CENTER FOR DRUG EVALUATION AND RESEARCH

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PHARMACOLOGY REVIEW(S)

DIVISION OF REPRODUCTIVE AND UROLOGIC DRUG PRODUCTS

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original submission

NDA No. 21-057 **Submission dated:** 1-28-1999

Reviewer: Krishan L. Raheja **Review completed:** 6-7-1999

Information to be conveyed to sponsor: Yes (*) No ( )

Sponsor: Organon Inc. West Orange, NJ

Drug name: Ganirelix acetate

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HFD-580 : DIVISION OF REPRODUCTIVE AND UROLOGIC DRUG PRODUCTS

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

ORIGINAL SUBMISSION

Key words: GnRH antagonist, ART, IVF, Toxicology, pharmacokinetics, rat, mouse, monkey, human

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Sponsor: Organon Inc. West Orange, NJ

Drug name: Ganirelix acetate

CAS Registry number: 124904-93-4

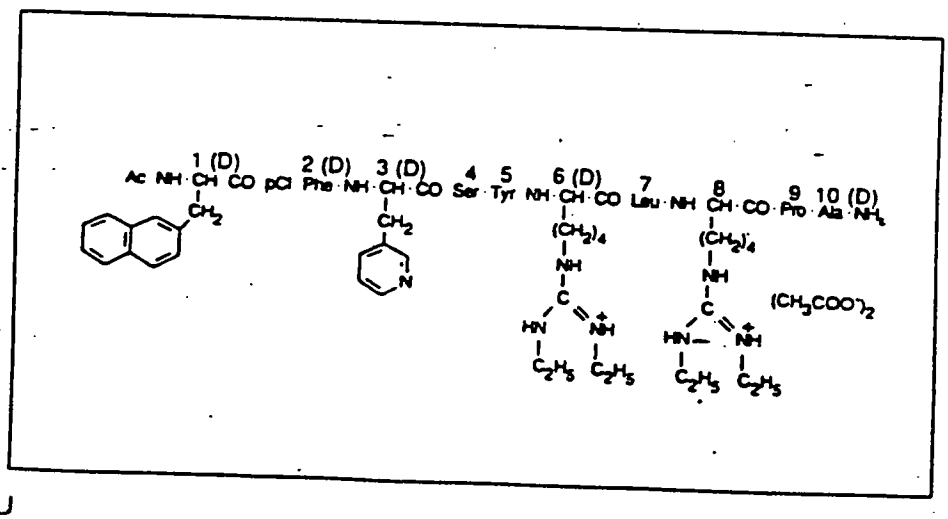
Laboratory code name: Org 37462

Chemical name: N-Acetyl-3-(2-naphtyl)-D-alanyl-4-chloro-D-phenylalanyl-3(3-pyridyl)-D-alanyl-L-seryl-L-tyrosyl-N^9,N^{10}-diethyl-D-homoarginyl-L-leucyl-N^9,N^{10}-diethyl-L-homoarginyl-Lprolyl-D-alaylamide acetate.

Molecular formula: $C_{80}H_{113}N_{18}O_{13}Cl$ (anhydrous free base)
$C_{80}H_{113}N_{18}O_{13}Cl.\ X\ CH_3CO_2H.\ y\ H_2O$ (hydrated salt)
where $2 < x < 3$ and $y < 10$.

Molecular mass: 1570.4 (anhydrous free base); 1690.5 (anhydrous diacetate) and 1750.6 (anhydrous triacetate).

Structural formula:



Related IND:

Class: GnRH antagonist

Indication: Prevention of premature LH surges in women undergoing controlled ovarian hyperstimulation.

Clinical formulation: Org 37462 is supplied as a colorless, sterile, ready-to-use, aqueous solution intended for **subcutaneous** administration only. Each pre-filled syringe contains 250 ug/0.5 ml of ganirelix acetate, 0.1 mg glacial acetic acid, 23.5 mg mannitol, and water for injection adjusted to pH 5.0 with acetic acid, NF and/or sodium hydroxide, NF. Thus the human daily dose will be approximately 4 ug/kg.

The clinical dose of 0.25 mg was selected based on the number of LH rises observed in the different dose groups and the overall clinical outcome such as the number of oocytes, the number of good quality embryos and the vital pregnancy rates at 5-6 weeks after embryo transfer (ET).

Route of administration: Subcutaneous

Proposed clinical program: This NDA is submitted with the data of 2 European pivotal clinical studies. As agreed by the Division (Pre-NDA package, serial submission #s 073 and 075 under IND), a US study was not required for submission of this NDA.

Previous clinical experience: Sponsor has extensive experience with ganirelix acetate. Volumes 1.34 to 1.84 contain results of numerous clinical pharmacology, pharmacokinetics and bioavailability and safety & efficacy studies.

Introduction and drug history: Org 37462 (ganirelix acetate) originally developed by is a synthetic decapeptide with high antagonistic activity against the action of naturally occurring gonadotropin-releasing hormone (GnRH).

