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ORIGINAL ARTICLE

Delayed administration of FK 506 is sufficient to suppress acute rejection changes after aortal transplantation in rats

Ivan Matia,[1] Alena Lodererova[2] and Miloš Adamec[1]

1 Department of Transplant Surgery, Institute for Clinical and Experimental Medicine, Prague, Czech Republic
2 Department of Transplant Pathology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic

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Correspondence
Ivan Matia MD, Department of Transplant Surgery, Institute for Clinical and Experimental Medicine, Videnska 1958/9, 140 21 Prague, Czech Republic. Tel.: 00420-2-6136-4105; fax: 00420-2-6136-2822; e-mail: ivmi@medicon.cz

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Summary

Arterial allografts are used now-a-days as a modality in the treatment of vascular prosthesis infections. Prolonged administration of immunosuppressive drugs seemed to be essential for long-time patency rates of alloarterial vascular reconstructions. Nevertheless, the use of immunosuppressives if there exist an acute infection is controversial. The experimental work described herein studied effects of a delayed low-dose FK 506 administration on the development of acute rejection changes 30 days after aortal transplantation in rats. The response of the recipient's immune system to aortal wall antigens of the donor in the field of no immunosuppression resulted in an intimal proliferation and its infiltration by immunocompetent cells of the recipient, necrosis of medial smooth muscle cells, including deposition of immunoglobulins, and a massive adventitial infiltration of CD4 and CD8 positive cells. On the other hand, all the principal histological signs of rejection listed above were suppressed by FK 506 administration, no matter whether the immunosuppressive was administered on day 0 or day 7 after the transplantation.

Introduction

The problem of unsatisfactory results in the treatment of the life-threatening vascular prosthesis infection has recently contributed to renewed interest in arterial allografts [1–3]. The procedure consists of an infectious prosthesis extraction and its *in situ* replacement by an allograft. Nevertheless, arterial grafts in the field of no immunosuppression are subject to degenerative changes, which may lead to their thrombosis or aneurysmal dilatation. This fact has been documented by experiments on animals, as well as in clinical practice [4–7].

The immunosuppressive therapy after alloarterial or allovenous reconstructions is not generally accepted. However, if the therapy is administered, the drug most frequently used (mainly in Europe) is cyclosporine A (CyA) [8–13]. However, it has been demonstrated that CyA can induce endothelial cell (EC) injury, as well as stimulate arterial wall fibrosis by triggering the production of the transforming growth factor-beta (TGF-beta) [14,15]. None of these properties is particularly suited for long-term patency rates of alloarterial reconstructions. An attractive alternative to CyA is tacrolimus (FK506). Tacrolimus when compared with cyclosporine-based immunosuppressive therapy in kidney transplantation, resulted in significantly reduced risk of graft failure, without an increase in the incidence of adverse events associated with long-term immunosuppression [16,17]. Moreover, the inhibition of TGF-beta production by FK 506 could make the drug eligible for use in vascular surgery as well [18,19].

However, the immediate use of immunosuppression in patients early after the replacement of an infectious prosthetic graft with an arterial allograft is not optimal as well.

A rat aortal allograft model is frequently used to study vascular rejection phenomena [20]. Different stages of the recipient's immune response were documented in detail in all three cell layers of an arterial graft: tunica intima, media and adventitia [21]. One of the features observed during the primary rejection of the arterial wall is a two-step process of denudation and regeneration of the endothelial layer. During the first 7 days, it is believed that the process is caused by a nonimmunological injury, followed

by an immunological reaction and an acute rejection of all three wall layers [21,22]. The administration of tacrolimus early after the aortal transplantation in rats proved to be able to prevent the arterial wall injury caused by the immunological rejection [22,23].

The aim of our experimental work was to study the possibility of delaying the administration of FK 506 for 1 week after the arterial transplantation to reduce its adverse effects in the early postoperative period in patients with a recent infection. The rejection changes of the arterial wall were studied using the Brown-Norway (BN) into Lewis (LEW) aortal transplantation model with a 30-day follow-up period.

Material and methods

Principles of laboratory animal care were followed, and applicable national laws observed during the study.

Animals

Male LEW rats ($RT1^l$) weighing 240–380 g (291.6 ± 32.1 g) ($n = 44$) were used as recipients of allogeneic or syngeneic abdominal aortal grafts. Male BN rats ($RT1^n$) weighing 220–320 g (264.2 ± 28.5) ($n = 18$) were used as donors of the allogeneic and LEW ($RT1^l$) weighing 280–320 g (305.01 ± 19.1) ($n = 4$) were used as donors of syngeneic abdominal aortal grafts. Animals were obtained from Charles River, Germany. All the rats were maintained according to the National Institute of Health Guidelines. Each transplanted animal was held separately in a confinement cage during the entire follow-up period.

