Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248–254

Inui, K., Okano, T., Maegawa, H., Kato, M., Takano, M., Hori, R. (1988) H^+ coupled transport of p.o. cephalosporins via dipeptide carriers in rabbit intestinal brush-border membranes: difference of transport characteristics between cefixime and cephradine. J. Pharmacol. Exp. Ther. 247: 235–241

Kramer, W., Girbig, F., Leipe, I., Petzoldt, E. (1988) Direct photoaffinity labelling of binding proteins for β-lactam antibiotics in rabbit intestinal brush-border membranes with [^{3}H] benzylpenicillin. Biochem. Pharmacol. 37: 2427 2435

Mine, Y., Sakamoto, H., Hirose, T., Shibayama, F., Kikuchi, H., Kuwahara, S. (1987) FK482, a new orally active cephalosporin: pharmacokinetics in animals. Abstract in 27th Intersci. Conference Antimicrob. Agents Chemother. Abstract 654

Muranushi, N., Yoshikawa, T., Yoshida, M., Oguma, T., Hirano, K., Yamada, H. (1989) Transport characteristics of ceftibuten, a new oral cephem, in rat intestinal brush-border membrane vesicles: relationship to oligopeptide and amino-β-lactam transport. Pharm. Res. 6: 308–312

Nakashima, E., Tsuji, A., Mizuo, H., Yamana, T. (1984) Kinetics and mechanism of in vitro uptake of amino-β-lactam antibiotics by rat small intestine and relation to intact-peptide transport system. Biochem. Pharmacol. 33: 3345–3352

Okano, T., Inui, K., Maegawa, H., Takano, M., Hori, R. (1986) H^+ Coupled uphill transport of aminocephalosporins via the dipeptide transport system in rabbit intestinal brush-border membranes. J. Biol. Chem. 261: 14130 14134

Sakamoto, H., Hirose, T., Mine, Y. (1985) Pharmacokinetics of cefixime, a new oral cephalosporin, in experimental animals. Chemother. (Tokyo) 33 (Suppl. 6): 157–168

Simanjuntak, M.T., Tamai, I., Terasaki, T., Tsuji, A. (1990) Carrier-mediated uptake of nicotinic acid by rat intestinal brush-border membrane vesicles and relation to monocarboxylic acid transport. J. Pharmacobiodyn. 13: 301–309

Simanjuntak, M.T., Terasaki, T., Tamai, I., Tsuji, A. (1991) Participation of monocarboxylic anion and bicarbonate exchange system for the transport of acetic acid and monocarboxylic acid drugs in the small intestinal brush-border membrane vesicles. J. Pharmacobiodyn. 14: 501–508

Sinko, P. J., Amidon, G. L. (1989) Characterization of the oral absorption of β-lactam antibiotics II: competitive absorption and peptide carrier specificities. J. Pharm. Sci. 78: 723 727

Tsuji, A., Hirooka, H., Terasaki, T., Tamai, I., Nakashima, E. (1987a) Saturable uptake of cefixime, a new oral cephalosporin without an α-amino group, by the rat intestine. J. Pharm. Pharmacol. 39: 272–277

Tsuji, A., Tamai, I., Hirooka, H., Terasaki, T. (1987b) β-Lactam antibiotics and transport via the dipeptide carrier across the intestinal brush-border membrane. Biochem. Pharmacol. 36: 565–567

Tsuji, A., Terasaki, T., Tamai, I., Hirooka, H. (1987c) H^+ gradient-dependent and carrier-mediated transport of cefixime, a new cephalosporin antibiotic, across brush-border membrane vesicles from rat small intestine. J. Pharmacol. Exp. Ther. 241: 594–601

Tsuji, A., Simanjuntak, M.T., Tamai, I., Terasaki, T. (1990) pH-dependent intestinal transport of monocarboxylic acids: carrier-mediated and H^+-cotransport mechanisms versus pH-partition hypothesis. J. Pharm. Sci. 79: 1123 1124

J. Pharm. Pharmacol. 1993, 45: 998–999
Communicated February 2, 1993

The effect of a nutritional model of chronic liver injury on the hepatic glucuronidation of morphine in rats

RICHARD CALLAGHAN*, PAUL V. DESMOND, MAURICE L. MASHFORD, *Departments of Clinical Pharmacology and Gastroenterology, St Vincent's Hospital, Victoria Parade, Fitzroy, Victoria, Australia 3065*

Abstract—The effect of a choline-deficient diet on the hepatic glucuronidation of morphine was investigated using a rat perfused liver system. Rats fed a choline-deficient diet developed a fatty liver with minimal necrosis. Despite the morphological changes, neither hepatic extraction ratio ($0{\cdot}51 \pm 0{\cdot}02$ in control; $0{\cdot}45 \pm 0{\cdot}04$ in the choline-deficient rats) nor intrinsic clearance ($0{\cdot}85 \pm 0{\cdot}05$ in control; $0{\cdot}77 \pm 0{\cdot}09$ in choline-deficient rats) were affected by this injury model. This finding suggests that glucuronidation is relatively resistant to this chronic liver injury.

