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Pharmacokinetics of phenazone (antipyrine) in rabbits with experimental common bile duct obstruction

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1 An altered functional state of liver due to experimental cholestasis could result in a change in the biotransformation of drugs. The aim of this study was to evaluate an influence of obstructive cholestasis on the pharmacokinetics of phenazone (antipyrine).

2 The investigation was carried out on male rabbits, randomly allocated into two groups: sham-operated and animals with biliary ducts ligation. Phenazone was administered intragastrically as a probe of drug metabolism.

3 Measurements, i.e. laboratory and pharmacodynamic tests, as well as pharmacokinetic assays, were performed before the operation as well as 10-12 days after the bile duct ligation. At the end of the study livers were examined macro- and microscopically and biochemical analysis of the liver microsomes was performed.

4 The measured pharmacokinetic parameters suggested an impaired biotransformation of phenazone in animals with obstructive cholestasis, leading to a slower drug elimination.

Keywords: Biliary obstruction; phenazone pharmacokinetics; liver function

Introduction

There are limited data on the pharmacokinetics of drugs in subjects with biliary obstruction and the results obtained are often conflicting (Hepner & Vessel, 1977; Fruncillo *et al.*, 1982; Gawrońska-Szklarz *et al.*, 1983). Drugs, such as phenazone, can be used as model substances to study the biotransformation capacity of a particular metabolic pathway, thus indicating the stage of liver damage and the extent to which the structural integrity of hepatocytes is maintained (Wójcicki *et al.*, 1990; Smilgin *et al.*, 1994). Phenazone is mainly metabolized in the liver microsomes via the cytochrome P-450 pathway (Danhof & Teunissen, 1984; Viktorov and Rybak, 1990).

The aims of the study were to evaluate the pharmacokinetics of phenazone in relation to the biotransformation of the drug in mechanical, extrahepatic cholestasis. The results obtained during the investigation may also refer to other drugs, metabolized by means of cytochrome P-450.

Methods

The study was carried out on 20 male mongrel rabbits, randomly allocated into two groups. The control group which were sham-operated rabbits, included 10 animals, weighing at the beginning of the study 3.20-4.30 (average 3.60 ± 0.04) kg. The experimental group were 10 rabbits which underwent bile drug ligation and which weighed during the same study period 3.30-4.30 (average 3.77 ± 0.03) kg. All animals were housed under constant conditions and maintained on a standard diet and water *ad libitum*.

The operation procedure was performed under pentobarbitone anaesthesia (Vetbutal-Biowet) - 12 mg kg^{-1}, given intravenously. The peritoneal cavity was opened by an oblique incision under the right costal arch. After dissection, the common bile duct was ligated 1 cm from its duodenal outlet, and after visualization, the cystic bile duct was also ligated. Following ligation of the bile ducts, the peritoneum, muscles and epidermal layers were each sutured.

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The following assays were performed in both experimental groups: pharmacodynamic test, laboratory assays, pharmacokinetics of phenozone as well as macro-, microscopic and biochemical homogenate evaluation of the liver during autopsy. The *in vivo* assessments were performed before the investigation and 12 days after the operation procedure, but the pharmacodynamic test was done 10 days after ligation of the bile ducts. All animals were autopsied on day 16 of the study.

The pharmacodynamic test involved measuring the duration of sleeping-time after the administration of pentobarbitone at a dose of 12 mg kg^{-1} i.v.

The following laboratory blood tests were performed by routine methods: concentration of bilirubin, urea, creatinine, cholesterol and glucose as well as the activity of aspartate- and alanine-aminotransferases and alkaline phosphatase.

