ABSTRACT:
Focused on enzyme activities and protein binding. Kinetic investigations, and from several in vitro studies primarily of absorption, distribution, metabolism, and excretion, from toxicological and pharmacological studies, namely mice, rats, rabbits, and macaques. In both rats and macaques, irbesartan was characterized by a rapid oral absorption, a large volume of distribution, a low plasma clearance, and a long terminal half-life. The oral bioavailability in macaques was notably higher than in rats. Irbesartan was highly protein bound in rats and macaques. A lower binding rate was found in mice and rabbits. In distribution studies performed in rats, mice, and rabbits, irbesartan was rapidly distributed into most organs and tissues including brain, intrauterine area, and milk. No retention of radioactivity in tissues other than liver and kidney was noted. Irbesartan was the main circulating compound in rats, rabbits, and macaques representing a maximum of 67, 68, and 80% of plasma radioactivity, respectively. The drug was metabolized mainly by glucuronidation (primarily on the tetrazole ring), hydroxylation, and additional oxidation. The overall pathways within the different species generated 18 metabolites identified from bile, urine, and feces samples. Irbesartan did not significantly induce or inhibit most of the isoenzymes commonly associated with drug metabolism in either rats or macaques after oral administration for 1 month. In most species irbesartan and its metabolites were mainly excreted in feces with more than 80% of a radioactive dose recovered within 24 or 48 h. Enterohepatic circulation was demonstrated in rats and macaques.

DISPOSITION OF IRBESARTAN, AN ANGIOTENSIN II AT1-RECEPTOR ANTAGONIST, IN MICE, RATS, RABBITS, AND MACAQUES

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Metabolism and disposition of irbesartan, an angiotensin II AT1 receptor antagonist, were investigated in mice, rats, rabbits, and macaques. In both rats and macaques, irbesartan was characterized by a rapid oral absorption, a large volume of distribution, a low plasma clearance, and a long terminal half-life. The oral bioavailability in macaques was notably higher than in rats. Irbesartan was highly protein bound in rats and macaques. A lower binding rate was found in mice and rabbits. In distribution studies performed in rats, mice, and rabbits, irbesartan was rapidly distributed into most organs and tissues including brain, intrauterine area, and milk. No retention of radioactivity in tissues other than liver and kidney was noted. Irbesartan was the main circulating compound in rats, rabbits, and macaques. In both rats and macaques, irbesartan was characterised by a rapid oral absorption, a large volume of distribution, a low plasma clearance, and a long terminal half-life. The oral bioavailability in macaques was notably higher than in rats. Irbesartan was highly protein bound in rats and macaques. A lower binding rate was found in mice and rabbits. In distribution studies performed in rats, mice, and rabbits, irbesartan was rapidly distributed into most organs and tissues including brain, intrauterine area, and milk. No retention of radioactivity in tissues other than liver and kidney was noted. Irbesartan was the main circulating compound in rats, rabbits, and macaques representing a maximum of 67, 68, and 80% of plasma radioactivity, respectively. The drug was metabolized mainly by glucuronidation (primarily on the tetrazole ring), hydroxylation, and additional oxidation. The overall pathways within the different species generated 18 metabolites identified from bile, urine, and feces samples. Irbesartan did not significantly induce or inhibit most of the isoenzymes commonly associated with drug metabolism in either rats or macaques after oral administration for 1 month. In most species irbesartan and its metabolites were mainly excreted in feces with more than 80% of a radioactive dose recovered within 24 or 48 h. Enterohepatic circulation was demonstrated in rats and macaques.

Irbesartan, a synthetic, nonpeptide antagonist of angiotensin II with chemical name 2-butyl-3[(2’(1H-tetrazol-5-yl) biphenyl-4-yl) methyl]-1, 3-diazaspiro [4, 4] naphthalen, is currently registered as a new orally active, potent, and safe antihypertensive drug (Aprovel, Karvea, Avapro). Irbesartan displays a high selectivity for the AT1 receptor subtype, which is the receptor involved in all the known physiological effects of angiotensin II, including the vasoressor action. Irbesartan does not show any affinity for the AT2 receptor subtype (Cazaubon et al., 1993). It showed long-lasting hypotensive effects in normotensive macaques (Lacour et al., 1993; Roccon et al., 1994) and an antihypertensive effect in hypertensive rats (Lacour et al., 1994).

The preclinical disposition of the active agent was investigated in the species and strains used in toxicological and pharmacological studies, namely mice, rats, rabbits, and macaques. The macaque was used as the nonrodent species because nonhuman primates have a basal renin-angiotensin system that is more active than in other animal species (Michel et al., 1984; Wood et al., 1985; DeGraaf et al., 1993) and make them a good and sensitive model, predictive of human response. Furthermore, in vitro studies with irbesartan on hepatic microsomal fractions from various animal laboratory species showed a metabolic rate in macaque closest to that in humans.

Pharmacokinetic data were obtained from studies designed in terms of absorption, distribution, metabolism, and excretion, from toxicokinetic investigations, and from several in vitro studies primarily focused on enzyme activities and protein binding.

Most of the pharmacokinetic studies were carried out using the 14C-isotope labeled on position 1 of the cyclopentane ring of the molecule. In vitro serum protein binding and erythroplasmatic distribution studies were conducted using the 3H compound labeled on positions ω and ω-1 of the butyl chain.

Materials and Methods

Chemicals. Unlabeled irbesartan was synthesized at Sanofi (Montpellier, France). 14C-Irbesartan was purchased from Amersham International (Cardiff, Wales). The specific radioactivity was 2.11 GBq/mmol and its radiochemical purity was ≥98% as determined by HPLC. The 3H-labeled was synthesized at the Isotope Laboratory of Centre Etude Atomique (Gif sur Yvette, France). The specific radioactivity was 1.85 TBq/mmol and its radiochemical purity ≥98% as determined by HPLC. Appropriate isotopic dilutions of irbesartan were made for use in animal studies.

Aminopyrine, o-nitrophenol, aniline, erythromycin, and bovine glucuronidase/aryl-sulfatase were obtained from Sigma Chemical Co. (St. Louis, MO). 7-Ethoxyresorufin was obtained from Pierce (Rockford, IL). Valproic acid was purchased from Sanofi. Most of the metabolites were synthesized (Sanofi) or isolated from human urine (Bristol-Myers Squibb, Princeton, NJ) and used as reference compounds. All other reagents were obtained from commercial suppliers and were of analytical grade.

