Galantamine is a tertiary alkaloid extracted from several Amaryllidaceae species and is an established competitive acetylcholine esterase inhibitor. Data from a number of clinical trials have shown that galantamine offers a significant therapeutic benefit in the management of patients with Alzheimer’s disease (Raskind et al., 2000; Tariot et al., 2000; Wilcock et al., 2000; Wilkinson and Murray, 2001). According to advances in analytical techniques, there was an interest in elucidating the metabolic profile of galantamine in both animals (Mihailova et al., 1985; Mihailova and Yamboliev, 1986; Bickel et al., 1991a) and humans (Westra et al., 1986; Mihailova et al., 1989; Bickel et al., 1991b), advances in analytical techniques have made further elucidation of the metabolic profile of galantamine in both compounds possible. The studies presented here were therefore performed to elucidate the metabolic profile of galantamine after oral dosing and to compare the metabolism and excretion of galantamine in rats, dogs, and humans.

**Materials and Methods**

**Test Item.** Galantamine base ([4αS-(4αS,6β,8αR*)]-4α,5,9,10,11,12-hexahydro-3-methoxy-11-methyl-6H-benzofuro[3a.3,2-c][2]benzazepin-6-ol) was 3H-labeled on the aromatic ring in the meta position to the methoxy group (Fig. 1). This 3H label was found to be metabolically stable because the extent of 3H exchange with water was ≤2% of the administered radioactivity dose in rats, dogs, and humans. Galantamine labeled with 14C at the methoxy or N-methyl position could not be used because the 14C label is lost following O-demethylation, N-demethylation, N-oxidation, and epimerization. All metabolic pathways observed in humans occurred in at least one animal species. In extensive metabolizers for CYP2D6, urinary metabolites resulting from O-demethylation represented 33.2% of the dose compared with 5.2% in poor metabolizers, which showed correspondingly higher urinary excretion of unchanged galantamine and its N-oxide. The glucuronide of O-desmethyl-galantamine represented up to 19% of the plasma radioactivity in extensive metabolizers but could not be detected in poor metabolizers. Nonvolatile radioactivity and unchanged galantamine plasma kinetics were similar for poor and extensive metabolizers. Genetic polymorphism in the expression of CYP2D6 is not expected to affect the pharmacodynamics of galantamine.

**ABSTRACT:**

Galantamine is a competitive acetylcholine esterase inhibitor with a beneficial therapeutic effect in patients with Alzheimer’s disease. The metabolism and excretion of orally administered 3H-labeled galantamine was investigated in rats and dogs at a dose of 2.5 mg base-Eq/kg body weight and in humans at a dose of 4 mg base-Eq. Both poor and extensive metabolizers of CYP2D6 were included in the human study. Urine, feces, and plasma samples were collected for up to 96 h (rats) or 168 h (dogs and humans) after dosing. The radioactivity of the samples and the concentrations of galantamine and its major metabolites were analyzed. In all species, galantamine and its metabolites were predominantly excreted in the urine (from 60% in male rats to 93% in humans). Excretion of radioactivity was rapid and nearly complete at 96 h after dosing in all species. Major metabolic pathways were glucuronidation, O-demethylation, N-demethylation, N-oxidation, and epimerization. All metabolic pathways observed in humans occurred in at least one animal species. In extensive metabolizers for CYP2D6, urinary metabolites resulting from O-demethylation represented 33.2% of the dose compared with 5.2% in poor metabolizers, which showed correspondingly higher urinary excretion of unchanged galantamine and its N-oxide. The glucuronide of O-desmethyl-galantamine represented up to 19% of the plasma radioactivity in extensive metabolizers but could not be detected in poor metabolizers. Nonvolatile radioactivity and unchanged galantamine plasma kinetics were similar for poor and extensive metabolizers. Genetic polymorphism in the expression of CYP2D6 is not expected to affect the pharmacodynamics of galantamine.
Dose Administration. Rat study. Five male and five female Wistar rats were dosed for the collection of urine and feces. Seven additional groups of three male rats each, seven groups of three female rats, and four groups of four female rats were dosed in an identical manner for the collection of plasma samples. All animals were dosed by gastric intubation, with 1.0 ml/100 g body weight of an aqueous solution of [3H]galantamine hydrobromide at a concentration of 0.25 mg base-Eq/ml to administer galantamine at a target dose of 2.5 mg base-Eq/kg body weight.

Dog study. Three male beagle dogs were dosed orally with 1.0 ml/kg body weight of an aqueous solution of [3H]galantamine hydrobromide at a concentration of 2.5 mg base-Eq/ml to administer galantamine at a target dose of 2.5 mg base-Eq/kg body weight.

Human study. Four healthy, male adult subjects drank 16.0 ml of an aqueous solution of [3H]-galantamine hydrobromide at a concentration of 0.25 mg base-Eq/ml, corresponding with a target dose of 4 mg base-Eq. The beaker used for dosing was rinsed twice with 25 ml of water and once with 50 ml of water; the rinsing solutions were consumed.

