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SHORT COMMUNICATIONS

Sulphation of *N*-hydroxy-4-aminobiphenyl and *N*-hydroxy-4-acetylaminobiphenyl by human foetal and neonatal sulphotransferase

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Abstract—Sulphation of the genotoxic compounds *N*-hydroxy-4-aminobiphenyl (N-OH-4ABP) and *N*-hydroxy-4-acetylaminobiphenyl (N-OH-4AABP) was determined in cytosolic preparations of human foetal, neonatal and adult liver and foetal and neonatal adrenal gland. Sulphotransferase (ST) activity capable of sulphating these compounds was present in foetal liver and adrenal gland by 14 weeks of gestation. Sulphation of N-OH-4ABP was higher in foetal and neonatal adrenal cytosol than was sulphation of N-OH-4AABP and in general, N-OH-4ABP ST activity was also greater than that towards 1-naphthol. In foetal and neonatal liver cytosol the sulphation of N-OH-4ABP was also higher than that of N-OH-4AABP (approximately 2-fold). In adult liver cytosols, however, N-OH-4AABP ST activity was higher than that for N-OH-4ABP and 1-naphthol sulphation. Aromatic hydroxylamines and hydroxamic acids are known to be converted by sulphotransferase into reactive, electrophilic compounds capable of reacting with DNA. Our data show that the human foetus and neonate have the capacity to sulphate these compounds and thus is able to produce the reactive mutagenic metabolites. Therefore, this class of genotoxic compounds may be bioactivated by humans during development—a time when they are most vulnerable to the effects of genotoxins.

Key words: carcinogens; bioactivation; liver; adrenal

Conjugation with sulphate is a key step in the detoxication of a large number of xenobiotics and endogenous compounds [1]. However, certain xenobiotics (e.g. hydroxamic acids [2–4], hydroxylamines [5] and benzylic alcohols [6–8]) generate reactive intermediates as a result of sulphate conjugation, which may covalently bind to cellular macromolecules. These sulphation reactions are catalysed by a family of STs*, located in the cytosol of most tissues. Thus far, STs metabolizing dehydroepiandrosterone, phenols, estrogens and monoamines have been purified, from adult human liver [9–11], brain [12] and platelets [13–15]. UDP-glucuronosyltransferases are expressed at low levels in the foetus compared to neonate and adult [16, 17], therefore sulphate conjugation may represent the main conjugation pathway in the foetus and we and others have demonstrated the presence of xenobiotic and endogenous STs in various human foetal tissues [e.g. 18–23]. To date, limited data have been presented on the sulphation of genotoxic hydroxylamines and hydroxamic acids in human tissues, however, adult human liver and placenta [24–26] and 30 week old foetal liver [26] are known to possess ST activity towards hydroxamic acids and hydroxylamines. Since we are exposed to these compounds or their precursors as a result of occupational contact, air pollution [27] and through the diet [28] it is important to know the capacity of the developing human to bioactivate such chemicals during a period where susceptibility to genotoxic compounds is greatest. We have therefore examined the sulphation of two model compounds, the hydroxylamine N-OH-4ABP and the hydroxamic acid N-OH-4AABP by human foetal and neonatal liver and adrenal cytosol preparations. The data presented demonstrate that foetal liver and adrenal have significant capacity for the sulphation of these compounds.

Materials and Methods

Chemicals. N-OH-4AABP was synthesized as described previously [29]. The corresponding hydroxylamine, N-OH-4ABP was synthesized immediately prior to use [29]. Purity was assessed as described by Kadlubar *et al.* [5] and was 90–95%. PAP was purchased from the Sigma Chemical Co. (Poole, U.K.) and PAPS was obtained from Pharmacia, (Milton Keynes, U.K.). 1-Naphthol was from Merck Ltd (Glasgow, U.K.). HPLC instrumentation (Beckman System Gold) was from Beckman (Palo Alta, CA, U.S.A.); HPLC column and precolumn (Nucleosil 120-5C18) were purchased from Machery-Nagel (Düren, Germany). All other reagents were of analytical reagent grade and used without further purification.

Tissue samples. Foetal tissue was obtained following routine termination of pregnancy using Gemeprost® vaginal pessaries (May and Baker, Dagenham, U.K.). Foetal development was carefully estimated based on the size, including crown-heel, crown-rump and heel-toe measurement [30], maternal menstrual history and ultrasound dating of pregnancy. Normality of foetuses was confirmed by autopsy. Neonatal tissue was obtained *post mortem* and histologically normal adult liver tissue was obtained during surgical resection for liver cancer or from organ donors. The study was approved by the Ethics Committee of Tayside Health Board.

Preparation of cytosols. Tissues were frozen in liquid nitrogen, and stored at −70° until used to prepare cytosols, which were harvested from 20% (w/v) homogenates made in 10 mM triethanolamine/HCl (pH 7.4) containing 250 mM sucrose and 5 mM 2-mercaptoethanol. The homogenates were centrifuged at 10,000 *g* for 10 min and the resulting supernatant for 1 hr at 105,000 *g*. The 105,000 *g* supernatant, designated cytosol, was aspirated carefully avoiding the lipid layer at the surface and stored at −70° in 1.0 mL aliquots before use.