The native GnRH is a decapeptide with the following structure:

(pyro)Glu-His-Trp-Ser-Trp-Gly-Leu-Arg-Pro-Gly-NH2

Replacement of the glycine in position 6 and/or position 10 by D-amino acids and ethylamide, respectively has produced agonistic analogs with significantly increased potency, partly as a result of enhanced receptor binding affinity and increased resistance to metabolic degradation. These agonists initially stimulate the release of pituitary gonadotropins but upon repeated administration, they reduce the number of receptors on pituitary gonadotrophs and reduce the ability of occupied receptors to produce an effective intracellular signal. As a result of the down-regulation, the secretion of LH and FSH decreases and gonadal function is reduced or completely abolished.

The initial stimulatory action and the long treatment period required to achieve down-regulation of gonadotropin secretion are the major disadvantages in the use of GnRH agonists. Sponsor stated that a safe antagonistic analog, which does not produce an initial increase in gonadotropin secretion and rapidly reduces circulating gonadal steroids to near castration levels, would be more desirable and implied that Ganirelix meets these criteria.

Mechanism of action: Synthetic analogs with a deletion or substitution of the histidine in position 2 have been shown to be competitive antagonists of the native hormone by virtue of their

ability to bind to but not activate the GnRH receptor. Sequential and multiple of D-amino acids at other positions in the molecule have resulted in progressive increase in antagonistic potency.

The first and second generation antagonistic analogs of GnRH were shown to release histamine that may cause unwanted effects ranging from local wheal and flare reactions at the site of the injection to generalized cardiovascular and pulmonary symptoms.

Org 37462 is a third generation GnRH antagonist. It is a synthetic decapeptide with high antagonistic activity against naturally occurring gonadtropin releasing hormone (GnRH or LH-RH). The amino acids at positions 1, 2, 3, 6, 8 and 10 in native GnRH have been substituted in Org 37462. The EC50 for Org 37462 for histamine release from rat peritoneal mast cells is significantly higher than the EC50 for detirelix, a second-generation antagonist.

Scientific rationale: Org 37462 was originally developed by for the long term treatment of various hormone-dependent disorders, such as endometriosis, leiomyomas and prostate cancer. Therefore, all nonclinical and 11 Phase 1 clinical studies in healthy male and female volunteers conducted by were aimed to evaluate the efficacy and safety of Org 37462 for the long-term suppression of gonadotropins.

Organon is now developing it for short-term treatment for the indication of prevention of LH surges in women undergoing controlled ovarian hyperstimulation (COH)

Current practice in COH combines stimulation by exogenous FSH with the suppression of endogenous gonadotrpins by GnRH agonists. Use of agonists, however, gives rise to an initial flare-up of endogenous gonadotropin secretion and therefore requires a 2-3 week pretreatment period to establish complete suppression. Also a relatively high dose of exogenous FSH is needed for adequate stimulation when combined with GnRH agonists.

Suggested potential clinical benefits with use of GnRH antagonists as compared to GnRH agonists are as follows:

1. the absence of initial LH and FSH release due to immediate pituitary suppression by receptor blockade

2. the shorter overall treatment will provide more patient convenience

3. shorter suppression of endogenous FSH, which may lead to lower overall dose of FSH for adequate ovarian stimulation

4. more rapid recovery of pituitary function following discontinuation of the treatment

5. Treatment with Org 37462 may allow administration of a GnRH agonist instead of hCG to induce final maturation and triggering of ovulation, because no desensitization of pituitary occurred with the antagonist. The absence of exogenous hCG may lower the incidence of ovarian hyperstimulation syndrome (OHSS).

Effect of ganirelix-induced COH on the off-spring: In a study entitled "Pregnancy and delivery follow-up of protocol 38602: A phase II, multiple-center, double-blind, randomized, dose-finding study to assess the efficacy of the GnRH antagonist Org 37462 to prevent premature LH surges in women undergoing controlled ovarian hyperstimulation with recombinant FSH", the sponsor concluded that the treatment was safe for both patients and their offspring.

In this study, a total of 68 vital intra-uterine pregnancies were established (52 singleton, 11 twins and 5 triplets). For one there was no follow-up data. In total 6 miscarriages occurred and karyotyping of the abortion material in one of the miscarriages identified trisomy 18 (Edwards syndrome).

In total 73 infants (33 boys and 40 girls) were born. One male with major congential malformation was diagnosed as suffering from Beckwith Wiedemann syndrome (exomphalos and macroglossia). Another infant had some minor (physical) abnormalities. In table 19 (Vol.1.78, p.56) fetal disorders mentioned were: clubfoot (1), exomphalos (1), fetal maturation impaired (2), malformation skull (1), Nevus (1), pyloric stenosis (1) and skin malformation (1).

None of the 54 infants included in the follow-up showed abnormal psychomotor development.

Severe adverse events (SAEs) were reported for 7 infants (neonatal and infancy disorders), of which 4 were reported for twins and related to prematurity and/or intrauterine growth retardation, one with sepsis neonatal and another with apnea neonatal and arrhythmia neonatal. It was stated that " the incidence of SAEs appears to be comparable to those in other IVF trials, although, no firm conclusion can be drawn due to the relatively low numbers".

