Arch Gynecol Obstet (2002) 266:12–17

ORIGINAL ARTICLE

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Plasma and follicular fluid concentrations of LHRH antagonist cetrorelix (Cetrotide®) in controlled ovarian stimulation for IVF

Received: 24 November 2000 / Accepted: 19 February 2001

Abstract Cetrorelix was administered in differing daily dosages for controlled ovarian stimulation. The dosage levels were 3 mg (9 cycles), 1 mg (19 cycles), 0.5 mg (43 cycles), 0.25 mg (46 cycles) and 0.1 mg (7 cycles). In the 3 mg, 1 mg and 0.5 mg group the respective median plasma concentrations of cetrorelix on the day of oocyte pick-up (OPU) were 2.10 ng/ml, 1.42 ng/ml and 0.88 ng/ml and 1.03 ng/ml, 0.46 ng/ml and 0.49 ng/ml on the day of embryo transfer (ET). In the 0.25 mg and 0.1 mg groups plasma cetrorelix levels were below the limit of quantification. The cetrorelix concentrations in follicular fluid (FF) in the 0.25 mg group were detectable in only 14 out of 44 samples, while in the 0.1 mg group no detectable concentrations could be obtained. We also examined 80 cycles after single doses of 5 mg (7 cycles), 3 mg (42 cycles), and 2 mg (31 cycles) cetrorelix. On the day of OPU the respective median plasma concentrations of cetrorelix were 0.57 ng/ml, 0.62 ng/ml, and 0.56 ng/ml, and 0.61 ng/ml and 0.28 ng/ml on the day of ET in the 5 mg and 3 mg groups. In the 2 mg group, the plasma concentrations fell to below limits of quantification in 8/9 samples on the day of ET. In 26 out of 27 FF samples cetrorelix was detectable in the 3 mg single dose group (median level: 0.69 ng/ml).

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Keywords LHRH antagonist · Controlled ovarian stimulation · Plasma concentrations · Follicular fluid · Cetrorelix

Introduction

The problem of a premature LH surge within controlled ovarian stimulation for assisted reproduction has been overcome by the introduction of LHRH agonists into stimulation protocols [22, 25].

LHRH antagonists have also been shown to be reliable in preventing a premature LH surge [13] but have advantages towards the long protocol [15, 16, 17]. The LHRH antagonist cetrorelix (Cetrotide®, ASTA Medica AG, Frankfurt, Germany, Serono International S.A., Geneva, Switzerland) has been used frequently in clinical studies in single, dual [18, 19] and multiple dose [8] protocols. In this study we present the results of cetrorelix plasma and follicular fluid concentrations in groups of patients previously described in dose-finding studies [2, 11, 21], using multiple dose protocols of cetrorelix (3 mg, 1 mg, 0.5 mg, 0.25 mg and 0.1 mg) as well as single dose protocols (5 mg, 3 mg, 2 mg). The concentrations in follicular fluid and plasma are analysed according to the dosage of cetrorelix.

Materials and Methods

Patients and stimulation procedure

The patient and treatment characteristics are shown in Table 1. All 204 patients in the study agreed to participate after all inclusion and exclusion criteria had been checked. The inclusion and exclusion criteria have been published before, as were the treatment characteristics of the patients in these studies [1, 2, 8, 11, 21].