Operative procedure

The donor animals were anesthetized by an i.m. injection of ketamine (Narkamon®; Spofa, Prague, Czech Republic) 100 mg/kg and xylazine (Rometar®; Spofa) 10 mg/kg. A 2–2.5 cm long segment of the infrarenal aorta was excised after the administration of heparin (100 IU/kg) via the inferior caval vein. The graft was gently flushed with 2 ml of histidine-tryptophan-ketoglutarate (HTK) solution (Custodiol®; Dr Franz Köhler Chemie GmbH, Alsbach-Hahnlein, Germany) containing 100 IU/ml of heparin, and divided into two pieces of an identical length for use in two recipient rats. Each of them was stored separately in 10 ml of Custodiol® solution at a temperature of about 4 °C. No antibiotics were added. The mean cold ischemic time (CIT) for the aortal grafts was about 25 h, the better to simulate clinical conditions of a fresh arterial transplantation. The specimen from each aortal graft was obtained at the end of the conservation period and prepared for a light microscopic histological evaluation as described below.

The recipient animals were anesthetized by an i.m. injection of sufentanil (Sufenta®; Janssen Pharmaceutica Inc.) 20 μg/kg and azaperone (Stresnil®, Janssen Pharmaceutica Inc., Titusville, NJ, USA) 1 mg/kg. The aortal grafts were transplanted orthotopically after midline laparotomy into the recipient's infrarenal aorta. Neither anti-coagulants nor anti-platelet drugs were administrated during the postoperative period.

Immunosuppressive therapy

FK 506 (Prograf®; Astellas Pharma Inc., Munich, Germany) suspended in normal saline solution was administered i.m. in daily doses of 0.2 mg/kg. The recipient animals were divided into four groups according to the postoperative immunosuppressive protocol: group A was that of isogeneic control (LEW to LEW), group B was that of allogeneic control (BN to LEW) with no administration of immunosuppression; animals in group C (BN to LEW) were immunosuppressed from day 1 to day 30; and animals in group D (BN to LEW) were immunosuppressed from day 7 to day 30 after the transplantation.

FK 506 blood levels were evaluated using an enzyme multiplied immunoassay technique (Emit® 2000 Tacrolimus assay; Dade Behring, Cupertino, CA, USA) on day 7, or day 30 after the transplantation. Blood samples were collected from the venous ophthalmic sinus under i.m. anesthesia.

The aortal grafts were removed after midline re-laparotomy in anesthetized and heparinized animals on day 7 (group A, B, C), or day 30 (group A, B, C, D), respectively. The animals were then killed by an intracaval administration of a lethal dose of thiopental (Thiopental®; Spofa). Removed grafts of the abdominal aorta were processed for histology and immunohistochemistry.

Histologic analysis

The removed grafts were fixed in a 10% natural buffered formalin. Paraffin cross sections (5 μm) were prepared and stained with hematoxylin and eosin and Van Gieson with elastica stain. The sections were taken from the midportion of the graft to avoid any iatrogenically induced response to the suture material.

Each section was photographed using an Olympus Cammedia C-5050 digital camera mounted on an Olympus BX51 microscope the magnification of which was ×400 (all from Olympus, Hamburg, Germany). The images were digitally captured in the TIFF format with no compression, and analyzed using Olympus DP-Soft software Version 3.2. Intimal thickness (from endothelial surface to the inner border of tunica media), as well as medial thickness (defined by internal and external elastic membranes), were measured up to 10 locations in each

section. The mean value and SD (mean ± SD) were calculated for each aorta as well as animal group.

Immunohistochemical analysis

As to the immunohistochemistry detection of antigens, the sections were obtained from the midportion of the graft as described above. After the harvest, they were embedded using Tissue Tek© Cryomold holders (Sakura, Japan) and Tissue Tek© O.C.D. compound, and subsequently frozen in 2-methylbutane (Fluka Chemika, Buchs, Switzerland) cooled by liquid nitrogen. The samples were then stored at −80 °C in freezers until the immunohistochemical analysis. Monoclonal and polyclonal antibodies used in the study are listed in Table 1.

