Biochemical Pharmacology, Vol. 46, No. 10, pp. 1775–1780, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

STEREOSELECTIVITY OF HUMAN LIVER AND INTESTINAL CYTOSOLIC FRACTIONS AS WELL AS PURIFIED HUMAN GLUTATHIONE *S*-TRANSFERASE ISOENZYMES TOWARDS 2-BROMOISOVALERYLUREA ENANTIOMERS

TITIA M. T. MULDERS,*† BEN VAN OMMEN,‡ PETER J. VAN BLADEREN,‡
DOUWE D. BREIMER* and GERARD J. MULDER*

*Leiden/Amsterdam Center for Drug Research, Divisions of Toxicology and Pharmacology, P.O. Box 9503, 2300 RA Leiden, and ‡TNO Toxicology and Nutrition Institute, Department of Biological Toxicology, P.O. Box 360, 3700 AJ Zeist, The Netherlands

(*Received* 7 *December* 1992; *accepted* 28 *July* 1993)

Abstract—Glutathione (GSH) conjugation of 2-bromoisovalerylurea (BIU) enantiomers is stereoselective in humans *in vivo*. Administration of racemic BIU results in a higher plasma elimination and urinary excretion of *R*-BIU and its mercapturate, respectively, than of *S*-BIU and its mercapturate. In order to relate the *in vivo* BIU pharmacokinetics to the activity of glutathione *S*-transferase (GST) isoenzymes, the GSH conjugation of BIU enantiomers was studied with human liver and intestinal cytosolic fractions as well as purified human class alpha (GSTA1-1, GSTA2-2), mu (GSTM1a-1a) and pi (GSTP1-1) GST isoenzymes. Stereoselective GSH conjugation of BIU enantiomers was observed for most human liver and intestinal cytosolic fraction. In general, the cytosolic fractions preferentially conjugated *S*-BIU. Stereoselective preference for GSH conjugation of *S*-BIU was also observed for GSTA2-2 and GSTM1a-1a, whereas GSTA1-1 was not selective for either of the BIU enantiomers. GSTP1-1 did not catalyse conjugation of *R*- and *S*-BIU. Quantification of the GST isoenzymes in the liver cytosolic fractions showed that the stereoselectivity towards *S*-BIU was related to the profile and amount of GST subunits in the cytosolic fractions. The discrepancy in stereoselectivity between the BIU pharmacokinetics *in vivo* and the GSH conjugation of BIU enantiomers *in vitro* is discussed. In addition, since in contrast to human GSTM1a-1a, rat class Mu isoenzymes prefer *R*-BIU, the present results indicate that related isoenzymes in different species may have a different stereoselectivity.

The glutathione *S*-transferases (GSTs§) are a group of isoenzymes best known for their role in the detoxification of electrophilic (alkylating) xenobiotics and intermediates. So far, four classes of cytosolic GSTs have been identified: alpha, mu, pi and theta. GST isoenzymes belonging to the same class exhibit similarities in, among others, substrate specificity and structural aspects [1–4]. The existence of multiple GSTs with their own but also overlapping substrate specificities enables the enzyme system to handle a wide range of compounds.

The role of glutathione (GSH) conjugation in the pharmacokinetics of drugs and in the aetiology of diseases has hardly been studied in humans *in vivo*, partly due to the lack of a suitable model substrate for the characterization of GSH conjugation. Since any model substrate will only be converted by a limited number of GST isoenzymes, the pharmacokinetics of the substrate will selectively reflect the properties of the isoenzymes involved. Recently, the sedative and hypnotic racemic drug 2-bromoisovalerylurea (BIU) (Fig. 1) was introduced as a model substrate for the characterization of GSH conjugation, by so far not clearly defined GST isoenzymes, in humans *in vivo* [5–7]. Studies with purified rat liver GST isoenzymes have shown that the class alpha isoenzymes 1-1 and 2-2 preferentially catalyse the conjugation of *S*-BIU, whereas the class mu isoenzymes 3-3 and 4-4 prefer *R*-BIU [8].

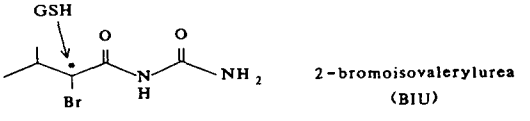

Fig. 1. Structure of BIU (* chiral centre).