Citing literature, sponsor stated that prospective and retrospective follow-up studies have been performed with IVF patients treated with combined GnRH agonists/FSH or hMG for COH. These studies indicated that pregnancies resulting from this treatment in combination with assisted reproductive technologies (ART), are not likely to lead to more complications or congenital abnormalities than regular pregnancies.

Preclinical pharmacology and toxicology: Except for one study i.e., sensitization assay in guinea pigs, all preclinical studies with Org 37462 were performed by These studies consisted of the following:

Pharmacology
Safety pharmacology
Pharmacokinetics
Single and repeated dose toxicity studies
single dose iv and sc in rats and monkeys
Repeated dose 2 weeks sc and oral in rat and monkeys
13 week SC in rats, mice and monkeys and
6 month SC in rats and monkeys
Special toxicity (guinea pig sensitization both with Syntex formulations, monkey and rabbit eye irritation and human blood compatibility studies)
Reproduction toxicity (rat male and female fertility, rat and rabbit teratology)
Mutagenicity (Ames, chromosomal aberration, mammalian cell gene mutation and mouse micronucleus assay).

All of the above studies have been submitted and reviewed previously under IND
In this review study numbers are indicated for significant findings for ease of location in the IND reviews.

The injectable formulation used by in preclinical studies contained the excipients acetic acid, mannitol, and water for injection adjusted to pH 5.0 with sodium hydroxide and/or hydrochloric acid.

Mouse micronucleus assay (AM 0398) which had not been reviewed under IND submissions, is summarized below:

In the mouse micronucleus assay a maximum dose of 10 mg/kg was given subcutaneously. The drug had no mutagenic potential under the conditions of the assay used. The dose selection was said to be based on the results of a dose range-finding study _. Detailed results of this study were not included in the submission and a statement was made that inactivity, labored respiration and one death occurred among 4 male and 4 female mice given 10 mg/kg of Ganirelix _ Probably this was the justification for the high dose of 10 mg/kg used in the definitive mouse micronucleus assay.

In this single-dose subcutaneous range-finding study (719-M-91) CD-1 mice were used. Mice were observed for 4 days. Doses used are expressed on the basis of _ free base. Formulation concentrations used were 5 and 20 mg/ml.

Dosage (mg/kg)	# of animals M/F	# died M/F	Dosage (mg/kg)	# of animals M/F	# died M/F
0 (vehicle)	4/4	0/0	40	4/4	0/1
10	4/4	1/0	80	4/4	0/1
20	10/4	3/0	160	4/4	0/0

Although sponsor's justification for the maximum dose in the definitive mouse micronucleus assay was based on one death in 4 male and 4 females at the 10 mg/kg dose, sponsor failed to state that the death rate was similar in the 40 and 80 mg/kg dose groups and no deaths were observed at the highest dose of 160 mg/kg. Sponsor also stated that clinical signs observed in groups given ganirelix included inactivity, labored respiration and/or rough coat which occurred within 30 minutes after treatment and abated within 3 hours postdosing among surviving animals. Changes at the injection sites, which included reddening, discoloration, encrustation and thickening of the skin, occurred predominantly in the 160 mg/kg group.

These results suggest that dose levels of 1, 3 and 10 mg/kg body weight used in the definitive micronucleus assay did not represent either the maximum tolerated dose (MTD) or maximum feasible dose (MFD). As such the conclusion drawn from the assay that was negative in the mouse bone marrow micronucleus test may not be justified and the study should be repeated following ICH guidelines.

Organon clinical formulation: Organon clinical formulation differs from the previous formulation which was used in all preclinical and clinical studies conducted by in that it does not contain _ Also the synthesis method was slightly different and may result in a slightly different impurity profile. Organon has conducted a guinea pig sensitization assay comparing the drug substances produced by The results did not show delayed sensitization with the clinical formulation (SDR RR 5263).

Note: Depending upon the Chemist's review of the submission with regards to the impurity profile, more toxicity studies may be requested with the new drug substance produced by _

Labeling:

NDA Summary

Following is a brief summary of the submissions pertaining to preclinical studies submitted by under IND from 9-26-1989 to 9-13-1993 for long-term use in the treatment of various hormone-dependent disorders. This information is available in reviews for serial submission #s 000, 002, 003, 008, 010-012, 015, 016, 018, 032, 036, 039, 041, 042, 045, 048, 051-055. Copies of these reviews are on file.

Preclinical pharmacology:

Reproductive pharmacology in females: Org 37462 treatment of females by SC administration in rats resulted in a dose-related inhibition of ovulation with an ED50 of 0.29 ug/rat (1.4 ug/kg) when administered at noon on pro-estrus. Inhibition of ovulation was thought to be due to inhibition of the preovulatory surge of gonadotropins (AT 4878). Treatment with 2.5 or 10 ug/kg/day for 8 weeks did not affect mating but fertility was significantly decreased at 10 ug/kg/day and returned to normal on cessation of treatment (AT6501).

Reproductive pharmacology in males: SC administration of Org 37462 reduced testosterone secretion in male rats, dogs and monkeys. There was a good correlation between plasma drug concentration and suppression of plasma testosterone levels. The suppression of release of endogenous gonadotropins was reversible without initial stimulation seen with GnRH agonists (AT4815).

