Ovulation induction with a single-blind treatment regimen comparing naltrexone, placebo and clomiphene citrate in women with secondary amenorrhoea

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Secondary amenorrhoea is often associated with emotional stress, weight loss, eating disorders or polycystic ovary-like disease. Involvement of the endogenous opioids in the pathophysiology of hypothalamic amenorrhoea by inhibition of hypothalamic GnRH secretion, has been demonstrated in some cases. Chronic blockade of the endogenous opioids with the long-acting opioid antagonist naltrexone could result in increased gonadotropin secretion and ovulation induction in these cases. A single-blind ovulation induction protocol comparing naltrexone, placebo and clomiphene citrate was evaluated in eight patients with secondary amenorrhoea. Naltrexone proved not to be more effective than placebo in our study. Only one patient ovulated on naltrexone, one on placebo and four on clomiphene citrate. The latter therapy caused a better endocrine response. In conclusion, although ovulation could be incidentally induced by naltrexone, this drug did not appear to be more successful than placebo and clomiphene citrate for ovulation induction in this population of patients.

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Secondary hypothalamic amenorrhoea is often associated with emotional stress, weight loss, excessive physical exercise and eating disorders like anorexia and bulimia nervosa. Decreased gonadotropin levels due to a decreased frequency and amplitude of pulsatile GnRH have been demonstrated in the majority of patients (1–3). There is evidence that an increased tone of the endogenous opioids may be involved in the inhibition of GnRH secretion, at least in some forms of hypothalamic amenorrhoea (2, 4–7).

The role of endogenous opioids in secondary amenorrhoea caused by polycystic ovary-like disease is less clear. Although the high LH pulse frequency, characteristic of this disease, may suggest a decreased central opioid inhibition, evidence exists that peripherally an increased level of ß-endorphin in plasma (8) and follicular fluid (9) may contribute to the endocrine disturbances found in polycystic ovary-like disease. Treatment with exogenous pulsatile GnRH successfully induces ovulation in 40–80% of patients (10–11). Based on these findings, we expected treatment with an opioid antagonist to stimulate the endogenous GnRH secretion and thus be suitable for ovulation induction in polycystic ovary-like disease.

Chronic blockade of the endogenous opioids with the oral opioid antagonist naltrexone has induced ovulatory cycles in women with hypothalamic amenorrhoea and normal body weight (12, 13). Because of its advantages of oral administration and good tolerance during chronic use, naltrexone seemed an attractive new drug for ovulation induction. However, the results of these studies were controversial and the effect of naltrexone may at least partly be attributed to placebo (13).

Furthermore, it is questionable whether naltrexone may have advantages above other oral treatments for induction of ovulation. For this reason we engaged in a single-blind study comparing three treatment forms of ovulation induction: naltrexone, placebo and clomiphene citrate in women with secondary amenorrhoea of different etiology.

Material and methods

Subjects

Eight women with secondary amenorrhoea participated in our study after informed consent. The protocol was approved by the Committee on the Ethics of Research on Human Subjects of the Free University Hospital.

Six patients with hypothalamic amenorrhoea associated with simple weight loss, stress, anorexia nervosa...
or excessive physical exercise participated in the study. The hypothalamic amenorrhea was characterized by low or normal LH and FSH. Anorexia and bulimia nervosa were diagnosed by the DSM III criteria (14). Five of the six patients had previously lost 8.3–37.5% of their initial body weight, but had partially regained weight before participating in the study, as expressed by the body mass index 17.5–29.3 kg/m² (Table 1). In two other patients the secondary amenorrhea was considered to be due to polycystic ovary-like disease, because their amenorrhea was associated with high LH levels (>6.5 IU/l) and with normal FSH. Obesity and hirsutism were absent in the two polycystic ovary-like disease patients. The ovaries had a polycystic aspect on ultrasound examination, containing many small cysts of ±3 mm diameter. All patients had normal PRL, testosterone and androstenedione values. The clinical and endocrine data of the eight patients are presented in Table 1.

None of the patients abused drugs or alcohol and none of them had taken any medication during at least three months prior to the study. None of the patients was seeking pregnancy. They were instructed to use mechanical contraception during the study period.

**Treatment**

A single-blind ovulation induction protocol was used, comparing three therapeutic cycles: *cycle 1* – naltrexone 50 mg qd (Trexan®, Du Pont Pharma, Bad Homburg, Germany), oral administration in the morning for 28 days; *cycle 2* – placebo, oral administration for 28 days; *cycle 3* – clomiphene citrate 50 mg qd (Serophene®, Serono, Geneva, Switzerland), oral administration from day 5 to day 9 in combination with a placebo on days 1–4 and 10–28. Each patient received three identical tablet strips for three cycles of 28 days. Before the start of the first cycle all patients were administered 100 mg progestin im in order to induce withdrawal bleeding.

