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Pharmacokinetics of Intragastrically Administered Digoxin in Rabbits with Experimental Bile Duct Obstruction

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Abstract

A change in the functioning of the liver as a result of experimental cholestasis could result in a change in the biotransformation of drugs. The aim of this study was to evaluate the effect of extrahepatic cholestasis on the pharmacokinetics of digoxin.

The investigation was performed on male rabbits randomly divided into two groups: sham-operated and animals with bile-duct ligation. Digoxin (0·02 mg kg^{-1}) was administered intragastrically as a single dose. Biomedical and anatomo-pathological tests and pharmacokinetic assays were performed before the operation and on the 6th day after surgery. A significant increase in area under the serum concentration–time curve and in mean residence time, a decrease in total body clearance, a reduction in the volume of distribution and increases in maximum concentration and the time to reach maximum concentration were observed in animals with the bile-duct ligation.

These results suggest reduced elimination of digoxin in animals with obstructive cholestasis.

Hospitalization as a result of obstructive cholestasis is not uncommon. It is observed in approximately 10% of patients suffering from choledocholithiasis and from neoplastic diseases of the pancreas or the biliary tract (Blamey et al 1983). Mechanical obstructive cholestasis induces many abnormalities in an organism; these alter the pharmacokinetics of drugs. After obstruction, modification of absorption, distribution, metabolism and elimination appears some time after the onset of cholestasis (Basseches & Digregorio 1982). Few data have yet been published on the pharmacokinetics of drugs in subjects with biliary obstruction, and almost all the studies have described drugs eliminated via the liver: theophylline (Fruncillo et al 1982), procainamide (Basseches & Digregorio 1982), tetracycline antibiotics (Gawronska-Szklarz et al 1983), phenazone (Elfstrom & Lindgren 1974; Hepner & Vesell 1975) and pentobarbital (Carulli et al 1975). The data suggest, although not in all cases unequivocally, impaired elimination under mechanical extrahepatic cholestasis. Because it has been reported that altered liver status might affect renal function (Ozawa et al 1979), it seems relevant to study the pharmacokinetics in obstructive cholestasis of drugs eliminated predominantly via the urinary tract. Digoxin is a drug which fulfils these criteria. It is mostly (50–80%) eliminated unchanged, via glomerular filtration, not more than 15% of administered digoxin undergoing hepatic biotransformation (Kramer & Reuning 1978; Beveridge et al 1978).

This study was therefore designed to assess the effect of mechanical extrahepatic cholestasis on the pharmacokinetics of intragastrically administered digoxin, the first time such an investigation has been performed.

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Materials and Methods

The study was performed on 17 male mongrel rabbits, randomly assigned to two groups: group 1, a control group of eight animals which were sham-operated, and group 2, an experimental group of nine rabbits with obstructive cholestasis. The average weights of animals in groups 1 and 2 before the operation were $3{\cdot}36 \pm 0{\cdot}14$ and $3{\cdot}62 \pm 0{\cdot}23$ kg, respectively. Both groups were housed identically at a controlled temperature (22°C) and in a 12-h light–12-h dark-cycle. During a 1-week acclimatization period, food (LSM, Motycz) and water were freely available.

The experiment was approved by Institutional Ethical Committee of the Pomeranian Medical Academy.

Surgery

The operation procedure was performed under anaesthesia induced with 12 mg kg^{-1} pentobarbital (Vetbutal, Biowet), given intravenously. The peritoneal cavity was opened using 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. After ligation of the bile duct, the peritoneum, muscles and epidermal layers were sutured. The same surgical procedure, except for ligation of the duct, was repeated in the control group.

Experimental procedures

The body weight of the animals was measured before the operation and at autopsy on the 7th day after surgery. Serum total bilirubin, creatinine, urea, glucose and albumin concentrations, and the activities of alanine, aspartate aminotransferase and alkaline phosphatase were measured by means of a standard laboratory Analyzer (Technicon RA1000) for all the animals, 4 days before the study and 6 days after the operation. Four days before the operation and 6 days after

surgery digoxin (Polfa) was administered intragastrically, as a single dose of 0·02 mg kg^{-1} suspended in 1 mL saline, to animals previously fasted for 18 h but with water freely available. Fasting was continued for 3 h after drug administration. Blood was collected from the ear marginal vein 15 and 30 min, and 1, 1.5, 2, 4, 6, 8, 12 and 24 h after administration of the drug; after clotting they were immediately centrifuged and the sera were stored at − 20°C until analysis.