Design of Studies. Species investigated were mice (Cobs-CD1, males and females) aged 5 to 6 weeks (30 g) and rats (Sprague-Dawley, males and females) aged 7 to 8 weeks (180–340 g), all purchased from Charles River (Cléon, France); rabbits (New Zealand, hybrid females) aged 18 weeks (3.3–4.1 kg) purchased from Les Dombes (Chatillons les Chalaronne, France); and macaques (macaca fascicularis or macaca mulatta, males and females) 3 to 7 kg, age unknown (captured in the wild) and purchased from Centre de Recherche Primatologique (Mahebourg, Mauritius). They were housed in a clean air-conditioned room (temperature 19–25°C; relative humidity 40–85%) under a 12-h light/dark cycle and an air renewal cycle of 10–12-fold per hour without recirculation. They had free access to water and were fed laboratory chow (Union pour une Alimentation Rationnelle, Villemeison Epinay-sur Orge, France) except when they were fasted overnight before...
dialysis was determined by the method of Biorad. The concentration of protein in the serum before and after dialysis and in the buffer after dialysis were used to count total radioactivity. Concentrations of irbesartan at concentrations ranging from 0.01 to 20 mg/liter. Serum was collected in vacutainers containing either sodium or lithium heparin as anticoagulant at 5 to 16 time points (predose to 24, 48, 72, 144, or 168 h) after single administration or at 1 to 10 daily selected time points in the multiple dose design. Plasma was obtained by centrifugation and stored at –20°C until analysis to assess the concentration of radioactive irbesartan and its related metabolites. Excreta were collected in portion of 0- to 6-h, 6- to 24-h, and subsequently in 24-h intervals up to 48, 72, 144, or 168 h after dosing to determine routes, rates, and balance of excretion of irbesartan and metabolites. The biliary excretion was measured over 48 h postdosing in bile duct-cannulated rats and macaques. Bile collected from orally and i.v. dosed rats was administered intraduodenally to other bile duct-cannulated rats from which urine and bile were collected to assess the enterohepatic circulation of radioactivity. Tissues were collected in mice and rats for periods of time ranging between 1 and 168 h after dosing. The placental transfer was studied in pregnant rats at days 11 and 18 of gestation and rabbits at day 28 of gestation. Milk excretion was assessed from suckling rats after treatment of nursing mothers at day 11 of the lactating period.

To determine the pharmacokinetic parameters, the study in macaques adopted a crossover design, with each group of two animals receiving in sequence i.v. or oral doses of irbesartan, with a 15-day washout interval between each dose. Serial blood samples were collected in each macaque over the entire period of the study. The study in rats adopted a destructive design where an individual animal was assigned to each blood-sampling time.

**Blood/Plasma Distribution Ratio.** To determine blood/plasma distribution ratio, [14C]irbesartan was added to freshly drawn blood samples collected on lithium heparin to give final concentrations in the range 0.01 to 20 mg/liter. After mixing and incubation for 30 min at 37°C, aliquots of blood were analyzed for total radioactivity, then the remainder of the sample was centrifuged. Plasma was examined visually to discard samples in which hemolysis was detected and aliquots were measured for radioactivity. The proportion of irbesartan in plasma was assessed with the ratio \( \frac{\text{dpm in plasma/dpm in plasma} + \text{dpm in erythrocytes}}{100} \).

**Semen Protein Binding.** The semen protein binding of [14C]irbesartan was determined in vitro by equilibrium dialysis. Experiments were carried out in 1-ml Teflon dialysis cells (Dianorm System). Blank 0.8-ml serum was dialyzed in duplicate or triplicate against 0.8 ml of Sörensen’s phosphate buffer (0.13 M, pH 7.4). Containing an isotopic dilution of 2H2O or 14C-labeled and unlabeled irbesartan at concentrations ranging from 0.01 to 200 mg/liter. Serum was separated from buffer by a membrane (Dichema, diameter 63 mm, reference no. 1014; Dianorm, München, Germany) with a molecular weight cutoff of 5000. Dialysis was carried out at 37°C for 3 h under slow rotation (12 rpm). The 3-h time period was established in preliminary experiments to be sufficiently long to ensure that equilibrium of concentrations had been achieved. Postdialysis serum and buffer samples were recovered in tared test tubes. Test tubes were weighed again to assess sample volumes. Three aliquots of 0.1 ml retentate (serum) and dialysate were used to count total radioactivity. Concentration of protein in the serum before and after dialysis and in the buffer after dialysis was determined by the method of Biorad.

Membranes were prepared for use by washing two times with water for 2 × 15 min and then rinsing for another 15 min before soaking overnight at 4°C in the dialyzing buffer. The binding rate was assessed using the equation of Boudinot and Jusko (1984):

\[
B\% = \frac{([R] - [D]) \times (\text{Vol}_{\text{dialysate}}/\text{Vol}_{\text{retentate}}) \times 100}{[R]} \times \frac{([R] - [D]) \times (\text{Vol}_{\text{dialysate}}/\text{Vol}_{\text{retentate}}) + [D]}
\]

with \([R]\) the concentration in the retentate, \([D]\) the concentration in the dialysate, \(\text{Vol}_{\text{dialysate}}\) the volume of the retentate at equilibrium, and \(\text{Vol}_{\text{retentate}}\) the initial volume of the retentate.

**Measurement of Radioactivity.** Aliquots of plasma (0.1–0.2 ml), urine (0.1–2 ml), and bile (0.05 ml) were mixed with 10 ml of liquid scintillator, Ready Safe (Beckman, Fullerton, CA) or Biofluor (NEN, Boston, MA) and counted for radioactivity in a TriCarb 300C liquid scintillation counter, (Packard, Downer’s Grove, IL). Aliquots of blood (0.3–0.6 g) and fecal homogenate samples (0.4–1 g) were combusted in a Packard model 306 sample oxidizer and the radioactivity was recovered in Carbosorb/Permafluor (Packard) and was counted in the liquid scintillation counter. Tissue samples were subjected either to solubilization (HCl 2–6 M, or soluene) or to combustion to count radioactivity. Counting efficiency was corrected by external standard procedure. The quantification limit of radioactivity was set at 50 dpm after subtraction of twice background (40 dpm). All measurements were done in triplicate.

