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UDP-GLUCURONOSYLTRANSFERASE ACTIVITIES IN HUMAN LIVER MICROSOMES AND IN SOME LABORATORY ANIMAL SPECIES

Jean A. Boutin, Alain Jacquier°, Anne-Marie Batt,
Philippe Marlière° and Gérard Siest

Faculté des Sciences Pharmaceutiques et Biologiques, E.R.A. C.N.R.S. n° 698
and °Service de Médecine H, Hôpital Central, F - 54000 NANCY

Information about the activity of UDP-glucuronosyltransferase (EC 2.4.1.17, acceptor unspecific) in human liver is rather scarce, except as regards the glucuronidation of bilirubin. Some other aglycones have already been tested in adult human liver homogenates for example : 1-naphthol and morphine, 4-methylumbelliferone, testosterone and steroids [see Dutton (1) for references] and chenodeoxycholic acid (2).

The activity of this transferase towards xenobiotics in purified microsomal fractions from adult human liver is poorly documented. In particular no information is available on the comparative rate of glucuronidation *in vitro* of aglycones, whether as drugs or as drug metabolites. The present experiment was designed to gain information on the glucuronidation of four aglycones in human liver microsomes in the optimal experimental conditions. A second aim was to find a good animal model for the glucuronidation rate of substrates administered to humans.

Materials and Methods. The human liver samples (200 - 700 mg) were obtained through laparotomy and immediately frozen in liquid nitrogen. Pig livers were obtained from adult castrated male animals (Large White, 90 kg) obtained from the Centre d'Elevage de I.N.R.A. (Jouy-en-Josas, France). Male Sprague-Dawley rats (180 - 200 g) and male C57 Bl/6 Rholco mice (18 - 20 g) were purchased from Iffa-Credo (St-Germain-sur-l'Arbresle, France). Male Wistar rats (180 - 200 g) were from the Centre de Sélection des Animaux de laboratoire, C.N.R.S. (Orléans, France).

The microsomal fractions were obtained by conventional ultracentrifugation (3). The following aglycones were used : eugenol and *l*-borneol (Fluka, Buchs, Switzerland), chloramphenicol (Boehringer Mannheim, Meylan, France), 4-hydroxyphenobarbital (Aldrich Chem. Co., Beerse, Belgium), and 4-nitrophenol (Merck, Darmstadt, Germany), and the glucuronidation rates were estimated by the Mulder and Van Doorn's kinetic procedure (4) after the detergent requirement for optimal activation had been determined with six different Triton X-100 / protein ratios. Appropriate control experiments without UDP-glucuronate were performed. The following concentrations were used : UDP-glucuronate (Boehringer Mannheim, Meylan, France), 4.5 mM ; aglycone, 0.5 mM ;

75 - 125 µg protein per assay in a final volume of 500 µl. The other components were as described by Mulder and Van Doorn (4). The reaction was followed at 37°C at 340 nm on a Beckman spectrophotometer or on a Cobas-Biocentrifuge analyser (Roche Inst.). For comparison, Frei's colorimetric procedure (5) was also used, with 0.14 mM 4-nitrophenol and 1.5 mM UDP-glucuronate. The protein content was determined according to the method of Lowry *et al.* (6) with serum bovine albumin as a standard.

Results and Discussion. Mulder and Van Doorn's procedure (4) must be used with care, to assess interfering reactions, notably any spontaneous oxidation of nicotinamide adenine dinucleotide (NADH), which is checked for, with a blank assay containing no UDP-glucuronate. Different authors [see Dutton (1) for references] recently reviewed the possible drawbacks of the method. We ourselves observed that Triton X-100 inhibited the activity of UDP-pyrophosphatase (EC 2.7.7.9) (results not shown). The method is useless without detergent in the medium because of the low activity of the transferase and the possible interfering enzymes activities present in microsomal fractions. The Triton X-100 concentration has to be strictly controlled in each assay and for each animal species.

The glucuronidation of five substrates in human liver microsomes is reported in Table 1. The activities decreased in the following order : eugenol, 4-nitrophenol, *l*-borneol, 4-hydroxyphenobarbital, chloramphenicol. Human hepatic drug-metabolizing enzymes and marker enzymes have been poorly documented in published reports owing to the difficulty of obtaining appropriate samples and the extremely limited amount of tissue available. Peters and Seymour (7) only described α-glucuronidase as a possible marker enzyme in the endoplasmic reticulum. Von Bahr *et al.* (8) reported data concerning cytochrome P-450 and related hydroxylations and epoxidations, but did not refer to UDP-glucuronosyltransferase.

