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Glucuronide Conjugates of 4-Aminobiphenyl and Its *N*-Hydroxy Metabolites

pH Stability and Synthesis by Human and Dog Liver

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ABSTRACT. Glucuronide conjugates of arylamines are thought to be important in the carcinogenic process. This study investigated the pH stability and synthesis of glucuronide conjugates of 4-aminobiphenyl and its *N*-hydroxy metabolites by human and dog liver. Both dog and human liver slices incubated with 0.06 mM [^{3}H]-4-aminobiphenyl produced the *N*-glucuronide of 4-aminobiphenyl as the major product. After 2 hr of incubation, the *N*-glucuronide of 4-aminobiphenyl represented 52 and 27% of the total radioactivity recovered by HPLC in dog and human, respectively. When 4-aminobiphenyl, *N*-hydroxy-4-aminobiphenyl, or *N*-hydroxy-*N*-acetyl-4-aminobiphenyl was added to human microsomes containing [^{14}C]UDP-glucuronic acid, a new product peak was detected by HPLC. At 0.5 mM, the rate of glucuronidation was *N*-hydroxy-*N*-acetyl-4-aminobiphenyl > *N*-hydroxy-4-aminobiphenyl > 4-aminobiphenyl. The rate of formation of the *N*-glucuronide of 4-aminobiphenyl was similar to that observed with benzidine and *N*-acetylbenzidine. The glucuronides of 4-aminobiphenyl and *N*-hydroxy-4-aminobiphenyl were both acid labile with $T_{1/2}$ values of 10.5 and 32 min, respectively, at pH 5.5. The glucuronide of *N*-hydroxy-*N*-acetyl-4-aminobiphenyl was not acid labile with $T_{1/2}$ values at pH 5.5 and 7.4 of 55 and 68 min, respectively. The glucuronide of 4-aminobiphenyl was the most acid labile conjugate examined. Thus, the glucuronide of 4-aminobiphenyl is a major product of dog and human liver slice metabolism and likely to play an important role in the carcinogenic process. BIOCHEM PHARMACOL 51;12:1679–1685, 1996.

KEY WORDS. 4-aminobiphenyl; *N*-hydroxy-4-aminobiphenyl; *N*-hydroxy-*N*-acetyl-4-aminobiphenyl; glucuronidation; *N*-glucuronides; acid lability; liver slices

4-Aminobiphenyl is an aromatic amine that causes bladder cancer in humans and dogs [1–3]. Exposure to this arylamine occurs in the chemical, dye, and rubber industries and from cigarette smoke [1, 4, 5]. Tobacco smoking causes a 2- to 3-fold increase in the relative risk of developing bladder cancer [6] with the presence of 4-aminobiphenyl in smoke thought to be a contributing factor.

Cytochrome P450 1A2 oxidizes 4-aminobiphenyl to *N*-hydroxy-4-aminobiphenyl [7, 8]. Following *O*-acetylation, the latter can form DNA adducts [9]. *O*-Acetylation reactions are catalyzed by (NAT)† enzymes. Two separate enzymes can catalyze this reaction, NAT1 and NAT2 [10, 11]. These enzymes can also *N*-acetylate 4-aminobiphenyl. Because *N*-acetylated products are difficult to oxidize, acetylation is considered a detoxification step for aromatic amines [12]. Slow and rapid acetylator phenotypes are present in humans with the slow phenotype genetically determined by the presence of two nonfunctional alleles of the *NAT2* gene [13, 14]. Slow acetylators would be expected to generate higher levels of *N*-hydroxy products than rapid acetylators [12]. This is consistent with the slow acetylator phenotype being associated with an increased formation of macromolecular adducts by arylmonoamines [15–17] and with a higher incidence of bladder cancer [18, 19].

