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Effect of Butylated Hydroxyanisole on Hepatic Glucuronidation and Biliary Excretion of Drugs in Mice

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Abstract—Inhibition of glucuronidation by depletion of UDP-glucuronic acid from liver impairs the hepatobiliary transport of glucuronidated xenobiotics. However, it is not known if enhancement of hepatic glucuronidation increases the biliary excretion of these compounds. Therefore, the effect of treatment with butylated hydroxyanisole (BHA), which increases hepatic glucuronidation capacity, on the biliary excretion of compounds undergoing glucuronidation was studied in mice. BHA-feeding (1% for 10 days) increased hepatic UDP-glucuronic acid content by 240% and enhanced hepatic UDP-glucuronosyltransferase activities (expressed per kg body weight) toward valproic acid, phenolphthalein, iopanoic acid and bilirubin 220, 180, 120 and 60%, respectively. BHA treatment did not influence the biliary excretion of unmetabolized cholephils, phenol-3,6-dibromphthalein disulphonate and phenolphthalein glucuronide, but enhanced that of phenolphthalein (+108%), iopanoic acid (+63%) and bilirubin (+33%) as glucuronides. However, these increases were apparent only in the initial phase of excretion. In contrast, BHA markedly decreased (−43%) the biliary excretion of valproic acid glucuronides. Simultaneously, BHA increased the urinary excretion of the glucuronides of phenolphthalein (+48%), iopanoic acid (+450%) and valproic acid (+150%). A shift in the distribution of iopanoic acid and valproic acid and metabolites from liver to kidney was also apparent in BHA-fed mice. Thus, enhanced glucuronidation does not facilitate the biliary excretion of all glucuronidated compounds and only transiently increases others. It is likely that this phenomenon is the result of the glucuronides readily entering the plasma and being excreted by the kidney.

Conjugation with glucuronic acid is dependent upon the catalytic activity of UDP-glucuronosyltransferase (EC 2.4.1.17) and the accessibility of UDP-α-D-glucuronic acid (UDP-glucuronic acid), the co-substrate of UDP-glucuronosyltransferase (Dutton & Burchell 1977). This biosynthetic reaction has long been considered important in influencing the hepatobiliary disposition of chemicals (Smith 1966; Millburn et al 1967). It has recently been shown that inhibition of the glucuronidation of cholephils by galactosamine- or diethyl ether-induced depletion of hepatic UDP-glucuronic acid reduces their excretion into bile (Gregus et al 1983; Dills & Klaassen 1984). Inherited deficiency of bilirubin-UDP-glucuronosyltransferase in Gunn rats severely decreases the biliary bilirubin excretion (Arias et al 1961). Thus, reduced glucuronidation capacity impairs the biliary excretion of compounds which undergo glucuronidation. It is not clear, however, if an increase in glucuronidation capacity would influence the hepatobiliary transport of chemicals that are glucuronidated. Investigations aiming to study this problem by examining the effect of inducers of UDP-glucuronosyltransferase have arrived at seemingly conflicting conclusions. For instance, phenobarbitone-induction of UDP-glucuronosyltransferase enhanced the biliary excretion of some glucuronidated xenobiotics (Levine et al 1970; Cooke et al 1973) but failed to influence (Watkins & Klaassen 1982a) or even decreased others (Peterson & Fujimoto 1973; Roerig et al 1974; Loser & Siegers 1985).

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The aim of the present study was to reinvestigate the effect of increased glucuronidation on the biliary excretion of xenobiotics using another inducer, butylated hydroxyanisole (BHA). BHA was chosen for several reasons: (i) it is an effective inducer of UDP-glucuronosyltransferase in mice (Hazelton et al 1985); (ii) BHA also elevates UDP-glucuronic acid in liver by increasing UDP-glucose concentrations and enhancing UDP-glucose dehydrogenase activity (Hazelton et al 1985; Watkins & Klaassen 1983); (iii) whereas phenobarbitone accelerates hepatobiliary transport, which results in an increase in the biliary excretion of unmetabolized compounds (Klaassen 1970a,b; Fischer et al 1978), earlier data suggest that BHA is devoid of such an effect (Gregus & Klaassen 1982).