Sulphotransferase enzyme assays. Sulphation of N-OH-4ABP, N-OH-4AABP and 1-naphthol were measured using an HPLC method which quantitates the formation of PAP as described previously [31–33].

Protein determination. Protein content of cytosols was estimated using the method of Lowry *et al.* [34], with bovine serum albumin (Fraction V, Boehringer, Mannheim, Lewes, U.K.) as standard.

* Abbreviations: N-OH-4AABP, *N*-hydroxy-4-acetylaminobiphenyl; N-OH-4ABP, *N*-hydroxy-4-aminobiphenyl; PAP, adenosine-3′,5′-bisphosphate; PAPS, 3′-phosphoadenylylsulphate; ST, sulphotransferase.

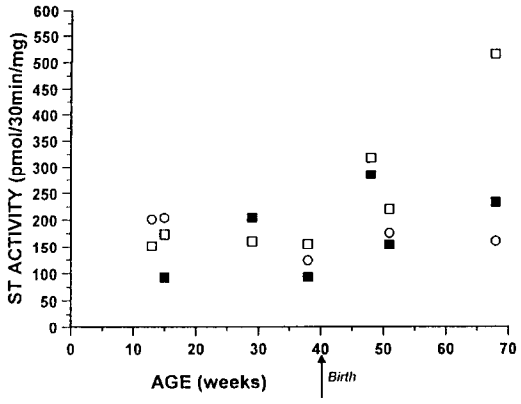

Fig. 1. Sulphation of N-OH-4-ABP, N-OH-4AAP and 1-naphthol in human foetal and neonatal liver. Liver cytosols were assayed for the ability to sulphate N-OH-4ABP (■), N-OH-4AABP (○) and 1-naphthol (□) as described in Materials and Methods. Assays were performed in duplicate.

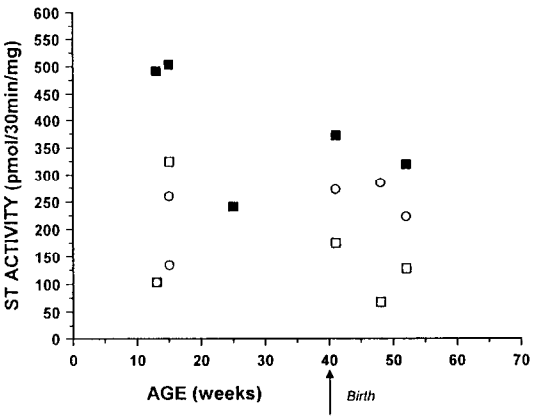

Fig. 2. Sulphation of N-OH-4-ABP, N-OH-4AAP and 1-naphthol in human foetal and neonatal adrenal. Adrenal cytosols were assayed for the ability to sulphate N-OH-4ABP (■), N-OH-4AABP (○) and 1-naphthol (□) as described in Materials and Methods. Assays were performed in duplicate.

Results

Sulphotransferases assay. Linearity of ST activity with respect to incubation time and cytosolic protein from human adult liver cytosol was tested with 0.2 mM N-OH-4AABP and 0.2 mM N-OH-4ABP. A linear relationship was observed between ST activity and time from 0 to 45 min and between ST activity protein concentration from 3.5 to 15 mg/mL of cytosolic protein at 37° (not shown). Therefore, determination of ST activity was carried out using an incubation time of 30 min and a protein concentration of approximately 5 mg/mL.

Rat liver AST IV catalyses three different reactions [35]: a sulphation reaction, an "exchange" reaction and the hydrolysis of the co-substrate PAPS. Similarly, a considerable PAPS hydrolysis by human ST preparations was observed in this study (10.1 ± 1.9 pmol PAP formed/min/mg protein, N = 6 adult human livers). A similar PAPS hydrolysis was found in cytosols of other organs and therefore all ST activities were corrected for this enzymatic PAPS hydrolysis. In cytosols where no ST activity was observed the PAPS hydrolysis was only 0.5–2.0 pmol PAP/min/mg protein.

Sulphation of N-OH-2AABP and N-OH-2ABP in human liver and adrenal cytosols during development. (a) *Liver.* ST activity towards these substrates was measured in human foetal, neonatal (Fig. 1) and in seven adult liver cytosols (Table 1). Sulphation of N-OH-4AABP, N-OH-4ABP and 1-naphthol occurred at very similar rates in the foetal and neonatal liver samples (Fig. 1), with a significant activity at the earliest age measured (approx. 15 weeks gestation). The highest ST activity in adult human liver preparations was towards N-OH-4AABP, where the sulphation of this compound was approximately twice than that for N-OH-4ABP and 3-fold higher than for 1-naphthol (Table 1). As would be expected, there was considerable inter-individual variation in the ST activities measured in adult liver samples.

