Pharmaceutical Rsesearch, Vol. 5, No. 6, 1988

Research Article

Disposition of Detirelix, A Potent Luteinizing Hormone-Releasing Hormone Antagonist, in Rats and Monkeys

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Received November 17, 1987; accepted January 14, 1988

The disposition of detirelix, a potent luteinizing hormone-releasing hormone (LHRH) antagonist, was studied in rats and monkeys. After a single 300-μg/kg intravenous dose in rats, the plasma elimination $t_{1/2}$ was 1.6 hr and the plasma clearance was 3.3 ml/min/kg. In the monkey, the mean $t_{1/2}$ and plasma clearance were 7.1 hr and 1.3 ml/min/kg, respectively, after an 80-μg/kg intravenous dose. Long plasma $t_{1/2}$ values of 18.7 and 31.6 hr were observed after single 0.2- and 1.0-mg/kg subcutaneous doses in the monkey, suggesting the possibility of subcutaneous depot formation at the injection site. Biliary excretion was the predominant route of elimination of detirelix in both species. Less than 10% of the detirelix was excreted renally. A major metabolite, isolated from the rat bile, was the 1–4 tetrapeptide fragment of detirelix. This metabolite was formed by enzymatic hydrolysis of the Ser[4]–Tyr[5] bond, one of the only two peptide bonds in detirelix not containing a D-amino acid.

KEY WORDS: luteinizing hormone—releasing hormone (LHRH) antagonist; detirelix; detirelix pharmcokinetics; rat; monkey.

INTRODUCTION

Detirelix (Fig. 1) is a synthetic decapeptide containing five D-amino acids (1). It is a potent antagonistic analogue of the naturally occurring hormone, luteinizing hormone-releasing hormone (LHRH). Detirelix can reversibly inhibit the pituitary–gonadal function in animals and humans at microgram-per-kilogram doses. In male monkeys, daily subcutaneous injection or continuous infusion of detirelix produces an immediate and sustained reduction of serum luteinizing hormone and testosterone, resulting in the suppression of spermatogenesis (2,3). In female animals, detirelix is luteal suppressive and has been shown to disrupt the course of gestation in rats and dogs (4–6).

By virtue of its inhibitory effect, detirelix is potentially useful in the clinic for fertility control and as a therapeutic agent in the treatment of precocious puberty and hormone-dependent neoplasms, such as prostatic cancer and endometriosis. Detirelix is currently undergoing Phase I clinical trials (7). The present study was conducted to investigate the single-dose disposition of detirelix in monkeys and rats, the two species used in the long-term safety evaluation of this compound.

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MATERIALS AND METHODS

Materials

Unlabeled detirelix and ^{14}C-labeled (sp act, 50.3 mCi/mmol) and ^{3}H-labeled (sp act, 44.6 Ci/mmol) detirelix were prepared by the Institute of Organic Chemistry, Syntex Research. Scintillation fluids used were BetaBlend (WestChem), Biofluor (NEN Research Products), and Permafluor (Packard). High-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from J. T. Baker. All other chemicals were obtained from common commercial suppliers.

Animals

Adult female Sprague Dawley-derived rats, body weight 174 ± 1 g (mean ± SE), were obtained from Simonsen Laboratories, Gilroy, Calif. Rats were acclimatized to the laboratory environment for at least 3 days prior to dosing. Food and water were allowed *ad libitum*.

Six adult male cynomolgus monkeys (*Macaca fascicularis*), body weight 4.6–9.1 kg, were selected from our animal colony. Food was withheld from the monkeys from about 16 hr before dosing until 4 hr after drug administration. Water was allowed *ad libitum*.

Plasma Level and Excretion Studies

Dose solutions were prepared either in 0.1 *M* phosphate buffer, pH 6.0 (rat studies), or in a vehicle containing pro-

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(A)

pGlu - His - Trp - Ser - Tyr - Gly - Leu - Arg - Pro - Gly - NH_2

(B)