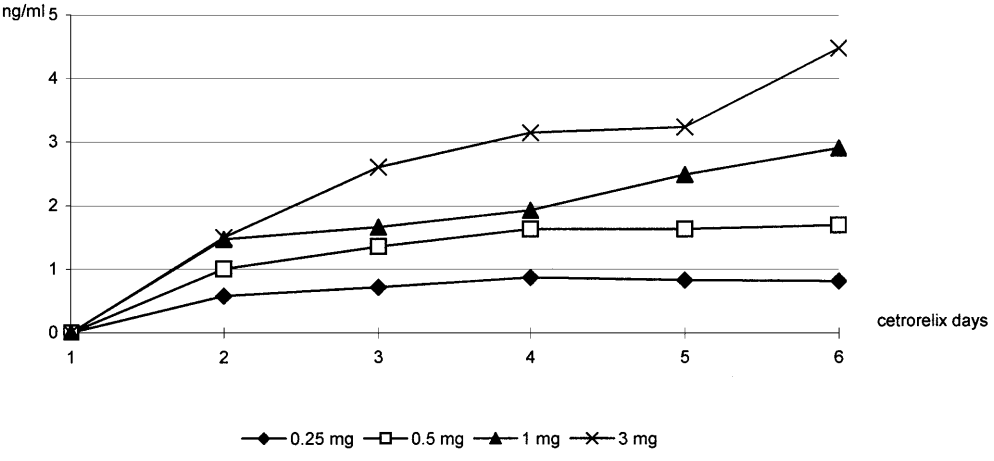
All studies were approved by the ethics committees of the Medical Campus of the Brussels Free University (Belgium), the University of Bonn (Germany), the Medical University of Lübeck (Germany) and/or the Hôpital Antoine Béclère (Clamart, France).

HMG (Pergonal®, Serono, Geneva, Switzerland; Humegon®, NV Organon, Oss, The Netherlands; Menogon®, Ferring Arzneimittel GmbH, Kiel, Germany) was used for controlled ovarian

Table 1 Patient characteristics in different dosage groups

Dosage groups	[mg]	Number of cycles	Age (mean±SD)	Weight (mean±SD)	Number of cetrorelix doses (mean±SD)
Multiple dose protocols	3.0	9	31.57±2.77	57.33±5.68	5.83±1.47
	1.0	19	31.89±3.08	62.73±8.60	5.25±0.97
	0.5	43	31.28±3.07	58.88±7.86	6.45±1.85
	0.25	46	30.09±3.86	61.65±7.67	5.22±1.30
	0.1	7	30.67±4.37	56.67±8.94	4.83±1.33
Single dose protocols	5.0	7	31.64±3.27	64.84±9.46	1
	3.0	42	31.64±3.79	57.43±7.05	1
	2.0	31	32.13±3.96	59.35±10.69	1

SD standard deviation. No statistical differences using Student's two-tailed t-test for different variances



n on day	1	2	3	4	5	6
0.25 mg	38	39	29	24	19	10
0.5 mg	43	43	42	42	38	29
1 mg	18	17	15	16	17	15
3 mg	13	13	13	13	12	7

Fig. 1 Predose plasma cetrorelix concentrations on the days of cetrorelix administration in a multiple dose schedule. Cetrorelix day 1 is stimulation day 6. Shown are the concentrations of 0.25 mg (*n*=46), 0.5 mg (*n*=43), 1 mg (*n*=19), and 3 mg (*n*=13). The plasma concentrations of 0.1 mg group are excluded, since only rarely were plasma cetrorelix concentrations above the limit of quantification. The median cetrorelix plasma concentrations are shown on the different stimulation days up to stimulation day 6. The median plasma concentrations in the 0.25 mg group were significantly lower ($p<0.05$) on days 2–6 compared to all other groups. On the following days, no difference could be shown, due to the low number of cases. Statistics were calculated using the Wilcoxon-rank-sum-test

stimulation in accordance with the stimulation protocols previously described [8, 18, 19]. Gonadotrophins were started on day 2 or 3 of a normal menstrual cycle. In the multiple dose protocols cetrorelix was given subcutaneously every 24 h starting on stimulation day 6 until and including the day of hCG administration. In the single dose protocols cetrorelix was given on stimulation day 7. Oocyte pick-up was performed 36 h after hCG administration and embryo transfer was undertaken 2 d after oocyte pick-up, 4 d after the last dose of cetrorelix in the multiple dose protocols.