The elimination of a number of drugs is impaired in patients with chronic liver injury (Wilkinson & Schenker 1975). The two most important factors responsible for this impairment are decreased hepatic metabolizing enzyme activity and alterations in hepatic circulation.

Several models of chronic liver injury in experimental animals have been established. The most frequently used model involves co-administration of CCl_4 and phenobarbitone to rats (McLean et al 1969). Another model of acute and chronic liver injury involves feeding rats a choline-deficient diet (Murray et al 1986). Both the CCl_4 (Villeneuve et al 1978) and nutritional (Murray et al 1986) models are associated with decreased content of total cytochrome P450 and reductions in activity of several P450 isozymes. The effect of either model of chronic liver injury on the glucuronidation pathway has received less attention.

The purpose of this study was to examine the effect of a chronic choline-deficient diet on the hepatic elimination of morphine, the major metabolic pathway of which is the formation of 3-*O*-glucuronide.

Materials and methods

The choline-deficient model of hepatic injury was based on published methods (Murray et al 1986). Rats, initially weighing 150 g, had free access to either a choline-deficient or choline-supplemented diet for 28 weeks. The diet consisted of 12% protein, 58% sucrose, 26% oils and fats, 4% salts and minerals and a vitamin supplement.

A rat non-recirculating isolated perfused liver system was used (Bartosek et al 1973). The perfusion medium was composed of 90% (v/v) Krebs–Henseleit buffer and 10% (v/v) washed human erythrocytes. Bovine serum albumin (10 g L^{-1}), glucose (3 g L^{-1}) and sodium taurocholate (75 mg L^{-1}) were dissolved in the medium. The viability of the preparation was assessed by the lactate/pyruvate ratio, perfusion back-pressure and bile flow. Each preparation was studied over four 30-min periods using perfusion rates of 7, 10 and 12 mL min^{-1} in control, and 10, 12 and 15 mL min^{-1} in choline-deficient preparations. Morphine was added to the perfusate to give an inflow concentration of

* Present address: Hospital for Sick Children, Cystic Fibrosis Research Program, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.

Correspondence: P. V. Desmond, Department of Gastroenterology, St Vincent's Hospital, Fitzroy, Victoria, Australia 3065.

5 μg mL^{-1}. Four inflow and outflow samples were taken in each period.

Morphine was assayed by HPLC. The HPLC system comprised a single piston pump, system controller and ultraviolet spectrophotometer (LC-6 Series, Shimadzu, Japan). Chromatography was carried out on a reverse-phase Ultrasphere ODS column (4·6 × 250 mm) with 5 μm particle size (Beckman Instruments, USA). The mobile phase was 15% (v/v) acetonitrile in buffer containing 0·046 M NaH_2PO_4, 0·015 M H_3PO_4 and 0·5 g L^{-1} octane sulphonic acid. The flow rate was 2 mL min^{-1} and peak detection was by UV absorption at 220 nm. Samples were prepared using a protein-precipitation method. Samples (1 mL) were mixed with 10·5% (w/v) perchloric acid, vortexed and centrifuged at 2000 *g* for 10 min. The supernatant was cooled to 5°C and 1 mL neutralized with cold 0·7 M K_3PO_4 (0·5 mL). This solution was vortexed and then centrifuged at 2000 *g* for 10 min at 0°C. The supernatant was collected and 20 μL injected onto the column.

Pyruvate and lactate were measured according to published methods (Czok & Lamprecht 1974), using a split beam spectrophotometer (Hitachi 2000, Japan).

All statistical comparisons of sample means were made using the unpaired Student's *t*-test. Histological sections of hepatic tissue were processed by the Department of Anatomical Pathology, St Vincent's Hospital, Australia.

Results

Total body weight (control 285 ± 19; choline-deficient 323 ± 12 g ($P < 0·01$)) and liver weights (control 4·1 ± 0·6, choline-deficient 5·9 ± 1·1 g/100 g ($P < 0·01$)) were increased by the choline-deficient diet, but spleen and kidney weights expressed as a proportion of body weight, did not differ from controls. To compare hepatic elimination between the groups, three sets of perfusion rates were employed. Perfusion rates of 7 and 10 mL min^{-1} in the control group represented normalized rates of approximately 0·5 and 0·8 mL min^{-1} (g liver)$^{-1}$, respectively. Similarly rates of 10 and 15 mL min^{-1} in the choline-deficient group represent approximately 0·5 and 0·8 mL min^{-1} (g liver)$^{-1}$, respectively.

Histological sections of livers from choline-deficient rats showed extensive fat accumulation by hepatocytes with displacement of nuclei and cytoplasmic components to the periphery. However, there was no evidence of fibrous septa extending through the parenchyma.

