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Incidence of experimental cirrhosis on hepatic disposition of [^{3}H] levamisole in rats

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Levamisole [phenyl-2 ^{3}H] was injected intravenously (4·7 mg kg^{-1}) into anaesthetized controls and rats in which cirrhosis had been induced by a combination of carbon tetrachloride and phenobarbitone. The biliary excretion (6 h) of the parent drug and its metabolites formed a significant part of the administered dose. Although bile flow did not vary, biliary excretion of levamisole and metabolites were respectively increased and decreased in cirrhotic compared with control animals. These differences could be the result of cirrhosis-induced decrease in the hepatic biotransformation of levamisole and also to limited active carrier transport for the output of metabolites into bile canaliculi.

Levamisole, the laevo-rotary isomer of tetramisole (2, 3, 5, 6-tetrahydro 6-phenyl imidazo (2,1-b) thiazole) is a broad spectrum anthelminthic active against most nematodes (Thienpont et al 1966). It also has immunostimulant effects and has recently been proposed for clinical use in man under specific conditions (Brunner & Muscoplat 1980) in the treatment of chronic active hepatitis (Chadwick et al 1977). In veterinary medicine the drug is largely used in parasite diseases.

We have used an animal model of cirrhosis (Mac Lean et al 1969) to investigate whether this induced liver condition modified biliary excretion and hepatic disposition of [^{3}H] levamisole in rats, as there appears to be no information on the fate of this drug in the liver of any animal species.

METHODS

Induction of cirrhosis

Male Sprague Dawley rats, ≃250 g, were used. Cirrhosis was produced by simultaneous administration of carbon tetrachloride and phenobarbitone (Mac Lean et al 1969). The carbon tetrachloride was administered by twice weekly inhalation of compressed air bubbled through the solvent and then into a plastic box (40 litres) at 1 litre min^{-1}. The solvent was carried into the box for 2 min on the first gassing, 3 min on the second and third gassing and 5 min thereafter. The rats were then left in the cage for the same time as the gassing period. Phenobarbitone was given in the drinking water at a concentration of 0·5 g litre; a fresh solution was prepared twice weekly. Control rats received water and were not gassed.

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Cirrhosis was histologically defined to be present when fibrous tissue bands completely encircled zones of hepatic parenchymal elements. Loss of hepatocytes corresponded to formation of regenerative nodules. Only rats which showed histological evidence of cirrhosis were included in the cirrhotic group. Furthermore, increases in glutamatic oxaloacetic transaminase and α-glutamyl transpeptidase levels were determined in the plasma of cirrhotic animals and the bromsulfonephthalein clearance assay (Toulet & Albot 1966), in these rats clearly demonstrated the cirrhotic syndrome (K_1 = 4·3% vs 21·3% in control rats).

For both biliary excretion and hepatic distribution studies, anaesthetized rats were given intravenously in 0·5 ml via the dorsal penile vein a dose of 4·7 mg kg^{-1} [^{3}H] levamisole. Each animal received around 2 μCi of labelled compound. The dosage corresponded to a diluted mixture of an injectable commercial form (Nemisol, Specia) and the tritiated levamisole [phenyl-2 ^{3}H] HCl distributed by I.R.E. (3·5 Ci mmol^{-1}).

Biliary excretion

Groups of six control and six cirrhotic rats were anaesthetized (ethyl carbamate i.p.). The common bile duct was exposed through a left side incision and cannulated with a 20 cm length of silastic medial grade tubing. Body temperature was maintained at 37·5 °C by means of a heating pad placed under animal. A 20 min equilibration period was allowed between the preparation of the animal and the initiation of the experiment.

Immediately after the intravenous administration of [^{3}H] levamisole, sampling of bile was begun. Bile samples corresponding to 30 min periods were col-

lected for 6 h in tared 1·5 ml vials presented by an automatic fraction collector. Volume was determined gravimetrically assuming a specific gravity of 1·0 of bile. At the end of the experiment blood samples and liver were removed for analysis.

Hepatic distribution

Groups of six control or cirrhotic rats were prepared and treated as for biliary excretion except that three bile samples were collected over 1 h after the drug had been injected corresponding to time 0–30, 30–55 and 55–60 min after which the animals were killed, the abdomen opened and blood drawn from the abdominal aorta. The liver was removed immediately, freed of extrahepatic tissue, blotted free of excess moisture and weighed (at 0–4 °C) according to De Duve et al (1955). The liver was homogenized with 35 ml of ice cold 0·25 M sucrose pH 7·0 in a Potter homogenizer with a Teflon pestle.

