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THE EFFECT OF RIFAMPICIN ON LIVER BLOOD FLOW, MICROSOMAL ENZYME ACTIVITY AND BILE FLOW IN THE RAT

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Abstract—Chronic rifampicin treatment (20 mg/kg/day orally, or 60 mg/kg/day intraperitoneally (i.p.)) had no effect on rat hepatic microsomal enzyme activity. Bile flow rate increased significantly after treatment (200 mg/kg/day orally) for 1, 2 or 4 days and returned to normal levels within 2 days of the final treatment of a 4 day period. Liver blood flow remained unchanged following rifampicin treatment and hence the increased bile flow was not dependent on an increased blood flow.

Reports on the effect of rifampicin on the liver of rats are conflicting. Barone *et al.* [1] and Pessayre and Mazel [2] were unable to detect any changes in hepatic drug metabolizing enzymes following chronic rifampicin treatment, whereas Otani and Remmer [3] and Bolt and Remmer [4] reported an increase in certain hepatic drug metabolizing enzymes. Similarly, bile flow has been reported to decrease [5] and increase [6] following rifampicin administration. Such equivocal data has prompted us to investigate the effect of chronic rifampicin treatment on both hepatic microsomal enzyme activity and bile flow. In addition, since we have previously reported the differential effects of hepatic microsomal enzyme inducers on liver blood flow [7], we wanted to know whether or not rifampicin could be classified with phenobarbitone in increasing liver blood flow in the rat.

MATERIALS AND METHODS

Animals. Mature male rats (220–350 g) of the Wistar strain were housed in groups in cages in well ventilated rooms at a temperature of approximately 24°.

Treatment. Solutions of rifampicin (Lepetit, Milan) were prepared immediately before use. For enzyme induction and blood flow studies the drug was suspended in physiological saline (60 mg/ml), and NaOH (4 M; 10 μl) added to achieve solution. Rats were injected intraperitoneally (i.p.) daily for 5 days at a dose of 60 mg/kg. Determinations of microsomal enzyme activity and liver blood flow were carried out on the 7th day. Rats were randomly allocated to either blood flow or enzyme studies. In some enzyme induction experiments, rifampicin (20 mg/kg) was administered orally (by gastric intubation) for 5 days and determinations of enzyme activity carried out on the 8th day. Control rats received the vehicle only. For bile flow studies, rifampicin was dissolved in physiological saline and NaOH and administered orally for 1, 2 or 4 days at a dose of 200 mg/kg. Control rats received the vehicle only. Bile flow was determined either 18 or 42 hr following the final drug administration.

Measurement of liver blood flow. Animals were anaesthetised with sodium pentobarbitone (50 mg/kg; i.p.). The right carotid artery was cannulated and with the aid of pressure monitoring the tip of the cannula was manipulated into the left ventricle. 60,000–80,000 carbonized microspheres (15 ± 5 μm diameter; 3M Company, St. Paul, Minnesota) labelled with ^{85}Sr were injected into the left ventricle over 20 sec in a total vol. of 0.6 ml of 0.01 per cent Tween 80 in physiological saline. Simultaneously, blood was withdrawn from a femoral artery at a constant rate (0.6 ml/min) for 90 sec with a syringe withdrawal pump (Perfusor IV, Braun, Melsungen). Arterial blood pressure was recorded from the other femoral artery by means of a pressure transducer (Bell & Howell type 4-422-0001) and a pen recorder.

Cardiac output and liver blood flow were determined by the method of McDevitt and Nies [8]. In this method, hepatic arterial flow is determined from the microspheres trapped in the liver and portal venous return is obtained indirectly by adding together the flows to the spleen, pancreas and gastrointestinal tract. Liver blood flow therefore refers to the sum of hepatic arterial and portal venous flows.

Measurement of enzyme activity. Rats were killed by cervical dislocation, the livers rapidly removed and homogenized in ice-cold 1.15% KCl using a Teflon in glass homogenizer. The 30% homogenate was centrifuged at 10,000 *g* for 20 min at 4°. The resultant supernatant was decanted without disturbing the pellet and centrifuged at 105,000 *g* for 60 min at 4°. The microsomal pellet was resuspended in 0.2 M phosphate buffer (pH 7.4; 10 ml). Microsomal protein, cytochrome P-450 and cytochrome *c*-reductase were determined respectively by the methods of Lowry *et al.* [9], Omura and Sato [10] and Williams and Kamin [11].

Pentobarbitone sleeping time was determined in groups of 4 rats as the time between loss and recovery of righting reflex after injection of pentobarbitone (40 mg/kg; i.p.).

