Metabolite Profiles of Degarelix, a New Gonadotropin-Releasing Hormone Receptor Antagonist, in Rat, Dog, and Monkey

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ABSTRACT:

Degarelix is a novel competitive gonadotropin-releasing hormone receptor blocker (antagonist). In this study, the nonclinical metabolism and excretion of degarelix was investigated in Sprague-Dawley rat, beagle dog, and cynomolgus monkey. Degarelix was found to be stable when incubated in microsomes and cryopreserved hepatocytes from animal liver tissue. Absorption, distribution, metabolism, and excretion studies in male rat, dog, and monkey showed that after a subcutaneous dose of tritium-labeled degarelix, the peptide was rapidly absorbed with 50% in each matrix, whereas in monkey the major route of excretion was fecal (50%) and renal (22%). In plasma and urine samples from all three species, mainly intact degarelix was detected. In bile and feces samples from rats and dogs, the same truncated peptides of the parent decapeptide were detected. The major metabolites identified represented the N-terminal tetrapeptide, the pentapeptide, and the heptapeptide. From the animal studies, it could be concluded that degarelix is subject to common peptidic degradation in the liver and bile and is fully excreted via metabolic and biliary (as metabolites and parent compound) and urinary (mainly as parent compound) pathways. Systemic exposure to metabolic products seems to be low.

Introduction

Degarelix (Fig. 1) is a decapeptide that shows a high affinity/selectivity to human gonadotropin-releasing hormone receptors (Jiang et al., 2001). Gonadotropin-releasing hormone (GnRH) blockers bind to GnRH receptors in the pituitary gland without any subsequent receptor activation, which results in a prompt suppression of luteinizing hormone (and follicle-stimulating hormone) secretion from this gland, which in turn results in decreased production of testosterone from the Leydig cells of the testes (Ortmann and Diedrich, 1999; Chengalvala et al., 2003). The suppression of testosterone occurs almost immediately after administration of the peptide drug and continues as long as degarelix is available at the receptors (de Pinieux et al., 2001). On the basis of the successful nonclinical findings, degarelix entered and underwent successful clinical development, which resulted in approval for the treatment of advanced prostate cancer by the U.S. Food and Drug Administration and European Medicines Agency. Degarelix can now be prescribed under the name Firmagon (Boccon-Gibod et al., 2009; Pommerville and de Boer, 2010).

The present study was aimed at elucidating the hepatic metabolism and excretion profile of this small peptide hormone analog with profound biologic activity on the pituitary gland and testicular function. Because of the presence of several non-natural amino acids in the molecule [D-2Nal, D-4Cpa, D-3Pal, 4Aph(L-Hor), D-4Aph(Cbm), ILys, and D-Ala; Fig. 1], degarelix was suspected not to follow the classical route of peptide hydrolysis, which made these explorations of particular interest. The metabolite profile of degarelix in plasma and excreta was compared with reported results of other luteinizing hormone-releasing hormone antagonists (Chan et al., 1991; Schwahn et al., 2000). Furthermore, it was also of interest to explore whether the compound is a substrate for the cytochrome P450 (P450) enzyme in hepatocytes with direct implications for the risk of drug-drug interactions.

This report summarizes studies conducted to investigate in vivo metabolism of degarelix in rat, dog, and monkey species as well as in vitro stability studies in liver microsomes and cryopreserved hepatocytes.

Abbreviations: GnRH, gonadotropin-releasing hormone; LC, liquid chromatography; MS, mass spectrometry; MS/MS, tandem mass spectrometry; P450, cytochrome P450; RAD, radiochemical detection; SPE, solid-phase extraction; Ac, acetyl; 2Nal, 2-naphtylalanine; 4Cpa, 4-chloro-phenylalanine; 3Pal, 3-pyridylalanine; 4Aph, 4-aminophenylalanine; Hor, hydroorotyl; Cbm, carbamoyl; ILys, N(alpha)-isopropyllysine; FE

200486, degarelix; ADME, absorption, distribution, metabolism, and excretion.
cytes from the same animal species as a part of the preclinical development performed in compliance with regulatory demands.