To investigate the pharmacokinetics of phenazone, the rabbits were given phenazone (Antypiryna-Polfa) intragastrically as a single dose of 50 mg kg^{-1}. Blood for pharmacokinetic assays was sampled from the marginal ear vein at the following intervals after the drug administration: 0.5, 1.0, 2.0, 4.0, 8.0, 12.0 h. Rabbits were fasted for 18 h before phenazone administration, and given water *ad libitum*. The method of Brodie *et al.* (1949) was employed for analysis of phenazone concentrations. In the analytical procedure of Brodie *et al.* (1949) phenazone is nitrosated in position 4 to give a green nitroso derivative, which is measured spectrophotometrically. A disadvantage of phenazone as a tool in drug metabolite studies is that it has several matabolites, but these metabolites are not detected with the procedure of Brodie *et al.* (1949). A one-compartment open model for extravascular administration was used for calculations. The following pharmacokinetic parameters were determined: area under the plasma concentration-time curve (AUC), maximum observed serum drug concentration (C_{max}), time to reach maximal concentration following drug administration (t_{max}), rate constant for elimination (λ_z), apparent volume of distribution (V_z) and relative apparent volume of distribution (V_z/BW), total (CL) and relative total body clearances (CL/BW) and the elimination half-life ($t_{1/2}$).

At the end of the experiment the animals were autopsied. The livers were weighed and examined macro- and microscopically. The microsomal fraction was prepared and analysed for cytochrome P-450 content by use of the method developed by Omura and Sato (1964), phospholipids (Bartlett, 1959), total cholesterol (Rudel & Morris, 1973) and protein concentration (Lowry, 1951). For microscopic study the liver was freeze-sectioned and stained with oil red 0 for the presence of neutral lipid. Other liver samples were placed into Schaffer's solution, and afterwards the prepared slices were stained with hematoxylin, eosin and paS.

Statistical evaluation was performed by use of paired Student's test within the same experimental group and unpaired Student's t test to compare the groups. A P value less than 0.05 was considered statistically significant.

Results

The first symptoms of jaundice appeared three days after the operation, in animals from the experimental group; activity was depressed and there was a yellow colour detectable in the ears and the conjunctivas. The intensity of these symptoms increased as the study period prolonged. Body weight of the jaundiced rabbits decreased by 18% ($P<0.001$) 14 days after the ligation in comparison to the control group.

The pentobarbitone sleeping-time in animals with bile duct obstruction was prolonged during the experimental period from 40 ± 1 min, before the operation, to 143 ± 3 min at the end ($P<0.001$), and was significantly longer in comparison to the controls [48 ± 2 min ($P<0.001$)] measured at the same study period.

There were no significant changes in the laboratory tests in the control animals, whereas in rabbits with ligated bile ducts, the following alterations in the blood serum were observed on the 12th day of the experiment (Table 1): a rise in bilirubin concentration from 1.19 ± 0.89 μmol l^{-1}, before the study, to 140 μmol l^{-1} ($P<0.001$), an elevation of activities of aspartate aminotransferase by 153% ($P<0.002$), alanine aminotransferase by 140% ($P<0.001$) and alkaline phosphatase by 95% ($P<0.05$) and in mean cholesterol concentration from 1.18 to 8.9 mmol l^{-1} ($P<0.001$). There were no alterations in the results of other laboratory tests performed, i.e. the blood serum concentration of glucose, creatinine and urea. There were no significant changes during the study in the sham-operated animals.

The concentrations of phenazone in animals with ligated bile ducts were elevated, as compared to the same animals prior to the operation (Figure 1). In contrast in the sham - operated animals (Figure 2) the levels of phenazone were similar or significantly lower (at 4, 6 and 8 h) compared with those before the operation.

The calculated pharmacokinetic parameters are presented in Table 2. There were no significant alterations in phenazone parameters for animals from the control group, measured before and after the operation. In contrast, all pharmacokinetic parameters were markedly changed in animals subjected to the bile duct ligation, compared with the preoperative results. The area under the plasma concentration-time curve (AUC) was increased by 141% ($P<0.001$), the maximal serum drug concentration (c_{max}) of phenazone was increased by 24% ($P<0.01$), the time to reach maximal concentration (t_{max}) was prolonged by 121% ($P<0.001$). The elimination rate constant, total body clearance (CL) and relative total body clearance

