Effect of laparoscopic ovarian diathermy on circulating inhibin B in women with anovulatory polycystic ovary syndrome

S.A. Amer1,3, S. Laird2, W.L. Ledger1 and T. C. Li1

1 Reproductive Medicine and Surgery Unit, University of Sheffield, Sheffield Teaching Hospitals and 2 Division of Biomedical Sciences, Sheffield Hallam University, Sheffield, UK
3 To whom correspondence should be addressed at: The Medical School, Derby City General Hospital, Derby, East Midlands, DE22 3NE, UK. E-mail: saad.amer@nottingham.ac.uk

BACKGROUND: Laparoscopic ovarian diathermy (LOD) frequently induces ovulation in patients with polycystic ovary syndrome (PCOS). The mechanism by which this effect occurs remains largely unexplained. The aim of this study was to measure changes in inhibin B production in response to LOD to see whether this could explain the mechanism of action of LOD. METHODS: This prospective study included 50 anovulatory women with PCOS. All women underwent LOD. Blood samples were collected before and after LOD to measure plasma concentrations of inhibin B, gonadotrophins and androgens. RESULTS: The pre-operative median plasma concentration of inhibin B was 110.0 pg/ml (range 19.0–567.0 pg/ml). There was a statistically significant inverse correlation ($r = –0.286; P < 0.05$) between body mass index (BMI) and inhibin B. Non-obese women with PCOS (BMI $\leq 30$ kg/m$^2$; $n = 37$) displayed significantly ($P < 0.05$) higher plasma inhibin B concentrations [122.0 pg/ml (range 19.0–567.0 pg/ml)] compared with those [51.0 pg/ml (range 27.0–201.0 pg/ml)] of obese PCOS women (BMI > 30 kg/m$^2$; $n = 13$). Following LOD, 39 women ovulated. No statistically significant change of inhibin B after LOD was observed in the overall group of women with PCOS or in the subgroup of non-obese PCOS women with higher pre-operative inhibin B. CONCLUSIONS: The lack of any change of inhibin B after LOD makes it unlikely that this hormone has any role to play in the mechanism of action of LOD.

Key words: polycystic ovary syndrome/laparoscopic ovarian diathermy/inhibin B/body mass index/ovulation

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrine disorder affecting 5–10% of women of reproductive age (Asuncion et al., 2000), accounting for at least 75% of cases with anovulatory infertility (Hull, 1987). It is characterized by a heterogeneous group of disorders that occur in varied combinations including clinical [oligomenorrhea/anovulation, hirsutism, acne and elevated body mass index (BMI)], biochemical (elevated circulating androgens and/or LH and evidence of insulin resistance) and/or ultrasound features of polycystic ovaries (increased ovarian volume $>10$ ml and/or number of small follicles $>12$). Although common, the pathophysiology of PCOS remains to be fully understood.

Inhibins are heterodimeric gonadal glycoproteins that suppress FSH release. They consist of an $\alpha$ subunit and one of two $\beta$ subunits, $\beta_A$ (inhibin A) and $\beta_B$ (inhibin B), which are secreted by the granulosa cells of the ovarian follicles. In women with normal ovulatory cycles, inhibin B rises from early follicular phase to reach a peak coinciding with the onset of the mid-follicular phase decline in FSH levels and then declines during the luteal phase. This suggests secretion by the developing cohort of follicles (Groome et al., 1996). It has therefore been suggested that follicular phase inhibin B may reflect the number of follicles present at baseline. This is further supported by studies of inhibin B in superovulated cycles, which have shown very high mid-follicular phase concentrations of inhibin B (Lockwood et al., 1996). Therefore, it is expected that inhibin B would be increased in PCOS subjects compared with normal women as a result of the increased number of small antral follicles. However, data from studies examining serum inhibin B in PCOS women are conflicting: whilst several reports revealed increased inhibin B concentrations in PCOS subjects compared with normal women (Anderson et al., 1998; Lockwood et al., 1998; Shen et al., 2005), many others have failed to demonstrate such an increase (Pigny et al., 2000; Elting et al., 2001; Laven et al., 2001; Norman et al., 2001; Cortet-Rudelli et al., 2002; Welt et al., 2002; Torgac et al., 2005). Some authors have reported that inhibin B concentrations are increased only in a proportion of PCOS women: non-obese PCOS patients (Pigny et al., 2000; Cortet-Rudelli et al., 2002) and in overall 23% of PCOS patients (Laven et al., 2001).
Although the underlying pathophysiology of PCOS remains uncertain, current evidence suggests that ovarian hypersecretion of androgens is the primary disorder in PCOS (Franks, 2002). It has been hypothesized that inhibin B is involved in the excess of intraovarian androgens by locally promoting theca-interstitial cells through autocrine/paracrine mechanisms (Hiller, 1991). It has also been proposed that increased inhibin B secretion by polycystic ovaries could suppress pituitary FSH secretion by an endocrine mechanism (Yen, 1991).

