Liver-Function Studies in Heart-Transplant Recipients Treated with Cyclosporin A


Cyclosporine (CsA) hepatotoxicity has been reported but has not been studied systematically. This study includes 17 patients undergoing heart transplantation (HTx) and being treated with CsA, azathioprine, and corticosteroids. We assessed liver function in these patients before HTx and during the following month by five biological tests: total bile acids (BA), alkaline phosphatase (AP), γ-glutamyltransferase (GGT), and total bilirubin in serum, and the aminopyrine breath test (ABT). With these tests we could classify the patients into three groups before HTx: normal (group I), mildly altered (group II), and severely altered (group III) liver function. During CsA therapy we did not observe any changes in any test results except for BA and GGT, and these only in group III. The ABT improved significantly in this group. During kinetic studies in patients without liver dysfunction, we confirmed a direct interference of CsA on BA secretion mechanism, but not the GGT increase, which remains to be explained.

Additional Keyphrases: bile acids • pharmacokinetics • hepatotoxicity

Treatment with cyclosporine (CsA) has greatly improved the results of kidney, heart, bone marrow, and liver transplantation. Well-known side effects of the drug include nephrotoxicity, neurotoxicity, hypertension, gingival hyperplasia, anorexia, nausea, and ileus. Hepatotoxicity has been reported in 10% to 50% of renal allograft transplantations and in 86% of bone marrow transplantations, and has also been suggested in heart transplantations. After heart transplantation, isolated or combined increases in bilirubin, bile acids, aminotransferases, and γ-glutamyltransferase have been observed in serum, and have usually been attributed to CsA hepatotoxicity. The clinical studies related to this problem have been hampered by the multiple (and possibly synergistic) causes of liver dysfunction, not only including toxicity of the associated drugs administered but also bacterial or viral infections and heart failure.

Because in our first patients results of various tests monitoring liver function generally improved after heart transplantation (HTx), we thought it necessary to investigate to what extent CsA was hepatotoxic. This study was designed to generate a better understanding of the effect of CsA on liver function.

Materials and Methods

Analytical Procedures

CsA assay. The "trough" concentration of CsA in serum (12 h after the last oral CsA dose) was determined with a radioimunoassay (RIA) kit provided by Sandoz Laboratories (Basel, Switzerland) that contained a polyclonal antibody cross-reacting to a certain degree with metabolites; the 3H-labeled CsA tracer was replaced by an 125I-labeled CsA tracer from Immunonuclear, Stillwater, MN. The blood samples were centrifuged after standing for 1 h at room temperature, then analyzed for CsA.

Enzyme and total bilirubin assays. We measured ALT AP, GGT, and total bilirubin in serum at 30°C by IFCC recommended methods, with commercially available kits (Boehringer-Mannheim, Mannheim, F.R.G.). All these tests were done in the Hitachi 705 multianalyzer.

Bile acids assay. We measured BA in serum with a fully enzymatic colorimetric test (cat. no. 14352; Merck Diagnostics, Darmstadt, F.R.G.).

Aminopyrine breath test. The aminopyrine breath test (ABT) measures excretion of labeled CO2 after oral administration of N-methyl-labeled aminopyrine, a drug demethylated almost exclusively by the hepatic controllobular microsomal mixed-function oxidase system. This test is very sensitive for detecting liver dysfunction related to heart failure. This test was performed both preoperatively and 30 days after HTx.

Upper reference limits. These were established as the above analytes in serum in our laboratory: ALT, 35 U/L; GGT, 40 U/L; AP, 100 U/L; bilirubin, 20.5 μmol/L; BA, 6 μmol/L; and ABT, >4.5% 2 h after ingestion.

Statistical analysis. This included an analysis of variance.

Patients

HTx. This was performed in 17 patients (two women, 15 men; ages 17 to 54 years; weight range 40 to 86 kg) with end-stage cardiac failure. Of these, 15 were ischemic, one was toxic, and one had postpartum cardiomyopathy. There was no previous history of liver disease and none had taken any hepatotoxic drug or had abused alcohol during the previous six months. Results of preoperative and postoperative serology tests for hepatitis A (anti-HA IgM) and B (HBs Ag and anti-HBc IgM), cytomegalovirus (IgM), and Epstein-Barr virus (IgM) were negative.

Preoperative liver function. This was assessed for the patients by measuring ALT, GGT, AP, bilirubin, BA, and ABT. Of the various tests performed, four (bilirubin, BA GGT, and ABT) were selected as the best indicators of liver dysfunction before HTx (Figure 1).

Classification of the patients. As shown in Figure 1, we classified the patients into three groups as scored for abnormal values for these four selected tests: e.g., AP <100 U/L = normal test = 0 point; AP >100 U/L = abnormal test = 1 point. Patients whose score was 0 or 1 were placed in group 1 (n = 7, normal liver function); score 2 or 3, in group II (n = 5 mildly altered liver function); and score 4 or 5, in group II...
ABT

Fig. 1. Operative values (mean and range) for serum bilirubin, bile acids, GGT, and ABT in patients with normal liver function (group I, n = 7), mildly altered liver function (group II, n = 5), and severely altered liver function (group III, n = 5).

(n = 5, severely altered liver function). By this evaluation, therefore, 10 of the 17 patients displayed preoperative liver dysfunction, presumably owing to heart-induced changes.

Immunosuppression. The immunosuppressive therapy consisted of treatment with CsA, corticosteroids, and azathioprine. CsA (5 mg per kilogram body weight) was given a few hours before surgery, followed by oral doses 12 or 24 h later according to the "trough" concentration of CsA in serum. Methylprednisolone was administered intravenously, 125 mg three times a day on day 0 to 2 only, followed by oral prednisolone, 20 mg three times a day, which was decreased by 5 mg every other day to achieve a daily maintenance dose of 10 mg on day 30. Azathioprine was administered orally, 3 mg per kilogram body weight each day during the first two weeks; it was discontinued in the absence of signs of rejection on examination of subendocardial biopsy.

