SCRIPTA MEDICA (BRNO) – 79 (3): 163–168, *July 2006*

# SOLUBLE FAS AND FAS-LIGAND PLASMA LEVELS IN HEART TRANSPLANT RECIPIENTS TREATED WITH NEORAL OR TACROLIMUS

HÖKL J.[1], ČERNÝ J.[1], ONDRÁŠEK J.[1], BEDÁŇOVÁ H.[1], VESPALEC J.[1], SIROTKOVÁ A.[2]

[1]Centre of Cardiovascular and Transplantation Surgery, St. Anne's Faculty Hospital, Faculty of Medicine, Masaryk University,Brno
[2]Department of Pathological Anatomy, St Anne's Faculty Hospital, Faculty of Medicine, Masaryk University, Brno

*Received after revision May 2006*

A b s t r a c t

The plasma levels of soluble antiapoptotic Fas (sFas) and proapoptotic Fas-ligand (sFasL) proteins were studied in heart transplant recipients at one-month follow-up. The patients were treated with Neoral (n=18) or Tacrolimus (n=12) and concomitant immunosuppressive drugs. The sFas and sFasL levels were detected by ELISA. The values were related to treatment with Neoral or Tacrolimus in stable patients and in patients with acute rejection episodes (treated with methylprednisolone; ≥IB), and also to antirejection treatment with methylprednisolone or ATG/OKT3. The results showed no significant changes in sFas and sFasL levels in the stable recipients and in the patients experiencing acute rejection treated with methylprednisolone, regardless of treatment with Neoral or Tacrolimus. However, with antirejection therapy, both sFas and sFasL levels increased in the patients given ATG or OKT3, but not in those treated with methylprednisolone.

K e y w o r d s

Soluble Fas, Soluble Fas-ligand, Neoral, Tacrolimus, Acute rejection episodes, Treatment, Heart transplantation

## INTRODUCTION

The soluble membrane-bound protein Fas (sFas) and its soluble Fas-ligand (sFasL) play a key role in maintaining tissue homeostasis via induction of apoptosis, i.e., programmed cell death (*1*). Soluble antiapoptotic Fas inhibits apoptosis by neutralising sFasL or anti-Fas antibody (*2*), while soluble proapoptotic FasL mediates apoptosis by binding to membrane-bound Fas molecules. While in humans sFas is

secreted by almost all cells, sFasL is predominantly released by activated cytotoxic T-lymphocytes.

In transplantation, apoptosis is involved in harmful events such as ischaemia, reperfusion injury or graft rejection, but it may also play a positive role – by depletion of alloreactive T-cells, apoptosis actively promotes transplantation tolerance or reduces the frequency of rejection episodes (*3*). In vitro studies have shown that immunosuppressive drugs, Neoral and Tacrolimus, are able to mediate apoptosis of thymocytes (*4,5*). In the therapy for acute rejection, polyclonal (ATG) and monoclonal (OKT3) anti-CD3 antibodies are, among other effects, associated with lymphocyte apoptosis (*6,7*), and corticosteroids also show apoptotic effects (*8*).
The aim of this study was to compare the plasma levels of antiapoptotic sFas and proapoptotic sFasL in the Neoral-treated transplant recipients with those in the Tacrolimus-treated recipients, as well as to relate sFas and sFasL levels to the therapy for acute rejection episodes in these patients.

## PATIENTS AND METHODS

The study comprised 30 adult heart transplant recipients followed up for 1 month. The immunosuppressive protocol included Neoral in 18 recipients and Tacrolimus in 12 recipients, as well as concomitant therapy (azathioprine or mycopfenofal mofetil + corticosteroids). All patients underwent endomyocardial biopsy at four weekly intervals and, on the basis of the results, were evaluated as stable cases (ISHLT grades 0 or IA) or requiring antirejection therapy (ISHLT grade equal to or higher than IB). The latter were given three daily doses of methylprednisolone intravenously (0.5 – 1.0 g/day). When the following biopsy results did not show a decrease in the ISHLT grade, the episode was regarded as steroid-resistant and ATG or OKT3 was administered for 10 days (3.0 mg/kg/day or 5.0 mg/day, respectively). The levels of sFas and sFasL were assessed 2- or 3-times after antirejection therapy had started. All recipients were free from any infection for the period of study.

The plasma levels of sFas (pg/ml) and sFasL (ng/ml) were determined by a commercial sandwich ELISA kit with the use of monoclonal antibodies (Bender, MedSystems, USA). The results were statistically evaluated using Student's *t*-test and were expressed as mean ± standard deviation and range values.

