Biochemical Pharmacology, Vol. 33, No. 14, pp. 2341–2344, 1984.
Printed in Great Britain.

0006–2952/84 $3.00 + 0.00

HIGH-RATE INTESTINAL CONJUGATION OF 4-METHYL-UMBELLIFERONE DURING INTRAVENOUS INFUSION IN THE RAT IN VIVO

Gerard J. Mulder, Sybolt Brouwer and Egbert Scholtens

Department of Pharmacology, State University of Groningen,
Bloemsingel 1, 9713 BZ Groningen, The Netherlands

(received: 12 March 1984; accepted: 2 May 1984)

In recent years it has become quite well established that the gut mucosa can actively conjugate orally administered drugs during absorption from the gut lumen (1,2). Indeed, isolated rat intestinal mucosa cells have been shown to conjugate various phenolic substrates with glucuronic acid or sulfate at appreciable rates (3,4). In addition, conjugation could also be demonstrated in perfused intestinal loop preparations from the rat, in which the substrate was supplied in the lumen (5-7); the conjugates can subsequently be excreted to the luminal (gut) or the contra-luminal (blood) side. However, to our knowledge the contribution of the gastro-intestinal region to the overall conjugation in vivo of intravenously administered substrates has not been studied. Experiments in which a substrate was supplied from the intravenous side in isolated perfused rat intestinal segments (6) and conjugation could be demonstrated, as well as the accumulation of L-DOPA conjugates in gut mucosa in the rat after i.v. administration of L-DOPA (8) suggested that indeed intestinal conjugation of substrates present in blood might be expected.

In the course of an investigation on the extraction of the phenolic compound, 4-methylumbelliferone, by rat liver in situ, we happened to find a very substantial contribution of the gastro-intestinal region to the clearance by conjugation of 4-methylumbelliferone. In the rat, 4-methylumbelliferone is mainly conjugated with glucuronic acid (9,10). We show in this communication that during gastro-intestinal passage 37% of the unconjugated 4-methylumbelliferone is extracted from the incoming arterial blood and converted into the conjugates.

MATERIALS AND METHODS

Rats (270 - 300 g body wt) which had free access to food and water, were anesthetized with sodium pentobarbital and kept on a heating pad to keep body temperature between 37.5 and 38.5 °C. Artificial respiration was applied through a trachea cannula. Infusions of 4-methylumbelliferone were given through a catheter in the jugular vein. Blood samples were drawn from the carotid artery and the portal vein through indwelling catheters. The surgical procedures have been extensively described elsewhere (11); the portal vein catheter was inserted by a new method which requires minimum surgical manipulation and leaves blood flow in the portal vein unaffected (12).

4-Methylumbelliferone (sodium salt, Sigma Chem. Comp., St. Louis, MO, USA) was dissolved in 0.9% (w/v) sodium chloride in water, containing 1% (w/v)

bovine serum albumin (Poviet Produkten, Oss, The Netherlands); the final pH was adjusted to 9.5 to keep the 4-methylumbelliferone in solution. The infusion rate was 6.2 μmol/min/kg; the infusion was preceeded by a priming dose in the tail vein of 45 μmol/kg. The solution was infused at 13.5 ml/kg/hour. The infusion lasted for 60 min; at that time blood was drawn from the carotid artery and the portal vein simultaneously.

Unconjugated 4-methylumbelliferone was extracted from whole blood with ethyl acetate. A 50 μl blood sample was added to 1.0 ml 75 mM sodium acetate buffer, pH 5.0. Unconjugated 4-methylumbelliferone was extracted with 4.0 ml ethyl acetate. A 1.0 ml sample of the organic layer was taken and the ethyl acetate was blown off with air. Subsequently, the 4-methylumbelliferone was redissolved in 3 ml 0.4 M sodium glycine buffer, pH 10.4, and fluorescence was measured on a Perkin Elmer 1000 M fluorimeter (13). Over the range of blood concentrations measured there was a linear relationship between fluorescence and blood concentration.

Conjugated 4-methylumbelliferone was determined by hydrolysis of the conjugates in blood, to measure the total concentration of 4-methylumbelliferone after hydrolysis (both the originally present unconjugated compound, and the aglycone released by hydrolysis). To a 50 μl blood sample was added 1.0 ml 75 mM sodium acetate buffer, pH 5.0. A 15 μl sample of an undiluted solution of β-glucuronidase/arylsulfatase from Helix pomatia (Boehringer Corp., Mannheim, Germany) was added, and the mixture was incubated for 60 min at 37 °C. After that, the unconjugated 4-methylumbelliferone was determined by ethyl acetate extraction as described above.