General pharmacology: In mice the drug produced a small dose-related increase in CNS stimulation at 0.001-0.1 mg/kg, pupil constriction at 1.0 mg/kg and hyperthermia at 0.1 mg/kg SC (AT4899). At doses up to 1000 ug/kg sc, ganirelix did not disrupt neurological or skeletal muscle coordination and function (AT4892), alter the onset or duration of the loss of righting reflex induced by hexobarbital (AT4891), did not protect mice against an electrically-induced tonic hind-limb extensor seizure (AT4893) and did not affect pentylenetetrazole-induced tonic flexor and extensor seizures (AT 4892).

Respiratory effects: RS-26306 had no significant respiratory effects when administered to pentobarbital-anesthetized dogs at doses of 1-1000 ug/kg (AT 4855).

Cardiovascular effects: Ganirelix had no significant cardiovascular effects in anesthetized rats at sc doses ranging from 1-1000 ug/kg or iv dose of 0.1-100 ug/kg (AT4884) or in conscious restrained monkeys at sc dose of 1-1000 ug/kg (AT 4882).

In the rat RS-26306 had about 600 fold separation between antiovulatory and hypotensive activities. The ED50 for hypotensive activity for the RS-36306 and detirelix (a second generation antagonist) when administered intravenously were 901 and 41 ug/kg respectively.

Renal effects: No adverse renal effects were reported in rats (AT 4901).

GI effects: When given SC at doses of 0.001-1.0 mg/kg in mannitol, ganirelix significantly increased volume and total mEq H acid secretion (AT 4717).

In-vitro histamine release: The ED50 for histamine release for RS-26306 and detirelix from rat peritoneal cells were 17.8 and 0.21 ug/kg respectively (AT 4917).

Note: Increased acid secretion may explain gastric erosions reported in drug treated rats.

In-vitro plasma binding of RS-26306: Binding to human, monkey and rat plasma was studied by equilibrium dialysis at 37 C (CL 5791).

Results: Binding of RS-26306 was independent of drug concentration (100 ng/ml to 10 ug/ml) and was 81.9%, 88.8% and 82.4% in human, monkey and rat plasma respectively. The extensive binding was attributed to hydrophobic interaction (due to hydrophobic amino acids, e.g. D-naphthylalanine and p-Cl-phenylalanine) and not to electrostatic interactions of RS-26306 with plasma proteins.

Pharmacokinetics and metabolism: AT 4845, AT 4846 an AT 4857

Pharmacokinetics for single dose IV and SC administered Org 37462 in mice, rats and monkeys

	Mouse		rat			Monkey[1]	
	Dose (mg/kg)/ route		Dose (mg/kg)/route			Dose (mg/kg)/route	
Parameter	1.0/ SC	10.0/ SC	1.0 / IV	1.0 / SC	10.0 / SC	1.0 / IV	1.0 / SC
Kinetic data[2]							
Tmax (h)	1	2		4	12		0.5[3]
Cmax (ng/ml)	161	2136	3747	437	915	7568 ± 362	1018 ± 297
CL (ml/min/kg)[4]			2.52			0.83 ± 0.24	
VdB (L/kg)[5]			0.29			0.32 ± 0.04	
T1/2 (h)	2.9	2.6	1.35	3.6	15.2	5.1 ± 0.8	> 19
AUC (ng.h/ml)	450	6863				17743 ± 3961	9883 ±2668
AUC 0- (ng.h/ml)	451	6879	6566	3549	26235	19756 ± 4495	

1. Mean + SEM for 4 monkeys.
2. Org 37462 plasma levels in mice determined by and in rats and by in monkeys.
3. Tmax for 3 monkey was 0.5 h, but was 24 h for the fourth monkey.
4. Systemic clearance rate.
5. Volume of distribution.

It was suggested that there was depot formation at the site of injection after sc administration since 11.9 + 4.0% of the administered labeled drug was recovered in the skin and muscle around the injection site 72 hours after dosing rats.

When Ganirelix was given sc as a single dose of 2.3, 11.5 or 57.5 mg of formulation containing polylactic acid (PLA) and N-methylpyrrolidone in 0.01, 0.05 and 0.25 ml as an injectable implant in male rats (AT 6356), plasma drug levels increased significantly by 6 hours (491, 854 &898

ng/ml). Cmax on day 1 was 704, 1975 & 3128 but at the end of study on day 63, values were 0.08, 1.21 and 2.81 ng/ml.

Plasma testosterone levels, which were around 3 ng/ml, decreased with all doses. At day 63, it was back to baseline with low dose but stayed around 0.3 ng/ml with mid and high doses.

Treatment caused marked, dose-related, irritation at the injection site and it was stated that the injectable system might not be well tolerated for controlled-release administration.

In study AT 6357, male rats were given a continuous infusion of 10, 100 or 1000 ug/rat/day for 28 days of ganirelix via implantation of [redacted] pumps. The 10 ug dose had the maximum effect on reducing plasma testosterone to 0.4 ng/ml from day 0 to end of the study. After 28 days of implantation, there was no clinical signs of irritation.

