

The LHRH antagonist Cetrorelix: a review

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In those clinical situations in which an immediate and profound suppression of gonadotrophins is desired, LHRH agonists have the disadvantage of producing an initial stimulatory effect on hormone secretion. Therefore, the use of GnRH antagonists which cause an immediate and dose-related inhibition of LH and FSH by competitive blockade of the receptors is much more advantageous. One of the most advanced antagonist produced to date is Cetrorelix, a decapeptide which has been shown to be safe and effective in inhibiting LH and sex-steroid secretion in a variety of animal species and in clinical studies as well. Clinical trials in patients suffering from advanced carcinoma of the prostate, benign prostate hyperplasia, and ovarian cancer are currently in progress and have already shown the usefulness of this new treatment modality. In particular, the concept that a complete suppression of sex-steroids may not be necessary in indications such as uterine fibroma, endometriosis and benign prostatic hyperplasia represents a promising novel perspective for treatment of these diseases. Following completion of phase III trials in controlled ovarian stimulation for IVF regimens, Cetrorelix was given marketing approval and, thus, became the first LHRH antagonist available clinically.

Key words: benign prostatic hyperplasia/cancer treatment/GnRH antagonist/gonadotrophins/ovarian stimulation

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Introduction

Analogues of the hypothalamic hormone, luteinizing hormone-releasing hormone (LHRH), which controls the secretion of the gonadotrophins, LH and FSH, from the anterior pituitary gland, belong to the standard medical armament for interfering with sex hormone production. The peptide hormone LHRH was isolated from hypothalamic extracts and its amino acid sequence was established by Schally *et al.* (1971), who also succeeded in synthesizing the hormone.

Replacement or deletion of different amino acids within the LHRH molecule resulted in the discovery of LHRH agonists, which possess an increased potency for the liberation of gonadotrophins. When agonistic analogues are applied continuously, following an initial stimulatory action, the opposite effect occurs, namely, an inhibition of gonadotrophin and sex-steroid

secretion. The mechanism of this effect is based on a desensitization of the gonadotrophic cells and a down-regulation of pituitary receptors leading to a selective medical hypophysectomy. Several LHRH agonists are currently available and are used for the treatment of prostate and breast cancer, endometriosis and female infertility (Mansfield *et al.*, 1983; Lemay *et al.*, 1984; Schally and Redding, 1987). However, situations where an immediate and dose-dependent suppression of the gonadotrophins is required, the drawback of the agonists is in their initial stimulatory effect on hormone secretion and the relatively long period (2–3 weeks) of chronic exposure before complete suppression can occur. Hence, antagonists which can produce an immediate and dose-related inhibition of gonadotrophin release by competitive blockade of the receptors is more desirable (Figure 1).

Several groups headed by Schally and Rivier began synthesis of antagonistic analogues of LHRH >20 years ago. However, an early generation of LHRH antagonists was too lipophilic and subsequent generation not suitable for clinical use because of oedematogenic side effects caused by histamine release (Schmidt *et al.*, 1984; Hahn *et al.*, 1985).

Since then, major improvements have been achieved by the synthesis of LHRH antagonists incorporating further amino acid substitutions (Bajusz *et al.*, 1988; Rivier, 1993). A modern

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antagonist which is devoid of oedematogenic effects is Cetrorelix, a decapeptide which has been shown to be safe and effective in inhibiting the secretion of gonadotrophins in a variety of species including man (Szende *et al.*, 1990; Behre *et al.*, 1992; Weinbauer *et al.*, 1993).

In this review, both the pharmaco-toxicological and the clinical results obtained with Cetrorelix will be summarised, including the indication of controlled ovarian stimulation for assisted reproduction techniques for which Cetrorelix (Cetrotide®; Asta Medica AG, Frankfurt, Germany) has recently been approved in Europe, thus making it the first GnRH antagonist to be marketed worldwide.

Summary of preclinical results

Chemistry

Cetrorelix is a decapeptide which was originally synthesized at Tulane University, New Orleans, USA, Bokser *et al.* (1990). Cetrorelix has a highly modified LHRH sequence, comprising 10 amino acids, five out of which are in a non-natural D-configuration (Table I). The C- and N-terminal protecting groups (acetyl, amide) provide stability and are mandatory for complete antagonistic activity. Cetrorelix was also investigated in terms of physico-chemical parameters, e.g. adsorption to surfaces and peptide aggregation. The tendency for aggregation and gel formation, as

well as adsorption phenomena in general, were reduced by handling the peptide in a properly acidified aqueous solution for product transfer before lyophilization (Reissmann *et al.*, 1994).

Stability

Peptides are subject to hydrolysis, oxidation, photo decomposition and enzymatic proteolysis among other processes. The stability of Cetrorelix in aqueous solution was investigated in the pH range 1.0–13.0. The peptide was found to be surprisingly stable for a period of 21 days at room temperature at pH 7.0 with significant decomposition at higher or lower pH values. It is resistant to oxidation with H₂O₂ under neutral conditions, but decomposes up to 15% within 30 min at 100°C at pH 7.0. When stored at refrigerator temperature (2–8°C) the substance as well as the lyophilisate are stable for at least 3 years. The reconstituted solution (lyophilisate dissolved in water for injection) is stable for at least 2 days when stored at room temperature (20°C). Cetrorelix is highly resistant to degrading enzymes, e.g. chymotrypsin, pronase and nargase (subtilisin), for up to 50 h at 37°C (Reissmann *et al.*, 1994). This is in sharp contrast to potent LHRH agonists which face almost complete degradation within several hours. The proteolytic stability of Cetrorelix is underlined in comparison with a diastereomeric analogue comprising L-configured citrulline in position 6, instead of D-citrulline as in Cetrorelix. This analogue is highly sensitive to degradation and lacks biological activity, probably due to this imminent enzymatic instability (Pinski *et al.*, 1995).

