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GONADOTROPIN RELEASING HORMONE ANALOGS FOR FEMALE CONTRACEPTION BY INHIBITION OF OVULATION

SVEN JOHAN NILLIUS
Department of Obstetrics and Gynecology University Hospital, S-751 85 Uppsala, Sweden

Summary—One hundred and one healthy regularly ovulating women used two superactive GnRH agonists, Buserelin and Nafarelin, for contraception for 3–26 months. The superagonists were administered once daily by a nasal spray. The treatment was initiated on one of the first 3 days of the menstrual cycle. Seventy-one volunteers used 200, 400 or 600 μg of Buserelin for contraception for 3–26 months. Normal ovulation was disturbed in all but 3 of 628 treatment months. No pregnancy occurred. Fifty-three women had regular or sparse menstrual-like bleedings during treatment while 18 developed amenorrhea. Normal ovulation and fertility rapidly returned after cessation of therapy. Thirty women received 125 or 250 μg of Nafarelin intranasally once daily for 3 months. The treatment consistently inhibited ovulation without serious side effects. The inhibition of ovulation by prolonged continuous intranasal GnRH agonist therapy is a promising new lead to peptide contraception in women.

INTRODUCTION

The isolation and structural elucidation of the hypothalamic gonadotropin releasing hormone (GnRH) by Schally and his co-workers in 1971 [1] provided the basis for development of novel approaches to fertility control in man. The discovery was followed by intense synthetic activity of both agonistic and antagonistic analogs of GnRH for pro- and anti-fertility purposes. Paradoxically, superactive agonists of GnRH proved to be anti-fertility by nature. The new approaches to female contraception are at present almost exclusively based on GnRH superagonists. The principal mechanism of the anti-reproductive action is pituitary desensitization caused by the repetitive stimulation of the pituitary gonadotropes by the superagonists of GnRH [2]. Until quite recently, the GnRH antagonists have not been potent enough for practical clinical contraceptive trials [3, 4].

Inhibition of ovulation in women by chronic GnRH agonist therapy was the first new lead to peptide contraception [5]. This new method of birth control has already proved to provide safe and effective contraception in women [6, 7]. Attempts to use superactive GnRH agonists for induction of deficient corpus luteum function, luteolysis or early abortion have been less successful. Since the contraceptive effectiveness of these approaches has not been demonstrated in women, they will not be reviewed in this paper. Here the promising new approach with ovulation inhibition by superagonists of GnRH will be presented and discussed. These studies have been published in detail elsewhere [7, 8, 9].

EXPERIMENTAL

Volunteers

Healthy regularly menstruating women of reproductive age volunteered for the contraceptive studies, which were approved by the Local Ethical Committee of the Medical Faculty at the University of Uppsala. In pre-treatment control cycles, ovulation was confirmed by basal body temperature (BBT) recordings and normal luteal phase progesterone levels in serum.

GnRH agonists

1. The stimulatory GnRH analog [D-Ser(tBU)^6, Pro^9-NHEt] GnRH (Buserelin, Hoe 766, Hoechst AG, Frankfurt/Main, F.R.G.) is 140 times as potent as GnRH for induction of ovulation in rats [10]. 2. The GnRH agonist [D-Nal(2)^6] GnRH (Nafarelin acetate, Syntex Corp, Palo Alto, CA, U.S.A.) is 200 or more times as potent as GnRH in an assay involving rat estrous cycle [11].

GnRH agonist therapy

The superactive GnRH agonists were administered once daily by means of a nasal spray. The treatment was initiated on one of the first 3 days of menstrual cycle and given continuously thereafter. Monitoring of the therapy was made by clinical examinations, basal body temperature (BBT) and uterine bleeding recordings and at least once weekly determinations of progesterone and estradiol in serum.

Hormone assay methods

Progesterone and estradiol in serum were assayed by radioimmunological methods, according to those originally described [12, 13]. In our laboratories, the normal mid-luteal phase progesterone levels exceed 30 nmol/l. The estradiol levels vary during the very early follicular phase between 60–200 pmol/l and during the preovulatory phase of the menstrual cycle between 500–1300 pmol/l.

