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Impact of diltiazem administration and cyclosporine levels on the incidence of acute rejection in heart transplant patients

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Abstract To identify the clinical factors associated with acute rejection (AR) in the first year after heart transplantation (HT), we analysed 112 patients. All patients received OKT3 and standard triple-drug therapy. We analysed the following variables to determine their relationship with AR: age and gender, panel-reactive antibodies, HLA-DR mismatch, use of Sandimmune vs Neoral, diltiazem administration, and cyclosporine levels in week 2 and months 1, 2, and 3 after HT. Fifty-two patients had no AR and 49 had at least one episode. The variables independently associated with absence of AR were diltiazem administration (odds ratio 0.306, confidence limit 0.102–0.921) and cyclosporine level in the first month after HT (odds ratio 0.996, confidence limit 0.992–0.999). Furthermore, a cyclosporine level greater than 362 ng/ml in the first month predicted the absence of AR. In conclusion, a cyclosporine level greater than 362 ng/ml and diltiazem administration in the first month after HT reduce AR during the first year. Both cyclosporine level and diltiazem show a large and independent protective effect.

Keywords Diltiazem · Cyclosporine · Pharmacokinetics · Immunosuppression · Rejection · Heart transplant

Introduction

Since the advent of immunosuppressive therapy, heart transplantation (HT) has emerged as a widely accepted therapeutic option for end-stage heart disease. Nevertheless, cellular acute rejection (AR) remains a major obstacle to long-term survival. Data from the International Society of Heart and Lung Transplantation (ISHLT) indicate that AR accounts for 20% of deaths early after transplantation [1].

The morbidity and mortality associated with AR is related to both the effect of rejection on left ventricular function and the result of anti-rejection therapy [2], which is associated with an increased risk of infection and subsequent malignancies [3]. However, with current methods of immunotherapy and rejection management,

over 40% of patients experience no clinical rejection for 6 months or more after HT [4].

Few analyses, though, have attempted to examine clinical factors that affect the frequency of AR [5]. Our aim was to identify the clinical factors associated with AR in the first year after HT.

Patients and methods

Study population

From January 1991 through June 1996, 112 orthotopic cardiac transplants (Lower's and Shumway's technique [6]) were performed at the Doce de Octubre Hospital. All patients suffered from advanced heart disease (New York Heart Association functional class III and class IV). The average age of the recipients (11 women and 101 men) was 51 ± 9 years (range 14–65 years). The native heart diseases that necessitated HT comprised ischaemic heart disease in 60 recipients (53.6%), idiopathic dilated cardiomyopathy in 34 (30.3%), valvular heart disease in 14 (12.5%), and other causes in four recipients (3.6%).

Eleven patients were excluded due to early death (15-day perioperative period) not related to AR. The presumed causes of death were early graft failure in eight cases, multi-systemic organ failures in two, and infection (*Aspergillus* sp.) in one.

The average age of the donor was 26 ± 8 years (range: 8–55 years), and the average ischaemia time was 192 ± 49 min (range: 93–350 min). Both donors and recipients were matched for weight and ABO blood group.

We used panel-reactive antibody (PRA) activity for histocompatibility testing. PRA activity was measured by lymphocytotoxic antibody screening against a 45–60 HLA-typed cell panel. The PRA value of each serum was calculated as percentage of positive reactions with panel cell. The number of mismatched antigens assessed the degree of donor–recipient histocompatibility for each of the HLA-DR.

Immunosuppressive protocol

Monoclonal antibody OKT3 was administered intravenously at a dosage of 5 mg/day for 14 days. Methylprednisolone was administered at 500 mg intravenously, before and during surgery and at 125 mg intravenously every 8 h for three doses after operation, followed by prednisone at 1 mg/kg per day orally, tapered by 0.1 mg/kg on alternate days to 0.2 mg/kg per day and reduced to 0.1 mg/kg per day after 1 year. Azathioprine was administered at 4 mg/kg intravenously before transplantation, followed by 2 mg/kg per day orally, to maintain white blood cell levels above 4,000/mm^3. Cyclosporin A (CyA) was administered at 5–8 mg/kg per day to maintain serum CyA levels within the range of 250–350 ng/ml during the first year, 150–200 ng/ml for the second year, and 100–150 ng/ml during the third and all following years. From 1991 till March 1995 we used Sandimmune; after this period, we used the Neoral formulation of CyA. Morning trough CyA concentrations (collected approximately 12 h post-dose) were measured in whole blood by selective radioimmunoassay (RIA). Intra-assay and inter-assay coefficients of variation were below 10% across the calibration range for this assay.