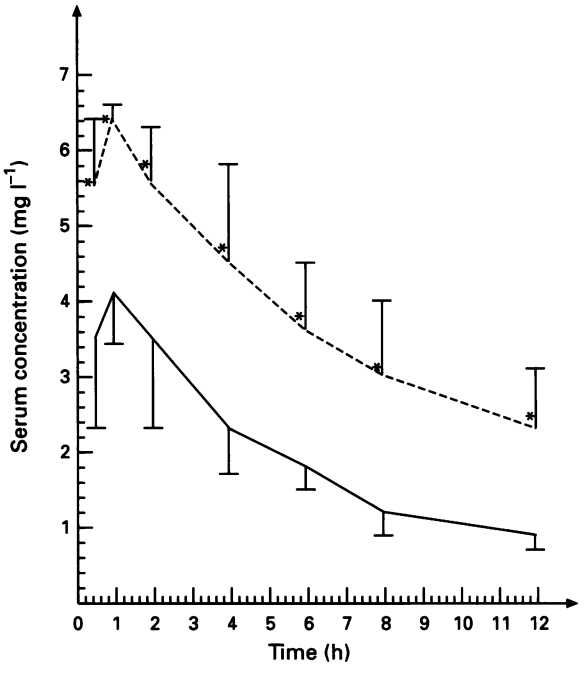

Figure 1 Mean serum concentrations of phenazone ($n=10$ at each point) after administration of 50 mg kg^{-1} (intragastrically) in bile duct ligated rabbits before the operation (————) and after the operative procedure (- - - - - -). Vertical lines show s.d. *Difference statistically significant.

Table 1 Basic laboratory biochemical tests before the operation (A, C) and 12 days after the operation (B, D)

	Sham-operated		*Ligated*		*Statistical significance (P)*		
Parameter	*A*	*B*	*C*	*D*	*A/B*	*C/D*	*B/D*
Total bilirubin (μmol l^{-1})	1.16±0.91	1.45±0.94	1.19±0.89	140±47	NS	<0.001	<0.001
Alkaline phosphatase (u l^{-1})	127±55	145±70	141±71	274±139	NS	<0.02	<0.02
Asparatate aminotransferase (u l^{-1})	58.6±28.9	35.3±20.6	50.5±20.1	127.0±66.7	NS	<0.002	<0.001
Alanine aminotransferase (u l^{-1})	12.7±59.9	65.8±39.0	85.8±69.0	205.7±62.1	NS	<0.002	<0.001
Urea (mmol l^{-1})	9.38±1.84	9.82±1.70	8.17±1.18	8.51±3.55	NS	NS	NS
Creatinine (μmol l^{-1})	122±25	129±17	117±14	167±78	NS	NS	NS
Glucose (mmol l^{-1})	6.63±0.43	6.49±0.56	6.68±0.42	6.16±0.75	NS	NS	NS
Total cholesterol (mmol l^{-1})	0.97±0.74	1.23±0.66	1.18±0.34	8.90±2.47	NS	<0.001	<0.001

Values shown are means ± s.d.
NS - denotes lack of statistical significance

(CL/BW) were decreased after the bile duct ligation by 38% ($P<0.001$), 59% ($P<0.001$) and 59% ($P<0.001$), respectively. The elimination half-life was prolonged by 55% ($P<0.001$). The apparent volume of distribution (V_z) and relative apparent (V_z/BW) volume of distribution were depressed significantly after the operative procedure by 37% ($P<0.001$) and 36% ($P<0.001$), respectively. The same alterations were observed when the results obtained in animals with obstructive jaundice were compared with the controls.

At the end of the study all animals were autopsied. The livers obtained from the animals with obstructive cholestasis were enlarged and more fragile, with a yellowish colour and strained capsules. Mean relative weight of the liver from the ligated group (37.89 ± 1.11 g) was significantly higher ($P<0.01$) in comparison with controls (28.04 ± 0.38 g). Cytochrome P-450 as well as cholesterol contents in the liver microsomes of animals with bile duct ligation decreased by 52% ($P<0.001$), from 2.03 ± 0.18 to 0.98 ± 0.19 mmol mg^{-1} protein and increased by 33% ($P<0.02$), from 0.097 ± 0.003 to 0.129 ± 0.002 mmol g^{-1} protein, respectively, as compared to the control rabbits. The other evaluated constituents of the endoplasmic reticulum were unchanged.

