women with PCOS. Naltrexone therapy led to a significant reduction of LH without marked changes of serum FSH, thus resulting in a decreased LH/FSH ratio. In a previous study of 10 subjects, Fruzzetti et al. (2002) observed a statistically non-significant trend toward lower LH following naltrexone treatment; this was likely due to the limited power of the study. Since hyperinsulinemia and hyperandrogenism may alter the secretion of gonadotrophins in favor of an increase in LH, naltrexone may lower LH secretion by reducing insulin and/or androgen levels. However, naltrexone may also act directly on the hypothalamo-pituitary axis. Fulghesu et al. (2001) proposed that naltrexone may directly inhibit GnRH release by suppression of opioid tone in the hypothalamus leading to a decreased basal level of LH. The effects of naltrexone on LH may depend on characteristics of individual patient populations; for example, naltrexone administration can reduce the LH response to GnRH in hyperinsulinemic women but fails to be effective in normoinsulinemic subjects (Lanzone et al., 1993).

Clinically, the most relevant observations of this study pertain to restoration of ovulatory response and to achievement of pregnancies in women who were previously not responding to CC. These findings are encouraging especially since the population studied (obese, hyperinsulinemic and hyperandrogenic women with CC resistance) is very challenging to manage. Usually, such patients receive gonadotrophins and are at a greatly increased risk of ovarian hyperstimulation syndrome and multiple pregnancies. Furthermore, naltrexone was generally very well tolerated with minimal or no significant side effects. Notably, although ovarian stimulation is a well-recognized complication of CC treatment, it was not observed in this study, possibly due to gradual, step-wise increases of the CC doses.

Data regarding the use of naltrexone during pregnancy are scanty and the risks are unknown. In laboratory animals, naltrexone has been shown to have an embryocidal effect when given at very high doses (~140 times the human therapeutic dose) (Christian, 1984). Naltrexone should be used in pregnancy only when the potential benefit justifies the potential risk to the fetus (Hulse et al., 2001).

In summary, we propose that naltrexone therapy either alone or in combination with CC may improve a broad range of clinical, endocrine and metabolic derangements characteristic in PCOS. Our data suggest that the use of naltrexone may be particularly helpful in infertile patients not responding to CC alone. However, the present observations should be verified by a randomized controlled trial. Concerns regarding possible teratogenicity of naltrexone also will need to be considered.

**Funding**

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**References**

