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THE EFFECT OF DANAZOL AND THE LHRH AGONIST ANALOGUE GOSERELIN (ZOLADEX) ON THE BIOLOGICAL ACTIVITY OF LUTEINIZING HORMONE IN WOMEN WITH ENDOMETRIOSIS

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SUMMARY

In an attempt to determine whether the suppression in oestradiol levels caused by danazol is due to an effect on the hypothalamic–pituitary axis, we compared the endocrine effects of danazol with those of the LHRH (GnRH) agonist analogue goserelin. Serum levels of immunoreactive LH (I-LH), FSH, 17β-oestradiol (E_2) and bioactive LH (B-LH) (using a mouse Leydig cell bioassay), were measured in ten and 20 women with endometriosis treated with danazol and goserelin, respectively. I-LH was measured both by radioimmunoassay (RIA) and immunoradiometric assay (IRMA). During 6 months of treatment with 600 mg of danazol daily, mean serum E_2 decreased ($P < 0{\cdot}05$) to levels near the upper limit of the post-menopausal range (to a mean (and 95% confidence interval of the mean) of 117 (65–169) pmol/l) whereas FSH, I-LH (both by RIA and IRMA) and B-LH levels were not significantly altered. During 6 months of treatment with monthly depot injections of 3·6 mg goserelin, mean serum E_2 decreased ($P < 0{\cdot}001$) to well within the post-menopausal range (to 23 (18–28) pmol/l). The mean FSH, I-LH and B-LH levels also decreased ($P < 0{\cdot}05$) during therapy with goserelin (from 3·9 (3·1–4·7) to 2·0 (1·6–2·4) IU/l for FSH, from 5·3 (4·5–6·1) to 1·9 (1·7–2·1) IU/l for RIA-LH, from 2·9 (2·5–3·3) to $< 0{\cdot}5$ ($< 0{\cdot}5$) IU/l for IRMA-LH and from 9·1 (7·1–11·1) to 2·9 (2·6–3·2) IU/l for B-LH). The bioactive to immunoreactive (B:I) RIA LH ratio was altered during the first month of treatment with both drugs, increasing with danazol from 1·6 (1·3–1·9) to 2·0 (1·7–2·3) ($P < 0{\cdot}05$) and decreasing with goserelin from 1·7 (1·5–1·9) to 1·4 (1·2–1·6) ($P < 0{\cdot}05$). The data indicate that danazol causes a relatively hypo-oestrogenic state which cannot be attributed to a decrease in the immuno-

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reactive gonadotrophin levels or to a decrease in the biological activity of LH and is associated with an increase in B:I LH ratio. In contrast, goserelin acts on the pituitary to produce suppression of gonadotrophin levels and effective down-regulation of the pituitary.

Danazol and luteinizing hormone-releasing hormone (LHRH) agonist analogues have similar efficacy in the treatment of endometriosis (*Lancet*, 1986; Henzl *et al.*, 1988; Fraser & Waxman, 1989). They both cause amenorrhoea, anovulation, and a hypo-oestrogenic state which makes them suitable agents for use in conditions which are oestrogen dependent or associated with menorrhagia and/or dysmenorrhoea (like endometriosis, fibromyomata, premenstrual syndrome and dysfunctional uterine bleeding).

Danazol, an isoxazole derivative of 17α-ethinyltestosterone, was originally described as having strong antigonadotrophic properties (Dmowski, 1979), but current evidence suggests that whilst it can eliminate the midcycle LH and FSH surge, danazol does not alter basal gonadotrophin levels (Barbieri & Ryan, 1981). However, E_2 levels are suppressed. Although there is evidence that danazol suppresses ovarian steroidogenesis (Barbieri *et al.*, 1977; Nilsson *et al.*, 1984; Carlstrom *et al.*, 1984; Olsson *et al.*, 1986), exogenous LHRH stimulation in danazol-treated patients leads to normal secretion of radioimmunoassayed gonadotrophins and E_2 (Rannevik, 1979; Braun *et al.*, 1983).

LHRH agonist analogues cause an initial stimulation of gonadotrophin and gonadal sex steroid secretion followed by a desensitization of the pituitary. This leads to suppression of gonadotrophin secretion and a reduction in ovarian steroidogenesis with a resultant decrease in serum oestrogen concentrations to levels which are similar to those found in women after the menopause or after surgical oophorectomy (Fraser & Waxman, 1989). Thus decreased I-LH levels have been widely reported in patients on LHRH agonist therapy (Schriock *et al.*, 1985; Thomas *et al.*, 1986; West & Baird, 1987; Matta *et al.*, 1988; Nicholson *et al.*, 1987), but others have noted increased levels of I-LH which have been reported to last up to 24 weeks (Meldrum *et al.*, 1982; Lemay *et al.*, 1984). These increased levels are associated with an increased release of LHα-subunit or of LH fragments (Lemay *et al.*, 1987; Meldrum *et al.*, 1984) which may cross-react with LH radioimmunoassays giving an erroneously high I-LH estimation. Furthermore, characterization of the serum I-LH by Sephadex chromatography demonstrated a change in the elution profile of I-LH on treatment with LHRH agonists indicating that LHRH agonists may induce the pituitary to release an immunoreactive LH with diminished bioactivity (Meldrum *et al.*, 1984; Evans *et al.*, 1984).