Pre- and Postdose Considerations. Rat study. During the acclimatization period of 6 days before dose administration and during the collection period after dose administration, the rats were housed in individual stainless steel cages. Immediately before dosing, a system for the separate collection of urine and feces was placed underneath the cages of the five male and five female animals identified for collection of excreta. Tap water and rat food were available ad libitum throughout the study.

Dog study. During the last 2 days of the 1-week acclimatization period before dose administration and during the collection period after dose administration, the dogs were housed in individual stainless steel cages equipped with a system for the separate collection of urine and feces. Tap water was available throughout the study. Dog food was presented daily at 10:00 AM and was withdrawn at 1:00 PM, except on the day of dose administration when it was presented 4 h after dose administration.

Human study. Before dose administration, subjects had fasted overnight for at least 10 h. Intake of water was allowed until 2 h before dose administration. After dose administration, the subjects had to remain in an upright position and during the collection period after dose administration. The weight of the feces samples was recorded. At the end of the study, the cages were rinsed with methanol and water. The washings were combined per cage, and the volumes were determined.

Blood was collected on heparin by decapitation from three male and three female rats at 20 min and 1, 3, 8, 24, 48, and 96 h after dose administration. In addition, blood was collected from four female rats at 1, 3, 8, or 24 h after dose administration. Plasma samples were prepared by centrifugation of the blood samples at approximately 1700g for approximately 10 min. Plasma samples were pooled per time point for radio-HPLC analysis.

Dog study. Urine was collected once before dose administration and in intervals of 0 to 4, 4 to 8, 8 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h after dose administration. At the end of the 0- to 4-h, 4- to 8-h, and 8- to 24-h intervals, the bladder was emptied with a probe. The pH and volume of the urine samples were measured. Feces were collected once before dose administration and in intervals of 0 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h after dose administration. The weight of the feces samples was recorded. At the end of the study, the cages, gratings, and metabolism pans were rinsed with water. The washings were combined per cage, and the volumes were determined.

Venous blood samples were collected from the jugular vein once before dose administration and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 24, 32, 48, 72, 96, and 168 h after dose administration. The blood samples were collected on heparin. Plasma samples were prepared by centrifugation of the blood samples at approximately 1700g for 10 min.

Human study. Urine was collected once before dose administration and in intervals of 0 to 2, 2 to 4, 4 to 6, 6 to 8, 8 to 24, 24 to 32, 32 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h after dose administration. The pH and volume of the urine samples were measured. Feces were collected once before dose administration and per stool for up to 168 h after dose administration or longer if necessary to ensure that at least seven stool samples were available for each subject. The time and date of delivery and the weight of each stool were recorded.

Venous blood samples were collected from an arm vein once before dose administration and at 0.5, 1, 2, 4, 6, 8, 10, 24, 32, 48, 72, 96, and 168 h after dose administration. The blood samples were collected on heparin. Plasma samples were prepared by centrifugation of the blood samples at approximately 1700g for approximately 10 min.

Sample Analysis. Blank samples of plasma, urine, and feces were spiked with known quantities of radiolabeled galantamine and stored during the period of the studies to allow verification of the stability of the drug in these media.

Radioactivity balance in urine. Levels of total radioactivity in urine samples from all three studies were determined by liquid scintillation counting of duplicate aliquots of the urine samples using Ultima Gold (Packard BioScience B.V., Groningen, The Netherlands) as scintillation cocktail. Levels of nonvolatile radioactivity in urine samples were determined after lyophilization of duplicate aliquots of the urine samples and subsequent reconstitution in water.

Radioactivity balance in feces. Feces samples of the three species were homogenized in methanol using an Ultra-Turrax homogenizer (Janke and Kunkel GmbHCo. IKA-Labortechnik, Staufen, Germany). After centrifugation of the homogenate, the supernatant methanolic extract was transferred into another vial, and the precipitate was extracted again with methanol. The homogenate was centrifuged; the supernatant methanolic extract transferred into another vial, and the precipitate extracted a third time. Hereafter, the extract was separated from the residue by filtration of the homogenate through a Büchner funnel (Mércz Eurolab Holding GmbH, Zaventem, Belgium). The methanolic extracts of the three extraction steps were combined, and the volume was determined. The radioactivity in duplicate aliquots of the fecal extracts was counted in duplicate with Ultima Gold as scintillation cocktail. The fecal residues were dried in air and ground in a Waring Blender (Snijders Scientific B.V., Tilburg, The Netherlands). Thereafter, four weighed aliquots of approximately 100 mg of each residue sample were combusted in a Packard Sample Oxidizer model 306. The radioactivity contained in the residues was measured in a liquid scintillation spectrometer with Monophase S (Packard BioScience B.V.) as a scintillation cocktail after collection of [3H]O.

