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SHORT COMMUNICATIONS

Sulphation and glucuronidation of paracetamol in human liver: assay conditions

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Paracetamol, the widely used analgesic, is mainly cleared from the body by formation of its sulphate and glucuronide conjugates. *In vitro* studies on the metabolism of paracetamol have been carried out with animal tissues [1–4] and human platelets [5, 6]. Most of the studies performed in humans have been *in vivo* [7–9]. We have therefore investigated the sulphation and glucuronidation of paracetamol in subcellular fractions obtained from human liver. Because of the lack of methodological details relating to the assay of sulphotransferase and glucuronyltransferase towards paracetamol in human liver, the assay conditions for the two enzymes are described in some detail.

Materials and methods

Samples (50–1000 g) of histologically normal livers were obtained from kidney transplant donors. Ethical approval for the study was granted and consent to removal of the liver samples was obtained from the donors relatives. The cause of death was head injury or subarachnoid haemorrhage. Donor 1 was an epileptic and had received phenytoin and phenobarbital for more than 20 years. Donors 4 and 10 were smokers.

The tissue (2.5–5.0 g) was finely chopped in 1/15 M sodium phosphate buffer (pH 7.4), containing KCl (1.15%) with an ultra-Turrax before homogenization with a Teflon in glass homogeniser. The clearance between the pestle and the glass chamber was 0.2–0.4 mm. The homogenate was centrifuged at 12,000 *g* for 20 min at 4°. The supernatant was decanted without disturbing the pellet and centrifuged at 105,000 *g* for 60 min at 4°. The ensuing supernatant was investigated as the cytosolic fraction. The pellet was resuspended in 50 mM Tris–HCl, pH 7.4, containing 30% glycerol and investigated as the microsomal fraction. Protein was determined according to the method of Lowry *et al.* [10]. Fractions were stored deep frozen at −70°.

Radiolabelled paracetamol (*p*-[^{3}H]-hydroxyacetanilide; 18.9 Ci/mmol) was obtained from New England Nuclear Corporation (Boston, MA) and purified by thin layer chromatography (chloroform:ethyl acetate:acetic acid, 6:3:1) before use. All fine biochemicals were obtained from Sigma (Poole, Dorset, U.K.). Other reagents were obtained from either BDH Ltd (Poole, Dorset, U.K.), or Fisons Ltd (Loughborough, U.K.).

The sulphotransferase assay was carried out in a final volume of 0.1 ml containing 0.1 M Tris–HCl (pH 9.0), 5 mM 2-mercaptoethanol, 0.5 mM paracetamol (150,000 cpm), an aliquot of the cystolic protein to give a final concentration of protein varying between 1.8 and 2.8 mg/ml. The reaction was started by addition of 0.4 mM PAPS (3′-phosphoadenosine-5′-phosphosulphate) in 5 μl of distilled water. The incubation was carried out at 37° for 20 min and was stopped by the addition of 0.9 ml ice-cold 0.4 M TCA and 0.6 M glycine. The samples were extracted twice in 10 ml of water-saturated ethyl acetate. Aliquots (0.2 ml) of the water phase residue were transferred to scintillation vials with 4 ml of scintillant (Aqualuma; LKB) and the radioactivity determined by liquid scintillation spectrometry (Packard model 4640).

The glucuronyltransferase assay was carried out in a final volume of 0.2 ml. The incubation mixture was as follows: 0.1 M Tris–HCl (pH 7.4), 5 mM $MgCl_2$, 0.5 mM paracetamol (300,000 cpm), an aliquot of microsomal protein to give a final protein concentration ranging between 2.8 and 5.1 mg/ml. The reaction was started by the addition of 5 mM UDPGA (uridine 5′-diphosphoglucuronic acid) in 10 μl distilled water. The incubation was carried out at 37° for 20 min and was stopped by the addition of 0.8 ml of 0.4 M TCA containing 0.6 M glycine. The tubes were centrifuged at 1500 *g* for 10 min. Aliquots (0.7 ml) of the supernatant were extracted (×2) in 7 ml ethyl acetate. After centrifugation at 1500 *g* for 5 min, 0.2 ml of the aqueous phase residue was transferred to scintillation vials and the radioactivity determined.