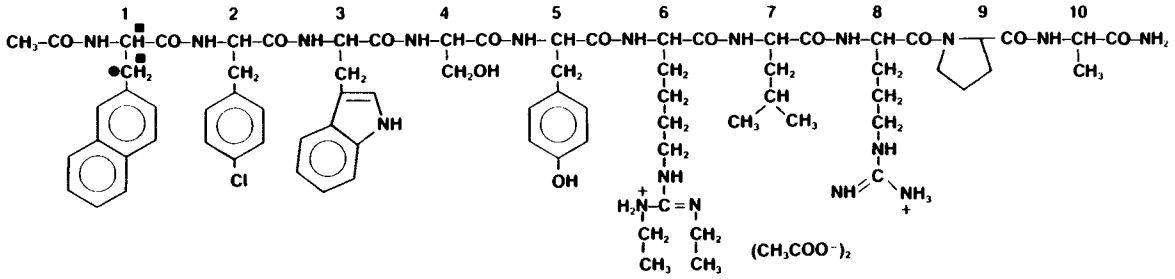

Fig. 1. Structures of (A) LHRH and (B) detirelix ([N-Ac-D-Nal(2)[1], D-pCl-Phe[2], D-Trp[3], D-hArg(Et_2)[6], D-Ala[10]]LHRH). Positions of ^{14}C in [^{14}C]detirelix and ^{3}H in [^{3}H]detirelix are indicated by ● and ■, respectively.

pylene glycol, mannitol, benzyl alcohol, and acetic acid, pH 5.0 (monkey studies). Radiolabeled dose solutions were diluted with appropriate amounts of unlabeled detirelix.

Rat. Each rat was given, via the tail vein, a bolus dose of 53 μg of detirelix containing 7.9 μCi of the ^{3}H label in a volume of 0.5 ml. At each of the following time points postdose, blood was collected from three rats by cardiac puncture: 2, 5, 10, 20, and 30 min and 1, 2, 3, 4, 6, 8, 24, and 48 hr. Plasma was separated after centrifugation at 4°C. An additional group of five rats was used for collection of excreta only. Plasma and excreta were stored at −20°C before assay.

Monkey. Three monkeys were each given an 80-μg/kg intravenous (iv) dose of [^{14}C]detirelix. Three different monkeys were given each of the following doses: 40 μg/kg [^{3}H]detirelix iv, 40 μg/kg [^{3}H]detirelix subcutaneously (sc), 0.2 mg/kg unlabeled detirelix sc, and 1.0 mg/kg unlabeled detirelix sc. [^{3}H]Detirelix was used in the second study so that the iv dose could be reduced. Washout periods of at least 2 weeks were allowed between dosings. Prior to dose administration, each monkey was fitted with indwelling catheters in the bladder (labeled drug study only) and the saphenous vein to facilitate urine and blood collection at appropriate time intervals during the first 8 hr of the experiment. After the initial 8 hr, blood was drawn by venipuncture and excreta were collected daily up to 7 days postdose in metabolic pans containing sodium azide.

Metabolite Isolation

Five rats were surgically prepared with one cannula inserted into the proximal end of the bile duct and another cannula inserted into the jugular vein. Twenty-four hours later, the rats were given a constant infusion of [^{14}C]detirelix dose solution via the jugular vein cannula at the rate of 100 μg (1.0 ml)/hr for 4 hr. Bile was collected via the bile duct cannula for up to 48 hr. One predominant metabolite, constituting about 40% of the biliary output, or 25% of the radioactivity in the dose, was isolated by repetitive reversed-phase HPLC after extracting a 1:1 mixture of the bile and 2 *M* KCl–0.1 *M* phosphate, pH 2.6, with a 1:1 mixture of chloroform and acetonitrile.

Analytical Procedures

HPLC. A Varian LC 5060 liquid chromatograph or a Rainin rabbit pump was used. Columns used were an Altex Ultrasphere 5 μ C_8 and a Rainin Microsorb C_{18} column. The mobile phase was a pH 2.6, 0.05 *M* phosphate buffer containing acetonitrile.

Total Radioactivity Assay. In studies employing radiolabeled detirelix, duplicate aliquots of plasma, urine, and bile samples were assayed for total radioactivity using a Packard Tri-Carb Model 3330 liquid scintillation counter. Samples of whole blood were decolorized with 30% H_2O_2 before assay. A Packard Model 306 sample oxidizer was used for combustion of fecal samples. Combusted samples containing ^{3}H label were assayed in Biofluor and those containing ^{14}C label were assayed in Permafluor. All other samples were assayed in BetaBlend.