**Determination of Irbesartan by HPLC-Fluorescence.** Concentrations of irbesartan in plasma were determined using prevalidated assays in terms of specificity, repeatability, accuracy, and precision. The sample cleanup involved a liquid phase extraction at pH 5 with a 10-ml solution of ethyl acetate-methylenyl chloride (50:50, v/v). The organic layer was dried by the addition of about 3 g of anhydrous sodium sulfate, then evaporated at 37°C under nitrogen, and the residue was dissolved in 0.3 ml HPLC mobile phase.

The concentrations of irbesartan were determined by HPLC, relative to calibration curves that ranged from 0.02 to 5 mg/liter, on a μ Bondapak C18 column, 3.9 mm i.d. × 300 mm, 10 μm (Waters, Milford, MA) with a mobile phase of acetonitrile/diethylamine-phosphate buffer at pH 3.5 (50:50, v/v), at room temperature and a flow rate of 1 ml/min with fluorescence detection (excitation wavelength 254 nm, emission wavelength 371 nm). The lower limit of quantification of irbesartan in plasma ranged from 0.02 to 0.05 mg/liter across the different species. This allowed the levels of irbesartan to be assessed over 72 h post dosing.

**Metabolite Profiles: Radio-HPLC.** Metabolite profiles in plasma, urine, bile, and feces were investigated by HPLC with radiomonitoring on a column 3.9 mm i.d. × 300 mm, 10 μm μ Bondapak C18 (Waters). A solvent gradient was formed using a solvent delivery system Varian LC 5000 pump (Palo Alto, CA) from solvent A, diethylamine 0.1% adjusted to pH 5.2 with acetic acid and solvent B, acetonitrile with a flow of 1 ml/min. The gradient started with 80% A and 20% B, changed linearly to 60% A and 40% B over 15 min, and ended with 60% A, 40% B isocratically at 30 min. Radioactivity was detected using a Berthold detector 506C (Berthold, Eirty, France) with a Z1000 liquid scintillation cell and Quicksint Flow 302 (Zinsser Analytic, Frankfurt, Germany) as scintillant.

Plasma samples (0.5 ml) were diluted by volume with acetonitrile followed by centrifugation for protein precipitation. The solid was washed with acetonitrile. The combined supernatants were concentrated down to 0.15 or 0.20 ml at room temperature under a stream of nitrogen and applied to radio-HPLC. Urine and bile samples were analyzed without any pretreatment except a filtration on a 0.45 μm-ulfrafilter filter (Millipore, St. Quentin en Yvelines, France). Fecal homogenate samples were extracted twice with 5 ml of methanol. The combined clear methanolic phase was kept dry under a stream of nitrogen. The residue was dissolved in 0.25 ml of water and subjected to radio-HPLC analysis. The recovery of radioactivity from sample processing was greater than 85%.

For enzymatic hydrolysis, portion of plasma, urine, bile, and fecal homogenate extracts were adjusted to pH 4.5 by addition of sodium acetate buffer and incubated with bovine β-glucuronidase/arylsulphatase (500,000 U/ml) for 16 h at 37°C. After incubation, small aliquots of the solutions were checked for excess of β-glucuronidase using phenolphthalein glucuronic acid as substrate.

**Liver Microsome Preparation.** Microsomes were prepared from the different species by tissue homogenization and centrifugation at 105,000g. Protein concentration was determined by the method of Pollard et al. (1978) using the Bio-Rad assay. Cytochrome P-450 (CYP) levels were determined by the method of Omura and Sato (1964).

**Liquid Chromatography/Mass Spectrometry (LC/MS) Analysis.** LC/MS analysis was performed using a HP 1050 chromatograph on line with a Berthold 506C radioactivity detector and a VG Instruments, AUTOSPEC/Q

1 Abbreviations used are: CYP, cytochrome P-450; AUC, area under the plasma concentration-time curve; LC/MS, liquid chromatography/mass spectrometry; \( V_d \), volume of distribution; \( C_{\text{peak}} \), peak concentrations of radioactivity and irbesartan in blood, plasma, and tissues; CL, clearance.
mass spectrometer (Manchester, UK) equipped with an on-line LC/MS Liquid Secondary Ion Mass Spectrometry (LSIMS)-FAB(+) ionization mode. MS experiments were performed using a continuous flow FAB+. The interface mass range was set between 50 and 1000 Da at a rate of 3 s/decade. The source temperature was set at 70°C. The mobile phase was as indicated above except that 1% glycerol was added in each solvent. Matrices (plasma, urine, bile, and feces) were pretreated as indicated for radio-HPLC metabolite profiling before processing with LC/MS.

**Autoradiography.** Sagittal whole body sections of 20 μm thickness were obtained at different levels of anatomical interest. They were prepared according to the technique of Ulberg (1954) using a large specimen cryomicrotome. The sections were placed in cassettes allowing close contact with Imaging Plates (Fuji, Tokyo, Japan). After 18 h of exposure under lead protection, the imaging plates were processed with a laser beam scanner Fujix Bas 2000 processor (Fuji) to obtain the true digital image. The image was then submitted to Tina software package (Raytest, Straubenhardt, Germany) to generate volume integration count from standard calibration sections.

**Pharmacokinetic Analysis.** The peak concentrations of radioactivity and irbesartan in blood, plasma, and tissues (C(max)) and the time to reach peak concentration (t(max)) were observed values. Plasma concentration profiles were fitted to a noncompartment model using the peeling algorithm available on Kintool program (Qualilab, Orleans, France), to calculate the slope of the apparent terminal phase (β) and the apparent volume of distribution (Vd). Area under the plasma concentration-time curve up to 24 h (AUC(t)), and up to time t (AUC(t)) with t as the last time point with measurable concentration, were calculated by the trapezoidal rule. AUC(max) was calculated by adding Cβ to AUC(t), with C(t) as the last measurable concentration. Plasma concentration at time 0 for calculating the AUC was estimated from the curve-fitting data. The bioavailability factor (F) was calculated assuming the linearity of plasma concentrations up to 30 mg/kg and using AUC(p.o./i.v.). The apparent terminal half-life of concentrations in the terminal segment of the concentration-time curves (T1/2) was calculated by logarithmic regression analysis. The clearance (CL) was calculated as dose/AUC. Dose proportions recovered in individual urine, bile, and feces samples were calculated to establish the mass balance over 24, 48, or 168 h. The proportions of the parent and individual metabolite peaks in plasma, urine, bile, and feces samples were determined by radio-HPLC. No statistical analysis was performed on the pharmacokinetic parameters and data were expressed as individual values or as mean ± S.D. (n = 2–6).

