The effects of buserelin microparticles on ovarian function in healthy women

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Objective. To investigate the tolerance, pharmacokinetics and pharmacodynamics of the microparticle formulation of buserelin, when it was administered subcutaneously.

Design. A single-blind, randomised, parallel-group design was used to investigate the duration of suppression of ovarian function associated with doses of 1,8, 3,6 and 7,2 mg buserelin administered subcutaneously as microparticles.

Setting. The study was carried out at the Hoechst Research Centre for Clinical Pharmacology, Department of Pharmacology, University of the Orange Free State, Bloemfontein.

Patients. Thirty-two healthy premenopausal female volunteers aged between 19 and 39 years and weighing between 52 and 85 kg completed the study.

Outcome measures. Serum progesterone and oestradiol concentrations were measured twice weekly until normal ovarian function resumed, i.e. when serum progesterone concentrations increased to at least 8 nmol/l (a sign of ovulation) and oestradiol concentrations increased to values above 300 pmol/l. Serum and urinary concentrations of buserelin were measured at the same times as those of progesterone and oestradiol.

Results. Doses of 1,8, 3,6 and 7,2 mg elicited anovulation for mean periods of 52, 77 and 113 days and suppressed ovarian production of oestrogen for 19, 38 and 69 days. Resumption of normal ovarian function occurred when serum buserelin concentrations decreased to between 0,03 and 0,05 µg/ml. The correlation coefficient between dose and duration of anovulation was 0,75; the correlation coefficient between dose and duration of suppression of oestrogen production was 0,76.

Conclusion. Apart from minor side-effects such as hot flushes, vaginal spotting and acne, the compound was tolerated well. We conclude that a good relationship exists between dose and duration of suppression of ovarian function. Doses of 3,6 - 7,2 mg buserelin should suppress oestrogen production for approximately 6 - 9 weeks and ovulation for 11 - 16 weeks.

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The luteinising hormone-releasing hormone (LHRH) agonists are antigonadotrophic agents used to elicit reversible gonadal suppression in gynaecology and oncology.[1] Buserelin, which is one of the LHRH agonists, has been tested in prostatic cancer patients and in patients with mammary carcinoma.[2] It can be administered as injections, nasal sprays and implants and has recently been formulated as microparticles to provide a subcutaneous depot with a view to improved compliance. The peptide is encapsulated in microparticles of polylactide co-polymer that can be suspended in water prior to subcutaneous injection. Buserelin microparticles are effective in rhesus monkeys, and completely suppress follicular maturation and oestrogen production for 4 - 6 weeks after a single dose of 3,6 mg.[1]

A long-lasting reversible hypogonadism was elicited in women by intramuscular injection of the hypothalamic releasing hormone, gonadorelin, in a microcapsule formulation.[3]

In another study gonadorelin microcapsules suppressed the pituitary testicular axis for at least 50 days.[4]

Subjects and methods

The primary objective of the study was to identify the dose of the microparticle formulation of buserelin that would provide release of the drug sufficient to suppress ovarian function for 4 - 6 weeks in humans. The secondary objective was to investigate the tolerability of buserelin microparticles in humans.

The study was conducted in accordance with the requirements of good clinical practice, with the permission of the Ethics Committee of the Faculty of Medicine of the University of the Orange Free State; volunteers gave informed consent.

During a control menstrual cycle preceding the administration of buserelin microparticles normal ovarian function had to be proved. Blood samples, for the measurement of serum progesterone concentrations, were taken on days 19, 23 and 26 of the cycle, and a progesterone concentration of 8 nmol/l or more on any of these days was considered proof of ovulation. Volunteers in whom ovulation was not confirmed were excluded from the study.

Thirty-two healthy premenopausal women (age range 19 - 39 years; weight range 52 - 85 kg) completed this single-blind, randomised study in 3 parallel groups.

Buserelin microparticles were administered as a single dose, injected subcutaneously through a 22G needle into the lateral abdominal wall. Doses were: group 1 (12 subjects) — 1,8 mg; group 2 (11 subjects) — 3,6 mg; group 3 (9 subjects) — 7,2 mg. Medication was given on the second or third day of the menstrual period following the control cycle.

Blood samples for measurement of serum oestradiol, progesterone and buserelin concentrations were taken twice weekly (Mondays and Thursdays) after injection; timing was arranged so that blood samples were taken as consistently as possible at the same time of day. Samples for determination of urinary buserelin concentrations were taken early in the morning on the same days as the blood samples.

Twice weekly blood and urine sampling continued until return of normal ovarian function was confirmed; a serum progesterone concentration of at least 8 nmol/l was used as a criterion of ovulation and a serum oestradiol concentration above 300 pmol/l as a sign of normal ovarian function.

Serum and urinary buserelin concentrations were measured by means of a radio-immunoassay technique, and urinary creatinine concentrations by means of an enzymatic method.

Serum progesterone and oestradiol concentrations were measured by means of the Coat-a-Count (Diagnostic Products Corporation) (detection limits: progesterone 0,3 nmol/l, 94 pg/ml; oestradiol 70 pmol/l, 20 pg/ml).

Results

Apart from minor side-effects such as hot flushes, vaginal spotting, acne and headaches the microparticles were tolerated well.

The mean duration of suppression of serum progesterone concentrations to values below 8 nmol/l and suppression of serum oestradiol concentrations to values below 300 pmol/l are shown in Tables I and II. The mean serum buserelin concentrations and urinary buserelin excretion (µg/g creatinine) are shown in Fig. 1; the corresponding mean serum progesterone and oestradiol concentrations for the three doses are shown in Fig. 2.