Assay of Detirelix. In studies using radiolabeled detirelix, plasma samples were passed through disposable C_{18} columns pretreated with methanol, 50% aqueous methanol, H_2O, and 0.1 *M* KH_2PO_4. Afterward the column was washed with 0.1 *M* KH_2PO_4 and H_2O. Detirelix was eluted with methanol/trifluoroacetic acid/ammonium hydroxide (100:0.1:0.16). The methanolic eluent was evaporated to dryness, reconstituted with the HPLC mobile phase, and analyzed on HPLC for detirelix. The HPLC eluents (20-sec fractions) were assayed for radioactivity.

Plasma concentrations of detirelix in studies using nonlabeled detirelix were assayed by a radioimmunoassay (RIA) procedure (8). This is a direct plasma assay requiring no prior sample purifications. The lower limit of quantitation was 150 pg/ml. Samples were assayed in duplicate. The assay was repeated when coefficients of variation exceeded 20%.

Amino Acid Analysis. Approximately 5 μg of the purified metabolite was hydrolyzed under vacuum with 6 *N* HCl at 115°C for 18 hr. Amino acid analysis was performed on a

HPLC by precolumn derivatization of the hydrolysate with 4-dimethylaminoazobenzene-4'-sulfonyl chloride (DABS-Cl) as previously described (9,10).

Pharmacokinetic Data. Areas under the plasma concentration vs time curves (AUC values) and plasma elimination half-lives ($t_{½}$) were calculated using a BIOP program on the HP 9816 microcomputer. The apparent volume of distribution in the terminal elimination phase was calculated as

$$V_d\beta = \frac{\text{dose iv}}{\text{AUC} \cdot \beta}$$

where $\beta = 0.693/t_{½}$ and AUC was from time 0 to infinity. The plasma clearance rate was defined as

$$Cl_s = \frac{\text{dose iv}}{\text{AUC}}$$

RESULTS

Plasma Profiles

Rats. The plasma level vs time curves of total radioactivity and of detirelix in rats after a single 300-μg/kg iv dose of [^{3}H]detirelix (Fig. 2) show a rapid distribution phase (0–1 hr) and a slower elimination phase (1–8 hr). Aqueous washings from the extraction of plasma on C_{18} BondElut columns contained very little radioactivity, indicating that there was little or no tritiated water in the plasma. Detirelix accounted for more than half of the total radioactivity at every time point. The plasma elimination $t_{½}$ of detirelix was 96 min.

Monkeys. After a single 80-μg/kg iv dose of [^{14}C]detirelix, the drug was still detectable in plasma 24 hr after dosing (Fig. 3A). After the single 40-μg/kg iv dose of [^{3}H]detirelix, however, plasma levels of detirelix and total ^{3}H decreased rapidly with time and no detirelix was detectable beyond the 7- or 12-hr time point (Fig. 3B). Plasma concentrations of detirelix and total radioactivity after the 40-μg/kg sc dose of [^{3}H]detirelix were lower and decreased less rapidly than after the iv dose (Fig. 3C). Plasma profiles after the sc dose show large animal-to-animal variations.

Plasma samples from monkeys after a single iv dose of [^{3}H]detirelix contained only small amounts of ^3H_2O (1–12% of total radioactivity). Whole blood-to-plasma ratios of total radioactivity averaged 0.56. Thus, there was no extensive partitioning of detirelix and/or its metabolites into red blood cells.

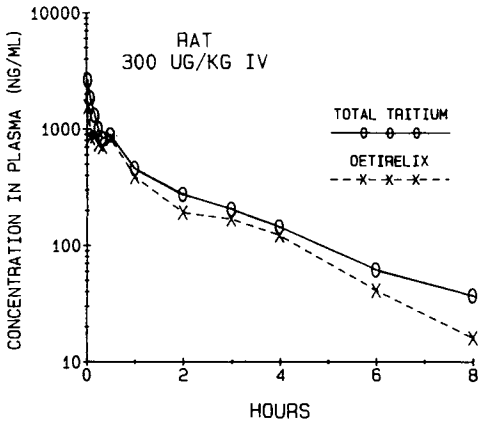

Fig. 2. Plasma concentrations of total radioactivity and of detirelix in female rats given a single iv bolus dose of [^{3}H]detirelix.

