In Vitro and In Vivo Human Metabolism of Degarelix, a Gonadotropin-Releasing Hormone Receptor Blocker

Anders Sonesson and Birgitte Buur Rasmussen

Ferring Pharmaceuticals A/S, Department of Bioanalysis, Copenhagen, Denmark

Received February 24, 2013; accepted April 15, 2013

ABSTRACT

Degarelix is a decapeptide that shows high affinity/selectivity to human gonadotropin-releasing hormone receptors and has been approved for the treatment of advanced prostate cancer in the United States, European Union, and Japan. To investigate the metabolism of degarelix in humans, in vitro metabolism was addressed in liver tissue and in vivo metabolism was studied in plasma and excreta samples collected in clinical studies. In addition, drug transporter interaction potential of degarelix with selected efflux transporters and uptake transporters was studied using in vitro membrane vesicle-based assays and whole cell-based assays. In vitro degradation was observed in fresh hepatocytes; less than 25% of the initial concentration of degarelix remained after incubation at 37°C for 2 hours. One metabolite was detected, representing a truncated nonapeptide of degarelix. The same metabolite was also detected at low concentrations in plasma. The in vivo investigations also showed that degarelix is excreted unchanged via the urine but is undergoing extensive sequential peptidic degradation during its elimination via the hepato-biliary pathway. No unique human metabolites of degarelix were detected in the circulation or in the excreta. Degarelix did not show any interaction with selected efflux transporters and uptake transporters up to concentrations representing 200 times the clinical concentration. Because degarelix does not seem to interact with the cytochrome P450 enzyme system as substrate, inhibitor, or inducer and does not show any interaction with hepatic and renal uptake and efflux transporters, the risk for pharmacokinetic drug-drug interactions with this compound is highly unlikely.

Introduction

Degarelix (Fig. 1) is a decapeptide that shows high affinity/selectivity to human gonadotropin-releasing hormone receptors (Jiang et al., 2001). Degarelix binds to gonadotropin-releasing hormone receptors in the pituitary gland, which in turn, results in decreased secretion of luteinizing hormone and, consequently, decreased production of testosterone from the Leydig cells of the testes (Ortmann and Diedrich, 1999; Chengalvala et al., 2003). The suppression of testosterone occurs rapidly after drug administration and continues as long as degarelix is available at its target receptors (de Pinieux et al., 2001). Degarelix has been approved for the treatment of advanced prostate cancer in the United States, Canada, Australia, European Union, and Japan and can now be prescribed under the name Firmagon (Boccon-Gibod et al., 2009; Pommerville and de Boer, 2010). In the clinic, degarelix is administered by subcutaneous injection, forming an in vivo depot at the injection site from which the peptide is released during >30 days (Frampton and Lyseg-Williamson, 2009).

Preclinical studies in rat, dog, and monkey showed that degarelix is subject to common peptidic degradation in liver/bile and is fully excreted via hepatic (as metabolites and parent compound) and urinary (as parent compound) pathways. In plasma from dogs and monkeys, only degarelix was detected, whereas in rat, small amounts of a truncated peptide of degarelix were detected in addition to the parent compound (Sonesson et al., 2011). Drug-drug interaction studies in human liver microsomes and primary hepatocytes have shown that degarelix does not act as an inducer or inhibitor of the cytochrome P450 enzyme system (Sonesson and Rasmussen, 2011).

Hepatic and renal transporters play a key role in drug clearance and can contribute to drug-drug interactions and toxicity (Glavinas et al., 2004; Sahi, 2005). Mass balance studies of degarelix in rat, dog, and monkey have shown that 50% of total administered radioactivity was excreted via the hepatic pathways, and the majority of the radioactivity excreted in the bile was truncated metabolites of degarelix. Taking into account that drug transporters could be involved in the elimination of degarelix via the hepatic pathways and that simultaneous prescription of more than one drug to treat one or more conditions is highly likely in patients with prostate cancer, the interaction of degarelix with selected ABC (efflux) transporters and selected uptake transporters was investigated in appropriate in vitro systems.

This article includes data from two clinical studies in which degarelix metabolism was studied and is the first on human degarelix metabolism, to our knowledge. The aim of the metabolism part of the studies was to evaluate whether the metabolite profile of degarelix in humans differed from that seen in nonclinical investigations by in vitro testing in human liver microsomes and hepatocytes and in vivo investigations in plasma and excreta from humans. Furthermore, the interaction of degarelix with major human transporter proteins was evaluated through in vitro testing.