Glucuronidation represents a major pathway for metabolizing carcinogenic aromatic amines [20, 21]. UGTs consist of isozymes in two distinct gene families that metabolize many endogenous and xenobiotic compounds. Polymorphism in humans may depend upon age, genetic incidence, and exposure to inducers (i.e. phenobarbital and tobacco). A specific human UGT catalyzes the formation of the *N*-glucuronide of 4-aminobiphenyl [22]. Because glucuronidation results in inactivation and excretion, *N*-glucuronidation competes with *N*-oxidation. While *N*-glucuronides of primary arylamines and *N*-OH-arylamines are found [20], arylamides are *not* *N*-glucuronidated [23]. Therefore, *N*-glucuronidation and *N*-acetylation are also competing pathways.

4-Aminobiphenyl is proposed to initiate bladder cancer

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† *Abbreviations*: NAT, *N*-acetyltransferase; and UGT, UDP-glucuronosyltransferase.

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by a mechanism involving hepatic *N*-oxidation and subsequent *N*-glucuronidation [24, 25]. This *N*-hydroxy arylamine *N*-glucuronide conjugate is thought to be excreted from the liver and to accumulate in urine within the lumen of the bladder. The acidic pH of urine then hydrolyzes this glucuronide conjugate to its *N*-hydroxy arylamine, which can be further metabolized by the bladder. While many studies have demonstrated acid lability of these glucuronides [26, 27], including those of *N*-hydroxy-4-aminobiphenyl [24, 27], their acid stability has not been evaluated quantitatively. Studies in the dog demonstrated that 70% of *N*-hydroxy-4-aminobiphenyl reaches the bladder unconjugated, while 30% is *N*-glucuronidated [25]. Although similar studies were not done in humans, these experiments establish that a significant amount of *N*-glucuronide metabolite forms. To further understand the role of glucuronidation in 4-aminobiphenyl-induced bladder cancer, this study investigated the pH stability and synthesis of glucuronide conjugates of 4-aminobiphenyl and its *N*-hydroxy metabolites by human and dog liver.

MATERIALS AND METHODS

[^{3}H]-4-Aminobiphenyl (110 mCi/mmol) and [^{14}C]-UDP-glucuronic acid (279 mCi/mmol) were purchased from Chemsyn (Lenexa, KS) and ICN (Irvine, CA), respectively. 4-Aminobiphenyl, UDP-glucuronic acid, paraoxon, ascorbic acid, and β-glucuronidase (*Escherichia coli*, Type VII-A) were purchased from the Sigma Chemical Co. (St. Louis, MO). *N*-Hydroxy-*N*-acetyl-4-aminobiphenyl was purchased from the Midwest Research Institute (Kansas City, MO). *N*-Acetyl-4-aminobiphenyl was prepared by acetylation of 4-aminobiphenyl with acetic anhydride as previously described [28]. *N*-Hydroxy-4-aminobiphenyl was synthesized by the reduction of 4-nitrobiphenyl (Sigma) using a modification of a published procedure [29]. The latter was accomplished by Dr. Shu Wen Li (Department of Biochemistry, St. Louis University Medical School, St. Louis, MO). The identity and purity (>95%) of the synthesized compounds were established by TLC, NMR and MS. Human tissue studies were approved by the St. Louis University Medical School Institutional Review Board. Tissues were obtained from the Department of Surgery at St. Louis University Medical School or from the Cooperative Human Tissue Network. Microsomes were prepared from frozen tissues.