Radioactivity in plasma. Plasma levels of total radioactivity were determined by liquid scintillation counting of duplicate aliquots of the plasma samples using Ultima Gold as a scintillation cocktail. Plasma levels of nonvolatile radioactivity were determined by liquid scintillation counting of du-
Bioanalysis of unchanged galantamine in plasma. To 1 ml aliquots of human plasma (or 0.5 ml aliquots of rat plasma), 1000 ng of internal standard (codeine phosphate contained in 100 μl of methanol) and 1 ml of a saturated KC1 solution were added. The samples were made alkaline by adding 100 μl of 1 M NaOH, vortex mixed, and extracted twice with 2.5 ml of toluene. The top organic layers from the two extractions were combined and evaporated to dryness under nitrogen at 65°C, and the residue was dissolved in 100 μl of methanol/0.01 M ammonium acetate (84:5:15.5) containing 1% diethylamine. The extracts were injected on an HPLC system (HP1100; Hewlett Packard, Palo Alto, CA) with fluorescence detection (Jasco FP-920; Jasco, Tokyo, Japan) at excitation and emission wavelengths of 280 and 310 nm, respectively. A 10-cm × 4.6-mm i.d. column, packed with Hypersil C18 BDS (3 μm) (Alltech Associates, Deerfield, IL), was used. The elution solvent was 0.01 M ammonium acetate, pH 7/acetoni trile (90:10), with a flow rate of 0.8 ml/min.

Radio-HPLC analysis. Where appropriate, individual or overall pools of the urine samples or the methanolic extracts of the feces samples were prepared by mixing constant fractions of the individual samples or the individual pools, respectively. Overall pools of the plasma samples were prepared by mixing equal volumes of the individual samples.

The metabolites in plasma, urine, and methanolic extracts were identified by HPLC cochromatography with authentic substances by enzymatic hydrolysis and/or liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis in the rat and human studies. For the identification of metabolites by HPLC cochromatography, a mixture of authentic substances was co-injected with the plasma samples, urine samples, and the methanolic fecal extracts. The authentic substances were monitored by UV-detection, whereas the radioactive metabolites were monitored by liquid scintillation spectrometry.

For the identification of glucuronic acid and sulfate conjugates of unchanged galantamine and/or its metabolites in plasma and urine, a comparison was made between the radio-HPLC chromatograms of samples before and after enzymatic hydrolysis with β-glucuronidase/β-arylsulfatase from Helix pomatia (Boehringer Ingelheim GmbH, Ingelheim, Germany; 10 μl/ml acetate-buffered sample, pH 5.0) and β-glucuronidase and β-arylsulfatase from Escherichia coli (Boehringer Ingelheim GmbH; 10 μl/ml phosphate-buffered sample, pH 7.0), or arylsulfatase from Aerobacter aerogenes (Sigma-Aldrich, St. Louis, MO; 10 μl/ml phosphate-buffered sample, pH 7.0). The incubations were performed at 37°C for 24 h. Saccharo-1,4-lactone (Sigma-Aldrich) at a final concentration of about 20 mM was used as a β-glucuronidase inhibitor to illustrate the specificity of the hydrolysis or, in the case of the combined β-glucuronidase/arylsulfatase preparation, to differentiate between glucuronic acid and sulfate conjugates.

In the rat and human studies, samples of plasma, urine, and methanolic feces extracts were analyzed by LC-MS/MS (LCQ; Thermo Finnigan MAT, San Jose, CA), using a Waters Alliance 2690 separation module with a Waters 996 photodiode-array detector to confirm the identity of the metabolites. The chromatographic conditions were identical to those described above for the radio-HPLC analysis. Electron spray ionization (ESI) was used in the positive mode, and the settings (ions voltages, quadrupole and octapole voltage offsets, etc.) were optimized for maximum intensity for galantamine using the auto-tune function within the LCQ Tune program. Following the instrument tune, the source voltage was maintained at +4.5 kV; the N2 sheath gas flow was set at 80 units, the auxiliary gas flow at 5.0 units, and the capillary (desolvation) temperature at 220°C. An isolation width of 2.00 Da was used, and the relative collision energy was set at 25%.

Data Analysis. The radioactivity excreted in urine and feces was expressed as a percentage of the administered radioactivity. The amount of tritiated water excreted in urine was calculated from the difference between the total radioactivity levels and the nonvolatile radioactivity levels and was expressed as a percentage of the sample radioactivity. The mass balance of galantamine and its major metabolites was presented as the percentage of the sample or dose radioactivity accounted for by these radiolabeled compounds.

Radioactivity levels in plasma were expressed as nanogram-equivalents to galantamine per milliliter. The concentration of tritiated water in plasma was calculated from the difference between the total radioactivity levels and the nonvolatile radioactivity levels. In plasma, the levels of unchanged galantamine and its major metabolites were presented as a percentage of the sample radioactivity and/or as nanogram-equivalents to galantamine base per milliliter.

Profiles of the plasma concentrations of total radioactivity (in rats, dogs, and humans) and of galantamine (in rats and humans) were analyzed by standard noncompartmental analysis. Based on the individual plasma concentration-time data, the following pharmacokinetic parameters were calculated as follows: AUCL (A), area under the plasma concentration-time curve from 0 to the time of the last quantifiable concentration found by linear trapezoidal summation; AUCC (t), area under the plasma concentration-time curve extrapolated to infinity by using β found by linear trapezoidal summation; t max , time to reach the peak plasma concentration found by visual inspection of the data; β, elimination rate constant determined by linear regression of the terminal points of the ln linear plasma concentration-time curve; t 1/2 , terminal half-life defined as 0.693/β. The results were expressed as mean ± standard deviation.

