DISPOSITION AND CHEMICAL STABILITY OF TELMISARTAN 1-O-ACYLGLUCURONIDE

T. EBNER, G. HEINZEL, A. PROX, K. BESCHKE, AND H. WACHSMUTH

Boehringer Ingelheim Pharma KG, Biberach, Germany

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ABSTRACT:
Telmisartan 1-O-acylglucuronide, the principal metabolite of telmisartan in humans, was characterized in terms of chemical stability and the structure of its isomerization products was elucidated. In addition, pharmacokinetics of telmisartan 1-O-acylglucuronide were assessed in rats after i.v. dosing. Similar to other acylglucuronides, telmisartan 1-O-acylglucuronide and diclofenac 1-O-acylglucuronide, which was used for comparison, showed the formation of different isomeric acylglucuronides on incubation in aqueous buffer. The isomeric acylglucuronides of telmisartan consisted of the 2-, 3-, and 4-O-acylglucuronides (α,β- anomers). First order degradation half-lives of 26 and 0.5 h were observed on incubation in buffer of pH 7.4 for the 1-O-acylglucuronides of telmisartan and diclofenac, respectively. This indicated that the 1-O-acylglucuronide of telmisartan was among the most stable acylglucuronides reported to date. The high stability of telmisartan 1-O-acylglucuronide was confirmed by in vitro experiments that indicated only very low covalent binding of telmisartan acylglucuronide to human serum albumin but a considerable amount of covalently bound radioactivity with the acylglucuronide of diclofenac. After i.v. dosing to rats, telmisartan 1-O-acylglucuronide was rapidly cleared from plasma with a clearance of 180 ml/min/kg, compared with 15.6 ml/min/kg for the parent compound. Because telmisartan 1-O-acylglucuronide exhibited a comparably high chemical stability together with a high clearance that resulted in low systemic exposure, the amount of covalent binding to proteins should be negligible compared with other frequently used drugs, such as furosemide, ibuprofen, or salicylic acid.

Conjugation of xenobiotic carboxylic acids with β-D-glucuronic acid to yield 1-O-acylglucuronides is a major metabolic pathway for many compounds including a range of hypolipidemic and nonsteroidal anti-inflammatory drugs (NSAIDs). They are also formed in the course of the metabolic degradation of endogenous compounds, e.g., bilirubin and bile acids. Telmisartan, a novel angiotensin II receptor antagonist, is extensively metabolized by conjugation to glucuronic acid in humans and various animal species, resulting in telmisartan 1-O-acylglucuronide as the major metabolite (Schmid et al., 1996) (Fig. 1). Besides minor amounts of a hexose glycoside in mice, no other metabolites of telmisartan, especially no phase I metabolites, were found in rats, dogs, rabbits, mice, or humans.

Acylglucuronides are ester-structured compounds that are chemically unstable in aqueous solution due to the susceptibility of the acyl group toward nucleophilic attack. Consequently, acylglucuronides undergo both spontaneous hydrolysis and intramolecular acyl migration. Acyl migration involves the transfer of the acyl group from the 1β position to the C-2, C-3, or C-4 position of the glucuronic acid ring, which results in the formation of isomeric acylglucuronides. In addition, α,β-anomers of the isomeric acylglucuronides are formed by mutarotation (Fenselau, 1994). Altogether, acylglucuronides form a complex equilibrium system in aqueous solution (Fig. 2).

Isomeric acylglucuronides, being reactive esters, have been shown to react with proteins to form covalent adducts. Because covalent binding is a general phenomenon for labile acylglucuronides, such protein binding could be a toxicological problem because these chemically modified proteins may be immunogenic in vivo (Gillette, 1974). There is a considerable amount of information available on immunologically based and clinically relevant adverse reactions of several drugs that are probably related to the formation of highly reactive acylglucuronides. These drugs include tolmetin, zomepirac, diflunisal, and diclofenac (Spahn-Langguth and Benet, 1992).

