Long-term efficacy and safety of conversion to tacrolimus in heart transplant recipients with ongoing or recurrent acute cellular rejection

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Abstract:

Background and Aims: Despite the widespread use of potent immunosuppressive drugs, such as cyclosporin A and mycophenolate mophetil, ongoing and recurrent cellular rejection remain a common problem after heart transplantation. We aimed to describe the long-term effects of conversion from cyclosporine A to tacrolimus in patients (pts) with ongoing and recurrent cellular rejection.

Methods: This was a single-centre retrospective analysis. 17 heart transplant recipients were switched from cyclosporine A to tacrolimus due to ongoing (5 pts) or recurrent cellular rejection (12 pts). We studied long-term efficacy and safety of this approach.

Results:

167 endomyocardial biopsies were performed during a mean follow-up of 69.1 ± 12.7 months. Thirteen biopsies (7.8%) in eight patients (47%) revealed higher grades of acute cellular rejection (Banff 2). However, they were not hemodynamically significant and did not require intravenous antirejection therapy. The mean rejection score was reduced significantly. Conversion to tacrolimus was tolerated in 82% pts without any significant side effects during a long-term follow-up.

Conclusion:

Conversion to tacrolimus in heart transplant recipients with ongoing or recurrent acute cellular rejection was safe and effective also during a long-term follow-up.

Keywords:

tacrolimus – heart transplantation – ongoing rejection – recurrent rejection

Abreviations:

EMB- endomyocardial biopsy

OHTx- orthotopic heart transplantation

Introduction:

Orthotopic heart transplantation (OHTx) remains the most effective therapy in patients with terminal heart failure. Introduction of cyclosporine A into routine prophylactic immunosuppressive regimen has led to significant improvement of long-term survival after OHTx. This success can be mainly explained both by reduced incidence and severity of acute rejection (Málek 2004). However, the risk of acute rejection continues to remain even in contemporary era of modern immunosuppressants. Acute rejection is common in the first months after transplantation - registry data show that approximately 40% of patients are treated for at least one acute rejection within the first post-transplant year (Taylor *et al.* 2007).

Earlier studies have shown that tacrolimus is effective in treatment of ongoing, steroid-resistant rejection (Onsager *et al.* 1999, Yamani *et al.* 2000, De Bonis *et al*. 2001). Less was known about the effect of tacrolimus in recurrent, low-grade rejection episodes. Such episodes do not cause extensive damage of allograft function immediately but may result in chronic vascular rejection. Our previous report demonstrated that recurrent acute cellular rejection can be significantly reduced after conversion to tacrolimus (Dufková *et al.* 2006). This short-term observation was extended and the aim of our new retrospective study was to evaluate efficacy and safety of tacrolimus therapy in long-term. We preferentially assessed tolerance of tacrolimus, acute rejection episodes and survival.

Materials and methods:

This was a single-centre retrospective analysis, which evaluated long-term effects of conversion to tacrolimus in heart transplant recipients with ongoing or recurrent acute cellular rejection. Ongoing rejection was defined as a rejection episode refractory to intravenous administration of methylprednisolon. The term recurrent rejection described a repetitive rejection episode following transitory regression of lymphocytic infiltrates induced by anti-rejection therapy.

Study group

17 heart transplant recipients were switched from cyclosporine A to tacrolimus due to ongoing or recurrent cellular rejection between April 2001 and January 2005. The study group characteristics were as follows: 5 women, 12 men, mean age 53 ± 11 years, mean time from OHTx 27± 32 months (ranging from 2 to 90 months). All patients presented with normal left ventricular systolic function. 8 patients (47%) had diabetes mellitus. Baseline immunosuppressive prophylaxis included cyclosporine A (in all patients), mycophenolate mofetil (14 patients) or azathioprine (2 patients) and prednisone (15 patients). Steroid-resistant rejection episodes were documented in five patients, while the remaining 12 patients had recurrent rejection of higher grades. These corresponded to Banff classification 2 and higher (Billingham *et al*. 1990) (Tab 1). Treatment of acute rejection before conversion was based on administration of intravenous methylprednisone in 11 patients, an increased dose of prednisone in 2 patients and antithymocyte globuline in one patient. The study group was followed until January 2009.

Protocol of conversion and follow-up assessment:

Upon conversion, the first dose of tacrolimus was administered 12 hours after the last cyclosporin A administration. The initial dosage of tacrolimus (0.1–0.15 mg/kg) was divided into two daily doses and plasma levels were determined following administration of five doses. Therapeutic blood levels (10–20 ng/mL) were obtained and tacrolimus was tolerated in all patients. The first follow-up endomyocardial biopsy (EMB) was scheduled 2-4 weeks after conversion. The following biopsies were planned according to the institutional protocol - during the first year post-transplant patients underwent EMB every week during first month, every 2 weeks until 3 months, every 1 month until 6 months, followed by EMB at 9 and 12 months. In the remaining time period, the study subjects followed a EMB protocol for patients at high-risk of rejection. They had usually the second EMB 4 months post-conversion.