ABBREVIATIONS: ADME, absorption, distribution, metabolism, and excretion; BCRP, breast cancer resistance protein; BSEP, bile salt export pump; LC, liquid chromatography; MDR, multidrug resistance; MOPS, 3-(N-morpholino)propanesulfonic acid; MRP, multidrug resistance-associated protein; MS, mass spectrometry; MXR, mitoxantrone resistance protein; m/z, mass-to-charge ratio; OATP, organic anion-transporting polypeptide; RAD, radiochemical detection; Sf9 cells, Spodoptera frugiperda ovarian cells; UPLC, ultra performance liquid chromatography; VT, vesicular transport.
Internal standard [Ile3(2H10)]barusiban was synthesized by Ferring Research (7-10) were synthesized by Polypeptide Laboratories (Wolfenbüttel, Germany). FE 200486(1-9)-OH, FE 200486(1-10)-OH, FE 200486(4-10), and FE 200486 compounds FE 200486(1-4)-OH, FE 200486(1-6)-OH, FE 200486(1-7)-OH, described previously (Sonesson et al., 2011). The unlabeled referencepared by a modification of the method by Parnes and Shelton (1986), as France). The liver microsome preparations were stored at

Liver Tissue

Liver microsomes obtained from human males (pool of 10), were purchased from XenoTech, LLC (Lenexa, KS), through Tebu-Bio (Le Perray-en-Yvelines, France). The liver microsome preparations were stored at ~80°C until use. Cryopreserved male human hepatocytes (three donors; M00995/EQB, EVY, and GIU) were purchased from In Vitro Technologies (Baltimore, MD) and stored in liquid nitrogen until use.

Fresh male human hepatocytes were purchased from Cytonet (Hannover, Germany) and Biopredic International (Rennes, France). The hepatocytes were used in vitro experiments directly after arrival to the laboratory. Cytonet and Biopredic International receive nontransplantable tissue from multiple organ donors. All donations of liver tissue were performed after gaining the consent of the individual's family. Five male batches were used. Cytonet and Biopredic International operate within the legal and ethical framework of the European Union.

In Vitro Studies

Microsomal Incubation Conditions. Degarelix was incubated in human liver microsomes with use of the same conditions as described previously (Sonesson et al., 2011) at concentrations 1, 10, and 40 μM in the incubation buffer. The samples were analyzed for degarelix substance stability and screening of metabolites with use of liquid chromatography–mass spectrometry (LC-MS).

Stability Studies with Hepatocytes. Received fresh hepatocytes were first washed with Krebs-Henseleit buffer (pH 7.4) containing 10 mM hydrogen carbonate, and 1% bovine serum albumin. The cells were counted and, thereafter, diluted to 2 million viable cells/ml in Krebs-Henseleit buffer. 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/ml in Krebs-Henseleit buffer. The metabolic activity of the hepatocyte suspensions was investigated by degragation studies of test substrates nal oxogluconic 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 using LC–tandem MS.

Substance stability studies were performed at concentrations of 10 μM [3H]degarelix. Hepatocyte suspensions were tempered at 37°C for 5 minutes before adding the test substrate. Samples were removed after 0, 10, 30, 60, and 120 minutes of incubation. Aliquots of 100 μl were withdrawn and terminated in 100 μl acetonitrile/glacial acetic acid 96:4 (v/v) in Bio-Rad (Hercules, CA) 1.5-ml 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-radiochemical detection (RAD) and LC-MS.

Interaction Studies with Transporter Enzymes

The interaction of degarelix with different human transporter enzymes was measured at concentrations of 0.05, 0.14, 0.41, 1.23, 3.70, 11.11, 33.33, and 100 μM. Each concentration was tested in duplicate. Degarelix was dissolved in dimethylsulfoxide.

ATPase Assays. ATPase activity was measured as described elsewhere (Sarkadi et al., 1992) by determining the liberation of inorganic phosphate from ATP with a colometric reaction. Membrane vesicles from Sf9 insect cells overexpressing human MDR1 transporter (SB-MDR1-Sf9-ATPase), MRP2 transporter (SB-MRP2-Sf9-ATPase), and MXR transporter (SB-MXR-HAM-Sf9-ATPase) were used for determining the effect of degarelix on the MDR1, MRP2, and MXR activities, respectively. In brief, membrane vesicles (20 μg/well) were incubated in 10 mM MgCl2, 40 mM 3-(N-morpholino)propanesulfonic acid (MOPS)–Tris, pH 7.0, 50 mM KCl, 5 mM dithiothreitol, 0.1 mM EGTA, 4 mM sodium azide, 1 mM ouabain, 5 mM ATP, and eight concentrations of degarelix. Two protocols were used: (i) activation study (incubation with or without 1.2 mM sodium orthovanadate) or (ii) inhibition
study (incubation with known activator, 40 μM verapamil for MDR1-ATPase activity, 10 μM sulfasalazine for MRP2-ATPase activity, and 100 μM sulfasalazine for MXR-ATPase activity, and/or without 1.2 mM sodium orthovanadate). All incubations were performed for 40 minutes at 37°C.

Calcine Assay. K562-MDR cells overexpressing MDR1 transporter (80,000 cells/well) were incubated in 100 μl Hanks’ balanced salt solution with the desired activator. The Calcein fluorescent dye was added to the membrane suspensions in a fluorimeter during 8 minutes (Glavinias et al., 2011). Reference inhibitor (positive control) was used at a concentration of 60 μM, and degarelix was investigated at eight concentration levels.