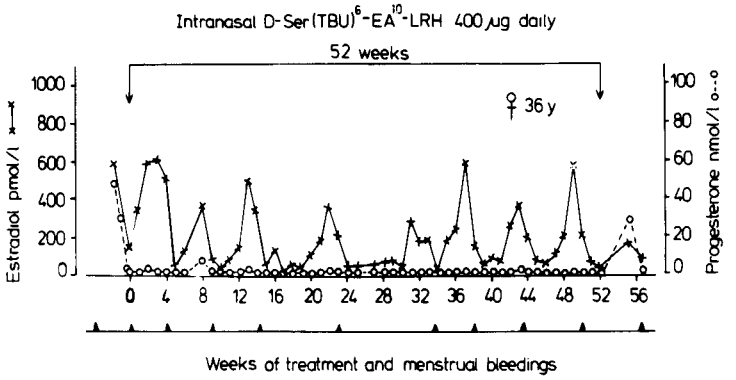

Fig. 1. Estradiol and progesterone concentrations in serum and bleeding patterns before, during and after 1 year of intranasal Buserelin therapy for contraception in a 36-year old woman who had 8 menstrual-like bleedings during treatment. From [14].

Statistical methods

The hormone values were logarithmically transformed before calculations of differences between mean values were performed using the standard Student's t-test for paired comparisons.

RESULTS

Buserelin

Seventy-one volunteers used one single daily dose of 200, 400 or 600 μg of Buserelin intranasally for contraception during 3–26 months. Only three initial treatment months were normal ovulatory. No pregnancy occurred during 628 months of treatment. The only observed side effects were bleeding irregularities caused by the interference with ovulation and corpus luteum function. Twenty-six women had fairly regular menstrual-like bleedings while 27 had oligomenorrhea and 18 developed amenorrhea during treatment. Spottings of short duration were observed by 5 volunteers only. No dysfunctional uterine bleeding occurred.

Representative hormone and bleeding patterns during about 1 year of continuous intranasal treatment with buserelin are shown in Figs 1 and 2. Normal ovulation was consistently inhibited during the prolonged therapy. One volunteer on daily 400 μg Buserelin did not have complete suppression of follicular growth, as evidenced by cyclical estradiol increases in blood and uterine bleedings (Fig. 1). The other volunteer on daily 600 μg Buserelin had more marked ovarian suppression with stable early follicular phase estradiol levels in serum and no uterine bleedings at all during treatment (Fig. 2). It is noteworthy that normal ovulation rapidly returned after the prolonged Buserelin treatment.

Nine of the 71 volunteers used the nasal Buserelin spray for contraception for 2 years or more. Hormone and bleeding patterns in two of these women are shown in Figs 3 and 4. A 29-year old volunteer had complete amenorrhea without symptoms of estrogen deficiency during 761 days of intranasal treatment with 400 μg of buserelin daily. She had prompt return of ovulation and menstruation after the long-term therapy (Fig. 3). The other woman treated for 2 years with 400 μg of Buserelin had 19 menstrual-like bleeding episodes during the treatment (Fig. 4).

The average serum concentration of estradiol dur-

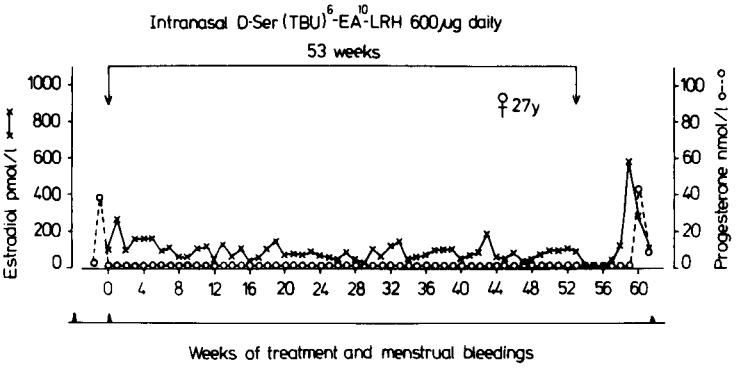

Fig. 2. Estradiol and progesterone concentrations in serum and bleeding patterns before, during and after more than 1 year of intranasal Buserelin therapy in a 27-year old volunteer with amenorrhea throughout treatment. From [14].

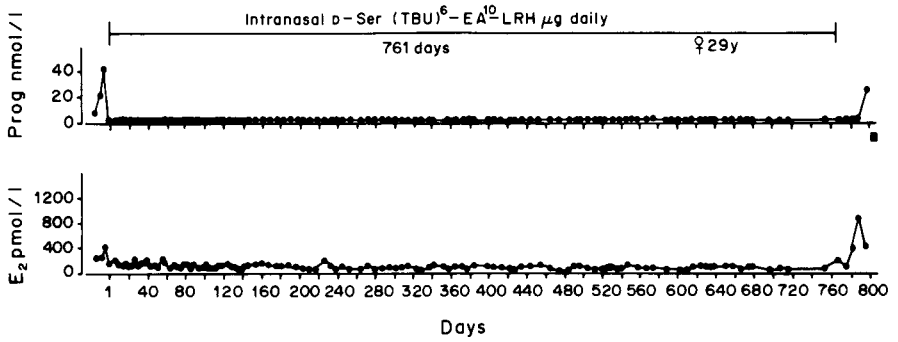

Fig. 3. Progesterone and estradiol concentrations in serum during more than 2 years of intranasal Buserelin therapy of a healthy volunteer with amenorrhea throughout treatment. The pre- and post-treatment control cycles were ovulatory. Menstrual bleeding. From [7].