The experiments were carried out on female mice as BHA increases glucuronidation in female mice more than in males (Hazelton et al 1985). Phenolphthalein, iopanoic acid, bilirubin and valproic acid were investigated because these compounds are glucuronidated and are excreted into bile in significant amounts.

Materials and Methods

Chemicals

Bilirubin, BHA (2[3]-tert-butyl-4-hydroxyanisole), diethylstilboestrol, β-glucuronidase (type B-1), phenolphthalein, phenolphthalein glucuronic acid sodium salt, urethane and UDP-glucuronic acid were purchased from Sigma Chemical Co., St. Louis, MO. CHAPS (3-[3-cholamidopropyl]-dimethylammonio)-1-propane sulphonate) was obtained from Calbiochem-Behring Corp., La Jolla, CA; iopanoic acid from Sterling-Winthrop Research Institute, Rensselaer,

NY; phenol-3,6-dibromphthalein disulphonate (DBSP) from Société d'Etudes et des Recherches Biologiques, Paris, France; and valproic acid from Saber Laboratories, Morton Grove, IL. Carrier-free [^{125}I]Na, [monoethyl-^{3}H]diethylstilboestrol (116 Ci mmol^{-1}) and [1-^{14}C]valproic acid (4·7 mCi mmol^{-1}) were purchased from Amersham/Searle Corp. (Arlington Heights, IL). [^{125}I]Iopanoic acid was synthesized as previously described (Gregus et al 1983).

Animals

Female ICR-Swiss mice (Sasco, Omaha, NE), 35–45 g, were used. For 10 days before the excretion studies, animals were fed ground Purina Laboratory chow (Ralston Purina Co.) containing 1% BHA. Control mice received only ground Purina Laboratory chow.

Determination of UDP-glucuronic acid and UDP-glucuronosyltransferase activities

Mice were decapitated. For the determination of hepatic UDP-glucuronic acid, 0·5 g of liver was heated at boiling temperature in 4·5 mL of water for 5 min and homogenized. The homogenate was centrifuged at 4000 *g* for 20 min. UDP-glucuronic acid was determined in the resultant supernatant by conjugation with [^{3}H]diethylstilboestrol as described by Watkins & Klaassen (1982b).

For the determination of UDP-glucuronosyltransferase activities, a 25% liver homogenate was prepared in 1·15% KCl at 0 to 5°C in a Potter-Elvehjem glass homogenizer using a Teflon pestle. The homogenate was centrifuged at 10 000 *g* for 10 min and the resultant supernatant was recentrifuged at 100 000 *g* for 60 min. The pellet from the second centrifugation was resuspended at a concentration of 2 mL g^{-1} liver in a buffer containing 0·25 M sucrose and 0·01 M Tris, pH 7·4, and stored at −50°C up to one week. Storage of microsomal suspension did not significantly influence UDP-glucuronosyltransferase activity. The UDP-glucuronosyltransferase assays utilized microsomes solubilized with 8 mM CHAPS at 0–5°C for 20 min and the following concentrations of aglycones: 0·1 mM bilirubin (VanRoy & Heirwegh 1968); 0·15 mM phenolphthalein (Winsnes 1969); and 0·5 mM valproic acid (Watkins & Klaassen 1982a). Glucuronidation of iopanoic acid (1·0 mM) was measured similarly to that of valproic acid (Watkins & Klaassen 1982a). Unconjugated iopanoic acid was separated from the glucuronide by solvent extraction with diethyl ether (Cooke & Cooke 1977). All enzyme assays were performed at 37°C in a medium (pH 7·4) containing 200 mM Tris, 10 mM $MgCl_2$, UDP-glucuronic acid and 1·25 mM D-saccharic acid 1,4-lactone. The reaction was initiated by the addition of UDP-glucuronic acid to the assay media. Microsomal protein was determined according to Bradford (1976). The enzymatic reactions were linear with respect to incubation time and protein concentration.