It has been postulated that the extent of covalent binding and thereby the incidence of potential immunotoxic reactions may be related to the rate of the initial 1-O-acyl to 2-O-acyl migration, which is the primary and irreversible step resulting in the formation of the highly reactive isomeric acylglucuronides. The formation of isomeric acylglucuronides via acyl migration is a prerequisite for covalent binding to proteins by the imine mechanism, in which the aldehyde group of the ring-open tautomer of the glucuronic acid moiety condenses with primary amino functional groups of proteins. There is direct (Ding et al., 1993) and indirect (Spahn-Langguth and Benet, 1992; Grubb et al., 1993; Kretz-Rommel and Boelsterli, 1994a) evidence that the imine mechanism plays an important role in the process of covalent binding of acylglucuronides to proteins. Because there is a relationship between the rate of degradation of 1-O-acylglucuronides (hydrolysis and acyl migration) in aqueous buffer and the extent of in vitro covalent binding to proteins (Benet et al., 1993; Bischer et al., 1995), data on the reaction rates of degradation of 1-O-acylglucuronides can serve as a measure for the potential of...
1-O-acylglucuronides to covalently bind to proteins. Because experiments on degradation are relatively straightforward and can be easily standardized, data on rate constants can be compared with respective data in the literature.

Another factor that contributes to the formation of covalent adducts in vivo is the extent of systemic exposure toward the acylglucuronides. Hence, the extent of irreversible binding observed for different drugs will be a function of both determinants, viz., the degradation rate of the acylglucuronide, as a measure of reactivity, and the systemic elimination rate.

It was the aim of this study to assess the reactivity of telmisartan 1-O-acylglucuronide by comparison with data of other acylglucuronides that were taken from the literature or by direct comparison with experiments using the 1-O-acylglucuronide of diclofenac. In addition, the pharmacokinetics of telmisartan 1-O-acylglucuronide were evaluated in rats to obtain data for an estimation of the systemic exposure toward this compound.

Materials and Methods

[14C]Telmisartan (position of radioactive label, see Fig. 1) and [14C]diclofenac, which was labeled in the methylene group of the phenylacetic acid moiety, were synthesized by the radiosynthesis group of the Department of Pharmacokinetics and Drug Metabolism, Boehringer Ingelheim Pharma KG (Biberach, Germany). The specific activities were 0.35 and 0.66 MBq/mg, respectively. Nonlabeled telmisartan was obtained from the Department of Medicinal Chemistry, Boehringer Ingelheim Pharma KG. All other reagents and solvents were reagent grade or better and were purchased from Sigma-Aldrich Chemical Co. (Steinheim, Germany) or Merck (Darmstadt, Germany). Human serum albumin (HSA; crystalline, essentially fatty acid-free) was also purchased from Sigma-Aldrich.

**Dosing to Rats and Collection of Bile Samples.** Solutions of the sodium salts of telmisartan and diclofenac were diluted with sterile saline to obtain i.v. formulations for dosing volumes of 1 and 3 ml/kg for telmisartan and diclofenac, respectively. [14C]telmisartan (30 mg/kg), with a specific radioactivity of 28 KBq/mg, was dosed to bile duct-cannulated male Wistar rats (320–400 g) via i.v. bolus injection. Bile was collected for 6 h (2-h fractions) over ice into sample tubes containing 50 ml of phosphoric acid (20%; w/v). Accordingly, diclofenac 1-O-acylglucuronide was isolated from rat bile after i.v. dosing of 20 mg/kg [14C]diclofenac. Several rats were also dosed with 50 mg/kg nonlabeled telmisartan to obtain the nonlabeled acylglucuronide for the assessment of its pharmacokinetics in rats.

**Isolation of Acylglucuronides from Rat Bile.** 1-O-Acylglucuronides were isolated from rat bile by semipreparative HPLC methods. Acylglucuronides were extracted automatically from bile samples by solid-phase extraction. This extraction was performed according to the column-switching technique (Roth et al., 1981). Three milliliters of bile were extracted on a 250 x 8 mm HPLC column filled with Bondesil C18 (40 μm) (ICT, Frankfurt, Germany), using 0.1 M ammonium acetate buffer (pH 6.9) with a flow rate of 2 ml/min for 10 min as enrichment buffer. Retained material was then transferred onto the analytical HPLC column (125 x 8 mm, Hypersil C18, 5 μm; Shandon Ltd., Astmoor, Runcorn, Cheshire, UK) by backflushing the extraction column. The transferred material was then chromatographed using 0.1 M ammonium acetate (pH 4.5) buffer-acetonitrile (70:30, v/v) as mobile phase, flow rate 5 ml/min. UV absorption of the eluent was monitored and the fractions containing the acylglucuronides were collected and subsequently evaporated under reduced pressure until the organic solvent was removed. After desalting and lyophilization.