Unbroken cells and nuclei were removed by centrifugation at 600 *g* for 10 min (nuclear fraction). The mitrochondrial and lysosomal fractions were obtained by centrifugation at 7000 *g* (9 min) and 35 000 *g* (9 min) respectively. The microsomal and the supernatant fractions were separated by centrifugation in the Ti 50 rotor of a Beckman L5 50 ultracentrifuge (35 min at 165 000 *g*). Each fraction was used for determination of radioactivity, levamisole and protein (Lowry et al 1951).

Analysis of biological samples

^{3}H radioactivity was estimated by liquid scintillation counting. Exchange of tritium from labelling with the general waterpool does not occur (verified by distillation assay from urine of injected animals). Liver homogenates, suspension of liver fractions, bile and plasma were diluted in liquid scintillation medium (Packard, Instagel). All results were corrected for quenching by automatic external standardization.

Levamisole was measured by high pressure liquid chromatography (Alvinerie et al 1981). 0·2 ml of biological samples were added to 1 ml NaOH 0·1 M and 10 ml of chloroform. After being shaken and centrifugation, the organic phase was evaporated and the residue dissolved in 100 µl of elution solvent which was further injected into the column (250 × 4·6 mm i.d. bondapack C18) of a model 202 chromatograph Waters equipped with an u.v. detector (λ = 225 nm); the mobile phase was 2 per cent acetic acid in water–methanol–heptane sulphonic acid (55:45:0·2 v/v/v). The detection limit of the method was 0·02 µg ml^{-1}).

RESULTS

Biliary excretion

Bile volume remained relatively constant for the duration of experiment: 2·95 ± 0·31 ml kg^{-1} h^{-1} and 3·08 ± 0·30 ml kg^{-1} h^{-1} for control and cirrhotic rats respectively; levamisole had no significant effect on bile flow. After intravenous injection of [^{3}H] levamisole, the biliary excretion of radioactivity reached a maximum in 0·5–2 h and subsequently decreased for all rats (Fig. 1). The excretion of the parent drug represents only a small percentage of the excreted radioactive materials for both treated and control animals (4·6 and 1·5% respectively).

Induction of experimental cirrhosis significantly decreased the biliary excretion of radioactivity for the first 3 h. Consequently, there was a significant difference in the excreted tritium for the duration of experiment between control (33·8 ± 6·4) and cirrhotic rats (18·3 ± 5·4% of the administered dose). In contrast in the first hour there was a slight increase in excreted levamisole from treated animals compared with controls. The choice of 1 h as the time for killing the rats used in the hepatic distribution study was because of the significant difference between control and cirrhotic rats for levamisole and tritium biliary excretion.

Hepatic distribution

Determination of protein contents of subcellular fractions clearly demonstrated the decrease in nuclear (34·3 ± 8·9 vs 69·5 ± 8·3 mg g^{-1} liver) and microsomal (10·7 ± 3·0 vs 16·4 ± 1·7) proteins for cirrhotic rats. Other contents were not significantly different, these were 21·1–23·2; 7·1–7·4 and 31·4–31·6 for mitochrondria, lysosomes and supernatant respectively.

Tritium and levamisole concentrations in plasma, liver homogenate and bile are given in Table 1, radioactivity levels correspond to [^{3}H] levamisole-equivalent as expressed in µg g^{-1} (or ml^{-1}) of each tissue or fluid. Plasma levels were similar between

Table 1. Respective tritium and levamisole concentrations in plasma, liver homogenate and bile of controls and cirrhotic rats (Mean ± s.e. of six experiments).

	Tritium equivalents (µg g^{-1})		Levamisole (µg g^{-1})	
	Controls	Cirrhotic	Controls	Cirrhotic
Plasma	2·13 ± 0·49	1·93 ± 0·40	1·31 ± 0·45	0·88 ± 0·33
Liver hom.	10·02 ± 0·68	*7·99 ± 0·92	3·53 ± 0·44	*6·18 ± 0·91
Bile	96·30 ± 11·84	*13·51 ± 3·36	0·95 ± 0·42	*2·89 ± 0·76

Each animal received an intravenous dose of levamisole (4·7 mg kg^{-1}) 1 h before death. The results have been analysed by Student's *t*-test and * indicates a statistically difference ($P < 0·05$) between the control and cirrhotic values.