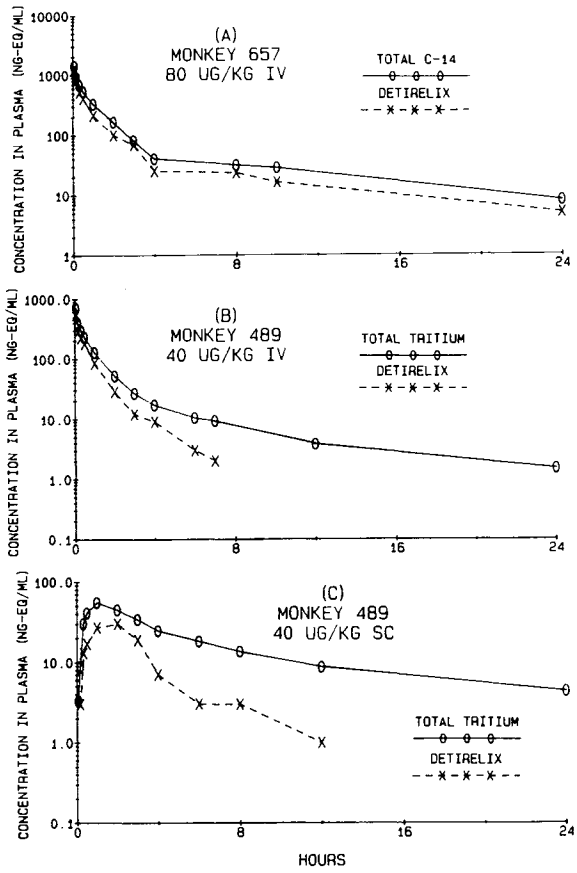

Fig. 3. Typical plots of plasma concentrations of total radioactivity and of detirelix in male cynomolgus monkeys after (A) a single 80-μg/kg iv dose of [^{14}C]detirelix, (B) a single 40-μg/kg iv dose of [^{3}H]detirelix, and (C) a single 40-μg/kg sc dose of [^{3}H]detirelix.

To determine whether the kinetics of sc detirelix in monkeys are dose dependent, two doses of unlabeled detirelix (0.2 and 1.0 mg/kg) were administered to the monkeys sc. Detirelix was still detectable in plasma 7 days after dosing (Fig. 4).

Metabolite Identification

The major metabolite of detirelix purified from the rat bile pool (Fig. 5b, inset) was hydrolyzed with 6 *N* HCl. The DABS derivatives of the hydrolysate were analyzed by reversed-phase HPLC as shown in Fig. 5b. Figure 5a is the HPLC chromatogram of standard DABS amino acids. Any tryptophan present in the metabolite would have been destroyed by the 6 *N* HCl treatment. Figure 5b shows that the metabolite contained Nal, p-Cl-Phe, and Ser. No Tyr was present. Thus, this metabolite was the 1–4 tetrapeptide fragment of detirelix formed by the cleavage of the Ser^4–Tyr^5 bond.

Pharmacokinetic Data

Rats. After a single 300-μg/kg iv dose of [^{3}H]detirelix,

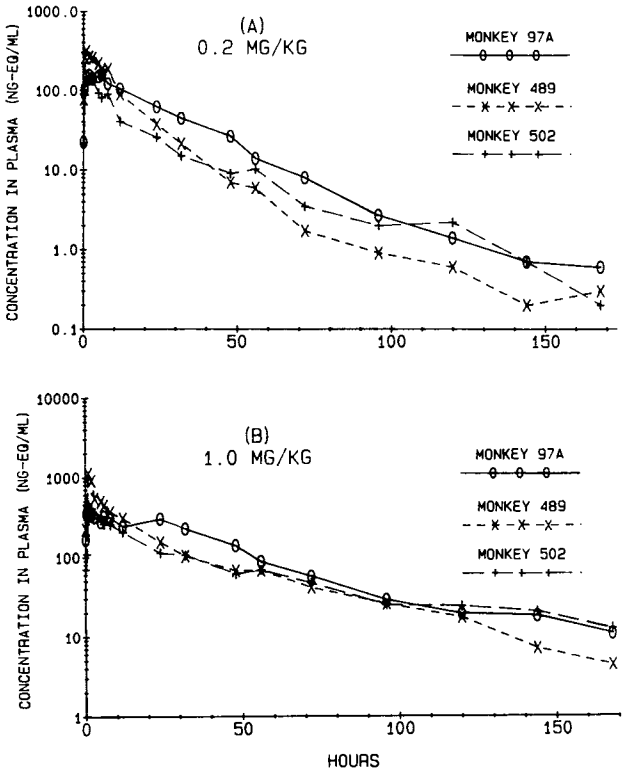

Fig. 4. Plasma concentrations of detirelix in male cynomolgus monkeys after single sc doses of (A) 0.2 mg/kg and (B) 1.0 mg/kg of unlabeled detirelix. Concentrations were determined by RIA.