Materials and Methods

Radiolabeled Test Material. Tritium-labeled degarelix ([H]degarelix; Fig. 1) was prepared by a modification of the method used for synthesis of the tritiated version of the structurally related GnRH antagonist in Parnes and Shelton (1986). In brief, 2-N-acetylamino-3-(2-naphthyl)acrylic acid was stereospecifically tritiated to N-acetyl-L-[2,3,4-3H]2-naphthylalanine with the homogeneous catalyst (2S,4S)-N-tert-butylxycarbonyl-4-diphenyl phosphino-2-diphenyl phosphinomethylpyrrolidine-Rhodium in methanol. After evaporation of the solvent, the crude product was coupled to the unprotected (2–10) decapeptide fragment D-2Nal-D-4Cpa-D-3Pal-Ser-4Aph(L-Hor)-D-4Aph(Cbm)-Leu-Lys-Pro-D-Ala-NH2 with benzotriazol-1-yloxytritylrolidinophosphonium/diisopropyl-ethylamine in dimethylformamide in 2 h at room temperature. During this coupling step, approximately 20% racemization of the N-acetyl-d-naphthylalanine occurred, but the resulting [Ac-2Nal]degarelix epimer could easily be separated during the final purification. No byproduct from coupling to the secondary nitrogen of isopropyllysine8 was found, possibly because of steric hindrance. Tritiated degarelix was obtained by reversed phase liquid chromatography (LC) (C18) purification using triethylamine phosphate (pH 2.3)/acetonitrile buffer followed by counterion exchange for acetate using the same column. The radiosynthesis was performed at Moravek Biochemicals (Brea, CA). In brief, 2-[3H]degarelix was synthesized by Polypeptide Laboratories (Torrance, CA). The unlabeled reference compounds were synthesized by Polypeptide Laboratories (Wolfenbüttel, Germany). Internal standard [Ile3(2H10)]barusiban was added to a concentration of 952 nM. The precipitated microsomal solutions were vortexed and put in ice for at least 30 min. Internal standard, [Ile3(2H10)]barusiban, was added to a concentration of 952 nM. The test tubes were centrifuged, and the supernatant was diluted with an equal volume of water in an LC autosampler vial. The samples were analyzed for degarelix substance stability and screening of metabolites using LC-MS.

Stability studies with cryopreserved hepatocytes. The stability of degarelix in hepatocytes was studied using cryopreserved preparations from male Sprague-Dawley rats and male beagle dog origin. Cryopreserved hepatocytes were thawed and prepared according to a protocol from In Vitro Technologies. The thawed cell suspensions were counted and diluted to 2 million viable cells per milliliter in a Krebs Henseleit buffer (pH 7.4) containing 2.2 g/l sodium hydrogen carbonate, 22.6 mM HEPES, and 1 mM calcium chloride. The metabolic activity of the hepatocyte suspensions was investigated by degradation studies of test substrates naloxone (glucuronic acid activity) (Wahlström et al., 1989), propranolol (CYP2D6 activity) (Masubuchi et al., 1994), and verapamil (CYP3A4 activity) (Kroemer et al., 1993). These activity control samples were analyzed by LC-MS/MS.

Substance stability studies were performed at concentrations of 40 µg of [H]degarelix. Hepatocyte suspensions were tempered at 37°C for 5 min before adding the test substrate. Samples were removed after 0, 10, 30, 60, and 120 min of incubation. Aliquots of 100 µl were withdrawn and terminated in 100 µl acetonitrile/glacial acetic acid [96:4 (v/v)] in Eppendorf polypropylene tubes. After centrifugation, 100 µl of supernatant was diluted with 100 µl Milli-Q water (Millipore Corporation, Billerica, MA) in an LC autosampler vial. The samples were stored at 8°C until analysis by LC-RAD and LC-MS.

In Vivo Samples. Animals and treatments. All animal studies were performed at contract laboratory facilities. The animal experiments were performed according to ethical guidelines of animal experimentation, and all animal work was performed in compliance with United Kingdom or Dutch legislation and was approved by a local ethical review process as appropriate. Sprague-Dawley rats, beagle dogs, and cynomolgus monkeys were used. All samples collected were frozen within 1 h after collection and stored at −80°C until the time of analysis. For metabolite screening analysis, the biologic samples were shipped on dry ice to the Ferring Pharmaceuticals laboratory facility and stored at −80°C until analysis, except for the samples from rat study R1 (see Rats), in which the sample analyses were performed at the contract research organization. All mass balance determinations were performed at the contract research organizations.