To assess intact liver metabolism of [^{3}H]-4-aminobiphenyl, slices (0.1 to 0.2 g) were placed in 20 mL plastic scintillation vials containing 1 mL of α-modified Eagle's medium at 37° and gassed with 5% CO_2/95% O_2 [23, 30, 31]. After 1 hr, the samples were regassed. After the incubation, 1 N NaOH was added to raise the pH above 8.5, along with 2 mL of ice-cold ethanol. The samples were sonicated for 10 sec and centrifuged, and supernatants were frozen at −70° for HPLC analysis. The radioactivity in the supernatant and pellet represented 94 and 6% of total recovered radioactivity, respectively. The HPLC system consisted of a Beckman 5 μm, 4.6 × 150 mm C-18 ultrasphere column attached to a guard column. The mobile phase for HPLC analysis started with 0.02 M potassium phosphate buffer (pH 6.8) and 25% methanol (v/v) (0–2 min), 25–35% linear gradient (2–9 min), 35–90% linear gradient (9–25 min), and 90% methanol (25–30 min) at a flow rate of 1 mL/min. Radioactivity in HPLC eluents was measured using a FLO-ONE radioanalytical detector. Chemical and enzymatic-prepared compounds were used as standards for HPLC analysis. Data were expressed as a percentage of the total amount of radioactivity recovered by HPLC.

Hepatic microsomal glucuronidation of 4-aminobiphenyl and its *N*-hydroxy metabolites was assessed. Microsomes were incubated in 100 mM potassium phosphate buffer (pH 7.4), containing 20 mM $MgCl_2$, 2 mM ascorbic acid, 0.1 mM paraoxon, 0.024 mM [^{14}C]-UDP-glucuronic acid, and 0.5 mM aromatic amine (final volume 0.1 mL) for 60 min at 37° [30]. The reaction was stopped by raising the pH to ≥8.5 with 1 N NaOH and addition of 0.1 mL methanol. Supernatant samples were analyzed by HPLC using the solvent system described below for the stability studies. To achieve maximum transferase activity, microsomes were pretreated with the detergent Emulgen 911 (0.5 mg/mg protein) for 60 min at 4° with continuous stirring [23]. Inclusion of 10 mM D-saccharic acid, 1,4-lactone, an inhibitor of β-glucuronidase, did not affect rates of metabolism. In some incubations, non-labeled UDP-glucuronic acid at 3.6 mM was used instead of [^{14}C]-UDP-glucuronic acid. The glucuronide of 4-aminobiphenyl was also prepared chemically. An ethanol/aqueous reaction mixture, containing 5 mM 4-aminobiphenyl and 200 mM D-glucuronic acid, was incubated at room temperature for 4 hr [23].

To determine the stability of glucuronide conjugates, ^{14}C-labeled glucuronides were prepared enzymatically using human microsomal incubations as described above. Reaction mixtures were applied directly to C-18 Bakerbond spe (1000 mg, 6 mL) extraction columns. Following a water wash, the glucuronides were eluted with 100% methanol. These partially purified ^{14}C-labeled glucuronides were dissolved in phosphate buffer at pH 5.5 or 7.4 with 1 mM ascorbic acid and incubated at 37° [30]. Incubations were stopped by raising the pH to >8.5 with 1 N NaOH. Samples were frozen at −70° and analyzed by HPLC. A Beckman HPLC system, consisting of a 5 μm, 4.6 × 150 mm C-18 ultrasphere column attached to a guard column, was used. Glucuronides were separated using the following solvent system: 25% methanol; 75% 0.02 M potassium phosphate buffer (pH 6.8), 0–2 min; 25–35% linear gradient, 2–7 min; 35–95% linear gradient, 22–32 min; 95% methanol, 32–37 min; flow rate, 1 mL/min. Radioactivity in the eluent was measured with a FLO-ONE Radioanalytical Detector. Incubation times overlapped the estimated $T_{1/2}$ with 5–6 time points assessed for each experiment [30]. The percent of radioactivity remaining as glucuronide conjugate was plotted versus time, and the $T_{1/2}$ was calculated.