**Results**

**Stability Tests.** No tritium exchange occurred during the study and the tritium-labeled compound was found to be stable in the used experimental conditions.

**Absorption and Bioavailability.** The urinary and fecal excretion of radioactivity after i.v. administration of irbesartan at 1 mg/kg to the rat and macaque was similar to that after oral administration of 10 mg/kg, suggesting good oral absorption and biliary involvement in the disposition of the compound in these species. Additional evidence for the good absorption of orally administered [14C]irbesartan in the rat and the macaque was obtained from bile duct-cannulated animals, which excreted 44 to 78 and 48 to 58% of the dose in the bile, respectively. More experiments in bile duct-cannulated rats receiving an intraduodenal dose of [14C]-labeled bile (from rats dosed with [14C]irbesartan) showed that the drug-related material secreted in the bile underwent an enterohepatic circulation involving at least 10% of the initial dose of irbesartan. The N2-glucuronide (metabolite E) of the parent drug, which represented a maximum of 54% of the administered dose into the bile of rat and macaque, accounted for most of the enterohepatic reprocessing of irbesartan (Fig. 1). Maximum plasma levels of irbesartan generally occurred 1 to 4 h after dosing, consistent with rapid absorption. The absolute bioavailability of irbesartan averaged 78% in macaques at 10 mg/kg oral dose and was similar to that found in humans (61–82%).

**Pharmacokinetics after Single Doses.** To determine the major pharmacokinetic parameters from plasma samples, rats and macaques received single oral 10-, 30-, and 90-mg/kg oral or 1-mg/kg i.v. doses of the test compound. Data obtained (Table 1) demonstrated that both species exhibit rapid absorption and long terminal half-life. This latter parameter results from low plasma CL and large Vd. However, the observation of relatively lower CL (≈1/40 hepatic blood flow) and Vd (≈2- to 3-fold total body water) in rats than in macaques (≈1/8 hepatic blood flow and ≈10-fold total body water, respectively) indicates that irbesartan behaved differently within each species. The lower oral bioavailability of irbesartan in rats than in macaques also illustrates this feature. The percentage of radioactivity recovered in the rat bile was greater than 70% of the administered radioactive dose. This led to rejection of the hypothesis of limited intestinal absorption to account for the low bioavailability in rats. AUC(0–24) values after oral administration of 10 mg/kg in rats and macaques were of a similar order. Conversely, AUC(0–24) values after i.v. administration of the 1-mg/kg dose appeared to be 3- and 5-fold higher in rats than in macaques, probably because of a lower Vd. This may explain the lower oral bioavailability recorded in rats. However, the bioavailability found in rats may be a less reliable estimate because it was derived from a less appropriate destructive study design.

**Pharmacokinetics after Multiple Doses.** Plasma concentrations after multiple (5–2000 mg/kg once daily from 1–53 weeks) oral doses of irbesartan were evaluated in mice, rats, and macaques. Typically, sampling times were 1, 2, 4, 8, and 24 h after daily administration. The increase of plasma concentrations generally appeared to be subproportional (data not shown). Deviations from the linearity appeared to be minimal in monkeys and highest in rats. Several mechanisms may explain a smaller than proportional increase in systemic irbesartan concentration with the high doses. One possibility is that intestinal absorption of irbesartan is dose limited, perhaps due to saturation of a carrier system at high drug concentrations or to a limited dissolution rate but more studies are necessary to substantiate this occurrence. Other possibilities may include saturation of protein binding or increased metabolism. Protein-binding studies clearly established that saturation occurred at concentrations of irbesartan corresponding to doses beyond 250 and 500 mg/kg per day in rats and macaques, respectively. On the basis of assessed enzymatic activity regulations (Table 2), autoinduction of the glucuronidation pathway might be also viewed as a factor altering the linearity in rats.
Pharmacokinetic parameters of irbesartan in plasma after single oral and i.v. administration of the drug to rats and macaques

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose Route</th>
<th>Sex</th>
<th>T_{max}</th>
<th>C_{max}</th>
<th>t_{1/2b}</th>
<th>AUC</th>
<th>CL</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>i.v.</td>
<td>M</td>
<td>12 h</td>
<td>9.0</td>
<td>18.9</td>
<td>24</td>
<td>8.7</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>M</td>
<td>19 h</td>
<td>18.0</td>
<td>13.5</td>
<td>21</td>
<td>18.0</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>F</td>
<td>14.3 h</td>
<td>14.3</td>
<td>14.3</td>
<td>17</td>
<td>14.3</td>
<td>0.71</td>
</tr>
<tr>
<td>Macaque</td>
<td>a)</td>
<td>M</td>
<td>24 h</td>
<td>5.8</td>
<td>15.0</td>
<td>38</td>
<td>5.8</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>b)</td>
<td>M</td>
<td>21 h</td>
<td>71.0</td>
<td>71.0</td>
<td>38</td>
<td>71.0</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Rats exhibited a considerable gender-related difference at dosage levels greater than 50 mg/kg per day. Although exposures were similar in both genders after multiple dosing at 50 mg/kg per day over 13 weeks, female rats had a 5-fold higher C_{max} and a 2- or 4-fold higher AUC_{0–24 h} than males at 250 and 500 mg/kg per day, respectively (data not shown). A higher rate of biliary excretion in male rats decreasing the amount of drug transferred to the systemic circulation may account for why plasma levels of irbesartan in rats were higher in females than in males. The highest concentration of cytochrome P-450 in male rats (Table 2) might also account for this discrepancy.

The pharmacokinetics of irbesartan did not change markedly between day 8 and 28. Exposures [C_{max}, C_{a帘} and AUC] values remained relatively steady from day 8 to 28 in both rats and macaques for each dosage up to 1000 mg/kg per day (data not shown).