The treatment monitoring consisted of daily measurements of total estrogens and pregnanediol in 24 h urine, measurements of follicular growth by ultrasound three times a week using a vaginal 5 MHz/10 mm transducer (ATL Ultramark 4, ATL, Inc., Bellevue, WA), monitoring of changes in the cervical mucus three times a week and measurements of LH and FSH levels three times a week. Additionally, PRL, testosterone and androstenedione were measured at weekly intervals in peripheral blood. Blood samples were collected 1 h after ingestion of the tablets during all three cycles. When a growing dominant follicle with a diameter larger than 14 mm was seen, daily ultrasound. LH and FSH monitoring were continued until ovulation had occurred. All patients kept a basal body temperature curve. Blood samples for the determination of liver and renal functions were taken at the start of each cycle and blood pressure was measured weekly. All patients were treated and monitored by the same gynecologist (MCA) on every occasion.

An ovulatory cycle had to satisfy the following criteria: disappearance of the dominant follicle on ultrasound preceded by an LH surge in blood and followed by a rise in pregnanediol above the level of 3 μmol/24 h during the luteal phase. Luteal phase defects could not be diagnosed in this study, as no endometrial biopsies were taken. An anovulatory cycle was defined when one or more growing follicles larger than 13 mm diameter were detected by ultrasound, but the LH peak in blood and/or the pregnanediol rise in urine were absent. No response was defined when either the follicle diameter remained below 13 mm or no follicle growth and no increase of estrogen production occurred during the cycle.

**Hormone assays**

Total estrogen was determined with an automated rapid fluorometric assay, based on the Ittrich reaction (15). The means of values per day were normalized to a standard excretion of 10 nmol of creatinine per day. Pregnanediol was determined by gas chromatography after mild acid hydrolysis (16).

LH and FSH were measured by a recently developed IRMA (LH-MAIAclone and FSH-MAIAclone, Serono Diagnostics Ltd., Woking, UK), which uses two mono-

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (yr)</th>
<th>BMI (kg m⁻²)</th>
<th>Amenorrhea (yr)</th>
<th>Weight loss (%)</th>
<th>Total estrogens (nmol/24 h)</th>
<th>LH (IU/l)</th>
<th>FSH (IU/l)</th>
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<td>9.3</td>
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<td>+</td>
<td>PCOD</td>
</tr>
</tbody>
</table>

*Previous weight loss expressed as % of the initial body weight.

*Withdrawal bleeding caused by 100 mg progestin im.

Reference values: LH follicular phase 1.5–6.5 IU/l, FSH follicular phase 3–12 IU/l, total estrogens follicular phase 87–362 nmol/24 h.
clonal antibodies, one labelled with $^{125}$I, the other with fluorescein. The method has been described in detail by Wennink et al. (17). The detection limit of both assays was 0.15 IU/l. The inter- and intra-assay coefficients of variation for LH were 13 and 10%, respectively for values < 2 IU/l and 5 and 2.8% respectively for values > 2 IU/l. The inter- and intra-assay coefficients of variation for FSH were 5%. The reference values are shown in Table 1.

Testosterone was determined by a solid phase RIA (Coat-A-Count. Diagnostic Products Corp., Los Angeles, CA), the lower limit of detection being 0.25 nmol/l. The inter- and intra-assay coefficients of variation varied between 5.1 and 8.6%; the interassay coefficient of variation was 9%. The reference values are 2–9 nmol/l.

Androstenedione was determined by RIA after extraction (Diagnostic Products Corp., Los Angeles, CA). The intra-assay coefficients of variation varied between 5.1 and 8.6%; the interassay coefficient of variation was 9%. The reference values are < 2.5 IU/l.

PRL was determined by IRMA (Medgenix, Belgium). The inter- and intra-assay coefficients of variation were 7% and 4%, respectively. The reference value is < 0.7 IU/l.

**Statistical analysis**

Statistical differences of endocrine parameters between the three cycles were calculated with one-way analysis of variance followed by Duncan’s multiple comparison test. A multivariate adaptation of the Kruskal-Wallis method was used in order to discriminate which cycle days showed significant differences. This non-parametric method was described by Koziol et al. (18). Statistical differences in clinical parameters between the three cycles were calculated with a chi-squared test. The criterion of significance was set at $p < 0.05$.

