EFFECT OF MYCOPHENOLATE MOFETIL IN HEART TRANSPLANTATION

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OBJECTIVE: To study the effect of mycophenolate mofetil (MMF), a new immunosuppressive drug that acts by inhibiting de novo pathways of purine synthesis, and rabbit antithymocyte globulin (RATG) on the lymphocyte subpopulation after heart transplantation.

DESIGN: A review of clinical and laboratory records.

SETTING: The Montreal Heart Institute.

PATIENTS: Thirty-one patients who underwent heart transplantation. In 9 patients, neoral cyclosporine, prednisone and azathioprine were administered (group 1). In 14 patients RATG was added during the first 3 postoperative days (group 2) and in 8 patients RATG and combination immunosuppression was given, but MMF was used instead of azathioprine (group 3). The demographic characteristics of donors and recipients were similar among the 3 groups.

MAIN OUTCOME MEASURES: The proportion of CD2, CD4 and CD8 receptor-positive lymphocytes, expressed as a mean (and standard deviation) percentage of the total lymphocyte population, measured at 7, 15 and 30 days and 6 months after transplantation.

RESULTS: At 7 days after transplantation, CD2 lymphocytes averaged 55% (18%), 16% (15%) and 14% (11%) in groups 1, 2 and 3 respectively (p < 0.05), CD4 averaged 36% (11%), 9% (12%) and 7% (8%) in groups 1, 2 and 3 (p < 0.05), and CD8 averaged 14% (6%), 4% (3%) and 4% (3%) in groups 1, 2 and 3 (p < 0.05). At 15 days after transplantation CD2 averaged 69% (10%), 42% (16%) and 47% (20%) in groups 1, 2 and 3 respectively (p < 0.05), and CD8 averaged 16% (7%), 16% (6%) and 19% (7%) (p = NS). At 30 days after transplantation the percentages of CD2, CD4 and CD8 lymphocytes were similar among the groups. The freedom rate from acute rejection averaged 22% (14%), 9% (8%) and 50% (18%) (p < 0.05) in groups 1, 2 and 3 at 6 months after transplantation, and the freedom rate from infection averaged 56% (17%), 36% (13%) and 38% (17%) for the 3 groups at this time period (p = NS).

CONCLUSIONS: A short course of RATG causes severe, transitory depletion of CD2, CD4 and CD8 lymphocyte subpopulations. MMF decreases the incidence of early acute rejection after heart transplantation without affecting the lymphocyte subpopulation when compared with azathioprine.

OBJECTIF: Étudier l’effet du mycophénolate mofétil (MMF), nouvel agent immunosuppresseur qui agit en inhibant les voies de synthèse de novo des purines, ainsi que des globulines antithymocytes de lapin (GATL), sur la sous-population des lymphocytes après une transplantation cardiaque.

CONCEPTION : Étude de dossiers cliniques et de laboratoire.

CONTEXTE : Institut de cardiologie de Montréal.

PATIENTS : Trente-et-un patients qui ont subi une transplantation cardiaque. On a administré de la cyclosporine n éorale, de la prednisone et de l’azathioprine à neuf patients (groupe 1). On a ajouté des GATL chez 14 patients au cours des trois jours qui ont suivi l’intervention (groupe 2) et l’on a administré à huit patients des GATL conjuguées à des immunosupresseurs, mais on a utilisé le MMF au lieu de l’azathioprine (groupe 3). Les caractéristiques démographiques des donneurs et des receveurs étaient semblables entre les trois groupes.
Mycophenolate mofetil in heart transplantation

MULTIPLE DRUG THERAPY BASED ON CYCLOSPORINE COMBINED WITH CORTIcOSTEROIDS AND AZATHIOPRINE HAS IMPROVED THE RESULTS IN SOLID ORGAN TRANSPLANTATION. Nevertheless, episodes of acute rejection are frequent and affect both short- and long-term prognosis after heart transplantation. The usefulness of these drugs is limited by their toxic side effects, such as renal dysfunction and bone marrow suppression.

