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Phenobarbitone effects on hepatic microsomal enzymes and liver blood flow in the guinea pig*

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Phenobarbitone is a potent inducer of liver microsomal enzymes and is known to increase liver blood flow in the rat [1–3] which is in contrast to other inducing agents such as 3,4-benzpyrene, phenytoin and chlordiazepoxide [1,2]. The increase in liver flow resulting from phenobarbitone pretreatment in the rat is dose-dependent and parallels the increase in liver weight [2]. This compensatory increase in hepatic blood flow is a result of increased portal venous return [1,2] and, together with the enhanced enzyme activity, could have important consequences for the rate of elimination of exogenous compounds [4].

As part of our investigation of the guineapig as a model of enzyme induction in man [5], we have studied the effects of phenobarbitone on hepatic microsomal enzyme activity and liver blood flow in this species.

Enzyme induction. Dunkin Hartley male guinea pigs weighing 320–420 g were housed in groups in rigid plastic cages with sawdust bedding. They were maintained on Oxoid Diet 18 and water (containing 50 mg ascorbic acid/l) *ad lib.* The animals were given intraperitoneal (i.p.) injections of phenobarbitone (40 mg/kg) once daily for 4 days. The phenobarbitone was injected in a volume 1 ml physiological saline/kg body weight (body wt.) and the control group of guinea pigs was injected with 1 ml physiological saline/kg body wt. The animals were randomly allocated to blood flow or enzyme studies.

Measurement of liver blood flow. The radioactive microsphere method was used [6]. Animals were anaesthetised with sodium pentobarbital (40–60 mg/kg i.p.; Sagatal, May & Baker). Artificial ventilation was given through a tracheal cannula. The right femoral artery was cannulated to allow withdrawal of blood at a constant rate (0.6 ml/min) during, and for 70 sec after, injection of the microspheres. Carbonized plastic microspheres of 15 ± 5 μm diameter labelled with ^{141}Ce (3M Co., St. Paul, MN, U.S.A.) were injected into the left ventricle over 20 sec through a cannula passed down the right carotid artery. The microspheres were suspended by ultrasonication in 0.6 ml physiological saline containing 0.02% (v/v) Tween 80. Cardiac output and liver blood flow were calculated as described by Nies *et al.* [1].

Preparation and assay of microsomal components. Animals were killed by cervical dislocation, their livers removed and placed immediately in ice-cold 1.15% KCl. The livers were blotted dry, weighed and roughly chopped over ice

Table 1. Effects of phenobarbitone on liver weight and blood flow in male guinea pigs

	Saline (n = 7)	Phenobarbitone (40 mg/kg/day) (n = 7)
Body weight (g)	373 ± 20	367 ± 10
Cardiac output (ml/min/100 g body wt.)	17.6 ± 0.6	17.5 ± 0.4
Mean arterial pressure (mmHg)	55.8 ± 3.5	56.8 ± 3.3
Liver weight (g/100g body wt.)	3.85 ± 0.23	4.91 ± 0.20**
Liver blood flow (ml/min/100 g body wt.)	5.17 ± 0.41	5.00 ± 0.34
Liver blood flow (ml/min/g liver)	1.36 ± 0.14	1.04 ± 0.11*
% Cardiac output to liver	29.7 ± 1.8	29.0 ± 2.3

Values given as mean ± S.E.M.
n = number of animals in groups.
Statistical significance from saline-treated animals using Student's *t*-test *P < 0.05, ** P < 0.01.

Table 2. Effects of phenobarbitone on liver weight, liver enzyme activity and pentobarbitone sleeping time in male quinea pigs

	Saline (n = 7)	Phenobarbitone (n = 4)
Body weight (g)	352 ± 28	358 ± 23
Liver weight (g/100 g body wt.)	3.54 ± 0.83	5.03 ± 0.19**
Microsomal protein (mg/g liver)	28.8 ± 8.2	64.3 ± 2.8***
Cytochrome P450 (nmol/mg protein)	0.87 ± 0.32	1.91 ± 0.19**
Cytochrome *c* reductase (nmol/g protein/min)	60.6 ± 13.8	167.9 ± 17.4**
Pentobarbitone sleeping time (min)	188 ± 22	112 ± 23**

Values are given as mean ± S.E.M.
n = number of animals in groups.
Statistical significance from saline-treated animals using Student's *t*-test: **P < 0.01 *** P < 0.001.

before being homogenized in 2 vol of 1.15% KCl using a motor driven Teflon in glass homogenizer (5 up-and-down strokes at 900 r.p.m.). The homogenate was then centrifuged at 10,000 *g* for 20 min at 4°. The supernatant was then further centrifuged at 105,000 *g* for 60 min at 4°. The resulting pellet was resuspended in 0.2 M phosphate buffer, pH 7.4. Protein concentration, cytochrome P450 content and cytochrome *c*-reductase activity were determined respectively by the methods of Lowry *et al.* [7], Omura and Sato [8] and Masters *et al.*[9].

Pentobarbitone sleeping time. Sleeping time was determined in groups of four guinea pigs as the time between loss and recovery of righting reflex after injection i.p. of 40 mg/kg pentobarbitone.

The results for liver blood flow and indices of induction are given in Tables 1 and 2 respectively. In contrast to the rat, phenobarbitone pretreatment did not result in an increase in liver blood flow relative to body weight. As a result of the hepatic enlargement liver perfusion (ml/min/g liver) was significantly reduced compared to saline-treated controls. Although cardiac output in the guineapig under pentobarbitone anaesthesia was lower than in the rat [1,2], liver blood flow in both species was similar at values of approximately 5 ml/min/100 g body wt. This is a result of a greater proportion of cardiac output being distributed to the hepatosplanchnic tissues in the guinea pig: 30 per cent rather than 25 per cent in the rat [10]. Since the increased liver blood flow after phenobarbitone treatment in the rat is a consequence of an increase in the proportion of the cardiac output passing through the splanchnic vascular bed, it may be that the guinea pig is unable to further increase splanchnic blood flow without a reduction in flow to other vital organs with a consequent compromise of their function.

The increased rate of drug elimination observed *in vivo* after phenobarbitone pretreatment in several species, including man, cannot be explained by changes in hepatic microsomal enzyme activity alone [11] and it has been postulated that increased liver blood flow also plays a part. The present study shows that increased blood flow does not accompany enzyme induction by phenobarbitone in all species. It can be seen from the results presented here that the guineapig provides a useful model of enzyme induction in which it is possible to investigate the effects of phenobarbitone on hepatic intrinsic clearance without the complication of increased liver blood flow.

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