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UDP GLUCURONYLTRANSFERASE AND PHENOLSULFOTRANSFERASE FROM RAT LIVER *IN VIVO* AND *IN VITRO*—IV*

SPECIES DIFFERENCES IN HARMOL CONJUGATION AND ELIMINATION IN BILE AND URINE *IN VIVO*

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Abstract—Harmol, (7-hydroxy-1-methyl-9H-pyrido-3,4b)-indol, is converted to harmol–sulfate and harmol–glucuronide when it is injected *in vivo* in the rat. Conjugation of harmol, and elimination of the conjugates in bile and urine were studied in cat, rabbit, mouse, guinea-pig and rat after an intravenous dose of 20 μmoles/kg. Rabbit and guinea-pig nearly exclusively glucuronidated harmol. The cat predominantly synthesized harmol–sulfate but harmol–glucuronide was also produced. Mouse and rat synthesized both conjugates to comparable amounts. After 2 hr about 20% of the dose was found in urine in the form of the conjugates. From 30–60% of the dose was present in bile after this time; the rabbit excreted only 9% in that time in bile and was a poor excretor in bile. The glucuronide conjugate is excreted to a higher extent in bile than the sulfate conjugate. The data suggest that biliary excretion requires a high liver concentration of the relevant compound for a high rate or excretion.

Considerable species differences in biotransformation of xenobiotics, and disposition of the resulting products in bile and urine have been found [1–3]. The reason for these differences in biotransformation in most cases will be a difference in the relative amounts of the enzymes involved in the various pathways of drug biotransformation; however, co-substrate supply may also become rate limiting. Thus, glutathion conjugation can become impaired when the hepatic amount of glutathion is reduced [4].

Two main conjugation reactions, sulfation and glucuronidation, can often accept the same substrates and, therefore, many compounds can be excreted as both the sulphate and glucuronide product. After injection and hepatic uptake, phenolsulfotransferase and UDP glucuronyltransferase, the enzymes responsible for these conjugations, will compete for the same substrate. Some consequences of this type of competition have been reported previously from this laboratory with the compound harmol [5–7], (7-hydroxy-1-methyl-9H-pyrido-3,4b)-indol, as the substrate of both enzymes. Harmol in the rat is conjugated both to harmol–sulfate and harmol–glucuronide; these conjugates are subsequently excreted, harmol glucuronide predominantly in bile and harmol–sulfate equally in bile and urine.

In the present paper we describe results of an investigation of harmol conjugation, and subsequent elimination *in vivo* of harmol–sulfate and harmol–glucuronide in several species, namely cat, mouse, rabbit, rat and guinea-pig.

* Part III of this series is Ref. 6.

MATERIALS AND METHODS

Animals. All animals had free access to food and water before the experiments. Bile and urine were collected fractionally during 2 hr after an intravenous injection of harmol (20 μmoles/kg); the cystic duct from the gall bladder was ligated in all animals. The temperature was kept at 37·5 ± 0·5°. Details for the various species are given below.

Mice. Male mice (Swiss, TNO, Zeist, the Netherlands) weighing 32–39 g were anesthetized with pentobarbital (Nembutal®), 90 mg/kg intraperitoneally. The bile duct was cannulated with polyethylene tubing through a midline incision and harmol was injected i.v. either in the tail vein or in the cannulated vena jugularis externa; both ways of administration provided the same results. Bile was collected in fractions of 30 min and urine was collected after termination of the experiment from the bladder with a syringe.

Guinea-pigs. Male guinea-pigs (500–703 g) were anesthetized with pentobarbital, 65 mg/kg intraperitoneally. Bile duct, vena jugularis externa and urethra were cannulated and harmol was injected i.v. in the vena jugularis externa. Bile and urine were collected in fractions of 30 min.

Rabbits. Male rabbits (2·8–5·3 kg) were anesthetized with pentobarbital injected i.v. in the ear vein (50 mg/kg). Bile duct and vena jugularis externa were cannulated and bile was collected in fractions of 30 min after injection of harmol. Urine was collected from the bladder at the end of the experiment with a syringe.

Cats. Male cats (2·7–3·4 kg) were anesthetized, after initial ether narcosis, with pentobarbital (30 mg/kg) intravenously). Bile duct, vena jugularis externa and

Table 1. Bile and urine production in the species*

Species	n	Mean wt (g)	Bile/30 min (mg)	Urine/30 min (ml)	Urine collected 120 min after injection from the bladder (ml)
Mouse	5	36	239 ± 13	—	0·66 ± 0·19
Guinea-pig	5	550	524 ± 51	0·06 ± 0·02	
Rabbit	4	3720	114 ± 14	—	0·45 ± 0·12
Cat	4	3050	45 ± 8	0·23 ± 0·05	
Rat†	7	314	228 ± 5	—	0·21 ± 0·07

* All values are expressed per 100 g body wt. Bile and urine fractions were collected after injection of harmol (20 μmoles/kg) during 2 hr. For bile, and for some species also for urine, production/30 min is given. For the other species the amount of urine aspired 2 hr after harmol injection from the bladder is given. n = number of animals, and means ± SEM are given.