In agreement with results obtained in rats *in vivo* [9, 10], a pronounced stereoselectivity in the pharmacokinetics of racemic BIU was found in humans. In healthy male volunteers, oral administration of 600 mg racemic BIU resulted in a much higher plasma elimination and urinary excretion of *R*-BIU and its mercapturate, respectively, compared to *S*-BIU and its mercapturate [6, 7]. In the present study, GSH conjugation of BIU enantiomers is studied with human liver and intestinal cytosolic fractions as well as the purified GST isoenzymes (which in the liver and intestine are probably most important [11–14]), GSTA1-1,

† Corresponding author. Tel. (31) 71 276223; FAX (31) 71 276 292.

§ Abbreviations: BIU, 2-bromoisovalerylurea; GSH, glutathione; GST, glutathione *S*-transferase; CDNB, 1-chloro-2,4-dinitrobenzene; AUC, area under curve.

Table 1. GST subunit characteristics of the human liver cytosolic fractions

Cytosol no.	Amount of subunit (μg/mg of cytosolic protein)						Specific activity (U/mg)
	GSTP	GSTM1a	GSTM1b	GSTA1	GSTA2	Total	
1	3.3 (6%)	19 (33%)	0	19 (35%)	14 (26%)	55 (100%)	1.31
2	3.7 (14%)	5.8 (22%)	0	14 (53%)	2.9 (11%)	27 (100%)	0.75
3	0.94 (2%)	9.9 (26%)	0	17 (45%)	10 (27%)	39 (100%)	1.22
4	1.8 (4%)	5.7 (13%)	0	24 (54%)	12 (29%)	43 (100%)	0.39

The specific activity is expressed as μmol of CDNB conjugated/min per mg of cytosolic protein. The amount of subunit is also expressed as percentage of total GST subunits.

Table 2. GST subunit characteristics of the human intestinal cytosolic fractions

Cytosol no.	Amount of subunit (μg/mg of cytosolic protein)			Specific activity (U/mg)
	GSTP	GSTA1	Total	
G1	51 (55%)	43 (45%)	94 (100%)	1.69
G2	24 (45%)	29 (55%)	53 (100%)	1.47
G3	6.3 (27%)	17 (73%)	23 (100%)	0.81
G4	40 (67%)	20 (33%)	59 (100%)	1.03
G5	59 (73%)	22 (27%)	81 (100%)	1.30

The intestinal fractions G1–G4 were derived from jejunal tissue and fraction G5 from colon. The specific activity is expressed as μmol of CDNB conjugated/min per mg of cytosolic protein. The amount of subunit is also expressed as percentage of total GST subunits.

GSTA2-2 (class alpha), GSTM1a-1a (class mu) and GSTP1-1 (class pi), in order to relate the *in vivo* GSH conjugation of BIU enantiomers to the GST isoenzyme activity.

MATERIALS AND METHODS

BIU was obtained from O.P.G. (Utrecht, The Netherlands). BIU enantiomers and its diastereomeric GSH conjugates (reference compounds for HPLC) were obtained as described previously [10, 15]. GSH was obtained from Boehringer (Mannheim, Germany).

The human liver cytosolic fractions (N = 4) and purified human GST isoenzymes were obtained as described previously [11]. The human intestinal cytosolic fractions were supplied by W. H. M. Peters (Division of Gastrointestinal and Liver Diseases, St Radboud University Hospital, Nijmegen, The Netherlands). Four of the intestinal cytosolic fractions were derived from jejunal tissue (No. G1–G4) and one (No. G5) was derived from colon tissue. Quantification of GST subunits in the human liver and intestinal cytosolic fractions was done as described previously [11].

The specific activity of the cytosolic fractions and purified GST isoenzymes towards 1-chloro-2,4-dinitrobenzene (CDNB) (expressed as μmol of CDNB conjugated/min per mg of protein) was determined according to Habig *et al.* [16]. Specific activities with CDNB as second substrate were 31, 22, 149 and 62 U/mg for GSTA1-1, GSTA2-2, GSTM1a-1a and GSTP1-1, respectively. Specific activities with CDNB of the human liver and intestinal cytosolic fractions are shown in Tables 1 and 2, respectively.