During this 5-year enrolment period, the therapeutic protocol varied. Between 1991 and 1993, transplant patients ($n = 45$) received standard triple-drug therapy regimen (CyA, azathioprine, and prednisone). From 1993, patients ($n = 56$) also received diltiazem from the first month to the first year (mean dose: 241 mg/day) to counteract the adverse effects of conventional immunosuppression, namely hypertension, accelerated atherosclerosis, and nephrotoxicity. Fifteen patients, after 1993, did not receive diltiazem because of sinus-node dysfunction.

Definition of acute rejection

AR was diagnosed by endomyocardial biopsy, which was performed on a routine schedule or when clinically indicated. Routinely, surveillance endomyocardial biopsies were obtained weekly during the first month, every 2 weeks until the third month, monthly until the sixth month, and every 3 months until the end of the first year. Biopsy specimens were graded in accordance with the ISHLT classification [7]. AR episodes (3A and above) were treated with pulse-therapy intravenous methylprednisolone (500 or 1,000 mg/day) for 3 days.

Furthermore, we considered the average biopsy score (ABS). We obtained the ABS by assigning a numerical score to each ISHLT grade of rejection (ISHLT grade 0 is ABS value 0, 1A is 1, 1B is 2, 2 is 3, 3A is 4, 3B is 5, and 4 is 6). Numerical values for biopsies that were performed during a certain period were then added and divided by the total number of biopsies reported during that time [8]. We selected the first 6 months after HT in order to avoid a dilution effect resulting from negative results of endomyocardial biopsies at a later time.

Investigated risk factors

The following clinical data were investigated as potential risk factors for AR: age and gender of donor and patient, PRA, HLA-DR mismatch, previous cardiac operation, use of Sandimmune vs Neoral formulation of CyA, diltiazem administration within the first month after HT, and CyA level (RIA) in week 2 and months 1, 2, and 3 after HT.

Statistical analysis

The patients' data were prospectively sampled and stored in a computerized database. They were summarized by descriptive statistics that characterized continuous variables by mean ± standard deviation and categorical variables by proportions. As a first step, a univariate analysis was performed of the association between single putative risk factors and the presence of AR in the first year (χ^2 test or Fisher's exact test for qualitative variables and Student's t-test or Wilcoxon's rank test for quantitative variables). All continuous and categorical variables that were significant in the univariate analysis were subsequently entered into multivariate stepwise logistic regression for the presence of AR and multiple regression for ABS. Also, using ROC curves, we investigated whether a particular level of CyA could predict AR after HT. Statistical significance was defined as a P value lower than 0.05. Version 6.09 of the SAS computer program (SAS Institute, Cary, N.C., USA) was used in the analysis.

Results

Acute rejection: frequency and mortality

During the first year after HT, 75 AR episodes were reported among 101 patients; 52 patients experienced no rejection episodes; 27 had one rejection episode, 19 had two rejection episodes, and three had three or

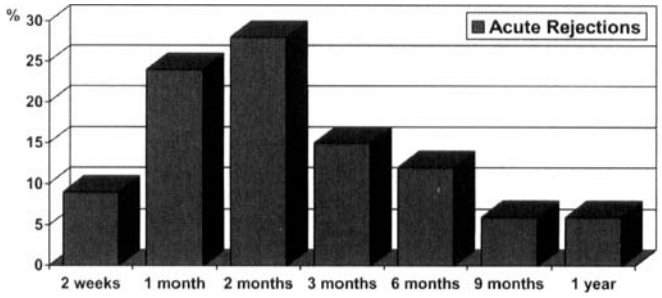

Fig. 1 Percentage of acute rejection episodes in each postoperative month

more rejection episodes. Of all AR episodes, 76% occurred within 3 months of transplantation, and 88% occurred within the first 6 months (Fig. 1). AR was the primary cause of death in five patients and accounted for 20% (five of 25) of total deaths at the end of follow-up.