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Table 1. The effect of rifampicin (60 mg/kg/day i.p.) on liver weight, liver enzyme activity and liver blood flow

	Control	Rifampicin	% Change
Body weight (g)	287 ± 16 (14)	312 ± 19 (6)	+ 8.7
Cardiac output (ml/min/100 g b.wt)	22.2 ± 3.2 (7)	22.4 ± 0.33 (6)	+ 0.9
Mean arterial pressure (mm Hg)	116 ± 7.0 (7)	118 ± 4.0 (6)	+ 1.7
Liver weight (g)	11.0 ± 0.32 (14)	*12.9 ± 0.53 (6)	+ 17.3
Liver weight (g/100 g b.wt)	3.83 ± 0.11 (14)	4.16 ± 0.17 (6)	+ 8.6
Liver blood flow (ml/min/g liver)	1.33 ± 0.15 (7)	1.17 ± 0.05 (6)	− 12.0
Liver blood flow (ml/min/100 g b.wt)	5.20 ± 0.15 (7)	4.70 ± 0.18 (6)	− 9.6
Microsomal protein (mg/g liver)	21.7 ± 1.84 (6)	20.3 ± 2.26 (5)	− 6.5
Cytochrome P-450 (nmol/mg prot)	0.80 ± 0.08 (6)	0.71 ± 0.08 (5)	− 11.3
Cytochrome *c*-reductase (nmol/mg prot/min)	43.0 ± 3.92 (6)	47.8 ± 8.75 (5)	+ 10.5
Pentobarbitone sleeping time (min)	104 ± 13.5 (4)	112 ± 12.6 (4)	+ 7.7

Each value is the mean ± S.E.M., with the number of animals in parentheses.
*Significantly different from control. $P < 0.05$ (Student's *t*-test).

Bile flow studies. Rats were anaesthetised with urethane (14% w/v in 0.9% saline: 10.0 ml/kg: i.p.). A polyethylene catheter was inserted into the common bile duct. Body temperature was maintained at 37° using a heated operating table and a warming lamp. Bile samples were collected in preweighed glass vials at 30 min intervals for 4 hr.

RESULTS

Table 1 shows the lack of effect of rifampicin (60 mg/kg/day) on liver weight, liver enzyme activity and liver blood flow. The significant increase ($P < 0.05$) in liver weight when expressed in grams was not seen when allowance was made for body weight (b.wt) and expressed as g/100 g b.wt. Rifampicin treatment resulted in a slight fall in liver blood flow/g liver and liver blood flow/100 g b.wt. There was no evidence of increased enzyme activity, and all parameters remained similar to controls.

Administration of rifampicin (20 mg/kg/day; orally) for 5 days followed by a 2 day drug-free period prior to the *in vitro* assays failed to increase microsomal enzyme activity (Table 2). A significant increase ($P < 0.05$) was seen in liver weight (g) but when allowance was made for body weight, the increase (5.6%) was statistically insignificant ($P > 0.05$).

There was a marked increase in bile flow rate in rats treated with rifampicin (200 mg/kg) orally for 1, 2 or 4 days compared to controls treated with vehicle only (Fig. 1). There was no significant difference in bile flow rate between individual treatment periods (e.g. between 1 and 2 days or 1 and 4 days). The foregoing bile flow studies were done approximately 18 hr after the final drug administration. When 42 hr were allowed to elapse after the final administration of a 4 day treatment period, bile flow rate was not significantly different from control values.

DISCUSSION

The findings of the present work do not support the claim of Otani and Remmer [3] and Bolt and Remmer [4] that treatment of rats with rifampicin specifically induces hepatic cytochrome *c*-reductase. Using the same dosing regimen as previously described to cause induction (20 mg/kg/day orally for 5 days followed by a gap of 2 days before animals were sacrificed) we were unable to detect any significant changes in microsomal enzyme activity. Treatment with a higher dose of rifampicin (60 mg/kg/day for 5 days i.p.) also failed to increase microsomal enzyme activity. Our results, therefore, concur with those of Pessayre and Mazel [2] who found no evidence of induction with chronic administration of rifampicin to rats.

The increase in bile flow rate following rifampicin treatment (200 mg/kg/day orally for 1, 2 or 4 days) is similar to that previously reported by Roze *et al.* [6]. These workers were able to demonstrate an increase in bile flow rate after both acute and chronic administra-

Table 2. The effect of rifampicin (20 mg/kg/day administered orally) on liver weight and liver enzyme activity

	Control	Rifampicin	% Change
Body weight (g)	222 ± 3.0 (12)	239 ± 12.3 (7)	+ 8.6
Liver weight (g)	9.54 ± 0.24 (12)	*10.86 ± 0.64 (7)	+ 13.8
Liver weight (g/100 g b.wt)	4.30 ± 0.10 (12)	4.54 ± 0.15 (7)	+ 5.6
Microsomal protein (mg/g liver)	23.8 ± 1.36 (12)	24.0 ± 1.55 (7)	+ 0.8
Cytochrome P-450 (nmol/mg prot)	0.90 ± 0.10 (12)	0.94 ± 0.05 (7)	+ 4.4
Cytochrome *c*-reductase (nmol/mg prot/min)	37.5 ± 4.76 (12)	40.5 ± 3.42 (7)	+ 8.0
Pentobarbitone sleeping time (min)	107 ± 14.0 (4)	102 ± 9.5 (4)	− 4.7

Each value is mean ± S.E.M. with the number of animals in parentheses.
*Significantly different from control. $P < 0.05$ (Student's *t*-test).