**Blood Distribution and Protein Binding.** Irbesartan had little affinity for red blood cells (usually less than 10% of whole blood radioactivity). Binding to serum proteins varied among species: 93 to 97% in rats, 91 to 94% in macaques, 85% in mice, and 68% in rabbits. The binding to human proteins was approximately 96%. Saturation generally occurred at concentrations of 50 to 100 mg/liter, which is 10- to 20-fold higher than those achieved at the 300-mg dose level in humans. These concentrations were found at doses beyond 250 mg/kg per day in rats, 500 mg/kg per day in macaques, or 2000 mg/kg per day in mice.

**Tissue Distribution in Normal and in Pregnant Animals and Milk Excretion.** Tissue distribution was investigated in mice (300 mg/kg), rats (nonpregnant, 30 mg/kg and pregnant, 150 mg/kg) and rabbits (pregnant, 10 mg/kg) using tissue dissection and/or whole body autoradioluminography. Dosages were chosen within the range of dosages tested in toxicology and teratology studies.

Both techniques and all doses investigated showed almost the same distribution pattern. Radioactivity appeared in all tissues sampled, including brain at the earliest observation time of 1 h after dosing. In terms of concentration, tissues fall into three main groups when compared with blood: levels were higher in the liver, gut, and kidneys; almost equal in lungs; and much lower in adrenals, myocardiun, salivary gland, spleen, ovary, bone marrow, Harder’s gland, thymus, muscle, and brain. The aortic wall appeared to be labeled only in the mouse. For all tissues, the radioactivity had significantly decreased by 24 h and was essentially quantitatively eliminated by 48 to 96 h although some residual radioactivity was found in the liver and kidneys.

Placental transfer of radioactivity into fetuses of rats and rabbits was low. Storage of radioactivity was observed in the gut and gall bladder. Radioactivity was also detected in the gut and liver of rat pups after treatment of lactating dams.

**Metabolism.** Mass spectrometric fragmentation of irbesartan and relevant fragment ions recorded in the mass spectra of metabolites derived from the main metabolic pathways are shown in Fig. 2. The proposed metabolic pathways of irbesartan with the distribution of the various metabolites in the different biological media across the different animals species are presented in Fig. 3 and Table 3.

Irbesartan was the main circulating compound (40–85% of plasma radioactivity across species and time points), and no major metabolites were found in plasma after oral and/or i.v. administration of [14C]-labeled irbesartan to rats, rabbits, and macaques. However, in mice, considerably lower levels of irbesartan (2.6–12.5%) were observed together with several plasma metabolites. The major routes of biotransformation involved N-glucuronidation and oxidation to hydroxylated and/or N-dealkylated (loss of tetrazolyl-diphenyl-methyl moiety) metabolites. These were further hydroxylated at different positions, further oxidized at the same position,
Effect of irbesartan on enzyme activities in rat and macaque liver microsomes after 1-month oral administration of raising doses of the drug

Microsomes (1 mg protein) were incubated with the marker enzyme activities in the presence of either NADPH or UDP-glucuronic acid fortified with their respective co-factors at pH 7.4 (potassium phosphate or Tris-HCl) in a final volume of 1 or 2 ml at 37°C. Irbesartan autoinduction test was carried out in Tris-HCl at pH 5.5.


<table>
<thead>
<tr>
<th>Species (sex)</th>
<th>Rat (Male)</th>
<th>S.D. (n = 5)</th>
<th>Rat (Female)</th>
<th>S.D. (n = 5)</th>
<th>Macaque (Male and Female)</th>
<th>S.D. (n = 6: three Males and three Females)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg/day)</td>
<td></td>
<td>0</td>
<td>30</td>
<td>70</td>
<td>150</td>
<td>0</td>
</tr>
<tr>
<td>Liver body weight</td>
<td>0.04</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.001-0.003</td>
<td>0.04</td>
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<tr>
<td>CYP-450 level</td>
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<td>0.73*</td>
<td>0.83</td>
<td>0.71*</td>
<td>0.03-0.13</td>
<td>0.45</td>
</tr>
<tr>
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<td>0.07</td>
<td>0.07</td>
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<tr>
<td>AND</td>
<td>4.60</td>
<td>5.00</td>
<td>4.90</td>
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<td>0.69</td>
<td>0.66</td>
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<tr>
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<td>1.42</td>
<td>1.61</td>
<td>1.41</td>
<td>1.11</td>
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<td>1.05</td>
</tr>
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<td>Na-G</td>
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<td>6.50</td>
<td>7.30</td>
<td>7.20</td>
<td>0.80-1.40</td>
<td>4.00</td>
</tr>
<tr>
<td>VPA-G</td>
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<td>39.0</td>
<td>45.0</td>
<td>40.0</td>
<td>3.0-9.0</td>
<td>31.0</td>
</tr>
<tr>
<td>Irbesartan-GR</td>
<td>1.00</td>
<td>1.28</td>
<td>1.64</td>
<td>1.64</td>
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<td>-</td>
</tr>
</tbody>
</table>

*, Difference to control statistically significant: P < .05 (Statistical analysis: Bartlett’s then ANOVA and Dunnett’s tests).

Values expressed as nanomoles per milligram of protein.

Values expressed as nanomoles per minute per milligram of protein.

Values expressed as nanomoles of glucuronide per minute per milligram of protein.

Values expressed as glucuronidation rate relative to untreated animal.

or further conjugated with glucuronic acid. The overall pathways led to at least 18 metabolites within the different species investigated.

**Metabolic pattern in mice.** After single oral dosage of [14C]irbesartan, the parent drug accounted for a small proportion of plasma radioactivity (12.5–2.6% from 5–30 min after dosing). The major circulating metabolite was the N-dealkyl derivative (metabolite F). The remaining plasma radioactivity was associated with several other metabolites from which two were characterized as monohydroxy N-dealkyl derivatives of irbesartan (metabolites G). Four isomers of metabolite G and one N-dealkyl-oxo-irbesartan metabolite (metabolite R) constituted almost all the urinary radioactivity, 27 to 38% of the administered dose over 6 and 24 h after dosing.