In an attempt to clarify these findings, and in particular to determine whether the suppression in oestradiol levels caused by danazol is due to an effect on the hypothalamic–pituitary axis, we compared the endocrine effects of danazol with those of the LHRH agonist analogue goserelin which is known to cause a state of hypogonadotrophic hypogonadism consistent with complete ovarian ablation (Thomas *et al.*, 1986; Nicholson *et al.*, 1987; Matta *et al.*, 1988).

MATERIALS AND METHODS

Subjects

Thirty normally menstruating women aged between 25 and 40 years (mean, 31 years) with laparoscopically diagnosed endometriosis were randomly allocated to 6 months of

medical therapy with either danazol 600 mg daily taken orally in three divided doses, or monthly subcutaneous injections of 3·6 mg of goserelin (D-SER [tBU]^6, Aza-GLY^{10}-LHRH) in the ratio of 1:2, respectively (10 patients received danazol and 20 patients received goserelin). All patients gave written consent and the study was approved by the hospital ethical committee. A blood sample was obtained from each patient during the early follicular phase (days 1–7) of the pretreatment cycle, at months 1 and 3 of treatment and 2 months post-treatment (i.e. 2 months after the last danazol capsule and 3 months after the last injection of goserelin). Serum was stored at −20°C until analysis.

Immunoassays

Serum LH, FSH and E_2 were analysed by radioimmunoassay using methods previously described (Ferguson *et al.*, 1982 (LH and FSH); Dowsett *et al.*, 1987 (E_2)). The within and between assay coefficients of variation (CV) were: for LH 6 and 12% at 4·8 IU/l respectively, with a sensitivity of 0·8 IU/l; for FSH 5 and 8% at 1·6 IU/l respectively, with a sensitivity of 0·4 IU/l, and for E_2 9 and 13% at 36 pmol/l respectively, with a sensitivity of 6 pmol/l.

LH was also measured using an immunoradiometric assay (Serono MAIAclone). The within and between assay CVs were 5 and 8% respectively, with a sensitivity of 0·5 IU/l.

LH Bioassay

The biological activity of LH was estimated using a dispersed mouse Leydig cell bioassay (Van Damme *et al.*, 1974) modified as previously described (Tsatsoulis *et al.*, 1987). The same pituitary LH reference standard (68/40) was used in this assay as for the RIA and IRMA LH assays. The within and between assay CVs were 7 and 12%, respectively, with a sensitivity of 2·5 IU/l.

Goserelin, danazol and its two major metabolites (ethisterone and 2-hydroxymethyl-ethisterone), did not interfere with the bioassay at concentrations of at least ten times greater than the expected maximum serum concentrations. Similarly, none of the drugs cross-reacted with the testosterone antibody employed in measuring testosterone secretion from Leydig cells.

Statistical analysis

Statistical analysis of E_2, FSH, I-LH (RIA and IRMA), B-LH, B:RIA LH ratio, B:IRMA LH ratio and RIA:IRMA LH ratio at each interval was performed using analysis of variance (ANOVA). Confirmatory *t*-tests were done using the mean squared error term from the ANOVA. Some of the B-LH and IRMA-LH assay values were less than the lower detection limit of the assay. In these cases the value allocated was 0·1 IU/l below the assay sensitivity (i.e. 2·4 IU/l for B-LH and 0·4 IU/l for IRMA-LH). This has the effect of underestimating any changes involving B-LH, IRMA-LH, B:RIA and B:IRMA LH ratios. In all analyses, $P < 0{\cdot}05$ was considered significant.

RESULTS

The results are presented in Table 1.