For both assays, each sample was assayed in duplicate. The activity of the sulphotransferase and glucuronyltransferase was determined on the basis of the specific radioactivity of paracetamol after correction for blanks. The radioactivity in the blank samples ranged between 160 and 190 cpm (sulphotransferase) and 300–350 cpm (glucuronyltransferase). The radioactivity in samples was always 2–5 times (sulphotransferase) and 2–3 times (glucuronyltransferase) higher than in blanks. The recovery of ^{3}H-paracetamol in the ethyl acetate was 94.5% and 99.5% after the first and second extraction, respectively. Ethyl acetate was better than 1,2-dichlormethane, chloroform and petroleum ether as extraction solvents.

Results

The methodology of this study does not rigorously identify by chemical means the products formed in each incubation. However, since the only difference from the blank samples (values of which were subtracted) was either PAPS or UDPGA we believe it justifiable to describe the radioactivity present in the aqueous phase as the respective conjugates.

The effect of incubation time, protein concentration and pH on the rate of paracetamol sulphation is shown in Fig. 1(a–c). Sulphotransferase activity increased with pH reaching a maximum at pH 9.0. When 0.1 M Tris–HCl was used the rate of paracetamol sulphation was higher than using 0.25 M sodium pyrophosphate buffer.

The effect of incubation time, protein concentration and pH on the activity of glucuronyltransferase is also shown in Fig. 1(d–f). The activity of both sulphotransferase and glucuronyltransferase towards paracetamol measured in 10 livers is summarised in Table 1. Sulphotransferase activity ranged between 52.5 and 121.7 pmol/min/mg protein. The mean enzyme activity (81.4 pmol/min/mg) was about 70% higher than glucuronyltransferase activity (47.8 pmol/min/mg). The coefficient of variation was 35.3% (sulphotransferase) and 28.1% (glucuronyltransferase). The addition of the β-glucuronidase inhibitor, D-saccharic-1,4-lactone (1.2 mM) did not increase the rate of paracetamol glucuronidation (data not shown). In contrast, Triton X-100 (0.02%) increased the activity of the glucuronyltransferase by 1.6 times (45.7 ± 12.5 to 73.0 ± 13.8 pmol/min/mg; N = 4).

Discussion

Sulphotransferase consists of various isoenzymes with different optimal pH values [11]. For example, in rat liver the optimal pH for the suplhation of paracetamol and 2-

