LONG TERM SUPPRESSION OF OESTRUS AND PREVENTION OF PREGNANCY BY DESLORELIN IMPLANT IN RATS

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Abstract

The experiments were designed to test the possibility to induce a down-regulation of pituitary GnRH-receptors and hence a suppression of oestrus by application of a long term deslorelin implant in female rats. Sixteen non pregnant female rats were randomly divided into two equal groups. The eight rats of the implant group (DESL) were subcutaneously implanted with long acting deslorelin implant, an analog of GnRH, at the dose of 4.7 mg, to inhibit oestrus. No treatment was applied to control group (CON). One adult male rat was added per cage of DESL and CON females after six weeks. Conceptions were diagnosed by inspection, palpation, and ultrasonography for one year. Conception rates were 0% and 100% in DESL group and CON group, respectively (P<0.001). An influence on the concentrations of progesterone and oestradiol - 17β was determined. Average hormone concentrations were statistically different between groups (P<0.01). In conclusion, the subcutaneous implantation of 4.7 mg of deslorelin is a practicable method for suppressing oestrus in rats. Further investigative studies will be required to determine upper time limit of down-regulation duration by deslorelin.

Key words: rat, contraception, deslorelin, oestrus, suppression.

Historically, fertility control has been achieved through surgical sterilisation. However, in some species the permanent nature of these procedures is disadvantageous. Other disadvantages include the associated trauma, production setbacks, and potential death. Gonadotropin releasing hormone (GnRH) has long been recognised as a potential target for the control and management of fertility in human and animal medicine. Similar to the actions of native GnRH, synthetic GnRH agonists like nafarelin, leuprolide, deslorelin, buserelin, and goserelin, stimulate production and release of gonadotropins from the pituitary gland. However, GnRH agonists, when used at sustained doses, reversibly inhibited the pituitary-gonadal axis after the initial period of stimulation (7, 9). Attempts to apply GnRH-based technology to manage fertility have focused on the development of GnRH agonists, antagonists and vaccines. Long term GnRH agonists have been used for ovarian protection, fertility preservation in non-malignant diseases, and prevention of menometrorrhagia during treatment in human reproduction (3).

Deslorelin, an agonist of GnRH, is administered by way of a subcutaneous controlled release implant, with its activity lasting for 6 or even longer than 12 months, depending on the formulation used. The potency is 40 to 144 times that of endogenous GnRH. Deslorelin implants have been previously used for manipulation of oestrus cycles, induction of ovulation, (1) and accessory corpora lutea in cattle (16), reducing early pregnancy loss in cattle (2), suppression of sexual activity in ewes (11) and boars (13), and enhancing uterine involution in postpartum dairy cows (19). The mechanism of action, leading to suppression of the pituitary-gonadal axis, is well known, and has been successfully employed in the prevention of cyclic activity of many species (21). Long-term fertility control agents based on GnRH are of interest for the management of reproduction in humans, domestic animals, and wildlife. However, the effects of deslorelin have not been investigated so far, regarding the suppression of oestrus and fertility in rats.

The aims of this study were to determine the oestrus suppression and contraceptive effects of the deslorelin over a period of one year after a single administration to female rats.
Material and Methods

Animals. The experiments were approved by the Ethics Committee for Animal Experiments of Yuzuncu Yil University. Sixteen non pregnant Wistar Albino rats, at various sexual cycles, 12-week-old, with an average weight of 200-250 g, were used. The rats were maintained in groups of four per cage, at a temperature-controlled animal care room (24°C) with a light/dark cycle (12/12 h). The animals had free access to tap water and commercial chow during the experiment.

Experimental design. The animals were randomly divided into two equal groups: implant group (DES) and non-treated control group (CON). To inhibit oestrus, rats of the DESL group were implanted subcutaneously with Deslorelin (Suprelorin®, Virbac, Germany), an analog of GnRH, at the dose of 4.7 mg. The implants were placed in the outer surface of the paraumbilical region using an implantator device after intensive cleaning of the area with alcohol gauze and avoiding blood vessels. One adult male rat was added to each cage of DESL and CON females after six weeks. Conceptions were diagnosed by inspection, palpation, and ultrasonographic methods for one year.

Blood sampling and hormone determination. Blood samples were collected at the end of one year from all rats by cardiac puncture under general anesthesia. The blood was spun down at 3,000 rpm at room temperature for 10 min, and the obtained serum was stored at 20°C until analysis. Serum progesterone (P4) and oestradiol - 17β (E2β) levels were determined by an in-house radioimmunoassays (RIA). Briefly, the samples were incubated with 3H-tracer in bovine serum albumin. For separation of free and bound P4/E2β, charcoal suspension was added; then scintillation fluid and 0.6 ml of supernatant were mixed and measured in a fluid scintillation counter (LS 5000 TD, Beckmann, Germany). Detection limits were 0.318 nmol/mL (P4) and 7.342 pmol/mL (E2β). Intra assay variation coefficient of the test was between 6.0% and 11.4%, inter assay variation coefficient between 13.1% and 13.2%.