Results

Dose Received. In the rat study, the actual dose received was 2.50 mg base-Eq/kg body weight (males) and 2.47 mg base-Eq/kg body weight (females). The total radioactive dose received was measured in the animals dosed for collection of urine and feces and averaged 1051 kBq (or 28.4 μCi) per male rat and 989 kBq (or 26.7 μCi) per female rat.
In the dog study, the actual dose was 2.47 mg base-Eq/kg body weight. The radioactive dose was 11.8 MBq (or 318.556 MANNENS ET AL. 10.5 MBq (or 282.5) (0.052 mg base-Eq/kg body weight), and the radioactive dose was calculated to result in a radiation exposure of less than 500 Sv. An effective dose between 100 and 1000 µSv is categorized as a category 2.5 per Sampling Time (extensive metabolizers)

TABLE 1

Excretion of radioactivity in urine and feces of rats, dogs, and humans after single oral administration of [3H]galantamine hydrobromide at 2.5 mg base-Eq/kg body weight (animals) and 4 mg base-Eq (humans)

<table>
<thead>
<tr>
<th>Species</th>
<th>Excretion in Urine</th>
<th>Excretion in Feces</th>
<th>Total Excretion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Wistar rats (n = 5)</td>
<td>60.04 ± 3.40</td>
<td>38.40 ± 3.18</td>
<td>99.39 ± 0.54</td>
</tr>
<tr>
<td>Female Wistar rats (n = 5)</td>
<td>64.71 ± 6.17</td>
<td>33.94 ± 5.65</td>
<td>99.39 ± 0.51</td>
</tr>
<tr>
<td>Male beagle dogs (n = 3)</td>
<td>90.67 ± 6.86</td>
<td>6.63 ± 1.50</td>
<td>98.82 ± 6.34</td>
</tr>
<tr>
<td>Healthy male subjects (n = 2)</td>
<td>94.13/92.26</td>
<td>4.39/2.9</td>
<td>98.52/98.55</td>
</tr>
<tr>
<td>Healthy male subjects (n = 2)</td>
<td>90.34/96.67</td>
<td>2.34/2.18</td>
<td>92.68/98.85</td>
</tr>
<tr>
<td>Healthy female (poor metabolizers)</td>
<td>90.34/96.67</td>
<td>2.34/2.18</td>
<td>92.68/98.85</td>
</tr>
</tbody>
</table>

In all species, radioactivity was predominantly excreted in urine and to a lesser extent in feces (Table 1). However, the urinary excretion in rats was much lower than that in dogs and humans. This difference was compensated for by the fecal excretion, which was higher in rats than in dogs and humans.

The rate of excretion was more rapid in rats and dogs than in humans, with 91 and 89% of total radioactivity being excreted within the first 24 h after dose administration, respectively, compared with approximately 73% in humans. Excretion was nearly complete at 96 h after dosing in all species. The urinary excretion rate half-life of total radioactivity in humans was 13.1 ± 2 h.

Plasma Kinetics of Total Radioactivity and of Unchanged Galantamine. In all studies, the total radioactivity in plasma was almost fully accounted for by nonvolatile radioactivity, indicating that no appreciable levels of tritiated water were formed and that the tritium label in the [3H]galantamine is metabolically stable.

The pharmacokinetic parameters determined on the basis of the plasma levels of total radioactivity and unchanged galantamine (rat and human study) are presented in Table 2. Plasma-concentration time profiles for nonvolatile radioactivity and for galantamine in humans are shown in Fig. 2.

In the human study, the pharmacokinetic parameters for the elimination of the nonvolatile radioactivity and unchanged galantamine from plasma did not seem to differ between poor and extensive metabolizers and were therefore averaged. Based on the AUC values for nonvolatile radioactivity and unchanged galantamine, unchanged galantamine accounted for 22.6 and 50.1% of nonvolatile radioactivity in plasma of male rats and female rats, respectively. In humans, based on AUC values for nonvolatile radioactivity and unchanged galantamine, unchanged galantamine accounted for 31.6% of total plasma radioactivity.

Metabolite Profile of Galantamine after Oral Administration. Unchanged galantamine was stable in the plasma and urine of all species and in the feces extracts of the dog, as indicated by the results of the radio-HPLC analyses of the blank samples fortified with the dosing solution. In feces extracts of the rat, partial degradation (9.7%) had occurred, predominantly to the N-oxide R117185. The stability of galantamine in feces extracts of humans was not examined because of the low radioactivity levels in the actual feces samples.

The metabolites of galantamine identified in these studies are listed in Table 3. These metabolites were formed directly from galantamine and/or its metabolites by glucuronidation, O-demethylation, N-oxidation, epimerization, N-demethylation, and sulfate conjugation.

Confirmation of the identities of the metabolites was achieved using LC-MS/MS, chromatography with authentic substances, and enzymatic hydrolysis. The separation of the authentic substances used for HPLC chromatography is shown in Fig. 3, together with the metabolite patterns obtained from the analysis of two human urine samples.

