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The Influence of Liver Dysfunction on Cyclosporine Pharmacokinetics —A Comparison between 70 per cent Hepatectomy and Complete Bile Duct Ligation in Dogs—

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ABSTRACT: The influence of experimentally induced hepatic dysfunction on the pharmacokinetics of Cyclosporine A (CsA) was determined in dogs. The pharmacokinetics of oral (PO) and intravenous (IV) CsA were studied before and after 70 per cent hepatectomy or complete bile duct ligation (CBDL). Changes in liver function were monitored by serial measurements of serum bilirubin, and by the maximum removal rate (Rmax) and plasma disappearance rate (ICG-K) of indocyanine green (ICG). Concentrations of CsA in whole blood were measured by HPLC. Seventy per cent hepatectomy caused significant liver dysfunction: the ICG-Rmax decreased by 47.7 ± 7.1 per cent (mean ± SD) and the ICG-K decreased by 61.3 ± 9.7 per cent during the first week after hepatectomy. At the same time, the systemic clearance (CLs) of IV-CsA decreased by 43.9 ± 8.2 per cent, the area under the concentration curve (AUC) of IV-CsA increased by 35.4 ± 20.8 per cent and the bioavailability of CsA decreased by 26.4 ± 14.8 per cent. CBDL also induced significant liver dysfunction: the ICG-Rmax decreased by 39.1 ± 12.8 per cent and the ICG-K decreased by 65.6 ± 3.6 per cent in the second week after the operation. During the same period, the AUC of PO-CsA decreased by 69.9 ± 10.7 per cent and the bioavailability of CsA also decreased markedly by 73.9 ± 15.6 per cent. These data indicate that hepatic impairment significantly influences the pharmacokinetics of CsA, not only by the changes in intestinal absorption, but also by those in hepatic metabolism. Dose adjustment is therefore necessary in the presence of hepatic dysfunction in order to maintain an adequate blood concentration of CsA without causing side effects.

KEY WORDS: liver dysfunction, cyclosporine pharmacokinetics, 70 per cent hepatectomy, complete bile duct ligation, kinetics of indocyanine green

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INTRODUCTION

Therapy with cyclosporine A (CsA) constitutes a major advance which has led to the success of orthotopic liver transplantation (OLTX) and other organ transplantations. OLTX in particular presents special problems regarding the maintenance of adequate pharmacological immunosuppression. The absorption, distribution, metabolism, elimination and excretion of CsA can be expected to be significantly altered by the transplanta-

tion procedure, as the liver is a major site of the metabolism and elimination of CsA, and also because this organ is related to its absorption. The status of the liver therefore greatly influences CsA pharmacokinetics. The assessment of dose adjustment necessary, however, is quite difficult because routine hepatic function tests do not correlate with CsA elimination.[1] It is therefore important to devise a more accurate quantitative method of assessing liver function, utilizing the kinetics of indocyanine green (ICG) removal from the blood. In the present study, we investigated the pharmacokinetics of intravenous and oral CsA following various degrees of hepatic dysfunction created by either 70 per cent hepatectomy or complete bile duct ligation in dogs.

Materials and Methods

Thirteen healthy adult mongrel dogs, weighing 19.0 to 24.0 kg, were fasted for 18 hours preoperatively. Anesthesia was induced with pentobarbital and respiration was maintained by a respirator with room air ventilated through a cuffed endotracheal tube. Resection of about 70 per cent of the liver was performed in 6 dogs, according to the procedure described by Sigel.[2] This procedure of approximately a 70 per cent hepatectomy was carried out by resecting the right central, quadrate, left central and left lateral lobes, according to the classification of Price et al.[3] The caudate and right lateral lobes were left. A complete bile duct ligation was performed in 7 dogs: The bile duct was ligated doubly and cut between the ties.

Cyclosporine administration, assay and pharmacokinetical analysis

CsA pharmacokinetics were studied in all 13 dogs. Each dog received 2 mg/kg of CsA as an intravenous continuous infusion over 1 hour, using a calibrated pump in order to maintain a constant rate of infusion, and 17.5 mg/kg of CsA (Sandimmune Oral Solution) orally, after an interval of a 3 day washout period. After the 70 per cent hepatectomy, intravenous or oral CsA was given for the pharmacokinetic study during the first postoperative week. After the complete bile duct ligation, the study of CsA kinetics was performed during the second postoperative week. Blood samples of 3 ml were collected from the jugular veins, before and 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12, 16 and 24 hours after drug administration, for the study of intravenous CsA kinetics, and 0, 1, 2, 3, 4, 6, 8, 12, 16 and 24 hours after drug administration for the oral CsA kinetics.