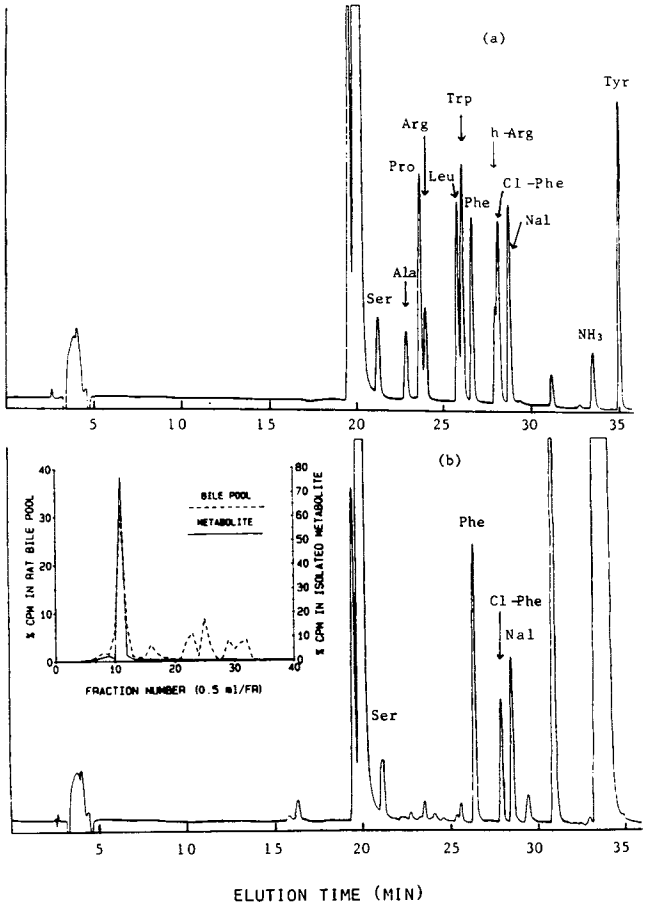

Fig. 5. HPLC chromatograms of (a) a mixture of DABS derivatives of amino acids constituting detirelix and (b) the acid hydrolysate of the major rat bile metabolite of detirelix (inset) derivatized with DABS-Cl. Phenylalanine (Phe) was added as an internal standard. h-Arg, *N,N'*-diethylhomoarginine; Cl-Phe, P-Cl-phenylalanine; Nal, 3-(2-naphthyl)alanine. Peak at 20 min was the reagent peak, DABS-OH. Peak at 31 min was probably from some unidentified endogenous compound in the rat bile.

the plasma elimination half-life of detirelix was 1.6 hr and the plasma clearance was 3.3 ml/min/kg (Table I). The apparent volume of distribution was 0.45 l/kg, suggesting that the drug distributes in total body water. Thus, detirelix probably does not bind extensively to body tissues.

Monkeys. A long plasma elimination $t_{1/2}$ of 7.1 ± 1.5 hr was observed in the monkey after a single 80-μg/kg iv dose of [^{14}C]detirelix (Table I). The $t_{1/2}$ value of 1.4 ± 0.3 hr after a single 40-μg/kg iv dose of [^{3}H]detirelix probably did not reflect the true terminal elimination $t_{1/2}$ because plasma concentrations beyond the 7-hr time point were below the detection limit by HPLC-radiometric assay. After the 40-μg/kg sc dose, large standard errors in the pharmacokinetic data (Table I) were indicative of large animal-to-animal variations. The bioavailability of the 40-μg/kg sc dose relative to the iv dose ranged from 29 to 89%, with a mean (± SE) value of 50 ± 20%. The T_{max} of detirelix after the sc dose was 1 to 2 hr.