Rats. Three studies in rats were performed. The first study (R1) was an absorption, distribution, metabolism, and excretion (ADME) study and comprised one group (A) dosed intravenously and two groups (B and C) dosed subcutaneously, respectively. Male and female rats were used. All rats (200–
400 g b.wt.) were dosed with 30 μg [3H]degarelix free base peptide/kg (−300 μCi/kg). Feces, hepatic plasma, and urine samples from groups A and B were collected up to 240 h after dosing. Bile sampling from cannulated rats (group C) as well as urine and feces were collected for up to 48 h. All samples in this study were analyzed by LC-RAD at the contract research organization.

The second rat study (R2) was a biliary excretion study. Three male rats under anesthesia had a flexible plastic cannula inserted in the common bile duct to allow for complete collection of bile. A second cannula was inserted into the stomach to be able to infuse sodium taurocholate in saline solution during the study as a replacement for lost bile salts. The animals were administered a single subcutaneous dose of [3H]degarelix (26 μg/kg b.wt. with nonradiolabeled degarelix added to a total dose of 30 μg/kg b.wt.) and placed in metabolism cages. Bile, urine, and feces samples were collected for 48 h after dosing. The collected samples were analyzed by LC-RAD and LC-MS.

The third rat study (R3) was performed to study the enterohepatic circulation of degarelix and its metabolites. Three pairs of male rats were surgically prepared under anesthesia. Bile duct cannulation was surgically established so that bile was directly and quantitatively transferred from the bile duct of the first (“donor”) rat to the duodenum of the second (“recipient”) rat. A further cannulation of the bile duct of the recipient rat allowed for the completion of collection of bile produced in the recipient animal. A single subcutaneous dose of [3H]degarelix (26 μg/kg b.wt. with nonradiolabeled degarelix added to a total dose of 30 μg/kg b.wt.) was administered to the donor rats, and during the following 48 h, urine and feces were collected from donor and recipient rats, and bile was collected from the recipient rats.

ADME Study in Dogs. Male and female dogs (10–15 kg b.wt.) were treated with either a single subcutaneous (group A) or intravenous dose (group B) of [3H]degarelix at 3 μg/kg b.wt. Feces, hepatic plasma, and urine samples from groups A and B were collected up to 240 h after dosing.

In addition, two male dogs had a surgically inserted catheter placed in the gall bladder via the bile duct for bile collection. Before surgery, the dogs fasted for at least 24 h and were allowed a recovery period of 3 days before dosing. The dogs were administered a subcutaneous injection of 1 mg of nonradiola-beled degarelix per kilogram body weight (injection volume 0.2 ml/kg), and hepatic plasma and bile samples were collected up to 24 h postdose for metabolite screening analysis.

ADME Study in Monkeys. Four male cynomolgus monkeys (4.2–7.5 kg b.wt.) were dosed for a disposition of radioactivity study. The animals were administered a single subcutaneous dose of 8.2 μg/kg (200 μCi/kg) [3H]degarelix. Urine and feces samples were collected quantitatively from each animal after dosing until the time of sacrifice. Blood samples were collected at the time of the sacrifice of the individual animal (6, 24, 48, and 240 h) into tubes containing EDTA as an anticoagulant.

Analysis of Total Radioactivity. Plasma, blood, urine, cage washings, homogenates of feces and tissue, and carcass digestes were combusted in oxygen using a Packard model 307 automatic sample oxidizer (PerkinElmer Life and Analytical Sciences, Waltham, MA). The products of combustion were absorbed into 15 ml Monophasic (Canberra Industries, Meriden, CT) for radioactivity measurement. Radioactivity was measured by liquid scintillation spectrometry using a Wallac 1409 automatic scintillation counter (PerkinElmer Life and Analytical Sciences). Radioactivity in hepatocyte uptake studies was measured using a Tricarb model 1600TR liquid scintillation analyzer (PerkinElmer Life and Analytical Sciences).