Risk factors of acute rejection

Patients were divided into two groups according to AR incidence: group 1 ($n=52$), patients without AR; group 2 ($n=49$), those with one or more AR episodes (ISHLT 3A, 3B, or 4) within the first year after HT. This served as the binary, dependent variable for logistic regression. Continuous and categorical variables that were investigated as potential risk factors for AR are characterized by descriptive statistics and depicted in Table 1. Univariate analysis shows a significantly higher proportion of patients receiving diltiazem and a significantly higher CyA level 1 month post-operation in the group without AR. A borderline significant influence was obtained from recipient age. Also, patients that received diltiazem had higher levels of CyA in month 1 after HT (349 ± 111 vs 251 ± 121 ng/ml in patients that did not receive diltiazem).

However, after adjustment by multivariate logistic regression analysis, the variables independently associated with AR were diltiazem administration (odds ratio 0.306) and CyA level in the first month after HT (odds ratio 0.996; see Table 2). Notice that CyA levels are measured in nanogrammes per millilitre, so the 0.996 odds ratio means a 0.4% increase in protective effect by each nanogrammes per millilitre of CyA. No interaction effect was observed between both variables. When ABS was entered as dependent variable in the model, only diltiazem administration was an independent predictor (linear multiple regression analysis, $P=0.0068$ and partial r^2, 0.076).

Table 1 Risk factors for AR within 1 year after HT: univariate analysis

Parameter	No AR	≥1 AR	*P*
No. of patients	52	49	
Acute rejection episodes	0	1.5 ± 0.6	0.001
Average biopsy score	0.39 ± 0.3	1.1 ± 0.4	0.0001
Recipient age (years)	52 ± 9	49 ± 9	0.07
Donor age (years)	26 ± 8	26 ± 9	0.88
Female recipients (%)	9.6	12.2	0.67
Female donors (%)	23	26.5	0.86
Panel-reactive antibodies (%)	1.1 ± 2.9	3.5 ± 8.9	0.16
HLA-DR match ≥1 (%)	43	50	0.5
Cardiac re-operation (%)	19.2	22.4	0.6
Neoral (%)	25	18.3	0.4
Diltiazem, 1 month (%)	73	34.6	0.0002
CyA level, 2 weeks (ng/ml)	255 ± 105	247 ± 142	0.4
CyA level, 1 month (ng/ml)	343 ± 121	266 ± 117	0.001
CyA level, 2 months (ng/ml)	340 ± 111	309 ± 135	0.2
CyA level, 3 months (ng/ml)	322 ± 134	295 ± 104	0.5
Overall mortality (%)	9.6	30.6	0.01

Table 2 Risk factors for AR within 1 year after HT: multivariate, stepwise logistic regression (*CL* confidence limit)

Variable	Odds ratio	Lower CL	Upper CL
Diltiazem, 1 month	0.306	0.102	0.921
CyA level, 1 month	0.996	0.992	0.999

Cyclosporine levels and acute rejection

A CyA level of greater than 362 ng/ml seems to be a good predictor of absence of AR: sensitivity 81%, confidence interval 69–90%, specificity 47%, confidence interval 35–59%, and area under the receiver operating characteristic (ROC) curve 0.6805 (Fig. 2).

Discussion

Acute allograft rejection after cardiac transplantation has remained an important complication, with a significant impact on morbidity and mortality [1]. However, current experience demonstrates that nearly one half of heart recipients have no clinical rejections during and after the first year of transplantation [4].

