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Stereoselective glucuronidation of (*R*)- and (*S*)-naproxen by recombinant rat phenol UDP-glucuronosyltransferase (UGT1A1) and its human orthologue

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Abstract—Recombinant rat phenol UDP-glucuronosyltransferase (UGT1A1) conjugates (*R*)-naproxen at a much higher rate (>17-fold) than its (*S*)-enantiomer, substantiating previous findings on stereoselective glucuronidation of racemic naproxen. In contrast, the recombinant human orthologue conjugated both enantiomers at equal rates. In line with high constitutive expression of UGT1A1 in extrahepatic tissues, a high *R*/*S* ratio of naproxen glucuronidation was found in rat testes, intestine, lung and kidney. The results demonstrate that (*R*)-naproxen represents a stereoselective substrate of rat UGT1A1, but not of the human orthologous UGT1A1.

Glucuronidation is a major biotransformation pathway of 2-arylpropionic acids or profens, which are widely used as non-steroidal anti-inflammatory drugs. Interestingly, they have a chiral center at C-2 of the propionic acid side chain, and their racemic forms can be resolved into (*R*)- and (*S*)-enantiomers, forming the corresponding diastereomeric glucuronides [1–3]. Previously racemic naproxen has been shown to be an overlapping substrate of 3-methylcholanthrene (MC*)- and phenobarbital-inducible UDP-glucuronosyltransferases (UGTs) (EC 2.4.1.7) with differing stereoselectivity [4]. MC-inducible rat UGTs appear to prefer the (*R*)-enantiomer.

A number of UGTs have been sequenced and two UGT families have been characterized both in rats and humans [5, 6]. The first family consists of at least four isozymes of the UGT1A or phenol/bilirubin UGT gene complex. The isozymes are formed by differential splicing of this large gene complex, localized on human chromosome 2. Due to inconsistencies in the proposed nomenclature [5], the previously used term UGT1A1 is maintained for the MC-inducible phenol UGT of the UGT1A gene complex and its human orthologue (= HlugPl, [5]). The other family consists of multiple steroid UGTs with broad substrate specificity. Availability of recombinant UGT1A1 [7] and of its human orthologue [8] prompted the present study which is part of an attempt to delineate the substrate specificity of various UGTs. MC-inducible UGT1A1 has been shown to conjugate a number of planar phenols including phenols of polycyclic aromatic hydrocarbons [7]. Since naproxen is conjugated at the propionic acid side chain and its glucuronidation appears to be catalysed by rat UGT1A1, it was of interest to study its glucuronidation utilizing recombinant UGT1A1. Stereoselectivity of naproxen glucuronidation was also investigated with the human orthologous UGT. In addition, naproxen glucuronidation was studied in extrahepatic rat tissues because of the wide tissue distribution of UGT1A1 [9, 10].

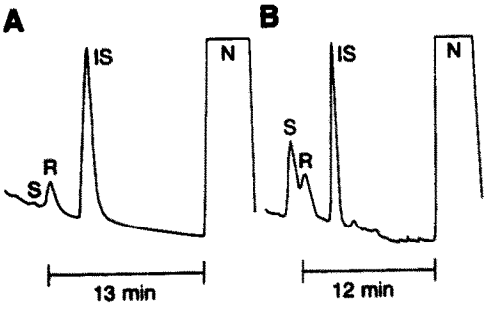

Fig. 1. HPLC separation of (*R*)- and (*S*)-naproxen glucuronides formed by recombinant rat UGT1A1 (A) and its human orthologue (B). Incubation was carried out with homogenates of transfected V79 cells as described in Materials and Methods. The deproteinized supernatant (100 μL) was injected into the HPLC system. UV absorption was monitored at 285 nm. R, (*R*)-naproxen glucuronide; S, (*S*)-naproxen glucuronide; N, racemic naproxen; IS, internal standard = 4-nitrophenol glucuronide.

* Abbreviations: MC, 3-methylcholanthrene; UGT, UDP-glucuronosyltransferase.

Materials and Methods

Chemicals. Enantiomers of naproxen were provided by the Syntex Corp. (Palo Alto, CA, U.S.A.). The (*S*)-enantiomer is dextrorotatory (+), and the (*R*)-enantiomer is levorotatory (−).

Treatment of animals and preparation of microsomes. Male Wistar rats (150–200 g) were treated with MC (40 mg/kg, dissolved in corn oil; once i.p.). Animals were killed 4 days after treatment. Liver microsomes were prepared as described [11]. In addition, microsomes were prepared from intestinal mucosa, kidneys, testes and lungs of untreated rats [9]. Protein concentration was determined using bovine serum albumin as a standard [12].

Human tissues. Human livers were obtained shortly after death from kidney transplant donors, HL15 from a boy (5 years), HL16 from a fatty liver of unknown etiology. Liver microsomes were prepared as described previously [13] and stored at −80°.

Recombinant UGT isozymes. Stable transfection of V79 cells was carried out with cDNA of rat and human UGT1A1 [7, 8]. The cells were cultured, homogenized and microsomes were prepared as described [7, 8]. V79 cells (a Chinese hamster lung fibroblast cell line) were chosen for transfection because of their lack of endogenous (*R*)- and (*S*)-naproxen UGT activity.

