LRH agonist buserelin  
as a post-partum contraceptive: lack of biological activity of buserelin in breast milk

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Abstract. To evaluate the possibility of using the LRH agonist buserelin as a contraceptive for lactating women we have investigated the passage of buserelin into breast milk and explored possible biological activity in the infant. Eleven mothers received 600 µg buserelin by nasal spray. Buserelin was measured by radioimmunoassay in the breast milk of these mothers, and values ranged from undetectable levels (less than 15 pg/ml) to 8800 pg/ml. The maximum amount of buserelin that an infant could ingest during an average feed would be 1–2 µg. In adult men ingestion of 600 µg buserelin dissolved in cows milk was without biological effect upon both serum and urinary levels of luteinizing hormone. There was no change in the levels of LH found in the urine of infants fed by women who had received 600 µg buserelin by nasal spray. We conclude that the small amount of buserelin passing into the breast milk of these volunteers was without biological activity when ingested by the infant.

The choice of contraceptive methods presently available to lactating mothers is limited. There is a need for a reliable, acceptable and easily administered method of contraception which does not interfere with lactation and is non-steroidal. To this end we are currently evaluating the effectiveness of luteinizing hormone releasing hormone agonist (LRH agonist), buserelin, as a contraceptive for lactating women. This report outlines the investigations that have been carried out prior to regular administration of buserelin to breast feeding mothers.

Breast feeding is nutritionally good for the infant (Buchanan 1975). The contraceptive effects of breast feeding have been investigated (Howie & McNeilly 1982; Short 1984), and it has been shown that while full breast feeding is associated with the suppression of ovarian activity in women post-partum when supplementary feeds are given (Howie et al. 1981) or when suckling activity declines to less than 50 min/day nursing (McNeilly et al. 1983), follicular development and ovulation resume. Steroidal forms of contraception are the most practical method currently available but are not ideal. Combined oral contraceptives may have an adverse effect upon breast milk production (Koetswang 1982). The progesterone-only pill is commonly prescribed to lactating women in developed countries, but troublesome side effects include irregular vaginal bleeding, nausea, vomiting and headache.

Numerous studies have demonstrated that daily administration of LRH agonist to women with normal menstrual cycles is a reliable and reversible method of suppressing ovulation (e.g. Bergquist et al. 1979; Schmidt-Gollwitzer et al. 1981). Our hypothesis is that buserelin administered to breast feeding women will reliably suppress ovulation
even when supplementary feeds are introduced and suckling activity declines.

The aim of this study was to determine the amount of buserelin that passes into the breast milk after the administration of 600 µg by nasal spray (the maximum dose proposed for contraceptive use) and to determine whether any buserelin passing into the breast milk could have a biological effect upon the breast feeding infant. To investigate the biological activity of orally ingested buserelin, adult males took buserelin dissolved in cows milk.

To avoid any risk to breast feeding infants, we carried out the initial work on quantifying the passage of buserelin into breast milk by recruiting mothers who were expressing and discarding their breast milk. Subsequently mothers who were breast feeding their infants received the buserelin nasal spray.

Patients and Methods

All aspects of this study were evaluated and approved by the local ethical committee. Informed consent was obtained from all volunteers.

Preliminary study

For the preliminary study, six healthy male volunteers, aged 25 to 40 years of age, were recruited. Four of these men received 600 µg buserelin by nasal spray. Each collected a 10 ml sample of urine prior to and at 2, 4, 6, 8 and 24 h after receiving the nasal spray. These samples were frozen at −20°C. A week later five of these men received 600 µg buserelin dissolved in 150 ml cows milk (to minimise possible enzymatic degradation of the buserelin prior to ingestion, the milk was drunk immediately after the buserelin was added). Venepuncture was performed on these volunteers prior to and at hourly intervals for 7 h from the time of buserelin ingestion. Each volunteer provided a 10 ml urine sample prior to and at 2, 4, 6, 8 and 24 h after receiving the buserelin. The serum and urine samples were stored at −20°C. The following week five of these men ingested 150 ml cows milk without buserelin added. They underwent the same blood and urine sampling regimen, thus acting as controls for the preceding two experiments.

Buserelin and its metabolites were measured in urine by radioimmunoassay. Luteinizing hormone (LH) in urine and serum was measured by radioimmunoassay. Urinary creatinine levels were estimated by the routine hormone laboratory within the Centre for Reproductive Biology.