Incubations were performed at 37° in 25 mM sodium phosphate buffer, pH 7.4. The incubations with human cytosolic fractions (liver and intestinal) contained 2.0 mM GSH and 2 mM acceptor substrate (*R/S*-, *R*- or *S*-BIU). Depending on the protein concentration of the cytosolic fraction, the final protein concentrations in the incubations were 3.71–6.08 mg/mL and 0.74–2.02 mg/mL for the liver and intestinal cytosolic fractions, respectively. The incubations with purified human GST isoenzymes contained 2.0 mM GSH, 0.25 mg/mL protein (GST)

and 0.05, 0.1, 0.2, 0.3, 0.5, 0.7, 1.0, 1.5 or 2.0 mM acceptor substrate (*R/S*-, *R*- or *S*-BIU). Total incubation volumes were 50 μL. For both (*R/S*)-BIU and its separate enantiomers, a 4 mM BIU stock solution was made in Millipore (Bedford, MA, U.S.A.) water. The limited solubility of BIU prevented the use of higher substrate concentrations. After pre-incubation at 37° for 0.5 min, the reaction was started by adding GSH. After incubation for 2.5 min, the reaction was stopped by adding 10 μL of 6 N hydrochloric acid immediately followed by freezing in liquid nitrogen. Samples were stored at −20° until analysis. In general, the incubations were performed in duplicate.

HPLC analysis of incubation samples to quantify the diastereomeric GSH conjugates of (*R/S*), *R* and *S*-BIU was performed as described elsewhere [17]. The data obtained with purified GST isoenzymes were analysed using Lineweaver–Burk plots.

RESULTS

Except for GSTP1-1, all GST isoenzymes studied (GSTA1-1, GSTA2-2 and GSTM1a-1a) as well as all hepatic and intestinal cytosolic fractions converted BIU to its GSH conjugates. In all cases, conjugation of *R*-BIU resulted exclusively in formation of the diastereomeric *S*-conjugate, whereas *S*-BIU was converted exclusively to the diastereomeric *R*-conjugate, confirming that GSH conjugation of BIU proceeds via an SN_2 mechanism with inversion of configuration at the chiral center. The spontaneous non-enzymatic GSH conjugation of BIU enantiomers was low. In general, the spontaneous reaction accounted for less than 10% of the total amount of conjugate formed.

Quantification of the GST subunits in the human cytosolic fractions demonstrated that in the liver the class alpha isoenzymes (GSTA1-1 and GSTA2-2) are predominantly present (about 66% of the total amount of GST subunits). All liver cytosolic fractions studied contained the polymorphic class mu isoenzyme GSTM1a-1a; the class pi isoenzyme was only present in minor amounts (Table 1). In the human intestinal cytosolic fractions only GSTA1-1 (class alpha) and GSTP (class pi) could be quantified (Table 2). The specific activities with CDNB were comparable for the liver and intestinal cytosolic fractions (Tables 1 and 2).

With the exception of one of the liver cytosolic fractions (No. 2), all other fractions studied showed stereoselectively in the conjugation of BIU enantiomers with GSH (Table 3). In general, the liver cytosolic fractions preferred conjugation of *S*-BIU (*R/S*-ratio < 0.5). Cytosol No. 2 did not demonstrate stereoselectivity towards either *S*- or *R*-BIU (*R/S*-ratio ≈ 1).

Three of the five intestinal cytosolic fractions preferentially conjugated *S*-BIU (Table 4). However, this stereoselectivity was less pronounced than that of liver cytosolic fractions. Cytosol No. G3 and G4 demonstrated a preference for GSH conjugation of *R*-BIU.

The incubations performed with purified human GST isoenzymes indicated that GSH conjugate formation by GSTA1-1 was not stereoselective. On the other hand, a pronounced stereoselectivity was observed for GSTA2-2 (Table 5, Fig. 2). The preference of GSTA2-2 for *S*-BIU is evident both at the level of the K_m and V_{max} values. GSTM1a-1a was also stereoselective towards *S*-BIU. This stereoselectivity of GSTM1a-1a is clearly demonstrated in the Lineweaver–Burk plot (Fig. 2), but is only marginally expressed in the enzyme kinetic parameters (Table 5). The latter may be related to the fact that due to the limited solubility of BIU, the enzyme activities could not be determined at higher substrate concentrations.