Measurement of cetrorelix in plasma and follicular fluid

The measurement of plasma and follicular fluid cetrorelix concentrations was done by the Department of Biochemistry, ASTA Medica AG, Frankfurt, Germany. Daily plasma samples were stored for measurement of cetrorelix if available. The follicular fluid of up to three large follicles without blood contamination was collected on the day of oocyte pick-up, pooled and frozen. Pooling was chosen to make measurement of mean follicular fluid concentrations possible, if follicular fluid from more than one follicle was possible. The concentration of cetrorelix was measured in the thawed samples after the studies had been finished. The radioimmunoassay (RIA) for cetrorelix in human plasma and human follicular fluid has been described in detail before [5, 23]. It consists of 2 d of incubation of cetrorelix antiserum [7], [^{125} J]cetrorelix and 20 µl human plasma (or human follicular fluid) in RIA buffer at 4°C. The separation of antibody-bound and non-bound radiolabelled cetrorelix was achieved by addition of anti-rabbit IgG and polyethylenglycol and centrifugation for 20 min at 4°C. The supernatants were aspirated and discarded. The pellets (antibody-bound fraction) were counted in a computer linked gamma counter. The samples were analysed as triplicates and the mean value was calculated from all three measurements.

The lower limit of quantification (loq) for human plasma or human follicular fluid was fixed at 81% B/B_0 (B: antibodybound radioactivity in presence of the analyte; B_0: B for blank plasma or follicular fluid, i. e. without the analyte). The acceptance criteria for the quality control samples are ± 25%. The range of loq was 0.2 ng/ml to 0.7 ng/ml depending on the tracer. For each RIA the loq was calculated. For each study several RIA series were used for the measurements.

The concentration coefficient was defined as the concentration of cetrorelix in plasma in relation to the concentration in follicular fluid, both sampled on the day of oocyte pick-up. It was calculated as the mean of all available coefficients in a certain dosing group. It was thought to serve as a parameter to show possible accumulation in the follicular fluid.

Table 2 Cetrorelix concentrations in plasma and in follicular fluid on the day of hCG, the day of oocyte pick-up and on the day of embryo transfer according to cetrorelix dosage levels in the multiple dose protocol. Shown are median values with lower and upper quartiles

Cetrorelix	Median cetrorelix concentration [ng/ml] (25% quartile/75% quartile)											
	In plasma on day of hCG			In plasma on day of oocyte pick-up			In plasma on day of embryo transfer			In follicular fluid on day of oocyte pick-up		
	Concentration	*n*	>loq	Concentration	*n*	>loq	Concentration	*n*	>loq	Concentration	*n*	>loq
3 mg/3 ml	3.37 (1.19/7.56)	12	12	2.1 (1.74/2.47)	6	6	1.03 (0.63/1.29)	5	5	1.04 (0.97/1.32)	12	12
1 mg/1 ml	2.32 (1.67/3.73)	12	12	1.42 (0.86/1.55)	17	16	0.46 (0.16/0.79)	15	8	0.89, 0.94[a]	2	2
0.5 mg/1 ml and 0.5 ml	1.65 (0.68/3.31)	33	33	0.88 (0.64/1.14)	41	38	0.49 (0.29/0.65)	28	28	1.19 (1.04/1.40)	41	20
0.25 mg/1 ml	0.71 (0.07/1.58)	43	43	blq[b]	41	6	blq[b]	45	2	blq[b]	44	14
minimal effective dose												
0.1 mg/1 ml	blq[b]	6	1	blq[b]	6	1	blq[b]	7	0	blq[b]	6	0
Sum[c]	–	106	–	–	111	–	–	100	–	–	105	–

n number of samples which were analysed, *>loq* number of values which were above the limit of quantification (range of loq: 0.2–0.7 ng/ml)

[a] Only 2 values were available, therefore no median could be calculated.

[b] In the 0.25 mg and 0.1 mg group most samples were below the limit of quantification, therefore these values could not be calculated and were assumed to be generelly below limit of quantification (blq)

[c] Not in all patients probes from each day were available, therefore the sum from day to day is different

Statistics

All cetrorelix measurements which were below the limit of quantification (blq) were calculated as 1/2 loq to make calculation of median and upper and lower quartiles possible.

Statistical analysis was done using Student's t-test and Wilcoxon-rank-sum-test.