Main study

The radioimmunoassay utilised for the measurement of buserelin and its metabolites in urine was adapted for use in measuring buserelin concentrations in samples of breast milk. The high fat concentration in these samples interfered with the radioimmunoassay; the breast milk was therefore defatted by ultracentrifugation prior to assay as described by Amarant et al. (1982). Buserelin is degraded in vitro by certain biological tissues e.g. liver homogenates (Sandow et al. 1981). The enzymatic degradation of LH by hypothalamic tissue extracts (Koch et al. 1974) is similar to that of LRH agonists. Bacitracin has been used to inhibit the degradation of LRH (McKelvey et al. 1976). To determine whether buserelin was degraded in breast milk, buserelin was added to freshly collected breast milk (250 µg/ml) and divided into 22 x 2 ml aliquots. One sample was immediately frozen to −20°C. Seven of these samples were incubated at 4°C, seven at 22°C and seven at 37°C. One sample from each group was frozen to −20°C at 1, 2, 4, 6, 8, 12 and 24 h after commencing the incubation. These samples were subsequently assayed for buserelin and its metabolites. This experiment was repeated with bacitracin added at a concentration of 10⁻³ M.

In vitro incubation of breast milk samples containing buserelin, as described above, demonstrated significant degradation, but the addition of bacitracin at a concentration of 10⁻³ mol largely overcame this degradation (Fig. 2).

Ten lactating mothers who were expressing and discarding their breast milk at 5–10 days post-partum (their infants having been temporarily admitted to the Special Care Baby Unit), were recruited from the post-natal wards of the Simpson Memorial Maternity Pavilion, Edinburgh. Six mothers received 600 µg buserelin by nasal spray, and 4 took a placebo spray. Samples of breast milk and urine were collected prior to and at 2, 4, 8 and 24 h after receiving the nasal spray. Bacitracin was added to the breast milk samples to give a final concentration of 10⁻³ M to inhibit degradation. All the samples collected were stored at −20°C until assayed. The breast milk and urine samples were assayed for buserelin and its metabolites, and the urine samples were also assayed for creatinine.

After establishing that only a small amount of buserelin and its metabolites passed into the breast milk of the volunteers, and that oral ingestion of large quantities of buserelin by the adult males was without biological effect, we proceeded to recruit mothers at 6 weeks post partum, who were breast feeding their infants, to receive the buserelin nasal spray. Fourteen breast feeding mothers were recruited from the post-natal wards. At six weeks post partum, 11 of these mothers received a single, 600 µg dose of buserelin by nasal spray.

Three mothers received a placebo spray. The fourteen mothers breast fed their infants 2 to 3 h after receiving the nasal spray.
Radioimmunoassays

Buserelin levels in urine and breast milk were determined by double antibody radio-immunoassays using a rabbit antibody (R104) raised against buserelin conjugated to human serum albumin (Fraser et al. 1983) in Edinburgh, and in Frankfurt using antibody 636 (Sandow et al. 1981, 1984, 1985). The antibodies are C-terminal specific, detecting intact buserelin and its metabolites. Breast milk samples were defatted by ultracentrifugation prior to being assayed. Parallelism was demonstrated for both urine and breast milk measurement of buserelin between 20 and 80% B/Bo. The intra-assay variation was 4%, the inter-assay variation was 18%. The lower limit of detection of buserelin in breast milk was 15 pg/ml and in urine 30 pg/ml.

Serum LH was measured by specific double antibody radioimmunoassay method of Hunter & Bennie (1975). Adults urinary LH was measured by the method of McNeilly & Hagen (1974). The creatinine and LH:creatinine ratio being determined as described by Metcalf & Livesey (1979). Intra-assay variation was 5%, the inter-assay variation 16%. Creatinine was measured by autoanalyser.

LH concentration in infants’ urine was determined using a solid phase immunoradiometric assay (LH MAIACLONE, Serono Diagnostics, Woking, Surrey) employing 1st IRP LH 68/40 as the assay standard. This kit makes use of two highly specific monoclonal antibodies for human LH (Soos & Siddle 1983) with magnetic particle separation technology (Forrest & Rattle 1983). Validation of the method for the present application included demonstration of parallelism by assaying serial dilutions of pooled infants urine. All specimens were assayed in duplicate in the same assay run; intra-assay variation was 6% (coefficient of variation) with a sensitivity (minimum detectable dose, \( P = 0.01 \)) of 0.41 IU LH/l. Of 48 infant urine samples assayed, 45 contained a measurable level of LH (mean 1.57 IU/l; range 0.79–6.22 IU/l).