Hoechst Assay. Hoechst dye transport assay was performed as described elsewhere (Kis et al., 2009). In brief, MCF7-MX cells overexpressing human MXR transporter (105 cells/well) were preincubated at 37°C in 150 μl Hanks’ balanced salt solution with degarelix. The Hoechst fluorescent dye 33424 was added in 50 μl at a final concentration of 50 μM. Fluorescence of accumulated Hoechst 33342 inside the cells was measured in real time at excitation and emission wavelengths of 355 and 460 nm, respectively, with use of a fluorescence spectrophotometer during 15 minutes. Reference inhibitor was K0.143 at a concentration of 1 μM.

Vesicular Transport Assay. Vesicular transport studies were performed as described elsewhere (Bodo et al., 2003); MRP2, MXR, and BSEP were expressed in Sf9 cells, from which inside-out membrane vesicles were prepared and incubated in the presence or absence of 4 mM ATP in a buffer containing 10 mM MgCl2, 40 mM MOPS-Tris, pH 7.0 and 70 mM KC1, substrate reporter, and eight concentrations of degarelix at 37°C. The inhibitory effect of degarelix on the transport of the reporter substrate was measured. Reporter substrates used were [3H]estradiol-17β-glucuronide (MRP2 activity; incubation time, 2 minutes), [3H]estrone-3-sulfate (MXR activity; incubation time, 1 minute), and [3H]taurocholate (BSEP activity; incubation time, 5 minutes). The transport reaction was stopped by addition of excess cold wash buffer (40 mM MOPS-Tris, pH 7.0, 70 mM KC1) to the membrane suspensions and then rapidly filtered through nitrocellulose membranes (pore size, 0.45 μM). After washing the filters with 10 ml of ice-cold wash buffer, the radioactivity associated with the filters was measured by liquid scintillation counting.

Inhibition of OATP-Mediated Transport. The inhibition experiments were performed as described by Taub et al. (2011). Stably transfected Chinese hamster ovary cells (106 cells/well), in 96-well culture plates, were first washed twice with prewarmed Krebs-Henseleit buffer. The cells were then incubated with 100 μM Krebs-Henseleit buffer containing degarelix at the target concentrations, and 0.1 μM [3H]estrone-3-sulfate (OATP1B1), 10 μM Fluo-3 (OATP1B3), or 1 μM [3H]estrone-3-sulfate (OATP2B1) were added to the wells. Cells were incubated at 37°C for 15 minutes. Uptake was stopped by aspirating the incubation buffer and washing each well 3 times with 0.2 ml of ice-cold Krebs-Henseleit buffer. The cells were lysed and analyzed. [3H]estrone-3-sulfate content in each well was measured by liquid scintillation counting, and Fluo-3 content of the lysate was analyzed using a fluorimeter.

In Vivo Studies

The clinical studies were approved by local Ethics Committees and conducted according to the principles of Good Clinical Practice and the Declaration of Helsinki. Subjects were informed of the purpose of the studies and had given their voluntary signed informed consent before being enrolled. Biologic samples were received from two clinical studies. Study H1 was a phase I study involving male Caucasian subjects, objectives of which were to compare the metabolic profile of degarelix among 8 healthy subjects and 16 patients with mild or moderate impaired hepatic function. A single dose of 1.0 mg degarelix added to 200 ml of 5% glucose solution was administered intravenously as a 1-hour continuous infusion. Intravenous administration was selected instead of subcutaneous administration to avoid sustained medical castration. Plasma samples were collected before administration and at 4, 12, 36, and 72 hours after administration. Urine samples were collected before administration and up to 72 hours after administration. Fecal samples were also collected up to 72 hours after administration.

Study H2 was a phase II/I study on Japanese patients with prostate cancer. The study design was dose-escalating single administration (subcutaneous injection). Samples were received from three dosing groups, each containing six subjects (in total, 18 patients). Group A was given 160 mg degarelix, group B as given 200 mg degarelix, and group C was given 240 mg degarelix. Four plasma samples were collected from each subject, one before administration and the other three 24 hours, 72 hours, and 7 days after administration. Urine samples were collected from the same subjects before and 24 and 72 hours after administration.

All plasma and excreta samples were frozen within 1 hour after collection and stored at ~80°C. The samples were shipped on dry ice to the Ferring Pharmaceuticals laboratory facility and stored at ~80°C until analysis.

Sample Preparation. Plasma and urine samples were prepared for analysis as described previously (Sonesson et al., 2011). Plasma volume precipitated was 1 ml, the volume of urine purified by solid phase extraction was 6–10 ml, and the amount of feces extracted was approximately 0.9–2.3 g.