In a previous study, Lockwood et al. (1998) reported on the impact of laparoscopic ovarian diathermy (LOD) on the pulsatility and concentrations of circulating inhibin B in 10 clomifene-resistant PCOS women. They demonstrated that inhibin B secretion in PCOS women is higher than that in normal subjects and lacks the normal pulsatile pattern. Following successful LOD in four women, inhibin B was reduced to normal values and resumed its pulsatility. They concluded that the normalization of inhibin B levels and pulsatility after LOD appeared to correlate with the occurrence of ovulation after LOD.

To establish the exact role of inhibin B in the pathophysiology of PCOS, we measured the plasma concentrations of inhibin B in PCOS women before and after LOD in a larger series of PCOS women. Our aim was to evaluate the impact of LOD on circulating inhibin B concentrations and to see whether this could explain the pathophysiology of PCOS and the mechanism of action of LOD.

Materials and methods

Subjects

Fifty women with anovulatory infertility associated with PCOS who were scheduled for LOD were prospectively included in this study between 2000 and 2004. All subjects had polycystic ovaries [ovarian stromal hypertrophy and multiple, small (6–8 mm) follicles arranged in the periphery] on transvaginal ultrasound scan according to criteria defined by Adams et al. (1985). In addition, each woman had biochemical evidence of PCOS including an elevated LH/FSH ratio (≥2) and/or raised serum concentrations of androgens [testosterone ≥2.5 nmol/l or free androgen index (FAI) >4]. These hormones were measured in the early follicular phase (days 2–4) of the menstrual cycle in 14 women. In women with marked oligomenorrhoea or amenorrhoea (n = 36), a random blood sample was accepted. FAI was calculated using the formula: testosterone × 100/sex hormone-binding globulin (SHBG) (Carter et al., 1983; Eden et al., 1988). Ten women had not received any treatment for ovulation induction before LOD. The remaining 40 subjects had previously failed to respond to incremental doses of clomifene citrate (50, 100 and 150 mg). All women gave informed consent for laparoscopic ovarian drilling using diathermy.

Collection of blood samples

Blood samples were taken from PCOS patients shortly before LOD at a random time in 46 women and early in the follicular phase (menstrual cycle day 2–4) in 4 women. Further blood samples were obtained 1 week after surgery.

Hormonal assays

The hormone assays for LH, FSH, testosterone, SHBG and progesterone were performed as per our established protocol described previously (Li et al., 2000). Inhibin B was measured using an ultrasensitive two-site-specific enzyme-linked immunosorbent assay (ELISA) (Oxford Bioinnovation, UK), according to the manufacturer’s instruction. The assay has a sensitivity of <15 pg/ml, and the coefficient of variation (both interplate and intraplate) was <7%.

LOD

The techniques of laparoscopic ovarian drilling used in this study have previously been published (Li et al., 1998; Amer et al., 2002, 2004). A specially designed monopolar electrocautery probe (Rocket of London, Watford, UK) was used to penetrate the ovarian capsule at several points with the aid of a short burst of monopolar diathermy. The electrosurgical unit used was the Force 2 Valleylab electrosurgical generator (Valleylab Inc., Boulder, CO, USA). A monopolar coagulating current was used to make three to four punctures per ovary at a power setting of 30 W applied for 5 s per puncture.

Post-operative monitoring for ovulation

Following LOD, blood samples were taken weekly to measure the serum concentrations of progesterone until menstruation occurred or a level of ≥25 nmol/l was detected. If the patient started a menstrual period within 6 weeks of the surgery, a blood sample was taken on day 2 of that cycle for measurement of serum concentrations of LH, FSH, testosterone, androstenedione and SHBG. Another blood sample was taken on day 21 of the same cycle for measurement of serum concentration of progesterone. Ovulation was diagnosed if any of the progesterone concentrations was ≥25 nmol/l. If spontaneous menstruation did not occur within 6 weeks from surgery, a random blood sample was taken to measure progesterone. If the patient did not ovulate, as evidenced by the low progesterone concentrations, clomifene citrate was started 6–8 weeks after surgery.