Analysis of Plasma Samples for Metabolites. Plasma samples were thawed and then centrifuged at 3000g for 20 min at 8°C. Plasma (0.5–1.0 ml) was transferred to a 6-ml glass vial and precipitated by adding two equivalents of acetonitrile/glacial acetic acid [96:4 (v/v)]. Internal standard [50 μl of 4 μg/ml [Ile3(2H10)]barusiban] was added to each tube. The tubes were vortexed and centrifuged at 3000g for 20 min at 8°C. The supernatants were transferred to a 6-ml glass vial and precipitated by adding two equivalents of acetonitrile/glacial acetic acid [96:4 (v/v)], and 0.05% (v/v) trifluoroacetic acid and transferred to a liquid scintillation analyzer. The column used was an Agilent HP Zorbax SB-C18, 5 μm (2.1 mm) with guard column SB-C8, 5 μm (2.1 mm). The LC flow rate was 0.22 ml/min. The gradient used was as followed: the Agilent system. The
agent/eluate ratio was 2.0; thus, 0.44 ml/min of scintillation cocktail was mixed with the LC column eluant. Samples were analyzed in nonStopFlow mode.

Metabolite Profiling and Metabolite Identification by Mass Spectrometry Detection. Plasma, bile, urine, and feces samples and liver microsome and hepatocyte samples were analyzed for metabolite identification using LC-MS. The LC instrument was a Waters 2690 Alliance system, and the MS system was a Micromass (Manchester, UK) Q-time of flight 1.0 upgraded to a 3.6-GHz time-to-digital converter. The column used was an YMC basic C18, 5 μm (150 × 2.1 mm) with guard column C18, 5 μm (10 × 2.1 mm). The LC flow rate was 0.22 ml/min. The samples were analyzed using a gradient starting at 5% acetonitrile isocratic for 5 min, and then a linear increase to 60% acetonitrile for 50 min. The mobile phase was acidified with 0.05% (v/v) trifluoroacetic acid. The MS interface used was an electrospray ionization interface operating in positive ionization mode.

The LC retention times of the synthesized standard truncated peptides were known, as were their MS and product ion spectra. In addition, all MS data files were screened for m/z values of possible truncated peptide ions as FE 200486(1–3)-OH, FE 200486(1–5)-OH, FE 200486(1–8)-OH, and FE 200486(1–6)–OH as well as oxidation products of degarelix and oxidation products of truncated metabolites of degarelix. The possible presence of a glucuronide derivative of degarelix was also investigated. Also, total ion current data from postdose samples were compared with the total ion current data from predose samples for any unique ions present in the postdose samples. ACD/Labs (Toronto, Ontario, Canada) software MS Manager was used for comparing data sets to detect unique differences. Product ion spectra of detected metabolite ions were recorded. The collision energy used was optimized for each metabolite.

Results

Radiolabeling of Degarelix. Tritium (two atoms per peptide molecule) was chosen as a radiolabel because of higher specific radioactivity than the alternative 14C. The label was placed on the alpha and beta carbon atoms of D-2Nal in position 1 of the peptide ([3H]degarelix; Fig. 1), where tritium would not be expected to exchange with hydrogen under physiologic conditions. The choice of D-2Nal as the tritiation site was dictated by ease of synthesis. At the same time, loss of tritiation site was dictated by ease of synthesis. At the same time, loss of radioactivity detected in void peak adds up to 100%.

Metylation of Degarelix. No degradation of degarelix was detected when the substrate was incubated in liver microsomes from male rat and male dog for up to 120 min at 37°C. The metabolic activity of the cryopreserved hepatocytes as evaluated using three test substrates demonstrated that all of the hepatocyte batches used expressed metabolic activities such as CYP3A4- and CYP2D6-mediated metabolism and glucuronic acid conjugation activity.

Recovery of Radioactivity in Rat. After a subcutaneous dose of 30 μg/kg (study R1), [3H]degarelix radioactivity appeared to be rapidly absorbed with a remainder of 10 to 14% of the radioactivity at the injection site after 10 days. Maximal plasma concentration was achieved within 1 to 2 h. The predominant routes of excretion were via the kidneys (50%) and the bile (40–50%). Most radioactivity was excreted within 48 h.