**Materials and Methods**

[14C]Telmisartan (position of radioactive label, see Fig. 1) and [14C]diclofenac, which was labeled in the methylene group of the phenylacetic acid moiety,

![Chemical structure of telmisartan 1-O-acylglucuronide.](image1)

The asterisk denoted the position of the 14C label.

![Reaction pathways of acylglucuronides in aqueous solution.](image2)

Fig. 2. Reaction pathways of acylglucuronides in aqueous solution. Mutarotation is possible in the 2-, 3-, and 4-O-acyl isomers. Hydrolysis of the 1-O-acylglucuronide to the aglycon is also shown.
zation of the remaining aqueous solution, solid acylglucuronide of telmisartan was obtained as amorphous powder that was essentially pure according to HPLC and UV detection. The radiochemical purity of the radiolabeled telmisartan 1-O-acylglucuronide was >99%. The acylglucuronide of diclofenac was isolated accordingly, however, with slight modifications concerning the HPLC separation: The HPLC column was filled with Kromasil 100 C 18 (5 μm, Knauer, Berlin, Germany) and a gradient elution was applied (flow rate 1 ml/min.) consisting of ammonium acetate buffer-acetonitrile (85:15) for 25 minutes, followed by a linear gradient from 70:30 to 45:55 for 5 minutes. After each separation, the HPLC columns were flushed with methanol for 2 minutes. After desalting and lyophilization, the crude diclofenac acylglucuronide was further purified by thin-layer chromatography on Kieselgel using chloroform-methanol 70:30 (v/v) as solvent. Radioactive bands were scraped off the plate and eluted with acetonitrile, which was subsequently evaporated to dryness. The identity of the isolated acylglucuronides was confirmed by NMR and liquid chromatography-mass spectrometry (LC-MS) for diclofenac 1-acylglucuronide. The identity of the isolated acylglucuronides was confirmed by NMR and liquid chromatography-mass spectrometry (LC-MS) for telmisartan and LC-MS and by glucuronidase treatment for diclofenac.

Treatment of Bile Samples or Incubation Experiments with β-Glucuronidase. Glucuronidase (type IX-A from Escherichia coli, 1 mg, approx. 740 U) was dissolved in 1 ml of pH 6.88 phosphate buffer. Rat bile (50–100 μl) or 50 μl of the purified 1-O-acylglucuronides of incubation experiments were added to 100 μl of the buffered β-glucuronidase solution, vortexed shortly, and subsequently incubated for 8 h (6 h for incubation experiments) at 37°C.

Covalent Binding of Acylglucuronides to HSA. 1-O-Acylglucuronides of 6.5 μM [14C]telmisartan or 3.3 μM [14C]diclofenac were incubated at 37°C in 1.4 ml of 0.1 M phosphate buffer of pH 7.4 containing 0.5 mM HSA. Samples of 200 μl were taken at various time points up to 8 h and were immediately pipetted into 5-ml polypropylene tubes containing 2 ml of acetonitrile-phosphoric acid (95:5). The tubes were vigorously vortex mixed to ensure complete precipitation, and the protein was pelleted by centrifugation at 10,000g for 10 min. The pellet was washed with 2 ml of methanol-acetonitrile (5:1) by vigorous vortex-mixing and centrifugation as above. The supernatant was removed and the procedure was repeated an additional five times. Radioactivity was measured in samples of the supernatants by liquid scintillation counting (LSC), which revealed that the protein pellets were essentially free of extractable radioactivity after five wash cycles. The resulting protein pellets were quantitatively transferred into 20-ml LSC polypropylene vials and were digested with 2 ml of 1 M sodium hydroxide solution for 2 h at 65°C. Twelve milliliters of scintillation cocktail was added (Ultima Gold; Canberra Packard, Dreieich, Germany) to each sample and radioactivity was quantified by LSC.