Statistical analysis

Data were entered into the Statistical Package for Social Sciences (SPSS) for PC version 12. Plasma inhibin B values did not follow a normal distribution and were therefore compared using non-parametric statistical tests (Mann–Whitney U-test and Wilcoxon-signed rank test). Other continuous data were normally distributed and were therefore compared using t-test. Comparisons of categorical data were carried out using 2 × 2 contingency table analyses. Relationship between inhibin B and other biochemical parameters and BMI was evaluated using Spearman’s correlation. P < 0.05 was considered the minimum level of significance.

Ethical considerations

This study was approved by the South Sheffield Ethics Committee.

Results

The characteristics of the 50 PCOS women included in the study are summarized in Table I.

The median plasma concentration of inhibin B in the 50 PCOS women included in this study was 110.0 pg/ml (range 19.0–567.0 pg/ml). There was a statistically significant inverse correlation (r = –0.286; P < 0.05) between BMI and inhibin B (Figure 1). Non-obese PCOS women (BMI ≤30 kg/m²; n = 37) displayed significantly (P < 0.05) higher plasma inhibin B concentrations [122.0 pg/ml (range 19.0–567.0 pg/ml)] compared with that [51.0 pg/ml (range 27.0–201.0 pg/ml)] of obese PCOS women (BMI > 30 kg/m²; n = 13) (Figure 2). There was no statistically significant correlation between inhibin B and age (r = –0.122; P = 0.4), LH (r = –0.167; P = 0.25), FSH (r = –0.246; P = 0.09), testosterone (r = –0.013; P = 0.93), FAI (r = –0.145; P = 0.36) or ovarian volume (r = –0.003; P = 0.9).
Following LOD, there was no statistically significant change of plasma concentrations of inhibin B from the pre-operative values in the overall group of 50 women with PCOS [110.0 pg/ml (range 19.0–567.0 pg/ml) versus 106.0 pg/ml (range 6.7–284.0 pg/ml)] (Figure 3). Further subanalysis in non-obese PCOS women (BMI ≤ 30 kg/m²; n = 37) with higher pre-operative plasma inhibin B concentrations revealed no statistically significant change of inhibin B after LOD [122.9 pg/ml (range 19.0–567.0 pg/ml) versus 117.5 pg/ml (range 7.0–284.0 pg/ml)] (Figure 4). Similarly, no statistically significant change was observed in obese women with PCOS (BMI > 30 kg/m²; n = 13) after LOD [51.0 pg/ml (range 27.0–201.0 pg/ml) versus 78.5 pg/ml (range 8.0–151.0 pg/ml)].

Women who ovulated after LOD (n = 39) had a higher median pre-LOD inhibin B level [113.0 pg/ml (range 27–567 pg/ml)] compared with that [71.0 pg/ml (range 19–210 pg/ml)] of subjects (n = 11) who did not respond, although the difference

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**Table 1.** Characteristics of 50 women with anovulatory infertility associated with polycystic ovary syndrome (PCOS)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SEM</th>
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<tr>
<td>Age (years)</td>
<td>29.0 ± 0.6</td>
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<tr>
<td>Body mass index (BMI) (kg/m²)</td>
<td>27.0 ± 0.7</td>
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<tr>
<td>LH (IU/l)</td>
<td>11.4 ± 0.9</td>
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<td>FSH (IU/l)</td>
<td>5.1 ± 0.1</td>
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<td>LH : FSH ratio</td>
<td>2.6 ± 0.2</td>
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<tr>
<td>Testosterone (nmol/l)</td>
<td>2.6 ± 0.2</td>
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<tr>
<td>Free androgen index (FAI)</td>
<td>8.2 ± 1.0</td>
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<tr>
<td>Ovarian volume</td>
<td>12.5 ± 1.1</td>
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<th>n (%)</th>
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<tr>
<td>Menstrual cycle pattern</td>
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<td>Regular: cycle length between 25 and 35 days</td>
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<td>Oligomenorrhea: cycle length between 35 days and 6 months</td>
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<td>Amenorrhoea: absence of the menstrual period for &gt;6 months</td>
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<td>Hirsutism</td>
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<td>Infertility</td>
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**Figure 1.** Correlation between body mass index (BMI) and plasma inhibin B concentration in 50 women with anovulatory polycystic ovary syndrome (PCOS). Spearman’s correlation coefficient (r) and P values are illustrated.

**Figure 2.** Box and whisker plots representing median inhibin B plasma concentrations with 25th and 75th quartiles (lower and upper borders of boxes) and ranges in obese (BMI >30 kg/m²; n = 13) and non-obese (BMI ≤30 kg/m²; n = 37) anovulatory polycystic ovary syndrome (PCOS) women. Mann–Whitney test was used for comparison. *P < 0.05.

