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APOPTOSIS OF PERIPHERAL T CELLS IN STABLE RECIPIENTS WITH NEORAL OR TACROLIMUS IMMUNOSUPPRESSION AFTER HEART TRANSPLANTATION

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A b s t r a c t

Relationships between two immunosuppressive regimens (Neoral or Tacrolimus) and in vivo T cell spontaneous apoptosis were studied in 18 heart transplant recipients during 18 months of follow-up. The results showed that there was no correlation between the whole blood levels of either Neoral or Tacrolimus and the rate of peripheral T cell apoptosis and that there were no significant differences in the total numbers of lymphocytes and rate of T cell apoptosis between the Neoral- and Tacrolimus-treated recipients. It is concluded that both Neoral and Tacrolimus, as part of an immunosuppressive regimen in heart transplant recipients, have the same effect on the level of T-cell apoptosis in the post-transplant period.

K e y w o r d s

Heart transplantation, Immunosuppression, Neoral, Tacrolimus, Apoptosis, T cells

INTRODUCTION

Apoptosis, genetically-controlled programmed cell death, plays an important role in the development and differentiation of tissues as well as in certain pathological conditions such as cancer, inflammation, AIDS or graft rejection. Although precise effector mechanisms responsible for injury to a transplanted organ during rejection are still poorly understood, application of effective immunosuppressive strategies can control the development of rejection by suppressing donor antigen-specific T cell responses or inducing apoptosis in donor antigen-stimulated, proliferating T cells of the recipient. Both Neoral and Tacrolimus are widely used to prevent allograft rejection. The immunosuppressive effect of these drugs has primarily been attributed to their ability of suppressing T cell activation.

In this study, the effects of Neoral (*1, 2,* 3) and Tacrolimus administration on the apoptosis of peripheral T cells in stable heart transplant recipients were investigated.

MATERIALS AND METHODS.

A group of 18 adult heart transplant recipients, followed up for 18 months, was divided into two subgroups according to the main immunosuppressive drug used; nine patients were treated with Neoral and nine with Tacrolimus. Concomitant therapy included azathioprine, mycophenolate mofetil, corticosteroids and Zenapax (monoclonal antibody against CD25). All the patients were free from any infection and none of them showed an acute rejection episode (>IA grade). Healthy adult volunteers served as a control group.

The patients were examined for acute rejection episodes, which were histologically diagnosed from endomyocardial biopsies on the basis of the International Society for Heart and Lung Transplantation (ISHLT) criteria. The whole blood levels of Neoral (ng/ml) and Tacrolimus (ng/ml) were determined by the competitive ELISA and the total lymphocyte number (elements x 10^9/l) was assessed by a routine method. Blood mononuclear cells were isolated from heparinised peripheral blood by Lymphoprep gradient centrifugation. Subsequently, T cells were negatively sorted by supermagnetic beads coated with antibodies (Dynal, Oslo, Norway), using a magnetically-activated cell sorter (purity of T cells > 85%). Spontaneous in vivo apoptosis (DNA fragmentation) was determined quantitatively by measuring cytosolic oligonucleosome-bound DNA with the use of an ELISA kit (Roche Diagnostic, Mannheim, Germany), as described by *Leist et al.* (*4*). Briefly, T cells were lysed and cytosolic fractions were obtained after centrifugation. These fractions served as a source of antigen in a sandwich ELISA, in which primary antihistone antibodies coated the microtitre plate and secondary anti-DNA antibodies were coupled with peroxidase. Absorbance values were used to measure the percentage of DNA fragmentation of T cells; the rate of apoptosis was calculated according to the formula (%):

absorbance of patient T cells/ absorbance of control T cells x 100.

Statistical evaluation was carried out using Student's *t*-test and correlation analysis; the results were expressed as mean ± standard deviation and range values.

RESULTS

No significant differences were found in the rate of apoptosis between the Neoral and Tacrolimus groups. No rejection episodes (ISHLT grade, >IA) were recorded and no infectious complications occurred. The total number of lymphocytes was not affected by the immunosuppressive drug used; the mean values and ranges were very similar in both the Neoral and Tacrolimus groups (*Table 1*).

Table 1

Apoptosis of peripheral T cells and the total number of lymphocytes in stable heart transplant recipients receiving Neoral or Tacrolimus

Apoptosis [%]		Total number of lymphocytes [elements x 10°/L]	
Tacrolismus (N=29)	Neoral (N=31)	Tacrolismus (N=40)	Neoral (N=34)
250 ± 85 80 - 415	265 ± 90 90 - 425	1,4 ± 0,7 0,6 - 3,0	1,5 ± 0,7 0,6 - 3,2

N, number of samples assessed; results are expressed as mean ± standard deviation and range values.

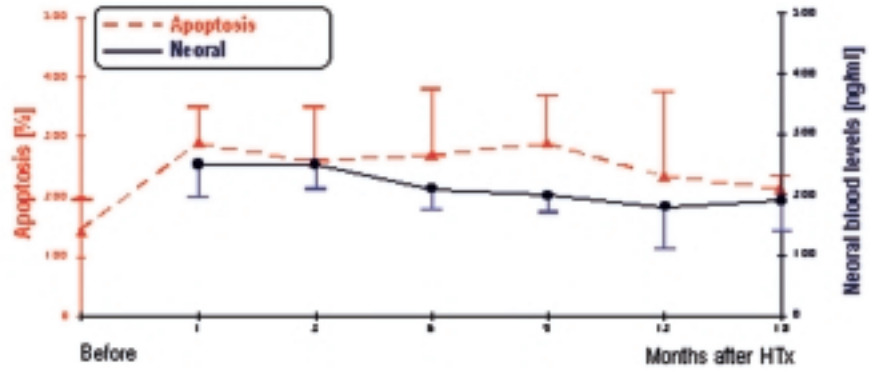

Fig. 1
Changes in Neoral blood levels and the rate of T-cell apoptosis in stable heart transplant recipients (N=9) during the 18-month period after transplantation.