Metabolite Profiling Using Radiochemical Detection

Hepatocyte samples were analyzed using a LC-RAD system consisting of a Waters (Milford, MA) 2695 Alliance system connected to an IN/US Systems (Tampa, FL) β-RAM model 3. FlowLogic 1:1 scintillation cocktail (AIM Research Inc., Hockessin, DE) was delivered by an ARC (AIM Research) StopFlow Model B unit. The column was used was an YMC (Kyoto, Japan) 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 system starting at 9% (v/v) acetonitrile and 91% aqueous 5 mM ammonium acetate (pH 4.6) buffer to 56% acetonitrile during 50 minutes. Agent to eluate ratio was 2.0; thus, 0.44 ml/min scintillation cocktail was mixed with the LC column eluant in the 100 μl flow-cell. Samples were analyzed in nonStopFlow mode.

Metabolite Profiling and Metabolite Identification by MS Detection

Plasma, urine, and fecal samples and liver microsome and hepatocyte samples were analyzed for metabolite identification by ultra performance liquid chromatography (UPLC)–MS. The UPLC instrument was a Waters ACQUITY UPLC, and the MS system was a Waters (Manchester, UK) quadrupole–time-of-flight Premier MS. The column used was an ACQUITY (Waters) UPLC BEH C18 1.7 μm, 2.1 × 100 mm, and the flow-rate was 0.3 ml/min. The samples were analyzed using a gradient starting at 5% acetonitrile isocratic for 2 minutes, then a linear increase to 22% acetonitrile during 2.5 minutes, and a linear increase to 60% acetonitrile during 7.5 minutes. The mobile phase was acidified with 0.05% (v/v) trifluoroacetic acid. The MS interface used was electrospray ionization operating in positive ionization mode. The time-of-flight resolution was approximately 12500 (m/z) at m/z; mass-to-charge ratio 816.5. Lock-mass was leucine enkephalin peptide (m/z 556.2771) 200 pg/ml dissolved in acetonitrile/water 1:1 (v/v) containing 0.5% (v/v) formic acid delivered by a pump at a flow-rate of 20 μl/min.

The detection limit of degarelix in plasma extracts was 50 pg/ml. Quantification was performed on base peak ion of the peptide. The UPLC retention times of the synthesized standard truncated peptides were known, as were their MS spectra and product ion spectra (Table 1). In addition, all MS data files were screened for m/z values of possible truncated peptide ions as FE 200486(1-3)-OH, (1-5)-OH and (1-8)-OH, and FE 200486(6-10), as well as oxidation products of degarelix and truncated metabolites of degarelix. The possible presence of glucuronol derivative of degarelix was also investigated. In addition, 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/Laboratories (Toronto, ON, Canada) software MS Manager was used for comparing data sets to detect unique differences. The MS response factors of the synthesized standards in relation to the response factor of degarelix were known. Product ion spectra of detected metabolite ions were recorded. The collision energy used was optimized for each metabolite.

Results

Stability in Liver Microsomes. No degradation of degarelix was detected when the substrate concentration was 1 and 10 μM. After increasing the substrate concentration to 40 μM, low amounts of six metabolites were detected. Five metabolites were assumed to represent...
Degarelix was degraded to the nonapeptide \([3H]FE\ 200486(1-9)-OH\) as detected by activity with an IC\(_{50}\) value of 13 μM. No other metabolites of \([3H]\)degarelix were detected. The metabolic stability of \([3H]\)degarelix in Hepatocytes was investigated with use of an authentic standard. In addition to the b-ions and y-ions present in the product ion spectrum of degarelix, the product ions -ions present in the product ion spectrum of degarelix \((m/z 121.1, 154.1, 171.2, 186.1 (y_8^-, 421.2 (b_2), 562.2 (y_8^-), 562.2 (b_2), 656.1 (b_4), 1055.4 (y_8^-), 1055.4 (b_4), 1179.5 (y_8^-), 1179.5 (b_4), 807.9 (b_10), 807.9 (b_10), 958.4 (b_5), 958.4 (b_5), 1292.7 (b_3), 1292.7 (b_3), 1393.0 (y_8^-), 1393.0 (b_4), 1409.0 (y_8^-))\) indicated that oxidation had occurred in the D-2NaI amino acid and in the D-3Pal amino acid, respectively.

### TABLE 1

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Precursor Ion</th>
<th>Characteristic Product Ions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Degarelix</td>
<td>([3H])FE 200486(1-9)-OH</td>
<td>m/z 121.1, 154.1, 171.2, 186.1 (y_8^-, 421.2 (b_2), 562.2 (y_8^-), 562.2 (b_2), 656.1 (b_4), 1055.4 (y_8^-), 1055.4 (b_4), 1179.5 (y_8^-), 1179.5 (b_4), 807.9 (b_10), 807.9 (b_10), 958.4 (b_5), 958.4 (b_5), 1292.7 (b_3), 1292.7 (b_3), 1393.0 (y_8^-), 1393.0 (b_4), 1409.0 (y_8^-))</td>
</tr>
<tr>
<td>FE 200486(1-10)-OH</td>
<td></td>
<td>m/z 121.1, 154.1, 171.2, 186.1 (y_8^-, 421.2 (b_2), 562.2 (y_8^-), 562.2 (b_2), 656.1 (b_4), 1055.4 (y_8^-), 1055.4 (b_4), 1179.5 (y_8^-), 1179.5 (b_4), 807.9 (b_10), 807.9 (b_10), 958.4 (b_5), 958.4 (b_5), 1292.7 (b_3), 1292.7 (b_3), 1393.0 (y_8^-), 1393.0 (b_4), 1409.0 (y_8^-))</td>
</tr>
</tbody>
</table>