Biliary excretion studies

A median laparotomy was performed under urethane (1·5 g kg^{-1} i.p.) anaesthesia and was followed by cannulation of the bile duct using a 30-gauge needle attached to a polyethylene (PE-10) tubing. Body temperature was maintained at 36·5°C with a heat lamp. The compounds were injected into the saphenous vein in a volume of 5 mL kg^{-1} at the following dosages: bilirubin 50 μmol kg^{-1}, [^{125}I]iopanoic acid 100 μmol kg^{-1} (10 μCi kg^{-1}), phenolphthalein, phenolphthalein-glucuronide and DBSP 200 μmol kg^{-1}, and [^{14}C]valproic acid 1000 μmol kg^{-1} (10 μCi kg^{-1}). Iopanoic acid, phenolphthalein-glucuronide and DBSP were dissolved in water. Bilirubin, phenolphthalein and valproic acid were dissolved in dimethyl sulphoxide (1 mL kg^{-1}) to which sodium hydroxide had been added in amounts equivalent to the molar quantities of the organic acids. These solutions were then diluted with water and injected at 5 mL kg^{-1}. Bile was collected for 15 min before administration of drugs to determine basal bile flow. After injection of the compounds, bile was collected in four consecutive 15 min periods. The volume of bile collected was measured gravimetrically using a specific gravity of 1·0 g mL^{-1}. Bile flow was calculated as μL min^{-1} kg^{-1}. Biliary excretion rates were calculated as the product of bile flow and bile concentration.

Urinary excretion studies

To stimulate urine production, mice were given saline via gavage (20 mL kg^{-1}). After administration of drugs into the tail vein, each mouse was placed in an inverted 250 mL beaker over a Petri dish covered with wire screen. This allowed excreted urine to be collected. Sixty min after injection of the compounds, the mice were killed. Urine which accumulated in the dishes during the 60 min period, as well as the urine remaining in the bladder, was collected. The liver and kidneys were removed so that drug concentrations could be determined in the respective tissues.

Chemical determinations

Bilirubin and its mono- and diglucuronide in bile were separated by high pressure liquid chromatography and detected spectrophotometrically at 436 nm (Chowdhury et al 1981) immediately after the end of each collection. Quantification was based on bilirubin standards, since bilirubin and its glucuronides have the same molar extinction coefficients (Ostrow & Murphy 1970). The determination of the foreign compounds in bile and urine as well as the separation of glucuronides was performed as described earlier (Gregus et al 1983). Radioactivity due to [^{125}I]iopanoic acid was determined in liver and kidney by counting weighed tissue samples in a Packard Auto-Gamma spectrometer (La Grange, IL). For the determination of [^{14}C]valproic acid-derived radioactivity in liver and kidney, 20–30 mg tissue samples were digested with 1 mL Protosol (New England Nuclear, Boston, MA) in glass scintillation vials and allowed to set overnight at room temperature (20°C). Twenty mL of complete counting cocktail 3a70 (Research Product International, Elk Grove, IL) was then added and the samples were counted in a liquid scintillation spectrometer (Packard model 3330 Tricarb). Quenching was corrected by automatic external standardization.

Statistics

The means of the BHA-treated and control groups were compared by the two tailed Student's *t*-test using $P < 0.05$ as the level of significance.

Table 1. Effect of butylated hydroxyanisole (BHA) on liver weight, hepatic concentration of UDP-glucuronic acid (UDP-GA) and hepatic UDP-glucuronosyltransferase (UDP-GT) activities in mice. Mice were fed with food containing 1% BHA for 10 days. Values are means ± s.e. of 8 mice.

	Liver weight ($g\ kg^{-1}$)	UDP-GA concn. ($nmol\ g^{-1}$)	UDP-GT activity ($nmol\ min^{-1}\ g^{-1}$ liver) toward PP	IOP	VPA	BI
Control	59·6 ± 1·8	251 ± 36	789 ± 59	3·26 ± 0·24	56·4 ± 4·9	121 ± 4·5
BHA	86·9 ± 4·0*	581 ± 60*	1538 ± 110*	4·89 ± 0·24*	124 ± 9·5*	132 ± 2·1

PP = phenolphthalein, IOP = iopanoic acid, VPA = valproic acid, BI = bilirubin.
*$P < 0·05$.