Metabolite Profiling in Rats. In the first study (R1), the samples were only analyzed by LC-RAD. The identity of the peaks detected was assigned by retention time comparison with standards, but the identities were not confirmed by LC-MS analysis. In plasma, most radioactivity detected was the parent compound [3H]degarelix, representing >94% of total radioactivity measured in female rats and >87% in male rats, respectively. One metabolite assumed to represent FE 200486(1–9)-OH that was detected in the plasma samples represented approximately 6% of the peak integrated radioactivity in female rats whereas in male rats approximately 13% was detected (Table 1). In urine, the major part of excreted radioactivity was the parent compound for both genders. Low levels of six metabolites were also detected. In bile and feces, a clear difference in metabolic profile was detected depending on the gender. In bile, the same five metabolites were detected in both genders, but in female rats most of the excreted radioactivity was the parent compound and the two metabolites were assumed to represent FE 200486(1–4)-OH and FE 200486(1–6)-OH, which were present in almost equal amounts. In bile collected from male rats, the amount of metabolite FE 200486(1–6)-OH was almost twice as large as the parent compound. In feces, the same six metabolites were detected in samples from female and male rats. The major part of excreted radioactivity in feces from male rats was metabolite FE 200486(1–4)-OH, whereas in female rats equal amounts of FE 200486(1–4)-OH and FE 200486(1–10)-OH were the largest metabolites detected.

In the second rat study (R2), bile, feces, and urine samples were analyzed by LC-RAD and LC-MS. In urine, most radioactivity (94%) detected was the parent compound [3H]degarelix (see Fig. 2). In addition, seven smaller metabolites were detected, representing <5% of total radioactivity detected. In bile, four metabolites were identified, and the metabolites were identified by LC-MS/MS to represent the truncated peptides of degarelix: FE 200486(1–4)-OH, FE 200486(1–5)-OH, FE 200486(1–6)-OH, and FE 200486 (1–8)-OH.

TABLE 1
Metabolite pattern in plasma, urine, bile, and feces after subcutaneous administration of 30 μg [3H]-degarelix/kg in rats (rat study R1)

<table>
<thead>
<tr>
<th>Gender</th>
<th>Sample</th>
<th>Sampling Period</th>
<th>Percentage of Component in Samplea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>[3H] Degarelix</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>FE 200486 (1–4)-OH</td>
</tr>
<tr>
<td>Male</td>
<td>Plasma</td>
<td>0–24</td>
<td>87.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>12.6</td>
</tr>
<tr>
<td>Male</td>
<td>Urine</td>
<td>0–48</td>
<td>96.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>Male</td>
<td>Bile</td>
<td>0–48</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25.9</td>
</tr>
<tr>
<td>Male</td>
<td>Feces</td>
<td>0–48</td>
<td>5.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25.7</td>
</tr>
<tr>
<td>Female</td>
<td>Plasma</td>
<td>0–24</td>
<td>94.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.75</td>
</tr>
<tr>
<td>Female</td>
<td>Urine</td>
<td>0–48</td>
<td>92.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.97</td>
</tr>
<tr>
<td>Female</td>
<td>Bile</td>
<td>0–48</td>
<td>28.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24.9</td>
</tr>
<tr>
<td>Female</td>
<td>Feces</td>
<td>0–48</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>26.6</td>
</tr>
</tbody>
</table>

U, unknown.

a Numbers represent mean values of four individual animals.

b Radioactivity detected in void peak adds up to 100%.
The product ion spectra data of metabolites detected as well as the product ion spectrum of degarelix (FE 200486) are summarized in Table 2. In addition, the product ion spectrum of degarelix is shown in Fig. 3. In addition to the b-ions and y-ions present in the product ion spectrum of degarelix, the product ions m/z 121.1, 154.1, and 170.1 represent immonium ions of amino acids 3Pal3, 4Cpa2, and Nal1, respectively. The origin of product ion m/z 171.2 is related to amino acid Ilys8.

A triethylamine phosphate buffer containing mobile phases was used for LC-RAD analysis in rat study R1, which enabled baseline separation of the metabolites. However, to use a mass spectrometer as a detector, ammonium acetate was the buffer salt used in the mobile phases for LC-RAD and LC-MS analysis of the samples from rat study R2. Using this mobile-phase mixture, the metabolites FE 200486(1–4)-OH and FE 200486(1–6)-OH unfortunately coeluted, as did the metabolites FE 200486(1–7)-OH and FE 200486(1–9)-OH (see Fig. 4). The peak representing the tetrapeptide and the hexapeptide was 46%, the peak representing the heptapeptide and the nonapeptide was 31%, and the peak representing [3H]degarelix was 22% of the total radioactivity detected, respectively, in the 0- to 6-h bile samples, which are close to the results found in study R1 for male rats (Table 1). Feces samples were investigated to confirm the completeness of the surgical procedure of bile-duct cannulation. No metabolites of [3H]degarelix or the parent compound were detected by LC-RAD or LC-MS in the feces extracts.