**Results**

Clinical results of the ovulation induction treatment are presented in Table 2. During the naltrexone treatment only one patient had a successful ovulation induction (patient no. 6). In this patient, one growing follicle with a maximal diameter of 21 mm was detected by ultrasound. Ovulation was proved by ultrasound, a maximal pregnanediol value of 8.7 μmol/24 h and a biphasic basal body temperature with a luteal phase of eight days. One patient ovulated on placebo (patient no. 7). One dominant follicle with a maximal size of 20 mm developed. Ovulation was proved by ultrasound, pregnanediol of 9.2 μmol/24 h and a biphasic basal body temperature with a luteal phase of 14 days. Two other patients (nos. 3 and 5) had anovulatory cycles on naltrexone as well as on placebo. A growing dominant follicle and an LH peak were detected in both patients in either cycle, but ovulation did not occur (no ovulation signs on ultrasound and pregnanediol < 3 μmol/24 h). Four patients responded to neither naltrexone nor placebo.

During treatment with clomiphene citrate ovulation was proved in four and anovulatory cycles occurred in two patients. Although it seems that clomiphene citrate gave better results than naltrexone and placebo in the individual patients, the differences were not significant ($\chi^2 = 1.886$; $p > 0.05$). Multiple follicular growth was observed, but only one dominant follicle (maximal diameter 21–28 mm) developed in the ovulatory cycles. The length of the luteal phase varied between 6 and 14 days. One patient did not respond to clomiphene. Only seven patients completed the three months’ protocol. One patient dropped out of the study after the second cycle (patient no. 6). Because she was not seeking pregnancy, she found the protocol too time consuming.

Mean values (±SEM) of total estrogen, pregnanediol, LH and FSH during the three treatment cycles are presented in Fig. 1 (A, B, C and D respectively). Treatment with clomiphene citrate caused significantly higher total estrogen concentrations than treatment with naltrexone or placebo. The differences were significant on cycle days 10–15 (Koziol analysis 0.004 < $p < 0.05$). Pregnanediol values in the luteal phase were significantly higher during clomiphene citrate than during naltrexone and placebo treatment ($p < 0.05$). The concentrations of LH and FSH did not differ significantly between the three cycles on any cycle day (Koziol analysis $p > 0.34$ for LH and $p > 0.14$ for FSH).

Because patient no. 6 dropped out, her endocrine data could not be considered in the statistical analysis of the three cycles illustrated in Fig. 1. Comparison of the naltrexone and placebo cycle, including the data from this patient ($N = 8$), did not reveal any significant difference in endocrine parameters between these two treatments (results not shown). In order to eliminate the possibility that a slight increment of LH and/or FSH concentration might not appear significant compared to the LH and FSH peaks, the data from cycle days 0–5 and 0–10 were also analysed separately. No significant differences were found (results not shown).

The medians and ranges of PRL, testosterone and

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Cycle 1 (naltrexone)</th>
<th>Cycle 2 (placebo)</th>
<th>Cycle 3 (clomiphene)</th>
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<tr>
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<tr>
<td>8</td>
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*Drop-out cycle.
OVUL = ovulatory cycles; ANOV = anovulatory cycles; NR = no response.
androstenedione levels during the three treatment cycles are presented in Table 3. There were no significant differences.

After discontinuing the treatment all patients were followed for at least two months. They all returned to their amenorrheic state.

Naltrexone was well tolerated by most of the patients and mild gastrointestinal complaints were reported by two. These side effects disappeared after discontinuation of naltrexone administration. There were no dropouts because of side effects. No change in blood pressure, liver and renal function occurred during the treatment with naltrexone. A positive side effect was the disappearance of bulimic attacks reported by the patient with bulimia nervosa during the treatment with naltrexone. Naltrexone did not affect eating behaviour in the other patients. No side effects were reported during placebo and clomiphene citrate treatment.

**Discussion**

This is the first controlled study comparing the effect of naltrexone and placebo for ovulation induction in secondary amenorrhea of different etiology. Naltrexone proved not to be more successful for ovulation induction than placebo. Our results are less optimistic than those published by Wildt and Leyendecker (12), who found a 100% response, and Remorgida et al. (13), who found 75% ovulatory cycles in 53% of the patients. This discrepancy in results could be attributed to the different patient populations. Hypothalamic amenorrhea is a heterogeneous disease, with different etiology and duration, which makes comparisons between patients very difficult. Although the number of patients is too small for statistical calculations we can see some
tendencies in the group of poor responders to naltrexone. It seems that patients who are still underweight or have still not completely recovered from anorexia and bulimia nervosa respond to naltrexone poorly. These results are consistent with earlier reports in which naloxone induced only minor changes in gonadotropin levels in weight loss related amenorrhea and anorexia nervosa (19, 20). In a larger study we have shown that naltrexone does not affect the LH pulse pattern in women with secondary amenorrhea who are still underweight (21).