Azathioprine is a purine analogue that slows the entire purine biosynthetic pathway. Its primary side effect is bone marrow suppression. Mycophenolate mofetil (MMF) is a new immunosuppressive drug that targets specific biochemical components of lymphocytes and does not have the toxicity of currently used agents. MMF inhibits inosine monophosphate dehydrogenase, an enzyme involved in the de novo pathway for purine biosynthesis. T and B lymphocytes are highly dependent on the de novo pathway for purine synthesis; however, other cell types can use the salvage pathway for the generation of purine. Therefore the selectivity of MMF can result in an inhibition of lymphocytes' proliferative response with less potential than azathioprine for inducing myelotoxicity. The objective of this study was to evaluate the effect of MMF on the lymphocyte subpopulation and its efficacy in preventing acute rejection after heart transplantation.

PATIENTS AND METHODS

A retrospective analysis of clinical and laboratory data from 31 consecutive patients who underwent heart transplantation at the Montreal Heart Institute between 1996 and 1998 was carried out. Three sequential treatment regimens were used and analysed. Nine patients were administered a triple immunosuppression combination, including cyclosporine (Neoral; Novartis Pharma Canada, Dorval, Que), corticosteroids and azathioprine (group 1); in 14 patients, rabbit antithymocyte globulin (RATG) was added during the first 3 days after transplantation to the triple immunosuppression (group 2), and in 8 patients RATG and triple immunosuppression was used, but MMF was substituted for azathioprine (group 3).

The following immunosuppressive protocol was used for each drug. Neoral cyclosporine was started on day 1 after transplantation, with the objective of maintaining a mean cyclosporine blood level between 200 and 400 µg/L. RATG (obtained from the Institut Merieux, Lyon, France), 125 mg, was infused intravenously for the first 3 consecutive days after transplantation. SoluMedrol 500 mg, was administered at the time of transplantation and for the next 2 days at a dosage of 125 mg/ d. Prednisone was then started and given following a standardized calendar at a maintenance dose of 5 to 10 mg/ d. Azathioprine was started 1 day after transplantation at a daily dose of 1 to 2 mg/kg and adjusted to maintain leucocyte counts between 5.0 and 7.0 × 10⁹/L. MMF, when used, was started 1 day after transplantation at a dose of 2 g/ d.

In all patients, cardiac biopsy for surveillance of acute rejection was performed every week during the first month and every other week for the next 3 months, and then monthly until the end of the first year after transplantation. Each biopsy specimen was studied, and rejection was graded according to the classification of Billingham and associates. Grades 3 and 4 rejection were considered significant and treated with an increase in immunosuppression. The infection rate included bacterial viral and protozoan infections.

Clinical and laboratory data were entered prospectively in a computerized database. All patients, whether elective, urgent or mechanically supported before transplantation were included, and none were lost to follow-up. Cyclosporine blood levels were...
measured using the Abbott TDX monoclonal specific assay (Abbott Axysym System; Abbott Laboratories, Abbott Park, Ill.).

The flow cytometric analysis of the lymphocyte subpopulation from whole blood was performed as follows. Briefly, within 30 minutes of blood collection, 100 µL of whole blood was added to 5 µL of the appropriate antibody (Cytostat Coulter klone liquid murine monoclonal antibody; Coulter Electronics of Canada, Burlington, Ont.). After stirring, the specimen was incubated for 10 minutes at room temperature in the dark, then immunoprep-treated (leukocyte preparation system; Coulter Electronics).

The samples were analysed in a Coulter EPIC Profile II flow cytometer within 2 hours of collection. The lymphocyte population was identified by its light-scatter characteristics and enclosed in a bit map. Five thousand lymphocytes per sample were analysed. Data are expressed as means and standard deviations. For comparison among groups, an analysis of variance was used. Kaplan–Meier analysis was used to examine freedom from rejection, infection and patient survival.

RESULTS

Lymphocyte subpopulations were expressed as the percentage of the total lymphocyte population. CD2 receptor-positive cells averaged 55%(18%), 16%(15%) and 14%(11%) at 7 days (p = 0.0001) and 69%(10%), 42%(16%) and 47%(20%) at 15 days (p = 0.0013) after transplantation respectively in groups 1, 2 and 3 (Fig. 1). CD4 lymphocytes averaged 36%(11%), 9%(12%), 7%(8%) at 7 days (p = 0.0001) and 46%(12%), 19%(12%) and 21%(21%) at 15 days after transplantation (p = 0.0006) respectively in groups 1, 2 and 3 (Fig. 2). CD8 lymphocytes averaged 14%(6%), 4%(3%) and 4%(3%) at 7 days (p = 0.0001) and 16%(7%), 16%(6%) and 19%(7%) at 15 days after transplantation (p = NS) respectively in groups 1, 2 and 3 (Fig. 3).