ing the prolonged therapy was in the early to mid-follicular phase range of the menstrual cycle. The endometrium was in a mild proliferative stage, as shown by repeated biopsies. Progesterone was able to induce a withdrawal bleeding during a short period of menostasis after 1 year of treatment (Fig. 4). Ovulation rapidly returned after cessation of therapy.

During the course of prolonged Buserelin treatment the estradiol concentrations in serum progressively decreased. The average estradiol concentration was in the early to mid-follicular phase range of the menstrual cycle. The predominant histological picture of 57 endometrial biopsies was inactivity or weak proliferation with slightly atrophic stroma. No signs of hyperplastic changes were recorded. The progesterone concentration in serum was slightly raised (mean 8.6 nmol/l, range 5–20 nmol/l) during 53 treatment months, indicating luteinized follicles or defective corpus luteum function.

Normal ovulatory cycles were rapidly regained in all the 71 volunteers with the first menstrual bleeding occurring 41.3 days after cessation of therapy. The first ovulatory menstrual bleeding appeared earlier in the 26 women with menstrual-like anovulatory bleedings than in the 18 women with amenorrhea during treatment (mean 36.9 and 55.7 days, respectively, $P < 0.01$).

The intranasal treatment was well accepted by all the volunteers even during extended periods of treatment. No serious side effects were observed.

Nafarelin

Thirty volunteers used one single daily dose of 125 or 250 µg of Nafarelin acetate intranasally for inhibition of ovulation during 3 months. All the 89 treatment months were anovulatory. After 4 weeks of treatment no additional contraceptives were used. No pregnancy occurred during the 59 months of treatment during which intranasal Nafarelin only were used for contraception.

During the initial 3 months of treatment, the number of uterine bleeding episodes per month was 0.34, an average. One woman had dysfunctional uterine bleeding after 6 weeks of treatment. Six women had 13 short periods of spottings during the 3-month study period. Fourteen women had short

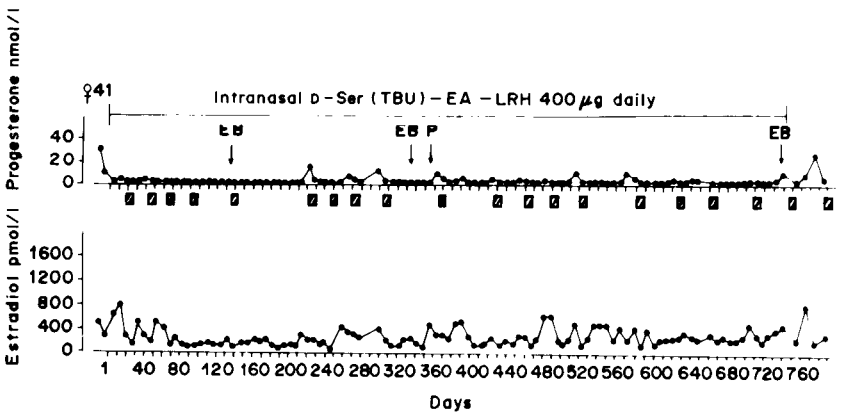

Fig. 4. Progesterone and estradiol concentrations in serum and bleeding pattern during 2 years of intranasal Buserelin therapy of a healthy volunteer with anovulatory menstrual-like bleedings during treatment. Uterine bleedings. EB = Endometrial biopsy. P = Progesterone challenge test (50 mg i.m.). From [7].