The aim of this study was to identify independent risk factors of AR during the first year of transplantation. Our analysis of 101 HT patients has identified two independent risk factors: CyA level and diltiazem administration in the first month after HT.

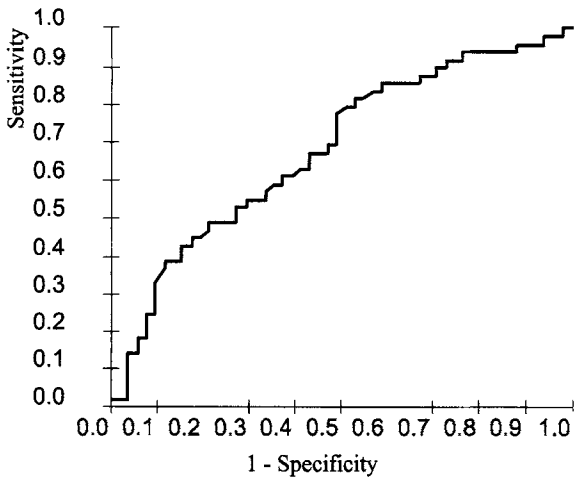

Fig. 2 Discriminative capacity of cyclosporine level in the first post-operative month for cellular acute rejection within the first year after heart transplantation. Area under ROC curve of 0.6805

Cyclosporine and acute rejection

At present, calcineurin inhibitors such as CyA and tacrolimus remain the cornerstone of immunosuppressive therapy in heart transplant recipients. The optimal dose of CyA that maximizes its immunosuppressive properties and minimizes its toxicity is poorly defined. A multicentre study suggested that in the first 6 months after transplantation a mean CyA level greater than 400 ng/ml (fluorescence polarization immunoassay) is associated with a lower incidence of rejection [9]. Another study suggested that levels less than 200 ng/ml (enzyme multiplied immunoassay technique) are associated with increased cellular rejection [10], whereas a third study found no correlation between CyA trough levels and the incidence of cellular rejection [11]. Therefore, the question of whether there is an optimal maintenance CyA trough level in heart transplant recipients remains inconclusively answered.

In our study, a CyA level greater than 362 ng/ml in the first month after HT seems to be a good predictor of absence of AR in the first year after HT. Although the CyA level associated with the lowest probability of AR in this study might not apply to other institutions, because of differences in protocols, practices, and assay methods, the concept that such a threshold exists still applies.

Diltiazem administration and acute rejection

In heart transplant patients, diltiazem is applied to counteract the adverse effects of conventional immunosuppression, namely hypertension and nephrotoxicity [12]. Moreover, it has been reported to slow the development of accelerated transplant coronary artery disease [13]. The findings of our study suggest that diltiazem, at conventional doses, reduces the incidence of AR.

A lower incidence of AR was reported previously to have been associated with diltiazem and CyA in kidney and liver transplantation [14, 15]. Also, studies with heterotopic heart transplant models have shown that diltiazem exerts immunosuppressive and immunomodulating effects [16, 17, 18]. Trans-membrane calcium movement plays a critical role in lymphocyte function, as it is recognized that removal of calcium from the surroundings of lymphocytes results in their complete inactivation. Numerous investigators have demonstrated that diltiazem inhibits lymphocyte functions and depresses the immune response.

This is the first clinical study to report a lower incidence of AR with the association of diltiazem and CyA in heart transplant patients. It has been observed that CyA levels are consistently increased in the presence of diltiazem [19]. Therefore, it remains unclear whether diltiazem has inherent immunosuppressive action or whether it interferes with the metabolism of CyA. Our study did not contemplate the pharmacokinetics of the drugs involved; however, statistical analysis did not find interaction between diltiazem and CyA. Therefore, from a statistical point of view, both diltiazem and CyA show a large and independent protective effect. The strength of association and the statistical analysis performed would not allow this protective effect to be explained by any relevant bias. However, the independent protective effect of diltiazem has to be confirmed in a clinical trial that is designed to answer this question.