GSH conjugate formation of a BIU enantiomer was influenced by the presence of an equimolar amount of the opposite BIU enantiomer (as in the racemate). The specific activities and *R/S*-ratios of the liver and intestinal cytosolic fractions (Tables 3 and 4) and the K_m and V_{max} values of the purified GST isoenzymes (Table 5) were higher for the separate BIU enantiomers compared to those obtained after incubations with racemic BIU as substrate. On the other hand, similar stereoselectivity was observed for incubations performed with either racemic BIU or its separate enantiomers.

DISCUSSION

Stereoselective GSH conjugation of BIU enantiomers was observed for the human liver and intestinal cytosolic fractions. The cytosolic fractions generally preferred conjugation of *S*-BIU. Preferential conjugation of *S*-BIU *in vitro* was not expected in view of the stereoselective BIU pharmacokinetics in humans *in vivo*. In healthy male volunteers, administration of racemic BIU (p.o.) resulted in a much lower plasma area under the curve (AUC) of *R*-BIU and a much higher urinary excretion of the mercapturate derived from *R*-BIU compared to those of *S*-BIU and its mercapturate [6, 7]. The GST class mu phenotype did not influence the BIU pharmacokinetics, as no difference could be detected in BIU pharmacokinetics between GST class mu deficient and non-deficient subjects [6].

GSH conjugation of BIU enantiomers was studied with the purified GST isoenzymes GSTA1-1, GSTA2-2, GSTM1a-1a and GSTP, which have been shown to be quantitatively the most important GST isoenzymes in the liver and gastrointestinal tract [11–14] (Tables 1 and 2). None of the GST isoenzymes studied preferred conjugation of *R*-BIU and especially GSTA2-2 was stereoselective towards *S*-BIU.

The stereoselective GSH conjugation of BIU enantiomers by human liver cytosolic fractions was related to the amount of class alpha and mu GST subunit. Based on the stereoselectivity of especially GSTA2-2 towards *S*-BIU, this relation is clearly reflected in the lack of stereoselectivity towards *S*-BIU of human liver cytosolic fraction No. 2 (Table 3). This liver cytosolic fraction contained, compared with the other liver cytosolic fractions, a relatively small amount of GSTA2 subunit (Table 1). Moreover, calculation of the total cytosolic specific activities by using the data obtained with purified human GST isoenzymes (GSTA1-1, GSTA2-2 and GSTM1a-1a) as well as the amount of corresponding

Table 3. Specific activities and *R*/*S*-ratios (mean, N = 2) of the human liver cytosolic fractions towards the BIU enantiomers and racemic BIU

Cytosol no.	Substrate	Specific activity (nmol/min per mg protein) *R*-BIU	*S*-BIU	*R*/*S*-ratio
1	*R*-BIU	0.91	—	—
	S-BIU		2.50	0.37
	(*R*/*S*)-BIU	0.50	2.13	0.23
2	*R*-BIU	0.43	—	—
	S-BIU	—	0.30	1.43
	(*R*/*S*)-BIU	0.25	0.26	1.00
3	*R*-BIU	0.75	—	—
	S-BIU	—	1.83	0.38
	(*R*/*S*)-BIU	0.43	1.49	0.29
4	*R*-BIU	0.74	—	—
	S-BIU	—	1.61	0.43
	(*R*/*S*)-BIU	0.34	1.02	0.34

Table 4. Specific activities and *R*/*S*-ratios (mean, N = 2) of human intestinal cytosolic fractions towards the BIU enantiomers and racemic BIU

Cytosol no.	Substrate	Specific activity (nmol/min per mg protein) *R*-BIU	*S*-BIU	*R*/*S*-ratio
G1	*R*-BIU	1.61	—	—
	S-BIU	—	2.19	0.75
	(*R*/*S*)-BIU	0.79	1.15	0.69
G2	*R*-BIU	0.98	—	—
	S-BIU	—	1.31	0.79
	(*R*/*S*)-BIU	0.37	0.60	0.62
G3	*R*-BIU	0.94	—	—
	S-BIU	—	0.46	1.72
	(*R*/*S*)-BIU	0.73	0.70	1.63
G4	*R*-BIU	0.88	—	—
	S-BIU	—	0.72	1.36
	(*R*/*S*)-BIU	0.33	0.29	1.12
G5	*R*-BIU	0.61	—	—
	S-BIU	—	0.80	0.79
	(*R*/*S*)-BIU	0.39	0.59	0.66