When male rats were administered Ganirelix (AT 6358) by daily SC injection vs SC infusion at similar doses (100, 33, 11, 3.7, 1.2 and 0.4 ug/rat/day), plasma Ganirelix levels were much higher with the infusion than with the daily injection. A dose of 11 ug by infusion was as effective as 100 ug by injection, and produced a maximum effect. Lower doses by infusion (0.4-3.7 ug/kg/day) and injection (1.2 –11.0 ug/kg/day) increased testicular volumes while higher dose levels decreased testicular volumes as expected, suggesting that suboptimal doses may have stimulatory effect on testosterone with resultant increased testicular volume.

Bioavailability of ganirelix administered intranasal at dose levels of 4 or 16 mg/monkey/day, was 2.4% and 1.4% for males and females on day 1 which decreased to 0.345 and 0.15% on day 26 respectively. Treatment resulted in irritation of the nasal septum and turbinate mucosa in both sexes (AT 5593).

It was pointed out that since the Vd was less in both monkeys and rats than the total body water content of 0.6 L/kg, it indicated that the drug does not bind extensively to tissue proteins in either species.

The extremely low oral absorption of RS-26306, combined with biliary excretion was suggested to contribute to the low oral bioavailability of this compound (AT 5774).

Excretion: Excretion pattern of a sc dose was similar in rats and monkeys. Over a 7-day interval, 58-69% of the label was recovered in feces and 16-22% in urine. 51% of the radioactivity was recovered in bile over 3 days in bile duct cannulated rats.

In rats, 12.7% and 84.1% of a 1 mg/kg iv dose was excreted in urine and feces respectively in 7 days. In monkeys the values were 25.9% and 62.1% in urine and feces.

SC bioavailability in monkey was determined to be 2 times higher compared to that in the rat (AT5010).

In monkeys after oral dosing, bioavailability was 0.23% relative to iv dose and 0.45% relative to sc dose (AT 5036).

Metabolic profile: Plasma from monkeys after sc dose contained mostly (87%) RS-26306 radioactivity. Some samples contained a metabolite which increased form 5% at 0.08 hour to 43% at 2 hours and then decreased to 13% at 8 hours with corresponding decrease and increase in RS-26306 radioactivity. Rat plasma contained RS-26306 and no metabolite. Urine from monkeys

and rats contained mostly RS26306. Bile pool contained very little (2-6%) RS-26306 and 2 or 3 metabolites which constituted 81% of the total radioactivity in 7-72 hr bile.

The metabolites detected in bile of rats administered 10 mg/kg sc dose of [^{3}H]-RS-26306 were 1-4 tetrapeptide, 1-6 hexapeptide and 1-7 heptapeptide fragments of RS-26306 (AT 5724).

A single metabolite in monkey plasma was identical in retention time on to 1-7 hepatapetide metabolite from the rat bile.

Tissue distribution of radioactivity in male rats after a single iv dose of 166.8 ug of tritiated RS-2636 (0.52 mg/kg) demonstrated that highest radioactivity was observed in kidneys, liver and urinary bladder and very low in brain and fat. At 0.25 hr only kidney had tissue/plasma (T/P) greater than 1 (i.e. 1.77). At 3 hr organs of elimination of the drug i.e. the small intestine, liver and urinary bladder had T/P substantially greater than 1 (up to 20 in urinary bladder). Beyond 3 hr, plasma levels dropped so that T/P was greater than 1 in most tissues (up to 117 in kidneys). Intestinal contents contained the highest amount of radioactivity (24.6% of the dose) at 7 hours. An average of 22.5% and 57.2% radioactivity was excreted in urine and feces respectively in 120 hours, mostly during the first day in urine and second day in feces. Total recovery including intestinal contents and organs averaged 80.7% (AT 5842).

Toxicology:

Acute sc toxicity in rats (AT 4877): Rats were given single injection of either RS-26306 or detirelix at dose levels of 1, 5, 15 or 40 mg/kg.

Clinical signs of local irritation consisting of discoloration, encrustations and thickening of skin were produced at the injection site by both drugs. Severity was mild to moderate and persisted for the duration of the study period. Systemic toxicity consisting of collapse, synosis, labored respiration, inactivity, erythema of the ears and swelling of the limbs, face and /or ears occurred only with detirelix at the 40 mg/kg dose.

Pathologic changes were related to local irritation and responses at the injection site and systemic effects representing pharmacological activity characterized by regressive/atrophic changes in reproductive organs.

Histopathologically, atrophic, degenerative, inflammatory and narcotizing changes that exceeded the frequency and severity of changes in vehicle control animals were present at injection sites in animals administered either compound. Reproductive organ atrophy was present in both sexes of animals receiving doses of 5 mg/kg or higher of either compound.

Acute iv toxicity in rats (AT 4865): Dose levels of 0.5, 1 and 2 mg/kg were used. Systemic changes were present primarily in the detirelix group. Clinical changes in the high dose RS-26306 consisted of discoloration of ears, decreased respiration, pallor and slight clonic convulsions, which were resolved in 3 days. Deaths were observed in the 2 mg/kg dose of RS-26306 and with all doses of detirelix.