To identify glucuronide conjugates, chemical and enzy-

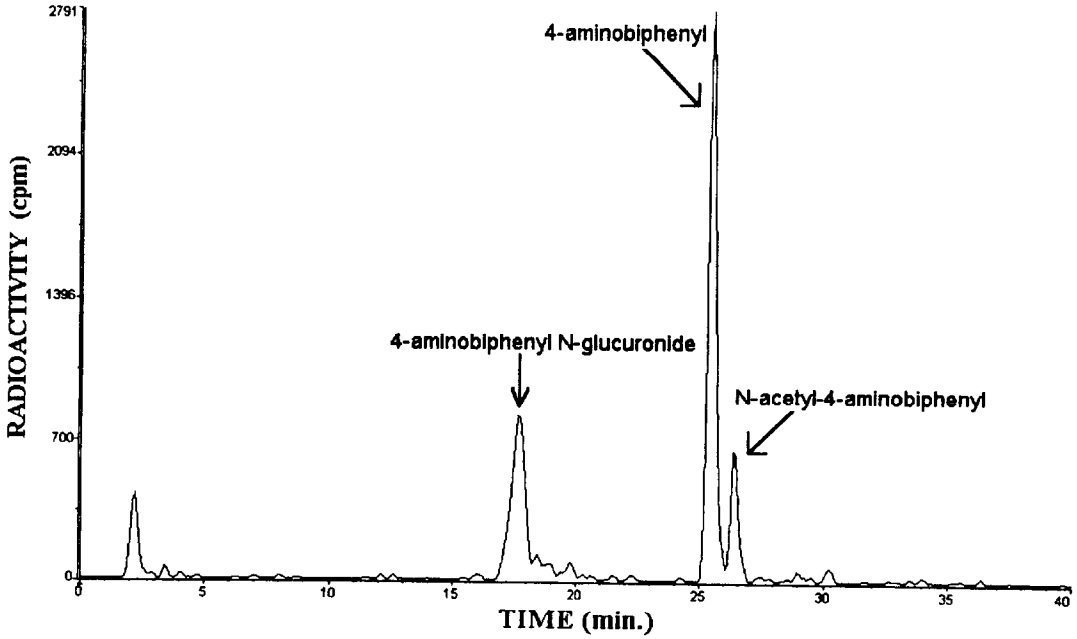

FIG. 1. HPLC profile [^{3}H]-4-aminobiphenyl metabolites produced by human liver slices. Human slices were incubated with 0.06 mM [^{3}H]-4-aminobiphenyl for 2 hr.

matic prepared samples were purified by C-18 Bakerbond spe (1000 mg, 6 mL) extraction columns. The identity of the glucuronide conjugates of 4-aminobiphenyl and *N*-hydroxy-4-aminobiphenyl was confirmed by acid hydrolysis to the amine and hydroxylamine, respectively. The glucuronide of *N*-hydroxy-*N*-acetyl-4-aminobiphenyl was confirmed by β-glucuronidase hydrolysis [32]. The $T_{1/2}$ values for the ^{14}C-labeled and unlabeled glucuronides were similar.

RESULTS

Human and dog liver slices were used to assess the relative importance of glucuronide formation in 4-aminobiphenyl metabolism. Using 0.06 mM [^{3}H]-4-aminobiphenyl, dog liver slices produced one major peak eluting at 18 min. After 1 hr of incubation, the peaks eluting at 18 and 25 min represented 37 and 31%, respectively, of the total radioactivity recovered by HPLC. Authentic standards of the *N*-glucuronides of both 4-aminobiphenyl and *N*-hydroxy-4-aminobiphenyl elute at 18 min. To determine the identify of the 18-min peak, samples were hydrolyzed during a 30-min (pH 5.5) incubation at 37°. This acid treatment completely eliminated the 18-min peak with corresponding radioactivity now eluting at 25 min. Because 4-aminobiphenyl and *N*-hydroxy-4-aminobiphenyl elute at 25 and 23 min, respectively, the *N*-glucuronide made by dog liver slices appears to be that of 4-aminobiphenyl. In two dogs, the average amounts of 4-aminobiphenyl *N*-glucuronide formed at 1 and 2 hr of incubation were 36 and 52%, respectively, of the total radioactivity recovered by HPLC. These results are consistent with the *N*-glucuronide of 4-aminobiphenyl being a major metabolite of dog liver.