In Vitro Stability of the 1-O-Acylglucuronides of [14C]Telmisartan and [14C]Diclofenac in Buffer. 1-O-Acylglucuronides of [14C]telmisartan or [14C]diclofenac were incubated at 37°C in 0.1 M phosphate buffer of pH 7.4 (telmisartan 1-O-acylglucuronide was also incubated at pH 8.5). pH of the incubation mixtures was assessed by direct measurements before and during the incubation experiments. Incubation mixtures contained 119 μg/ml [14C]telmisartan 1-O-acetylglucuronide (equivalent to 1 × 10⁶ dpm/ml) or 88.6 μg/ml of [14C]diclofenac 1-O-Acetylglucuronide (equivalent to 3.5 × 10⁶ dpm/ml). The total volume of incubation experiments was 100 μl. Sample aliquots of 10 μl were taken at time points 0, 0.5, 1, 2, 4, 6, and 8 h, or at time points 0, 1, 2, 8, 24, 48, 72, and 96 h from incubation experiments with 1-O-acylglucuronides of [14C]diclofenac or [14C]telmisartan, respectively. Samples were directly injected into the HPLC system without delay or any additional processing. Radioactivity of each collected peak was quantified by collection of 330 μl (equivalent to 18 s) fractions of the eluent of the analytical column. UV signals of the eluent were also recorded.

HPLC Analysis of Buffer Samples Containing Isomeric Acylglucuronides. Incubation samples (10 μl) were directly injected onto a 125 × 4 mm HPLC column (Kromasil 100 C 18, 5 μm, Knauer, Germany). Telmisartan acylglucuronides were analyzed using a gradient consisting of 0.1 M ammonium acetate (pH 8.5)-acetonitrile going from 75:25 to 65:35 within 22 min and then to 55:45 within 5 min. This HPLC column was also used for diclofenac acylglucuronides using the same mobile phase gradient as for the isolation of diclofenac acylglucuronide from rat bile.

Isolation of Isomeric Acylglucuronides of Telmisartan. Two hundred microliters of an aqueous solution of [14C]telmisartan 1-O-acylglucuronide (equivalent to 1 × 10⁶ dpm, 600 μg) was diluted with 250 μl of phosphate buffer (0.1 M, pH 7.4). This solution was incubated for 1 h at 80°C. Thereafter, the solution was divided into 10 aliquots and stored frozen at −20°C. The peak fraction eluates of the individual peaks of 10 HPLC runs were combined and were then concentrated under reduced pressure at room temperature after addition of 50 μl of acetic acid to remove the acetonitrile. This solution was injected onto an HPLC column (600 × 4.6 mm) filled with 40 μm Bondelut C 18. Ten milliliters of water were pumped through the column to remove salts and the acylglucuronides were then eluted with acetonitrile using reverse flow. Eluates were collected and subsequently lyophilized to complete dryness.

NMR Spectroscopy of Telmisartan Acylglucuronides. 1H-NMR spectra were recorded on a Bruker DRX 600 (Bruker, Rheinstetten, Germany) using 2H2O pyridine with a trace of trifluoroacetic acid as solvent according to (Kuo and Dulik, 1995) to attain a downfield shift of the water signal.

Pharmacokinetics of Telmisartan 1-O-Acylglucuronide after i.v. Injection to Rats. Telmisartan 1-O-acylglucuronide was dissolved in 200 μl of polyethylene glycol 200 and 1300 μl of sterile saline to a final concentration of 4.02 mg/ml and was then injected i.v. into the jugular vein at a volume of 1 ml/kg. Blood samples (approx. 0.3 ml) for the measurement of telmisartan 1-O-acylglucuronide were taken from the jugular vein of the anesthetized (pentobarbital 60 mg/kg) rats at time points 0 h (predose) and 1.5, 4.5, 14.5, and 29.5 min after i.v. dosing. Plasma was prepared by centrifugation (5 min at 12,000g) of the blood samples. The plasma was then diluted with an equal volume of 0.2 mol/liter hydrochloric acid.