The lactate/pyruvate ratio did not differ between groups at any perfusion rate.

The effects of a choline-deficient diet on hepatic extraction ratio and hepatic clearance of morphine is shown in Table 1. There were no significant differences in either parameter between control and choline-deficient livers. If the data for extraction ratio (control 0·55 ± 0·01; choline-deficient 0·45 ± 0·04 mL min^{-1} (g liver)$^{-1}$) and hepatic clearance (control 0·46 ± 0·08; choline-deficient 0·37 ± 0·11 mL min^{-1} (g liver)$^{-1}$) and intrinsic hepatic clearance (control 0·85 ± 0·05; choline deficient 0·77 ± 0·09 mL min^{-1} (g liver)$^{-1}$) are normalized for liver weight, by comparing controls at a flow rate of 10 mL min^{-1} with choline deficient at 15 mL min^{-1}, there were again no significant differences. Intrinsic hepatic clearance (a measure of hepatic enzyme activity) was calculated on the basis of the venous equilibrium model (Wilkinson & Shand 1975).

Table 1. The effects of a choline-deficient diet on morphine extraction ratio and hepatic clearance at various flow rates.

Flow rate	Control		Choline-deficient	
	Extraction ratio	Hepatic clearance	Extraction ratio	Hepatic clearance
7	0·65 ± 0·02	4·5 ± 0·1	N.D.	N.D.
10	0·51 ± 0·02	5·1 ± 0·2	0·53 ± 0·01	5·3 ± 0·1
12	0·45 ± 0·03	5·4 ± 0·4	0·51 ± 0·04	6·0 ± 0·5
15	N.D.	N.D.	0·45 ± 0·04	6·8 ± 0·6

N.D. = not detectable.

When all studies were considered, there was a significant correlation between hepatic clearance and perfusion rate ($y = 0·41 \times + 0·106$, $r = 0·726$ for control; $y = 0·38 \times + 0·073$, $r = 0·745$ for choline-deficient) and between hepatic clearance and intrinsic clearance ($y = 0·22 \times + 0·226$, $r = 0·816$ for control; $y = 0·39 \times + 0·074$, $r = 0·901$ for choline-deficient). There was no effect of the liver injury on these relationships.

Discussion

The choline-deficient diet provides high amounts of fat and eliminates lipotropic agents such as choline and methionine. Consequently, rats fed this diet for prolonged periods accumulate triglycerides in hepatocytes. As the triglycerides accumulate, the hepatocyte cytoplasm becomes distorted and loses its functional capacity. Hepatic necrosis and subsequent replacement with fibrosis tissue has also been previously reported (Murray et al 1986), but was not seen in this study.

Despite these gross morphological changes, hepatic elimination of morphine was not significantly affected. The choline-deficient diet did not alter either extraction ratio or intrinsic clearance of morphine. This suggests that glucuronidation is spared in this nutritional model of chronic liver injury. This is in contrast to the reports of impaired metabolism of several oxidized substrates (Murray et al 1986).

Choline-deficient diet has been shown to alter the structure and composition of microsomal membranes (Tinoco et al 1979); this may be responsible for the preserved glucuronidation of morphine in this model, i.e. reduced enzyme concentration but increased availability of the active site to the substrate.

References

Bartosek, I., Guaitani, A., Miller, L. L. (1973) Isolated Liver and Its Applications. Raven Press, New York

Czok, R., Lamprecht, W. (1974) Pyruvate, phosphoenolpyruvate and D-glycerate-2-phosphate. In: Bergenmeyer, H. U. (ed.) Advances in Enzymology. Academic Press, New York, p. 1446

McLean, E. K., McLean, A. E. M., Sutton, P. M. (1969) An improved method for producing cirrhosis of the liver in rats by simultaneous administration of carbon tetrachloride and phenobarbitone. Clin. Pharmacol. Ther. 25: 161–166

Murray, M., Zaluzny, L., Farrell, G. C. (1986) Drug metabolism in cirrhosis. Selective changes in cytochrome P-450 isozymes in the choline-deficient rat model. Biochem. Pharmacol. 35: 1817–1824

Tinoco, J., Endeman, G., Medwadowski, B., Miljanich, P., Williams, M. A. (1979) Ethanolamine kinase activity and compositions of diacylglycerols, phosphatidylcholines and phosphatidylethanolamines in livers of choline-deficient rats. Lipids 14: 968–974

Villeneuve, J. P., Wood, A. J., Shand, D. G., Rogers, L., Branch, R. A. (1978) Impaired drug metabolism in experimental cirrhosis in the rat. Biochem. Pharmacol. 27: 2577–2581

Wilkinson, G. R., Schenker, S. (1975) Drug disposition and liver disease. Drug Metab. Rev. 4: 139–175

Wilkinson, G. R., Shand, D.G. (1975) A physiological approach to hepatic drug clearance. Clin. Pharmacol. Ther. 18: 377–390