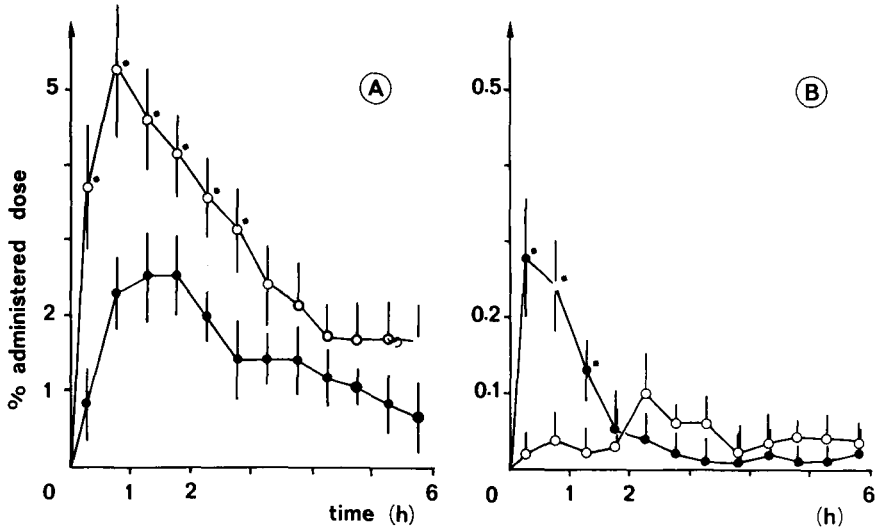

FIG. 1. The effect of experimental cirrhosis on the biliary excretion of radioactivity (A) and levamisole (B). The labelled levamisole was given by intravenous injection at 0 h. Data are expressed in terms of the administered compound. Statistically significant differences ($P < 0.05$) between normal (○) and cirrhotic (●) rats are indicated by asterisks as compared by Student's *t*-test.

control and cirrhotic rats although levamisole represented only 45 to 60% of the plasma radioactivity. In liver homogenate and bile of cirrhotic animals, there was a decrease in tritium related to an increase in levamisole content; effectively, as shown in Fig. 1, 1 h after administration of the drug there was significant decrease in excretion of radioactivity and increase in biliary levamisole for cirrhotic animals compared with control rats. Under these conditions, the levamisole liver/plasma ratio is greater for cirrhotic rats (7·02 vs 2·69) whereas bile/liver ratio for radioactivity is lower in the same animals (1·69 vs 9·61).

Table 2. Respective tritium and levamisole concentrations in nuclear, mitochrondrial, lysosomal, microsomal and supernatant fractions of controls and cirrhotic rat livers.

	Tritium equivalents (μg g^{-1})		Levamisole (μg g^{-1})	
	Controls	Cirrhotic	Controls	Cirrhotic
Nuclei	11·99 ± 0·79	10·25 ± 1·51	6·31 ± 0·60	7·91 ± 2·66
Mitochondria	16·39 ± 2·61	14·20 ± 1·64	5·25 ± 0·66	*7·81 ± 1·17
Lysosome	13·00 ± 3·42	15·42 ± 2·53	3·02 ± 0·48	4·41 ± 1·08
Microsome	24·90 ± 4·13	29·47 ± 3·04	4·26 ± 0·49	3·77 ± 0·47
Supernatant	6·73 ± 0·38	*5·67 ± 0·71	1·68 ± 0·78	*4·67 ± 0·56

For legends, see Table 1.

The comparisons in tritium and levamisole levels in liver subcellular fractions are given in Table 2. There was a significant decrease in supernatant tritium content whereas levamisole concentrations were significantly greater in mitochondrial and supernatant fractions from cirrhotic animals.

DISCUSSION

In contrast to analgesics (Neal et al 1979) and propranolol (Branch et al 1977), the hepatic clearance of which was decreased by experimental cirrhosis with a correlative enhancement in blood bioavailability, the biliary excretion of levamisole was increased in cirrhotic rats. A decrease in hepatic biotransformation of the drug could be responsible for this change, this could be due either to a pathological shunting of the drug within the liver or to a reduction in liver enzyme activity. Effectively in the rat, levamisole metabolism consists essentially of oxidative reactions such as the introduction of a double bond into the imidazole ring, the conversion of the sulphur to sulphoxide and hydroxylation in the *para*-position of the phenyl ring (Graziani & De Martin 1977). The experimental cirrhosis produced by a combination of carbon tetrachloride and phenobarbitone administration also led to both reduced cytochrome P450 levels (Marshall & Mac Lean 1969) and inhibition of the microsomal oxidation enzyme system (Miyamoto et al 1977) in rat livers.