The CsA blood concentration in whole blood was measured using a high-performance liquid chromatographic assay (HPLC) by the method of Sawchuk and Cartier.[4]

Pharmacokinetic parameters were calculated using a model-independent analysis. The terminal disposition rate constant (Kel) was determined by the least square linear regression analysis of the terminal phase of the blood concentration *versus* time curve. The area under the blood concentration *versus* time curve (AUC) was calculated using a trapezoidal rule. The computer "Moments" was used to calculate the model-independent parameters, which included the total body clearance (CLs), the mean residence time and the steady-state volume of distribution (Vss). The bioavailability (percentage F) of oral CsA was calculated using the following equation.

$$\text{Bioavailability (percentage F)} = \frac{\text{AUC of oral CsA}}{\text{AUC of intravenous CsA}} \times \frac{\text{Dose of intravenous CsA}}{\text{Dose of oral CsA}} \times 100$$

Kinetics of ICG removal from the blood

In order to estimate the ICG plasma disappearance rate (ICG-K) using the ICG dose of 0.5 mg/kg body weight, and to measure the ICG maximal removal rate (ICG-Rmax) using ICG doses of 0.5 mg/kg and 5.0 mg/kg body weight, Moody and Rikkers' method[5] was used with some modification. ICG-K relates to effective hepatic blood flow, and ICG-Rmax is one of the most accurate parameters of hepatic functional reserve.[6] The

study of the kinetics of ICG removal was performed on a different day from that of CsA kinetics. During the analysis of ICG kinetics, each dog was placed in a Pavlov frame. ICG in doses of 0.5 or 5.0 mg/kg was then injected intravenously into one foreleg, while sequential blood samples were obtained from the opposite leg 3, 5, 7, 10 and 15 minutes after the injection. The ICG concentration of the plasma fraction of these samples was determined spectrophotometrically at a wave length of 805 nm, after appropriate dilution with normal saline. Based on these data, ICG-K was determined by the least square linear regression analysis of the ICG blood concentration *versus* time curve. ICG-Rmax was calculated by applying the Michaelis Menten equation and a Lineweaver-Burk plot of the double-reciprocal relationship between the administered dose and the removal rate of ICG was used.[7] Regression analysis was performed to test the applicability of this formula to our data.

Serum chemical studies

Serum chemical studies, such as serum bilirubin, glutamic oxaloacetic transaminase (SGOT) and prothrombin time, were measured in all dogs. The body weight and hematocrit values were checked before and after surgery, and on the day of investigation.

Data analysis

Results are represented by the mean ± standard deviation and analyzed by the paired Student's t-test for ICG kinetics and the unpaired Student's t-test for CsA kinetics. A p-value of less than 0.05 was considered statistically significant.

Results

The 70 per cent hepatectomy did not result in immediate mortality of any of the 6 dogs, although the complete bile duct ligation resulted in an over-all mortality rate of 14 per cent (1/7) of the 7 dogs, occurring within 2 weeks after the operation.

The CsA concentration-time curves were adequately described by a two-compartment first order pharmacokinetic model. Tables 1, 2 and 3 summarize the pharmacokinetic parameters of CsA and the kinetics of ICG removal, derived from the blood concentra-

Table 1. The Kinetics of ICG Removal and Serum Bilirubin Levels According to the Liver Function after 70 Per cent Hepatectomy in Dogs

Parameters of ICG kinetics	Control (n=6)	70% Hepatectomy (n=6)
ICG-Rmax (mg/kg/min)	0.377±0.046	0.158±0.028 p<0.001
ICG-K (/min)	0.092±0.011	0.035±0.008 p<0.001
Serum Bilirubin (mg/dl)	0.30 ±0.08	0.71 ±0.17 p<0.05

ICG-Rmax: the maximal removal rate of indocyanine green
ICG-K: the plasma disappearance rate of indocyanine green
Results are represented by mean ± standard deviation and analysed by the paired Student's t-test. A p-value of less than 0.05 was considered statistically significant, when comparing control and hepatectomy results.