Stability in Hepatocytes. In fresh hepatocytes, \([3H]\)degarelix was degraded to the nonapeptide \([3H]FE\ 200486(1-9)-OH\) with use of an authentic standard. 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 171.2 represent the monoisotopic mass, compared with that of degarelix, whereas the sixth and most abundant metabolite had a mass in loss of 70 Da. Product ion spectra were successfully recorded for two of the assumed mono-oxidated metabolites, and the data were compared with the product ion spectrum of degarelix (Table 1). The product ion spectra indicated that oxidation had occurred in the D-2NaI amino acid and in the D-3Pal amino acid, respectively. The metabolite with a mono-isotopic mass of 1560.6 (70 Da less than degarelix) was identified as FE 200486(1-9)-OH with use of an authentic standard. 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 171.2 represent the monoisotopic mass, compared with that of degarelix, whereas the sixth and most abundant metabolite had a mass in loss of 70 Da. Product ion spectra were successfully recorded for two of the assumed mono-oxidated metabolites, and the data were compared with the product ion spectrum of degarelix (Table 1). The product ion spectra indicated that oxidation had occurred in the D-2NaI amino acid and in the D-3Pal amino acid, respectively. The origin of the product ion m/z 171.2 is related to amino acid Iys^3.

### Stability in Hepatocytes.

In fresh hepatocytes, \([^{3H}]\)degarelix was degraded to the nonapeptide \([^{3H}]FE\ 200486(1-9)-OH\) as detected by LC-RAD (Fig. 2) and identified by UPLC-MS. Less than 30% of the original concentration of 10 μM \([^{3H}]\)degarelix remained in the buffered hepatocyte solution after incubation for 120 minutes at 37°C. No other metabolites of \([^{3H}]\)degarelix were detected. The metabolic stability of the hepatocyte preparations, as evaluated using three test substrates, demonstrated that all of the hepatocyte batches expressed metabolic activities, such as CYP3A4- and CYP2D6-mediated metabolism and glucuronic acid conjugation activity.

### Transporter Interactions.

Degarelix was investigated as a potential substrate and inhibitor of MDR1, MRP2, breast cancer resistance protein (BCRP), BSEP, OATP1B1, OATP1B3, and OATP2B1 (Table 2). In the MDR1, MRP2, and BCRP ATPase assays, degarelix showed no stimulation of baseline activity. Degarelix demonstrated inhibition of MDR1 ATPase activity with an IC\(_{50}\) value of 19 μM, but no inhibition of MDR1-mediated efflux of calcein was detected at concentrations up to 100 μM. Inhibition of MDR2 ATPase activity was measured with an IC\(_{50}\) value of 76 μM degarelix, and inhibition of MRP2-mediated efflux of \([^{3H}]\)estradiol-17β-glucuronide in the VT assay indicated a IC\(_{50}\) value > 100 μM. Inhibition of BCRP was investigated with three different assays, degarelix showed inhibition of BCRP ATPase activity with an IC\(_{50}\) value of 13 μM, inhibition of BCRP mediated efflux of \([^{3H}]\)estrone-3-sulfate in a VT assay occurred with an IC\(_{50}\) value of 48 μM, whereas no inhibition of the BCRP-mediated efflux of Hoechst 33342 was detected at concentrations up to 100 μM. Degarelix showed no effect on BSEP-mediated efflux of \([^{3H}]\)taurocholate. There was no inhibitory potency of degarelix on OATP1B1 and OATP2B1 uptake transporter, and the OATP1B3 cellular uptake of Fluo-3 was inhibited with an IC\(_{50}\) value of 10 μM degarelix.

### Metabolite Profiling in Plasma Samples.

In plasma samples, mainly intact parent peptide was detected. In samples from study H1 (Caucasian population), low amounts of the metabolite FE 200486(1-9)-OH were detected in about 90% of the total amount of degarelix and FE 200486(1-9)-OH determined. No other metabolites of degarelix were detected in the urine samples. Three metabolites, identified as \([^{3H}]\)degarelix and FE 200486(1-9)-OH, were also detected in 19 of total 42 postdose plasma samples from Japanese patients (study H2) (Table 4). In patients from the groups administrated 200 and 240 mg degarelix, the amount of FE 200486(1-9)-OH was 3.5–3.7% of the total amount of degarelix and FE 200486(1-9)-OH determined. No other metabolites of degarelix were detected in the plasma samples analyzed.