**Enterohepatic Circulation of Degarelix in Rat (Study R3).** After a single subcutaneous dose of 30 μg/kg, [3H]degarelix radioactivity was almost entirely absorbed from the dose site, and approximately one half of the dose was excreted in bile. Only approximately 1% of this biliary excreted fraction was reabsorbed from the gastrointestinal tract; thus, enterohepatic recirculation of [3H]degarelix and/or its metabolites was demonstrated.

### TABLE 2

Identifications of degarelix and metabolites of degarelix using product ion mass spectrometry data

<table>
<thead>
<tr>
<th>Analyte</th>
<th>m/z</th>
<th>Characteristic Product Ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degarelix</td>
<td>816.4</td>
<td>121.1, 154.1, 170.1, 171.2, 186.1 (y3), 240.1 (b1), 356.3 (y5), 421.2 (b2), 469.4 (y8), 569.2 (b2), 606.3 (y8), 656.1 (b2), 674.4 (y3), 694.9 (y3), 794.9 (y3), 807.9 (b2), 958.4 (b2), 976.6 (b2), 1063.6 (y8), 1163.6 (b2), 1211.7 (y8), 1276.5 (b2), 1393.0 (y8)</td>
</tr>
<tr>
<td>FE 200486(1–10)-OH</td>
<td>816.9</td>
<td>121.1, 154.1, 170.1, 171.2, 187.1 (y3), 240.1 (b2), 357.3 (y5), 421.2 (b2), 470.4 (y8), 569.2 (b2), 606.8 (y8), 656.1 (b2), 675.4 (y8), 697.4 (y8), 795.4 (y8), 807.9 (b2), 958.4 (b2), 977.6 (b2), 1064.6 (y8), 1163.6 (b2), 1212.7 (y8), 1276.5 (b2)</td>
</tr>
<tr>
<td>FE 200486(1–9)-OH</td>
<td>781.4</td>
<td>121.1, 154.1, 170.1, 171.2, 240.1 (b2), 286.3 (y5), 399.4 (y5), 421.2 (b2), 569.2 (b2), 604.6 (y5), 656.1 (b2), 662.0 (y5), 760.1 (y5), 773.6 (b2), 906.8 (y5), 958.4 (b2), 993.8 (y5), 1141.9 (y5), 1163.6 (b2), 1323.2 (y5), 1276.5 (b2)</td>
</tr>
<tr>
<td>FE 200486(1–7)-OH</td>
<td>1294.5</td>
<td>121.1, 154.1, 240.1 (b2), 337.2 (y3), 421.2 (b2), 569.2 (b2), 639.3 (y3), 656.1 (b2), 726.3 (y3), 874.4 (y3), 958.4 (b2), 1055.4 (y3), 1163.6 (b2), 1251.5 (y3), 1276.5 (b2)</td>
</tr>
<tr>
<td>FE 200486(1–6)-OH</td>
<td>1181.4</td>
<td>121.1, 154.1, 240.1 (b2), 421.2 (b2), 562.3 (y3), 569.2 (b2), 613.2 (y3), 656.1 (b2), 761.3 (y3), 942.3 (y3), 958.4 (b2), 1139.4 (y3), 1163.6 (b2)</td>
</tr>
<tr>
<td>FE 200486(1–5)-OH</td>
<td>976.4</td>
<td>121.1, 154.1, 240.1 (b2), 321.2 (y3), 408.1 (y3), 421.2 (b2), 556.2 (y3), 569.2 (b2), 656.1 (b2), 737.4 (y3), 934.3 (y3), 958.4 (b2)</td>
</tr>
<tr>
<td>FE 200486(1–4)-OH</td>
<td>674.3</td>
<td>121.1, 154.1, 240.1 (b2), 254.1 (y3), 421.2 (b2), 435.1 (y3), 569.2 (b2), 632.2 (y3), 656.1 (b2)</td>
</tr>
</tbody>
</table>
metabolites plays virtually no part in the disposition of the drug in the rat.

**Recovery of Radioactivity in Dogs.** Beagle dogs were dosed with a single subcutaneous dose of 3 μg/kg [3H]degarelix. The radiolabeled compound reached a maximum in plasma after 2 h. The radioactivity was excreted rapidly, with most excreted within 48 h. The routes of excretion were via the kidneys (40–42%) and the bile (50–52%).