Detection of $CD4^+$, $CD8^+$ cells and Von Willebrand factor
Detection was performed on 8 μm thick sections using a two-step indirect method. Briefly, the sections were fixed in cold acetone for 10 min. After rinsing in 0.2% Triton X-100 and phosphate-buffered saline, the specimens were incubated with a primary antibody for 60 min. After blocking of endogenous peroxidase in 0.3% H_2O_2 in 70% methanol for 30 min, the antibody was detected by incubation with a secondary antibody (Histofine®; Simple Stain Rat MAX PO, Nichirei, Japan) for 30 min. After the incubation for 5 min with Dako Liquid DAB+ Substrate-Chromogen System (DakoCytomation, Glostrup, Denmark), the specimens were counterstained and dipped in Entellan (Merck, Darmstadt, Germany).

The slides were then scored in a blinded fashion. The cells were counted at five locations at the original magnification, ×1000. The cellularity was defined as the mean value of the cells counted.

Detection of immunoglobulins
The detection was performed on 8 μm thick sections using a direct method. After being rinsed in phosphate-buffered saline, the tissues were air-dried and incubated with an antibody directly conjugated with fluorescein isothiocyanate for 30 min. The specimens were then dipped in glycerin medium and then immediately analyzed by a fluorescence microscope.

Detection of $RT1.B^u$ positive cells
The detection was performed on 8 μm thick sections using a three-step indirect method. The sections were fixed for 10 min in cold acetone. After being rinsed in 0.2% Triton X-100 and phosphate-buffered saline, endogenous biotin was blocked using the Biotin blocking system (DakoCytomation). The tissues were then incubated in 10% horse serum to prevent unspecific binding, and then using a primary antibody for 60 min. After blocking of endogenous peroxidase in 0.3% H_2O_2 in 70% methanol for 30 min, the antibody was detected by incubating the specimen with a secondary biotinylated horse anti-mouse (Vector Lab, Burlingame, CA, USA) antibody, followed by an incubation with R.T.U. Vectastain Elite ABC Reagent (Vector Lab). After the incubation for 5 min with Dako Liquid DAB+ Substrate-Chromogen System (DakoCytomation), the specimens were counterstained and dipped in Entellan (Merck).

Statistical analysis

The values in the text and figures are expressed as the mean ± SD. Comparisons of intimal and medial thicknesses and numbers of lymphocytes between the experimental groups were performed using the analysis of variance, followed by the Tukey HSD Multiple Comparisons test. The Kruskal–Wallis one-way analysis was used to compare FK 506 blood levels between the groups. P-values <0.05 were considered significant.

Results

Animals

Forty-eight aortal grafts were successfully transplanted. Two animals died in group B because of a hemorrhage resulting from anastomosis and anastomotic stenosis, respectively. One animal was overdosed and died during blood sampling in group C.

Table 1. Antibodies for immunohistochemical staining.

Specificity	Origin	Company	Dilution (×)	Clone	Detection
Von Willebrand factor	Rabbit	DakoCytomation	5000		Histofine
$CD4^+$ cells	Mouse	Cymbus Biotechnology Ltd	800	W3/25	Histofine
$CD8^+$ cells	Mouse	Cymbus Biotechnology Ltd	500	OX-8	Histofine
Immunoglobulins	Sheep	Chemicon International	10		FITC
$RT1.B^u$*	Mouse	Cedar Lane	50	MRC-OX3	Vector

FITC, fluorescein isothiocyanate.
List of monoclonal and polyclonal antibodies used for immunohistochemical staining of aortal grafts.
*MHC class II positive cells of Lewis origin.

A decrease in the preoperative weight was observed in all animal groups on day 7. After that day, the weight of animals was increasing in all the experimental groups.

Immunosuppression

The mean blood level of FK 506 during the entire experiment was 5.0 ± 0.7 ng/ml, with no statistical difference between the immunosuppressed groups. No adverse effects of the FK 506 administration, such as diarrhea or a persisted weight loss, were observed in any of the experimental animals.

Tunica intima

The mean CIT during the entire experiment was 25.8 ± 8.0 h, with no statistical differences between the experimental groups. No significant changes in the vascular wall were observed after the 25-h period mentioned above.

The moderate intimal thickening was observed in all the groups on day 7 (Table 2). The ECs were underlying with small disparate clumps of mononuclear cells. The higher extent of the leukocyte adhesion was observed in the allogeneic nonimmunosuppressed group. The mean value of the intimal reaction in this group reached a peak value of 20.3 ± 15.0 μm.

The process of intimal proliferation continued then in all the experimental groups. There was no statistical difference in the intimal thickness between group A, group C and group D on day 30 (Table 3). However, the intimal proliferation in the allogeneic nonimmunosuppressed group B was statistically higher when compared with all the other groups (Fig. 1).