Data for the 0.2- and 1.0-mg/kg sc doses (Table I) clearly demonstrate the dose dependency of the plasma elimination $t_{1/2}$ of detirelix. The values were 18.7 ± 1.3 and 31.6 ± 3.6 hr, respectively, for the 0.2- and 1.0-mg/kg doses. The T_{max} was 1 hr in every case except for two monkeys after the 0.2 mg/kg sc dose which had T_{max} values of 2 and 6 hr. Plasma clearance was 1.3 to 1.6 ml/min/kg.

Routes of Excretion

Table II shows that elimination in feces was the major route of excretion of detirelix in both rats and monkeys, accounting for over four-fifths of the radioactivity in the dose. Only a few percent of the total administered radioactivity was excreted in urine.

DISCUSSION

LHRH antagonists, because of their ability to suppress immediately the pituitary–gonadal axis without the initial stimulatory phase characteristic of the LHRH agonists, might have greater potential than the agonists for the treatment of hormone-dependent diseases of the reproductive organs. Development of the antagonists for clinical uses, however, lags far behind that of the agonists due to the low potency of most LHRH antagonists synthesized. It was only within the last few years that antagonists of sufficient potency became available for extensive pharmacologic evaluation. Consequently, data on the metabolism and pharmcokinetics of LHRH antagonists are limited compared with data on the agonists (12). Detirelix is one of the most potent LHRH antagonists synthesized to date, and we report here the results from our preclinical studies of this compound.

Our data on the plasma $t_{1/2}$ of detirelix (Table I) support the observed long duration of action of this compound in

Table I. Pharmacokinetic Data of Detirelix in Monkeys and Rats

Species	Dose (mg/kg)	Label	Dose route	T_{max} (hr)	C_{max} (ng/ml)	Plasma $t_{1/2}$ (hr)	$AUC_{0-\infty}$ (ng/ml · hr)	Cl_s (ml/min/kg)	$V_d\beta$ (liters/kg)
Rat	0.3	^3H	iv	0.03	1550	1.6	1564	3.3	0.45
Monkey[a]	0.08	^{14}C	iv	0.08	1128 ± 177	7.1 ± 1.5	1109 ± 212	1.3 ± 0.3	0.86 ± 0.32
	0.04	^3H	iv	0.08	488 ± 60	1.4 ± 0.3	471 ± 127	1.6 ± 0.3	0.18 ± 0.03[b]
	0.04	^3H	sc	1.7 ± 0.3	32 ± 11	4.1 ± 1.9	284 ± 180	—	—
	0.2		sc	3 ± 1.5	201 ± 55	18.7 ± 1.3	3334 ± 591	—	—
	1.0		sc	1.0	662 ± 232	31.6 ± 3.6	14417 ± 1399	—	—

[a] Values are mean ± SE of three monkeys.
[b] This value was probably an underestimation because the plasma $t_{1/2}$ might not be the terminal elimination $t_{1/2}$.

animals and in humans (2–7). Thus in the rat, the plasma elimination $t_{1/2}$ of detirelix was 1.6 hr, which is much longer than those (11) of endogenous LHRH (6.7 min) and a "long-acting" LHRH agonist, nafarelin (33.6 min). Likewise, in the monkey, the plasma elimination $t_{1/2}$ of detirelix after a single 80-μg/kg iv dose averaged 7 hr, which is long compared with the corresponding values of 33 min and 2 hr for LHRH and nafarelin, respectively (11).

Several factors can contribute to the long plasma $t_{1/2}$ of detirelix. Detirelix was designed by substituting several amino acids in the LHRH amino acid sequence with more hydrophobic residues, such as Nal(2)[1], pCl-Phe[2], and Ala[10] (Fig. 1). The increase in hydrophobicity may enhance the binding of detirelix to membrane receptors on the pituitary gonadotrophs. Increased hydrophobicity has been suggested to cause prolonged duration of action in LHRH agonists and antagonists due to formation of hydrophobic depot in the body (13,14). Furthermore, the positively charged diethylhomoarginine residue (Fig. 1) at position 6 may increase the interaction of detirelix with sialic acids on the receptors (15). The increased affinity of detirelix with membrane receptors might prolong the plasma $t_{1/2}$. Enhanced receptor binding probably plays a more significant role in enhancing the biological activity of LHRH antagonists than the agonists because the antagonists must be present continuously to block the receptors from binding with endogenous LHRH. The agonists, on the other hand, exert their paradoxical suppressive effect by desensitization of pituitary receptor molecules (16).