**Metabolite Profiling in Dogs.** In plasma, only the parent compound degarelix was detected in postdose plasma samples; no metabolites of degarelix were detected. In bile samples, intact degarelix and five metabolites of degarelix were detected in postdose bile samples. The identity of the four metabolites FE 200486(1–4)-OH, FE 200486(1–6)-OH, FE 200486(1–7)-OH, and FE 200486(1–9)-OH were confirmed by retention time comparison and MS/MS product ion spectra data with synthesized compounds. The fifth metabolite was identified to be FE 200486(1–5)-OH on the basis of the product ion spectrum recorded (Table 2). The heptapeptide, the largest metabolite detected, constituted 47% of total integrated peak area, whereas the other peaks detected were smaller: the pentapeptide (26%), the hexapeptide (10%), the nonapeptide (6%), the tetrapeptide (4%), and degarelix (7%).

**Recovery of Radioactivity in Monkey.** Four male monkeys were dosed with a single subcutaneous dose of 8.2 μg/kg [3H]degarelix.
The major route of excretion was fecal, with 50% of the radioactivity recovered at 240 h. Excretion by the fecal route was slow, with only 1% excreted at 6 h postdose. This rose to 30% at 48 h postdose and 50% at 240 h postdose. Urinary excretion was lower but more rapid. At 6 h postdose, 10% of the radioactivity was excreted, rising to 20% at 240 h postdose. The mean overall recovery of radioactivity from the time of dosing up to the time of sacrifice, including excreta, tissues, and remaining carcass, was 83.3 ± 5.9% of dose.

**Metabolite Profiling in Monkeys.** In plasma samples, only the parent compound was detected. No metabolites of [3H]degarelix were detected in the postdose plasma samples. Also, most of the radioactivity detected (92%) in the urine samples collected during the first 24 h after dosing was the parent compound. At least five low-abundant metabolites of [3H]degarelix were detected in the urine samples. The major metabolite peak detected (5% of total radioactivity detected) was identified to be a coelution of the metabolites FE 200486(1–9)-OH and the FE 200486(1–10)-OH by retention time comparison with synthesized standards.

**Discussion**

This is the first detailed report on the nonclinical testing of degarelix that investigates the metabolic and excretion profile of the compound in numerous animal species using in vitro and in vivo approaches. On the basis of previous experiences with peptide drug candidates containing less than 10 amino acids and that the degarelix molecule contains 7 non-natural amino acids, the peptide was subjected to stability studies in liver microsomes and hepatocytes as performed for a small molecule drug. However, degarelix was found to be a very poor substrate to any degrading enzymes, e.g., P450 enzymes (Mabic et al., 1999) present in the buffered liver microsome suspensions or in hepatocyte suspensions. The minor loss of degarelix in the rat microsomes could not be explained and was not further pursued. Similar results have been reported for cetrorelix (ganirelix), a luteinizing hormone-releasing hormone receptor antagonist developed by ASTA Medica AG that has several structural similarities to degarelix. In incubations with liver fractions from rat and dog, no degradation of cetrorelix was detected (Schwahn et al., 2000).

The ADME studies of degarelix performed in rat, dog, and monkey showed that degarelix and its metabolites were fully excreted via the biliary and urinary pathways in almost equal amounts. The total recovery of administered radioactivity was >90% in rat and dog. The overall recovery of radioactivity was somewhat lower than expected in the monkey; however, it was considered likely that incomplete collection of excreta from the primates accounted for most of the radioactivity not recovered.

Other peptide-based GnRH blockers have been reported to be mainly excreted by the biliary route in nonclinical studies. An ADME study of cetrorelix in rats and dog (Schwahn et al., 2000) showed that rats excreted 70% of radiolabeled drug via feces and 24% into urine, whereas in dogs equal amounts were excreted into feces and urine. In bile collected from bile-duct-cannulated female rats, 70% of the radioactive dose of cetrorelix administered was recovered. A disposition study of RS-26306 (ganirelix), a luteinizing hormone-releasing hormone antagonist developed by Syntex Research, showed that 16% and 55 to 69% of the radioactive dose was recovered in urine and feces, respectively, in rat and monkey (Chan et al., 1991).

Enterohepatic circulation was found to play no part in the disposition of degarelix or metabolites of degarelix in the rat. The same result has been reported for cetrorelix in the rat (Schwahn et al., 2000).