Results

Cetrorelix was administered dissolved in 5 ml (5 mg dose), 3 ml (3 mg dose) and 1 ml water for injection (1 mg, 0.5 mg, 0.25 mg, 0.1 mg dose), respectively. In some patients doses of 0.5 mg were administered in 0.5 ml water for injection (*n*=11). The measured cetrorelix concentrations in plasma and follicular fluid are shown in Table 2 as are the median concentrations, as well as the lower and upper quartiles.

The plasma concentrations of cetrorelix in patients treated with 0.1 mg cetrorelix/day was above the limit of quantification only in 12 out of 80 samples (15%) in 5 patients at different time points during the stimulation procedure. For this reason no calculation of median values was possible. In the 0.25 mg group the cetrorelix follicular fluid concentration was below the limit of quantification in 30 out of 44 cases. In the 0.1 mg group cetrorelix concentration was below the limit of quantification in all six cases, in which follicular fluid was sampled.

Figure 1 shows the plasma cetrorelix predose concentrations according to the duration and dosage of cetrorelix administration in a multiple dose schedule. The median cetrorelix plasma concentrations are shown on the different stimulation days. A slight increase can be seen in the 3 mg and 1 mg group.

After stopping the administration of cetrorelix on the day of hCG, the plasma concentrations of cetrorelix fell in all groups. The concentration coefficient betweeen plasma and follicular fluid was in the same range in all groups (0.57–1.57).

Table 3 shows plasma cetrorelix concentrations on the days of hCG administration, oocyte pick-up and embryo transfer in the single dose protocol as well as in the follicular fluid cetrorelix concentration on the day of oocyte pick-up. In all groups there was a clear fall from the day of hCG to the day of oocyte pick-up and the day of embryo transfer. Follicular fluid cetrorelix concentrations above loq were available in only 26 patients of the 3 mg group (median: 0.69 ng/ml). They were similiar compared to the plasma concentrations on that day in the 3 mg group (median: 0.62 ng/ml).

Although if they seem to be slightly higher than the concentrations in the multiple dose protocol using 0.25 mg/d, these concentrations are in fact in the range of loq (0.2–0.7 ng/ml) of the cetrorelix assay.

Table 3 Cetrorelix concentration in plasma and in follicular fluid on the day of hCG, the day of oocyte pick-up and the day of embryo transfer according to cetrorelix dosage levels in the single dose protocol. Shown are median values with lower and upper quartile

Cetrorelix	Median cetrorelix concentration [ng/ml] (25% quartile/75% quartile)									
	In plasma on day of hCG			In plasma on day of oocyte pick-up			In plasma on day of embryo transfer			In follicular fluid on day of oocyte pick-up		
	Concentration	*n*	>loq	Concentration	*n*	>loq	Concentration	*n*	>loq	Concentration	*n*	>loq
5 mg/5 ml	1.65 (1.04/2.17)	7	6	0.57 (0.47/1.05)	6	5	0.61 (0.29/0.87)	6	4	not measured		
3 mg/3 ml	1.48 (1.04/1.95)	40	40	0.62 (0.49/0.87)	31	27	0.28 (0.22/0.63)	25	11	0.69 (0.55/0.90)	27	26
2 mg/2 ml	1.10 (0.84/1.56)	31	31	0.56 (0.33/0.80)	22	18	blq[a]	9	1	blq[a]	26	5
Sum[b]	–	78	–	–	59	–	–	40	–	–	53	–

n number of samples which were analysed, *>loq* number of values which were above the limit of quantification (range of loq: 0.2–0.7 ng/ml)
[a] In the 2 mg group most samples were below the limit of quantification, therefore these values could not be calculated and were assumed to be generelly below limit of quantification (blq)
[b] Not in all patients probes from each day were available, therefore the sum from day to day is different

Discussion

The lowest doses and concentrations used in multiple dose protocols with cetrorelix were at 0.25 mg and 0.1 mg per day. However, the dosage of 0.1 mg/d did not prevent a premature LH surge in all cases [2]. Therefore, 0.25 mg/d is the minimal effective dose in clinical practice.