Acute SC toxicity study in monkeys (AT 4842): Using the same doses as used in the rat study i.e., 1, 5, 15 and 40 mg/kg, no clinical signs of systemic toxicity were observed. Swelling and thickening of the site of injection occurred at doses of 5 to 40 mg/kg and increased skin thickness persisted upto 14 days. Hematological changes consisted of slight increases in leukocytes and neutrophil counts. Serum chemistry changes consisted of slightly increased transaminases levels

with dose of 15-40 mg/kg and decreased serum protein at the 40 mg/kg dose. These changes were mostly reversed by day 14 after treatment. Plasma estradiol and testosterone levels were decreased and stayed suppressed in a dose-dependent manner.

Acute iv toxicity in monkeys (AT 4841): Dose levels of 0.25, 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg of ganirelix were used. Inactivity was observed in 1/4 animals at 1.5 and 2 mg/kg. At 3 mg/kg dose, facial redness occurred in all animals and 2/4 exhibited inactivity. Similar changes were observed with 0.5 mg/kg dose of detirelix.

Two-week SC toxicity in rats (AT 4864): 15 rats/s were given daily either vehicle or 0.1, 1.0 or 10.0 mg/kg of RS-26306 and 5/s were followed for 4-week recovery period.

Following were the significant findings:

Dose-related severity of local irritation. In the 10 mg dose group, total RBC counts, Hb and Hct were decreased and WBC count increased. Also SGPT and SGOT values were increased and total protein and albumin decreased.
Gastric mucosal erosions were observed in drug treated animals. Increased spleen weight was reported in treated females. All other findings were due to drug's pharmacological effects.

Two week SC toxicity in monkeys (AT 4864): With same doses as used in the 2 week rat SC study, local irritation was slight to moderate in the 10 mg/kg dose. Slight decrease was observed in RBC counts, Hb and Hct but no effect on serum chemistry or urinalysis.

2-week oral toxicity in monkeys (AT 5420): With dose levels of 2.5, 10 and 40 mg/kg/day, no adverse effects were observed in either sex. However, similar treatment in rats resulted in a significant increase in weight of spleen in both males and females and that of liver in females in the 10 mg/kg dose groups. These weight increases were not explained by histological changes.

Thirteen-week SC toxicity in monkeys (AT 5612): Administration of ganirelix at dose levels of 0.1, 0.7 and 5 mg/kg/day resulted in no systemic toxicity. Signs of irritation at site of injection were some times severe in high dose group and histologic inflammatory changes were present at the end of 20-week recovery period.

Amenorrhea occurred in 0/4, 1/3, 5/5 and 5/5 females in the control and 3 treated groups during the last 7 weeks of dosing. Menstruation occurred after 5-9 weeks of recovery.
One male and one female in treatment group had high spleen weight at the end of treatment and one male and one female out of 2 in the mid and high dose had spleen weight at the end of recovery period. Effects on reproductive organs were expected pharmacological effects.

13-week SC toxicity study in rats (AT 5635): Using doses levels of 0, 0.1, 0.7 and 5 mg/kg/day (25 females/g), the following significant observations were made:

Local irritation was dose/concentration dependent in incidence and severity. By the 12th week of treatment none of the females in the mid and high dose were cycling. Cyclic estrus activity occurred by 5th and 12th week after cessation of treatment in the mid and high dose groups.

High dose females had lower Hb, Hct, MCV and MCHb and higher platelet counts than controls. Spleen weight was higher in mid and high dose groups and stayed higher after recovery period.

When the study was repeated using male rats (AT 5648), similar local irritation and increased spleen weights were seen in mid and high dose groups.

In both monkeys and rats, disproportionately higher trough plasma levels were observed at the high dose. Sponsor stated that the increase in concentration of plasma trough levels with repeated dose was consistent with depot formulation at the subcutaneous site.

3-month dose range-finding study with Ganirelix in mice (AT 6233): Doses levels of 0.3, 1.0, 3.0 and 10.0 mg/kg/day were used. Significant treatment-related findings were:

Dose-related local irritation, decreased MCV and MCH and increased leukocyte and lymphocyte counts in high dose males and high platelet count in females, increased liver weight in high dose males and spleen in mid and high dose groups.
Histological changes consisted of midzonal hepatocytic hypertrophy in high dose males and all treated females. Splenic changes included increased extramedullary hematopoiesis and lymphoid hyperplasia. Adrenal cortical zona fasiculata hypertrophy was increased in males given 3 or 10 mg/kg and zona reticularis hypertrophy was present in females given 0.3 or 1.0 mg/kg and in males given 3 or 10 mg/kg/day.

6-month toxicity study with Ganirelix in monkeys (AT 6322): Dose levels of 0.1, 0.5, or 2.5 mg/kg/day were used. No systemic toxicity was observed. Local irritation was greater in treated compared to control group.

6-month SC toxicity in rats (AT 6426): Dose levels of 0, 0,02, 0.2 or 2.0 mg/kg/day were used. Significant findings were injection site irritation in high dose group. Compared to controls, SGPT and SGOT values were significantly elevated and total protein, albumin and TG were decreased in high dose male rats. In high dose females, SGOT and alkaline phosphatase were increased. Also myeloid/erythroid ratio in the sternal bone was increased in both sexes given high dose. No toxic dose was suggested to be 0.02 mg/kg/day and even at this dose level injection site reaction was seen.