Table II. Recovery of Radioactivity in the Excreta of Female Rats and Male Cynomolgus Monkeys After a Single Dose of Radiolabeled Detirelix[a]

Species	N	Route	Label	Dose (mg/kg)	Radioactivity (%) recovered in Urine	Feces
Rat	5[b]	iv	^3H	0.3	4.2 ± 0.3	94.3 ± 2.7[d]
						97.1 ± 1.2[e]
Monkey	3[c]	iv	^{14}C	0.08	2.8 ± 0.8	80.2 ± 3.9[e]
	3[b]	iv	^3H	0.04	4.5 ± 0.5	77.4 ± 11.9[e]
	3[b]	sc	^3H	0.04	10.2 ± 6.0	87.9 ± 10.6[e]

[a] Values are means ± SE of N animals.
[b] Collection period, 0–7 days.
[c] Collection period, 0–5 days.
[d] From combustion of wet homogenates.
[e] From combustion of dried samples.

An equally important factor contributing to the long plasma $t_{1/2}$ of detirelix is its reduced susceptibility to enzymatic degradation. The principal mechanism of inactivation of peptide drugs is by the action of proteolytic enzymes. Many LHRH agonists contain a D-amino acid at position 6. This single substitution was able to retard enzymatic degradation significantly since most endogenous peptidases are specific for L-amino acids. The major sites of cleavage of LHRH were the pGu[1]–His[2], Tyr[5]–Gly[6], Gly[6]–Leu[7], and Pro[9]–Gly[10] bonds (12,17). In the detirelix molecule, one or both amino acids at these preferred sites of cleavage were substituted with D-amino acids (Fig. 1), thereby protecting the molecule against enzymatic cleavages.

The major metabolite of detirelix, isolated from the rat bile, was formed by the cleavage of the Ser[4]–Tyr[5] bond. This is reasonable since the Ser[4]–Tyr[5] bond is one of the only two peptide bonds in detirelix not containing a D-amino acid. Although we failed to detect any 1-8 octapeptide fragment of detirelix, it is conceivable that this peptide might also have been generated but was subsequently cleaved further at the Ser[4]–Tyr[5] bond.

Consistent with the lack of extensive metabolism of LHRH is the observation of little or no formation of ^3H_2O when animals were given [^{3}H]detirelix and, also, the predominant biliary excretion (Table II) of this compound. The lack of extensive degradation of detirelix coupled with the hydrophobic nature of the molecule does not favor renal excretion of detirelix.

Of particular interest is our pharmacokinetic data in monkeys given detirelix subcutaneously (sc). Blood levels after sc doses are often influenced by proteolytic enzymes in tissues, the volume of injection, and regional differences in blood flow and, therefore, exhibit large animal-to-animal variations. Our data for the 40-μg/kg doses (Table I) implicated prolonged and delayed absorption of the sc depot and reduced bioavailability via the sc dose route. Similar observations on the sustained-release nature of sc dose regimens have been reported for other peptide drugs such as LHRH (18) and insulin (19). The plasma $t_{1/2}$ of sc detirelix was dose dependent (Table I), being longer with larger doses. Nevertheless, ratios of $AUC_{0-\infty}$ and C_{max} for the 0.2- and 1.0-mg/kg doses indicate fairly linear kinetics in this dose range.

The peptide nature of LHRH and its agonists precludes their administration by the oral route. Most LHRH antagonists, like detirelix, contain several D-amino acids. Therefore, it was anticipated that the antagonists might be able to survive degradation by enzymes in the gastrointestinal tract and might be orally active. So far, studies along this line

have been disappointing. Oral absorption of only about 1%, estimated from dose and biological activity relationships, have been reported for several antagonists (20–22). We have also demonstrated the poor absorption of [^{14}C]detirelix in rhesus monkeys (unpublished data). Nasal administration, which has been successfully used for delivery of several LHRH agonists (23), is not practical for LHRH antagonists because of the relatively large doses required. Subcutaneous dosing appears to be the most practical route of administrating LHRH antagonists. Our data on detirelix, especially those after the sc doses, will be valuable for future research on this compound and on other LHRH antagonists.

ACKNOWLEDGMENTS

We thank Brian G. Rice and Ruben Silva for their expert assistance in animal studies and Dr. Howard Parnes for supplying us with radiolabeled detirelix.

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