The lymphocytes subpopulation for CD2, CD4 and CD8 positive cells were similar 30 days after transplantation in all groups (p = NS). Leukocyte counts at 1 month averaged 8(2), 7(2) and 8(3) × 10^9/L respectively in groups 1, 2 and 3 (p = NS).

Actuarial freedom from acute rejection was significantly higher in group 3 when compared with the 2 other groups, averaging 22%(14%), 9%(8%) and 50%(18%) respectively (p = 0.02) 6 months after transplantation (Fig. 4). Freedom rate from infection was similar in all groups, averaging 56%(17%), 36%(13%) and 38%(17%) respectively (p = NS) 6 months after transplantation (Fig. 5). There was no difference in terms of the type of infecting agents: bacterial and viral. Patient survival averaged 89%(10%), 86%(9%) and 100% respectively in groups 1, 2 and 3, 6 months after transplantation (p = NS).

Cyclosporine blood levels averaged 313(77), 292(77) and 363(42) µg/L respectively in groups 1, 2 and 3 (p = NS) 1 month after transplantation.

DISCUSSION

Many immunosuppressive drugs impair lymphocyte formation. Cyclosporine is known to decrease total lymphocyte count in patients after heart transplantation. In this study, the most important decrease in the lymphocyte subpopulation occurred in RATG-treated patients. Lymphocytes were decreased throughout the first 15 days in groups 2 and 3, receiv-
Mycophenolate mofetil is a specific inhibitor of the de novo pathway used for the biosynthesis of guanosine nucleotides. Lymphocytes are highly dependent on the de novo pathway for the generation of purine. In vitro studies have demonstrated that MMF inhibits the proliferative response of T and B lymphocytes. Azathioprine, a purine analogue, blocks several enzymes involved in the purine biosynthesis pathway. Thus, the immunosuppressive action of azathioprine is through a non-selective inhibition of the leukocytes’ proliferative response. Lymphocyte subpopulations for CD2-, CD4- and CD8 receptor-positive cells were similar in the azathioprine and MMF-treated patients throughout the first month after transplantation. The safety and efficacy of MMF in heart transplant recipients has been initially evaluated in the treatment of acute rejection. MMF succeeded in controlling histologic rejection in the majority of patients. The clinical efficacy of MMF for preventing acute rejection has been evaluated in renal allograft recipients. In a randomized study comparing MMF to azathioprine as a part of a triple immunosuppression regimen with cyclosporine and corticosteroids, MMF (2 g/d)-treated patients experienced a significantly lower proportion of acute rejection episodes than patients treated with azathioprine. In the present study, heart transplant recipients treated with MMF experienced a significantly lower proportion of acute rejection episodes over 6 months compared with patients treated with azathioprine. Episodes of infection after heart transplantation remain a major problem. The degree of immunosuppression, prior latent infection and the underlying medical conditions of the recipient can predispose to opportunistic infections after transplantation. RATG induced a transitory but severe lymphopenia, and treated patients experienced a high rate of infection 6 months after the transplantation, but the difference was not statistically significant. It has been reported that patients treated with MMF (2 or 3 g/d) had an increase in the number of cytomegalovirus invasive infections. However, freedom rates from infection 6 months after transplantation were similar in azathioprine- and in MMF-treated patients in our study.

MMF with cyclosporine and prednisone is effective in preventing acute rejection after heart transplantation in
patients who receive a 3-day course of RATG postoperatively. Although the percentage of lymphocytes with positive CD2, CD4 and CD8 receptors was similar in patients treated with M M F to those treated with azathioprine, the rate of acute rejection was significantly reduced with M M F. M M F also appears to be safe when used with the polyclonal antibody RATG. The effect of M M F on the appearance of transplant vasculopathy and long-term patient survival remains to be studied.

References


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