(b) *Adrenal.* Foetal and neonatal adrenal cytosolic preparations (a total of 10 preparations) were used to study the sulphation of N-OH-4ABP, N-OH-4AABP and 1-naphthol (Fig. 2). With four samples, no ST activity was found at all for any of the substrates. In five of the other samples conversion of the hydroxylamine N-OH-4ABP to its sulphate ester was higher than that of N-OH-4AABP and 1-naphthol. Levels of ST activity were comparable to those observed with the foetal and neonatal liver samples in Fig. 1, although the activity towards N-OH-4ABP appeared to be greater in the foetal adrenal than in foetal liver.

Discussion

Sulphate conjugation is a major pathway in the biotransformation of aromatic hydroxylamines and hydroxamic acids and the liver plays an important role in the metabolism of these compounds. Sulphation of hydroxamic acids in the rat is sex and age dependent [32]; no such sex difference was observed for hydroxylamines. Hydroxamic acids are mainly sulphated by aryl sulphotransferase IV (AST IV), while most of the hydroxylamines are not

Table 1. Sulphotransferase activity towards N-OH-4ABP, N-OH-4AABP and 1-naphthol in adult human liver cytosols

Substrate	ST activity (pmol/30 min/mg)							
	HuLi3	HuLi4	HuLi5	HuLi6	HuLi7	HuLi8	L92G5	mean ± SEM
200 μM N-OH-4ABP	ND	165	180	186	129	ND	66	145 ± 50
200 μM N-OH-4AABP	264	231	255	435	237	123	162	244 ± 99
200 μM 1-Naphthol	81	57	24	66	105	57	162	79 ± 44

ND, not determined.

substrates for this enzyme [32]. Since AST IV is a liver-specific enzyme, sulphation of hydroxamic acids occurs in this organ only. Sulphation of N-OH-2AAF and N-OH-2AF was determined in human foetal and adult liver and placenta cytosols [24, 25, 36], and no sex dependency for ST activity towards N-OH-2AF and N-OH-2AAF was observed in adult human liver cytosols. In the present report we found that the human foetus and neonate has significant sulphotransferase activity towards N-OH-4ABP and N-OH-4AABP.

The human liver ST activities towards N-OH-4ABP and N-OH-4AABP were 50 to 100 times lower than in juvenile and adult rat liver cytosol [32]. Catalytic efficiency (V_{max}/K_m) of the partially purified human enzymes involved in the sulphation of N-OH-2AAPP (0.2 mM/min, Ref. 33) were very much lower than the catalytic efficiency of AST IV (110 mM/min, R. Gilissen and D. Ringer, unpublished results). This indicates why human hydroxamic acid and hydroxylamine ST activity is lower in cytosols.

Limited information is available on the developmental pattern of sulphotransferase expression in animals and human. In rats [37–39] the ontogeny of ST is substrate dependent and in general develops postnatally, whereas the presence of substantial ST activity in human foetal tissue has been described [18–22, 26]. We demonstrate here that N-OH-4ABP and N-OH-4AABP ST activities have developed by the 14th week of gestation, and were expressed in adrenal and liver cytosols—activity in foetal and neonatal kidney samples was in general very low or not detected (not shown). For human foetal, neonatal and adult liver cytosols the ST activities towards N-OH-4ABP and N-OH-4AABP were similar, indicating that no significant age-dependent expression of the ST activity towards these compounds occurs in humans and that the ST(s) converting these compounds are expressed continuously in the liver during human development. In foetal adrenal cytosols substantial ST activity towards N-OH-4ABP and N-OH-4AABP was observed, while with 59 and 120 weeks old neonatal cytosols no activity was measured. Since only a limited number of neonatal and no adult adrenal cytosols were used our results do not exclude an altered ST expression in adrenal tissue during development.

Our results show that in foetal adrenal tissue N-OH-4ABP ST activity is usually twice as high as N-OH-4AABP and 1-naphthol ST activities. In foetal liver, however, the sulphation of N-OH-4AABP and 1-naphthol was higher than for N-OH-4ABP. These data and those previously described [21] suggest that the sulphation of N-OH-4AABP and N-OH-4ABP in the foetal adrenal gland and liver may be catalysed by different STs. In adult liver, we have shown that two forms of P-PST and possibly DHEA ST are able to sulphate a range of hydroxylamines and hydroxamic acids, and it is thus known that multiple ST isozymes are involved in the metabolism of these compounds [36]. Elucidation of the specific isozymes of ST expressed in different human foetal tissues during development will finally determine the basis for the observations made in this report. This work is underway.

In conclusion, our results indicate that in humans ST activity towards hydroxylamines and hydroxamic acids develops prior to the 14th week of gestation. In the human tissues examined, the ST activity towards these aromatic hydroxylamines and hydroxamic acids remains constant during development. Since ST activity towards N-OH-4ABP and N-OH-4AABP is observed in foetal, neonatal, and adult human tissues and the resulting sulphate esters represent ultimate, reactive compounds capable of inducing DNA lesions, the presence of STs in developing human tissues indicates that bioactivation of these compounds can occur in the foetus—this may be particularly dangerous at a time when susceptibility to genotoxic compounds is enhanced.

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