Thereafter, EMB was repeated every 4-6 months until 2-3 years and once a year until 4-6 years post-conversion. Biopsies were performed in all patients for a minimum of 4 years post-conversion. All subjects had a routine echocardiographic follow-up, which was carried out during EMB visits and later every 4-6 months of follow-up.

Methods of analysis:

To evaluate efficacy of tacrolimus in the treatment of ongoing or recurrent acute cellular rejection, a score of acute cellular rejection was calculated in each patient. It was based on the grading of the Banff classification (Billingham *et al.* 1990). Three EMB results before and all biopsies after conversion were classified using a specific number of points according to a scale (Table 1.). The score was calculated as the mean value of points from three biopsies before conversion and separately from biopsies performed during the first, second, third and fourth year post-conversion. All subjects were informed about retrospective data processing and gave their informed consent.

Statistics:

Continuous data were expressed as a mean ± SD. Paired T-test was used to assess repeated measurements, considering values of $p < 0.05$ as significant.

Results:

Efficacy

The mean follow- up of the study group was 69.1 ± 12.7 months after conversion (range 48-92 months). 167 endomyocardial biopsies were performed during this period. Just thirteen biopsies (7.8%) in eight patients (47%) revealed higher grades of acute cellular rejection after conversion to tacrolimus. These biopsies were classified as Banff 2 (moderate) acute cellular rejection. Median time period from conversion to the first Banff 2 rejection episode was 12.5 months (IQR 7.8 and 18.8 months). Nevertheless, they did not induce systolic dysfunction of the graft and did not require intravenous steroids or antithymocyte globulin. The mean

rejection score was 2.9 ± 2.5 points before conversion. It was significantly reduced during the first year (0.7 ± 0.4 points), the second year (0.7 ± 0.6 points) , the third year (0.4 ± 0.5 points) and the fourth year (0.5 ± 1.0) post-conversion as compared with the baseline value, all $p< 0.001$ (Figure 1). Immunosuppressive prophylaxis at the end of the study included tacrolimus (in all patients), mycophenolate mofetil (14 patients) or azathioprine (1 patient) and prednisone (16 patients).Tacrolimus through levels decreased during follow-up (Table 2). They were comparable between patients with and without acute rejection after conversion to tacrolimus, except for the lower values in individuals with acute rejection in the 3rd year. This difference was associated with three episodes of acute cellular rejection and may indicate the need of higher target levels of tacrolimus in this time period. Discontinuation of steroids was no feasible in this study group due to high risk of rejection.

Safety

At the end of follow-up, sixteen patients (94%) were still alive with normal systolic function of the graft. One patient with several comorbidities died of septicaemia complicated by renal and liver failure in January 2008 (81 months after conversion). Another patient was switched from tacrolimus to sirolimus because of significant impairment of renal function in June 2008 (61 months after conversion). The remaining fifteen subjects (88%) tolerated the administration of tacrolimus without any significant side effects. Moderate increase in creatinine level was registered only in the first year after switching to tacrolimus. However, in the following years the creatinine level was comparable with baseline value (Table 2). There was no registered new-onset of diabetes mellitus. Eight patients had diabetes mellitus before conversion (four patients on intensive insulin therapy, four patients on diet or per oral medication). After conversion to tacrolimus six patients were on insulin therapy. There was no case of malignancy during follow-up.

Discussion:

Conversion to tacrolimus in heart transplant recipients with ongoing or recurrent acute cellular rejection was safe and effective during a mean follow-up of 69 months. It resulted in significant reduction of the incidence and severity of acute cellular rejection. Although 47% of subjects experienced a recurrent Banff 2 (moderate) rejection, these episodes were not hemodynamically significant and did not require subsequent use of intravenous steroids or antithymocyte globuline.

Comparison with previous studies and clinical implications

These findings are in accordance with previous reports about conversion to tacrolimus in heart transplant recipients with ongoing (Onsager *et al.* 1999, Yamani *et al.* 2000, De Bonis *et al.* 2001) and recurrent cellular rejection (Dufková *et al.* 2006). The current paper provides new evidence especially in patients with recurrent rejection who were not studied in reports from other groups. Another contribution of this study seems to be a longer follow-up after conversion. The mean follow-up in previous studies was in range of 11 to 27 months.

The main clinical benefit of conversion to tacrolimus seems to be reduced need for repeated use of non-selective immunosuppressants, which are required for the management of moderate and severe grades of cellular rejection. Both high-dose methylprednisolon and antithymocyte globulin may be associated with significant adverse effects (Vymětalová and Málek 2005), such as infection, osteoporosis, diabetes, etc. The risk is higher, when this therapy is used repeatedly. Furthermore, episodes of ongoing or recurrent acute cellular rejection represent a risk factor for the development of chronic rejection (cardiac allograft vasculopathy) (Valantine 2004). Conversion to tacrolimus thus provides a new approach, which might reduce both short-term and long-term complications of ongoing and recurrent cellular rejection.