Table I. Mean number of days after medication during which serum progesterone concentration was below 8 nmol/l (i.e. duration of inhibition of ovulation)

Dose (mg)	Days	CV%	Range
1,8	52	51,0	16,0 - 88,0
3,6	77	29,2	21,0 - 99,0*
7,2	113	13,9	91,0 - 144

* Excluding one value of 21 days, the range was 62 - 99 days.

Table II. Mean number of days after medication during which serum oestradiol concentration was below 300 pmol/l (i.e. duration of suppression of ovarian function)

Dose (mg)	Days	CV%	Range
1,8	19,3	87,8	0,00 - 49,0
3,6	38,3	47,0	0,00 - 56,0*
7,2	69,2	27,9	39,0 - 101

* Excluding one value of 0 days, the range was 21 - 56 days.

Regression analysis of the dose of buserelin microparticles and the duration of suppression of ovulation (measured by the number of days that serum progesterone levels were below 8 nmol/l) yielded a correlation coefficient of 0,75. The correlation coefficient between dose and duration of suppression of serum oestradiol concentrations to values below 300 pmol/l was 0,76.

The end of the effect of buserelin on ovulation was defined as the point in time when two consecutive serum progesterone concentrations were above 8 nmol/l. The mean serum buserelin concentrations associated with resumption of ovulation and with oestradiol concentrations above 300 pmol/l are given in Table III.

Table III. Mean serum buserelin concentrations (ng/ml) associated with resumption of ovulation (column A) and with oestradiol concentrations above 300 pmol/l (column B).

Dose (mg)	A	B
1,8	0,04	0,03
3,6	0,03	0,03
7,2	0,04	0,05

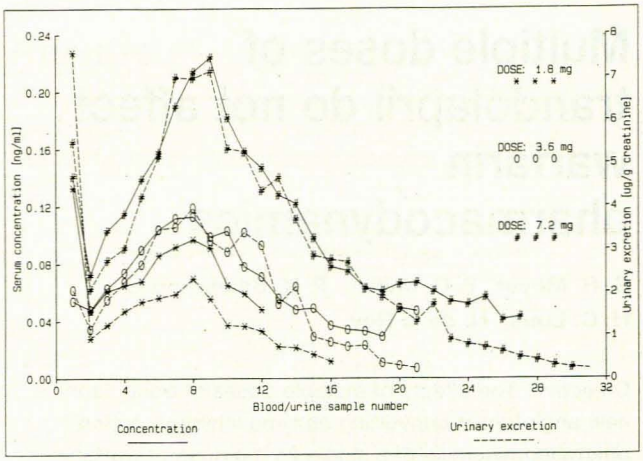

Fig. 1. Mean serum buserelin concentrations (——) and urinary buserelin excretion per gram urinary creatinine (– – –) for doses of 1,8 (*), 3,6 (0) and 7,2 (#) mg.

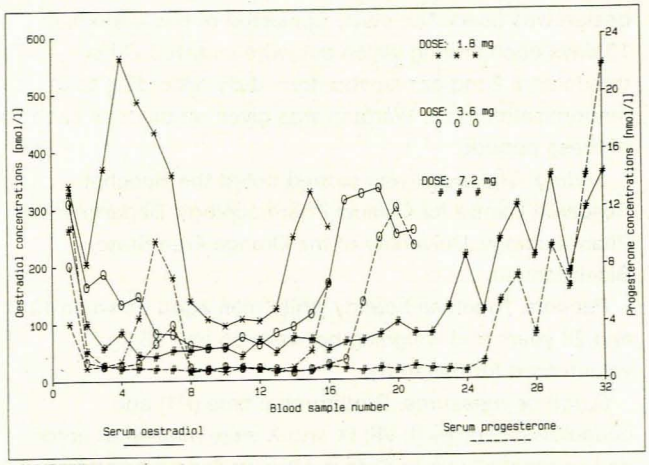

Fig. 2. Mean serum oestradiol (——) and progesterone (– – –) concentrations for doses of 1,8 (*), 3,6 (0) and 7,2 (#) mg buserelin.

Discussion

Both the duration of suppression of ovulation and reduction of oestradiol serum concentration correlated well with the dose administered. Inhibition of ovarian function ceased when buserelin serum concentrations fell below 0,04 µg/ml and urinary excretion of the compound fell below approximately 0,4 µg/g creatinine.

We conclude that the microparticle formulation of buserelin was tolerated well, and that a good relationship exists between dose administered and duration of suppression of ovarian function. A dose of between 3,6 mg and 7,2 mg of the microparticles should be ideal for suppression of ovarian function and should suppress oestrogen production for a period of between 6 and 9 weeks, and ovulation for between 11 and 16 weeks.

REFERENCES

1. Sandow J, Stoeckemann K, Jerabek-Sandow G. Pharmacokinetics and endocrine effects of slow release formulations of LHRH analogues. *J Steroid Biochem* 1990; **37:** 925-931.
2. Kramer M. Long-term treatment with new therapeutic systems concerning releasing hormones in steroid-dependent diseases. *Dtsch Aerztebl* 1988; **85:** C647-C648.
3. Zorn JR, Tanger C, Roger M, *et al.* Therapeutic hypogonadism induced by a delayed-release preparation of microcapsules of D-Trp-6-luteinizing hormone-releasing hormone: a preliminary study in eight women with endometriosis. *Int J Fertil* 1986; **31(1):** 16-27.
4. Gonzalez-Barcena D, Perez-Sanches PL, Graef A, *et al.* Inhibition of the pituitary-gonadal axis by a single intramuscular administration of D-Trp-6-LH-RH (decapeptyl) in a sustained-release formulation in patients with prostatic carcinoma. *Prostate* 1989; **14**(4): 291-300.

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