Naproxen UGT activity. Racemic naproxen (0.5 mM, dissolved in dimethyl sulfoxide, 2% final concentration) was incubated at 37° for 15 and 30 min with transfected V79 cell homogenates (1 mg protein) or tissue microsomes (*ca*. 2 mg protein) in the presence of 50 mM Tris-maleate, pH 6.5 (to avoid acyl migration), 5 mM $MgCl_2$, 0.02% bovine serum albumin, 0.1 mM dithiothreitol, 1.5 mM saccharolactone and digitonin (0.5 mg/mg protein). The reaction was started by the addition of 3 mM UDP-glucuronic acid. Total volume of the incubation mixture was 210 μL. The incubation was stopped with 20 μL 0.5 M HCl. After precipitation of the protein and centrifugation,

Table 1. (*R*)- and (*S*)-naproxen UGT activity with recombinant rat UGT1A1 and its human orthologue

Enzyme source	UGT activity (*R*)-naproxen (nmol/min/mg protein)	(*S*)-naproxen	*R/S* ratio
Rat			
Liver microsomes (MC treatment)	6.5 ± 1.4	3.6 ± 0.5	1.8
Homogenates of rat UGT1A1-transfected V79 cells	0.34 ± 0.15	<0.02 ± 0.01	>17
Human			
Liver microsomes (HL15)	0.97 ± 0.01	0.66 ± 0.08	1.5
Liver microsomes (HL16)	0.84 ± 0.05	0.42 ± 0.10	2.0
Homogenates of human UGT1A1-transfected V79 cells	0.06 ± 0.01	0.09 ± 0.01	0.7

Data represent means ± SD (N = 4).

Table 2. (*R*)- and (*S*)-naproxen UGT activity in rat tissues

Tissue	UGT activity (*R*)-naproxen (nmol/min/mg protein)	(*S*)-naproxen	*R/S* ratio
Liver	2.6 ± 0.2	2.4 ± 0.5	1
Testes	0.4 ± 0.03	0.02 ± 0.01	20
Intestine	0.13 ± 0.02	<0.005	>26
Lung	0.07 ± 0.03	0.005 ± 0.002	14
Kidney	0.03 ± 0.02	0.005 ± 0.003	6

Microsomal fractions were incubated with 0.5 mM racemic naproxen in the presence of 3 mM UDP-glucuronic acid.
Data represent means ± SD (N = 4).

the incubation mixture was filtered through 0.45 μm membrane filters and analysed by HPLC.

Under these conditions the reaction was linear with time and protein concentration. With untransfected V79 cells no UGT activity toward naproxen could be detected. With UGT1A1-transfected V79 homogenates, with human liver microsomes and with extrahepatic rat tissues, the addition of digitonin did not lead to UGT activation but slightly decreased the enzyme activity (<20%). However, with liver microsomes of MC-treated rats naproxen UGT activity was activated *ca.* 4-fold.

HPLC determination of diastereoisomeric naproxen glucuronides. (*S*)- and (*R*)-naproxen glucuronides were separated by reversed-phase HPLC as described [3, 4]. As an internal standard 5 mM 4-nitrophenol glucuronide (5 μL) was added to the clear supernatant. The supernatant was injected into a Waters HPLC system (Millipore, Bedford, MA, U.S.A.) with variable UV detector. Separation of metabolites was achieved on a 4.6 × 250 mm steel column packed with Spherisorb ODS2 (5 μm). It was eluted with 0.05 M ammonium acetate/acetonitrile (80:20), adjusted to pH 6.0. UV absorption was monitored at 285 nm. Identification of (*S*)- and (*R*)-naproxen glucuronides was achieved on the basis of separate incubations carried out with the individual naproxen enantiomers. The ratio of areas of naproxen glucuronide and internal standard peaks was utilized for quantitative measurements, as described previously [13].

Results and Discussion

Racemic naproxen has been suggested previously to be an overlapping substrate of several rat UGT isozymes, the (*R*)-enantiomer being more selective for MC-inducible UGTs [4]. These observations were substantiated utilizing recombinant rat UGT1A1 (Fig. 1 and Table 1). Glucuronidation of the (*R*)-enantiomer was >17-fold higher than that of the (*S*)-enantiomer. *R/S* ratios in microsomes were lower than those observed in UGT1A1-transfected V79 cells because of the contribution of other isozymes with high activities toward the (*S*)-enantiomer. As shown previously [4], (*R*)-naproxen glucuronidation was found to be higher with microsomes from MC-treated rats (Table 1) in comparison with untreated controls (Table 2). Naproxen UGT activities with *R/S* ratios >1 were also found in a number of extrahepatic rat tissues (Table 2), an observation consistent with their high basal expression of UGT1A1 [9, 10]. Utilizing selective cDNA probes for exon I of rat UGT1A1 it has been demonstrated recently that, in contrast to liver, there is a high constitutive expression of UGT1A1 in extrahepatic tissues [10, 14]. In contrast to rat UGT1A1, the human orthologue conjugated both enantiomers with similar activity. Recently, glucuronide formation of (*S*)-naproxen and of other carboxylic acid-containing drugs has been found with other human UGT isozymes [15, 16].

Evidence has been obtained previously that some UGT isozymes are stereoselective [1–4, 17–22]. However, the potential use of stereoisomeric substrates for characterization of different UGT isozymes has not been fully explored. Stereoselective glucuronidation of (*R*)-naproxen by rat UGT1A1 may be an interesting tool for several reasons: first, naproxen is conjugated at the propionic acid side chain indicating limitations of nomenclature such as phenol UGT to describe UGT1A1. Moreover, the much lower activity toward the (*S*)-enantiomer may shed some light on the geometry of the active site of rat UGT1A1 since only one of the enantiomers is conjugated by the rat enzyme. It is also interesting that

the human orthologous UGT1A1 (= HlugPl, [5]) conjugates both (*S*)- and (*R*)-naproxen at equal rates.

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