Table 2. The Kinetics of ICG Removal According to the Liver Function and Serum Bilirubin Level after Complete Bile Duct Ligation in Dogs

Parameters of ICG kinetics	Control (n=6)	Bile duct ligation (n=6)
ICG-Rmax (mg/kg/min)	0.357±0.049	0.215±0.047 p<0.005
ICG-K (/min)	0.088±0.009	0.029±0.004 p<0.001
Serum bilirubin (mg/dl)	0.29 ±0.09	5.80 ±2.80 p<0.001

ICG-Rmax: the maximal removal rate of indocyanine green
ICG-K: the plasma disappearance rate of indocyanine green
Results are represented by mean ± standard deviation and analysed by the paired Student's t-test. A p-value of less than 0.05 was considered statistically significant when comparing control and bile duct ligation results.

Table 3. Pharmacokinetic Parameters of CsA Following 70 Per cent Hepatectomy or Complete Bile Duct Ligation in Dogs

Pharmacokinetic parameters of intravenous CsA	Control	Hepatectomy	Bile but ligation
t1/2 (hrs)	5.06±1.16*	7.65±1.44** p<0.05	8.32±3.41
CLs (ml/min/kg)	7.09±2.10*	3.85±0.61** p<0.05	5.37±0.92*** p<0.05
Vss (l/kg)	2.80±1.10	2.57±0.61	2.64±0.69
AUC (ng·hr/ml)	5100±1471*	7311±1462** p<0.05	5842±1124
Pharmacokinetic parameters of oral CsA			
t1/2 abs (hr)	2.74±1.68	5.35±2.17	12.7±9.50
AUC (ng·hr/ml)	8382±1815*	10037±656	2602±898*** p<0.001
%F (%)	21.8±4.4*	16.0±3.2** p<0.05	5.7±3.4*** p<0.001

CsA pharmacokinetic parameters, t1/2; terminal half life, CLs; total body clearance of CsA, Vss; steady state volume of distribution, AUC; area under CsA concentration *versus* time curve, %F; per cent bioavailability of oral CsA.
Results are represented by mean ± standard deviation and analysed by the unpaired Student's t-test. A p-value of less than 0.05 was considered statistically significant, when comparing control and hepatectomy results (* VS **), and when comparing control and bile duct ligation results (* VS ***).

tion *versus* time. The control value was taken from the data in the preoperative periods. Each parameter was examined during the first postoperative week after hepatectomy in 6 dogs, and in the second week after bile duct ligation in 6 dogs.

In the preoperative control study, the mean CLs of intravenous CsA was 7.09 ± 2.10 (ranging from 4.38 to 8.90) ml/min/kg and the mean bioavailability of oral CsA was 21.8 ± 4.4 (ranging from 15.5 to 26.3) per cent. The maximal CsA concentration of intravenous and oral CsA were 1671 ± 715 (ranging from 654 to 2600) ng/ml and 1105 ± 174 (ranging from 800 to 1293) ng/ml, respectively. The time taken to achieve the peak concentration of oral CsA was 2.17 ± 1.0 (ranging from 1.0 to 4.0) hours.

During the first week (4 days postoperatively) after hepatectomy, ICG-Rmax and ICG-K were reduced by 57.7 ± 7.1 per cent ($p<0.001$) and 61.3 ± 9.7 per cent ($p<0.001$), respectively, and serum bilirubin was elevated slightly by 0.71 ± 0.17 mg/dl ($p<0.05$). This liver impairment was associated with a marked decrease in the CLs of intravenous CsA by 46.4 ± 7.8 per cent ($p<0.01$). The AUC of intravenous CsA increased by 43.4 ± 28.7 per cent ($p<0.05$). Although the bioavailability of oral CsA decreased by 30.9 ± 15.2 per cent ($p<0.05$), the AUC of oral CsA did not change significantly.

Complete bile duct ligation also induced significant liver dysfunction. ICG-Rmax was reduced by 39.1 ± 12.8 per cent ($p<0.005$) and ICG-K was reduced by 65.6 ± 3.6 per cent ($p<0.001$) in the second week after surgery with a marked increase in serum bilirubin (5.80 ± 2.80 mg/dl). During the same period, the CLs of intravenous CsA decreased by 21.6 ± 9.3 per cent ($p<0.05$). The AUC of intravenous CsA, however, did