LHRH receptor binding

Binding affinities of Cetrorelix and the agonist (D-TRP⁶)-GnRH to membrane receptors on cells from male rat pituitary glands were estimated using labelled GnRH (Fekete *et al.*, 1989). Results indicated that LHRH binds to two classes of membrane receptors on pituitary cells, one with low, the other with high affinity. LHRH is displaced by Cetrorelix from both receptors, which has an affinity constant ~5 times higher for the first and 1.4 times higher for the second receptor class.

These data were confirmed in a mouse fibroma cell line model which was transfected with the gene for the human LHRH receptor protein and showed a stable expression (Beckers *et al.*, 1995). The binding and affinity of Cetrorelix to this receptor was

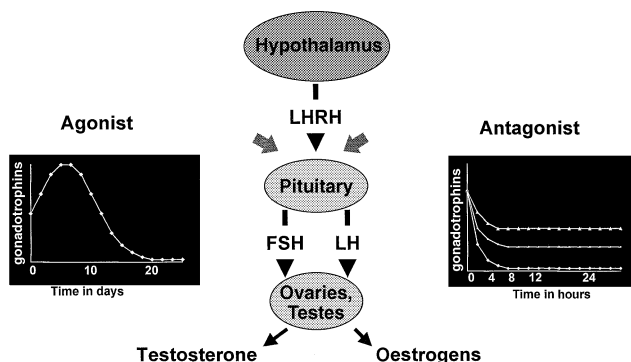


Figure 1. Luteinizing hormone-releasing hormone (LHRH) analogues – mode of action.

Table I. Sequence of amino acids in luteinizing hormone-releasing hormone (LHRH) and Cetrorelix

	1	2	3	4	5	6	7	8	9	10
GnRH	Glu	His	Trp	Ser	Tyr	Gly	Leu	Arg	Pro	Gly-NH ₂
Cetrorelix	D-Nal	D-Phe	D-Pal	4	5	D-Cit	7	8	9	D-Ala

Molecular weight = 1431.07.

Table II. Dissociation constants of luteinizing hormone-releasing hormone (LHRH) analogues for the human LHRH receptor

Peptide	LHR	Cetrorelix	Antide	Buserelin
KDa × 10 ⁹ (mol/l)	1.47 ± 1.23	0.19 ± 0.03	0.36 ± 0.13	0.30 ± 0.12

determined in comparison to Antide, an antagonist which has already been tested in phase I clinical trials, and to the agonist buserelin (Suprefact® Hoechst AG, Frankfurt, Germany). Cetrorelix has a binding affinity ~20 times higher than the native LHRH and about twice as high as the agonist, buserelin, or the antagonist, antide (Table II).

Preclinical safety evaluation

In the past, the problems in the development of LHRH antagonists included the property to induce a systemic liberation of histamine. The introduction of D-Cit in position 6 of an antagonistic decapeptide results in a less intense histamine release, as demonstrated in rat mast cells in an in-vitro assay system (Bajusz *et al.*, 1988). Subsequently, the histamine releasing activity of Cetrorelix was determined during the safety pharmacological evaluation at first in this assay system. The results indicated that the ED₅₀ value obtained for Cetrorelix was >1000-fold higher than the pharmacological effective plasma concentrations and, therefore, can be regarded as being of no clinical relevance. However, in view of the high variability, this assay has a limited predictivity if the test compounds to be compared are not evaluated within the same assay and if they have different solubilities in the assay medium. Therefore, in-vivo experiments appeared to be more suitable as a test method. Injections of Cetrorelix into rats at a dose of 1.5 mg/kg showed no oedematous reaction. In addition, the examination of systemic effects revealed that with i.v. injections at doses of 1 and 4 mg/kg no apparent changes in respiratory and cardiovascular functions were observed. In both pharmacological and long-term toxicological safety studies in rats and dogs, Cetrorelix exerted no systemic side-effects. A variety of organ changes, related to the pharmacodynamic effects did not show any progressive properties or were morphologically and/or functionally reversible after cessation of treatment. No direct target organ toxicity was found in acute, subacute or chronic toxicity experiments. No contact sensitizing properties, teratogenic potential, or influence on the early embryonic development and implantation were detected at clinically relevant doses. Mutagenicity tests were unequivocally negative for genotoxic and chromosomal aberration endpoints.

Animal experiments

Effects on LH, FSH and testosterone concentrations

In most of the experiments performed on animals, Cetrorelix was tested alone or in comparison with GnRH agonists, since a standard antagonist did not exist. In order to assess its hormone suppressive potency, treatment with Cetrorelix was carried out in mice, rats and monkeys. In what follows, some selected results are described illustrating the mode of action and efficacy of Cetrorelix. In order to determine whether Cetrorelix also competes with LHRH agonists *in vivo* for the same binding sites on the pituitary cells, investigations were conducted as to whether the antagonist-induced suppression of LH can be overcome by the administration of the agonist Decapeptyl® (D-Trp⁶-GnRH). The results showed that a stimulation of LH was indeed achieved by Decapeptyl during the suppressive treatment with Cetrorelix. This stimulation was of lower amplitude than in the control group, but was consistent at different time points indicating that both compounds compete for the LHRH binding sites. When Cetrorelix was given

shortly before the agonist, the stimulated LH response was significantly lower and could even be nullified (Pinski *et al.*, 1992). Under these conditions, the binding sites available for the agonist were obviously not sufficient for the induction of gonadotrophin release. The pituitary receptors occupied by Cetrorelix appear to be identical to those required for D-TRP⁶-binding. Based on these results, it can be concluded that Cetrorelix is a LHRH antagonist which specifically and competitively binds to high-affinity GnRH receptors, thereby inhibiting the release of gonadotrophins from the pituitary gland.