The blood serum concentration of digoxin was determined by the immunofluorescence polarization method by means of Abbott's TDx system digoxin II assay (abnormal levels of total bilirubin up to 20 mg L^{-1} result in less than 10% error in levels of digoxin measured using this system). In our experiment the levels of total bilirubin in the group with ligated bile duct were from 1–42·3 mg L^{-1}). The lowest measurable level which can be distinguished from zero with 95% confidence is 0·2 ng mL^{-1}. Some metabolites of digoxin (digoxigenin, digoxigenin mono-digitoxoside and digoxigenin bis-digitoxoside) were also detected by use of this system.

Pharmacokinetic analysis

Mean maximum serum digoxin concentration (C_{max}) and time (t_{max}) required to reach C_{max} were calculated from the individual peak serum digoxin concentration. The area under the plot of serum concentration against time (AUC) was measured by the trapezoidal rule and was extrapolated to infinity. The apparent elimination rate constant (λ_z) was calculated by non-linear least squares regression analysis of serum levels in the terminal phase. Elimination half-life ($t_{\frac{1}{2}\lambda z}$) was calculated from $t_{\frac{1}{2}\lambda z} = \ln 2/\lambda_z$. The mean residence time (MRT), apparent volume of distribution per kg body weight (BW) (V_z/BW) and total body clearance (CL/BW), were calculated on the basis of non-compartmental analysis because of the small number of blood samples. As bioavailability (F) might not be unity, the volume of distribution and clearance are reported as V_z/F/BW and CL/F/BW.

Anatomo-pathological procedure

All animals were autopsied at the end of the study, i.e. on the 7th day. Kidneys and livers were weighed and examined macro- and microscopically. For microscopic studies pieces of liver were freeze-sectioned and stained with oil red 0 for the presence of neutral lipid. Other liver samples were placed into Schafer's solution, and afterwards the prepared slices were stained with haematoxylin, eosin and pas. Slices of the kidney were placed into Carnoy's solution, and in a further step stained with haematoxylin and eosin.

Statistical analysis

Data from each group are presented as mean ± s.e. The significance of differences between groups was analysed using the non-parametric Wilcoxon test (the Wilcoxon matched-pairs test inside the group and the Wilcoxon two-sample test between groups). $P < 0·05$ was considered as indicative of statistical significance.

Results

General observations

The first symptoms of jaundice appeared in animals from the experimental group 3 days after the operation. The motor activity of the rabbits was depressed and a yellow colour could be observed on the ears and the conjunctivas. The intensity of these symptoms increased throughout the study period. The body weight of the jaundiced animals decreased by 26% ($P < 0·02$), whereas that of the controls did not change significantly.

Laboratory tests

There were no significant changes in the results from laboratory tests on the control rabbits, except for a drop in glucose concentration from 14·33 ± 0·26 mM before the operation to 9·53 ± 0·71 mM after ($P < 0·05$). In animals with ligated bile ducts, the following changes in blood serum levels were observed on the 6th day after surgery: a 55-fold rise in total bilirubin concentration from 1·31 to 72·28 μM ($P < 0·001$); an increase in aminotransferase activity; an increase in alanine from 28·33 ± 4·15 to 125·11 ± 14·30 units L^{-1} ($P < 0·001$); an increase in aspartate from 32·33 ± 1·76 to 188·44 ± 52·35 units L^{-1} ($P < 0·001$); an increase in alkaline phosphatase activity from 53·89 ± 4·54 to 385·22 ± 95·79 units L^{-1} ($P < 0·01$) (control group from 160·5 ± 7·27 to 181·00 ± 8·32); significant increases in the concentrations of serum creatinine from 153·44 ± 6·20 to 386·22 ± 16·07 mM and of urea from 4·15 ± 0·17 to 10·76 ± 3·40 mM (control group 4·38 ± 0·15 to 4·23 ± 0·20); and pronounced decreases in the blood serum concentrations of albumin from 43·49 ± 0·67 to 33·57 ± 0·63 g L^{-1} ($P < 0·01$) and of glucose from 13·17 ± 0·99 to 9·50 ± 0·63 mM.

Pharmacokinetics

Concentrations of digoxin in the blood serum (Table 1) of rabbits with experimental bile duct ligation were higher than the levels measured for control animals and higher than the pre-operative values (Fig. 1). In the control group there were no pronounced differences in the blood serum concentration of digoxin except for the last two points of assay.

The pharmacokinetic parameters calculated are presented in Table 2. A significant increase in AUC from 12·25 ± 1·01 to 19·67 ± 2·57 ng mL^{-1} h and decrease in CI/F/BW from 1·77 ± 0·24 to 1·14 ± 0·14 L^{-1} h^{-1} kg^{-1} were observed in the control animals. In contrast, most pharmacokinetic para-

Table 1. Mean (± s.e.) blood serum concentrations of digoxin in controls (group 1) before (A) and after (B) the sham operation, and in the jaundiced animals (group 2) before (C) and after (D) bile duct ligation.