**Metabolic pattern in rats.** Most of the radioactivity present in the plasma of rats represented unchanged drug. After oral (single or repeated) or i.v. doses of [14C]irbesartan, the parent drug accounted for more than 67% of the total plasma radioactivity over 24 h after dosing. The remaining radioactivity was associated with several other quantitatively minor metabolites from which one was identified as N2-glucuronide (metabolite E) and one as a ring-opened irbesartan derivative (metabolite J). The four monohydroxy N-dealkyl irbesartan derivatives (metabolites G) and the N-dealkyl-oxo-irbesartan derivative (metabolite R) constituted almost all the urinary radioactivity (8% of the administered dose) in male rats. The metabolic pattern was different in female rats (6% of dose) with small amounts of parent drug (about 10% of excreted material), monohydroxy irbesartan metabolites (metabolites C, three isomers) and metabolite J as excreted compounds. Repeated dosing did not modify the urinary metabolite pattern. No major qualitative or quantitative differences were observed in the biliary metabolite profiles between males and females rats after oral or i.v. routes. Over 48 h after dosing, the parent drug accounted for at most 21% of the excreted radioactivity whereas proportions of the N2-glucuronide ranged between 29 and 69%. After repeated oral dosing for 2 weeks, irbesartan accounted for 20, 51, and 54% of the total radioactivity recovered in feces on days 1, 7, and 14, respectively. Several other unconjugated metabolites were also detected.

**Metabolic pattern in rabbits.** Most of the radioactivity present in plasma represented unchanged drug. Irbesartan accounted for 68 to 40% in 1- to 8-h plasma samples obtained after a single oral dose and declined to about 14% at 24 h. The major circulating metabolites were monohydroxy derivatives (metabolites C). The main excreted urinary compounds (22% of dose) were two different cyclopentane monohydroxy derivatives (metabolites C), the parent drug and its N2-glucuronide (metabolite E). Several other quantitatively minor metabolites were also identified.

**Metabolic pattern in macaques.** Irbesartan accounted for 85 to 41% in 0.17- to 8-h plasma samples after single i.v. administration, for 80 to 50% in 1- to 4-h samples after single oral administration and for 79 to 55% in 0- to 24-h samples after 14 days of daily oral administration. The remaining radioactivity was associated mainly with metabolite J, and two glucuronide derivatives (metabolites E and I). In urine, the parent drug accounted for 0.6 and 9% of total radioactivity in pooled 0- to 6- (3% of dose) and 24- to 48-h (2% of dose) samples, respectively. The main excreted compound after oral administration was the monohydroxy N-dealkyl derivative (metabolite G) whereas i.v. route, the main excreted compound was the N2-glucuronide. Repeated dosing for 14 days did not modify the metabolic pattern. In bile, the N2-glucuronide accounted for about 94% of the radioactivity excreted over 48 h after oral administration and about 81% after i.v. administration. In feces, the parent drug was the main compound accounting for 48 to 66% of the total radioactivity. The remaining radioactivity was associated with several other metabolites.

**Liver Enzyme Activity Regulation.** The catalytic activities of microsomes prepared from specimen of rat and macaque livers obtained after 1 month treatment with irbesartan were tested toward test substrates as 7-ethoxresorufin, aminopyrine, aniline, and erythromycin for specific phase I (CYP1A, CYP2C, CYP2E, and CYP3A, respectively) and O-naphthyl and valproic acid for phase II (UGT1 and UGT2 UDP-glucuronosyltransferase, respectively) isozyme subfamilies. As a broad generalization, CYP activities appeared preserved after multiple dosing in both rats and macaques (Table 2). In rare cases, some CYP activities appeared to change slightly when compared with microsomes from untreated animals. CYP total level decreased by 33% in macaques. Although statistically significant (P ≤ .05, ANOVA and Dunnett’s test) these slight changes could be
considered as biologically insignificant. Moreover, the isoforms affected were not significantly involved in the process of irbesartan metabolism. On the other hand, multiple dosing caused a slight increase, statistically not significant, in UGT1 (19% in female rat and 11% in macaque), in UGT2 (16% in male rat, 40% in female rat, and 14% in macaque), and in irbesartan autoglucuronidation rate (60–90% in male and female rats). No autoinduction of the glucuronosyltransferase activities were noticed in macaques.

These screens indicated that irbesartan did not act as an inducer or an inhibitor of phase I isozymes, although some minimal variation on CYP content, CYP1A and CYP3A activities were noticed. However a slight increase of glucuronosyltransferase activities might be expected after multiple dosing in rats.

**Excretion.** Except in mice, which excreted similar amounts of radioactivity in urine and feces, the major route of excretion of radioactivity was the feces. The urinary excretion was fairly constant (generally less than 15% of the dose) across the different studies (Table 4). In all cases, the elimination was rapid with most of the excreted material appearing in the feces within 24 or 48 h. The presence of considerable amounts of radioactivity in the feces after oral administration was a consequence of extensive biliary excretion (44–78% of the oral dose over 24 or 48 h after dosing) as shown in bile duct-cannulated rats and macaques.

The results of the study in which the bile of irbesartan-treated rats was infused into the duodenum of bile duct-cannulated rats showed the occurrence of substantial enterohepatic recirculation (9–14% of the 10-mg/kg initial oral dose). Radioactivity remaining in the carcass 7 days after single oral dosage in rats was less than 2%, indicating that xenobiotic elimination was essentially complete.