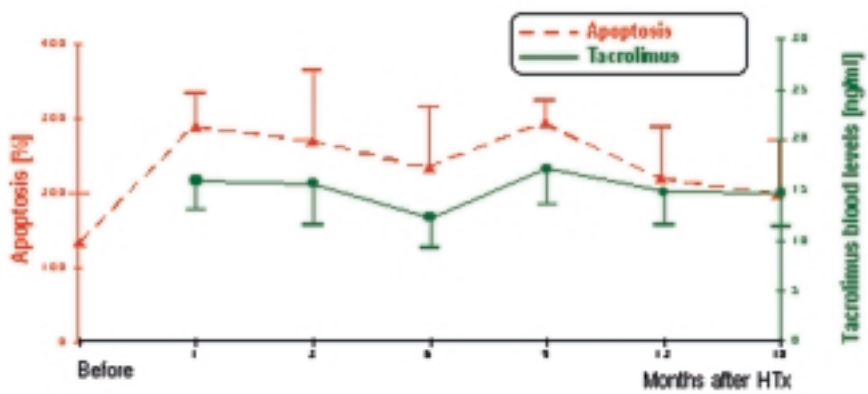

Fig. 2
Changes in Tacrolimus blood levels and the rate of T-cell apoptosis in stable heart transplant recipients (N=9) during the 18-month period after transplantation.

The dynamics of apoptosis was recorded during 18 months of follow-up. In the Neoral group, after a mild increase in the first post-transplant month, the rate of apoptosis remained more or less at a constant level, with only slight fluctuation. The level of Neoral in the whole blood also remained stable, with an average value of about 200 ng/ml for the follow-up period. There was no correlation between these two variables (*Fig. 1*).

In the Tacrolimus group, changes in the dynamics of apoptosis were similar. After an increase in the first post-transplant month, the rate of T cell apoptosis varied between 200 and 300% throughout the follow-up. Tacrolimus blood levels were maintained at about 15 ng/ml, particularly later in the period; this was slightly higher than the recommended concentration (10 ng/ml). There was no correlation between the blood levels of Tacrolimus and the rate of peripheral T cell apoptosis (*Fig. 2*).

DISCUSSION

Apoptosis plays an important role in damage, to which organs after transplantation are subjected, such as alloreactivity and ischaemic or reperfusion injury. On the other hand, it may be beneficial to the recipient by mediating the action of immunosuppressive drugs and thus supporting the elimination of donor-specific T cells. This process reduces the severity of acute cellular rejection episodes or may even prevent them (*5*).

The effect of Neoral and Tacrolimus, the most commonly used immunosuppressive drugs, is based on suppression of the expression of donor antigen-activated T cells in the recipient. On the other hand, these drugs may induce apoptosis in antigen-stimulated T lymphocytes. Pre-clinical studies have shown that Tacrolimus, in contrast to Neoral, enhances the anti-CD3-induced apoptosis of peripheral T cells (*6*) and increases the rate of apoptosis induced by steroids (*7*) or bacterial super-antigens (*6*). Therefore, it was expected that Tacrolimus would enhance the rate of apoptosis more than Neoral. However, the results of our study did not corroborate this assumption, although we used the same method of assessing the rate of apoptosis, as described by other authors. This fact may be accounted for by differences in experimental designs between this and the other studies (*6, 7*). Further investigations are necessary before a plausible explanation can be provided.

The effects of concomitant immunosuppressive drugs, such as steroids or mycophenolate mofetil, on the development of apoptosis have also been studied and this therapy was reported to increase the rate of apoptosis (*8,* 9).

In conclusion, the results of our study comparing Neoral and Tacrolimus effects are in agreement with the studies on peripheral T cell (lymphocytes) apoptosis in heart transplant recipients who were treated with Neoral *(1,2,3,10).*

A c k n o w l e d g e m e n t

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APOPTÓZA PERIFERNÍCH T BUNĚK U STABILNÍCH PŘÍJEMCŮ PO TRANSPLANTACI SRDCE PŘI IMUNOSUPRESI NEORALEM vs. TACROLIMUS

S o u h r n

U osmnácti stabilních příjemců 18 měsíců po transplantaci srdce, kteří byli na imunosupresi Neoralem vs. imunosupresi s Tacrolimus byla sledována spontánní in vivo apoptóza periferních T buněk. Bylo zjištěno, že hladiny Neoralu nebo Tacrolimus v plné krvi nekorelují s apoptózou periferních T buněk a že se celkový počet lymfocytů a apoptóza periferních T buněk významně neliší u příjemců s imunosupresí Neoralem nebo Tacrolimus. Z toho vyplývá, že Neoral i Tacrolimus, jako součást imunosupresivní léčby, mají v období po transplantaci stejný účinek na apoptózu T buněk.

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