Table 5. Enzyme kinetic parameters of purified human GST isoenzymes towards the BIU enantiomers (N = 1) and racemic BIU (mean ± SD)

Enzyme	Substrate	*R*-BIU K_m (mM)	*R*-BIU V_{max} (nmol/mg/min)	*S*-BIU K_m (mM)	*S*-BIU V_{max} (nmol/mg/min)
GSTA1-1	*R*-BIU	0.51	19	—	—
	S-BIU	—	—	0.37	18
	(*R*/*S*)-BIU*	0.12 ± 0.06	8.7 ± 0.2	0.13 ± 0.08	9.2 ± 0.4
GSTA2-2	*R*-BIU	3.9	26	—	—
	S-BIU	—	—	1.7	109
	(*R*/*S*)-BIU*	1.2 ± 0.1	7.8 ± 3.6	0.63 ± 0.05	39 ± 5
GSTM1a-1a	*R*-BIU	2.6	30	—	—
	S-BIU	—	—	1.7	31
	(*R*/*S*)-BIU†	1.0 ± 0.4	10 ± 1	0.73 ± 0.2	14 ± 2

* N = 2, † N = 3.

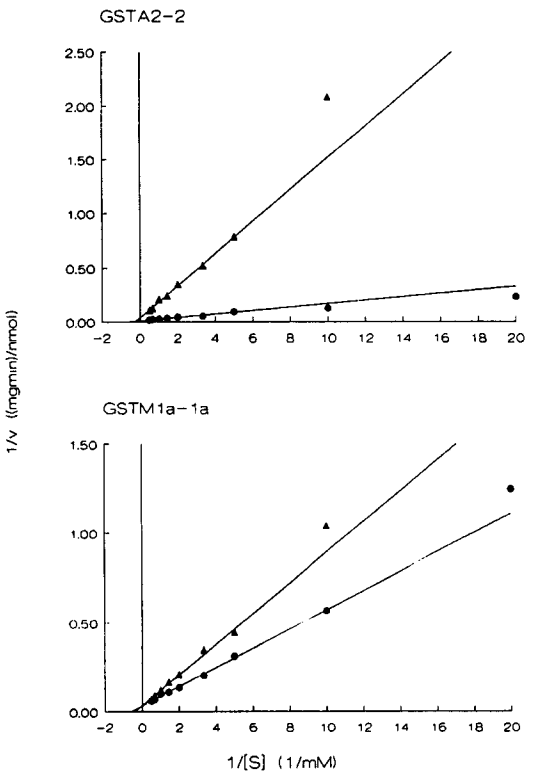

Fig. 2. Lineweaver–Burk plots of GSTA2-2 (upper) and GSTM1a-1a (lower) with *R*- and *S*-BIU as substrates at 2.0 mM GSH. Triangles = GSH conjugate derived from *R*-BIU, circles = GSH conjugate derived from *S*-BIU.

GST subunit per cytosolic fraction, showed that the activities towards the BIU enantiomers and *R*/*S*-ratios of the liver cytosolic fractions were predictable. This also indicates that other possible GST isoenzymes present in the liver (e.g. class theta isoenzymes) are unlikely to have a major influence on the stereoselective conjugation of BIU enantiomers. This assertion is supported by the observation that for only one of the four liver cytosolic fractions (No. 4) specific activity (1.03 nmol/min per mg cytosolic protein) towards dichloromethane (substrate class theta) could be detected.