**Comparison with Humans.** In humans, irbesartan was rapidly and virtually completely absorbed after oral administration. The peak plasma concentrations were generally achieved within 1 to 2 h. The

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>MH⁺</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<th>5</th>
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<td>235</td>
<td>401</td>
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<td></td>
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</tr>
<tr>
<td>G</td>
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</tr>
<tr>
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</tbody>
</table>

**Fig. 2.** Mass spectrometric fragmentation of irbesartan and characterization of relevant fragment ions of metabolites derived from the main metabolic pathways. MH⁺ indicates the protonated molecular ion for the compounds. These data were obtained using on-line LC/MS (LSIMS) analyses on crude (urine, bile) or extracted (plasma, feces) matrices. 1 to 7: indicate major fragment ions observed in the mass spectra of irbesartan and its metabolites. Structural elucidation of metabolites C, E, H, I, and O was also based on similarity of mass spectral data with either reference standard or with metabolites isolated from human urine (Chando et al., 1998).
absolute bioavailability was 60 to 80% and was not affected by food. Plasma concentrations over the therapeutic dose range increased in a dose-proportional manner and accumulation did not occur after multiple doses as expected for a drug with a half-life of about 16 h. The pharmacokinetics of irbesartan was not influenced by gender. The apparent $V_d$ was approximately 2-fold the total body water. Total body CL was about 1/10 of hepatic blood flow. Of the radiolabeled oral dose, approximately 20% was recovered in the urine and approximately 55% in the feces, indicating that elimination is primarily via biliary excretion (Necciari et al., 1994; Gillis and Markham, 1997; Marino et al., 1998). This pharmacokinetic profile has been found similar to that in the macaque. The rat differed with a much lower
bioavailability, a dose-dependent exposure, a lower CL, a marked gender effect, and a rather smaller urinary excretion.

With regard to metabolism, unchanged irbesartan in humans accounted for about 80% of total plasma radioactivity whereas the glucuronide conjugate (metabolite E, Fig. 3; Table 3), the major metabolite, accounted for 6 to 8%. None of the remaining metabolites exceeded 4% of the total plasma radioactivity (Chando et al., 1998). This pattern more closely resembled that in macaque than in mouse, rat, and rabbit. In humans, irbesartan and its glucuronide metabolite each accounted for about 10% or less of the urinary radioactivity. The predominant metabolite excreted in human urine (26% of the urinary radioactivity) was the ω-1 monohydroxylated metabolite (metabolite C in Fig. 3 and Table 3). The other major metabolites (17–20% of the urinary radioactivity) were metabolites B, C isomers, N, and O (Fig.
The studies described here with mice, rats, rabbits, and macaques have provided a survey of the disposition of irbesartan in animal laboratory species. Irbesartan was rapidly and almost completely absorbed after oral administration. The food effect on absorption was not specifically examined in these animals. However, high exposure to irbesartan was achieved at high dosage in fed animals, suggesting that food would not significantly affect the absorption of the compound. No food effect on the pharmacokinetic parameters has been found in humans (Necciari et al., 1994). Clinically insignificant food effects have been reported for candesartan (candesartan cilexetil (Atacand) product information, 1998), losartan (Iosartan (Cozaar) product information, 1998), and valsartan although administration with food reduced the absorption of valsartan by 40% [valsartan (Diovan) product information, 1998]. In rats and macaques, the pharmacokinetics of irbesartan was characterized by a large V<sub>p</sub>, a low plasma CL, and a long terminal half-life. These findings could be explained by the large uptake of the compound in the liver, substantial excretion into the bile, and subsequent reabsorption from the gut. Similar statements are reported for losartan in the dog although the V<sub>p</sub>, the systemic CL, and the elimination half-life are of different values (Christ et al., 1994). Irbesartan AUC and C<sub>max</sub> generally increased less than dose proportionally.

Steady state of plasma concentrations in rats and macaques was achieved within the first week of treatment and minimal accumulation was observed. These results indicated that animals were under a relatively constant exposure to the compound during mid- and long-term treatments.

The bioavailability factor was notably greater in macaques than in rats. At high dosages, rats showed gender-specific differences in the plasma levels of parent compound, with greater exposure to drug in females than in males. These differences were less marked in mice and no sex-related difference was observed in macaques. It is not at all uncommon for a chemical to have different pharmacokinetic behavior in males versus females in small species. This feature generally results from the well-described differences in microsomal multifunction oxidase activities, which have been frequently and convincingly demonstrated in rats as opposed to other species (Cox Gad and Chengelis, 1992). The microsomal mixed function oxidase is under a variety of hormonal controls that are responsible for the sex-related differences. Rates of microsomal metabolism are generally higher in males than in females with several model substrates including aminopyrine, a marker of CYP2C mainly involved in the metabolism of irbesartan (Table 2). Gender-related differences observed on urinary metabolite profiles in rats with the presence of monohydroxy N-dealkyl derivatives in males and the presence of parent drug (suggesting lower metabolism) and monohydroxy irbesartan derivatives in females corroborate this statement. As reviewed by Mulder (1986) and consistent with the data in Table 2, hepatic UDP-glucuronosyltransferase activities in rats were also higher in males than in females. However, no significant sex-related difference was observed in the N<sub>3</sub>-glucuronide formation of irbesartan. Like losartan (Christ et al., 1994), valsartan (Waldmeier et al., 1997), and candesartan [candesartan cilexetil (Atacand) product information, 1998], irbesartan showed little affinity for red blood cells and was essentially confined to plasma. The binding of irbesartan to serum protein in mice and rabbits was relatively low compared with that found in rats, macaques, and humans. The [14C]irbesartan-related material was rapidly distributed into most organs and tissues including intrauterine area and milk. Milk excretion in rats has been also reported for valsartan (Fachinformation, 1996) and candesartan (candesartan cilexetil (Atacand) product information, 1998).

The current studies show that irbesartan generally accounts for most of the total circulating components and it is probable that irbesartan also