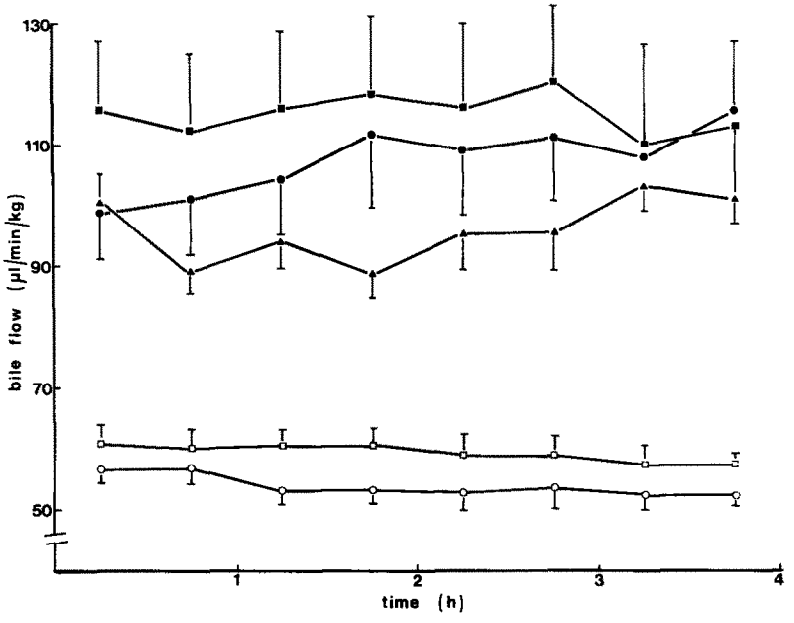

Fig. 1. The effect of rifampicin (200 mg/kg; orally) administered for 1 (●——●), 2 (▲——▲) or 4 (■——■) days on bile flow rate (μl/min/kg).
○——○, Control rats treated with vehicle alone. ┆——┆, Rats treated with rifampicin for 4 days with a gap of 1 day prior to bile flow determination. Each point is the mean + S.E.M. of at least 5 experiments.

tion of rifampicin. The present findings and those of Roze *et al.* [6] are therefore at variance with the claim of Keberle *et al.* [5] that a single dose of rifampicin (200 mg/kg) given orally has a cholestatic effect.

Since bile flow is an important determinant of the excretion of certain compounds into bile [12, 13] it is relevant to ascertain whether or not increases in bile flow rate correlate with other alterations in hepatic physiology. Klaassen [14] studied the effect of various known enzyme inducers (phenobarbitone, nikethamide, chlordane, chlorcyclizine, phenylbutazone, 3-methylcholanthrene and 3,4-benzpyrene) on biliary flow, liver weight and microsomal enzyme activity. Only phenobarbitone produced a significant increase in bile flow rate and this correlated with an increase in liver weight and microsomal enzyme activity. However, since other of the agents (e.g. 3-methylcholanthrene and 3,4-benzpyrene) increased both liver weight and enzyme activity without altering bile flow rate it was concluded that microsomal enzyme induction itself does not produce a choleresis.

We have previously examined the relationship between hepatic microsomal enzyme activity and liver blood flow [7]. All the drugs studied (phenobarbitone, amylobarbitone, antipyrine, chlordiazepoxide and phenytoin) caused an increase in liver weight and microsomal enzyme activity, but only phenobarbitone and amylobarbitone increased liver blood flow.

Phenobarbitone alone of drugs known to alter hepatic physiology has been shown to increase both bile and liver blood flow. It is highly probable therefore, that a direct correlation exists between the increased blood flow and bile flow. Previous studies with isolated perfused livers [15] have indicated the dependence of bile flow on blood flow rates.

With these considerations in mind and since rifampicin is such a potent choleretic we examined the effect of rifampicin on liver blood flow. When expressed in terms of either liver or body weight results in rifampicin treated animals were not significantly different from controls. Thus the increase in bile flow produced by rifampicin is unrelated to changes in liver blood flow.

Following phenobarbitone administration for seven days, bile flow remained significantly elevated for five days after the last dose [14]. In contrast, in the present study bile flow returned to control levels after only one day had elapsed following the final rifampicin treatment. Since, in addition, a comparable increase in bile flow was seen after 1, 2 or 4 days treatment with rifampicin it is suggested that the choleresis is at least in part dependent on the excretion of rifampicin and/or metabolites into the bile.

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