Statistical evaluation was performed using paired or unpaired Student’s t-test and analysis of variance.

Results

Preliminary study

The levels of LH measured in the urine of the men are shown in Fig. 1a. A significant (\( P < 0.005 \)) by analysis of variance) rise in urinary LH levels occurred 4–8 h after the administration of 600 \( \mu g \) buserelin by nasal spray, but no such rise was present after the oral ingestion of 600 \( \mu g \) buserelin. These findings were further substantiated by the finding that serum levels of LH were not affected by the oral ingestion of 600 \( \mu g \) buserelin (Fig. 1b).

Furthermore, levels of buserelin were significantly (\( P < 0.01 \) t-test) lower in urine after oral administration compared to its application with the nasal spray (Fig. 1c).

<table>
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<tr>
<th>Volunteer No.</th>
<th>Buserelin pg/ml breast milk</th>
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Table 1. Buserelin concentration in breast milk of mothers at 5–10 days post partum (volunteers 1–6) and 6 weeks post partum (volunteers 7–17) who received 600 \( \mu g \) buserelin by nasal spray at time 0 (volunteers 18–20 are controls).
Main study

The levels of buserelin present in breast milk, collected from mothers between 5–10 days post partum, are shown in Table 1. The maximum amount of buserelin present after the administration of 600 µg buserelin by nasal spray was 214 pg/ml. LH could not be measured in their urine because of the persistence of human chorionic gonadotropin which interferes with the radioimmunoassay used in our laboratory.

The maximum amount of buserelin present in breast milk samples collected from mothers at 6 weeks post partum, who received 600 µg buserelin by nasal spray and who were actively feeding their infants, was 8800 pg/ml (Table 1). This is higher than that found in the breast milk of mothers who...
took a comparable dose of buserelin at 5–10 days post partum. However, immunoreactive buserelin present in the urine of the mothers at 6 weeks post partum did not differ significantly when compared to that measured in mothers at 5–10 days post partum, suggesting that absorption and urinary excretion was similar between the two groups. Urinary buserelin levels at 2 and 24 h after taking the 600 µg buserelin nasal spray were 26,300 ± 10,900 pg/mg and 145 ± 42 pg/mg, respectively, at 5–10 days post partum and 28,200 ± 7,800 pg/mg and 136 ± 31 pg/mg, respectively, at 6 weeks post partum, all values measured in pg buserelin/mg creatinine and expressed as mean ± SEM. The urine samples collected from mothers at 6 weeks post partum were assayed for LH. A 3–12-fold increase in urinary LH (measured in IU/g creatinine) was observed at 4–8 h after taking 600 µg buserelin by nasal spray as compared to controls, this increase was highly significant $P < 0.01$ by unpaired $t$-test. The results obtained in the buserelin radioimmunoassay in Edinburgh were confirmed by radioimmunoassay in Frankfurt (results not shown).

The urine samples collected from the infants who breast fed from mothers who had received buserelin by nasal spray showed no evidence of a rise in levels of LH (Fig. 3). In one infant’s urine a small amount of buserelin immunoactivity was detectable (62 pg/ml urine) in the sample collected at 6 h after breast feeding from the mother who had 8,800 pg/ml in her breast milk, but there was no evidence of a rise in urinary LH. There was no detectable buserelin in any of the other samples collected from these infants.

Discussion

We have demonstrated that only a small amount of buserelin passes into the breast milk of lactating mothers after administration of 600 µg buserelin.

![Degradation of buserelin in breast milk at 4°C (Δ—Δ), 22°C (○—○) and 37°C (■—■) in the absence (top panel) or presence of bacitracin at $10^{-3}$ mol (bottom panel).](image)
Infants urinary LH levels before and after breast feeding from a mother who had received 600 µg buserelin by nasal spray (n = 11) at the time marked by the arrow. Values expressed as means ± s.e.m.