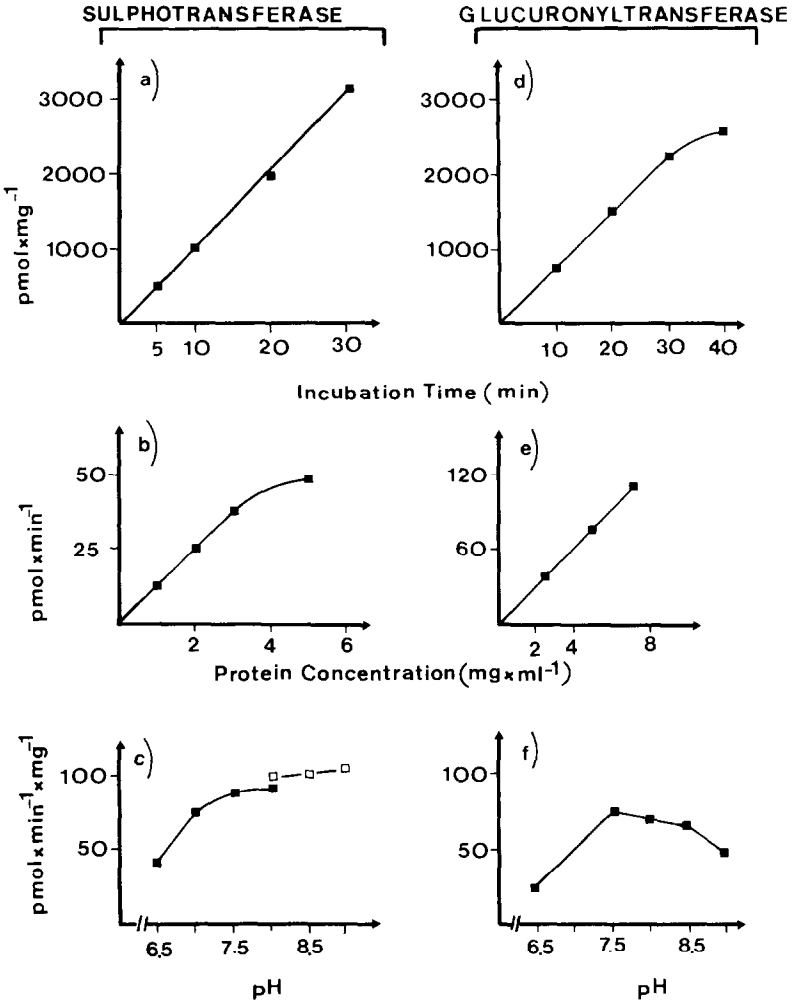

Fig. 1. Effect of the incubation time, protein concentration and pH on the activity of sulphotransferase (panels a, b and c) and glucuronyltransferase (panels d, e and f) of paracetamol. The liver was from donor 9 (male 27 years old): (a) protein concentration was 3.4 mg/ml, pH 9.0; (b) incubation time 20 min, pH 9.0; (c) protein concentration 3.4 mg/ml, incubation time 20 min. Closed squares phosphate buffer; open squares Tris buffer; (d) protein concentration 5 mg/ml, pH 7.4; (e) incubation time 20 min, pH 7.4; (f) protein concentration 5 mg/ml, incubation time 20 min.

Table 1. Rate of sulphation and glucuronidation of paracetamol in human liver

Donors	Sex	Age	pmol × min^{-1} × mg^{-1} Sulphotransferase	Glucuronyltransferase
1	F	46	55.2	67.9
2	M	60	57.9	45.4
3	F	17	52.5	40.4
4	M	18	53.0	35.3
5	M	35	71.4	38.3
6	F	66	60.9	55.7
7	M	27	118.2	25.9
8	M	29	121.7	49.6
9	M	27	106.4	71.1
10	M	54	117.2	48.6
Mean			81.4	47.8
± SD			28.7	13.4
C.V.%			35.3	28.1

naphthol is 9 [4] and 5.5–6.5 [11], respectively. A similar picture is also observed in human liver (this study; and Ref. 12). This indirect evidence suggests that paracetamol and 2-naphthol are sulphated by independent isoenzymes in both rat and human liver. Sekura and Jakoby [13] have observed that the forms I and II isolated from rat liver catalyse the sulphation of paracetamol.

The activity of the sulphotransferase and glucuronyltransferase varied only by a factor of 2 despite the wide range of the donors' ages (17–66 yr). There was no correlation between age and activity for either enzyme. There was no correlation between the two enzymes suggesting that they are independently regulated.

On the basis of these *in vitro* data sulphation is apparently predominant over glucuronidation. However, caution is needed in relating enzyme activities measured at standard concentrations of substrate *in vitro* to the *in vivo* situation where concentrations rapidly change. Indeed *in vivo*, Clements *et al.* [7] have observed the urinary excretion of paracetamol glucuronide to exceed that of paracetamol sulphate following both oral and intravenous administration. In another study Siegers *et al.* [8] recovered identical amounts of both the glucuronide and sulphate in the bile of six subjects. When comparing *in vitro* and *in vivo* data a number of additional points should also be considered. There is *in vivo* the presence of non-hepatic transferase enzymes and also non-microsomal glucuronyltransferase [14, 15]. The presence of Triton X-100 did increase glucuronyltransferase activity by 60% and *in vivo* the latent enzyme is at least partially activated by the regulator UDPNAG. Also, a non-physiological pH (9.0) was used in the assay of sulphotransferase.