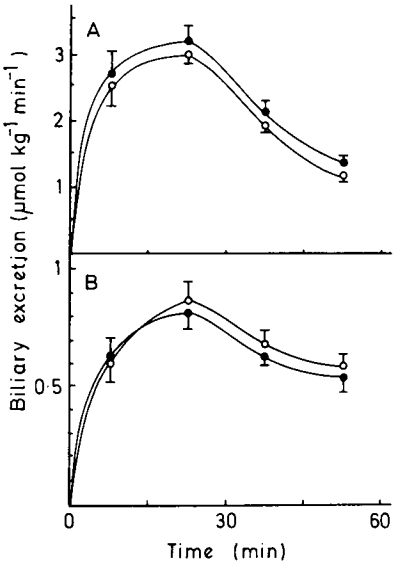

FIG. 1. Effect of butylated hydroxyanisole (BHA) on the biliary excretion of (A) phenol-3,6-dibromphthalein disulphonate (200 $\mu mol\ kg^{-1}$ i.v.) and (B) phenolphthalein glucuronide (200 $\mu mol\ kg^{-1}$ i.v.). Food containing 1% BHA was fed to mice for 10 days before the experiments. Symbols represent means ± s.e. of 6–10 mice; ○ control, ● BHA.

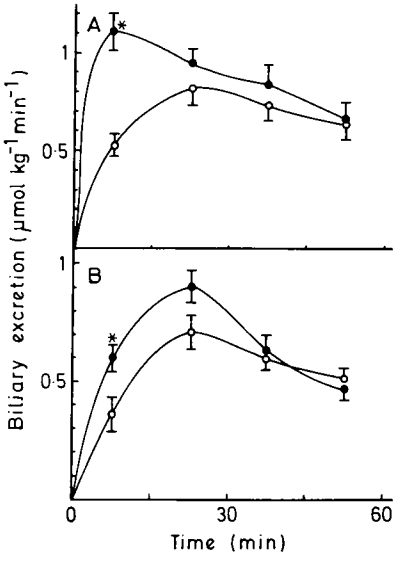

FIG. 2. Effect of butylated hydroxyanisole (BHA) on the biliary excretion of (A) phenolphthalein (200 $\mu mol\ kg^{-1}$ i.v.) and (B) iopanoic acid (100 $\mu mol\ kg^{-1}$ i.v.). Food containing 1% BHA was fed to mice for 10 days before the experiments. Symbols represent the biliary excretion rates of glucuronides of the drugs (means ± s.e.) in 7–12 mice. Only trace amounts of the parent compounds were present in bile. * Significantly different ($P < 0·05$) from controls; ○ control, ● BHA.

Results

Hepatic UDP-glucuronic acid concentration and UDP-glucuronosyltransferase activity

The effect of pretreatment with BHA on hepatic UDP-glucuronic acid concentrations and UDP-glucuronosyltransferase activities are shown in Table 1. Mice treated with BHA had significantly higher hepatic UDP-glucuronic acid levels (+131%) than the controls. BHA also markedly increased UDP-glucuronosyltransferase activities toward phenolphthalein (+94%), iopanoic acid (+50%) and valproic acid (+119%) but not toward bilirubin (+9%). In addition, BHA-treatment produced an increase in liver weight (+46%) and hepatic microsomal protein concentration (+11%).

Biliary excretion of unmetabolized compounds

As illustrated in Fig. 1, BHA treatment did not influence the biliary excretion rates of two unmetabolized cholephilic model compounds, DBSP and phenolphthalein-glucuronide.

Biliary excretion of compounds undergoing glucuronidation

The biliary excretion of phenolphthalein and iopanoic acid was enhanced by BHA (Fig. 2). However, the increased excretion of the glucuronides of phenolphthalein (+110%) and iopanoic acid (+67%) was apparent only in the initial 15 min period after administration. Later the excretion rates of control and treated mice did not differ significantly.