Table 1. UDP-glucuronosyltransferase activity in liver microsomes from seven human control patients

Patients			Microsomal protein ($mg.g^{-1}$ liver)	Glucuronidation ($nmol.min^{-1}.mg^{-1}$ protein)				
	Sex	Age (yrs)		Eugenol	*l*-borneol	4-hydroxyphenobarbital	Chloramphenicol	4-Nitrophenol*
A	F	79	38.0	22.5	4.8	2.0	1.0	5.1
B	F	56	39.8	21.4	4.8	1.6	0.9	3.7
C	M	45	35.3	23.6	4.5	2.1	1.1	-
D	M	74	27.3	22.2	3.8	2.4	-	-
E	M	67	25.3	-	-	2.8	1.3	3.9
F	M	17	41.2	16.8	2.9	2.4	1.7	4.6
G	M	59	42.8	18.0	1.3	1.6	0.9	3.8

* Results obtained with the Frei assay (5).
All other results by Mulder and Van Doorn assay (4).

The samples obtained through laparotomy were from the peripheral area of the liver, which is the last to change in the course of a pathological process (9). The samples were large enough to permit biological examinations, which indicated histologically normal livers. The reproducibility of the data was also a function of the size of the sample (10). The determination of 4-nitrophenol glucuronidation by the Frei colorimetric method (5) produced a mean value of 159.7 ± 38.3 $nmol.min^{-1}.g^{-1}$ liver, which is in accordance with the results of Okolicsanyi *et al.* (11) and with previous findings (12) in this laboratory for human liver homogenates. In homogenates again, Auclair *et al.* (13) obtained higher values in their controls, using digitonin-activated samples and Pogell and Leloir's assays (14). Wishart, using rats (15) also obtained higher glucuronidation activities after digitonin activation than after Triton X-100. In human

and various other animal species examined, the glucuronidation of chloramphenicol *in vitro* was very poor. This may account for the relative toxicity of chloramphenicol, as Young and Lietman (16) previously suggested. Other authors (17, 18, 19) have also reported chloramphenicol to be a poor substrate in rats.

The data obtained in control specimens of different species were compared (Table 2) in a search for a model animal for glucuronidation. Among the mammals tested, pig liver microsomes exhibited the highest activities, which might be related to the deficiency of this species in the sulfation pathway (20). In 2 months old Hanford miniature pigs, activities of 48 ± 9 nmoles 4-nitrophenol.min^{-1}.mg^{-1} protein have been reported (21) in good agreement with the data presented here. Except with *l*-borneol in rats, the order of decreasing activities was the same. In a similar comparison Boobis *et al.* (22) concluded that differences do exist between the glucuronosyltransferase activities in human and rat liver. But describing a model would also require that the regulation of the enzyme(s) will be compared.

From this work it appears that the UDP-glucuronosyltransferase activities in microsomes from both human liver and the livers of the laboratory animals used, varied greatly (16-fold) with the aglycone to be glucuronidated. As the activities seemed more dependent on the aglycones than on the species, perhaps, potentially "good" and "poor" substrates might be predictable from biophysical constants of the aglycones, for example the Van der Waals volume, and hydrophobicity parameter of the aglycone molecule (23).

For rats (24) and mice (25) the substrates have been classified into two groups on the basis of the surge of glucuronidation in developing animals, induction by phenobarbital and 3-methylcholanthrene (26, 27), purification (18, 28), and tissue distribution (29).

Table 2. UDPGT activities in liver microsomes from humans and four other mammals

Aglycones	Glucuronidation Activity (nmol.min^{-1}.mg^{-1} microsomal protein)				
	Humans (Caucasian)	Pigs (Large White)	Rats (Sprague Dawley)	Rats (Wistar)	Mice (C57 Bl/6 Rholco)
Eugenol	20.7 ± 2.7	59.5 ± 9.0	41.9 ± 1.0	33.5 ± 6.0	33.2 ± 2.1
4-Nitrophenol	4.2 ± 0.6⁂ 12.2 ± 2.5°	55.5 ± 7.7	12.7 ± 0.4	36.3 ± 8.0	11.8 ± 0.6
l-borneol	3.7 ± 1.4	11.4 ± 1.7	23.7 ± 0.6	14.6 ± 2.3	5.7 ± 0.4
4-hydroxypheno-barbital	2.1 ± 0.4	10.8 ± 1.1	6.8 ± 0.6	5.7 ± 1.0	3.3 ± 0.03
Chloramphenicol	1.2 ± 0.3	8.6 ± 1.2	3.3 ± 0.5	4.05 ± 1.3	2.9 ± 0.2

The data in the various columns were calculated as follows : humans, from Table 1 ; pig, mean ± SD from a triplicate determination in one pig ; Sprague-Dawley rat and Wistar rat, means ± SD from 5 determinations ; mouse, mean ± SD of 5 determinations in microsomes obtained from 10 pooled livers.
⁂ Results obtained with Frei colorimetric method (5), UDPGA 0.15 mM.
° Mudler and Van Doorn assay (4), UDPGA 0.5 mM.

Except 4-nitrophenol, the substrates studied here have been described as group-2 substrates in rats and mice. The relevance of this classification has not yet been demonstrated in humans. Recently, Onishi *et al.* (30) and Pacifici and Rane (31) reported data obtained during fetal (as well as neonatal) development in human. Their results suggested that if two groups of such substrates do exist in humans, they may be split differently than in rats. Experiments also have to be performed with a larger number of aglycones, before a tentative classification of these substrates can be proposed for humans.

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