The castrated male rat is a commonly used model which allows the measurement of even low suppressive effects on gonadotrophins, since increased LH and FSH plasma concentrations are present due to the absence of a negative feed-back of testosterone. In these animals a dose-dependent suppression of LH was achieved and a single dose as low as 2.5 µg/rat (~10 µg/kg) significantly decreased plasma LH concentrations immediately after a s.c. injection. The nadir was reached after four h with a fall in LH concentrations of >80%, with serum LH returning to normal after 24 h. Further increase in dosage prolonged the duration of LH suppression (Bokser *et al.*, 1991). The efficacy of these low doses already indicates the high suppressive potency of Cetrorelix, which can also be expected in intact animals. In intact female rats the dose of 2.0 µg/rat was sufficient to completely inhibit the ovulation and even lower doses significantly reduced the rate of ovulation. All doses were devoid of macroscopically visible side-effects; furthermore, the recovery of hormone values and gonadal function after prolonged treatment was confirmed (Bokser *et al.*, 1990).

The gonadotrophin suppression induced by Cetrorelix was also tested in the sub-human primate *Macaca fascicularis*. As had been shown previously in rats, an immediate suppression of LH concentrations could be observed in male castrated monkeys as well. There were no differences between the treatment groups consisting of single doses of 250, 625 and 1250 µg/kg Cetrorelix. The nadir was reached at ~12 h post-injection and surprisingly, the LH concentrations remained suppressed for at least 96 h. Even at the lowest dose, there was no tendency for a LH rebound within this time interval, thus indicating again the high LHRH antagonistic potency (Weinbauer and Nieschlag, 1993). A dose of 225 µg/kg given daily for 14 days to intact monkeys produced a continuous suppression of testosterone to castration concentrations (Weinbauer *et al.*, 1993). Haematology and clinical chemistry parameters showed no pathological alterations. During prolonged treatment with a dose of 450 µg/kg s.c. daily for 7 weeks, a continuous suppression of LH and testosterone was induced in these intact monkeys. Additionally, starting at week 2 of treatment serum concentrations of inhibin were suppressed and testicular volume decreased; following continuous Cetrorelix injections, all animals became azoospermic (Weinbauer *et al.*, 1994). After termination of treatment all effects were reversible.

Investigations have shown that FSH concentrations are not affected by Cetrorelix as strongly as LH during short-term treatment, which might be due to differences between bioactive and immunoactive FSH, differential regulation of LHβ and FSHβ subunit expression and/or the prolonged plasma half-life of FSH (Matikainen *et al.*, 1992). In rats, the

pituitary LH and FSH content was not altered by the treatment with Cetrorelix, indicating lack of effect on the synthesis of gonadotrophins by the gonadotroph cells of the anterior pituitary gland (Ayalon *et al.*, 1996). In agreement with these results, single high doses resulted in an immediate arrest of the oestrous cycle in rats with the duration being dose-dependent (Reissmann *et al.*, 1996). In-vitro tests revealed that plasma concentrations achieved in these experiments do not interfere with epidermal growth factor (EGF)-stimulated human granulosa cell proliferation, which was only inhibited by concentrations that were ~100 times higher (Yano *et al.*, 1997). These results prove that Cetrorelix effectively and dose-dependently suppresses the secretion of gonadotrophins, especially LH, from the pituitary gland. As a result of this hormone-withdrawal, pre-ovulatory LH peaks can be inhibited. When given at sufficiently high doses, a cessation in reproductive function in female and male animals is observed, which is reversible after treatment termination.

Summary of clinical results

Phase I studies

Based on the preclinical efficacy and a favourable safety profile, clinical phase I studies were initiated in volunteers. In 15 phase I studies, including single and multiple s.c. injections as well as single i.v. infusions, Cetrorelix was administered to a total of 236 healthy subjects of both sexes (161 male and 75 female). The dose range tested for single doses was 0.25–20.0 mg s.c. The results of representative studies are summarized below.

The first administration of Cetrorelix to man was performed as single s.c. doses in healthy male volunteers (Klingmüller *et al.*, 1991; Behre *et al.*, 1992). Compared with the placebo group, the extent and duration of suppression increased in parallel with increasing doses. After the administration of 1.0 mg, a maximal testosterone suppression of 73% in comparison with baseline was seen 8 h after injection; the suppression was 80 and 91% after single doses of 2.0 and 5.0 mg respectively. By 48 h after the injection of the 5 mg dose, testosterone values were no longer different from those in the placebo group and reached serum concentrations in the lower normal range. As in animal studies, the suppression of FSH did not reach a statistical significance. Linear kinetics were found, with a calculated plasma $t_{1/2}$ of 30 h after single doses of 5 mg Cetrorelix.