BHA treatment elevated the biliary excretion rates of exogenously administered bilirubin (Fig. 3) in form of its monoglucuronide (+33%) and diglucuronides (+41%). A decreased excretion of the parent compound (−54%) was observed. Again, these effects were seen only in the initial phase of bilirubin excretion.

No increase in the biliary excretion of valproic acid was observed after BHA-treatment (Fig. 4). Moreover, the rate of biliary excretion of valproic acid glucuronide in the BHA-fed mice declined 30 min after its administration and was considerably lower than in the controls in the third and fourth 15 min bile collection periods (−49 and −56%, respectively).

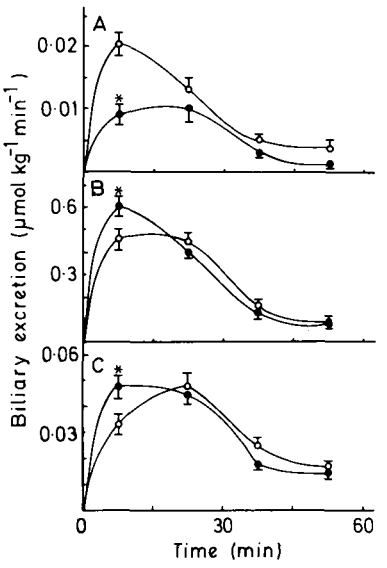

FIG. 3. Effect of butylated hydroxyanisole (BHA) on the biliary excretion of (A) bilirubin, (B) its monoglucuronide and (C) diglucuronide. Food containing 1% BHA was fed to mice for 10 days before the experiments. Bilirubin was injected intravenously in a dose of 50 μmol kg^{-1}. Symbols represent means ± s.e. of 7–10 mice. * Significantly different ($P < 0.05$) from controls; ○ control, ● BHA.

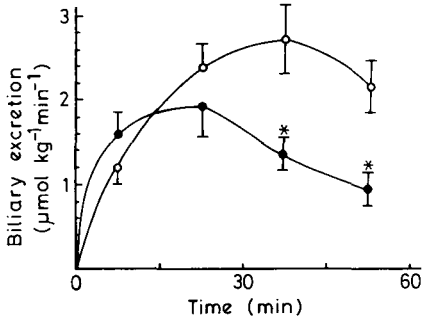

FIG. 4. Effect of butylated hydroxyanisole (BHA) on the biliary excretion of valproic acid (1000 μmol kg^{-1} i.v.). Food containing 1% BHA was fed to mice for 10 days before the experiments. Symbols represent the biliary excretion rates of valproic acid glucuronide (means ± s.e.) in 7–9 mice. Only trace amounts of the unconjugated drug was present in bile. * Significantly different ($P < 0.05$) from controls; ○ control, ● BHA.

Urinary excretion

BHA-treatment increased the hepatic glucuronidation of the xenobiotics studied but decreased the biliary excretion of valproic acid and only transiently enhanced the excretion of phenolphthalein and iopanoic acid into bile. Therefore, it was of interest to determine if BHA enhanced the elimination of the glucuronidated drugs into urine.

Table 2 demonstrates that BHA increased the urinary excretion of phenolphthalein (+48%), iopanoic acid (+450%) and valproic acid (+150%) as their respective glucuronides. In addition, a marked increase in the renal concentration and a decrease in hepatic concentration of iopanoic acid (and metabolites) was observed in BHA-treated animals with respect to controls. A similar tendency is seen with valproic acid.

Discussion

This study confirmed that BHA is an effective inducer of hepatic glucuronidation. The BHA-induced changes in liver which may result in enhancement of hepatic glucuronidation of a compound are (i) an increase in UDP-glucuronosyltransferase activity, (ii) an elevation of UDP-glucuronic acid concentration, and/or (iii) an increase in liver weight. By expressing the changes in hepatic enzyme activity and co-substrate levels on a per kg body weight basis, it can be seen that BHA enhanced hepatic UDP-glucuronosyltransferase activity toward valproic acid, phenolphthalein, iopanoic acid and bilirubin by 220, 180, 120 and 60%, respectively, while the hepatic UDP-glucuronic acid content was increased by 240%.