The decreased biliary excretion of radioactive materials is not related to a reduced bile flow because this did not change between control and cirrhotic rats. Since there was no exchange of tritium, the radioactivity should correspond to levamisole and its phenyl [2-^{3}H]-containing metabolites (Graziani & De Martin 1977). So, a biotransformation process takes place in the liver of normal rats as indicated by comparison of levamisole/radioactivity ratios

obtained 1 h in plasma, (0·615), liver (0·352) and bile (0·14) after intravenous injection. In cirrhotic animals, the corresponding ratios were significantly increased for liver and bile because of the decreased hepatic biotransformation of the drug. The increased levamisole liver/plasma ratio in treated animals can be related to the corresponding enhanced liver homogenate concentration. The observed increase in drug concentration in the supernatant might be due to the increased biliary levels of levamisole. Furthermore with the cirrhotic liver there was no impaired ability to remove levamisole from blood, unlike findings with propanolol in man (Pessayre et al 1978).

The hepatic distribution of radioactivity demonstrated the decrease in liver homogenate levels. This would also correspond with the decrease in bile concentrations and simultaneous increase in levamisole in homogenate and bile. Since the total radioactivity should correspond to levamisole and its major metabolites, the decrease in the bile/liver ratio of radioactivity for cirrhotic rats might be due to a decrease in the transfer of metabolites from the hepatocyte into the bile; effectively this transport did not vary for the parent drug and the data did not identify which metabolite had a reduced biliary excretion (an unpublished complementary experiment has demonstrated that biliary radioactivity consisted of highly water-soluble materials that would not be either glucoronide or sulphate conjugates). It would be also required to determine the molecular mechanism for this inhibition of the transport process into the bile canaliculi of cirrhotic rats. Some investigators have suggested that biliary output, like hepatic uptake, might be dependent on active carrier-mediative transport as determined for acetyl procainamide ethobromide (Hwang & Schanker 1973), tetracycline (Lanman et al 1973) or amethopterin (Strum & Liem 1977); so cirrhosis might induce a decreased activity of some carrier proteins required for the active biliary output of watersoluble metabolites.

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REFERENCES

Alvinerie, M., Galtier, P., Escoula, G. (1981) J. Chromatogr. 223: 445–448

Branch, R. A., Kornhauser, D. M., Shand, D. G., Wilkinson, G. R., Wood, A. J. J. (1977) J. Clin. Pharmacol. 4: 630 p

Brunner, C. J., Muscoplat, C. C. (1980) J. Am. Vet. Med. Assoc. 176: 1159–1162

Chadwick, R. G., Jain, S., Thomas, H. C. (1977) Gut 18: A 979

De Duve, C., Pressman, B. C., Gianetto, R., Wathiaux, R., Appelmans, F. (1955) Biochem. J. 60: 604–168

Graziani, G., De Martin, G. L. (1977) Drugs Exp. Clin. Res. 2: 221–233

Hwang, S. W., Schanker, L. S., (1973) Am. J. Physiol. 225: 1437–1443

Lanman, R. C., Muranishi, S., Schanker, L. S. (1973) Ibid. 225: 1240–1246

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Mac Lean, E. K., Mac Lean, A. E. M., Sutton, P. M. (1969) Br. J. Exp. Pathol. 50: 502–506

Marshall, W., Mac Lean, A. E. M. (1969) Ibid. 50: 578–583

Miyamoto, J., Okuno, Y., Kadota, T., Mihara, K. (1977) J. Pest. Sci. 2: 257–269

Neal, E. A., Meffin, P. J., Gregory, P. B., Blaschke, T. F. (1979) Gastroenterol. 77: 96–102

Pessayre, D., Lebrec, D., Descatoire, V., Peignoux, M., Benhamou, J. P. (1978) Gastroenterol. 74: 566–571

Strum, W. B., Liem, H. H. (1977) Biochem. Pharmacol 26: 1235–1240

Thienpont, D., Vanparijs, O. F. J., Rayemaekers, A. H. M., Vandenberk, J., Demoen, P. J. A., Allewijn, F. T. N., Marsboom, R. P. H., Niemegeers, C. J. E., Schellekens, K. H. L., Jansen, P. A. J. (1966) Nature (London) 209: 1084–1086

Toulet, J., Albot, G. (1966) Arch. Fr. Malad. Appar. Dig. 55: 999–1014