Biochemical Pharmacology, Vol. 25, pp. 613–616. Pergamon Press, 1976. Printed in Great Britain.

GLUCURONIDATION OF PARACETAMOL, MORPHINE AND 1-NAPHTHOL IN THE RAT INTESTINAL LOOP

Dorothee Josting, Dietrich Winne and Karl Walter Bock
Institut für Toxikologie and Institut für Pharmakologie
74 Tübingen, West Germany

(Received 1 December 1975; accepted 15 December 1975)

Many phenolic compounds are known to be glucuronidated by the intestinal mucosa (1). When 1-naphthol is instilled into the jejunal loop it is rapidly conjugated and appears in venous blood mainly as the glucuronide (2). The intestinal loop system offers the possibility of investigating, in close to in vivo conditions, the extent to which conjugation reactions reduce the amount of free phenolic drugs reaching blood in the unconjugated form. Therefore the previous studies with naphthol were extended to paracetamol and morphine. In addition it was attempted to compare 1-naphthol glucuronidation in the intestinal loop and in mucosal homogenates or microsomes.

Radioactive compounds were obtained from the following sources: ^{3}H-paracetamol (generally labeled, 370 mCi/mmol) from New England Nuclear Co., Boston; (N-methyl-^{14}C)morphine hydrochloride (57 mCi/mmol) and 1-(1-^{14}C)naphthol (20.8 mCi/mmol) from Radiochemical Center, Amersham. Paracetamol (5 μCi), morphine hydrochloride (2.4 μCi) or 1-naphthol (0.25 μCi), 100 nmol of each compound, was dissolved in 0.5 ml 0.9% (w/v) NaCl containing 0.3% (v/v) dimethylsulfoxide and instilled into the closed intestinal loop of male Wistar rats (300–350 g) as described previously (2). Venous blood from the jejunal loop was collected at 10 min intervals for 30 min. The recovery of free drug and its conjugates was determined from the radioactivities in the intestinal content and in venous blood. The ratio of free paracetamol or morphine to their conjugates was then determined in the intestinal content and in blood plasma. It was assumed that the latter ratio also applied to the erythrocyte layer, since the radioactivity in this layer corresponded approximately with the amount of contaminating plasma. In the experiments with 1-naphthol the high concentration of naphthol in the erythrocyte layer had to be taken into account. The partition ratio between blood cells and plasma is about 4:1 (2). 1-Naphthol and its conjugates were analyzed as described (2). Only trace amounts of 1-naphthol sulfate could be detected. Free ^{3}H-paracetamol was

separated from conjugates by extraction with ethylacetate and the radioactivity counted.Conjugates were determined in the remaining water phase from the amounts of free paracetamol extracted after hydrolysis with 2.5 mg/ml sulfatase-free ß-glucuronidase (Serva,Heidelberg) or with 0.05 mg/ml arylsulfatase (Boehringer,Mannheim).Enzyme treatment was performed in 0.1 M acetate buffer pH 4.5 for 16 h at 37°C.Morphine and its conjugates were analyzed by thin-layer chromatography on silica-gel plates developed with ethanol-1 M ammonium acetate (9:1,v/v).Radioactive peaks were scraped off the plates and the radioactivity was counted.Glucuronide and sulfate ester conjugates were not separated in this system.Therefore the amount of morphine glucuronide was estimated by subtracting the radioactivity of conjugates remaining after hydrolysis with ß-glucuronidase from the radioactivity of total conjugates.

Table 1. Absorption and glucuronidation of paracetamol, morphine and 1-naphthol in the rat jejunal loop

Compound	Recovery of phenolic compounds 30 min after administration			
	Intestinal content		Venous blood	
	(% of dose)			
Paracetamol				
Free	6.2	(3.7-8.0)	75.6	(74.7-76.5)
Total conjugates	0.5	(0.3-0.7)	4.4	(4.3-4.5)
Glucuronide	0.2	(0.1-0.3)	3.0	(2.9-3.1)
Morphine				
Free	73.9	(68.3-82.3)	6.9	(3.5-9.4)
Total conjugates	5.1	(4.7-5.7)	6.1	(3.0-7.7)
Glucuronide	3.6	(3.4-4.1)	4.9	(2.3-5.8)
1-Naphthol				
Free	7	(2-12)	23	(21-25)
Total conjugates	15	(10-20)	55	(52-58)
Glucuronide	15	(10-20)	55	(52-58)

The mean of 3-6 experiments is shown.Ranges are given in parenthesis. The difference between total conjugates and glucuronide represents mainly the sulfate ester conjugate.

Table 1 shows recoveries of phenolic compounds and their conjugates in the intestinal content and in venous blood obtained 30 min after instillation of the free substance into the closed jejunal loop.Paracetamol is rapidly absorbed from the intestinal lumen.About 76% appeared in venous blood

within 30 min,51% in the first 10 min.Conjugation is of minor importance.In contrast morphine is absorbed slowly.1.4%,2.5% and 3% of free morphine appeared in venous blood in successive 10 min periods.Approximately equal amounts of free morphine and its conjugates were released into the blood. A similar amount of morphine conjugates was found in the intestinal lumen. 1-Naphthol is conjugated in the intestinal mucosa at a very high rate.Hence 1-naphthol which is rapidly absorbed appeared in blood mostly as the glucuronide.It appears therefore that intestinal glucuronidation markedly reduces the amount of free morphine and 1-naphthol reaching general circulation by competition with the absorption process.