### Metabolite Profiling in Urine Samples.

In both studies (Tables 3 and 4), degarelix was detected in all postdose samples and accounted for about 90% of the total amount of degarelix and metabolites detected in the urine samples. Three metabolites, identified as FE 200486(1-4)-OH, FE 200486(1-5)-OH, and FE 200486(1-6)-OH, were present in most postdose samples from both studies. In addition, low levels of FE 200486(1-10)-OH and FE 200486(1-7) were detected (Tables 3 and 4). The total amount of metabolites present in the postdose urine samples constituted 6–10% of the total amount of degarelix and metabolites detected. The amount of metabolite FE 200486(1-5)-OH was calculated with the same MS response factor as FE 200486(1-4)-OH and FE 200486(1-6)-OH (largely identical), because no synthesized standard of the pentapeptide was available.

### Metabolite Profiling in Fecal Samples.

In extracts of fecal samples collected in clinical study H1, six metabolites of degarelix were identified (Table 3). FE 200486(1-5)-OH, the largest metabolite detected, constituted 59% of the total amount of degarelix and
metabolites detected in samples from healthy subjects and 48% in samples from hepatically impaired subjects. The second largest metabolite was the tetrapeptide (27% in healthy subjects and 41% in hepatically impaired subjects), whereas the other four metabolites identified, the hexapeptide, the heptapeptide, the nonapeptide, and the hydrolysis product \([\text{FE 200486 (1-10)-OH]}\), were present in lower amounts (0.4–5.9%). Intact degarelix constituted 3.1% in samples from healthy subjects and 0.8% in samples from hepatically impaired subjects of the total amount of degarelix and metabolites detected in the fecal extracts.

**Discussion**

To our knowledge, this is the first report on the metabolism of degarelix in humans. These investigations were part of the safety package of degarelix and intended to explore (1) whether degarelix is metabolized by the human cytochrome P450 enzymes or other degrading enzymes in in vitro hepatic tissue assays, (2) the presence of circulating metabolites to ensure that no unique human metabolites were present at a systemic exposure greater than 10% of parent drug during an exposure to a 1-month depot, (3) to ensure that human metabolite pattern in excreta was not different from that observed in animals used in toxicology studies, and (4) to investigate whether degarelix has the potential to interact with human drug transporters that could cause drug-drug interactions.

Only very minor in vitro degradation of degarelix was detected when incubating the peptide in male liver microsomes; at a high concentration (40 \(\mu\)M), low levels of oxidative metabolites and the truncated metabolite \([\text{FE 200486(1-9)-OH}]\) were detected. Similarly, no degradation of degarelix was detected when the peptide was incubated in liver microsomes of dog, guinea pig, monkey, rabbit, and rat origin (Sonesson and Rasmussen, 2008). Collectively, these observations indicate that degarelix is a very poor substrate for the cytochrome P450 enzyme system.

When incubations of degarelix were performed in suspensions of hepatocytes from cryopreserved preparations, no degradation of degarelix was detected when incubating the peptide in male liver microsomes; at a high concentration (40 \(\mu\)M), low levels of oxidative metabolites and the truncated metabolite \([\text{FE 200486(1-9)-OH}]\) were detected. Similarly, no degradation of degarelix was detected when the peptide was incubated in liver microsomes of dog, guinea pig, monkey, rabbit, and rat origin (Sonesson and Rasmussen, 2008). Collectively, these observations indicate that degarelix is a very poor substrate for the cytochrome P450 enzyme system.

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**TABLE 2**

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Assay</th>
<th>Activation Values</th>
<th>Inhibition Values</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>(\text{EC}_{50})/Compound’s Maximal Efficiency</td>
<td>(\text{IC}_{50})/Compound’s Maximal Efficiency</td>
</tr>
<tr>
<td>MDR1</td>
<td>ATPase</td>
<td>—</td>
<td>19/76</td>
</tr>
<tr>
<td>MRP2</td>
<td>ATPase</td>
<td>—</td>
<td>76/56</td>
</tr>
<tr>
<td>BCRP</td>
<td>ATPase</td>
<td>—</td>
<td>&gt;100/8</td>
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<tr>
<td></td>
<td>VT</td>
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<td>VT</td>
<td>48/63</td>
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<td>BSEP</td>
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<td>OATP1B1</td>
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<td>OATP1B3</td>
<td>Uptake</td>
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</tr>
<tr>
<td>OATP2B1</td>
<td>Uptake</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\(\text{EC}_{50}\), the concentration of the compound tested to reach 50% of the compound’s own maximal efficacy; \(\text{IC}_{50}\), the concentration required for inhibiting the transport of the reporter substrate by 50%.

* Mean of duplicate determinations.
investigate whether oxidative metabolites could be detected as in the microsome incubations performed.