Microscopic evaluation of the liver demonstrated abnormalities in rabbits with cholestasis. The hepatocytes in rabbits from the control group were of normal morphology and contained glycogen granules both in the portion surrounding the terminal hepatic venules (zone 3) and in the intermediate tissue (zone 2) of the lobules. Hepatocytes in rabbits with obstructive jaundice contained grains of glycogen in small quantities, mostly in zone 3 and in zone 2. The Disse's spaces were filled with lipid droplets and a few, solitary lipid droplets were seen in the cytoplasm of the hepatocytes. Hepatocytes and Kupffer's cells in animals with ligated bile ducts contained small droplets of bile, predominantly within the portal space. Bile deposits were observed in the bile canaliculi. An enlargement of portal spaces, hypertrophy of bile ducts as well as dilatation of interlobular ducts were seen. Hyperplasia of the connective tissue in the portal spaces was noted. Some lymphoidal-cell infiltrations were also observed.

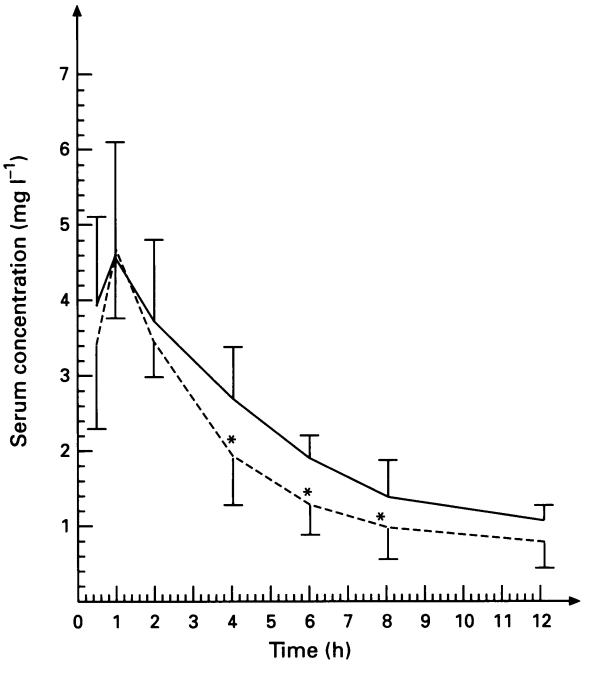

Figure 2 Serum concentrations of phenazone after administration of 50 mg kg^{-1} (intragastrically) in sham-operated rabbits before the operation (———) and after the operation procedure (- - - - - -). Values are means and vertical lines show s.d. ($n=10$ at each point). *Difference statistically significant.

Discussion

The influence of liver cirrhosis on the parmacokinetics of drugs has been presented in many papers, whereas any effects of acute and chronic cholestasis have not been well documented. There are available data, often contradictory, on the pharmacokinetics of tetracycline antibiotics (Gawrońska-Szklarz *et al.*, 1983), procainamide (Basseches & Digregorio, 1982), theophylline (Fruncillo *et al.*, 1982) as well as phenazone (Elfstrom & Lindgren, 1974; Hepner & Vesell, 1975), meprobamate, and pentobarbitone (Carulli *et al.*, 1975). The data suggest impaired elimination under mechanical, extrahepatic cholestasis. The present results show that the pharmacokinetics of phenazone are altered in rabbits with ligated bile ducts leading to a slower drug elimination. Phenazone is mostly metabolized in the liver via the cytochrome P-450 pathway (Danhof & Teunissen, 1984). Thus observed changes can be attributed to an impaired biotransformation in the liver microsomes.

One of the constituents of bile, deoxycholic acid has been found to disrupt and destroy certain microsomal structures (McLuen & Fouts, 1961). Therefore it was postulated that animals with obstructive jaundice might have an enhanced response to a given dose of drug. In the present study the cytochrome P-450 content in the liver microsomes was decreased, confirming that it could be the cause of the observed changes. The activity of integral membrane enzymes such as cytochrome P-450 often depends on the membrane lipid

Table 2 Pharmacokinetic parameters of phenazone in the control animals prior to the operation (A) and 12 days after operative procedure (B) and in animals with obstructive cholestasis (group 2) before operation (C) and after bile duct ligation (D)