Comparison of the present data with those obtained for purified rat liver GST isoenzymes [8] shows that neither the human nor rat class pi isoenzyme catalysed the GSH conjugation of BIU enantiomers. In addition, the stereoselectivity of the human class alpha isoenzyme GSTA2-2 is similar to that of purified rat class alpha GST isoenzymes 1-1 and 2-2, which preferentially catalyse the conjugation of *S*-BIU. However, the stereoselectivity of human GSTM1a-1a (preference for *S*-BIU) is opposite to that of rat class mu GST isoenzymes 3-3 and 4-4, which prefer *R*-BIU. Therefore, despite the similarities in structural and catalytic properties between rat and human cytosolic GST isoenzymes [18], a species difference in stereoselectivity was found for the GSH conjugation of BIU enantiomers by the human and rat class mu GST isoenzymes.

Previous studies with rat liver cytosolic fractions, isolated rat hepatocytes, perfused rat liver and the intact rat showed that *R*-BIU was conjugated two to three times faster than *S*-BIU [10, 19]. Based on the stereoselectivity of purified rat GST isoenzymes [8] and the predominance of GST subunits 1 and 2 (class alpha) and 3 and 4 (class mu) in the rat liver (the concentration of class mu isoenzymes are slightly higher than those of class alpha isoenzymes) [20], preferential conjugation of *R*-BIU in rats *in vivo* is most likely related to the faster conjugation of *R*-BIU by rat liver 3-3 and 4-4. In contrast to rats, involvement of human class mu isoenzymes in the *in vivo* GSH conjugation of especially *R*-BIU is not likely, since GSTM1a-1a preferentially conjugated *S*-BIU and *in vivo* the BIU pharmacokinetics were not influenced by the GST class mu phenotype [6]. Furthermore, although all human liver cytosolic fractions contained GSTM1a subunits, they preferentially conjugated *S*-BIU.

The present *in vitro* results would suggest preferential GSH conjugation of *S*-BIU in humans *in vivo*. However, after oral administration of racemic BIU to healthy male volunteers, both the plasma clearance of *R*-BIU and the urinary excretion of the mercapturate derived from *R*-BIU in urine were higher compared to that of *S*-BIU and its mercapturate [6, 7]. As stereoselective or incomplete absorption of BIU enantiomers in humans is unlikely [6], the stereoselective BIU pharmacokinetics in humans seem to be caused by a more extensive GSH conjugation of *R*-BIU.

A possible explanation for the *in vitro–in vivo* difference in GSH conjugation of BIU enantiomers in humans may be the involvement of other GST isoenzymes, outside the liver and gastrointestinal tract, than those presently studied in the conjugation of BIU enantiomers. More likely, other metabolic routes, including presystemic metabolism, may be involved in the BIU pharmacokinetics *in vivo*. The latter is supported by the fact that in humans only 30–40% of the total racemic BIU dose could be recovered as BIU mercapturates in urine in 24 hr. Furthermore, in the rat, hydrolysis of BIU by amidase activity to 2-bromoisovaleric acid (BI) has been observed [10, 19]. Nevertheless, the tissues and/or GST isoenzymes involved in the stereoselective BIU pharmacokinetics, i.e. preferential GSH conjugation of *R*-BIU, in humans are still open for discussion.

Acknowledgements—This work was financially supported by the Dutch "Praeventiefonds". Den Haag, The Netherlands: Project number 28-1962. The authors thank W. H. M. Peters (Division of Gastrointestinal and Liver Diseases, St Radboud University Hospital, Nijmegen, The Netherlands) for the supply of human intestinal cytosolic fractions.

REFERENCES

1. Ketterer B and Mulder GJ, Glutathione conjugation. In: *Conjugation Reactions in Drug Metabolism* (Ed. Mulder GJ), pp. 307–364. Taylor and Francis Ltd, London, 1990.
2. Mannervik B, The isoenzymes of glutathione transferase. *Adv Enzymol* **57**: 357–417, 1985.