Human liver slices also produced 4-aminobiphenyl *N*-glucuronide (Fig. 1). The peak eluting at 18 min represented 27% of the total radioactivity recovered by HPLC and was hydrolyzed completely by acid treatment. 4-Aminobiphenyl (25 min) and *N*-acetyl-4-aminobiphenyl (26 min) represented 49 and 11%, respectively, of the total radioactivity (Fig. 1). Thus, the *N*-glucuronide of 4-aminobiphenyl is a major metabolite formed during human liver slice incubations.

Human liver microsomal formation of the *N*-glucuronides of 4-aminobiphenyl and its *N*-hydroxy metabolites was assessed (Table 1). Detergent Emulgen 911 pretreatment was shown previously to increase the synthesis of *N*-glucuronides of benzidine and *N*-acetylbenzidine [23, 30]. In each liver, the amount of glucuronide formed was the following: *N*-hydroxy-*N*-acetyl-4-aminobiphenyl > *N*-hydroxy-4-aminobiphenyl > 4-aminobiphenyl. The rate of formation of the *N*-glucuronide of 4-aminobiphenyl was similar to that observed with benzidine and *N*-acetylbenzidine. The microsomal synthesized *N*-glucuronide of 4-aminobiphenyl had an HPLC elution profile identical to that of the slice-derived material.

The stability of ^{14}C-labeled glucuronide conjugates of 4-aminobiphenyl and its *N*-hydroxy metabolites was assessed (Table 2). The glucuronides of 4-aminobiphenyl and *N*-hydroxy-4-aminobiphenyl were both acid labile. At pH 5.5, the glucuronides of 4-aminobiphenyl and *N*-hydroxy-4-aminobiphenyl had $T_{1/2}$ values of 10.5 and 32 min, respectively. At pH 7.4, these $T_{1/2}$ values increased to 185 and 210 min, respectively. The glucuronide of *N*-hydroxy-*N*-acetyl-4-aminobiphenyl was not acid labile. The $T_{1/2}$ values at pH 5.5 and 7.4 were 55 and 68 min, respectively. Similar $T_{1/2}$ values were obtained when non-radiolabeled

TABLE 1. Glucuronidation of 4-aminobiphenyl, *N*-hydroxyl-4-aminobiphenyl, and *N*-hydroxy-*N*-acetyl-4-aminobiphenyl by human liver microsomes

Sample No.	BZ	ABZ	ABP	NABP	NAABP
	(% of radiactivity recovered)				
A	6	2.3	4.5	17	45
B	10	8	11	28	48

Microsomes were pretreated with Emulgen 911 (0.5 mg/mg protein) and incubated for 1 hr at 37°. Relative activity was expressed as the percent of total radioactivity recovered by HPLC. All amine substrates were 0.5 mM with [^{14}C]-UDP-glucuronic acid at 0.024 mM. Values represent the averages of duplicate determinations from a representative experiment. Abbreviations: BZ, benzidine; ABZ, *N*-acetylbenzidine; ABP, 4-aminobiphenyl; NABP, *N*-hydroxy-4-aminobiphenyl; and NAABP, *N*-hydroxy-*N*-acetyl-4-aminobiphenyl.

glucuronides of 4-aminobiphenyl and its *N*-hydroxy metabolites were analyzed. Thus, the glucuronide conjugate of 4-aminobiphenyl was much less stable at acid pH than its *N*-hydroxy metabolites. At pH 7.4, the glucuronide of *N*-hydroxy-*N*-acetyl-4-aminobiphenyl was the least stable.