One interesting observation was the relatively high activity of glucuronyltransferase in donor 1. This patient had been treated with phenobarbital and phenytoin for more than 20 years. Prescott *et al.* [16] have previously reported that anticonvulsant drugs enhance paracetamol glucuronidation *in vivo*.

In conclusion, simple assays are described for determining sulphotransferase and glucuronyltransferase activity of human liver using paracetamol as substrate. Sulphotransferase activity measured in hepatic cytosol was 70% greater than glucuronyltransferase activity assayed in native microsomes.

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Department of Pharmacology and Therapeutics
University of Liverpool
P.O. Box 147
Liverpool, L69 3BX, U.K.

GIAN M. PACIFICI*
DAVID J. BACK
MICHAEL L'E. ORME

* Present address: Associate Professor in Pharmacology at the Department of General Pathology, Medical School, University of Pisa, Italy.

REFERENCES

1. Dybing E, Inhibition of acetaminophen glucuronidation by oxazepam. *Biochem Pharmacol* **25**: 1421–1425, 1976.
2. Moldeus P, Paracetamol metabolism and toxicity in isolated hepatocytes from rat and mouse. *Biochem Pharmacol* **27**: 2859–2863, 1978.
3. Sweeney DJ and Weiner M, Metabolism of acetaminophen in hepatocytes isolated from mice and rats of various ages. *Drug Metab Dispos* **13**: 377–379, 1985.
4. Mizuma T, Araya H, Hayashi M and Awazu S, Multiple forms of aryl sulphotransferase for acetaminophen. *J Pharm Dyn* **7**: 784–789, 1984.
5. Reiter C and Weinshilboum RM, Acetaminophen and phenol: Substrates for both a thermostable and a thermolabile form of human platelet phenol sulfotransferase. *J Pharmacol Exp Ther* **221**: 43–51, 1981.
6. Anderson RJ, Weinshilboum RM, Phillips SF and Broughton DD, Human platelet phenol sulphotransferase: Assay procedure, substrate and tissue correlations. *Clin Chim Acta* **110**: 157–167, 1981.
7. Clements JA, Critchley JAJH and Prescott LF, The role of sulphate conjugation in the metabolism and disposition of oral and intravenous paracetamol in man. *Br J Clin Pharmacol* **18**: 481–485, 1984.
8. Siegers C-P, Loeser W, Gieselmann J and Oltmanns D, Biliary and renal excretion of paracetamol in man. *Pharmacology* **29**: 301–303, 1984.
9. Howie D, Adriaenssens PI and Prescott LF, Paracetamol metabolism following overdosage: Application of high performance liquid chromatography. *J Pharm Pharmacol* **29**: 235–237, 1977.
10. Lowry OH, Rosebrough, NJ, Farr AL and Randall RJ, Protein measurement with the folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
11. Jakoby WB, Duffel MW, Lyon ES and Ramaswamy S, In: *Progress in Drug Metabolism*, Vol. 8 (Eds. Bridges JW, Gibson GG and Chasseaud LF), pp. 11–33. Taylor & Francis, London, 1984.
12. Pacifici GM, Franchi M, Colizzi C, Giuliani L and Rane A, Sulfotransferase in humans: Development and tissue distribution. *Pharmacology* **36**: 411–419, 1988.
13. Sekura RD and Jakoby WB, Phenol sulfotransferases. *J Biol Chem* **254**: 5658–5663, 1979.
14. Pacifici GM, Benchini C and Rane A, Presystemic glucuronidation of morphine in humans and rhesus monkeys: subcellular distribution of the UDP-glucuronyltransferase in the liver and intestine. *Xenobiotica* **16**: 123–128, 1986.
15. Pacifici GM, Giuliani L and Calapeina R, Glucuronidation of 1-naphthol in nuclear and microsomal fractions of the human intestine. *Pharmacology* **33**: 103–109, 1986.
16. Prescott LF, Critchley JAJH, Balali-Mood M and Pentland B, Effects of microsomal enzyme induction on paracetamol metabolism in man. *Br J Clin Pharmacol* **12**: 149–153, 1981.