Single dose administration of Cetrorelix to healthy premenopausal female subjects was performed in view of the planned clinical use of Cetrorelix in IVF programmes. Following single doses of 3 and 5 mg administered between days 6 and 10 of the menstrual cycle, serum LH, FSH and oestradiol decreased immediately (Leroy *et al.*, 1994). A nadir was reached 24 h after injection with a reduction of 56 ± 19 , 29.5 ± 16 and $85 \pm 17\%$ (48 h) compared with baseline respectively. No significant differences with regard to the extent of suppression were seen between the two different doses. The LH surge was postponed in all cases, occurring 6–17 days after the Cetrorelix injection. When Cetrorelix was administered during the late follicular phase during which time plasma oestradiol concentrations were >150 pg/ml, spontaneous LH surges were also postponed in all women. Again,

the suppression of FSH was less pronounced, giving early reason for speculations that a reduced stimulation procedure could be applied during controlled ovarian stimulation cycles.

Single i.v. administration of Cetrorelix to healthy men was performed in order to determine pharmacokinetics, absolute bioavailability, pharmacodynamic effects, safety and tolerability. Six healthy male subjects randomly received single doses of 3 mg Cetrorelix i.v. and s.c. with a wash-out period of 21 days between each single administration. The extent of suppression was the most pronounced for testosterone, reaching mean decreases from baseline of 93% (i.v.) and 95% (s.c.). Compared with the baseline, LH was reduced by 82% (i.v.) and 80% (s.c.), whereas FSH values were influenced less, as seen by a decrease from baseline of 41 and 49% after i.v. and s.c. administration respectively (Hermann *et al.*, 1996).

Multiple dose administration to healthy men ranged from 0.25 to 10 mg given daily for 7, 8 or 14 days. During daily doses for 8 days (Behre *et al.*, 1994), a dose-dependent suppression of gonadotrophins and testosterone was found, but interestingly only a dosage as high as 10 mg/day was able to maintain castration concentrations of testosterone over the period of administration. When lower doses were used, an increase of testosterone concentrations was found between days 2–4 of treatment. Therefore, based on results from animal studies and pharmacokinetic considerations, an investigation was conducted to determine if the suppression of LH, FSH, and testosterone can be achieved by an initial high-dose and thereafter be maintained by continued low-dose injections. A loading-dose schedule using 10 mg/day for 5 days followed by a maintenance dose of 1 mg/day was then tested in male volunteers (Behre *et al.*, 1997) which resulted in a continuous suppression of testosterone to castration range. In addition, it was found in these studies that treatment with Cetrorelix induced a pronounced and reversible reduction (~40%) of the prostatic volume within 2 weeks of treatment.

Female volunteers were observed during three consecutive menstrual cycles consisting of pre-treatment, treatment and a post-treatment control cycle. Subjects received 3 mg Cetrorelix s.c. daily for 1 week with the first injection administered on day 8 of the individual cycle. By 24 h after the first application, LH was strongly suppressed and oestradiol reached post-menopausal values. The mean duration of the suppressive effects of Cetrorelix after the last Cetrorelix injection compared to baseline values was 13.0 days for LH, 9.4 days for FSH and 14.6 days for oestradiol. In the nadir (day 15) LH was reduced to 16.1% and FSH to 63.5% of the respective baseline values and oestradiol to post-menopausal values (Gonzalez-Barcena *et al.*, 1994b; Sommer *et al.*, 1994). After termination of treatment, an LH surge followed by post-ovulatory progesterone values was found in all women. Pharmacokinetic analyses of a study in female volunteers receiving daily doses of 0.25, 0.5 and 1 mg from cycle days 3 to 16 revealed dose-linearity and a single dose of 3 mg resulted in a plasma half-life of ~8 h after iv.-injection and 25 h after s.c. injection with a bioavailability of 92% (Hermann *et al.*, 1996).

Tolerability in phase I studies

Since the histamine releasing potential and subsequent severe local and systemic side-effects, e.g. oedema, anaphylactoid

reactions had been previously recognized with the use of former LHRH antagonists, an intradermal skin test was usually performed prior to a systemic administration of therapeutic doses. Thus, in most of the initial studies an intracutaneous test with 10 µg Cetorelix was performed which would lead to exclusion of a patient in case of severe local and/or systemic allergic reactions. However, no systemic adverse reactions were reported in any of these trials. Mild local reactions were reported with symptoms such as redness at the injection site, but these reactions were mild and transient. During further investigations, 25 healthy volunteers of each sex received five consecutive intracutaneous tests (10 µg Cetorelix). It became apparent that the intracutaneous test did not result in reproducible effects on the skin and was not at all predictive of the occurrence of local or systemic side-effects in subsequent s.c. treatments. The most frequent local reaction following s.c. single doses was redness/erythema and was classified as being of slight to moderate intensity and subsided spontaneously and completely within minutes. The inter- and intra-subject (day-to-day) variability of the erythema size was remarkably high. There was no evidence that the frequency of local reactions depends on the number of consecutive administrations. Local reactions at the site of injection occurred independently of dose or sex and also occurred after placebo (Hermann *et al.*, 1996). Other side-effects were based on the pharmacodynamic action of Cetorelix, i.e. suppression of testosterone or oestradiol, and consisted of a decreased libido and hot flushes. Laboratory parameters showed no alterations following single dose, whereas time and dose dependent increases of high-density lipoprotein (HDL) cholesterol were observed during multiple dose treatment. No significant change was seen in serum concentrations of low-density lipoprotein (LDL) cholesterol or triglycerides (Behre *et al.*, 1994).