Pre- and Post-Transplantation Evaluations of Liver Function

Pre-transplantation kinetics studies of liver function. Twenty-four-hour kinetic studies of ALT, GGT, AP, bilirubin, and BA concentrations in serum were measured in specimens collected 1, 2, 3, 4, 6, 8, 10, 12, and 24 h after administration of a placebo or of CsA in an oral dose of 5 mg per kilogram body weight to eight patients without liver dysfunction (group I). The areas under the curve (AUC) of all the biochemical variables and the ratio AUC BA/AUC CsA were calculated.

Post-transplantation follow-up of liver function. Concentrations of ALT, GGT, AP, BA, bilirubin, and CsA in serum were followed for all the patients: before operation, daily during the first 14 postoperative days, and on day 30 (at 0800, 12 h after the last oral dose of CsA).

Results

Pre-transplantation effect of CsA on a normal liver function. Compared with placebo, CsA administration significantly increased bile acids in serum, while ALT, GGT, bilirubin, and AP did not increase significantly in any case. The areas under the curve (mean and range) for concentrations of bile acids (BA AUC) were significantly increased (P <0.05) after CsA administration, 220 (78-516) mg/m²/24 h, compared to these during placebo, 68 (67-73) mg/m²/24 h. Whatever the CsA concentration in serum, high (AUC 5100) or low (AUC 722), the BA increase was proportional, as confirmed by the ratio BA/CsA AUC, which remained within narrow limits in all eight patients: 0.101 to 0.111, mean 0.105. Figure 2 shows an example of the 24-h pharma-

Fig. 2. Pretransplantation kinetics of liver function variables over 24 h in a patient with normal liver function after placebo (x-x) and after a single oral dose (5 mg per kilogram body weight) of CsA (— —). Broken line shows upper limits of normal values for each test.

cokinetic evaluation of the variables studied in one of these patients.

Peak CsA concentrations were observed at 2 h in each patient, whereas BA concentrations were greatest at 3 h or 6 h.

Post-transplantation evaluation of liver function. Based on estimation of the ABT, liver function remained stable in group II, but improved during the postoperative period in patients of group I and (particularly) of group III (mean and range). The preoperative values compared with the values on day 30 were: group I from 4.8% (3–6%) to 6.7% (3.2–9.6%); group II from 4% (3–5%) to 3.6% (3–7%); group III from 1.8% (1.2–2.6%) to 3.8% (3–5%) (P <0.01). ALT, AP, and bilirubin concentrations in serum did not change significantly during the postoperative period in any of the three groups.

On the other hand, increased concentrations of bile acids in serum were observed from day 4 to day 14, and on day 30 only in group III (Figure 3); GGT values were also significantly increased when compared with pre-HTx values, but only on day 30 for all the patients of this group (data not shown). These increases were not observed in any of the other groups. CsA doses decreased significantly (P <0.001) from group I to group III (mean and SD), respectively—6.5 ± 0.8, 4.5 ± 0.9, and 2.9 ± 0.9 mg per kilogram body weight per day—to achieve the same "trough" concentration of CsA in serum, 238 ± 90 μg/L (by RIA), for the three groups during the 4th to 14th day period, and on day 30 as well.

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Discussion

Hepatotoxicity of CsA has been claimed or suspected in numerous articles (1–9).

At the CsA doses given to our patients, we did not observe such toxicity. No significant changes in ALT, AP, or bilirubin were observed in any of the patients of the three groups. Moreover, liver dysfunction as assessed by ALT showed no alteration in group I or II, and a significant improvement was observed in group III (with preoperative dysfunction). This postoperative improvement favors the hypothesis that the liver dysfunction was essentially attributable to cardiac failure, and it argues against any detectable hepatotoxicity of the treatment. These observations are at variance with other studies (5–9) showing modifications of bilirubin, AP, or ALT. Reasons for these discrepancies could include other immunosuppressive therapy (no azathioprine and higher "tough" concentration of CsA) and thus potential hepatotoxicity at higher concentrations of CsA.

On the other hand, serum BA and GGT significantly increased after HTx in patients with severely impaired liver function preoperatively. One could suspect a direct effect on bile acids uptake and on enzyme induction (GGT) without hepatotoxic effect or alteration of the functional cell mass.

Impairment of bilirubin and BA excretion induced by CsA has been demonstrated by Schade et al. (13) in heart-transplant recipients and by Rotolo et al. (14) in the isolated perfused rat liver model, but high amounts of CsA were used in both studies. Increases in serum BA under our conditions, with lower "tough" concentrations of CsA in serum and without bilirubin changes, suggest that CsA impedes BA excretion long before that of bilirubin.

Increase of GGT in serum was also observed in our patients, but only in group III and on the 30th postoperative day. Histochernically, GGT can be mainly demonstrated in the ductular cells (15). This enzyme is a very sensitive indicator of cholestasis, but its concentration is also increased in the serum of patients treated with enzyme-inducing drugs. GGT was also reported to be irregularly and inexplicably increased for months after heart transplantation in 20 patients receiving CsA (6). Because no clear-cut effect on GGT was demonstrated in our kinetic studies, a direct relationship between CSA and postoperative increase in GGT in group III patients cannot be postulated.

In conclusion, our heart-transplant recipients treated with CsA did not show signs of hepatotoxicity. However, a direct effect of CsA on bile acids excretion was clearly demonstrated. Further experiments will be needed to clarify the physiological mechanism responsible for the effect of CSA on bile clearance, albeit already described on animal models (13, 14–16).

References