## RESULTS

A total of 374 sFas and sFasL samples from 30 patients were evaluated. In the stable cases (ISHLT grade ≤ IA, no infection), no significant differences between the Neoral – and Tacrolimus-treated patients were found in either sFas or sFasL levels during the first post-transplantation month (*Table 1*). In the patients who developed acute rejection episodes (ISHLT grades IB, II or IIIA) and received methylprednisolone, no significant differences in sFas and sFasL concentrations in relation to the main immunosuppressive drug therapy were found (*Table 2*). When the acute rejection was steroid-resistant (a repeated biopsy showed ongoing rejection of similar or worse intensity) and required treatment with ATG or OKT3, the levels of both Fas and sFasL showed a marked increase in comparison with the levels in patients receiving methylprednisolone (*Table 3*). These results were not statistically

*Table 1*
Levels of sFas and sFasL during Neoral or Tacrolimus immunosuppressive therapy in stable (0 + IA) recipients

| Immunosuppressive therapy | No. of stable cases | sFas (pg/ml) | sFasL (ng/ml) |
|---|---|---|---|
| Neoral + concomitant drugs | 18 | 60.7 ± 12.9<br>38.0 – 99.0 | 0.54 ± 0.19<br>0.12 – 0.91 |
| Tacrolimus + concomitant drugs | 12 | 73.2 ± 11.7<br>58.0 – 110.0 | 0.56 ± 0.14<br>0.12 – 0.78 |

*Table 2*
Levels of sFas and sFasL in methylprednisolone-treated recipients with acute rejection episodes (IB + II + IIIA)

| Antirejection therapy | No. of acute rejection episodes | sFas (pg/ml) | sFasL (ng/ml) |
|---|---|---|---|
| Methylprednisolone + Neoral + concomitant drug | 8 | 72.6 ± 10.6<br>49.0 – 98.0 | 0.62 ± 0.09<br>0.42 – 0.79 |
| Methylprednisolone + Tacrolimus + concomitant drug | 7 | 76.1 ± 14.4<br>58.0 – 112.0 | 0.65 ± 0.13<br>0.43 – 0.88 |

*Table 3*
Levels of sFas and sFasL in recipients with acute rejection episodes (IB + II + IIIA) receiving antirejection therapy

| Antirejection therapy | No. of acute rejection episodes | sFas (pg/ml) | sFasL (ng/ml) |
|---|---|---|---|
| Methylprednisolone + Neoral / Tacrolimus + concomitant drug | 15 | 74.3 ± 12.5<br>49.0 – 112.0 | 0.63 ± 0.11<br>0.42 – 0.88 |
| ATG / OKT3 + Neoral / Tacrolimus + concomitant drug | 3* | 153.0 ± 37.7<br>92.0 – 199.0 | 1.31 ± 0.17<br>1.08 – 1.63 |

*Of the three steroid-resistant acute rejection episodes two received ATG and one OKT3.

evaluated because of the low number of ATG-/OKT3- treated recipients. The therapy resulted in reduction of the acute rejection grade, as shown by the results of endomyocardial biopsy performed within 7 days of the rejection episode onset. shown by the results of performed within 7 or 10 days of the rejection episode onset.

## DISCUSSION

Immunosuppressive therapy with Neoral or Tacrolimus is used to suppress donor-specific T-cell responses or to induce apoptosis in donor antigen-stimulated expanding T-cells. In vitro studies (*4,9*) showed that Tacrolimus can induce T-lymphocyte apoptosis in activated cells and that apoptosis is higher than when it is induced by *Neoral* (*10*). On the other hand, Neoral and Tacrolimus, which inhibit T-cell activation, block the susceptibility of activated T-cells to apoptosis (*11*). *Hökl et al.* (*12*) showed that there was no difference between Neoral and Tacrolimus in their effects on the apoptosis of peripheral T-cells in stable heart transplant recipients.
In the maintenance of homeostasis, apoptosis is associated, among other mechanisms, with soluble Fas molecules for inhibition and soluble Fas-ligand molecules for activation. In relation to immunosuppressive treatment in this study, there was no difference in sFas and sFasL levels between the Neoral – and Tacrolimus-treated patients, regardless of whether they were stable recipients or had an acute rejection episode higher than IA. *Arkensmit et al.* found significantly elevated serum sFasL and decreased sFas levels in heart transplant recipients treated with *Neoral* (*13*). *Seino et al.* reported that the recipients of related liver transplants had elevated serum sFas concentrations, but not sFasL levels (*14*). Methylprednisolone, used in this study as an anti-rejection drug, has been reported to induce apoptosis of peripheral T-lymphocytes shortly after intravenous administration (*6*). It has also had a stabilising effect on the plasma membrane, which may inhibit the release of soluble sFas and sFasL molecules from the membranes. In a study of soluble apoptotic markers in liver transplant recipients, serum sFas levels were significantly enhanced in acute rejection patients and were brought to normal values by immunosuppressive therapy (*17*). The action of both ATG and OKT3 may involve several mechanisms, such as complement-dependent lysis, apoptosis associated with activation, Fas-ligand apoptosis or modulation of functional molecules, to produce lymphopenia. Some of these mechanisms may both induce the release of soluble apoptotic mediators and maintain their increased levels in circulation. The role of increased levels of soluble Fas and Fas-ligand molecules in relation to immunosuppressive therapy is not clear and, together with the mechanism of their regulation, warrants further studies.