Infants urinary LH levels before and after breast feeding from a mother who had received 600 µg buserelin by nasal spray (n = 11) at the time marked by the arrow. Values expressed as means ± s.e.m.

by nasal spray. By monitoring levels of LH in the urine of infants before and after suckling from mothers who received buserelin nasal spray, it seems that the small amount of buserelin that passes into breast milk does not have any biological activity when ingested by the infant. This observation is supported by the findings of the study in adult men that oral administration of 600 µg buserelin dissolved in cows milk is without biological effect. Assuming that the average volume of breast milk ingested at a single feed is 150 ml breast milk and that the maximum concentration of 8.8 ng/ml of buserelin is present throughout the whole feed, then the maximum amount of buserelin that could be ingested by an infant would be 1–2 µg, which is less than a hundredth of the dose ingested by the adult males without signs of biological activity. It has been tested previously and shown that 5 mg buserelin administered orally in gelatine capsules has no effect upon LH or FSH release (J. Sandow, unpublished observations).

A possible reason for the difference in the amount of buserelin detected in the breast milk at 5–10 days compared to 6 weeks post partum, is that the women at 5–10 days post partum were expressing a relatively small amount of breast milk each day, approx 150–200 ml, whereas the mothers who were breast feeding their infants at 6 weeks were producing up to one litre of milk a day. We hypothesise that if milk is being rapidly secreted into the breast, higher concentrations of circulating buserelin may be carried into that milk.

It is difficult to explain the wide individual variation in buserelin content in the breast milk. All possible precautions were taken to avoid degradation (addition of bacitracin and prompt freezing of collected samples). One possible explanation of the wide variation is that the amount of buserelin absorbed from the nasal spray may have been greater in those women with the higher concentrations of buserelin in their breast milk i.e. a dose-dependent phenomenon. This is supported by the observation that there tended to be higher urinary levels of buserelin in the women with the highest concentration in their breast milk, but this tendency was not statistically significant. Individual differences in metabolism and urinary clearance of buserelin may be an important factor.

Whilst these results do not exclude the possibility of accumulation of buserelin within the breast after administration of repeated doses, recent observations of women taking 300 µg buserelin nasal spray daily from 6 weeks post partum, collecting regular breast milk samples over a 4–6 week period, show no evidence of accumulation (P. J. Dewart, unpublished observations).

The pituitary of an infant is maximally responsive to LRH at 4–12 weeks post partum (Tapannainen et al. 1982). A 3–7-fold increase in serum levels of LH were found after an iv injection of 50 µg LRH/m² body surface area, given to a group of infants in this age range, but they were unable to demonstrate a significant rise in serum levels of testosterone. These findings suggest that the levels of buserelin which pass into the breast milk of women in this study, are not sufficient to exert a biological effect on the infant when he or she feeds on that milk, a view substantiated by the failure to demonstrate a rise in the levels of LH in the infants.

Endogenous LRH and other small peptides are present in breast milk in low concentrations (Amarant et al. 1982; Sarda et al. 1981), and it has been suggested that they may have biological effects on the infant in the early days of life when
the gastrointestinal enzyme system is not fully matured, and when the infant gut may be permeable to small peptides. Since we do not intend to begin medication until the sixth post partum week this problem should not arise.

One infant had a small amount of immunoreactive buserelin in his urine. This does not reflect the presence of active agonist, because the radioimmunoassay used in this study detects metabolites of buserelin provided the C-terminal remains intact. At low concentrations of immunoreactive buserelin, only inactive metabolites are excreted in the urine. It is well established that only intact buserelin has biological activity (Sandow et al. 1978).

We have demonstrated in vitro degradation of buserelin in breast milk to undetectable metabolites. It follows that degradation of buserelin also occurs in stored milk within the breast, but this would be difficult to demonstrate because it is uncertain as to whether the buserelin entering the breast milk is intact or already partially degraded. Buserelin is primarily degraded in the liver and kidneys, the degradation products being excreted in the urine (Sandow et al. 1984, 1985a,b). The addition of bacitracin to the breast milk samples ensures that further degradation cannot occur in vitro before the samples are assayed.

The rise of the levels of LH seen in the urine of mothers who received 600 µg buserelin by nasal spray at 6 weeks post partum is consistent with other reports of a return of pituitary responsiveness to LRH by the 6th week after delivery (Keye & Jaffe 1976).

In conclusion, we have demonstrated that when buserelin is administered in the periperrum at a dose of 600 µg by nasal spray, it can be detected in breast milk, but that the levels present in the milk have no biological effect on the infant and are significantly lower than the levels known to be needed to exert an effect in adults. We conclude from this study that longer term studies of the use of buserelin as a possible post partum contraceptive are warranted.

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