Routine "Cardiac" and "Hepatic" Serum Enzyme Profiles in Cardiac-Transplant Patients Treated with Cyclosporine A: Operative and Post-Operative Findings

F. N. McKenzie,¹ G. C. Moses,² and A. R. Henderson²

We report representative serum enzyme changes after cardiac transplantation in 20 patients receiving post-transplant therapy with cyclosporine. In general, the changes resembled those after acute myocardial infarction or coronary artery bypass surgery, but were more prolonged. Cardiac biopsy or episodes of cardiac rejection did not usually alter serum enzyme activities. Cyclosporine A toxicity appeared to be responsible for increases in serum transaminases (alanine and aspartate) and lactate dehydrogenase-5 activities. Serum γ-glutamyltransferase activities were intermittently, and inexplicably, increased for months after the transplant.

Lott and Stang (1) have reviewed the major serum enzyme findings in myocardial ischemia and necrosis. Neumeier and Kemkes (2) have summarized the creatine kinase (EC 2.7.3.2; CK) changes in serum during the peri-operative phase of coronary artery bypass surgery.⁵ There have also been many articles describing the general enzyme changes in serum after coronary artery bypass surgery; for example, Ström and his colleagues (3–5) have thoroughly defined the CK, aspartate aminotransferase (EC 2.6.1.1; AST), and lactate dehydrogenase (EC 1.1.1.27; LD) patterns during and after coronary bypass operations. However, there appears to be little information on enzyme changes in serum after cardiac transplantation—a procedure that is likely to become increasingly common with the advent of immunologic suppression by cyclosporine A (6). We therefore report some representative findings on the peri- and post-operative serum enzyme profiles of cases of cardiac transplantation treated with cyclosporine A.

Materials, Methods, and Clinical Procedures

Enzyme Assays

Enzyme activities in serum were determined at 37 °C by recommended methods (7, 8). We used commercially available kits (supplied by Boehringer Mannheim Canada Ltd., Dorval, Quebec) and the LKB 2086 Mark II Reaction Rate Analyzer (LKB Produkter AB, Bromma 1, Sweden) for determination of CK, AST, alanine aminotransferase (EC 2.6.1.2; ALT), and LD. γ-Glutamyltransferase (EC 2.3.2.2; γ-GT) and alkaline phosphatase (EC 3.1.3.1; AP) were determined with Enzyme Analyzer System TR (Beckman Instruments Inc., Fullerton, CA 92634).

LD isoenzymes were separated on agarose gels (Universal Electrophoresis Film; Corning Medical and Scientific, Palo Alto, CA 94306) and quantified by measuring the fluorescence of NADH, as previously described (9). CK isoenzymes were separated on agarose gels with the Beckman Reagent System (Beckman Instruments Inc., Brea, CA 92821) and quantified densitometrically. Total bilirubin was determined by a continuous-flow Jendrassik–Grof procedure (SMA II, Technicon Instruments Corp., Tarrytown, NY 10591). Cyclosporine A was radioimmunoassayed (10); we initially assumed ideal therapeutic trough concentrations to be 100 to 400 μg/L, but later decreased them to 100 to 200 μg/L.

Upper reference limits for serum established for this laboratory were: ALT and AST, 30 U/L; total LD 378 U/L; γ-GT, 30 U/L; total CK, 174 (for males) and 140 (for females) U/L; LD-1/LD-2 ratio, 0.75 (11); LD-5, 16% of total LD activity (9); CK-2 (CK-MB), 10 (for males) and 8 (for females) U/L; and total bilirubin, 2–10 mg/L.

Clinical Procedures

Cardiac transplantation was performed on 20 patients (17 males, three females, age range 14–52 years). Six patients had ischemic cardiomyopathy; 13 others had congestive cardiomyopathy; another had restrictive cardiomyopathy. All had terminal heart disease, with a life expectancy of less than a year. There was no significant secondary organ dysfunction. All had failed trials of medical therapy or had undergone previous unsuccessful cardiac surgery.