Statistical analysis. Significance of differences in hormone levels between groups was determined with the use of the Student’s t-test. Pregnancy rates between groups were analysed by the χ² test using the SPSS 13.0 software.

Results

It was demonstrated that conception rates were 0% and 100% in DESL group and CON group, respectively (P<0.001). Average and per animal E2β and P4 values for DESL and CON group were shown in Table 1 and Fig. 1. Average concentrations of the hormones were statistically different between both groups (P<0.01).

Discussion

The prevention of breeding in pet animals and contraception in humans using GnRH analogues have been the main focus of research over many years. Gonadotropin-releasing hormone agonists were investigated as contraceptive agents from the late 1970's to the mid-1980's in humans. They were abandoned as they appeared to offer no advantage over conventional oral contraceptives (20). This conclusion appears to be incorrect. The literature pointed out that agonist of GnRH effectively inhibited the pituitary-gonadal axis for extended intervals (21). Many studies reported that GnRH agonists have very high level of safety associated with treatment (15, 21). No unfavourable effects were observed in this study and deslorelin implants could be used safely in rats. Biochemical castration induced by GnRH agonist administration is a safe, effective, complete, and reversible method of removing the overlay of gonadal steroids from a variety of diseases, which are known to exacerbate in humans (4, 20).

In male dogs, complete infertility caused by deslorelin implant starts 6 weeks after implantation. We have previously reported that effect of deslorelin implant on the prevention of conception by down-regulation was not effective enough in female rats 4 weeks after its application (18). However, in this study, 6 weeks after application the implant was effective for GnRH receptor down-regulation and conception did not occur in DESL group for one year.

Administration of GnRH agonist formulations can achieve long-term reversible suppression of the pituitary–ovarian axis in a wide range of species and the effect is dose-dependent (12). Continuous administration or long term release formulations of GnRH agonists reversibly suppressed reproductive function in male and female dogs for periods exceeding 1 year in some studies (6). In male dogs, there was a positive relationship between the dose of agonist and the duration of suppression (21). Deslorelin has been found to be effective and safe in the domestic cat, and only the limited availability restricts more widespread use. It was demonstrated that the treatment of cats with implants containing 6 and 12 mg of deslorelin suppressed oestradiol secretion for at least 14 months in 80% of the animals (14).

Table 1

<table>
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<th>E2β (pmol/L)</th>
<th>P4 (nmol/L)</th>
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<tbody>
<tr>
<td>DESL</td>
<td>10.32 ± 5.72²</td>
<td>10.26 ± 7.05²</td>
</tr>
<tr>
<td>CON</td>
<td>43.57 ± 33.55²</td>
<td>78.16 ± 50.85²</td>
</tr>
</tbody>
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Different superscripts in the same column indicate statistical significant differences (P<0.01); E2β - oestradiol-17β, P4 – progesterone; ± - standard deviation
Large-scale trials of similarly formulated deslorelin in female cattle have demonstrated that implants containing higher doses (12 mg) can inhibit reproduction for >300 d in 100% of animals and >400 d in 90% of animals (5). In a similar study, Rubion and Driancourt (17) reported that azagly-nafarelin (a GnRH agonist) prevented ovulation in queens for 3 years. In the current study, deslorelin implant (4.7 mg) suppressed oestrus and prevented pregnancy over a period of one year after a single administration to female rats. Higher doses or additional deslorelin implants may provide longer suppression in rats and other species.

In this study we observed that levels of E2β and P4 in DESL group were significantly lower than in CON group at the end of the experiment. Low E2β and P4 levels resulted probably from suppression of follicular growth and ovulation. In previous studies (10), it was shown that long-term treatment with GnRH agonists can induce down-regulation of GnRH receptors on gonadotrops, desensitize the anterior pituitary gland to GnRH, and abolish the pulsatile release of FSH and LH in cattle. Such effects lead to suppression of follicular growth and an arrest of follicles at 2 or 3 mm in diameter after continuous treatment for 28 d (10). The ability of deslorelin to suppress follicular growth in cows and heifers has been demonstrated previously (1, 16). Amounts of GnRH receptor mRNA and concentrations of GnRH receptors in ewes treated continuously with GnRH decreased by 48% and 69%, respectively (22).

Therefore, the subcutaneous application of 4.7 mg deslorelin implant (GnRH-analogue) represents a practicable method for suppressing oestrus in rats. In respect to the various indications underlying the desired elimination of ovary function, down-regulation via treatment with GnRH is an elegant method to check for possible ovari-o hysterectomy related side effects, to treat sex hormone dependent diseases without permanently eliminating reproductive capacity or to eliminate ovary function over a longer period in case of risks of anaesthesia. Further studies will be required to determine upper time limit of down-regulation duration by deslorelin.

Fig.1. Serum E2β and P4 values for all rats in the study.

References