DISCUSSION

This is the first study to assess quantitatively the acid stability of the glucuronide conjugates of 4-aminobiphenyl and its *N*-hydroxy metabolites and to evaluate the metabolism of 4-aminobiphenyl by dog and human liver slices. The *N*-glucuronide of 4-aminobiphenyl was the major metabolite observed during dog and human liver slice metabolism. Neither *N*-hydroxy-4-aminobiphenyl nor its glucuronide was detected. *N*-Hydroxy-4-aminobiphenyl formation should reflect the amount of cytochrome P450 1A2 in liver [7, 8]. This parameter was not measured in the present study. In a previous study with dog, only low levels of *N*-hydroxy-4-aminobiphenyl were observed. Following a p.o. dose of [^{3}H]-4-aminobiphenyl [25], dog urine contained only 1% of the dose as *N*-hydroxy-4-aminobiphenyl or its glucuronide. While it is difficult to compare the results from *in vivo* experiments [25] with slice incubations, both protocols detected significant amounts of the glucuronide of 4-aminobiphenyl in dog. Our results would suggest that the glucuronide of 4-aminobiphenyl is a major metabolite in dog and human.

A significant amount of *N*-hydroxy-4-aminobiphenyl appears to be present in its unconjugated form. Oxidation of the latter compound to nitrosobiphenyl in red cells causes it to react and form a covalent bond with the cysteine residue at position 93 of hemoglobin [33, 34]. This stable sulfinamide adduct is used as a marker of exposure to 4-aminobiphenyl and was reported to be higher in cigarette smokers than non-smokers [34, 35]. As much as 5% of the dose of 4-aminobiphenyl administered to rats form this adduct [33], suggesting a significant amount of unconjugated *N*-hydroxy-4-aminobiphenyl. Studies in dog have demonstrated that 70% of *N*-hydroxy-4-aminobiphenyl reached the bladder in the unconjugated form [25]. Similar urinary measurements have not been made in humans.

Human microsomes elicited significant formation of glucuronides of 4-aminobiphenyl and *N*-hydroxy-4-aminobiphenyl. Formation of the glucuronide of *N*-hydroxy-4-aminobiphenyl exceeded that of 4-aminobiphenyl. However, the glucuronidation of 4-aminobiphenyl was similar to that observed for benzidine and *N*-acetylbenzidine. Previous studies have demonstrated significant glucuronidation of benzidine by humans and dogs [31, 36] and *N*-acetylbenzidine by humans [37]. For benzidine, the K_m and V_{max} values in dog are 0.142 ± 0.006 mM and 0.65 ± 0.1 nmol/mg protein/min, respectively [31], and in human 0.8 ± 0.06 mM and 4.2 ± 0.7 nmol/mg protein/min, respectively [36]. Similar parameters were not evaluated for 4-aminobiphenyl and its *N*-hydroxy metabolites.

Previous studies have assessed UGT metabolism of 4-aminobiphenyl and *N*-hydroxy-4-aminobiphenyl [38–40]. These studies used human and rat liver microsomes as well as cells expressing the human and rat *UGT1* gene complex (UGT1.6 and UGT1.7). With human microsomes and human UGT1.7 [40], the rates of metabolism of *N*-hydroxy-4-aminobiphenyl and 4-aminobiphenyl were similar. With rat microsomes [38, 40] and with rat or human UGT1.6 [40], the rate of metabolism of *N*-hydroxy-4-aminobiphenyl exceeded 4-aminobiphenyl. In these studies, the concentration of UDP-glucuronic acid was saturating, while our experiments used a limiting concentration of [^{14}C]-UDP-glucuronic acid. Similar results were observed in our study with the rate of glucuronidation of *N*-hydroxy-4-aminobiphenyl greater than that of 4-aminobiphenyl (Table 1). Thus, our results with human microsomes are consistent with those reported in other studies.