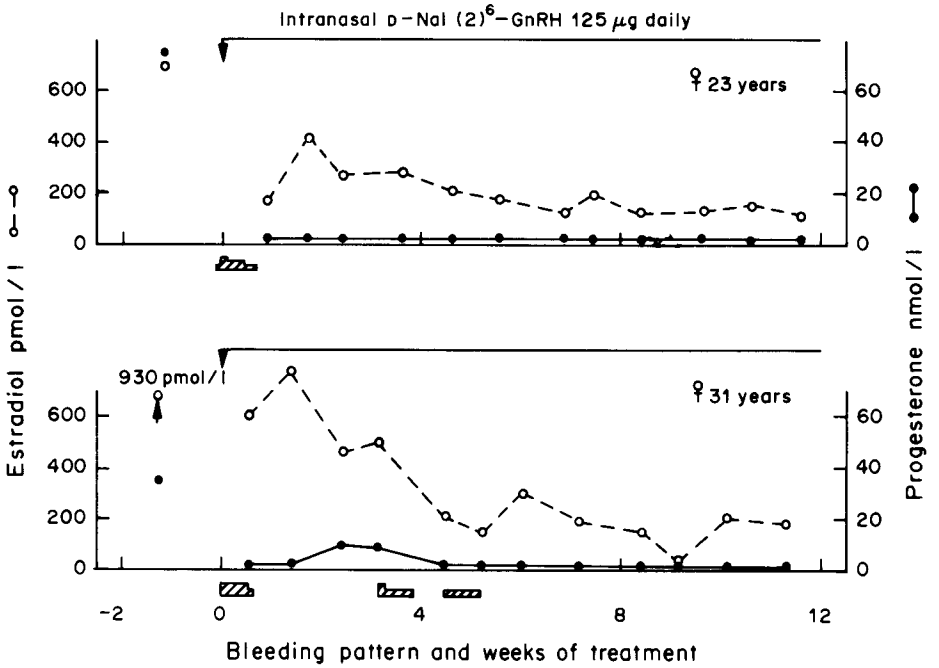

Fig. 5. Estradiol and progesterone concentrations in serum and bleeding patterns in a 23-year old volunteer with amenorrhea (top panel) and a 31-year old woman with uterine bleedings (lower panel) during 3 months of daily intranasal treatment with 125 μg of Nafarelin acetate. From [8].

and light menstrual-like bleeds while 9 women developed amenorrhea during the treatment.

Hormone and bleeding patterns during 3 months of intranasal nafarelin treatment of four of the volunteers are shown in Figs 5 and 6. The mean estradiol concentrations in serum during the first 3 months of therapy are shown in Fig. 7. The estrogen concentration gradually decreased during the treatment with the greatest reduction between the first and second month of treatment.

None of the women had any local symptoms of estrogen deficiency, e.g. vaginal discomfort or dyspareunia. Hot flushes, however, appeared in 6 of the 30 volunteers after 5–10 weeks of treatment. The

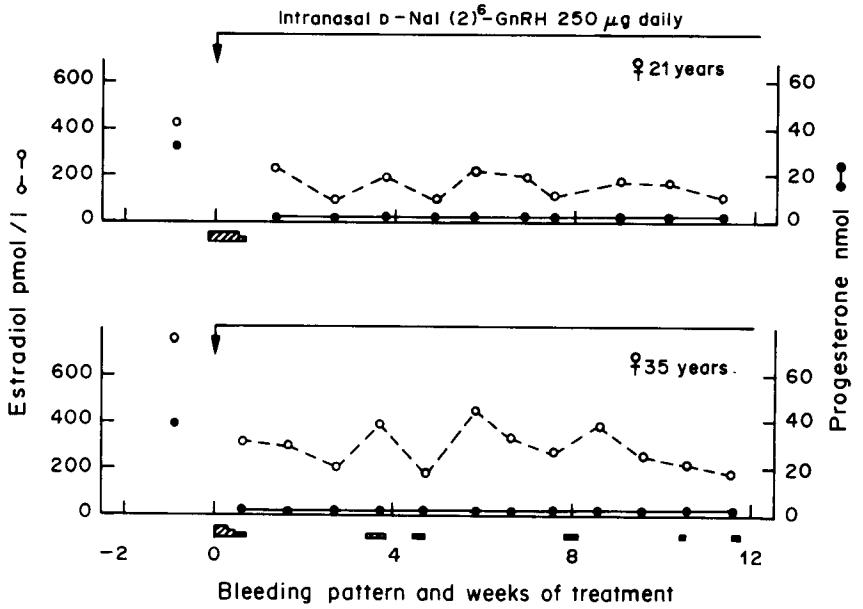

Fig. 6. Estradiol and progesterone concentrations in serum and bleeding patterns in a 21-year old woman with amenorrhea (top panel) and a 35-year old woman with uterine bleedings during 3 months of daily treatment with 250 μg of Nafarelin acetate. From [8].