Phase II studies in different indications

Controlled ovarian stimulation for assisted reproductive techniques

For ovarian stimulation, either human menopausal gonadotrophin (HMG) or, more recently, recombinant (r)FSH is used followed by ovulation induction with human chorionic gonadotrophin (HCG), which is injected when a sufficient number of mature follicles are present. Thereafter, assisted reproductive techniques, such as IVF or intracytoplasmic sperm injection (ICSI) are used in order to obtain embryos for replacement in the uterine cavity. The classical stimulation procedure with gonadotrophins has the disadvantage of an unpredictable ovarian reaction and the occurrence of a premature LH surge, caused by the positive feedback of rising oestradiol in up to 25% of the cases (Schmutzler and Diedrich, 1990). The high LH concentrations during this surge may have deleterious effects on the quality of oocytes and the increasing progesterone concentrations may have a negative effect on the endometrium, both reducing pregnancy rates and thus leading to the cancellation of the treatment cycle. LHRH agonists were added to the stimulation protocols to prevent premature LH surges by inducing a suppression of endogenous LH, thereby achieving a reduction in the frequency of premature luteinization to ~2%. Presently, the so-called long protocol in which the treatment with agonists starts at least 14 days prior to stimulation with gonadotrophins, appears to be the most effective

procedure. However, there are some disadvantages of this treatment schedule: (i) a long treatment period before the suppression of gonadotrophins occurs and ovarian stimulation with exogenous gonadotrophins can be started (~14 days in the long-protocol); (ii) a relatively long exposure to hormonal medication; (iii) a strong suppression of both gonadotrophins and oestradiol resulting in the occurrence of hormone-withdrawal symptoms, e.g. hot flushes, and the requirement for high doses of HMG/FSH for stimulation; and (iv) as a result of the induced down-regulation of the receptors and a depletion of gonadotrophin storage vesicles, a recovery period is necessary for the restoration of pituitary responsiveness which might contribute to the requirement of luteal phase support.

New treatment modalities are now available based on the use of Cetorelix in these procedures, since because of the immediate suppression of gonadotrophins the unwanted stimulatory phase produced by the LHRH agonists can be avoided and the duration of treatment duration can be significantly reduced by administration of Cetorelix only during the period of increased risk for premature LH surges.

Phase II clinical trials including 294 patients were conducted to investigate the efficacy and safety of Cetorelix (Cetrotide) in controlled ovarian stimulation for assisted reproduction techniques using HMG, since rFSH had not been registered in Europe when the study was initiated. Two different dose regimens of Cetorelix were applied consisting of multiple doses of 3, 1, 0.5, 0.25 and 0.1 mg/day starting on cycle day 5 or 6 until and including the day of HCG administration, and a single or dual dose of 5, 3 and 2 mg given primarily on stimulation day 7. For both types of treatment, a minimal effective dose for the prevention of premature LH surges (defined by LH \geq 10 IU/l and progesterone \geq 1 ng/ml), was determined (Felberbaum and Diedrich, 1999) (Figure 2).

Diedrich *et al.* (1994) included a total of 20 patients with primary or secondary tubal sterility undergoing controlled ovarian stimulation. In all, 15 patients were treated with 3 mg of Cetorelix (Cetrotide) daily s.c. starting on day 7 of the menstrual cycle until the application of HCG. Since no endogenous LH surge was seen, five additional patients were treated with a dose of 1 mg/day of Cetorelix using the same treatment schedule; however, once again no LH surge was observed. Following the first Cetorelix dose, LH

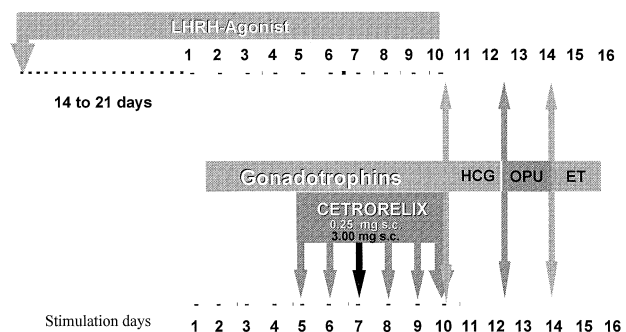


Figure 2. Cetorelix in controlled ovarian stimulation for assisted reproduction techniques: treatment schedule for daily 0.25 or single 3 mg dose. HCG = human chorionic gonadotrophin; OPU = oocyte retrieval; ET = embryo transfer.

values fell immediately and this suppression was evident for both the 3 mg/day dose and 1 mg/day dose of Cetrorelix. Mean oestradiol concentrations were unaffected and underwent normal changes indicative of continuous follicular development. The quantity and quality of oocytes was comparable with the use of the long protocol with agonists as applied in the corresponding institution. In all patients the oocytes could be collected and fertilized (61.5% fertilization rate), and embryo transfer was subsequently performed in all cases. As could be expected from the results of phase I trials in women, the number of gonadotrophin ampoules needed could be reduced to 27, compared with 35–40 ampoules with the long protocol with agonists in this centre. The responsiveness of the pituitary was also examined in this study by performing a LHRH test 3 h before ovulation induction. Administration of 25 µg of LHRH and measurement of the LH release 30 min later revealed that a mean increase in serum LH was 10 mIU/ml for the 3 mg group, while the average maximum in the 1 mg group was ~32.5 mIU/ml, thus indicating a preserved pituitary function (Felberbaum *et al.*, 1995).

In subsequent studies (Albano *et al.*, 1997) a further reduction in the amount of Cetrorelix to daily injections of 0.5, 0.25 and 0.1 mg was evaluated in a total of 90 patients. In this study a daily s.c. dose of 0.25 mg of Cetrorelix proved to be the minimal effective dose to prevent premature LH surges and to obtain good quality of oocytes (Figure 3). Analysis of Cetrorelix in serum during the entire administration period and in follicular fluid obtained at the day of oocyte retrieval showed concentrations at or below the detection limit (0.3 ng/ml). On the day of embryo transfer, no plasma concentrations of Cetrorelix were detected.