Quantification of Telmisartan 1-O-Acylglucuronide in Plasma Samples of Rats. Telmisartan and telmisartan 1-O-acylglucuronide were extracted from plasma samples by automated solid-phase extraction on 17 × 4.6 mm enrichment columns. The sample injection volume was 100 μl, wash buffer was 50 mM ammonium acetate, pH 4.5. After 3 min wash time at a flow of 1 ml/min the analytes were transferred (backflush) onto the analytical column with 50 mM ammonium acetate-acetonitrile (90:10, v/v). The analytical column (12.5 × 4.6 mm) was filled with Nucleosil 100–5 C 18, after 3 min at a linear gradient was used that went from 70:30 to 40:60 within 12 min. The analyte peaks were detected by fluorescence (excitation 305 nm, emission 365 nm), peak area data were used for quantification together with a calibration curve ranging from 5 to 1000 ng/ml (external calibration). Quality control samples were used to establish accuracy and precision of the analytical assay. The lower limit of quantitation was 2.5 and 5 ng/ml for telmisartan and telmisartan 1-O-acylglucuronide, respectively. The accuracy of the assay was within 4.6% and the maximum imprecision over a period of 3 days was 3.3%.

Data Analysis. Calculations of kinetic data of degradation of acylglucuronides were performed by iterative nonlinear regression analysis of the measured data using the equation for first order reaction kinetics: C = C(0) e −kt. The program used was the Solver subprogram implemented in the program Microsoft Excel 97. Weights 1/y were used for nonlinear regression analysis. Pharmacokinetic parameters were determined by noncompartmental or compartmental analysis of telmisartan 1-O-acylglucuronide plasma concentration time profiles after i.v. bolus injection using the program TopFit (Heinzel et al., 1993). A two-compartment disposition model was used for compartmental pharmacokinetic model calculations.

Results

Telmisartan 1-O-acylglucuronide was isolated from rat bile, which is a convenient source for this metabolite because rat bile contains no other telmisartan-related material apart from the acylglucuronide and small amounts of parent drug. Telmisartan 1-O-acylglucuronide (non-labeled and 14C-labeled) was obtained as a white amorphous solid material. According to HPLC analysis, the isolated telmisartan 1-O-acylglucuronide contained 0.3% of an isomerized acylglucuronide and less than 0.1% of parent compound. The radiochemical purity of the radiolabeled telmisartan 1-O-acylglucuronide, was >99%. The identity was confirmed by LC-MS and 1H-NMR (Schmid et al., 1996). Diclofenac 1-O-acylglucuronide was also purified to obtain essen-
UDP-glucuronic acid or diclofenac and [14C]UDP-glucuronic acid was not possible. However, LC-MS data together with incubation experiments with β-glucuronidase gave evidence that the isolated glucuronide metabolite was the 1-O-acylglucuronide. In addition, incubation experiments with rat liver microsomes in the presence of [14C]diclofenac and UDP-glucuronic acid or diclofenac and [14C]UDP-glucuronic acid resulted in the formation of a single radioactive metabolite with retention time identical with the compound that was isolated from rat bile (not shown). When telmisartan 1-O-acylglucuronide was incubated at aqueous buffer of pH 7.4, the formation of parent compound and three other compound peaks was observed, which according to liquid chromatography-tandem mass spectrometry analysis consisted of telmisartan glucuronides (Fig. 3). In subsequent experiments, each of the isomeric telmisartan glucuronides was isolated and purified for 1H-NMR measurements. NMR data of the rearrangement products, partially pure (radiochemical purity >98%) material. The amount of this compound was not sufficient for proof of structure by 1H-NMR measurements. HPLC data of the rearrangement products, together with signal assignments of the 1-O-acylglucuronide of telmisartan, a separation of the α,β-anomers by HPLC was not possible. α,β-anomers of glucuronides of structurally less complex aglycons were successfully separated by HPLC (Sidelmann et al., 1995). However, HPLC separation is difficult for larger, structurally more complex aglycon molecules (Lenz et al., 1996). Similar to the results with telmisartan 1-O-acylglucuronide, incubation experiments with the 1-O-acylglucuronide of diclofenac also resulted in the formation of three isomeric acylglucuronides besides parent diclofenac (Fig. 4).