	Group 1		Group 2	
Time (h)	A	B	C	D
0·25	0·66 ± 0·13	1·15 ± 0·28	0·89 ± 0·12	1·08 ± 0·13
0·5	1·18 ± 0·32	1·79 ± 0·31	1·45 ± 0·20	2·13 ± 0·27
1·0	1·65 ± 0·26	2·12 ± 0·41	1·98 ± 0·25	3·09 ± 0·42
1·5	1·93 ± 0·38	1·93 ± 0·40	1·98 ± 0·26	3·54 ± 0·57‡
2·0	1·66 ± 0·16	1·58 ± 0·27	1·68 ± 0·26†	3·73 ± 0·58‡
4·0	0·96 ± 0·07	1·13 ± 0·18	1·07 ± 0·16†	2·84 ± 0·34‡
6·0	0·53 ± 0·05	0·71 ± 0·10	0·69 ± 0·09†	2·08 ± 0·29‡
8·0	0·34 ± 0·05	0·53 ± 0·10	0·50 ± 0·08†	1·72 ± 0·22‡
12·0	0·21 ± 0·03*	0·39 ± 0·07*	0·31 ± 0·05†	1·11 ± 0·16‡
24·0	0·11 ± 0·02*	0·24 ± 0·05*	0·13 ± 0·02†	0·69 ± 0·07‡

*$P < 0·05$ compared with result for group A. † $P < 0·05$ compared with result for group D. ‡ $P < 0·05$ compared with result for group B.

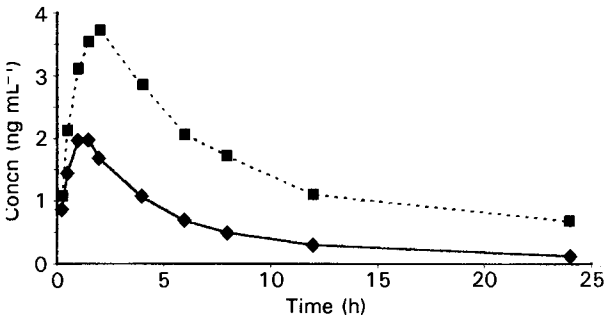

FIG. 1. Mean blood serum concentrations of digoxin in the jaundiced animals, before (◆) and after (■) bile duct ligation.

meters changed much more markedly in animals subjected to bile duct ligation; compared with the preoperative values AUC increased by 247% ($P < 0.01$), MRT and t_{max} were prolonged by 94% ($P < 0.05$) and by 78% respectively, C_{max} increased by 79% ($P < 0.02$), $V_z/F/BW$ decreased by 51% ($P < 0.01$) and CL/F/BW dropped by 69% ($P < 0.01$).

Anatomo-pathological findings

The livers from the animals with obstructive cholestasis were enlarged and more fragile, with yellowish coloration and strained capsules. The mean relative weight of the livers from the ligated group (38.31 ± 3.48 g kg^{-1}) was significantly higher ($P < 0.001$) than for controls (23.15 ± 0.86 g kg^{-1}); the same was true for the kidney — in controls it was 4.75 ± 0.25 g kg^{-1} and in animals with cholestasis 6.86 ± 0.67 g kg^{-1}. The histological structure of the liver from the control group was normal whereas some abnormalities were detected in animals with common bile duct obstruction. Hepatocytes and Kupfer's cells in these animals contained small droplets of bile predominantly within the portal space. Bile deposits were observed on the bile canaliculi. Enlargement of portal spaces, hypertrophy of bile ducts and dilation of the interlobular ducts was seen, as was hyperplasia of the connective tissue in the portal spaces and some lymphoidal cell infiltration. Hepatocytes in rabbits with obstructive jaundice showed grains of glycogen in small quantities. Macroscopic examination of the kidney did not reveal any abnormality in either study group whereas microscopic appearance was normal in the control animals only. In animals with mechanical cholestasis the kidneys showed contraction of the glomeruli, dilation of the Bowman's space and thickening of the Bowman's capsule. Within the cortex many of the convoluted tubules had flat epithelium and dilated lumen. In some the epithelium was totally absent. Degeneration of the renal bodies was also seen, but only rarely, in the jaundiced rabbits.