## CONCLUSIONS

The levels of soluble Fas and Fas-ligand proteins were recorded in the plasma of heart transplant recipients treated with Neoral or Tacrolimus, who were stable or had

an acute rejection episode and received antirejection therapy (methylprednisolone). No significant differences were found between these two immunosuppressive protocols. However, an increase in both sFas and sFasL levels was found in the patients with acute rejection episodes who received antirejection therapy with ALG/OKT3, as compared with those treated with methylprednisolone.

*Hökl J., Černý J., Ondrášek J., Bedáňová H., Vespalec J., Sirotková A.*

## PLASMATICKÉ HLADINY ROZPUSTNÝCH FAS A FAS-LIGANDY U PŘÍJEMCŮ PO TRANSPLANTACI SRDCE, KTEŘÍ BYLI LÉČENI NEORALEM NEBO TACROLIMEM

S o u h r n

Plasmatické hladiny rozpustné antiapoptotické Fas (sFas) a proapoptotické Fas-ligandy (sFasL) byly sledovány u příjemců po transplantaci srdce během prvního měsíce po transplantaci. Pacienti byli léčeni Neoralem(n=18) nebo Tacrolimem (n=12) s dalšími imunosupresivy. Metodou ELISA byly stanoveny hladiny sFas a sFasL. Tyto hodnoty byly vztaženy na léčení Neoralem nebo Tacrolimem u stabilních příjemců a pacientů s akutními rejekčními episodami (léčenými methylprednisolonem; ≥ IB) a také na antirejekční léčbu ATG/OKT3. Výsledky neukázaly signifikantní změny sFas a sFasL u stabilních příjemců a pacientů s akutní rejekcí (léčených methylprednisolonem) bez ohledu na léčbu Neoralem nebo Tacrolimem. Při antirejekční léčbě ATG nebo OKT3 došlo ke zvýšení u sFas i sFasL.

## REFERENCES

1. *Nagata S*. Fas and Fas Ligand: a death factor and its receptor. Adv Immunol 1994; 57: 129–144.
2. *Cheng J, Zhou T, Liu C, et al.* Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. Science 1994; 263: 1759–1762.
3. *Wells A, Turka LA*. The role of apoptosis in the induction and maintenance of tolerance. Curr Opin Organ Transplant 2001; 6: 1–6.
4. *Migita K, Eguchi K, Kawabe Y, et al. FK* 506 augments activation-induced programmed cell death of T lymphocytes. J Clin Invest 1995; 96: 727–732.
5. *McCarthy SA, Cacchione RN, Mainwaring MS, Cairns JS*. The effect of immunosuppressive drugs on the regulation of activation-induced apoptotic cell death in thymocytes. Transplantation 1992; 54: 543–547.
6. *Bonnefoy-Berard N, Genestier L, Preville X, Revillard J-P*. TNF and CD95-L contribute to apoptosis of activated lymphocytes triggered by ATGs. Transplant Proc 1999; 31: 775–777.
7. *Janssen O. Wesselborg S, Kabelitz D.* Immunosuppression by OKT3-induction of programmed cell death (apoptosis) as a possible mechanism of action. Transplantation 1992; 53: 233–234.
8. *Migita K, Eguchi K, Kawabe Y, et al.* Apoptosis induction in human peripheral blood T lymphocytes by high-dose steroid therapy. Transplantation 1997; 63: 583–587.
9. *Migita K, Eguchi K, Kawaba Y, et al.* FK 506 potentiates steroid induced T cell apoptosis. Transplantation 1997; 64: 1364–1369.
10. *Kimball PM, Rhodes SA*. Changes in cell cycling and apoptosis contribute to reduced cell generation along long-term heart survivors. Transplant Proc 1999; 31: 109–110.
11. *Li Y, Li XC, Zheng WW, et al.* Blocking both signals 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. Nat Med 1999; 5: 1298–1302.
12. *Hökl J, Černý J, Ondrášek, J, et al.* Apoptosis of peripheral T cells in stable recipients on Neoral or Tacrolimus immunosuppression after heart transplantation (Abstract). Transplantation 2004; 78 (Suppl 1): 418.

13. *Ankersmit HJ, Moser B, Zuckermann A, et al.* Activation-induced T cell death, and aberrant T cell activation via TNFR1 and CD95-CD95 ligand pathway in stable cardiac transplant recipients. Clin Exp Immunol 2002; 128: 175–180.
14. *Seino K, Kayagaki N, Yamaguchi N, et al.* Soluble forms of CD95 and CD95 ligands after living related liver transplantation. Transplantation 1999; 67: 634–636.
15. *Rivero M, Crespo J, Mayorga M, et al.* Soluble Fas in acute rejection in patients with liver transplantation. Response to treatment. Gastroenterol Hepatol 2000; 23: 215–218.