The time course of rearrangement/hydrolysis of telmisartan 1-O-acylglucuronide and diclofenac 1-O-acylglucuronide are shown in Fig. 5. At early time points, the formation of the 2-O-acylglucuronide was the predominant reaction for telmisartan and diclofenac, whereas hydrolysis to parent compound was negligible. Therefore, the rate of degradation of the 1-O-acylglucuronide was analyzed by nonlinear regression analysis (Fig. 6). Apparent first order degradation constants, half-lives, and r² values for incubation experiments are listed in Table 2. On incubation at pH 7.4 diclofenac 1-O-acylglucuronide exhibited a 52-fold higher degradation rate constant compared with telmisartan 1-O-acylglucuronide. The marked effect of pH on the degradation rate was shown by the 3.3-fold higher degradation rate for telmisartan 1-O-acylglucuronide after increasing the pH from 7.4 to 8.5.

Addition of [14C]diclofenac 1-O-acylglucuronide to buffer containing HSA resulted in covalent binding of radioactivity. After 1h at 37°C, 3.8% of the radioactivity present in the incubation was covalently bound to HSA. The amount of covalently bound radioactivity declined at later time points, which was probably due to the limited stability of the adducts. In contrast, no radioactivity was bound to HSA after 1 or 4 h using [14C]telmisartan 1-O-acylglucuronide. After 8 h of incubation time, covalent binding was observed that accounted for 0.4% of total radioactivity.

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**Table 1**

<table>
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<tr>
<th>Isomers</th>
<th>H¹</th>
<th>H²</th>
<th>H³</th>
<th>H⁴</th>
<th>H⁵</th>
<th>( \delta ) in ppm</th>
<th>J in Hz</th>
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<td>4.21</td>
<td>4.36</td>
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<td>4.71</td>
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</table>

*Arbitrary.

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**FIG. 3.** HPLC chromatograms of analytical samples of authentic telmisartan 1-O-acylglucuronide (bottom) and the decomposition/isomerization after 96-h incubation in aqueous buffer (pH 7.4, 37°C) (top).

**FIG. 4.** Analytical sample of diclofenac 1-O-acylglucuronide (bottom) and the decomposition/isomerization after 8-h incubation in aqueous buffer (pH 7.4, 37°C) (top).

U₁, U₂, and U₃ consist of isomeric acylglucuronides of diclofenac. U₃ was tentatively assigned to the 2-O-acylglucuronide based on the observation of its rapid formation at early time points.
Telmisartan 1-O-acylglucuronide was i.v. administered to rats \((n = 6)\) at a dose of 4.02 mg/kg, the molar equivalent to 3 mg/kg of telmisartan. An HPLC assay with fluorescence detection was used for quantification of telmisartan 1-O-acylglucuronide and parent compound in plasma samples of rats. At early time points after i.v. dosing of telmisartan 1-O-acylglucuronide, very high plasma concentrations, which were in the micrograms per milliliter range, of telmisartan 1-O-acylglucuronide were measured. No parent compound telmisartan was observed. Noncompartmental pharmacokinetic analysis revealed a terminal half-life of approximately 0.17 h (Fig. 7). The results of the compartmental pharmacokinetic analysis using a two-compartment disposition model are listed in Table 3.

Telmisartan 1-O-acylglucuronide was rapidly cleared from the plasma. It exhibited a markedly higher clearance of 180 ml/min/kg compared with the parent compound, which was 15.6 ml/min/kg after an i.v. dose of 1 mg/kg (data on file). The mean residence time of telmisartan 1-O-acylglucuronide was 0.05 h.

There was also a clear reduction in the apparent volume of distribution for telmisartan 1-O-acylglucuronide, which was 0.46 liter/kg (after dosing of 1.34 mg/kg) compared with the parent compound. According to earlier results (data on file), telmisartan had an apparent volume of distribution of 2.36 liter/kg after i.v. dosing of 1 mg/kg.

**Discussion**

The formation of acyl glucuronides in the metabolism of drugs, and especially new drug entities, requires some additional consideration. This is because the formation of acyl glucuronides can be of concern for the therapeutic safety of drugs. In this respect, the experimental approach to assess stability in aqueous buffer offers an uncomplicated and straightforward experimental system that can be easily standardized. In contrast, in vitro assays on covalent binding to proteins can be expected to be highly variable. It has been shown that the extent of covalent binding varied greatly depending on the particular albumin preparation used (Williams and Dickinson, 1994). In addition to this

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**TABLE 2**

<table>
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<tr>
<th>Compound</th>
<th>pH</th>
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<th>(T_{1/2})</th>
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<td>1.35</td>
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<td>0.9997</td>
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**Fig. 5.** Time course of the degradation of telmisartan 1-O-acylglucuronide (top) and diclofenac 1-O-acylglucuronide (bottom) by hydrolysis to parent compound or acyl-migration yielding isomeric acylglucuronides.