Discussion

Digoxin is eliminated almost entirely via the kidney, 60–80% in the unchanged form by glomerular filtration and to some extent by active tubular secretion (Sumner & Ressel 1979; Mooradian 1988), although some reports suggest that approximately 30% of intravenously administered digoxin is excreted with the bile, undergoing enterohepatic circulation (Doherty et al 1961). A small fraction of the drug is metabolized in the liver with formation of the derivatives 3b-digoxigenin, b-digoxigenin and mono and bis-digitoxoside (Gault & Longerich 1983). Peters et al (1978) reported a close relationship between digoxin and creatinine clearance, because of the predominant role of glomerular filtration in the elimination of digoxin.

The mean concentrations of digoxin in animals with ligation of the bile duct were significantly higher than concentrations measured before the operation and for controls. The induced mechanical cholestasis impaired not only liver function, as was indicated by the pronounced increase in the activities of alanine, aspartate aminotransferase, and alkaline phosphatase, the increase in serum bilirubin and the drop in serum albumin concentrations. The function of the kidney was also disturbed. This was manifested by a marked increase in serum creatinine, urea and bilirubin and was confirmed by abnormalities observed during anatomo-pathological examination. The observations are in keeping with reports suggesting altered kidney status in the presence of concomitant liver disease (Fogarty et al 1995). So, impaired function of the kidney resulted in accumulation of the drug in the jaudiced animals. Disruption of the enterohepatic circulation, which plays, to some extent, a role in the behaviour of digoxin, could be a further factor in the impaired elimination of the drug. Altered values of pharmacokinetic parameters confirmed the impaired elimination of digoxin in animals with the bile ducts obstructed. Significant increases were observed in AUC, MRT, C_{max} and t_{max}, and decreases in the apparent clearance and the volume of distribution. The change in clearance associated with the induced cholestasis can probably be explained by the reduction in the renal clearance of digoxin.

Table 2. Mean (± s.e.) values of the pharmacokinetic parameters for digoxin in controls (group 1) before (A) and after (B) the sham operation, and in the jaundiced animals (group 2) before (C) and after (D) bile duct ligation.

	Group 1		Group 2	
Parameter (unit)	A	B	C	D
Area under plasma concentration–time curve (ng mL^{-1} h)	12·25 ± 1·01*	19·67 ± 2·57	15·77 ± 1·10†	54·88 ± 6·54‡
Mean residence time (h)	10·33 ± 1·19	18·01 ± 3·59	12·99 ± 2·11†	25·25 ± 9·08
Maximum concentration of drug in serum (ng mL^{-1})	2·02 ± 0·36	2·51 ± 0·36	2·26 ± 0·23†	4·04 ± 0·61‡
Time required to reach maximum drug concentration (h)	1·75 ± 0·13	1·34 ± 0·41	1·06 ± 0·15†	1·89 ± 0·32
Elimination constant (h^{-1})	0·08 ± 0·02	0·08 ± 0·02	0·09 ± 0·01	0·08 ± 0·01
Elimination half-life (h)	11·48 ± 2·55	11·96 ± 3·01	9·43 ± 2·47	10·79 ± 1·69
Volume of distribution (L kg^{-1})	17·69 ± 2·21	20·34 ± 5·02	16·89 ± 2·72†	8·40 ± 1·74‡
Clearance (L h^{-1} kg^{-1})	1·77 ± 0·24*	1·14 ± 0·14	1·32 ± 0·09†	0·41 ± 0·05‡

*$P < 0.05$ compared with result for group B. † $P < 0.05$ compared with result for group D. ‡$P < 0.05$ compared with result for group B.

All biochemical tests, except for serum glucose, and the anatomo-pathological findings performed in the control group showed no changes during the study; results were within the normal range.

Changes seen in the controls after surgery, i.e. an increase in digoxin concentration 12·0 and 24·0 h after intragastric application and an increase in AUC, and CL, could be ascribed to a post-operative period. Elfstrom (1979) reported altered blood flow and metabolic capacity of the liver, and impaired renal blood flow, after surgery. The intensity of the observed changes in the controls is not so great as in animals with mechanical cholestasis, so in the group with mechanical cholestasis two factors could modify the pharmacokinetics of digoxin, i.e. surgery, to a lesser extent, and cholestasis, which seems to be the major factor inducing impaired elimination of digoxin.

The disposition of digoxin in rabbits was studied by Buss & Routledge (1985) and by Ochs et al (1978). They suggested that in the rabbit the drug is largely metabolized. However, they performed examinations during repeated administration of digoxin in the intact rabbits and assumed that chronic drug administration stimulated its own clearance. Our rabbits received digoxin as a single dose, and their liver function was impaired. According to Bansinath et al (1986) the bioavailability of digoxin can be tested in rabbits and the results obtained correspond to those obtained by testing in man. The current results suggest that mechanical, extrahepatic cholestasis impairs the elimination of digoxin, a drug predominantly excreted via the kidney.

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