N-Hydroxy-*N*-acetyl-4-aminobiphenyl can be activated by *N*,*O*-acetyltransferases to yield *N*-acetoxy-4-aminobiphenyl [41]. The latter can react with DNA and form an adduct. The possible glucuronidation and detoxification of *N*-hydroxy-*N*-acetyl-4-aminobiphenyl have been evaluated. Results with human microsomes suggest that this metabolite is glucuronidated more readily than either 4-ami-

TABLE 2. Stability of glucuronide conjugates of 4-aminobiphenyl and its *N*-hydroxy metabolites

Compound	$T_{1/2}$ (min)
4-Aminobiphenyl	
pH 5.5	10.5
pH 7.4	185
N-Hydroxy-4-aminobiphenyl	
pH 5.5	32
pH 7.4	210
N-Hydroxy-*N*-acetyl-4-aminobiphenyl	
pH 5.5	55
pH 7.4	68

[^{14}C]-Glucuronide conjugates of 4-aminobiphenyl and its *N*-hydroxy metabolite (3–5 μM) were incubated at the indicated pHs in phosphate buffer with 1 mM ascorbic acid and analyzed by HPLC. Data were plotted as percent remaining versus time, and the $T_{1/2}$ values were estimated. Two to four experiments were conducted for the calculations of each $T_{1/2}$.

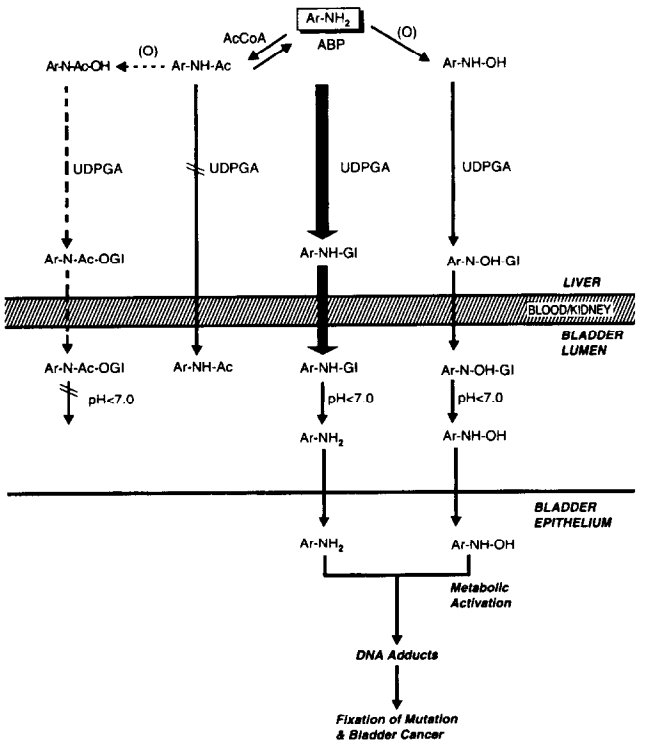

FIG. 2. Model depicting the role of glucuronidation, acetylation, and oxidation pathways in 4-aminobiphenyl human bladder carcinogenesis. 4-Aminobiphenyl (Ar-NH_2, ABP) is metabolized by oxidation (O), *N*-glucuronidation using UDP-glucuronic acid (UDPGA) as cosubstrate, or *N*-acetylation using acetyl CoA (AcCoA) as cosubstrate. *N*-Acetyl-4-aminobiphenyl (Ar-NH-Ac) is not glucuronidated, but can be oxidized, to a limited extent, to *N*-hydroxy-*N*-acetyl-4-aminobiphenyl (Ar-N-Ac-OH). The latter can be metabolized to an *O*-glucuronide (Ar-N-Ac-OGl), which is not acid labile. Glucuronidation, acetylation, and oxidation are competing pathways in 4-aminobiphenyl metabolism. Hepatic glucuronidation results in detoxification and excretion. *N*-Glucuronides of 4-aminobiphenyl (Ar-NH-Gl) and *N*-hydroxy-4-aminobiphenyl (Ar-N-OH-Gl) can be hydrolyzed by acidic urine to their corresponding arylamines, which can then enter the bladder and undergo further metabolism by peroxidation and/or *O*-acetylation to form DNA adducts [9, 45, 47].