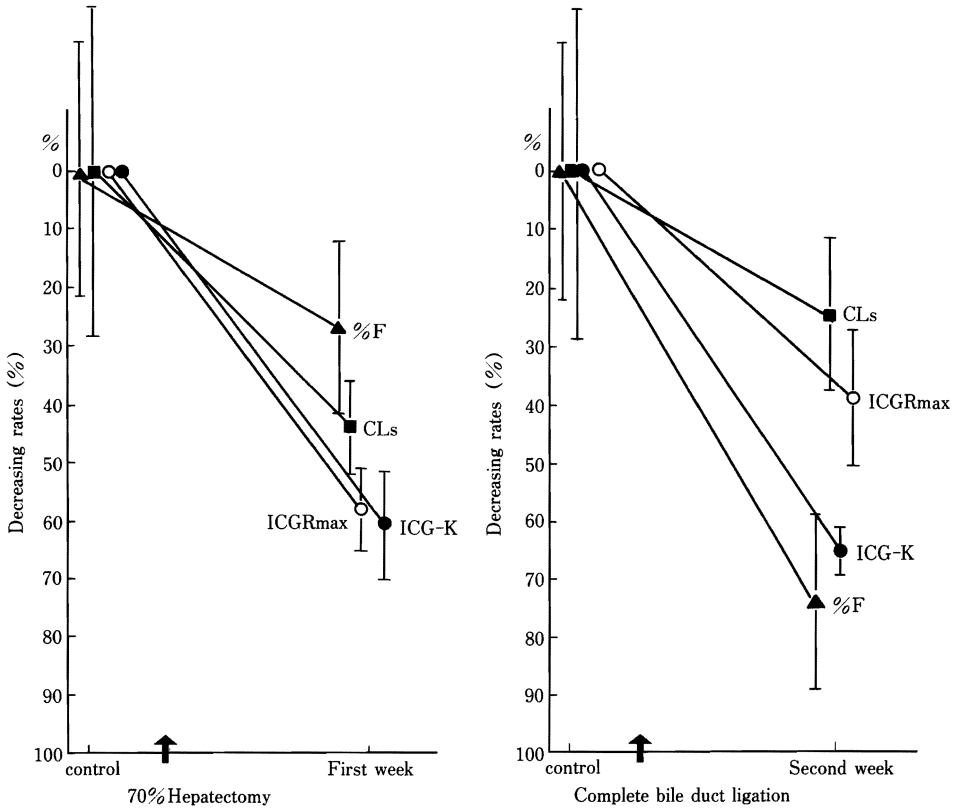

Fig. 1. Comparison between the per cent changes in CsA kinetic parameters and ICG kinetic parameters following 70 per cent hepatectomy (4 days after operation) or complete bile duct ligation (10 days after operation) in dogs. Although the ICG kinetics showed severe hepatic dysfunction after hepatectomy or complete bile duct ligation respectively, the systemic clearance of CsA have a significantly lower decreasing rate than that of the ICG kinetics. After bile duct ligation, the percentage bio-availability reduced markedly. (mean ± standard deviation)
○—○; the decreasing rates of maximal removal rate of indocyanine green (ICG-Rmax)
●—●; the decreasing rates of plasma disappearance rate of indocyanine green (ICG-K)
■—■; the decreasing rates of total body clearance of CsA (CLs)
▲—▲; the decreasing rates of per cent bioavailability (%F)

not change significantly, even though the ICG kinetics indicated severe hepatic dysfunction. The AUC of oral CsA decreased by 69.0 ± 10.7 per cent ($p<0.001$) and the bioavailability also decreased markedly, by 79.4 ± 6.1 per cent ($p<0.001$) (Fig. 1).

Discussion

The liver is the major site of CsA metabolism and CsA is extensively metabolized and eliminated in the bile. An analysis of bile by HPLC, however, revealed that less than 1 per cent of an administered dose of CsA is excreted unchanged in the bile.[8] For the patient treated with CsA, therapeutic drug monitoring is necessary as it is difficult to assess individual variations in drug absorption, distribution, metabolism and elimination, which prevent CsA concentrations from being directly estimated by dose alone. This suggests that when a patient has hepatic

dysfunction, it will be much more difficult to establish a near-basal level of CsA and to maintain a steady condition because the patient needs CsA determination rapidly to change the actual amounts and inter-dose intervals of the CsA regimen in relation to the degree of the liver impairment and the cause of hepatic dysfunction, such as toxicity of CsA, rejection, ischemic damage, arterial thrombosis or biliary complication.

Regarding the influence of liver dysfunction on CsA pharmacokinetics, Kahan[1] reported that patients with impaired hepatic function cleared the drug one third more slowly, leading to an increased AUC, from a demographic analysis using radioimmunoassay measurement. However, since primary metabolites of CsA do not possess significant immuno-suppressant activity in animals[9] and the deterioration of hepatic function secondary to rejection or hepatic thrombosis frequently produced a disproportionate rise in the radioimmunoassay blood concentration, the use of radioimmunoassay in the setting of altered hepatic function grossly underestimates the CsA dosage required to achieve the usual therapeutic level of the parent CsA compound.[10] For these reasons, in our experiments, we measured the CsA blood concentration using the HPLC method and whole blood to evaluate the parent compound.

