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Influence of hepatic dysfunction on cyclosporine metabolism in the pig

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Abstract. Cyclosporine (CyA) is eliminated from the body via biliary excretion at a rate directly proportional to bile production and the functional status of the liver. Previous reports demonstrated that disturbances in the hepatic excretory function with a rise in the plasma bilirubin level are positively correlated with high blood concentrations of CyA and CyA plus metabolites (CyA + M). Less information is available about the blood concentrations of the CyA parental substance or CyA metabolites in the case of liver dysfunction when there was no elevation of serum bilirubin content. To answer this question, we compared the pharmacokinetic profile of CyA in a cholestatic and in a ischemic model in pigs. Our results show that in pigs receiving a single dose of CyA after liver ischemia, the blood concentrations of CyA and CyA + M are significantly increased independently of the serum bilirubin concentration, probably through a slow down of CyA metabolism by impairment of cytochrome P450 III A.

Key words: Cyclosporine metabolism – Liver dysfunction – Serum bilirubin

Cyclosporine (CyA) has been widely used in numerous transplantation protocols. This drug is extensively distributed in the body, as reflected by the large apparent volume of distribution. CyA is primarily metabolized in the liver through N-demethylation and mono- and dihydroxylation mediated by cytochrome P450 III A isoenzyme. Many metabolites of CyA have already been isolated, but none has an important immunosuppressive activity.

As the metabolism of CyA depends upon the activity of microsomal liver enzymes and its elimination upon the bile production [3, 5], the fact that some impairment of the liver function, a situation frequently encountered after orthotopic liver transplantation (OLT), induces large variations of the CyA blood concentrations is not surprising.

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Because drug is liposoluble, it needs bile components in order to be absorbed from the upper small intestine after oral administration. Therefore, in the case of cholestatic icterus, very high blood concentrations of CyA and CyA metabolites can be expected when the drug is injected (i.v. route) and very low ones when it is orally administered.

The CyA parent drug can be measured by high performance liquid chromatography (HPLC) or by immunoassays RIA and fluorescence polarization immunoassay, FPIA based on a monoclonal antibody (MRIA) that only recognises the parental substance. The CyA metabolites are quantitated with the same immunoassays except that a polyclonal antibody (PRIA) is used which crossreacts with a number of metabolites plus the parental drug (CyA + M).

Previous reports showed a positive correlation between the serum bilirubin level, and impaired CyA and CyA + M excretion, as reflected in an elevation of the CyA PRIA/HPLC ratio [2, 4].

However, less information is actually available about whether or not liver cell damage, without a rise in the serum bilirubin level, is followed by the same variations of CyA and CyA + M blood level ratio.

To answer this question, we compared the pharmacokinetic profile of CyA in a cholestatic and in an ischemic liver model of pigs over 24 h after a single i.v. administration of CyA.

Pigs were chosen for this study because the interspecies differences between humans and pigs in cyclosporine disposition have been previously reported [1].

Materials and methods

Twelve pigs weighing between 25 and 35 kg were investigated. The pigs received a single i.v. dose of CyA (8 mg/kg). Blood samples were obtained before the injection and after 5, 15, 30, 60, 90, 120, 180, 240, 360, 540, 660, 780, 960, 1200, and 1440 min (24 h). Each pig was his own control. After a week, all pigs underwent surgery.

Under general anesthesia, exposition of the hepatoduodenal ligament was obtained through a midline incision. In the cholestatic

Table 1. Liver tests performed in the 3 groups

Liver tests	Control group	Gr A	Gr B
AST (U/l)	46 ± 12	157 ± 46	1211 ± 1113
ALT (U/l)	54 ± 16	61 ± 10	164 ± 69
GGT (U/l)	18 ± 4.75	38 ± 28	23 ± 12.67
ALP (U/l)	168 ± 47	310 ± 66	395 ± 121
BIL (μmol/l)	3.11 ± 0.3	63 ± 21	7.4 ± 4.6
PT (%)	87 ± 19	96 ± 6	67 ± 14

GrA, cholestatic group; GrB, ischemic group; AST, ALT, aspartate and alanine aminotransferases; GGT, γglutamyltransferase; ALP, alkaline phosphatase; BIC, bilirubin; PT, prothrombin time

group (A; $n = 6$), the common bile duct was distally ligated and divided. In the ischemic group (B; $n = 6$), the hepatoduodenal ligament was completely dissected and the hepatic artery ligated. At the end of the dissection, only the portal vein and the biliary tract, denuded, are conserved. Then, the falciform and the left and right triangular ligaments were divided. No drains were left in place.

At 48 h after surgery each pig received a second i. v. dose of CyA (8 mg/kg). Blood samples were obtained between 0 and 24 h, as indicated previously. Liver tests were performed before and 48 h after surgery.

Analytical methods. Both CyA and CyA + M were determined with commercial kits (Sandoz, Basel, Switzerland) of radioimmunoassay technique (MRIA and PRIA) and confirmed with FPIA (Abbott, Chicago, USA).