Even though BHA produced marked changes that are favourable for enhanced hepatic glucuronidation of compounds, e.g. increases in both the enzyme and the co-substrate responsible for conjugation with glucuronic acid, only transient and moderate increases were observed in the biliary excretion of bilirubin, phenolphthalein and iopanoic acid, whereas the excretion of valproic acid into bile was actually decreased.

There are a number of possible reasons why BHA is relatively ineffective in enhancing the biliary excretion of compounds that are glucuronidated. One possible reason is that BHA interferes with the biliary excretion of the

Table 2. Effect of butylated hydroxyanisole (BHA) on urinary excretion and hepatic and renal concentration of phenolphthalein, iopanoic acid and valproic acid in mice. Mice were fed with food containing 1% BHA for 10 days. Urine was collected for 60 min after administration of phenolphthalein (200 μmol kg^{-1} i.v.), iopanoic acid (100 μmol kg^{-1} i.v.) and valproic acid (1000 μmol kg^{-1} i.v.). Only glucuronides of the drugs were detected in the urine. Tissue samples were taken 60 min after drug administration. Tissue concentrations represent parent compound and metabolites. Values are means ± s.e. of 6–12 mice.

		Urinary excretion (μmol kg^{-1})	Tissue concentration (nmol g^{-1})	
			Kidney	Liver
Phenolphthalein	control	25·8 ± 2·5	n.d.	n.d.
	BHA	38·2 ± 4·0*	n.d.	n.d.
Iopanoic acid	control	1·10 ± 0·11	67·3 ± 7·8	157 ± 14
	BHA	6·06 ± 1·6*	227 ± 49*	99·6 ± 7·1*
Valproic acid	control	275 ± 31	15 500 ± 1170	44 900 ± 7480
	BHA	690 ± 52*	20 100 ± 2480	38 200 ± 5550

*$P < 0.05$. n.d. = not determined.

compounds tested. However, our findings that BHA-treatment did not influence the biliary excretion of unmetabolized cholephils, dibromphthalein and phenolphthalein-glucuronide, suggest that BHA does not interfere with the hepatobiliary transport function.

Another possible reason for the relative ineffectiveness of BHA in enhancing the biliary excretion of compounds undergoing glucuronidation, could be that their rate of biliary excretion is not limited by their normal rate of glucuronidation. In fact, the in-vitro hepatic UDP-glucuronosyltransferase activity for phenolphthalein and bilirubin in the untreated mice (47 and 72 μmol min^{-1} kg^{-1}; calculated from data in Table 1) exceed 60- and 15-fold, respectively, their maximal biliary excretion rates in-vivo (Figs 2, 3). In addition the similarities of the biliary excretion rates of phenolphthalein (Fig. 2) and the exogenously given phenolphthalein-glucuronide (Fig. 1), also suggest that glucuronidation of phenolphthalein is not the rate-limiting step in its hepatobiliary transport. However, these observations may not necessarily mean that the glucuronidation of these compounds in-vivo cannot limit the velocity of their excretion into bile. It should be noted that the UDP-glucuronosyltransferase activities were measured in detergent-solubilized (activated) microsomes in the presence of an excess amount of UDP-glucuronic acid. These conditions may not reflect the in-vivo situation where UDP-glucuronosyltransferase functions in a lipid constraint (Bock & White 1974; Vessey & Zakim 1978) and the supply of UDP-glucuronic acid may be limited. In addition, the fact that the biliary excretion rate of the exogenously administered phenolphthalein glucuronide was not significantly higher than when phenolphthalein was administered, may be due to a lower hepatic uptake of the glucuronide compared with that of the parent compound (Clark & Cooke 1978). It has been concluded that circulating glucuronides are less available for biliary excretion than those synthesized in liver (Gessner & Hamada 1974; Mehendale 1976). In the transport of phenolphthalein from blood to bile, the parent compound is taken up by the liver and its glucuronide is formed intracellularly. The question of whether or not the normal glucuronidation rate of cholephilic compounds is the rate-limiting factor in their hepatobiliary transport is difficult to determine. Nevertheless, our results suggest that the glucuronidation may be rate-limiting at least in the initial phase of substrate loading, because the BHA-induced elevation of hepatic UDP-glucuronic acid level and UDP-glucuronosyltransferase activity were associated with facilitated biliary excretion of glucuronides of phenolphthalein, iopanoic acid and bilirubin.