Table 1. The effect of danazol and goserelin therapy on E_2, FSH, RIA LH, IRMA LH, B-LH, B:RIA LH and B:IRMA LH

	Danazol				Goserelin			
Hormone	Pre	M1	M3	Post	Pre	M1	M3	Post
E2	356	160	75	305	282	18	30	476
(pmol/l)	169; 543	55; 265	56; 94	188; 422	198; 366	14; 22	12; 48	326; 626
FSH	3·5	4·4	3·7	3·7	3·9	1·5	2·2	4·0
(IU/l)	2·3; 4·6	3·4; 5·4	2·9; 4·5	2·8; 4·5	3·1; 4·7	1·2; 1·8	1·7; 2·7	2·6; 5·4
RIA LH	6·8	7·3	6·5	7·1	5·3	2·2	1·7	10·2
(IU/l)	5·3; 8·3	5·6; 9·0	3·9; 9·1	4·2; 11·0	4·5; 6·1	2·0; 2·4	1·5; 1·9	4·6; 15·8
IRMA LH	3·9	4·6	3·7	3·8	2·9	0·5	·4	5·8
(IU/l)	3·0; 4·8	3·5; 5·7	3·1; 4·2	1·4; 6·2	2·5; 3·3	0·4; 0·6	0·4; 0·4	2·8; 8·8
B-LH	11·3	14·9	12·5	13·9	9·1	2·9	2·9	14·6
(IU/l)	7·2; 15·4	10·0; 19·8	6·6; 18·4	5·3; 22·5	7·1; 11·1	2·7;3·1	2·6; 3·2	9·7; 19·5
B:RIA LH	1·6	2·0	1·9	1·8	1·7	1·4	1·7	1·9
	1·3; 1·9	1·7; 2·3	1·8; 2·0	1·7; 1·9	1·5; 1·9	1·2; 1·6	1·5; 2·0	1·6; 2·2
B:IRMA LH	2·9	3·3	3·4	3·7				
	2·3; 3·5	2·8; 3·8	2·9; 3·9	3·2; 4·2				

Pre, Pretreatment; M1, month 1; M3, month 3; Post, post-treatment. All values are given as mean and 95% confidence interval.

Danazol treatment led to a reduction ($P < 0.05$) in E_2 concentration at 1 and 3 months (to a mean (and 95% confidence interval of the mean) of 160 (55–265) and 75 (56–94) pmol/l, respectively), but hormone levels returned to within the pretreatment range (356 (169–543) pmol/l) 2 months after therapy was completed. Goserelin therapy led to a reduction ($P < 0.001$) of E_2 levels at 1 month to 18 (14–22) pmol/l. This level of E_2 was only 6% of the pretreatment concentrations and was far lower ($P = 0.01$) than that found with danazol. Secretion of E_2 remained severely suppressed after 3 months of therapy but in contrast to that found with danazol, increased ($P < 0.05$) to higher than pretreatment levels 2 months post-therapy.

The level of FSH was unchanged during danazol therapy whereas it decreased ($P < 0.01$) drastically during goserelin treatment, returning to pretreatment levels 2 months post-therapy.

The levels of RIA, IRMA or B-LH levels were unchanged during or after danazol treatment. The B:RIA LH ratio rose ($P < 0.05$) after 1 month of danazol treatment but returned to the upper limit of the pretreatment range at 3 months of treatment and remained within the pretreatment range 2 months post-treatment. The B:IRMA LH ratio also rose during danazol treatment but the increase was significant ($P < 0.05$) only at 3 months of treatment.

Mean RIA-LH and IRMA-LH levels fell ($P < 0.001$) during treatment with goserelin and rose after the end of therapy to above pretreatment levels ($P < 0.05$). B-LH levels also fell ($P < 0.001$) during goserelin therapy. The B:RIA LH ratio was lower ($P < 0.05$) during the first month of treatment compared to the pretreatment figure but rose to pretreatment levels by the third month of treatment with a further (not significant) rise by 2 months post-treatment. The B:IRMA LH ratio was not calculated in the case of goserelin therapy

because a large number of values in both the bioassay and the IRMA were below the lower detection limit of the assays.

For both groups, the RIA:IRMA LH ratio at pretreatment and post-treatment was similar (1·8 (1·5–2·1)). This was not altered by danazol therapy whereas goserelin therapy resulted in a greater decrease in LH IRMA compared to LH RIA values such that the RIA:IRMA LH ratio was increased to 4·4 (4·0–4·8) and 4·3 (3·9–4·7) at 1 and 3 months of treatment respectively ($P < 0·001$).

DISCUSSION

This is the first report relating bioactivity with radioimmunoreactivity of LH in women treated with danazol. Comparisons were made with goserelin-treated patients since, like other LHRH agonists, this compound is known to be effective through suppression of gonadotrophin-stimulated ovarian stereoidogenesis (West & Baird, 1987). We have examined differential changes in immunoreactivity and bioactivity as reflected in the B:I ratios since these ratios provide a sensitive index of qualitative changes in LH (Dufau & Veldhuis, 1987).