Parameter (unit)	*Sham-operated*		*Ligated*		*Statistical significance (P)*		
	A	*B*	*C*	*D*	*A/B*	*C/D*	*B/D*
AUC (mg l^{-1} h)	35.74 ± 5.96	30.79 ± 5.83	27.49 ± 7.89	66.31 ± 15.28	NS	< 0.001	< 0.001
C_{max} (mg l^{-1})	4.92 ± 1.35	5.14 ± 1.01	4.64 ± 0.97	5.78 ± 0.97	NS	< 0.01	NS
t_{max} (h)	0.80 ± 0.02	0.75 ± 0.02	0.70 ± 0.02	1.55 ± 0.05	NS	< 0.001	< 0.001
λ_z (h^{-1})	0.14 ± 0.04	0.15 ± 0.02	0.17 ± 0.04	0.11 ± 0.02	NS	< 0.001	< 0.001
$t_{1/2}$ (h)	5.46 ± 1.86	4.75 ± 0.82	4.21 ± 0.90	6.54 ± 1.31	NS	< 0.001	< 0.01
V_z (l)	42.48 ± 10.73	35.41 ± 7.29	41.33 ± 10.18	26.12 ± 5.96	NS	< 0.001	< 0.01
V_z/BW (l kg^{-1})	11.09 ± 3.26	11.48 ± 2.79	11.47 ± 2.51	7.35 ± 1.62	NS	< 0.001	< 0.001
Cl (l kg^{-1})	5.60 ± 1.32	5.20 ± 0.99	7.04 ± 2.06	2.90 ± 1.11	NS	< 0.001	< 0.001
Cl/BW (l h^{-1} kg^{-1})	1.44 ± 0.32	1.67 ± 0.31	1.97 ± 0.59	0.81 ± 0.26	NS	< 0.001	< 0.001

Values shown are means ± s.d.
AUC = area under the plasma concentration-time curve; C_{max} = maximal serum drug concentration; t_{max} = time to reach maximal concentration; λ_z = rate constant for elimination; V_z/BW = relative apparent volume of distribution; Cl = total body clearance; Cl/BW = relative total body clearance; $t_{1/2}$ = elimination half-time; NS - denotes a lack of statistical significance.

composition. It was demonstrated, that purified cytochrome P-450IIIA (CYP3A) from rat and man required specific lipids for functional reconstitution *in vitro* (Imuoka *et al.*, 1992). The lipid composition of microsomes of cirrhotic rats has been shown to be altered (Reichen *et al.*, 1992), and this may also occur in ligated animals. The increase of serum and liver microsomes cholesterol in the bile duct-ligated group is in agreement with previous work by other investigators (Bengochea *et al.*, 1987).

The reduced metabolizing capacity of the liver is supported by the results on pentobarbitone sleeping-time which was prolonged significantly in animals with obstructive cholestasis. This drug is also metabolized in the liver microsomal fraction, thus supporting the conclusion that the activity of these enzymes is impaired under mechanical cholestasis. The foregoing data are consistent with those of other authors (McLuen & Fouts, 1961).

Phenazone has been extensively used in man and animals as a probe for hepatic mixed-function oxidase activity *in vivo*. When administered orally it is rapidly and completely absorbed and undergoes extensive hepatic metabolism. The rate of metabolism is slow and is unaffected by hepatic blood flow changes.

Phenazone is metabolized by different cytochrome P-450 izoenzymes forming three major metabolites (Buters *et al.*, 1993). Formation of 4-OH-phenazone has been ascribed to cytochrome P-450 IA2 (CYPIA2), but other isozymes involved in phenazone metabolism have yet to be identified and might differ between animals and man (Loft, 1990). Major oxidation products of phenazone are formed in approximately equal amounts. A meaningful value for the clearance for production of each metabolite is only obtained when the kinetics are linear (Danhof & Teunissen, 1984). Caffeine has been established as a probe for cytochrome P-450 IA2 by measurement of 3-demethylation, the main route of its metabolism in man (Kalow & Tang, 1991).

Based on the results of this study it is clear that obstructive cholestasis impairs the activity of the liver microsomes and affects the metabolism of phenazone and possibly other drugs metabolised via the same biotransformation pathway.

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