All patients underwent standard orthotopic cardiac transplantation. Total ischemic time for the donor heart varied between 30 and 80 min. Immunosuppression was begun immediately before operation with an oral dose of cyclosporine A. In early patients in this series cyclosporine A was given orally in a dose of 50 mg/kg body weight, 4 to 6 h before surgery, to maintain concentrations of at least 100 to 400 μg/L. This pre-operative dose has since been steadily reduced, and currently is 5 mg/kg.

After surgery, cyclosporine A is continued in a dose sufficient to maintain serum concentrations of at least 100 to 200 μg/L.

A single 500-mg dose of methylprednisolone is given during the operation, and thereafter 1 mg of prednisone per kilogram is given daily for the first week post-operatively. The prednisone dose is decreased rapidly thereafter to a maintenance dose of 10 to 15 mg per day, usually achieved by the end of the first post-operative month.

Endomyocardial biopsies are obtained weekly after transplantation, regardless of the patient's course. Rejection, as evidenced by myocardialysis, is treated by pulse (i.e., single dose) steroid therapy for three days, followed by a further cardiac biopsy. Cyclosporine A concentrations are adjusted to the therapeutic trough quoted earlier, if they have been abnormal.
**Results**

**Creatine Kinase**

Total CK activity typically was greatest 24 to 48 h after the operation (Figure 1), values ranging between two- and 10-fold the upper normal reference limit. CK-2 (CK-MB) isoenzyme activity usually peaked 6 h before total CK activity did, values for CK-2 being between eight- and 15-fold the upper normal reference limit. Both activities declined to within their respective reference limits by five to seven days. Neither cardiac biopsy nor episodes of transplant rejection was ever accompanied by increased creatine kinase isoenzyme activities.

**Lactate Dehydrogenase**

Total LD activity usually was greatest within the first two days, then declined slowly during the next 20 days, although we have observed persistent increases for as long as 60 days.

The LD-1/LD-2 ratio, indicating myocardial damage or hemolysis, remained increased for between 20 to 40 days, depending on the number of blood transfusions and whether or not there were complications. The procedure of endocardial biopsy or the onset of episodes of transplant rejection were not usually associated with increases in either LD or LD-1/LD-2 ratio. However, in one of our cases (Figure 2) endocardial biopsy, done on post-operative days 7, 14, and 21 (none showed histological evidence of rejection), appeared to be associated with changes in LD activity: the first two biopsies were associated with increases in total LD activity, the third with an increased LD-1/LD-2 ratio. As noted earlier, we detected no changes in CK-3 or CK-2 activities in this patient.

“Liver” Enzyme Profiles

Once the initial trauma to the patient’s cardiac remnant and to the transplanted heart is over, it is appropriate to consider serum AST activity together with ALT, γ-GT, AP, and LD-5 activities as “liver” enzymes and to review their changes together. AST usually remains increased for the first four to five days after the operation, then returns to normal. ALT and LD-5 are usually increased only on post-operative days 1 and 2, and then only marginally. However, if the trough concentrations of cyclosporine A are too high, as occurred in case 4 during the first week (in μg/L: day 1, 784; day 2, 712; day 3, 457; day 4, 516; day 5, 417; day 6, 178; day 7 and onward, <200) after transplant (Figure 3), then both AST and ALT will be above normal and LD-5 activity may increase slightly. In the first case to be transplanted in this hospital, the trough cyclosporine A concentrations were much higher than is now considered optimal during the first 14 days after transplant (in μg/L: day 0, 300; day 1, 360; day 2, 600; day 3, 513; day 4, 756; day 5 to day 16, 567 to 251; day 17 and onward, <200). During that period AST, ALT, and LD-5 were clearly increased, between five- and 10-fold, only returning to the reference interval when the cyclosporine A concentration decreased (Figure 4).