3. Meyer DJ, Coles B, Pemble SE, Gilmore KS, Fraser GM and Ketterer B, Theta, a new class of glutathione transferases purified from rat and man. *Biochem J* **274**: 409–414, 1991.
4. Mannervik B, Awasthi YC, Board PG, Hayes JD, Di Ilio C, Ketterer B, Listowsky I, Morgenstern R, Muramatsu M, Pearson WR, Pickett CB, Sato K, Widersten M and Wolf CR, Nomenclature for human glutathione transferases. *Biochem J* **282**: 305–308, 1992.
5. Mulders TMT, Breimer DD and Mulder GJ, Glutathione conjugation in man. In: *Human Drug Metabolism from Molecular Biology to Man* (Ed. Jeffery EH), pp. 133–142. CRC Press Inc., Boca Raton, 1992.
6. Mulders TMT, Venizelos V, Schoemaker R, Cohen AF, Breimer DD and Mulder GJ, Characterization of glutathione conjugation in man: stereoselectivity in plasma elimination kinetics and urinary excretion of (*R*)- and (*S*)-2-bromoisovalerylurea in healthy volunteers. *Clin Pharm Ther* **53**: 49–58, 1993.
7. te Koppele JM, Schipper C, Breimer DD and Mulder GJ, stereoselectivity in the urinary excretion of the mercapturates of (*R*)- and (*S*)- α-bromoisovalerylurea in man. *Br J Clin Pharmacol* **28**: 151–154, 1989.
8. te Koppele JM, Coles B, Ketterer B and Mulder GJ, Stereoselectivity of rat liver glutathione transferase isoenzymes for α-bromoisovaleric acid and α-bromoisovalerylurea enantiomers. *Biochem J* **252**: 137–142, 1988.
9. Polhuijs M, Kuipers F, Vonk RJ and Mulder GJ, Stereoselectivity of glutathione conjugation: blood elimination of alpha-bromoisovalerylurea enantiomers and biliary excretion of the conjugates in unanesthetized normal or congenitally jaundiced rats. *J Pharmacol Exp Ther* **249**: 874–878, 1989.
10. te Koppele JM, Esajas SW, Brussee J, van der Gen A and Mulder GJ, Stereoselective glutathione conjugation of the separate α-bromoisovalerylurea and α-bromoisovaleric acid enantiomers in the rat *in vivo* and in rat liver subcellular fractions. *Biochem Pharmacol* **37**: 29–35, 1988.
11. van Ommen B, Bogaards JJP, Peters WHM, Blaauboer B and van Bladeren PJ, Quantification of human hepatic glutathione *S*-transferases. *Biochem J* **269**: 609–613, 1990.
12. Hayes PC, Bouchier IAD and Beckett GJ, Glutathione *S*-transferase in humans in health and disease. *Gut* **32**: 813–818, 1991.
13. Peters WHM, Roelofs HMJ, Nagengast FM and van Tongeren JHM, Human intestinal glutathione *S*-transferases. *Biochem J* **257**: 471–476, 1989.
14. Peters WHM, Kock L, Nagenkast FM and Kremers PG, Biotransformation enzymes in human intestine: critical low levels in the colon? *Gut* **32**: 408–412, 1991.
15. te Koppele JM, van der Mark EJ, Olde Boerrigter JC, Brussee J, van der Gen A, van der Greef J and Mulder GJ, α-Bromoisovalerylurea as model substrate for studies on pharmacokinetics of glutathione conjugation in the rat. I. (Bio-)synthesis, analysis and identification of diastereomeric glutathione conjugates and mercapturates. *J Pharmacol Exp Ther* **239**: 898–904, 1986.
16. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferases, the first step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
17. te Koppele JM, van der Mark EJ and Mulder GJ, Liquid chromatographic determination of diastereomeric glutathione conjugates and futher derivatives of α-bromoisovalerylurea in rat bile and urine by electrochemically generated bromine. *J Chromatogr* **427**: 67–77, 1988.
18. Mannervik B, Ålin P, Guthenberg C, Jensson H, Tahir MK, Warholm M and Jörnvall H, Identification of three classes of cytosolic glutathione transferase common to several mammalian species: correlation between structural data and enzymatic properties. *Proc Natl Acad Sci USA* **82**: 7202–7206, 1985.
19. te Koppele JM, De Lannoy IAM, Pang KS and Mulder GJ, Stereoselective glutathione conjugation and amidase-catalyzed hydrolysis of α-bromoisovalerylurea enantiomers in isolated rat hepatocytes. *J Pharmacol Exp Ther* **243**: 349–355, 1987.
20. Bogaards JJP, van Ommen B and van Bladeren PJ, An improved method for the separation and quantification of glutathione *S*-transferase subunits in rat tissue using high performance liquid chromatography. *J Chromatogr* **474**: 435–440, 1989.