In order to simplify the stimulation protocol further, the suitability of a single dose injection was evaluated. Frydman *et al.* included 17 patients in a study to assess a duration of action of a single 5 mg dose injected when oestradiol concentrations were between 150–200 pg/ml per follicle >14 mm (Olivennes *et al.*, 1994). A second dose was injected 48 h later if the triggering of ovulation was not decided upon. As a result, six patients were injected with a single dose and 11 patients received two injections, the mean day of the first injection being day 9.6. At 24 h after injection a mean decrease in LH to 0.2 mIU/ml (5.5 mIU/ml baseline) occurred and hence no premature LH surge was

observed until the day of ovulation induction which was performed on cycle day 11.7.

Thereafter, 11 women were included in a IVF protocol and received the single dose of 3 mg Cetrorelix on day 8 of the menstrual cycle. In eight patients a single injection was sufficient and ovulation induction was performed on cycle day 11 ± 1 . In the remaining three patients a second injection of 3 mg Cetrorelix was necessary 72 h after the first dosing and ovulation was triggered on cycle day 12.3 ± 0.6 . Subsequently, it was confirmed that the 3 mg dose represents the minimal effective dose for preventing premature LH surges (Figure 4) in the majority of patients when given on cycle day 8 (Olivennes *et al.*, 1995, 1998). Overall, 294 patients were included into the phase II development of Cetrorelix in controlled ovarian stimulation for assisted reproduction, in which a pregnancy rate per embryo transfer of 30% was achieved.

In order to confirm these results, a phase III trial programme was initiated including three multicentric, multinational studies. Multiple doses of 0.25 mg/day of Cetrorelix starting on stimulation day 5 or 6 were tested. Buserelin nasal spray (0.6 mg/day) starting on pre-cycle day 20 was given to a control group of patients. In addition, the use of a single dose of 3 mg Cetrorelix on stimulation day 7 was evaluated as well as a single dose of 3.75 mg triptorelin depot in the control group. The results of these studies are not yet published, but it is expected that with respect to number of follicles, oocytes retrieved, fertilization rate and pregnancy rates, the results with Cetrorelix will be comparable to those obtained in patients under LHRH agonist treatment. This assumption is based on preclinical findings showing that Cetrorelix does not affect steroid biosynthesis of granulosa-lutein cells or growth-factor induced granulosa cell proliferation (Yano *et al.*, 1997) in an in-vitro setting. Furthermore, no concentrations of Cetrorelix can be measured in follicular fluid or plasma at the time of oocyte retrieval.

The prevention of premature LH surges with Cetrorelix offers the possibility of using different options to induce final oocyte maturation and ovulation. Due to the preserved pituitary responsiveness to LHRH under Cetrorelix treatment (Felberbaum *et al.*, 1995), the idea was conceived of administering a single injection of an LHRH agonist or recombinant LH (Sills *et al.*, 1999) for the induction of

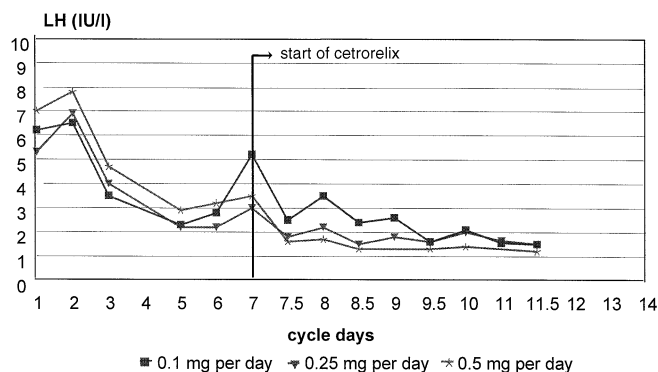


Figure 3. Cetrorelix in controlled ovarian stimulation for assisted reproduction techniques: mean LH concentrations during treatment with daily s.c. doses of 0.1, 0.25 and 0.5 mg.

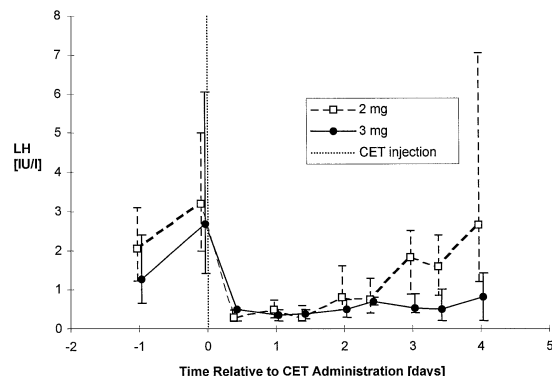


Figure 4. Cetrorelix (CET) in controlled ovarian stimulation for assisted reproduction techniques: mean LH concentrations after treatment with single s.c. doses of 2 and 3 mg.