The MS/MS product-ion ESI mass spectrum of galantamine and of galantaminone shows characteristic fragment ions useful for metabolic identification (Fig. 4, A and B). The fragmentation behavior is dominated by cleavages in the azepine ring. The MS/MS product-ion ESI spectra of the radioactive peak unchanged drug (MH+ 288) and the retention time of the reference compound R113675 (galantamine) are identical (Table 3).

The ESI mass spectra of metabolite 2 and metabolite 3 display the protonated molecular ion at m/z 450 (Table 3). A mass shift of 162 units compared with galantamine points to glucuronidation, with concomitant demethylation of the molecule. The base peak at m/z 274 in the MS/MS product-ion ESI spectra corresponds to the protonated aglycon. The shifted fragment ions b (m/z 231–217) and c (m/z 213–199) point to O-demethylation (Fig. 4A). Fragment ion m/z 432,
arising from the loss of a water molecule directly from the protonated molecular ion, indicates a free hydroxyl group at C6, and therefore, glucuronidation at C3 is suggested. The other fragment ions d, e, and f (m/z 225, 197, and 209) (Fig. 4A) confirm the proposed structures. Based on these spectral data and on the retention time of the aglycons, metabolite 2 and metabolite 3 are identified as a glucuronide of O-desmethyl-galantamine and glucuronide of O-desmethyl-epigalantamine, respectively.

The ESI mass spectrum of metabolite 5 reveals the protonated molecular ion at m/z 464 (Table 3). A mass shift of 176 units compared with galantamine points to glucuronidation of the molecule. The fragment ions m/z 270 and b through f (Fig. 4A), arising from the protonated aglycon at m/z 288, and cochromatography of the aglycon confirm the proposed structure of a glucuronide of galantamine.

The MS/MS product-ion ESI spectra of metabolite 6 (MH+ 274) and the retention time of the reference compound R119729 (O-

Fig. 2. Plasma concentration-time curves of nonvolatile radioactivity (upper chart) and unchanged galantamine (lower chart) after a single oral dose of 4 mg base-Eq of [3H]galantamine hydrobromide in four male subjects.

Subjects 1 and 2 were poor CYP2D6 metabolizers and subjects 3 and 4 extensive metabolizers.
desmethyl-galantamine) are identical (Fig. 4A). Metabolic O-desmetylation is confirmed by the shifted fragment ions β and ω (Fig. 4A) compared with galantamine (m/z 231→217 and m/z 213→199, respectively).

The MS/MS product-ion ESI spectra of metabolite 8 (MH+ 274) and the retention time of the reference compound R117455 (N-desmethyl-galantamine) are identical (Table 3). Metabolic N-demethylation is confirmed by the unchanged fragment ions β and ω (m/z 231 and 213, respectively). The MS/MS product-ion ESI spectra of metabolite 16 (MH+ 274) and metabolite 8 display identical fragment ions (Table 3); however, they have different relative intensities, as also seen for galantamine and epigalantamine (see below for metabolite 13). Also the retrentions times are different. Based on these spectral data, metabolite 16 is identified as N-desmethyl-epigalantamine.

The MS/MS product-ion ESI spectra of metabolite 10 (MH+ 304) and metabolite 17 display identical fragment ions (Table 3), with different relative intensities pointing to the presence of N-oxide of epigalantamine or N-hydroxy-methyl galantamine (or N-hydroxy-methyl-epigalantamine). N-Oxidation is confirmed by the unchanged fragment ions β and ω (m/z 231 and 213, respectively). Cochromatography confirmed that metabolite 10 was an N-oxide of galantamine, and metabolite 17 was proposed as an N-oxide of epigalantamine, analogous to the comparison of the relative intensities of the fragment ions of galantamine and epigalantamine (see below for metabolite 13).

The MS/MS product-ion ESI spectra and the retention time of metabolite 13 (MH+ 288) and the reference compound R117172 (epigalantamine) are identical (Table 3). The fragmentation behavior is dominated by cleavages in the azepine ring, as presented in Fig. 4A. Distinction is made between galantamine and epigalantamine by the comparison of the relative intensities of their MS fragment ions.

The MS/MS product-ion ESI spectra of metabolite 14 (MH+ 286) and the retention time of the reference compound R118218 (narwedine, galantaminone) are identical. The formation of the most diagnostic fragments is proposed in Fig. 4B.

The ESI mass spectra of metabolite 15 and metabolite 22 display the protonated molecular ion at m/z 368. A mass shift of 80 units compared with galantamine indicates that metabolite 15 and metabolite 22 are sulfate conjugates of galantamine or its isomer. The base peak at m/z 270 in the MS/MS product-ion ESI spectra arises from the expulsion of a water molecule from the protonated aglycone at m/z 288. Fragment ion β (m/z 231, arising from the protonated aglycone), ω (m/z 213), and ι (m/z 209) confirm the proposed structure. Based on the comparison of the relative intensities of the fragment ions of galantamine and epigalantamine, metabolite 15 and metabolite 22 are identified as sulfate conjugates of galantamine and epigalantamine, respectively.

Metabolite 20 has the same retention time as the aglycon of metabolite 3. Metabolite 3 was identified as a glucuronide of O-desmethyl-galantamine. The MS/MS product-ion spectra of metabolite 20 and the protonated aglycon (m/z 274) of metabolite 3 are similar (Table 3). Consequently metabolite 20 is identified as O-desmethyl-galantamine.