**Fig. 6.** Nonlinear regression analysis of the degradation of the 1-O-acylglucuronides of telmisartan (top) and diclofenac (bottom) according to a first order rate equation.

**Fig. 7.** Pharmacokinetic compartmental analysis of individual plasma concentration-time data of telmisartan 1-O-acylglucuronide after i.v. bolas injection of 4.02 mg/kg to male rats. A two-compartment model was used.
effect, which was probably due to differences in the extent and nature of the reversible binding (noncovalent) of acylglucuronides to albumin, the magnitude of in vitro covalent binding may be also dependent on the stability of the formed protein adducts (Barber et al., 1994).

There is, however, a problem using experiments on the chemical stability of acylglucuronides in buffer systems as a measure of reactivity toward protein adduct formation. Such indirect experiments may not give consistent results in those cases where covalent binding does not occur via the imine mechanism, i.e., where covalent binding is not entirely dependent from the initial rate of acyl migration. The covalent binding of oxaprozin acylglucuronide was reported to occur predominantly via direct nucleophilic displacement of the glucuronic acid moiety of the 1-acylglucuronide (Fenselau, 1994). A marked discrepancy between the extent of covalent binding to albumin and the chemical stability has also been reported for the acylglucuronide of mefenamic acid (McGurk et al., 1996). For telmisartan, the low amount of covalent adduct formation to HSA was consistent with its high chemical stability.

Telmisartan 1-O-acylglucuronide exhibited a half-life of 26 h at pH 7.4, which was considerably longer than 0.5 h, the apparent degradation half-life of diclofenac 1-O-acylglucuronide. Consequently, the potential for covalent binding to proteins should be much smaller for telmisartan 1-O-acylglucuronide compared with diclofenac 1-O-acylglucuronide. This conclusion, which was confirmed by experiments on the covalent binding to HSA. If literature data were taken into consideration (Table 4), it was evident that telmisartan 1-O-acylglucuronide was one of the most stable acylglucuronides that were investigated to date. It was more than an order of magnitude more stable than several of the most frequently used NSAIDs, such as naproxen, indomethacin, or salicylic acid. It was also less reactive than the acylglucuronide of bilirubin, a major endogenous metabolite of bilirubin. With respect to degradation half-lives, there was a marked difference to those compounds (zomepirac, tolmetin) that clearly showed covalent adducts during drug therapy in humans. These two NSAIDs were withdrawn from the market because of severe adverse events that were most likely due to the formation of new antigens followed by autoimmune responses.

Diclofenac, in contrast, ranked among those drugs that had the highest potential for covalent adduct formation. Indeed, there are several reports that deal with rare, but severe adverse events based on autoimmune reactions (Salama et al., 1996; Banks et al., 1997). In addition, the different biochemical steps that precede such adverse events are well described for diclofenac by a series of publications dealing with the formation of protein adducts (Kretz-Rommel and Boelsterli, 1994a), localization and characterization of target liver proteins (Hargus et al., 1994; Kretz-Rommel and Boelsterli, 1994b), and mechanisms of autoimmune cytotoxicity (Kretz-Rommel and Boelsterli, 1995). Therefore, our data on the high degradation rate of diclofenac 1-O-acylglucuronide nicely dovetail these findings and give additional evidence for the correlation between chemical stability and extent of covalent protein binding of 1-O-acylglucuronides. To our knowledge, the apparent degradation half-life of diclofenac 1-O-acylglucuronide in aqueous buffer has not been reported in the literature, despite the wealth of information considering covalent binding of diclofenac 1-O-acylglucuronide to proteins.