Hepatic dysfunction created by hepatectomy produced a 46 per cent reduction in CLs, a 43 per cent increase in AUC after intravenous CsA administration, and a prolongation of terminal half life by 51 per cent. The bioavailability of oral CsA, however, decreased by 31 per cent, even though the AUC of oral CsA administration did not change significantly, which suggested that the oral CsA dosage regimen and the inter-dose interval may not need to be changed immediately to intravenous administration.

On the other hand, the hepatic impairment created by bile duct ligation resulted in a different change in CsA kinetics compared to that created by hepatectomy. In spite of a 22 per cent depression in systemic clearance, which correlated well with the ICG-Rmax decreasing rate due to the hepatic dysfunction, the bioavailability and AUC of oral CsA administration markedly decreased, presumably due to the lack of sufficient bile salt for CsA absorption. Treatment should therefore be given rapidly by constant intravenous drugs or by double route therapy, in order to achieve the therapeutic level of the parent CsA compound.

In fact, CsA malabsorption is common in the period immediately following OLTX and is associated with such problems as diarrhea, liver failure and externalized bile drainage. In a pediatric liver transplant patient, a bioavailability of less than 5 per cent was reported when a large amount of bile was eliminated from the T-tube and rejection occurred in the postoperative period. This patient needed an intravenous CsA dosage of up to 4 mg/kg and oral CsA 13.3 mg/kg (double route therapy) for maintaining therapeutic blood levels of CsA.[10]

The assessment of hepatic dysfunction which influenced CsA kinetics has been reported, using elevated bilirubin levels. In this experiment, however, the serum bilirubin levels of the bile duct ligation dogs were about eight-times higher than the levels of the hepatectomized dogs, even though the hepatic dysfunction created by bile duct ligation was not as severe as that created by hepatectomy (the ICG-Rmax decreasing rate was 39 per cent in the bile duct ligation dogs and 58 per cent in the hepatectomized dogs, $p<0.05$). These data thus suggested that the serum bilirubin level is not a specific marker of liver dysfunction or drug metabolism. Moreover, we observed no significant difference in the prothrombin time or SGOT levels after either 70 per cent hepatectomy or bile duct ligation on the liver function test and there was no correlation between the prothrombin time or SGOT levels and the changes in CsA parameters.

For the assessment of hepatic dysfunction, especially after hepatectomy, to study the

regeneration of the liver, ICG-Rmax is known to be one of the most accurate parameters of hepatic function and a highly sensitive index to hepatic cell damage.[6] ICG-K is also known as an index of functional hepatic blood flow which becomes reduced due to shunting or impaired hepatocellular function.[14] In this experiment the ICG-Rmax and ICG-K decreasing rates correlated well with the change in CsA kinetics. After 70 per cent hepatectomy, the ICG-Rmax decreasing rate correlated well with the systemic clearance ($r=0.668$, $p<0.01$) and the ICG-K decreasing rate also correlated well with the bioavailability of oral CsA ($r=0.667$, $p<0.01$).

Concerning the correlation between the ICG kinetics and CsA kinetics after these hepatic injuries, although ICG kinetics showed severe hepatic dysfunction, the systemic clearance of CsA was observed with a significantly lower decreasing rate than the ICG kinetic parameters (Fig. 1). This significant discrepancy suggests that the pathway of metabolism and elimination between ICG and CsA could be different. ICG does not exhibit extrahepatic removal or enterohepatic circulation and it is not chemically altered during its transit through the liver,[15,16] whereas CsA metabolism on the other hand, is circumstantially implicated by the monooxygenase cytochrome P450.[17] This comparatively low decreasing rate in the systemic clearance of CsA, however, can not be attributed only to an increase in the capacity of remaining hepatocytes to metabolize CsA. There is also the possibility that CsA might be extensively distributed into peripheral body regions in the case of hepatic injuries, and the influence of the entero-hepatic circulation of CsA should also be considered.[18]

The data in this experiment demonstrates that liver impairment significantly influences the pharmacokinetics of CsA, not only by changes in the intestinal absorption but also by changes in hepatic metabolism. Safe and effective therapy will require an improved understanding of how CsA pharmacokinetics very with the degree and cause of hepatic dysfunction. The dosage regimen should therefore be individualized in patients with hepatic impairment in order to maintain an adequate blood concentration of CsA.

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