Pathophysiology of acute cellular rejection and its interaction with tacrolimus

Acute rejection is mediated either by cytotoxic antibodies (humoral rejection) or more commonly by activated lymphocytes (cellular rejection). The process of acute cellular rejection is triggered by T- lymphocytes that can recognize incompatible human leukocyte antigens (HLA) of the allograft either directly or indirectly (Sheldon and Poulton 2006). Recognition of an alloantigen is followed by activation of T-lymphocytes, their clonal expansion, differentiation into effector cells and migration into the allograft (Ingulli 2008). This reaction can be suppressed by both cyclosporine A and tacrolimus. These drugs inhibit expression of interleukin-2 (IL-2) and IL-2 receptor by blocking calcium-dependent signal transduction via calcineurin. They also bind to different intracellular proteins, such as cyclophilin and FK-binding protein (Schreiber *et al*. 1992, Thomson *et al.* 1993). In vitro studies suggested that both drugs have a similar mode of action. On the contrary, several clinical studies have demonstrated that only tacrolimus has an ability to reverse ongoing acute cellular rejection in heart transplant recipients (Jiang *et al.* 2001, Ebbs *et al.* 2002). Inhibition of interleukin-10 (IL-10) production has been advocated as a specific mechanism that can explain the above difference (Jiang *et al.* 2002). IL-10 is involved in up-regulation of functional CD8+ T-cell and NK-cell local infiltration with release of cytotoxic cytokines such as granzyme B and perforin 1 (Jiang *et al.* 2002). However, gene expression profiles in the rat heart transplantation model showed that drug-specific effect of tacrolimus may include reversed expression of fourteen other genes except of IL-10 (Erickson *et al.* 2003). Exact molecular mechanism explaining clinical differences between both immunosuppressants thus remains unclear.

Conclusion:

In conclusion, conversion to tacrolimus in heart transplant recipients with ongoing or recurrent acute cellular rejection was safe and effective also during a long-term follow-up. It resulted in significant reduction of the incidence and severity of acute cellular rejection.

Although 47% of subjects experienced a recurrent Banff 2 (moderate) rejection, these episodes were not hemodynamically significant and did not require subsequent use of intravenous steroids or antithymocyte globuline.

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Table 1: Banff classification and point score

		Point score
Banff 0	No rejection	0
Banff 1A	focus infiltration by lymphocytes, no myocytes damage	1
Banff 1B	diffuse infiltration by lymphocytes, no myocytes damage	2
Banff 2	focus infiltration by lymphocytes, myocytes damage	3
Banff 3A	multifocal infiltrations, myocytes damage	4
Banff 3B	diffuse infiltration, myocytes damage	5
Banff 4	diffuse mixed infiltration with myocytes damage, vasculitis, hemorrhage, edema	6

Table 2: Freedom from acute rejection and number of rejection episodes during four years after conversion, one episode of acute rejection was documented in the sixth year of follow-up. Tacrolimus through levels and serum creatinine as averaged from values obtained at each endomyocardial biopsy. Continuous data are shown as a median and interquartile range.

	Before conversion	1st year	2nd year	3rd year	4th year
Freedom from acute rejection after conversion (number of patients)	----------	12 (71%)	11 (65%)	10 (59%)	9 (53%)
Number of acute rejection episodes after conversion	----------	5 (38%)	3 (23%)	3 (23%)	1 (8%)
Tacrolimus through levels in the whole study group (µg/l) (n=17 pts)	----------	12.2 (11; 14.2)	11.6 (10.5; 13)	11.4 * (8.8; 12)	9.0 ** (7.3; 11.5)
Tacrolimus through levels in individuals with acute rejection after conversion (µg/l) (n= 8 pts)	----------	12.4 (11.1; 12.9)	12.1 (11.3; 13.1)	9.7 * † (8.6; 10.4)	9.8 (6.8; 11.6)
Tacrolimus through levels in individuals without acute rejection after conversion (µg/l) (n= 9 pts)	----------	11.6 (11; 15.5)	10.9 (9.1; 11.9)	12.0 (11.5; 12.8)	8.9 * (8.8; 11.8)
Serum creatinine (µmol/l)	107.7 (93.3; 126)	121.8 * (101; 130)	106.5 (90; 126.5)	114.4 (95; 129)	105.0 (101; 123)

P-value for pairwise comparison between the first time period and follow-up data was coded: $p<0.05$ *, $p<0.01$ **. P-value for comparison of tacrolimus through levels in individuals with and without acute rejection within each year of follow-up was coded: † $p< 0.05$.

Figure 1: Changes in the rejection score after conversion to tacrolimus. Rejection score was compared between baseline and follow-up measurement.