ovulation, thus avoiding the administration of HCG. Therefore, attempts were made to induce ovulation by a single s.c. administration of the LHRH agonist, Triptorelin, in five patients treated according to the single dose regimen in a controlled ovarian stimulation programme (Olivennes *et al.*, 1996). Following the injection of Triptorelin, an LH surge was observed in all five patients and 12 h later mean plasma LH concentrations had increased from 1.3 ± 1.0 to 56.3 ± 40.0 IU/l, followed by a significant rise of plasma progesterone concentrations to 17.27 ± 4.12 ng/ml 72 h after Triptorelin injection. Thus, Cetorelix treatment allows ovulation induction by LHRH or one of its agonists instead of HCG, which could be beneficial in patients at high risk of ovarian hyperstimulation syndrome (OHSS) and those suffering from polycystic ovarian disease (PCOD) (Felberbaum and Diedrich, 1999). Clinical studies comparing the use of HMG versus rFSH in controlled ovarian stimulation for assisted reproduction techniques with Cetorelix are ongoing. In addition, the concept of the so-called 'natural cycles' which include a minimal ovarian stimulation can be applied with the use of Cetorelix (Rongières-Bertrand *et al.*, 1999). In this study, Cetorelix was given as a single sc. injection of either 1 or 0.5 mg in the late follicular phase of 44 cycles, when oestradiol values were 100–150 pg/ml. A mean of 4.7 ampoules of HMG were used for stimulation and treatment resulted in a successful inhibition of the LH surge in all available patients and in a pregnancy rate of 32% per transfer.

Uterine myoma

Endometriosis and uterine fibroids are diseases dependent on oestrogens. In both conditions the therapeutic use of LHRH agonists is well established, but again the initial flare-up is undesirable and the necessity of achieving castration concentrations of oestradiol is questionable. Therefore, the potential advantage of a LHRH antagonist is obvious. Results of pilot trials with Cetorelix indicate an effective reduction in myoma size, a shorter treatment period in comparison with LHRH agonists and indeed, shrinkage of the myoma without castration (oestradiol >50pg/ml) can be obtained.

An explorative study was conducted to investigate the efficacy and safety of Cetorelix in the medical management of uterine leiomyomata (Gonzalez-Barcena *et al.*, 1997). Cetorelix was administered to 18 pre-menopausal women with myomata who had been scheduled for hysterectomy. The initial dose of Cetorelix was 5 mg s.c. twice daily for the first 2 days and thereafter 0.8 mg s.c. twice daily for at least 3 months until the day of surgery. The mean duration of treatment was 4.4 months. The mean baseline uterine volume was 395.4 ± 69.2 ml, and after 3 months 16 patients showed a progressive reduction in uterine volume to a mean of 230.8 ± 52.6 ml. All patients became amenorrhoeic under these conditions. After termination of treatment with Cetorelix, a myomectomy was performed in 12 women. Further (unpublished) studies suggest that the reduction in size and vascularity of the uterus make surgical extirpation of the myomata much more feasible. Promising results were also obtained in a clinical trial (Felberbaum *et al.*, 1998), which used a Cetorelix depot formulation, and showed that a complete suppression of oestradiol might not be necessary to obtain a maximum reduction of myoma size already after a 4 week

treatment period. Preliminary results from an ongoing multicentre study in Japan also indicate that a short treatment period and incomplete oestradiol suppression are sufficient for reducing the volume of uterine fibroids substantially within 4 weeks of treatment using a dose of 3 or 5 mg Cetorelix once a week. Gynaecological studies with Cetorelix have been summarized recently by Schally (1999a).

Ovarian cancer

LHRH receptors are expressed in ~80% of ovarian cancers and there is experimental evidence that the growth of these receptor-positive cells can be inhibited by LHRH analogues (Emons and Schally, 1994; Srkalovic *et al.*, 1998). Since Cetorelix was shown to be more active than agonistic compounds in some models of ovarian cancer (Shirahige *et al.*, 1994), a phase I/II clinical trial was started in patients with advanced disease after first-line chemotherapy. Patients showing progressive disease were treated with Cetorelix in a dose of 10 mg s.c. daily. Presently, 17 patients are available for response; of these, three have shown a partial remission and four a stabilization of their disease (no change). In these patients the time to progression was between 125 and 196 days, whereas for all patients treated this value was 59 days. These results are very encouraging since 15 patients had progressive disease after third to fifth line therapies before starting Cetorelix. Currently, patients are being treated according to a stratification consisting of platinum-resistant and platinum-sensitive groups (Emons *et al.*, 1999).

Benign prostatic hyperplasia (BPH)

The standard therapy to achieve total testosterone suppression is orchidectomy with the occurrence of hormone-withdrawal symptoms such as hot flushes and impotence, which should be avoided in a benign disease such as BPH. Since total testosterone suppression might not be mandatory in BPH, theoretically it should be possible to apply a short-term treatment and produce the degree of testosterone suppression as low as necessary using an LHRH antagonist, e.g. Cetorelix. Therefore, several studies have been performed including 114 patients who were treated with various dosages and dose schedules of Cetorelix. These studies have shown the effectiveness of Cetorelix as shown by an increase of peak urinary flow rate, a reduction of prostate volume and improvement in other BPH symptoms. The efficacy initially observed in uncontrolled pilot studies (Gonzalez-Barcena *et al.*, 1994a) was confirmed in a double-blind placebo-controlled study (Lepor *et al.*, 1997). In this trial patients received s.c. injections of Cetorelix at a dose of 1 mg/day for 4 weeks. At the end of the treatment period a shrinkage of the prostate and a major improvement of urine flow were noted. Surprisingly, these effects were obtained although the administered dose of Cetorelix suppressed the serum concentrations of testosterone only by ~50%. The improvement in BPH symptoms lasted for several months and was independent of prostate size at study entry. These results were substantially extended by Comaru-Schally *et al.* (1998), who treated patients with moderate to severe symptomatic BPH with Cetorelix. In this study a significant (53%) reduction in urinary symptom score, a 46% improvement in the quality of life and increase in urinary flow-rate was found, although testosterone was only suppressed by 64–74% during maintenance therapy. During the follow-up, the effects

proved to be long-lasting with a decline in the international prostate symptom score (IPSS) of 72% still being present at study week 85. The fast onset and long duration of efficacy indicate that growth factors not yet identified may play a role in the mechanism of Cetrorelix action. Thus, beneficial clinical effects could be obtained without the negative consequences associated with a classical anti-androgen therapy. These results indicate that an intermittent treatment schedule, possibly in combination with an α -receptor blocker, could be elaborated in subsequent trials, thus opening the possibility of achieving a sustained improvement in symptoms using a few short-term treatment cycles per year.