γ-GT is often above normal at the time the transplant is done, because of the preceding, often prolonged, period of cardiac failure—but activity appears to decrease to relatively normal values within two to 10 days after the transplant. However, many of our transplant patients subsequently showed varying, and often transient, degrees of increase in γ-GT activity during the following months, some as great as 41-fold the upper reference limit, but most two- to 10-fold. Of our 18 cardiac transplants, 10 showed clearly increased γ-GT on a long-term basis. Values for γ-GT and AP showed no correlation; AP consistently remained normal or nearly normal during the entire course of study in each patient.
Discussion

The surgical assault on the transplanted heart causes increases in cardiac enzymes activity in serum that are very similar to, but more prolonged, than those seen after a myocardial infarction or coronary artery bypass operation. The secondary increase (Figure 3) in the cardiac transplant patient is unique to the cyclosporine A-suppressed host and probably is ascribable to hepatotoxicity. Cyclosporine A has toxic effects on the liver that have not always been clearly defined. For example, in a group of 21 bone-marrow transplant recipients, 86% had one or more episodes of hyperbilirubinemia, and of 10 patients with defined cyclosporine A toxicity, ALT increases were between two- and seven-fold the upper reference limit. By contrast, AP showed only a minimal elevation (13). Another group (14) who undertook bone-marrow transplants described only hyperbilirubinemia. In 30 cardiac-transplant recipients immunosuppressed with cyclosporine A and prednisone, hyperbilirubinemia and slight increases (up to two-fold) in ALT were observed but no increases in either AST or AP (15). The Canadian Transplant Study Group (16) recorded complications in 142 recipients of cadaveric renal transplants treated with cyclosporine A. Only five episodes of (undefined) clinical hepatotoxicity were reported, all of which resolved on decreasing the minimum concentration of cyclosporine A to (presumably much) less than 700 μg/L. In our hospital's study of the treatment of early type 1 (insulin-dependent) diabetes mellitus (17) with cyclosporine A, the serum bilirubin had increased, at 30 days after commencing treatment, by 42% and serum AP activity by 36%; values for both subsequently returned to baseline as treatment progressed.

It is becoming evident that prolonged administration of cyclosporine is nephrotoxic. Myers et al. (18) recently reported cyclosporine-induced nephropathy in cardiac-transplant patients. Accordingly, we examined pre- and post-operative values for serum creatinine in our group of patients and found increases, some of them significant (e.g., 10 to 35 mg/L) during days 1 through 10 in all 19 patients. In most, this initial increase was followed by a slow but steady decrease to near pre-operative values, but with considerable individual variation. For only two of the 20 patients did values remain above pre-operative values (and above the upper limits for the appropriate age/sex reference range) for 500 days or longer after the operation (e.g., 30 and 26 mg/L).

Clearly, serum enzyme evidence of cyclosporine A hepatotoxicity can be transient (see Figure 3). It is therefore essential, when seeking evidence for this side effect, to obtain frequent specimens for analysis. The post-operative increase in serum γ-GT activity, which is persistent in many cardiac-transplant patients, does not appear to have been observed by other groups, and we cannot explain this increase. The most likely mechanism would appear to be enzyme induction. We therefore compared values for serum γ-GT in patients with cadaveric renal transplants, who had been treated with cyclosporine A, with a matched group of patients treated with azathioprine and prednisolone in this hospital. Neither group showed increased γ-GT. This contrasts with other reports (19, 20) in which several renal-transplant patients were found to have increased serum γ-GT activities. Some of our cardiac transplant patients received, post-operatively, drugs such as phenobarbital or...
phenytoin, which are known to induce microsomal enzyme activity, but γ-GT activities did not appear to be related to administration of these drugs, and other patients who did not receive these drugs also had increased γ-GT activities. A more plausible explanation is that, after a myocardial infarction, serum γ-GT may be increased during myocardial tissue repair (21), but this explanation may not account for the long-term elevation seen in many of our patients. Clearly, this problem needs further study.

A pertinent study on hepatic dysfunction after open-heart surgery in 93 patients has recently appeared (22); early post-operative findings suggested a leak of enzymes from both myocardium and liver. Even after two weeks, half the patients had abnormal values for ALT, 28% abnormal AP, and 45% abnormal γ-GT. Interestingly, our own experience suggests that hepatic dysfunction is not a feature of the post-cardiac-transplant state unless cyclosporine A toxicity is present.

There are many contradictions in the current literature regarding the effects of routine procedures. Evidently, more work is required to elucidate the reasons for these contradictions.

This work was supported by grant AN047 from the Ontario Heart and Stroke Foundation.

References