† Calculated from experiments reported in Ref. 5.

urethra were cannulated. Harmol was injected in the vena jugularis externa and bile and urine was collected in fractions of 30 min duration. Throughout the experiment anesthesia was maintained by small doses of pentobarbital.

Harmol determination. Harmol and its conjugates were determined fluorimetrically after separation of harmol, harmol–sulfate and harmol–glucuronide by applying bile or urine samples to t.l.c. as described in detail previously [8].

RESULTS

Comparison of bile and urine production between the species. A great difference exists between the species in the amount of bile produced per 100 g of body wt (Table 1). Between the cat (the lowest) and guinea-pig (the highest) more than an 11-fold difference was found. Mouse and rat showed similar bile production rates and rabbit produced at about half this rate. Smaller differences, about 4-fold, were found in urine production between guinea-pig (lowest) and cat (highest). No correlation was found between rate of bile production and biliary excretion of the harmol conjugates, nor between rate of urine production and urinary excretion rate of the conjugates.

Conjugation of harmol in the species. Rabbit and guinea-pig nearly exclusively conjugated harmol with glucuronate, whereas the cat predominantly synthesized the sulfate conjugate (Table 2). In the cat the glucuronide conjugate was also excreted to a small extent; similarly, small amounts of the sulfate conjugate were produced in rabbit and guinea-pig. Mouse and rat synthesized both conjugates at considerable rates, the mouse producing more of the glucuronide conjugate and the rat more of the sulfate conjugate.

Biliary and urinary excretion of harmol conjugates. In all species investigated in the present study, about the same percentage of the dose, namely about 21%, is excreted in urine in the form of harmol–sulfate and harmol–glucuronide. Considering biliary excretion of the harmol conjugates, the low biliary excretion in the rabbit is striking: whereas the other species investigated showed 33–58% of the dose excreted in bile, in rabbit this was only 9%. In the rabbit, however, a much lower over-all recovery of harmol was observed after 2 hr (Table 2).

Figure 1 gives data on biliary and urinary excretion of harmol–sulfate and harmol–glucuronide separately. The rabbit is the only species excreting more of the conjugates in urine than in bile. The other species excreted most of the injected harmol in bile (at least during the 2 hr after injection). Harmol–glucuronide is excreted predominantly in bile (the rabbit being an exception), but harmol–sulfate also to a considerable extent in urine. Finally, the results of Fig. 1 show that in most cases biliary excretion of harmol–glucuronide and harmol–sulfate took place during the first 30 min after injection of harmol; the cat is the notable exception in that the biliary excretion rate is the same for two consecutive 30-min periods.

DISCUSSION

In the present work species differences in conjugation pattern and excretory pathways of harmol were found. Factors determining the amount of harmol–sulfate and harmol–glucuronide in bile and urine respectively are e.g. molecular weight of the conjugates,

Table 2. Glucuronidation and sulfation of harmol in some species *in vivo*

Species	n	Harmol–glucuronide (%)	Harmol–sulfate (%)	Total recovery of conjugates (%)
Rabbit	4	34·6 ± 3·9	1·1 ± 0·2	35·6 ± 4·0
Guinea-pig	5	50·5 ± 2·4	3·8 ± 0·3	54·3 ± 2·5
Cat	4	8·8 ± 1·8	53·5 ± 2·6	62·3 ± 2·2
Mouse	5	44·3 ± 2·3	34·2 ± 2·4	78·5 ± 2·4
Rat†	7	17·6 ± 1·3	48·4 ± 3·5	66·1 ± 7·8

Harmol (20 μmoles/kg) was injected i.v. Bile and urine were collected during 2 hr after the injection. The total amounts, expressed as % of the dose, of harmol–glucuronide and harmol–sulfate, excreted in bile and urine in that period are given. The recovery gives the % of the dose recovered after these 2 hr in the form of harmol–glucuronide and harmol–sulfate. n = number of animals used and the mean ± SEM is given.

† Data from Ref. 5.