The in vivo metabolism of degarelix was similar in all species investigated. In plasma, mainly intact degarelix was detected. Low levels of circulating metabolites were detected in plasma from rats,
whereas no circulating metabolites could be detected in plasma from
dogs and monkey. Also in urine, mainly intact degarelix (>90%) was
detected. Only low levels of truncated peptide metabolites were de-
tected in the urine samples from animals. In bile and feces samples,
mainly degradation products of degarelix (i.e., truncated peptides)
were detected. In rat and dog, the tetrapeptide, the pentapeptide, and
the heptapeptide metabolites of degarelix were found to represent the
major part of the radioactivity excreted in feces.

Similar metabolite profiles have been reported for rats and dogs
receiving cetrorelix or RS-26306 (Chan et al., 1991; Schwahn et al.,
2000). For both compounds, plasma and urine samples were found to
mainly contain intact parent, and in bile and feces samples the four
main metabolites were truncated peptides of the parent decapeptide.
The metabolites detected were (1–4), (1–6), (1–7), and (1–9) for
cetrorelix (Schwahn et al., 2000) and the (1–4), (1–6), and (1–7) form
of RS-26306 (Chan et al., 1991), i.e., the same size truncated peptides
of degarelix found in bile from in vivo rat and dog studies in this
study.

The in vivo excreta results reported in this study show that degare-
lix is mainly excreted unchanged via the urine and is subject to
extensive sequential peptidic degradation during its elimination via
the hepatobiliary pathway. The results are in line with data for other
compounds of similar size and structure as degarelix (Chan et al.,
1991; Schwahn et al., 2000) and in line with the fact that compounds
of this size (molecular weight 1632) are more likely to be eliminated
by the hepatobiliary pathway (Sahi, 2005). Peptidases that play an
important role in the metabolism of several other peptide drugs (Lin,
2009; Malm-Erjefält et al., 2010) do not degrade degarelix in the
blood circulation.

Seven of the 10 amino acids in degarelix are unnatural; despite
this, proteolytic fragments from cleavage at most positions were
found with two notable exceptions. Proteolysis of the very unnat-
ural all D and lipophilic (1–3) partial sequence does not appear to
have occurred. In addition, no products of oxidative metabolism of
the (1–4) fragment were observed, suggesting that it may not be a
substrate of the P450 enzymes. The fragment by cleavage between
ILys and ProD was not observed either, suggesting that none of
the proteases that came into contact with degarelix were able to
productively bind the partial sequence around these amino acids. It
is noteworthy that the (1–5) and (1–6) fragments were found in
high proportion in the bile, urine, or feces of several species, which
would require proteases capable of accommodating in their S1
pocket a bulky side chain (Aph(Hydroorotyl))D or a D-amino acid
(φ-Aph(Cbm)D). Because all mass spectrometry data collected were
screened for the ions representing all theoretical cleavages of the
peptide backbone, we most likely can rule out that other proteolytic
cleavages should have occurred to a great extent because they were
not detected by LC-MS. No other metabolites of degarelix than the
truncated peptide metabolites reported above were detected when
the LC-MS data were evaluated using the ACD/Labs compare MS
data set software function.

The metabolite FE 200486(1–10)-OH was only detected in feces.
FE 200486(1–10)-OH was not detected in the bile of rats. The
metabolite might have been formed by two possible mechanisms:

![Diagram](https://example.com/diagram.jpg)

**Fig. 5.** Outline of the in vivo metabolism of degarelix based on animal data. b, bile; f, feces; p, plasma; u, urine, matrix.
proteases present in the duodenum and jejunum of the rat, or bacteria in the large intestine (cecum/colon) of the rats (Langguth et al., 1994). However, incubations of degarelix with rat jejunal juices or with colonic contents/feces have not been performed to confirm if any of these mechanisms is responsible for the formation of FE 200486(1–10)-OH.

As outlined in the summary figure (Fig. 5), degarelix is subject to proteolysis by endopeptidases, and unchanged degarelix and metabolites are fully excreted via the hepatic and urinary pathway. Systemic exposure to any metabolic products seems to be low.

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Authorship Contributions
Participated in research design: Sonesson, Koechling, and Buur Rasmussen.
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Performed data analysis: Sonesson and Buur Rasmussen.
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