### TABLE 4

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose</th>
<th>Route</th>
<th>Sex</th>
<th>Urine 0-24 h</th>
<th>Urine 0-16 h</th>
<th>Bile 0-24 h</th>
<th>Bile 0-48 h</th>
<th>Feces 0-24 h</th>
<th>Feces 0-16 h</th>
<th>Number of Animals</th>
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<tbody>
<tr>
<td>Rat</td>
<td>10</td>
<td>P.o.</td>
<td>M</td>
<td>7.3 ± 1.4</td>
<td>7.9 ± 13</td>
<td>70.2 ± 8.6</td>
<td>81.7 ± 11</td>
<td>73.8 ± 2.8</td>
<td>77.7 ± 2.1</td>
<td>n = 4</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td>5.1 ± 0.7</td>
<td>6.1 ± 0.8</td>
<td>56.9 ± 12.0</td>
<td>85.1 ± 2.0</td>
<td>65.0 ± 6.7</td>
<td>71.6 ± 6.3</td>
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<tr>
<td></td>
<td>30</td>
<td>P.o.</td>
<td>M</td>
<td>3.4 ± 1.0</td>
<td>3.5 ± 10</td>
<td>93.2 ± 5.5</td>
<td>99.0 ± 3.9</td>
<td>85.3 ± 5.5</td>
<td>86.9 ± 4.8</td>
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</tr>
<tr>
<td></td>
<td>F</td>
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<td></td>
<td>4.2 ± 0.7</td>
<td>4.6 ± 0.8</td>
<td>74.0 ± 5.4</td>
<td>86.9 ± 4.8</td>
<td>53.1 ± 6.2</td>
<td>85.4 ± 5.8</td>
<td>n = 4</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>I.v.</td>
<td>F</td>
<td>4.8 ± 1.8</td>
<td>5.4 ± 18</td>
<td>51.6 ± 5.7</td>
<td>64.6 ± 5.5</td>
<td>51.6 ± 11.3</td>
<td>64.6 ± 11.3</td>
<td>n = 3</td>
</tr>
<tr>
<td>Macaque</td>
<td>10</td>
<td>P.o.</td>
<td>M</td>
<td>7.1 ± 1.4</td>
<td>10.1 ± 3.3</td>
<td>115 ± 9.3</td>
<td>81.5 ± 8.4</td>
<td>44.3 ± 20.0</td>
<td>48.1 ± 18.9</td>
<td>n = 3</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>I.v.</td>
<td>M</td>
<td>21.5 ± 20.7</td>
<td>57.5 ± 45.1</td>
<td>0.1 ± 0.2</td>
<td>82.6 ± 8.2</td>
<td>20.7 ± 57.5</td>
<td>14.2 ± 44.3</td>
<td>n = 3</td>
</tr>
</tbody>
</table>

Urine, bile, and feces were collected in portions of 0- to 6-, 6- to 24-, and subsequently in 24-h intervals up to 48 or 168 h after dosing. Bile was collected from conscious bile duct-cannulated animals. Radiolabeled contents were determined by direct liquid scintillation counting (urine and bile) or after combustion (feces).
accounts for most of the radioactivity in the target tissues. In all animal species, the primary route of elimination was via N-glucuronidation followed by biliary excretion and ultimately by fecal excretion. When expressed as percentage of the given dosages, irbesartan N\textsubscript{2}-glucuronide in bile amounted to at least 37 to 40% in rats and 45% in monkeys. The high levels of radioactivity in the liver and kidneys suggest that the radioactivity is highly cleared. Consistent with this statement, the blood and tissue concentrations of radioactivity declined rapidly, and more than 60% of the administered dose was generally excreted in urine and feces via the bile within 24 h. The excretion balance was effectively complete (\(\simeq 90\%\)) in 168 h. The long half-life of irbesartan could be explained by the biliary excretion of its N\textsubscript{2}-glucuronide, its hydrolysis back to irbesartan in the gut, and its subsequent reabsorption. Indeed, the reprocessing of irbesartan from its glucuronide was demonstrated after oral administration of the latter to monkeys. Although the intestinal microflora is more likely to hydrolyze the N\textsubscript{2}-glucuronide back to the parent compound, there is no experimental basis to exclude the intrinsic activity of the mucosa. Despite a long terminal half-life, minimal accumulation of irbesartan was observed after repeated oral administration in accordance with the substantial amount of drug eliminated by 24 h. This is consistent with rapid establishment of steady state in plasma concentrations.

The major compound generally detected in plasma was the parent drug, responsible for pharmacological activity, but no less than 18 metabolites resulting from hydroxylation, N-dealkylation, additional oxidation, glucuronidation, and imidazole ring opening, were shown to be present in urine or bile. Irbesartan N\textsubscript{2}-glucuronide accounted for most of total radioactivity in the bile whereas irbesartan was ultimately found in the feces. This is in agreement with the enterohepatic recirculation of irbesartan discussed above. All metabolites identified in humans (Chando et al., 1998) were detected in animals in the different biological matrices (plasma, urine, bile, and feces), suggesting that animals in toxicological studies were exposed to all human metabolites. Irbesartan did not induce or inhibit the activity of isozymes commonly associated with drug metabolism. CYP2C9 has been shown to be the main isoform involved in the oxidative metabolism of irbesartan in humans (Bourrè et al., 1999). This isozyme has been shown to be also involved in the biotransformation of the other best studied angiotensin antagonists, losartan (Spiegelberg et al., 1996; Williamson et al., 1997) and candesartan (Miwa et al., 1998). Waldmeier et al. (1997) tentatively explained the involvement of CYP2C9 by the established preference of this enzyme for anionic substrates. CYP2C9 is one of the predominant CYP isoforms expressed in humans (Shimada et al., 1994) and has been shown to be implicated in genetic polymorphism (Spiegelberg et al., 1996). Genetic polymorphism and/or modulation of CYP2C9 activities by specific inducers or inhibitors are expected to result in intersubject variability in the pharmacokinetics, as well as in the efficacy of these drugs. Unlike losartan (Wong et al., 1996) and candesartan cilexetil (Nishikawa et al., 1997), and similar to valsartan (Waldmeier et al., 1997), irbesartan does not generate an active metabolite. Some of the metabolites of irbesartan displaced 125\textsuperscript{i}-angiotensin II from the AT\textsubscript{1} receptor in vitro (J. Gougat, in house data), but because they were detected only in very small quantities in human plasma, they are unlikely to contribute to the pharmacological activity of irbesartan. Therefore, irbesartan does not depend on active metabolite formation for its sustained pharmacological activity. In contrast to irbesartan, the efficacy of losartan in subjects with genetically determined deficient CYP2C9 activity is expected to be decreased because little of the active metabolite would be formed. This suggests also the possibility of decreased metabolism and thus decreased activity of losartan in the presence of specific CYP2C9 inhibitors.

In conclusion, irbesartan was well absorbed in all species. Although cleared mainly metabolically, irbesartan circulated primarily as unchanged compound with persistent concentration in plasma of rats, rabbits, and macaques. The macaque exhibited pharmacokinetic parameters close to human values. Therefore, reliable clinical predictions can be made from data from this nonhuman primate species that was used extensively for pharmacological and toxicological testing. Rats revealed greater difference in bioavailability and showed gender-related differences not observed in humans (Vachharajani et al., 1995). Mice and rabbits demonstrated different levels of protein binding.

References