The PRIA/MRIA ratio, CyA half-life ($t_{1/2}\beta$; min), area under the curve (AUC; ng/ml · min), total clearance (tot CL; ml/min · kg), distribution volume (VD; ml/kg), and relation with liver tests were studied. Enzymes activities and serum bilirubin concentration were determined with a Technicon RA-1000 analyzer according to IFCC recommendations for alanine aminotransferase (ALT), aspartate aminotransferase (AST), γglutamyltransferase (GGT), alkaline phosphatase (ALP), and prothrombin time (PT).

Results

The serum bilirubin concentration was significantly higher in the cholestatic group than in the ischemic group or control group ($P < 0.01$). The ALT concentrations were significantly higher in the ischemic group than in the cholestatic group or control group ($P < 0.01$). Results are shown in Table 1.

In both groups A and B, the $t_{1/2}\beta$ of CyA and CyA + M, and the AUC increased and the tot CL decreased significantly compared with the control group ($P < 0.01$). The VD remained unchanged in the three groups (Table 2).

We found no difference between groups A and B except for the ratio of metabolites to total cyclosporine as a function of elapsed time [(AUC PRIA–AUC MRIA)/AUC PRIA], which is significantly more important in the cholestatic group.

Discussion

CyA is eliminated from the body via biliary excretion at a rate directly proportional to bile production and to the functional status of the liver [3]. The biliary concentration of CyA metabolites exceeds that of the parent drug, suggesting that most of the CyA is excreted in the bile [3, 5]. In clinical OLT, high levels of CyA expose the patient to the drug's side-effects and toxicity [2]. Conversely, a low

Table 2. Results of the CyA and CyA + M in the 3 groups

	CyA			CyA + M		
	Control	Gr A	Gr B	Control	Gr A	Gr B
$t_{1/2}\beta$	368 ± 31	634 ± 162	636 ± 110	373 ± 37	751 ± 60	740 ± 177
AUC	728 ± 110	1137 ± 428	1331 ± 659	842 ± 146	1176 ± 587	1700 ± 856
Tot CL	11.18 ± 1.87	7.64 ± 2.59	7.77 ± 4.93	9.71 ± 1.71	4.80 ± 1.57	6.29 ± 4.43
VD	5931 ± 1112	6619 ± 1361	6549 ± 279	5211 ± 1688	5175 ± 1688	5848 ± 2219

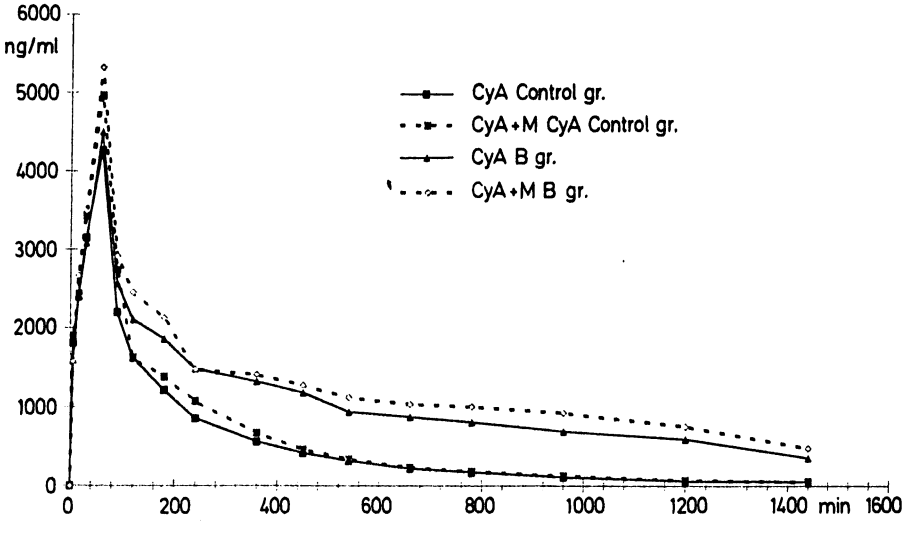

Fig. 1. Cyclosporine (CyA) and CyA + M (metabolites) blood concentrations (determined by radioimmunoassay) before and after liver desarterialization (group B)

concentration of the parental substance, even in the case of a high blood concentration of metabolites, expose the patient to rejection (metabolites have a undefined, but poor, immunosuppressive activity). It is thought that the accumulation of metabolites in the presence of liver dysfunction is due to impaired excretion. An increase in the plasma bilirubin level is known to be positively correlated with an increase of the ratio PRIA/MRIA [4].

The results of the present study show that in pigs receiving a single dose of CyA after liver ischemia, the blood concentrations of CyA and CyA + M are significantly increased (Fig. 1), although there is no elevation of serum bilirubin level (Table 1). A decrease in the CyA metabolism through dysfunction of the cytochrome P450 III A probably explains this increased CyA blood concentration. This fact indicates that in liver transplant patients, whatever the cause of the liver dysfunction and the plasma bilirubin level, careful monitoring of the CyA and CyA + M blood levels is mandatory.

In conclusion, this work supports the view that a sudden rise in the CyA levels in blood can take place independently of the serum bilirubin concentration.

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