Another possible reason for the relative ineffectiveness of BHA in enhancing the excretion of glucuronides into bile is that BHA might simultaneously decrease the availability of glucuronides for biliary excretion. This could happen by enhancing the hydrolysis of glucuronides, by facilitating the urinary excretion of glucuronides or by altering the tissue distribution of the compounds leading to lower hepatic concentrations.

Although we did not determine the effect of BHA-treatment on the activity of β-glucuronidase, it is not likely that BHA-feeding increased the hydrolysis of glucuronides. If glucuronide hydrolysis were enhanced in the liver by BHA, we would have expected a diminution in the biliary excretion rate of exogenously injected phenolphthalein-glucuronide, because the parent compound is not excreted into bile.

The assumption that BHA could decrease the availability of glucuronides for biliary excretion by enhancing their urinary excretion was examined. It was found that BHA treatment did increase the excretion of compounds as glucuronic acid congugates into urine (Table 2). However, it appears that the enhancement of the urinary excretion of phenolphthalein and iopanoic acid may have little influence on their biliary excretion because both drugs are excreted preferentially via bile (the biliary and urinary excretion in the first hour after administration were, respectively, 21 and 13% of the dose of phenolphthalein and 33 and 1% of iopanoic acid), and the net increase in their urinary excretion after treatment with BHA is only 5–6% of the dose. In contrast, valproic acid is preferentially excreted into urine in mice (28% of the dose appears in urine and 13% in bile in 1 h), and the net BHA-induced increase in its urinary excretion was 41% of the dose. Thus, it can be assumed that this marked elevation in the urinary excretion of valproic acid is at least partly responsible for the decrease in its biliary excretion after BHA-feeding.

An alteration in the tissue distribution of a compound, e.g. its shift from liver to extrahepatic tissues, may also reduce its availability for biliary excretion. In fact, we observed a significant decrease in the hepatic concentration of iopanoic acid with a concomitant increase in its renal concentration in BHA-fed mice (Table 2). A similar tendency for change in tissue distribution was noted for valproic acid. In previous studies on the effect of induction of UDP-glucuronosyltransferase by phenobarbitone and *trans*-stilbene oxide on the hepatobiliary disposition of morphine, it was concluded that increased morphine glucuronidation resulted in an increased release of the glucuronide from liver to plasma (Peterson & Fujimoto 1973; Fuhrman-Lane & Fujimoto 1982). Furthermore, in isolated perfused liver about twice as much naphthol glucuronide is released into the perfusate as into bile (Bock & White 1974). When glucuronidation of naphthol was increased by phenobarbitone or 3-methylcholanthrene, the release of naphthol glucuronide into the perfusate was markedly enhanced. All these data support our assumption that, although glucuronidation is essential for the biliary excretion of most compounds undergoing conjugation with glucuronic acid, when increased above a certain level, glucuronidation may actually decrease the availability of glucuronides for biliary excretion through an increased release of conjugates into the systemic circulation. Enhancement of extrahepatic glucuronidation of drugs may also result in a decrease in their hepatic concentration, because circulating glucuronides are less available for biliary excretion than those formed in the liver (Gessner & Hamada 1974; Mehendale 1976). Indeed, BHA has been shown to increase UDP-glucuronosyltransferase activity and UDP-glucuronic acid levels in the small intestine of the mouse (Hjelle et al 1985). Thus, a BHA-induced enhancement in the extrahepatic glucuronidation of the compounds studied, may lead to the diversion of the extrahepatically formed glucuronides from the liver, resulting in a reduced availability for biliary excretion and an increased availability for excretion into urine.