It was attempted to compare 1-naphthol glucuronidation in the intestinal loop and in subcellular systems.Experiments with liver indicate that full potential UDP-glucuronyltransferase activity is constrained by the microsomal membrane (3,4,5).In the constrained form UDP-N-acetylglucosamine is a positive allosteric effector which may be of physiological importance (4). The constrained enzyme is also activated by a variety of treatments altering membrane integrity such as addition of Triton X-100 (6,7).

Table 2. Activation of intestinal UDP-glucuronyltransferase by UDP-N-acetylglucosamine and Triton X-100

Additions to assay	UDP-glucuronyltransferase (1-naphthol as substrate)	
	Homogenate (μmol/g tissue/min)	Microsomes (nmol/mg protein/min)
	(Assay 0.5 h after sacrifice)	
None	0.08 ± 0.01 (6)	-
+ UDP-N-acetylglucosamine	0.22 ± 0.04 (6)	-
+ Triton X-100	0.34 ± 0.06 (6)	-
	(Assay 4 h after sacrifice)	
None	0.30 ± 0.01 (4)	6.3 ± 1.1 (4)
+ UDP-N-acetylglucosamine	0.20 (2)	6.0 ± 1.5 (4)
+ Triton X-100	0.34 (3)	5.2 ± 1.2 (4)

The small intestine,from the pylorus to 5 cm proximal to the ileocecal valve,was rapidly removed under ether anesthesia.The intestine was rinsed extensively with ice-cold saline.The mucosa was scraped off with a spatula, and homogenates (20%,w/v) were prepared in 0.25 M sucrose.The homogenates were centrifuged at 20 000 x g for 20 min.Microsomes were obtained from the ensuing supernatant after centrifugation at 100 000 x g for 45 min.All procedures were carried out at 0-4 °C.UDP-glucuronyltransferase was assayed with a radio-assay as described (3) using 0.5 mM 1-naphthol and 3 mM UDP-glucuronic acid.The mean ± standard deviation is shown.The number of experiments is listed in parenthesis.UDP-N-acetylglucosamine and Triton X-100 were used at concentrations of 3 mM and 0.05%(w/v),respectively.

In contrast to the enzyme in microsomes from liver,kidney,lung and spleen (unpublished results),1-naphthol glucuronidation could not be activated in intestinal microsomes (Table 2).In liver microsomes the enzyme is activated about 15-fold by Triton X-100 and 4-fold by 3 mM UDP-N-acetylglucosamine under similar conditions.The difference in activation of the intestinal and liver enzyme was also noted with other substrates (8,9).However the intestinal enzyme could be markedly activated when homogenates of intestinal mucosa were studied 0.5 h after sacrifice of the animal.When homogenates were left for 4 h at 0-4°C the activation was no longer detectable.In fact full potential enzyme activity was found without addition of activating agents.Since it takes 2-4 h to prepare microsomes it is conceivable that the enzyme is spontaneously activated during the isolation procedure,possibly by intestinal lipases and proteases.Addition of trypsin inhibitor (Boehringer,Mannheim;0.5 mg/g mucosa wet weight) or 5 mM EDTA did not prevent spontaneous activation.

In the intestinal loop perfused with 0.07 mM 1-naphthol a glucuronidation rate of 7 nmol/g mucosa/min was determined (2).When mucosal homogenates were assayed 0.5 h after sacrifice of the animal with 0.07 mM 1-naphthol and 0.3 mM UDP-glucuronic acid a glucuronidation rate of 8 nmol/g mucosa/min was found. The rate was much higher in fully activated homogenates and at substrate concentrations near saturation of the enzyme (Table 2).Although the level of UDP-glucuronic acid in the intestinal mucosa is not known the comparative data on naphthol glucuronidation in the intestinal loop and in the mucosal homogenate suggest that intestinal UDP-glucuronyltransferase is constrained or latent in the intact tissue,similar to the liver enzyme (3).

Acknowledgement:The authors wish to thank the Deutsche Forschungsgemeinschaft for financial support.

References

1. K.Hartiala,Pharmacol.Rev.33,496 (1973).
2. K.W.Bock and D.Winne,Biochem.Pharmacol.24,859 (1975).
3. K.W.Bock and I.N.H.White,Eur.J.Biochem.46,451 (1974).
4. D.Zakim,J.Goldenberg and D.A.Vessey,Eur.J.Biochem.38,59 (1973).
5. A.Winsnes and G.J.Dutton,Biochem.Pharmacol.22,1765 (1973).
6. K.K.Lueders and E.L.Kuff,Arch.Biochem.Biophys.120,198 (1967).
7. G.J.Mulder,Biochem.J.125,9 (1971).
8. A.Aitio,Int.J.Biochem.5,617 (1974).
9. E.Del Villar,E.Sanchez and T.R.Tephly,Drug Metab.Disp.2,370 (1974).