nobiphenyl or *N*-hydroxy-4-aminobiphenyl. The hydrolysis of this glucuronide by β-glucuronidase is consistent with it being an *O*-glucuronide rather than an *N*-glucuronide. Because 4-aminobiphenyl and *N*-Hydroxy-4-aminobiphenyl form *N*-glucuronides [32], a different UGT may be metabolizing *N*-hydroxy-*N*-acetyl-4-aminobiphenyl than 4-aminobiphenyl or *N*-hydroxy-4-aminobiphenyl. Compared to the other two glucuronides, the glucuronide of *N*-hydroxy-*N*-acetyl-4-aminobiphenyl was about 2–3 times more labile at neutral pH (Table 2). This is consistent with previous studies demonstrating base lability of *O*-glucuronides [42].

Neither *N*-hydroxy-*N*-acetyl-4-aminobiphenyl nor its glucuronide was formed during our slice incubations. Because dog is a non-acetylator [43, 44], *N*-acetyl-4-aminobiphenyl was not formed, and, thus, its oxidation product would not be produced. However, while human slices made *N*-acetyl-4-aminobiphenyl, its oxidation product was still not detected. The lack of formation of *N*-hydroxy-*N*-acetyl-4-aminobiphenyl by human slices may be due to the difficulty in oxidizing an amide, like *N*-acetyl-4-aminobiphenyl [12]. This is consistent with a recent rat hepatocyte study [32]. Rats with increased levels of cytochrome P450 1A2, induced by 3-methylcholanthrene, did not produce *N*-hydroxy-*N*-acetyl-4-aminobiphenyl. Thus, *N*-acetyl-4-aminobiphenyl may be a detoxification product of 4-aminobiphenyl metabolism.

The glucuronide of 4-aminobiphenyl was the most acid labile conjugate examined. This glucuronide was 3–5 times more acid labile than the glucuronides for *N*-hydroxy-4-aminobiphenyl and *N*-hydroxy-*N*-acetyl-4-aminobiphenyl (Table 2). The glucuronide of 4-aminobiphenyl would be hydrolyzed readily by acidic urine to yield 4-aminobiphenyl. Because this glucuronide is a major product of liver slice metabolism, hepatic glucuronidation, glucuronide excretion, and acidic urine hydrolysis provide a mechanism for delivering carcinogenic 4-aminobiphenyl to bladder epithelium (Fig. 2). To initiate carcinogenesis, 4-aminobiphenyl would have to be metabolized further and activated by the epithelium. Bladder epithelium contains peroxidase and *O*-acetyltransferase enzyme activities capable of initiating carcinogenesis [45, 46].

This study provides additional support for and extends the current hypothesis [24] (Fig. 2). *N*-Glucuronide conjugates of *N*-hydroxy arylamines are hypothesized to accumulate in the bladder and be hydrolyzed by acidic urine [24, 25]. This study has demonstrated quantitatively that the glucuronide of *N*-hydroxy-4-aminobiphenyl is acid labile. Thus, *N*-hydroxy-4-aminobiphenyl would be formed from its *N*-glucuronide in acidic urine. *N*-Hydroxy-4-aminobiphenyl would probably require further metabolism by bladder *O*-acetyltransferases before binding DNA [9, 47]. The *N*-glucuronide of 4-aminobiphenyl is a major metabolite of dog and human liver slices (Fig. 1). Previous studies with dog and human liver [23, 30, 36, 37] have also demonstrated that benzidine and its monoacetylated metabolite form *N*-glucuronides that are acid labile and capable of accumulating in bladder, as illustrated for 4-aminobiphenyl in Fig. 2. Thus, our results extend the current hypothesis by demonstrating that primary arylamines, like 4-aminobiphenyl and benzidine, may also undergo hepatic detoxification and accumulation in acidic urine (Fig. 2). *N*-Glucuronides of carcinogenic arylamines may play a more important role in carcinogenesis than previously hypothesized.

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