Miscellaneous toxicity studies

Intradermal sensitization study in guinea pigs (AT 4853): No increased erythema or edema was seen in treated groups when compared to controls in either study previously conducted by with batch K or the one repeated by Organon with modified clinical formulation batch E synthesized by

Eye irritation study in rabbits (AT 5569): 0.1 ml of 20 mg/ml RS-26306 produced moderate circumcorneal hyperemia and slight conjunctival redness which disappeared within one day after dosing.

One week intranasal irritation study in monkeys (AT 5936): 4.5 mg of RS-26306 formulation containing propylene glycol, demonstrated that both vehicle and drug formulation caused minimal to moderate focal to diffuse leukocytic infiltration and minimal focal intraepithelial cyst formation involving the mucosa.

In vitro blood compatibility (CL 4955): Prototype clinical formulations of RS-26306 for parenteral injection (1 and 10 mg/ml) were compatible with human blood and plasma in vitro.

Reproductive toxicity studies

Ganirelix Teratogenic potential: Studies conducted in rats with daily SC doses of 0, 1, 3 and 10 ug/kg from gestation days 7 through 16 (AT 4980) and in rabbits (AT 5276) with doses of 3, 10 and 30 ug/kg from gestation day 7 through 19 showed no teratogenic effects.

Effect of 9 weeks of sc injections of 2.5 ug/kg/day of _____ followed by 7 weeks without treatment, on mating and fertility in female rats as measured by ovulation, implantation and delivery of pups (AT 5596).

Results showed no significant differences between drug-treated and vehicle control groups with regards to % of rats mated (76 vs 90%), ovulating (each 90%), with implants (100 vs 90%), and delivered pups (89 vs 80%) as well as number of ova recovered, implantations present and pups delivered.

Two generation female fertility and reproduction study in rats using SC doses of 0.0, 0.1, 0.5 and 2.5 ug/kg/day beginning 14 days before cohabitation with untreated males and continued through postpartum day 20 (AT 5775).

Results showed that no drug-related clinical changes occurred except for prolonged littering in some P1 and P2 females and deaths in these females was attributed to incomplete littering.

Compared with controls, the number of females with prolonged estrous (equal or greater than 4 consecutive days of estrus) was greater in 2.5 ug/kg group P1 females during the first 15 to 18 days of treatment, the numbers being 0, 0, 1 and 7.

Relative to controls, the pregnancy rate, number of c.l. and implantations, live litter size and implantation index were lower in females given 2.5 mg/kg dose.

No treatment-related effects on postnatal development parameters were seen in offspring after initial or first recovery breeding phases.

Results of pups found dead revealed common variations and sporadic malformations (sternebrae reduced ossification, sternebrae dysmorphic ribs, rudimentary 14th, and thoracic centrum reduced which was in 3 pups from 2 high dose animals).

It was concluded that decreased fertility was seen in females of the 2.5 ug/kg dose group and it was reversible on cessation of treatment.

Effect of daily injections of _____ (2.5 or 10.0 ug/kg, sc) for 8 weeks on mating and fertility of female rats during treatment and at 7 weeks after cessation of treatment (AT 6101).

Results of this study showed that the % of rats mating in the low and high dose groups (85% and 90%) was not significantly different from 85% for the vehicle control group. The 53% of the mated rats becoming pregnant in the low dose group was not significantly different but 6% for the high dose group was different from 88% for the vehicle controls. The number of fetuses/pregnant rat ranged from 13-16 in the control and treated groups and differences were not significant. Seven weeks after cessation of treatment only the number of mated rats becoming pregnant in the 2 treated groups (33% and 55%) was different from the controls (100%).

The % of rats that mated on first day of cohabitation was 82% and 100% for low and high dose groups compared to 23% for the vehicle controls and similar findings were observed in rats after cessation of treatment.

Mutagenicity: Ames test, Chromosomal aberration and Mammalian cell gene mutation assays conducted with the maximum feasible dose of RS-26306 were negative (AM 0395, AM 0396, AM 0397). Although it was reported that RS-26306 had no mutagenic potential in the mouse micronucleus assay, the doses used were neither MTD nor MFD. **This test may have to be repeated unless sponsor can justify the doses used.**

Clinical experience with Ganirelix:

Human Pharmacokinetics:

Mean (SD) pharmacokinetics parameters of 250 ug of Org 37462 following a single subcutaneous (SC) injection (n=15) and daily SC injections (n=15) for 7 days is given in table below:

	Tmax h	T1/2 h	Cmax ng/ml	AUC ng.h/ml	CL L/hr	Vd L
Org 37462 Single dose	1.1 (0.3)	12.8 (4.3)	14.8 (3.2)	96 (12)	2.4 (0.2)	43.7 (11.4)
Org 37462 Multiple dose	1.1 (0.2)	16.2 (1.6)	11.2 (2.4)	77.1 (9.8)	3.3 (0.4)	76.5 (10.3)

Comparison of the pharmacokinetics parameters obtained for rats, mice and monkeys demonstrate that Cmax and AUC values in these species represent a high multiple of those obtained in humans with proposed clinical dose of 4 ug/kg/day.