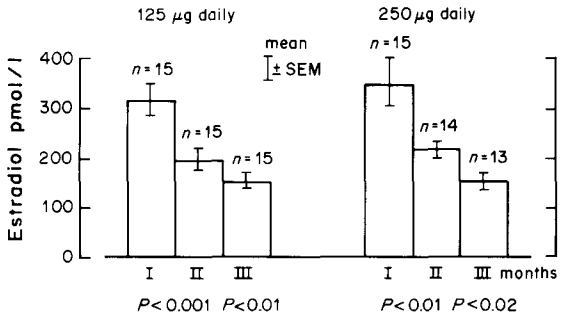

Fig. 7. Mean serum estradiol concentrations during the first (I), second (II) and third (III) month of daily intranasal Nafarelin therapy of 30 female volunteers treated with two different doses of the GnRH agonist Nafarelin acetate. From [8].

symptoms were normally mild and temporary. However, 1 woman treated intranasally with 250 μg Nafarelin had symptoms severe enough to reduce the daily intranasal dose to 125 μg resulting in improvement of the symptoms. None of the women discontinued therapy because of hot flushes.

No severe side effects appeared, except for the acceptable bleeding irregularities caused by induced anovulation. Only 1 woman discontinued therapy prematurely because of diverse psychosomatic symptoms unrelated to the Nafarelin treatment.

DISCUSSION

The present studies with two superactive GnRH agonists, Buserelin and Nafarelin, show that repetitive intranasal administration of microgram doses of these superagonists consistently inhibit ovulation and provide safe and effective control of fertility in women. This new method of birth control holds great promise as a future contraceptive in women. The induced anovulation is associated with acceptable bleeding irregularities but no other adverse effects. The suppressive effect on ovulation is rapidly reversible after cessation of long-term superagonist therapy and fertility returns.

The ovulation inhibition approach to control of fertility by superactive GnRH agonists seems to be the most promising new lead to contraception to date. Luteolytic approaches to peptide contraception are less encouraging. The contraceptive effectiveness of these approaches in women remains to be demonstrated [3].

The advantage of using peptides instead of steroids for inhibition of ovulation seems to be considerable. The peptides exert a specific action on the hypothalamo–pituitary–gonadal–uterine system. They have a short half-life and have proved to be remarkably atoxic in acute and chronic toxicological studies in several animal species [4]. The exogenous synthetic steroids have, apart from their ovulation-inhibiting effect via the hypothalamo–pituitary system, act on the liver and thus also on many other metabolic processes in the body. These effects may occasionally cause severe, sometimes even life-threatening, side effects during steroidal contraception.

A major concern for the method of inhibiting ovulation by GnRH superagonists is the fact that follicular growth is not completely suppressed by the intranasal doses presently used for peptide contraception. Our volunteers on long-term Buserelin therapy, therefore, never complained of clinical symptoms of estrogen deficiency (hot flushes, vaginal discomfort etc.). On the other hand, the majority are faced with a situation of "unopposed estrogen secretion" with weak proliferation of the endometrium. Hyperphasic changes have never been observed in endometrial biopsies from our volunteers [7, 14]. Our biopsies have been thoroughly reviewed by American pathologists, confirming the results [15].

On intranasal Buserelin therapy Schmidt-Gollwitzer *et al.*[16] have described occasional hyperplastic changes, which regressed with continuous treatment. Clearly, the endometrium has to be carefully monitored in further studies of continuous superagonist therapy for contraception. This was pointed out already in the first report on this potential new method of birth control [5]. In our opinion, the endometrial results available to date do not warrant introduction of synthetic progestogens for potential endometrial protection from progressive hyperplasia during prolonged GnRH superagonist therapy for inhibition of ovulation.

Combined oral contraceptives seem to afford protection from endometrial and ovarian cancer [17]. However, recent results suggest that long-term use, particularly from an early age in nulliparous women, may increase the risk of breast and cervical cancer [18, 19]. The results on breast cancer are extremely worrying. Despite later reassuring results [20], this issue must be thoroughly followed-up by well-planned epidemiological studies. It would be a disaster for steroidal contraception in the future should eventually the results of Pike *et al.*[19] be confirmed.

There is without doubt a need for intensive research for alternative methods of contraception. GnRH analogs provide an excellent basis for development of fundamentally new birth control methods in man. Superactive GnRH agonists for paradoxical inhibition of ovulation have already proved to provide safe and effective control of fertility in women. Daily intranasal application of the peptide is well accepted. However, alternative routes of administration (osmotic minipumps, biodegradable implants or injectable microspheres etc) may be advantageous for prolonged therapy. Further large-scale clinical investigations of this new promising lead to peptide contraception clearly seem to be strongly indicated.

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