Table 1. Concentrations of 4-methylumbelliferone and its conjugates in arterial and portal blood in the rat in vivo during intravenous infusion of 4-methylumbelliferone.[a]

	Carotid artery	Portal vein	Difference
Total concentration of 4-methylumbelliferone (μM)	457 ± 50	463 ± 54	6
Unconjugated (μM)	119 ± 16	72 ± 13	47
Conjugated (μM)	338	391	53
Percentage 4-methylumbelliferone extracted across intestinal region[b]	40 ± 3		

[a] Infusion of 6.2 μmol/kg/min in the jugular vein, after bolus injection of 45 μmol/kg. The blood samples were taken 60 min after start of the infusion, during steady-state. The mean ± S.E.M. is given; n = 6.

[b] The percentage extraction during steady state was calculated for each rat individually, by dividing the concentration of 4 MU in the portal vein by that in the artery (x 100).

RESULTS AND DISCUSSION

When 4-methylumbelliferone is infused at a high rate as used in the present series of experiments, it is almost completely converted to the glucuronide, and only little (less than 6%) is excreted as the sulfate conjugate (Weitering and Mulder, unpublished data). In the course of experiments to delineate the role of the liver in the elimination of 4-methylumbelliferone in situ, we have sampled blood from the carotid artery and the portal vein simultaneously. To our surprise, we found a marked decrease in the concentration of unconjugated 4-methylumbelliferone across the intestinal area: an extraction percentage of 40% was calculated (Table 1). In order to find out what had happened to the eliminated 4-methylumbelliferone, we have also determined the concentration of the conjugates at both sites. The results show that the substrate that disappeared in steady-state was quantitatively recovered in the form of its conjugates (Table 1): 112 $\pm$ 15% of the eliminated 4-methylumbelliferone reappeared as the conjugates.

Under the conditions of the experiment, the elimination of 4-methylumbelliferone is in steady-state (Weitering and Mulder, unpublished data), so that conjugation in the intestinal region plays a major role in the elimination of the substrate within the gastrointestinal/liver circulation. Assuming a portal blood flow of approx. 60 ml/kg/min a total conjugation rate by the gastrointestinal region of 2.8 μmol/kg/min is observed, which is approx. 40% of the total body clearance under these conditions (52 ml/kg/min).

One of the main sites of conjugation presumably is the intestinal mucosa Since total 4-methylumbelliferone in arterial blood was quantitatively recovered in the portal blood (in terms of concentrations) it seems that excretion of the formed conjugates directly into the gut lumen does not take place; the latter has been demonstrated to occur with other phenols (14).

This report shows that conjugation in the gastrointestinal region may play a rather important role in the elimination of substrates present in blood. Further investigation will be required to find out whether this also occurs with other substrates of both phase I and phase II metabolism.

Acknowledgements. We are indebted to Mr. B. Kort for excellent technical assistence.

REFERENCES

1. G.M. Powell, J.J. Miller, A.H. Olavesen and C.G. Curtis, Nature 252, 234 (1974).
2. C.F. George, Clin. Pharmacokinet. 6, 259 (1981).
3. A.S. Koster and J. Noordhoek, Biochem. Pharmacol. 32, 895 (1983).
4. J.R. Dawson and J.W. Bridges, Biochem. Pharmacol. 28, 3299 (1979).
5. J.R. Dawson and J.W. Bridges, Biochem. Pharmacol. 28, 3291 (1979).
6. A.S. Koster and J. Noordhoek, J. Pharmacol. exptl. Therap. 226, 533 (1983).
7. P. Wollenberg, V. Ullrich and W. Rummel, Biochem. Pharmacol. 32, 2103 (1983).
8. L. Landsberg, M.B. Berardino and P. Silva, Biochem. Pharmacol. 24, 1167 (1975).
9. D. Robinson, J.N. Smith and R.T. Williams, Biochem. J. 53, 125 (1953).

10. G.J. Mulder, Biochem. Pharmacol. 22, 1751 (1973).
11. G.J. Mulder, E. Scholtens and D.K.F. Meijer, Methods Enzymol. 77, 21 (1981).
12. E. Scholtens and G.J. Mulder, Experientia 39, 1176 (1983).
13. G.M.J. van Kempen and G.S.I.M. Jansen, Anal. Biochem. 46, 438 (1972)
14. P. Wollenberg and W. Rummel, Biochem. Pharmacol. 33, 205 (1984).