As the first plasma sampling after the administration of cetrorelix was done 24 h after its administration and as only few plasma samples were collected after the last cetrorelix administration, no evaluations of area under the curve and elimination half-life could made. Only few samples after 0.1 and 0.25 mg cetrorelix were above the limit of quantification. Thus reliable data were only obtained in the higher dosage groups.

In contrast to the multiple dose approach Olivennes et al. [18] gave a single or dual dose of 5 mg cetrorelix in the late follicular phase. A second injection was given 48 h later if ovulation did not occur. No spontaneous LH surge was observed. To improve the timing, to simplify the protocol, and to reduce the single dosage to 3 mg, a second study was done [19]. In a follow-up phase II study to investigate the minimal effective single dose, 3 mg proved to reliably prevent premature ovulation for at least 4 d whereas in the 2 mg group this time period was not covered and LH surges were observed in 2 patients [21].

Comparing both cetrorelix protocols, the plasma and follicular fluid concentrations of cetrorelix were comparably low after the single dose protocol using 3 mg and the multiple dose protocol using 0.25 mg – which are the minimal effective dose groups. From this aspect both protocols are equivalent. There is no accumulation of cetrorelix, if only 0.25 mg/d are given. This confirms the observations of a phase I study with multiple injections of 1 mg, 0.5 mg and 0.25 mg of cetrorelix in healthy volunteers [10]. There is no higher plasma or follicular fluid concentration compared to the single dose regimen, if the range of loq for interpretation of the values is considered.

As for plasma cetrorelix concentrations in the multiple dose 0.5 mg, 1 mg and 3 mg groups, one can find a slight dose dependency on the days of oocyte retrieval or embryo transfer. An approximately 1:1 concentration equilibrium is observed between the plasma and follicular fluid cetrorelix concentrations. Therefore, no accumulation in follicular fluid and later release from this compartment should be expected.

The extremely low cetrorelix plasma and follicular fluid concentrations on the day of hCG and embryo transfer is a clear advantage over the use of LHRH agonist depot preparations. Sommer et al. [24] have previously shown, that multiple doses of 3 mg cetrorelix in healthy volunteers did not alter the length of the following cycle. However, it is well known from clinical practice, that LHRH-agonist depot preparations induce cycle instability after an unsuccessful IVF cycle. This effect may be mainly due to persisting plasma concentrations after administration of an LHRH-agonist depot preparation like triptorelin [12].

Earlier studies have found no deleterious effect of LHRH antagonists on the luteal phase [9]. However, when a dosage of 0.5 mg was used in the multidose protocols in six patients, and no luteal phase support was given, the luteal phase was shortened and no pregnancies occurred [2]. Nothing is yet known about the outcome of non-supplemented luteal phases, especially after multiple doses of only 0.25 mg. However, in a recent analysis from Albano et al. [3] it could be shown, that similiar hormone patterns in the luteal phase were observed following multiple midfollicular doses of 0.5 mg and 0.25 mg cetrorelix, respectively. Very low LH concentrations were found throughout the luteal phase. Since cetrorelix was almost never present in the plasma of these patients on the day of ET, a prolonged effect of this drug on the luteal phase is unlikely. Therefore, it may be, that the administration of hCG for ovulation induction has of itself a deleterious effect on the luteal phase.

Since the follicular fluid cetrorelix concentration was around the lower limit of quantification, no oocyte toxicity should be expected. The plasma concentrations around the lower limit of quantification following a single dose of 3 mg or multiple doses of 0.25 mg on the day of embryo transfer is reassuring in terms of no embryo toxicity. By giving cetrorelix in the midfollicular phase one avoids anxieties about giving an LHRH analogue during early pregnancy – like it is possible in the long luteal agonist protocol.

Plasma and follicular fluid concentrations of the LHRH antagonist should be kept as low as possible. This aim can be achieved by the multiple and the single dose protocols using the LHRH antagonist cetrorelix. Cetrorelix is a safe drug, since it is at the lower limit of quantification in plasma on the day of ET in most patients when the minimal effective dose is used for blocking the LHRH receptors of the gonadotrophic cells in the pituitary.

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