The in vivo metabolites of degarelix detected in humans were the same as those previously detected in rat, dog, and monkey absorption, distribution, metabolism, and excretion (ADME) studies (Sonesson et al., 2011), as shown in the summary figure (Fig. 3). Mainly intact degarelix was detected in the plasma samples. In plasma from rats, low levels of a circulating metabolite were detected, representing the truncated nonapeptide FE 200486 (1-9)-OH, whereas no circulating metabolites could be detected in plasma from dogs and monkey. In plasma samples from Caucasian and Japanese humans, low levels of the truncated nonapeptide were detected in most plasma samples collected up to 12 hours after administration. No apparent differences were seen between Caucasian and Japanese subjects. Because degarelix has been shown to be stable when incubated in vitro in freshly prepared human plasma (unpublished data), the origin of FE 200486(1-9)-OH could be from enzymatic degradation by endopeptidases located in the hepatic tissue, as also indicated by the degradation in fresh hepatocytes.

In urine samples, mainly (>90%) intact degarelix was detected together with low levels of the truncated peptide metabolites. In fecal samples, mainly degradation products of degarelix (i.e., truncated peptides of degarelix) were detected. The major metabolites detected were the tetrapeptide and the pentapeptide. The same metabolite patterns have been detected in excreta samples from dog, monkey, and rat ADME studies (Sonesson et al., 2011). Because all mass spectrometry data collected were screened for all possible truncated forms of the degarelix peptide, we most likely can rule out that other proteolytic cleavages should have occurred to a great extent, because they were not detected by UPLC-MS. No other metabolites of degarelix than the truncated metabolites reported above were detected when UPLC-MS data were evaluated using ACD laboratories compare MS data set software function. Thus, the in vivo excreta results showed that degarelix is mainly excreted unchanged via the urine and is subject to extensive sequential peptidic degradation during its elimination via the hepato-biliary pathway. The results are in line with data for other compounds of similar size and structure as degarelix and in line with the fact that compounds of this size (molecular weight, 1632 Da) are more likely to be eliminated by the hepato-biliary pathway (Sahi, 2005).

No interactions were detected with selected ABC efflux transporters and OATP uptake transporters by degarelix at clinically relevant concentrations. The interaction with OATP1B3, detected at a degarelix concentration of 10 μM, and MDR1, MRP2, and BCRP at degarelix concentrations >10 μM represent a concentration 100–2000-times higher than the clinical relevant concentration (Frampton and Lyseng-Williamson, 2009); thus, the detected inhibitions have no clinical relevance.

Mass balance studies in rat, dog, and monkey have shown that 50% of total administered radioactivity was excreted via the hepatic pathways and that the majority of the radioactivity excreted in the bile was truncated metabolites of degarelix, indicating that transporters could be involved in the elimination of degarelix. Brush border membrane of intestinal mucosal cells contains a peptide carrier system with rather broad substrate specificity and various endo- and exopeptidase activities (Bai and Amidon, 1992; Bernkop-Schnürch and Schmitz, 2007). Similar membrane-bound enzymes might be responsible for the transport and the degradation of degarelix. The other 50% of the total radioactivity documented in the ADME studies was excreted by urinary pathways mainly as intact degarelix (Sonesson et al., 2011).

In conclusion, degarelix was shown to be a poor substrate and a poor inhibitor of ABC efflux transporters, such as P-glycoprotein (MDR1) and OATP uptake transporters. Because degarelix has been shown not to induce CYP3A4 in vitro, no further tests of P-glycoprotein induction

<table>
<thead>
<tr>
<th>Sample</th>
<th>Subjects</th>
<th>Sampling Period</th>
<th>Degarelix</th>
<th>FE 200486 (1-4)-OH</th>
<th>FE 200486 (1-5)-OH</th>
<th>FE 200486 (1-6)-OH</th>
<th>FE 200486 (1-7)-OH</th>
<th>FE 200486 (1-9)-OH</th>
<th>FE 200486 (1-10)-OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>Healthy*</td>
<td>0–72 hours</td>
<td>96.3 (3.0)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3.7 (3.1)</td>
<td>—</td>
</tr>
<tr>
<td>Plasma</td>
<td>Impaired*</td>
<td>0–72 hours</td>
<td>93.7 (4.9)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>6.3 (5.0)</td>
<td>—</td>
</tr>
<tr>
<td>Urine</td>
<td>Healthy*</td>
<td>0–72 hours</td>
<td>89.6 (4.4)</td>
<td>5.6 (3.1)</td>
<td>4.3 (1.4)</td>
<td>0.5 (0.4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Urine</td>
<td>Impaired*</td>
<td>0–72 hours</td>
<td>90.6 (6.4)</td>
<td>3.6 (4.3)</td>
<td>3.1 (3.1)</td>
<td>0.5 (0.8)</td>
<td>0.1 (0.2)</td>
<td>2.1 (2.0)</td>
<td>—</td>
</tr>
<tr>
<td>Feces</td>
<td>Healthy*</td>
<td>0–72 hours</td>
<td>3.1 (4.6)</td>
<td>27.0 (15)</td>
<td>59.2 (15)</td>
<td>5.9 (10)</td>
<td>1.4 (4.2)</td>
<td>0.7 (2.6)</td>
<td>2.7 (3.8)</td>
</tr>
<tr>
<td>Feces</td>
<td>Impaired*</td>
<td>0–72 hours</td>
<td>0.8 (2.8)</td>
<td>41.2 (19)</td>
<td>47.9 (21)</td>
<td>3.6 (4.5)</td>
<td>2.6 (5.8)</td>
<td>0.4 (1.7)</td>
<td>1.5 (3.2)</td>
</tr>
</tbody>
</table>