**Figure 3.** Box and whisker plots representing median inhibin B plasma concentrations with 25th and 75th quartiles (lower and upper borders of boxes) and ranges in 50 polycystic ovary syndrome (PCOS) women before and after laparoscopic ovarian diathermy (LOD). Wilcoxon-signed ranks test was used for comparison.
did not reach statistical significance. On the other hand, although neither the responders nor the non-responders showed any statistically significant change of median plasma inhibin B concentrations after LOD, there was a trend towards a reduction in the responders only.

Table II summarizes other endocrine changes after LOD. The results show a statistically significant reduction in FAI, LH : FSH ratio and the plasma concentrations of LH and testosterone. Plasma concentrations of FSH did not change after LOD.

Discussion

In this study, we have measured the circulating concentrations of inhibin B in 50 women with anovulatory infertility associated with PCOS and studied the effect of LOD on these levels.

Our data showed that most PCOS women in this study had plasma inhibin B concentrations (19–217 pg/ml) comparable with those (22–213 pg/ml) found in regularly cycling women as previously reported by Laven et al. (2001). Only four PCOS women in our study had inhibin B levels >217 pg/ml. This is in accordance with several other previous studies (Pigny et al., 2000; Elting et al., 2001; Norman et al., 2001; Cortet-Rudelli et al., 2002; Welt et al., 2002; Torgac et al., 2005), which showed no increase in inhibin B levels in PCOS women. Three previous studies have reported increase in inhibin B concentrations in PCOS women compared with normal control subjects (Anderson et al., 1998; Lockwood et al., 1998; Shen et al., 2005), although the concentrations reported by Lockwood et al. (1998) and Anderson et al. (1998) were within the ‘normal range’ reported by Laven et al. (2001). The study of Shen et al. (2005) included mostly non-obese PCOS women with a mean BMI 23 ± 3.8 kg/m². This may explain their relatively higher inhibin B concentrations (mean ± SD, 290 ± 86 pg/ml) compared with our study, which included women with higher BMI (28 ± 4.2 kg/m²).

Theoretically, inhibin B might be expected to be elevated in women with PCOS compared with normal subjects as a result of the increased number of small antral follicles characteristic of this syndrome. The absence of this elevation of inhibin B in PCOS women could be explained by the confounding effect of obesity on inhibin B concentrations (vide infra). In other words, although PCOS women might overproduce inhibin B as a result of increased numbers of small antral follicles, this effect is counteracted by the increased BMI which occurs in a large number of PCOS women. This balancing effect would not occur in non-obese PCOS women.

On the basis of the results of this study and those of previous reports, it seems that inhibin B is not elevated in women with PCOS and may therefore not be involved in the pathogenesis of PCOS.

Inhibin B and BMI

We have confirmed the previous finding of an inverse correlation between inhibin B and BMI (Pigny et al., 2000; Cortet-Rudelli et al., 2002). It is not clear why obesity is associated with a reduction of the circulating inhibin B concentrations in women with PCOS. It has been hypothesized that inhibin B secretion may be reduced in obese women as a result of functional impairment of the granulosa cells because of insulin resistance (Franks et al., 1996). Hyperinsulinaemia, which is more common in obese women, may reduce inhibin B production either by a direct effect on the granulosa cells or through impairment of insulin-like growth factor-I (IGF-I) action on these cells (Cortet-Rudelli et al., 2002).

Inhibin B and LH

In this study, there was no correlation between inhibin B and LH. This is consistent with a previous report by Pigny et al. (2000) and in disagreement with other reports (Laven et al., 2001; Norman et al., 2001; Cortet-Rudelli et al., 2002), which showed a modest but statistically significant positive correlation between inhibin B and LH. However, despite the positive correlation in these studies, a cause-and-effect relationship between inhibin B and LH is unlikely. This is evidenced by the inability of LH to stimulate inhibin B in vivo in normal subjects (Welt et al., 2002) and in vitro in polycystic and normal ovaries (Welt and Schneyer, 2001).
Inhibin B and FSH

Inhibin B suppresses FSH secretion and, in theory, should therefore be negatively correlated with serum FSH concentrations. The current data showed a trend towards a negative correlation between inhibin B and FSH. The lack of statistical significance may be due to the relatively small number of subjects included in this study and the various other possible regulators of pituitary FSH secretion [e.g. estradiol (E₂), LH and follistatin] which are integrated at the hypothalamus and pituitary.