Prostate cancer

Several open label phase I/II studies in patients with advanced prostate cancer were performed using different dose schedules of Cetrorelix acetate (Gonzalez-Barcena *et al.*, 1994a, 1995, 1996; Ayalon *et al.*, 1996). In a first explorative study, six patients with biopsy-proven prostatic cancer (two stage C and four stage D2) received Cetrorelix daily in terms of s.c. injections of 0.5 mg twice daily for 6 weeks. The treatment resulted in an immediate fall of testosterone to subnormal concentrations, reaching a nadir after 6–12 h. After 6 weeks of therapy total serum testosterone was below castration concentration (2 nmol/l). At the end of treatment the values of acid prostatic, total acidic and alkaline phosphatases reached normal values in all patients, whereas PSA concentrations were in the normal range in three patients. After the first week of treatment, a significant decrease in bone pain, relief of urinary flow obstruction and reversal of signs of prostatism was observed. Subjective improvement continued during the following weeks and ultimately the patients no longer needed analgesics. A progressive decrease in prostate volume was obtained starting in the second week of treatment.

Since this dose regimen did not achieve castration concentrations of testosterone from the beginning, an initial loading dose schedule was applied (Gonzales-Barcena *et al.*, 1996; Ayalon *et al.*, 1996) which had been shown to be effective in a preceding Phase I study (Behre *et al.*, 1997). The results obtained with respect to dose finding for continuous castration are in accordance with these phase I results and showed that a loading dose of 10 mg daily for 2–5 days is required to achieve suppression of testosterone to castration concentrations in all patients from the beginning. Thereafter a daily dose of 1–2 mg is effective in maintaining castration concentrations. Despite the fact that not all dose schedules tested resulted in castration concentrations of testosterone from the beginning, the treatment proved to be clinically effective as evidenced by reduction of serum PSA, regression of metastatic lesions and fast improvement of disease-related symptoms, e.g. bone pain, paraesthesia and paraplegia and hence, no co-medication with anti-androgens is required in symptomatic patients.

Conclusions

The decapeptide Cetrorelix has been extensively characterized as a potent antagonist of LHRH in various in-vitro animal models. The hormone-suppressing effects are dose-dependent and can be

induced immediately after the start of the administration thereby avoiding the 'flare-up effect' seen with the LHRH agonists. All treatment related effects are reversible and no teratogenic, mutagenic or contact sensitizing properties were found in toxicological studies. The human results on the LHRH antagonist Cetrorelix clearly demonstrate its safety profile and potential usefulness in the clinic. Therefore, Cetrorelix is also suitable for the use in controlled ovarian stimulation for assisted reproduction techniques and treatment of benign conditions including leiomyoma, endometriosis and BPH.

In patients undergoing controlled ovarian stimulation an excellent efficacy and safety profile was shown. Prevention of LH surges with Cetrorelix offers the possibility of using different options for the development of follicles, the induction of final oocyte maturation and ovulation. This in turn permits the simplification of procedures and reduces the risk and duration of exposure of patients to hormonal treatment. This could be beneficial in patients at high risk of OHSS and those suffering from PCOD. The results from clinical phase IV studies are expected to further substantiate the advantages of Cetrorelix compared with the use of LHRH agonists for this indication.

Preliminary results obtained following Cetrorelix treatment of patients with leiomyoma show a rapid reduction of uterus and myoma volumes within a 4 week treatment period. Therefore, the LHRH antagonist Cetrorelix should also be indicated for the treatment of endometriosis. In both conditions the possible advantages of LHRH antagonists still have to be demonstrated in large-scale studies; however, based on the clinical efficacy already demonstrated for LHRH agonists, their benefits may be deduced.

All studies performed to date in BPH patients reveal that Cetrorelix is effective in this condition. A daily treatment for 4 weeks produces a clinically significant and long-lasting improvement in BPH symptoms for at least 3 months. It is important that this can be achieved without testosterone suppression to castration concentration and related side-effects. This indicates that Cetrorelix may be used for intermittent therapy in BPH based on 1–3 treatment cycles per year.

Because of a strong suppression of sex-steroids, Cetrorelix can be utilized for the treatment of hormone-dependent cancer such as prostate and ovarian cancer. Oncological studies with Cetrorelix have been summarized recently (Schally, 1999b). In prostate cancer patients, treatment with Cetrorelix proved to be clinically effective as evidenced by reduction in serum PSA, regression of metastatic lesions and fast improvement of disease-related symptoms, e.g. bone pain, paraesthesia and paraplegia. Since this antagonist successfully inhibits spermatogenesis, it might also be useful for the prevention of gonadal damage by cytostatic agents during chemotherapy or radiotherapy (Ataya *et al.*, 1988; Schally *et al.*, 1989; Meistrich, 1998).

Currently, daily injections of Cetrorelix are used, however, by increasing the dose new treatment schedules, e.g. 3 mg per week, can be applied in indications requiring treatment for several weeks as may be the case for uterine fibroids and BPH. For long-term treatment such as for various cancers, a depot formulation is desirable and different approaches towards a clinically suitable depot formulation are under evaluation.

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