*Mean (S.D.) of 8 subjects.

**Mean (S.D.) of 16 subjects.

**TABLE 3**

Metabolite pattern in plasma, urine, and fecal samples after continuous infusion by intravenous administration of 1 mg degarelix in healthy Caucasian subjects and Caucasian subjects with mild or moderate impairment of hepatic function (clinical study H1)

Time normalized values: plasma (4, 12, 36, and 72 hours), urine (12, 24, and 72 hours), and feces (3–4 samples/subject during 72 hours).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dose Group</th>
<th>Sampling Period</th>
<th>Degarelix</th>
<th>FE 200486 (1-4)-OH</th>
<th>FE 200486 (1-5)-OH</th>
<th>FE 200486 (1-6)-OH</th>
<th>FE 200486 (1-7)-OH</th>
<th>FE 200486 (1-9)-OH</th>
<th>FE 200486 (1-10)-OH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>160 mg</td>
<td>0–7 days</td>
<td>97.6 (4.0)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.4 (4.1)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Plasma</td>
<td>200 mg</td>
<td>0–7 days</td>
<td>96.3 (6.7)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3.7 (6.7)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Plasma</td>
<td>240 mg</td>
<td>0–7 days</td>
<td>96.5 (4.5)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>3.5 (4.5)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Urine</td>
<td>160 mg</td>
<td>0–72 hours</td>
<td>89.2 (5.8)</td>
<td>5.7 (6.6)</td>
<td>4.3 (2.4)</td>
<td>0.8 (0.4)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Urine</td>
<td>200 mg</td>
<td>0–72 hours</td>
<td>93.1 (3.8)</td>
<td>2.4 (2.0)</td>
<td>3.7 (1.8)</td>
<td>0.7 (0.5)</td>
<td>0.02 (0.06)</td>
<td>—</td>
<td>0.1 (0.2)</td>
</tr>
<tr>
<td>Urine</td>
<td>240 mg</td>
<td>0–72 hours</td>
<td>93.5 (2.8)</td>
<td>2.5 (2.0)</td>
<td>3.3 (1.4)</td>
<td>0.6 (0.4)</td>
<td>—</td>
<td>—</td>
<td>0.1 (0.1)</td>
</tr>
</tbody>
</table>

**TABLE 4**

Metabolite pattern in plasma and urine samples after subcutaneous administration of degarelix in Japanese patients (clinical study H2)

Numbers represent mean (S.D.) values for six subjects. Time-normalized values: plasma (24 hours, 72 hours, and 7 days) and urine (24 and 72 hours).
in vivo are necessary (US Food and Drug Administration, 2006; http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM072101.pdf). Together with previously reported data on cytochrome P450 enzyme interaction studies and cytochrome P450 enzyme induction studies (Sonesson and Rasmussen, 2011), our data show that degarelix is highly unlikely to cause any drug interaction with coadministered drugs when used for the treatment of patients with prostate cancer. Similar to other peptide drugs of this size (Sahi, 2005), degarelix is subject to proteolysis by endopeptidases, and unchanged degarelix and its metabolites are fully excreted via the hepatic and urinary pathway. Therefore, systemic exposure to any metabolic products also seems to be low and/or negligible. All detected metabolites of degarelix in plasma and excreta from humans have been observed in samples from toxicological studies in rat and dog; thus, no unique human metabolites were detected.

Acknowledgments
The authors thank Claudio Schteingart, Ferring Research Institute, for valuable discussions on peptide metabolism.

Authorship Contributions
Participated in research design: Sonesson, Rasmussen.
Performed data analysis: Sonesson, Rasmussen.
Wrote or contributed to writing of the manuscript: Sonesson, Rasmussen.

References

Fig. 3. Outline of the in vivo metabolism of degarelix, based on human data (present report) and animal data (Sonesson et al., 2011). b, bile; f, feces; p, plasma; u, urine, matrix.


Address correspondence to: Anders Sonesson, Ferring Pharmaceuticals A/S, Bioanalysis Department, Kay Fiskers Plads 11, DK-2300 Copenhagen S, Denmark. E-mail: anders.sonesson@ferring.com