A mass shift of 14 units of the protonated molecular ion of metabolite 21 (m/z 272) with respect to metabolite 14 implies demethylation of the molecule (Table 3). The shifted fragment ion ω (m/z 231, arising from the protonated aglycone) at m/z 213 and ι (m/z 209) confirm the proposed structure. Based on the comparison of the relative intensities of the fragment ions of galantamine and epigalantamine, metabolite 15 and metabolite 22 are identified as sulfate conjugates of galantamine and epigalantamine, respectively.

The ESI mass spectrum of metabolite 23 displays the protonated molecular ion at m/z 354 (Table 3). A mass shift of 66 units compared with galantamine indicates a sulfate conjugate of desmethyl galantamine. The base peak at m/z 274 in the MS/MS product-ion ESI spectrum corresponds to the protonated aglycon. The shifted fragment ion β (m/z 231→217) points to O-demethylation. Fragment ion m/z 336, arising from the loss of a water molecule directly from the protonated molecular ion, proposes a free hydroxyl group at C6, and therefore, sulfate conjugation at C5 is in favor. Based on these spectral data and on the comparison of the relative intensities of the fragment ions of O-desmethyl-galantamine and O-desmethyl-epigalantamine, metabolite 23 is identified as a sulfate conjugate of O-desmethyl-galantamine.

Metabolic profile in urine. The metabolites identified in the urine samples are listed in Table 4.

In the rat study, some gender differences were observed in the amount of unchanged drug excreted (15.9% in males and 32.6% in males).
females) and in the metabolites formed. For example, metabolite 10 represented 6.4% in male rats but only 2.4% in females, whereas metabolite 15, the major metabolite in female rats (8.2%), was not detected in male rats. Metabolites 14 and 17 were two minor metabolites only detected in male rats.

In the dog study, the major metabolites found, in addition to unchanged drug which accounted for 46.2%, were metabolite 5 (14.7%) and 10 (18.6%). There were five minor unidentified metabolites, together accounting for less than 1.4% of total dose radioactivity.
In the human study, a difference was found between poor and extensive metabolizers. Metabolites that were formed by \(O\)-demethylation (Table 7; especially metabolites 2, 3, 6, and 23) were formed much more in extensive metabolizers (33.1 versus 5.2%); this was compensated for primarily by the urinary excretion of unchanged galantamine and its \(N\)-oxide (metabolite 10) and to a lesser extent the glucuronide of unchanged galantamine (metabolite 5), \(N\)-desmethyl-galantamine (metabolite 8), \(N\)-desmethyl-epigalantamine (metabolite 16), the \(N\)-oxide of epigalantamine (metabolite 17), epigalantamine (metabolite 13), and \(O\)-desmethyl-epigalantamine (metabolite 20) in poor metabolizers.

**Metabolite profile in feces.** The metabolites identified in the methanolic fecal extracts from the rat and dog studies are listed in Table 5. A quantitative evaluation of the metabolite profile in humans was not possible due to the low levels of radioactivity in the methanolic fecal extracts.

In the rat study, the extraction recovery of fecal radioactivity was 73 to 80%. For feces fortified with \([3H]\)galantamine, the extraction recovery was 87%.

Although the bulk of the radioactivity was excreted as unchanged drug in the feces of female rats, metabolites 18 and 20 were present in equal amounts to unchanged drug in male rats. In female rats, metabolite 15 accounted for 8.5% of the total radioactivity. The \(N\)-oxide R17185, which was a degradation product of galantamine in blank rat feces fortified with galantamine, was detected in minute concentrations in the actual samples.

In the dog study, only 6.6% of the total dose radioactivity was recovered in feces. In addition to the metabolites listed in Table 5, there were 12 minor metabolites in dog feces, each constituting less than 0.6% of total dose radioactivity and together accounting for 2.1% of the total excretion.

**Metabolite profile in plasma.** The percentages of the sample radioactivity accounted for by the various metabolites in the 1-h plasma of rats, dogs, and humans are listed in Table 6.

In rats, the metabolite pattern in plasma corresponded well with that in urine, with unchanged drug accounting for the major part of the radioactivity at least up to 8 h after dosing. In the 1-h plasma samples, the glucuronide of galantamine (metabolite 5) was the major metabolite, whereas in the 8-h samples, the polar metabolites 2 and 3 were more abundant.

In the dog study, the major plasma metabolite was the \(N\)-oxide of galantamine (metabolite 10), especially 8 h after dosing, although the glucuronide of galantamine (metabolite 5) was also present at high levels, especially in the 4-h samples.

In the human study, unchanged galantamine accounted for the bulk of the sample radioactivity in both poor and extensive metabolizers. The remainder of the radioactivity was accounted for by the glucuronide (metabolite 5) in poor metabolizers, whereas both metabolite 5 and the glucuronide of \(O\)-desmethyl-galantamine (metabolite 2) were found in extensive metabolizers. Unconjugated \(O\)-desmethyl-galantamine, a pharmacologically active metabolite, was not detected in either poor or extensive metabolizers.