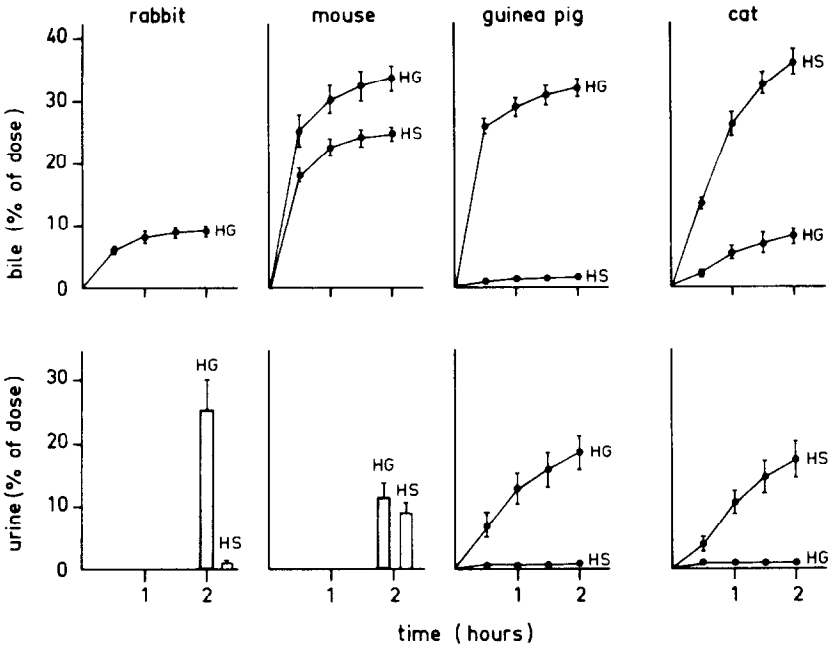

Fig. 1. Biliary and urinary excretion of harmol–sulfate (HS) and harmol–glucuronide (HG) in various species after an intravenous injection of harmol. Bile was collected in fractions of 30 min after injection of harmol (20 μmoles/kg). Urine was collected similarly in guinea-pig and cat; in rabbit and mouse urine was collected from the bladder 2 hr after injection of harmol. The percentages of the dose excreted as sulfate or glucuronide conjugate are given cumulatively in time. Means ± SEM are indicated in the figure.

the presence of carrier systems transporting the conjugate from liver cell into blood or bile, or from blood into urine, and the concentration of the conjugate attained in the liver cell.

The minimum mol. wt required for extensive biliary excretion of organic anions has been determined by Hirom *et al.* [10] in some species: for the rat 325 ± 50, for the guinea-pig 400 ± 50 and for the rabbit 475–50. Harmol–sulfate has a mol. wt of 278, and harmol-glucuronide of 374. Therefore, due to this difference in mol. wt one would expect [2] harmol–glucuronide to be excreted better in bile than harmol sulfate. This is the case in the species studied; similar findings were reported by Miller *et al.* [9] for 2,6-dimethoxyphenol in the rat. Only in the mouse the same percentage of the total excreted amount of harmol–sulfate and harmol–glucuronide is found in bile (75%). Millburn (Ref. 2, p. 21) reported that sulfate conjugates below a mol. wt of 275 are rather poorly excreted in bile in the rat; therefore, harmol–sulfate seems an exception because it is present in bile to a considerable extent in all species that synthesize the sulfate conjugate extensively.

Recently Curtis *et al.* [11] reported that sulfate conjugates are actively excreted in urine in the rat. If this applies also to harmol–sulfate this might imply that harmol–sulfate leaking from liver will quickly be excreted in urine.

It is well known that the cat has a poor glucuronidating capacity, whereas sulfation is more important in this species [3, 12]. Our results are in agreement with this. Similarly, high glucuronidation and low sulfation rates in rabbit are not unexpected [13] and are confirmed in the present work.

A low recovery of harmol was found in the rabbit (35%); this may be due to a low excretory rate of the harmol metabolites in the rabbit (especially into bile), or to the formation of a non-fluorescent metabolite which would escape our detection method. Previously [5] we have shown that in the rat only harmol–sulfate and harmol–glucuronide were found in a 100% recovery after 6 hr.

The ratios of sulfate to glucuronide conjugates we find with harmol agree well with those reported for phenol in several species by Capel *et al.* [14]. However, the rabbit excreted in their experiments equal amounts of phenyl-sulfate and phenyl-glucuronide in urine; these authors used female animals.

Species differences in pharmacokinetic behavior of harmol may determine (along with other factors) sulfate:glucuronide ratio, and may be the cause of both the low recovery of harmol in the rabbit, and the constancy of biliary and urinary excretion rates of the conjugates in the cat. The results presented in Fig. 1 suggest that in rabbit, mouse and guinea-pig (and in rat for harmol–glucuronide [5]), supply of the harmol conjugates to the presumed carriers in the liver cell for transport of the conjugates from liver cell into bile is highest in the first period of 30 min. Contrary to this, the urinary excretion of harmol–glucuronide in the guinea-pig is constant during the first two periods of 30 min. It may be that the concentration of harmol–glucuronide in the liver cell has fallen below a value were high biliary excretion is possible, whereas leakage from liver cell into blood is still unimpaired. More detailed pharmacokinetic studies of the conjugates should increase our understanding of this process.

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