For the assessment of the potential risk of covalent adduct formation by acylglucuronides in vivo, the reactivity of a given acylglucuronide and the extent of systemic exposure toward the acylglucuronide must be considered. Literature data suggest that acylglucuronides of even lower reactivity may result in substantial amounts of covalent protein adducts in vivo, if relatively high concentrations of the acylglucuronides are present over longer time periods. Although the AUC of tolmetin acylglucuronide after oral dosing of tolmetin in human volunteers is significantly less than for the structurally related NSAID zomepirac, both drugs exhibit comparable covalent adduct formation to plasma proteins in vivo (Hyneck et al., 1988). This observation could be due to the higher reactivity of tolmetin acylglucuronide. In monkeys, the AUC of ibuprofen acylglucuronide is approximately twice as high compared with the AUC for ibufenac acylglucuronide, each after an oral dose of 100 mg of the respective aglycon. Yet, the extent of in vivo covalent binding to plasma proteins was clearly higher after ibufenac dosing, probably because of the differences in reactivity (Castillo and Smith, 1995). Because of a marked increase of the AUC of salicylic acid acylglucuronide in rats with renal failure compared with control rats, the amount of covalently bound salicylic acid metabolites to plasma protein of rats was also markedly elevated (Liu et al., 1996).

The pharmacokinetic parameters of telmisartan 1-O-acylglucuronide in rats suggested that it was rapidly cleared from the circulation. This could be confirmed during the preclinical development of telmisartan. After i.v. or oral dosing of telmisartan to rats, the AUC of

### Table 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>CV</th>
</tr>
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<tbody>
<tr>
<td>MRT&lt;sub&gt;tot&lt;/sub&gt;</td>
<td>h</td>
<td>6.9</td>
</tr>
<tr>
<td>V&lt;sub&gt;c&lt;/sub&gt;</td>
<td>liters/kg</td>
<td>20.3</td>
</tr>
<tr>
<td>V&lt;sub&gt;re&lt;/sub&gt;</td>
<td>liters/kg</td>
<td>25.6</td>
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<tr>
<td>CL</td>
<td>ml/min/kg</td>
<td>19.3</td>
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<tr>
<td>C(0)</td>
<td>µg/ml</td>
<td>23.5</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;model&lt;/sub&gt;</td>
<td>µg - h/ml</td>
<td>18.4</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>µg/ml</td>
<td>23.5</td>
</tr>
</tbody>
</table>

Pharmacokinetic parameters: V<sub>c</sub>, the apparent volume of distribution of the central compartment after intravascular dosing; V<sub>re</sub>, the apparent volume of distribution at steady state; MRT, mean residence time; CL, total body clearance from plasma after intravascular dosing; AUC<sub>model</sub>, the area under the fitted curve from zero time to infinity; C(0), the back-extrapolated plasma concentration after i.v. bolus injection.

### Table 4

<table>
<thead>
<tr>
<th>Compound</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tolmetin</td>
<td>0.26</td>
<td>(Fenselau, 1994)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1.4</td>
<td>(Fenselau, 1994)</td>
</tr>
<tr>
<td>Naproxen, S enantiomer</td>
<td>1.8</td>
<td>(Bischer et al., 1995)</td>
</tr>
<tr>
<td>Salicylic acid</td>
<td>1.3</td>
<td>(Liu et al., 1996)</td>
</tr>
<tr>
<td>Furosemide</td>
<td>5.3</td>
<td>(Fenselau, 1994)</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>7.3</td>
<td>(Fenselau, 1994)</td>
</tr>
<tr>
<td>Mefenamic acid</td>
<td>16.5</td>
<td>(McGurk et al., 1996)</td>
</tr>
<tr>
<td>Telmisartan</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Genfibrozil</td>
<td>44</td>
<td>(Sallustio and Foster, 1995)</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>79</td>
<td>(Fenselau, 1994)</td>
</tr>
</tbody>
</table>
telmisartan metabolites was very small compared with the parent compound. Consequently, the elimination rate of telmisartan 1-O-acylglucuronide markedly exceeded its degradation rate.

In conclusion, telmisartan 1-O-acylglucuronide with an apparent half-life of 23 h was found relatively stable when compared with other acylglucuronides. It should therefore pose only minimal risk of covalent adducts formation during therapy. Systemic exposure toward the telmisartan 1-O-acylglucuronide during drug therapy can be expected to be low. This low systemic exposure together with the high chemical stability will multiply to a relatively low risk of covalent adduct formation.

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References