Local adverse effects in humans: Sponsor has stated that clinical phase 1 experience has shown that local reactions occurs in humans but they are generally mild (Investigation Brochure on Org 37462. Peter M & F. Rotteveel, July 1997. SDG-RR No. 4397).

Local tolerance data from Organon's two completed control clinical studies was summarized as follows:

At one hour after injection, the incidence of itching, pain, bruising, swelling, and redness was experienced by 81.1%, 77.4%, 87.0%, 55.3%, and 44.7% of the subjects, respectively. In subjects who experienced an injection site reaction at any assessment time, the reaction was primarily of mild intensity. No injection site reaction was reported by 26.1% of the subjects at 1 hour after injection, by 56.5% of subjects at 4 hours after injection, and 73.6% of the subjects at 24 hours after injection. Overall, there was a low incidence of moderate or severe local tolerance reactions.
At 1, 4 and 24 hours after injection, 18.8%, 2.0% and 3.4% of subjects reported moderate or severe reactions, respectively.

Correspondence with the sponsor: The mouse micronucleus assay was conducted at low dose levels, the highest dose being much lower than the dose found to be non toxic in the dose range-finding study. In the dose range-finding study the high dose of 160 mg/kg was non-lethal, yet in the definitive assay a high dose of only 10 mg/kg was used.

After discussion with Dr. Jordan, the sponsor (Mr. Albert P. Mayo, Executive Director, Regulatory Affairs) was informed on 3-19-1999 that the assay would need to be repeated according to ICH guidelines. Mr. Mayo said he would look at the data and get back to me soon. Since I did not hear from the sponsor, on 3-29-1999 I again called Mr. Mayo.

On 4-13-1999, I received a response from Dr. H. Joosten, Toxicology Expert, Dept. of Toxicology & Drug Disposition. The justification for acceptance of the assay was suggested on multiples of human dose and exposure based on a very small and extremely variable data.

On 4-24-1999, I called Dr. Lohmann, Department Head, Toxicology & Drug Disposition and spoke with him. Dr. Joosten called me immediately after and discussed at length the mouse micronucleus assay. He agreed that the assay was not conducted at the MTD or MFD and that it will be repeated. I told him that they could repeat it using the limit dose of 2000 mg/kg according to OECD guidelines. It was emphasized and agreed that the test will be conducted and a draft report submitted for review before drug approval. I called back Dr. Lohmann to tell him of my conversation with Dr. Joosten.

On 5-4-1999, Dr. Joosten faxed a draft protocol for the mouse micronucleus assay to be conducted according to the OECD guidelines. On 5-10-1999, Dr. Joosten was informed that the protocol is fine and the draft results of the test should be submitted as soon as possible.

Summary and conclusion: Significant preclinical toxicological findings were:

1) a dose-related irritation at the injection site in rats, mice and monkeys;
2) possibility of depot formation at the SC injection site in rats and monkeys;
3) decreased erythroid parameters and increase in WBC and platelet counts in rats and mice;
4) elevated serum SGOT and SGPT and decreased total protein and albumin in rats; and
5) increased spleen weight in rats, mice and monkeys.

Most of the changes noted were severe in rats and minimal in monkeys suggesting species difference in sensitivity to treatment. These adverse effects mostly occurred at dose level above 5 mg/kg, which represented very high multiples of the clinical dose of 4 ug/kg/day, both on body weight as well as on systemic exposure basis.

Most other findings characterized by regressive/atrophic changes in reproductive organs were due to the drug's pharmacological activity.

Human risk assessment: Proposed treatment regimen seems reasonably safe base on the following preclinical observations:

1. systemic adverse effects observed in animals occurred at a very high multiples of the human dose expressed both on body weight basis as well as on actual systemic drug exposure basis,

2. effects were of minimal severity in monkeys compared to rats suggesting species differences in sensitivity, and

3. effects were mostly reversible on cessation of the treatment in all species tested.

Recommendations and regulatory action: Since sponsor's proposed use of Org 37462 is of very short duration (less than 6 weeks) and the dose level proposed will be much lower than that anticipated by for long term studies for other indications, the submitted preclinical data, except for the mouse micronucleus assay is more than sufficient for the proposed use in assisted reproductive technology (ART). Pharmacology has no objection to the approval of the NDA for the proposed indication. If available, the results of the repeat mouse micronucleus assay will be included in the labeling.

Sponsor however, should be informed regarding the following review and labeling comments:

Review comments

As agreed upon in telephone conversation on 4-24-1999, the mouse micronucleus assay should be repeated using the maximum tolerated or feasible dose or a limit dose. A draft report of the results should be submitted for review as soon as possible.

Labeling comments

Please include the significant reproductive toxicity findings in the draft package insert. Doses should be expressed as mg/kg and the multiples of the human therapeutic dose should be based on actual systemic exposure basis.

APPEARS THIS WAY
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