Endometriosis is an oestrogen-dependent condition not seen before puberty or after the menopause (in the absence of hormone replacement therapy). The desired pharmacological effect of any drug suitable for the treatment of endometriosis is, therefore, oestrogen deprivation. The marked suppression of E_2 levels seen with goserelin therapy was consistent with a complete ovarian ablation and was significantly greater than that seen with danazol. Despite this, danazol appears to have similar efficacy to LHRH agonists in the treatment of endometriosis (*Lancet*, 1986; Henzl *et al.*, 1988; Fraser & Waxman, 1989). The increase in free, biologically active testosterone seen with danazol therapy (Dowsett *et al.*, 1986; Forbes *et al.*, 1986) may contribute to the therapeutic efficacy of danazol. Direct inhibition of endometrial cell growth by danazol (Rose *et al.*, 1988), may also play a role.

The lack of suppression of the FSH and LH levels by danazol treatment is in agreement with Barbieri and Ryan (1981) and suggests that the observed decrease in E_2 levels is due to the inhibitory effect of danazol on ovarian steroidogenesis (Barbieri *et al.*, 1977; Nilsson *et al.*, 1984; Carlstrom *et al.*, 1984; Olsson *et al.*, 1986). This, however, does not exclude a direct effect of the drug on the hypothalamo–pituitary axis which prevents a measurable compensatory rise of gonadotrophin levels and also abolishes the mid-cycle gonadotrophin surge. The effect of danazol on LH pulsatility (Braun *et al.*, 1983; Dmowski *et al.*, 1983) may also result in reduced E_2 secretion as in women with hypothalamic dysfunction.

The marked suppression of FSH and RIA-LH levels during goserelin treatment is in agreement with most previous studies involving this agonist (Thomas *et al.*, 1986; West & Baird, 1987; Matta *et al.*, 1988; Nicholson *et al.*, 1984) and other LHRH agonists (Schriock *et al.*, 1985; Franssen *et al.*, 1989; Tummon *et al.*, 1989). The decrease in B-LH during goserelin therapy is in agreement with the findings of all the previous published studies on LH bioactivity using other LHRH agonist analogues (Meldrum *et al.*, 1984; Evans *et al.*, 1984; Chiang *et al.*, 1985;) and confirms that the decrease seen in the I-LH is of biological significance. This is reflected in the observed marked decrease in E_2 levels.

The increase in the E_2, RIA-LH, IRMA-LH, and B-LH seen at 2 months after the end of goserelin treatment appears to be due to one-quarter of the patients being at mid-cycle at the time of the post-treatment blood sample.

The increase seen in the B:RIA LH ratio and the B:IRMA LH ratio at 1 and 3 months of danazol treatment may reflect an effect of low E_2 levels on the B:I-LH ratio similar to that seen in postmenopausal women and patients with Turner's syndrome (Dufau *et al.*, 1976), an androgenic effect of the drug as seen in studies in animals (Solano *et al.*, 1980) and in men (Beitins *et al.*, 1981; Carani *et al.*, 1987), or an effect on the LH pulsatility resulting in differential secretion of LH isohormones (Dufau & Veldhuis, 1987). In contrast, the decrease in the B:RIA LH ratio seen at 1 month on goserelin treatment is in agreement with previous studies on other LHRH agonists (Meldrum *et al.*, 1984; Chiang *et al.*, 1985; Lemay *et al.*, 1987). However, discordance between B-LH and RIA-LH was much greater in these last studies. This may be attributed to the incomplete suppression of the I-LH in those studies, the production of immunoreactive LH fragments or forms with reduced bioactivity (Meldrum *et al.*, 1984; Evans *et al.*, 1984), or differences in the polyclonal antibodies used in the RIAs.

Bischof and Herrmann (1988) demonstrated that LHRH agonist therapy in women with endometriosis produces a much greater decrease in LH as measured by an IRMA based on two different monoclonal anti-LH antibodies compared to LH measured by the RIA based on a rabbit polyclonal anti-LH antiserum.

Similarly, our data show a marked elevation in the RIA:IRMA LH ratio during goserelin therapy. This and other work (UK EQAS Report, 1988; Haavisto *et al.*, 1989) suggest that the RIA is unreliable at low levels ($< 2{\cdot}0$ iU/l). For samples where LH levels are expected to be of a low order an IRMA rather than the RIA may be the assay of choice.

We conclude that the divergent effect of the two drugs on the B:RIA LH ratio reflects the fundamentally different mechanisms of action of these drugs on the pituitary. The data indicate that danazol causes a relatively hypo-oestrogenic state which cannot be attributed to a decrease in the immunoreactive gonadotrophin levels or to a decrease in the biological activity of LH and is associated with an increase in B:I LH ratio. In contrast, goserelin acts directly on the pituitary to produce suppression of gonadotrophin levels and effective down-regulation of the pituitary.

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