Inhibin B and age

No statistically significant correlation was found between inhibin B and age, although inhibin B is expected to decrease with age as a result of the declining number of antral follicles. This is in agreement with several previous studies (Laven et al., 2001; Norman et al., 2001; Cortet-Rudelli et al., 2002). A possible explanation of this lack of correlation is that most women in the current study were relatively young (only three patients were 35–38 years old). A study by Bili et al. (2001), which included many PCOS women with a wider age range (17–42 years), showed a modest inverse correlation (r = −118; P < 0.05) between inhibin B and age.

The impact of LOD on inhibin B

In this study, we have demonstrated that serum inhibin B concentrations do not change after LOD in the overall group of 50 women with PCOS as well as in non-obese PCOS women who have relatively higher inhibin B. Also, no statistically significant change was observed in the responders or the non-responders following LOD. This is in disagreement with a previous study by Lockwood et al. (1998), who reported a fall in inhibin B after LOD in eight PCOS women. This discrepancy may be due to the different timing of measurement of inhibin B before and after LOD between the current study (mostly at random times) and that of Lockwood et al. (1998) (on day 5 of a spontaneous or progestogen-induced withdrawal bleed). Although early follicular phase assessment of baseline hormonal profile is valid in regularly cycling women, it is unlikely to be so in oligomenorrheic women. It is well established that ovulatory cycles alternate with anovulatory cycles in oligomenorrheic women (Franks, 1995). Several studies have shown temporary normalization of the hormonal profile after ovulation in oligomenorrheic women, an effect that continues for 14 days after the post-ovulation menstruation (Homburg et al., 1988; Minakami et al., 1988; Preleiv et al., 1990; Anttila et al., 1992; Taylor et al., 1997). A similar normalization of hormonal profile occurs after progestagen administration (Taylor et al., 1997). Therefore, a blood sample in the first few days of a spontaneous or an induced bleed may not be representative of the true hormonal profile of PCOS women. It is therefore possible to conclude that a random blood sample, in the absence of ovulatory activity, is more accurate than an early follicular phase one in oligomenorrheic women (van Hooff et al., 1999). In the present study, because ovarian activity has not been excluded at the time of collecting the blood sample, it is possible that samples may have been taken in the periovulatory period in some cases. However, this is likely to have occurred only in a small minority of cases and may therefore not have a significant impact on the overall results.

It is important to note that the lack of a statistically significant difference between pre- and post-operative inhibin B may represent a β error as a result of the relatively small number of subjects in this study. Given the high variations in baseline inhibin B, a larger sample will be required to show statistical significance for any changes in this hormone after LOD.

Mechanism of action of LOD

On the basis of the results of this study, it is difficult to implicate post-operative inhibin B changes in the mechanism of action of LOD. The reduction of androgens and LH observed after LOD in this study (Table II), which is consistent with several other previous studies (Gjonnaess and Norman, 1987; Greenblatt and Casper, 1987; Armar et al., 1990; Ligouri et al., 1996; Felemban et al., 2000; Amer et al., 2002), supports the hypothesis that LOD exerts its effects through the destruction of androgen-producing tissue in the ovarian. The decreased androgen concentrations may result in a fall in estrone (E₁) because of decreased availability of androgen precursors for estrogen production. This fall in E₁ may result in decreased positive feedback on LH and decreased negative feedback on FSH at the level of the pituitary. This effect, coupled with a decrease in local androgen concentrations, would convert the intrafollicular environment from being androgen dominant to one that is estrogenic. This may remove an intraovarian block to follicular growth, allowing follicular recruitment and development to proceed to subsequent ovulation.

It has also been hypothesized that the response of the ovarian to injury during LOD leads to a local cascade of growth factors (such as IGF-1), which sensitizes the ovarian to circulating FSH resulting in the stimulation of follicular growth.

The prognostic value of inhibin B in women undergoing LOD

There was an obvious trend towards higher pre-operative concentrations of circulating inhibin B in PCOS women who responded to LOD compared with the non-responders (median 113 versus 71 pg/ml, respectively). The lack of statistical significance may be due to the relatively small number of subjects included in the study (vide supra).

In conclusion, plasma inhibin B concentrations in women with PCOS seem to be comparable with those of regularly cycling women, indicating that this hormone does not appear to be involved in the pathogenesis of PCOS. The lack of any significant change of circulating inhibin B concentrations after LOD in women with PCOS suggests that inhibin B does not seem to play any role in the mechanism of action of LOD.

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