In conclusion, diltiazem administration and high levels of CyA in the first month after HT are associated with reduced AR in the first year after HT. The CyA level in the first month after HT is independently associated with AR, and the cut-off point level that best predicts the absence of AR is at greater than 362 ng/ml, although its discriminative capacity is moderate.

References

1. Hosenpud JD, Bennett LE, Keck BM, Boucek MM, Novick RJ (2001) The Registry of the International Society for Heart and Lung Transplantation: 18th Official Report 2001. J Heart Lung Transplant 20:805
2. Reid KR, Menkis AH, Novick RJ, et al. (1991) Reduced incidence of severe infection after heart transplantation with low-intensity immunosuppression. J Heart Lung Transplant 10:894
3. Swinnen LJ, Costanzo-Nordin MR, Fisher SG, et al. (1990) Increased incidence of lymphoproliferative disorder after immunosuppression with the monoclonal antibody OKT3 in cardiac transplant recipients. N Engl J Med 323:1723
4. Kobashigawa JA, Kirklin JK, Naftel DC, et al. (1993) Pretransplantation risk factors for acute rejection after heart transplantation: a multi-institutional study. J Heart Lung Transplant 12:355
5. Kirklin JK, Naftel DC, Bourge RC, et al. (1992) Rejection after cardiac transplantation. A time-related risk factor analysis. Circulation 86 [Suppl II]:236
6. Stinson EB, Dong E Jr, Iben AB, Shumway EN (1969) Cardiac transplantation in man: III. Surgical aspects. Am J Surg 118:182
7. Billingham ME, Cary NRB, Path MRC, et al. (1990) A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: Heart Rejection Study Group. J Heart Transplant 9:587
8. Kobashigawa JA, Miller L, Young A, et al. (1995) Does acute rejection correlate with the development of transplant coronary artery disease? A multicenter study using intravascular ultrasound. J Heart Lung Transplant 14:S221
9. Miller L, Kobashigawa J, Hauptman P, et al. (1996) Definition of critical cyclosporine level for preventing rejection in heart transplant patients. J Heart Lung Transplant 15:S77
10. El Gamel A, Keevil B, Rahman A, et al. (1997) Cardiac allograft rejection: do trough cyclosporine levels correlate with the grade of histologic rejection. J Heart Lung Transplant 16:268
11. Hausen B, Demertzis S, Rohde R, et al. (1994) Low-dose cyclosporine therapy in triple drug immunosuppression for heart transplant recipients. Ann Thorac Surg 58:999
12. Kelly JJ, Walker RG, d'Apice AJF, et al. (1990) A prospective study of the effect of diltiazem in renal allograft recipients receiving cyclosporin A: preliminary results. Transplant Proc 22:2127
13. Schroeder JS, Gao SZ, Alderman EL, et al. (1993) A preliminary study of diltiazem in the prevention of coronary artery disease in heart-transplant recipients. N Engl J Med 328:164
14. Kunzendorf U, Walz G, Brockmöller J, et al. (1991) Effects of diltiazem upon metabolism and immunosuppressive action of cyclosporine in kidney graft recipients. Transplantation 52:280
15. Mies S, Massarollo PCB, Figueira ERR, et al. (1998) Lower incidence of liver graft rejection in patients on diltiazem plus cyclosporine therapy. Transplant Proc 30:1347
16. Dumont L, Chen H, Daloze P, et al. (1993) Immunosuppressive properties of the benzothiazepine calcium antagonists diltiazem and clentiazem, with and without cyclosporine, in heterotopic rat heart transplantation. Transplantation 56:181
17. Libersan D, Marchand R, Montplaisir S, et al. (1997) Cardioprotective effects of diltiazem during acute rejection on heterotopic heart transplants. Eur Surg Res 29:229
18. Lapointe N, Chen H, Qi S, et al. (1999) Immunomodulating effects of second-generation calcium channel blockers on experimental heart transplantation. Eur Surg Res 31:259
19. Brockmöller J, Neumayer HH, Wagner K, et al. (1990) Pharmacokinetic interaction between cyclosporine and diltiazem. Eur J Clin Pharmacol 38:237