Furthermore, it is well known that even in patients with normal weight or with considerable weight regain after a history of weight loss opiate antagonism is not always effective. About half of the patients with hypothalamic amenorrhea were positive responders and the other half non-responders in experiments with naloxone (2, 4). Even the presence of a normal night-time LH pulsatility cannot always predict the responsiveness to opiate blockade in the individual patient (2). In our patients with hypothalamic amenorrhea the response to opiate blockade with naltrexone was unrelated to the initial levels of gonadotropins or total estrogen excretion.

Although naltrexone is a longer acting opioid antagonist than naloxone, chronic treatment for 28 days with 50 mg naltrexone qd does not seem to be sufficient for ovulation induction in most patients with hypothalamic amenorrhea. Either the endocrine disturbance of these patients is more severe than that of naltrexone responders or their amenorrhea is not related to an increased tone of endogenous opioids, but to other inhibitors of GnRH secretion. From the results of our ovulation induction protocol we cannot draw further conclusions regarding the cause of GnRH inhibition in the naltrexone non-responders. Whatever the mechanism of this GnRH inhibition may be, it can be successfully overcome by exogenous pulsatile GnRH, resulting in ovulation induction with a mean conception rate of 30% per cycle, even in underweight patients with hypothalamic amenorrhea (22).

Habituation to naltrexone was probably not the cause of its ineffectivity for ovulation induction. Chronic administration of naltrexone induced a marked increase of hypothalamic opioid receptors in the rat (23), but there is no evidence that such a phenomenon may operate in humans. We could not find any transient changes in LH and FSH concentrations during treatment with naltrexone. We cannot exclude the possibility that naltrexone treatment lasting longer than 28 days could give better results for ovulation induction, as some patients with severe long-standing secondary amenorrhea might need a longer triggering period before an ovulatory response is seen.

With our experience, limited to only two patients, it is difficult to draw conclusions on the effectiveness of naltrexone for ovulation induction in polycystic ovary-like disease. No changes in LH or FSH secretion were observed during treatment with naltrexone in these two patients. However, subtle changes in GnRH secretion may not always be reflected in an increased LH and FSH secretion, or may have failed to be detected by the method used in this study. Thus further research in polycystic ovary-like disease is warranted.

In contrast to Judd et al. (6), who found that clomiphene citrate caused a greater acceleration of LH pulse frequency and a greater increment of LH concentration than naloxone in women with hypothalamic amenorrhea, we found no significant difference in the LH level between the clomiphene and naltrexone cycle. However, clomiphene citrate caused significantly higher total estrogen and pregnanediol levels than naltrexone, as a result of the higher prevalence of ovulatory and anovulatory cycles during clomiphene citrate. It is questionable whether the better response to clomiphene may have been partly triggered by the preceding treatment with naltrexone and placebo. This mechanism is at least suggested in patient no. 3, by the fact that ovulation on clomiphene citrate was preceded by anovulatory cycles during naltrexone and placebo treatment. Anovulatory cycles followed by ovulatory ones have also been observed during ovulation induction with pulsatile GnRH (24), providing theoretical support for this mechanism. The position of placebo between the naltrexone and clomiphene citrate cycles was chosen in order to avoid interactions between these treatments. However, evaluation of the placebo effect is limited by this design of the study.

It is unlikely that successful ovulation induction with one of the three treatment regimens was due to a spontaneous return of the menstrual cycle, as all women returned to their amenorrheic state after discontinuing the protocol.

In conclusion, although ovulation could be induced incidentally by naltrexone, this drug did not appear to be more successful than placebo for ovulation induction in this population of patients. Clomiphene citrate caused a significantly better endocrine response and still remains the first choice of treatment in women with secondary amenorrhea. The role of endogenous opioids in the pathophysiology of secondary amenorrhea still remains controversial. Because of its long action, its oral administration form and its good tolerance, naltrexone is a suitable opioid antagonist for endocrine research in female reproduction.

Acknowledgments. We like to thank Du Pont Pharma, Bad Homburg, Germany, for kindly providing us with naltrexone (Trexan®) for this clinical study. We are also grateful to the staff of Endocrinology Laboratory of the Free University Hospital for performing the hormone assays.

References

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