**Discussion**

The overall metabolism of galantamine in rats, dogs, and humans after single oral administration was evaluated on the basis of the metabolite profiles in urine, feces, and plasma. Metabolite profiles in the methanolic fecal extracts from the human study could not be determined because of the very low levels and were not taken into account. However, given the high recovery of the administered galantamine dose in human urine, the overall metabolism could be adequately characterized on the basis of urine and plasma metabolites only.

After a single oral dose of galantamine, 24 to 33% of the dose was excreted unchanged in the urine of female rats and extensive human metabolizers, 39% in poor human metabolizers, and 46% in dogs. In male rats, this figure was 16%. The overall metabolism of galantamine is summarized in the metabolic scheme presented in Fig. 5.

Multiple metabolic pathways and renal excretion are involved in the elimination of galantamine. Galantamine is metabolized by the
hepatic cytochrome P450 enzymes, glucuronidated, and excreted unchanged in the urine. No single pathway seems predominant. Important metabolic pathways were glucuronidation, O-demethylation, N-demethylation, N-oxidation, and epimerization. Although the metabolic scheme suggests that the epimerization occurs first, it is also possible that epimerization occurs after glucuronidation, O-demethylation, N-demethylation, and N-oxidation. The stereoisomeric conversion of the alcohol group of galantamine has been reported before. It is suggested to result from dehydrogenation of the alcohol group to an intermediate ketone, galantaminone, followed by hydration (Bachus et al., 1999). In vitro incubations clearly demonstrate that the epimerization and the hydration (Bachus et al., 1999). In vitro incubations clearly demonstrate that the epimerization and the hydration (Bachus et al., 1999). In vitro incubations clearly demonstrate that the epimerization and the hydration (Bachus et al., 1999).

The relative contribution of the various metabolic pathways to the overall metabolism and excretion of galantamine is shown in Table 7. There is considerable interspecies variation and differences between sexes in the rat and between poor and extensive CYP2D6 metabolizers. However, all metabolic pathways observed in humans occurred in at least one animal species.

In rats, O-demethylation was the most important metabolic pathway, accounting for approximately 23% of the dose in males and 11% in females, whereas in dogs, N-demethylation played a more important role, accounting for 20% of the dose.

In the human study, there were some differences in metabolism between poor and extensive metabolizers for CYP2D6. In extensive metabolizers, six metabolites resulting from O-demethylation (metabolites 2, 3, 6, 20, 21, and 23) represented over 33% of the dose, whereas in poor metabolizers, these metabolites represented only 5% of the dose. The lower level of excretion of metabolites formed by O-demethylation in poor metabolizers was compensated for primarily by higher levels of unchanged galantamine and the N-oxide of galantamine (metabolite 10) and to a lesser extent by higher levels of the glucuronide of unchanged galantamine (metabolite 5), N-desmethyl-galantamine (metabolite 8), N-desmethyl-epigalantamine (metabolite 16), the N-oxide of epigalantamine (metabolite 17), and epigalantamine (metabolite 13).

After a single oral dose of 10 mg of galantamine in healthy male volunteers, Bachus and coworkers (1999) identified galantamine and three metabolites in urine, namely the glucuronide of O-desmethyl-galantamine, N-desmethyl-galantamine, and epigalantamine. Some 25.1% of the dose was excreted as galantamine, 19.8% as the glucuronide of O-desmethyl-galantamine, 5% as N-desmethyl-galantamine, and 0.8% as epigalantamine. This quantitative contribution corresponded very well with what was detected in the present trial (Table 7). No glucuronide conjugates of galantamine, epigalantamine, galantaminone, and N-desmethyl-galantamine were detected. Bachus et al. (1999) also showed that by inhibition of CYP2D6 with quinidine, virtually no glucuronide of O-desmethyl-galantamine was excreted in urine, but the excretion of galantamine, N-desmethyl-galantamine, and epigalantamine was increased in a compensatory manner.

The use of radiolabeled drug enabled us to gain some new information on the metabolic fate of galantamine. In addition to what has been published before, we were able to identify some major (galan-
tamine glucuronide, the N-oxide of galantamine, and the sulfate conjugate of O-desmethyl-galantamine) and minor metabolites (glucuronide of O-desmethyl-epigalantamine, O-desmethyl-galantamine, N-desmethyl-epigalantamine, the N-oxide of epigalantamine, and O-desmethyl-epigalantamine) that have never been detected as such in in vivo trials (Table 4).

**TABLE 6**

Relative amounts of unchanged galantamine (UD) and its major metabolites in deproteinized 1-h plasma samples of rats and of three healthy male dogs after single oral administration of [3H]galantamine hydrobromide at 2.5 mg base-Eq/kg body weight and of four healthy male subjects after a single oral dose of 4 mg base-Eq [3H]galantamine hydrobromide.

The data for human subjects are separately presented for poor and extensive metabolizers of CYP2D6. Figures represent the percentage of sample radioactivity.