In conclusion, our results indicate that enhancement of

glucuronidation can increase the biliary excretion of glucuronidated compounds. Simultaneously, however, the enhanced glucuronidation may accelerate processes (e.g. release of glucuronides from liver to blood, escape of extrahepatically formed glucuronides from hepatic extraction, urinary excretion) which counteract the increase in biliary excretion or may even result in a diminished excretion into bile. The ultimate outcome of these competing pathways appears to be determined by the distribution and/or elimination characteristics of the compound in question.

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References

Arias, I. M., Johnson, L., Wolson, S. (1961) Am. J. Physiol. 200: 1091–1094

Bock, C. W., White, I. N. H. (1974) Eur. J. Biochem. 46: 451–459.

Bradford, M. M. (1976) Anal. Biochem. 72: 248–254

Chowdhury, J. R., Chowdhury, N. M., Wu, G., Shouval, R., Arias, I. M. (1981) Hepatology 1: 622–627

Clark, A. G., Cooke, R. (1978) J. Pharm. Pharmacol. 30: 382–383.

Cooke, W. J., Cooke, L. (1977) Drug Metab. Dispos. 5: 368–376.

Cooke, W. J., Berndt, W. O., Mudge, G. H. (1973) J. Pharmacol. Exp. Ther. 187: 158–168.

Dills, R., Klaassen, C. D. (1984) Biochem. Pharmacol. 33: 2813–2814

Dutton, G. J., Burchell, B. (1977) Prog. Drug Metab. 2: 1–70

Fischer, E., Varga, F., Gregus, Z., Gogl, A. (1978) Digestion 17: 211–220

Fuhrman-Lane, C., Fujimoto, J. M. (1982) J. Pharmacol. Exp. Ther. 222: 526–533

Gessner, T., Hamada H. (1974) Life Sci. 15: 83–90

Gregus, Z., Klaassen, C. D. (1982) J. Pharmacol. Exp. Ther. 221: 242–246

Gregus, Z., Watkins, J. B., Thompson, T. N., Klaassen, C. D. (1983) Ibid. 225: 256–262

Hazelton, G. H., Hjelle, J. J., Klaassen, C. D. (1985) Toxicol. Appl. Pharmacol. 78: 280–290

Hjelle, J. J., Hazelton, G. A., Klaassen, C. D. (1985) Drug Metab. Dispos. 13: 68–70

Klaassen, C. D. (1970a) J. Pharmacol. Exp. Ther. 175: 289–300

Klaassen, C. D. (1970b) Biochem. Pharmacol. 19: 1241–1249

Levine, W.G., Millburn, P., Smith, R. L., Williams, R. T. (1970) Ibid. 19: 235–244

Loser, W., Siegers, C. P. (1985) Arch. Int. Pharmacodyn. Ther. 275: 180–188

Mehendale, H. M. (1976) Drug Metab. Dispos. 4: 124–134

Millburn, P., Smith, R. L., Williams, R. T. (1967) Biochem. J. 105: 1275–1281

Ostrow, J. D., Murphy, N. H. (1970) Ibid. 120: 311–327

Peterson, R. E., Fujimoto, J. M. (1973) J. Pharmacol. Exp. Ther. 184: 409–418

Roerig, D. L., Hasegawa, A. T., Peterson, R. E., Want, R. I. H. (1974) Biochem. Pharmacol. 23: 1331–1339

Smith, R. L. (1966) Prog. Drug Res. 9: 299–360

VanRoy, F. P., Heirwegh, K. P. M. (1968) Biochem. J. 107: 507–518

Vessey, D. A., Zakim, D. (1978) in: Drug Biotransformation, (eds) Elsevier/North-Holland, Biomedical Press, Amsterdam, pp 247–255

Watkins, J. B., Klaassen, C. D. (1982a) J. Pharmacol. Exp. Ther. 220: 305–310

Watkins, J. B., Klaassen, C. D. (1982b) J. Pharmacol. Methods 7: 145–151

Watkins, J. B., Klaassen, C. D. (1983) Drug Metab. Dispos. 11: 37–40

Winsnes, A. (1969) Biochim. Biophys. Acta 191: 279–291