<table>
<thead>
<tr>
<th>Metabolite Code</th>
<th>Rats</th>
<th>Male</th>
<th>Female</th>
<th>Male Dogs</th>
<th>Poor Metabolizers</th>
<th>Extensive Metabolizers</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UD</td>
<td>43.9</td>
<td>83.2</td>
<td>22.6</td>
<td>68.4</td>
<td>62.1</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.5</td>
<td>3.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>14.3</td>
<td>11.4</td>
<td>23.3</td>
<td>20.3</td>
<td>23.8</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5.9</td>
<td>2.4</td>
<td>3.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>4.1</td>
<td>0.8</td>
<td>47.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>13</td>
<td>10.6</td>
<td>2.8</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>14</td>
<td>2.6</td>
<td>0.9</td>
<td>0.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>15</td>
<td>—</td>
<td>6.9</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>P9</td>
<td>—</td>
<td>—</td>
<td>2.2</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sum</td>
<td>92.8</td>
<td>115.5</td>
<td>100.0</td>
<td>88.7</td>
<td>99.3</td>
<td></td>
</tr>
</tbody>
</table>

——, not detected or below the limit of quantification.

*Metabolite fraction comprising 1 or 2 metabolites.

**FIG. 5.** Metabolic pathways of galantamine after a single oral dose in the rat, dog, and human.

The metabolite code is given in parentheses. R, rat; D, dog; H, human.
Bickel et al. (1991b) excluded the presence of pharmacologically active plasma metabolites based on pharmacokinetic-pharmacodynamic analysis. The present trial demonstrates the presence of galantamine and two plasma metabolites, namely the glucuronide of O-desmethyl-galantamine (metabolite 2) and the glucuronide of galantamine (metabolite 5). The glucuronide of O-desmethyl-galantamine has been shown to be inactive, whereas O-desmethyl-galantamine was more potent than galantamine (Bachus et al., 1999). Probably glucuronidation of galantamine itself will also result in substantial loss of activity, and this could all fit into the finding that galantamine alone is the pharmacological active compound in plasma.

The role of CYP2D6 in the O-demethylation of galantamine has been demonstrated before in vivo and in vitro (Bachus et al., 1999). In vitro incubations showed that CYP3A4 plays a role in the N-oxidation of galantamine [Reminyl (galantamine hydrobromide) package insert; Janssen Pharmaceutica, Titusville, NJ; March 2001]. In the present article, the difference between poor and extensive metabolizers was also evident from the metabolic profile in plasma. The glucuronide of O-desmethyl-galantamine (metabolite 2) represented up to 19% of the administered dose and were also observed in the present human trial.

**TABLE 7**

The relative contributions of various metabolic pathways of galantamine in rats, dogs, and humans after single oral administration of [3H]galantamine hydrobromide at 2.5 mg base-Eq/kg body weight (animals) and 4 mg base-Eq (humans)

The figures represent the percentage of the administered dose and were derived from the mass balance of galantamine and its metabolites in urine and faeces. The metabolites have been assigned to one or another group on the basis of their primary metabolic pathway.

<table>
<thead>
<tr>
<th>Group</th>
<th>Primary Metabolic Pathway</th>
<th>Metabolites</th>
<th>Rats Male</th>
<th>Rats Female</th>
<th>Dogs</th>
<th>Poor metabolizers</th>
<th>Extensive metabolizers</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Unchanged drug (UD)</td>
<td>UD</td>
<td>21.5</td>
<td>44.9</td>
<td>47.3</td>
<td>39.1</td>
<td>24.4</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>Oxidation/reduction</td>
<td>13, 14, 22, (17*, 21*)</td>
<td>4.6 (8.2)</td>
<td>5.3 (5.4)</td>
<td>1.6 (1.6)</td>
<td>3.5 (6.2)</td>
<td>8.2 (6.2)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>N-Oxidation</td>
<td>10, 17*</td>
<td>7.8</td>
<td>2.9</td>
<td>2.7</td>
<td>19.2</td>
<td>8.8</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>N-Demethylation</td>
<td>8, 16*</td>
<td>10.8</td>
<td>7.4</td>
<td>19.8</td>
<td>7.0</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>O-Demethylation</td>
<td>6, 2*, 3*, 20*, 21*, 23*</td>
<td>22.7</td>
<td>11.4</td>
<td>1.3</td>
<td>5.2</td>
<td>33.1</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>Sulfate conjugation</td>
<td>15, (22*, 23*)</td>
<td>1.3 (1.3)</td>
<td>16.7 (17.3)</td>
<td>— (—)</td>
<td>— (0.4)</td>
<td>— (8.9)</td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>Glucuronidation</td>
<td>5, (2*, 3*)</td>
<td>4.1 (16.9)</td>
<td>4.5 (13.6)</td>
<td>14.7 (14.7)</td>
<td>13.4 (13.4)</td>
<td>10.2 (31.2)</td>
<td></td>
</tr>
<tr>
<td>TOTAL*</td>
<td></td>
<td></td>
<td>72.8</td>
<td>93.1</td>
<td>87.4</td>
<td>87.4</td>
<td>81.0</td>
<td></td>
</tr>
</tbody>
</table>

* Secondary metabolite (derived from a primary metabolite).
* Metabolites in parentheses have not been included in the numerical total for that group to avoid double counting. The totals for these pathways are, however, included in parentheses.

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**References**