A typical feature observed in all the groups was a nonconcentric form of intimal lesions. The regions with massive intimal thickening alternate with those showing a lower intimal response.

The luminal surface of the thickened intima was covered by a monolayer of ECs in all the groups on day 30 (Fig. 2).

Lewis major histocompatibility complex (MHC) class II positive cells in the neointima of BN were detected only in the allogeneic, but not in any of the immunosuppressed groups, on day 30 (Fig. 3).

Table 2. Parameters under study – day 7.

			Intima†		Media†		Adventitia‡	
Group	Immunosuppression	Day	Thickness	ECs layer	Thickness	IgG deposition	$CD4^+$ cells	$CD8^+$ cells
A	No	7	10.6 ± 5.2	–	77.5 ± 9.3	–	21.4 ± 7.5	3.0 ± 2.8
B	No	7	20.3 ± 15.0	–	75.2 ± 10.6	–	32.6 ± 8.8	8.7 ± 7.4
C	FK 506 day 1–30	7	14.3 ± 5.1	–	81.2 ± 14.2	–	15.0 ± 4.9	3.9 ± 3.2
D*	FK 506 day 7–30	7	20.3 ± 15.0	–	75.2 ± 10.6	–	32.6 ± 8.8	8.7 ± 7.4

ECs, endothelial cells.

Parameters of aortal wall injury 7 days after transplantation.

*Values of group D on day 7 are identical to those of allogeneic no immunosuppressed group B.

†Intimal and medial thicknesses are expressed in μm.

‡Numbers of CD4 and CD8 positive cells in one eyepiece at original magnification ×1000.

Table 3. Parameters under study – day 30.

			Intima*		Media*		Adventitia†	
Group	Immunosuppression	Day	Thickness	ECs layer	Thickness	IgG deposition	$CD4^+$ cells	$CD8^+$ cells
A	No	30	35.5 ± 23.4	+	80.2 ± 14.2	–	25.3 ± 8.8	9.6 ± 7.1
B	No	30	121.6 ± 33.2‡	+	61.3 ± 11.1	+	108.8 ± 24.0‡	59.8 ± 12.2‡
C	FK 506 day 1–30	30	41.0 ± 19.2	+	73.5 ± 11.8	–	23.8 ± 9.3	6.0 ± 5.0
D	FK 506 day 7–30	30	40.5 ± 23.6	+	82.9 ± 13.7	–	23.7 ± 6.5	8.3 ± 4.5

Effects of a delayed administration of FK 506 on main features of an aortal wall injury 30 days after the transplantation.

*Intimal and medial thicknesses are expressed in μm.

†Numbers of CD4 and CD8 positive cells in one eyepiece at original magnification ×1000.

‡$P < 0.05$.

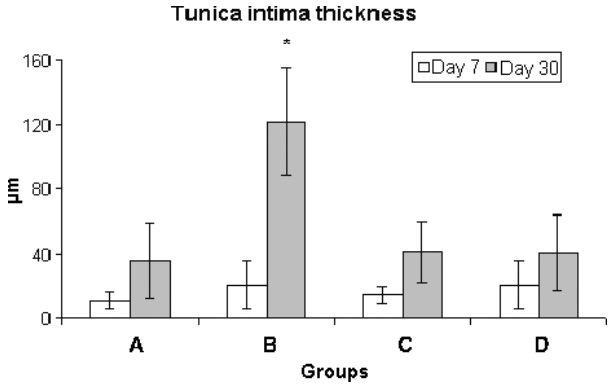


Figure 1 The extent of the intimal reaction on day 30 in both immunosuppressed groups (C, D) was comparable with that of the isogeneic group (A). The intimal thickness in the allogeneic group B on day 30 was significantly higher ($P < 0.05$) when compared with groups A, C and D.

Tunica media

No statistical difference in the tunica media thickness was noticed in the experimental groups on day 7. The medial layer of none of the aortal grafts showed any morphological alterations or IgG depositions.

The delayed administration of FK 506 had no effect on the medial thickness of aortal allografts on day 30. Values of the tunica media thickness on day 30 did not differ between group A, group C and group D, respectively. The histological findings were comparable with those observed on day 7. However, the tunica media thickness in the allogeneic animals was significantly lower when compared with all the other groups. The degeneration and necrosis of medial smooth muscle cells was not detected in most of the allogeneic grafts. The maximum medial degenerative changes were localized beneath regions of the most significant intimal proliferation.

No medial IgG depositions were detected in the isogeneic or in any of the immunosuppressed allogeneic groups on day 30. Immunofluorescent staining revealed IgG depositions as small fluorescent granular deposits

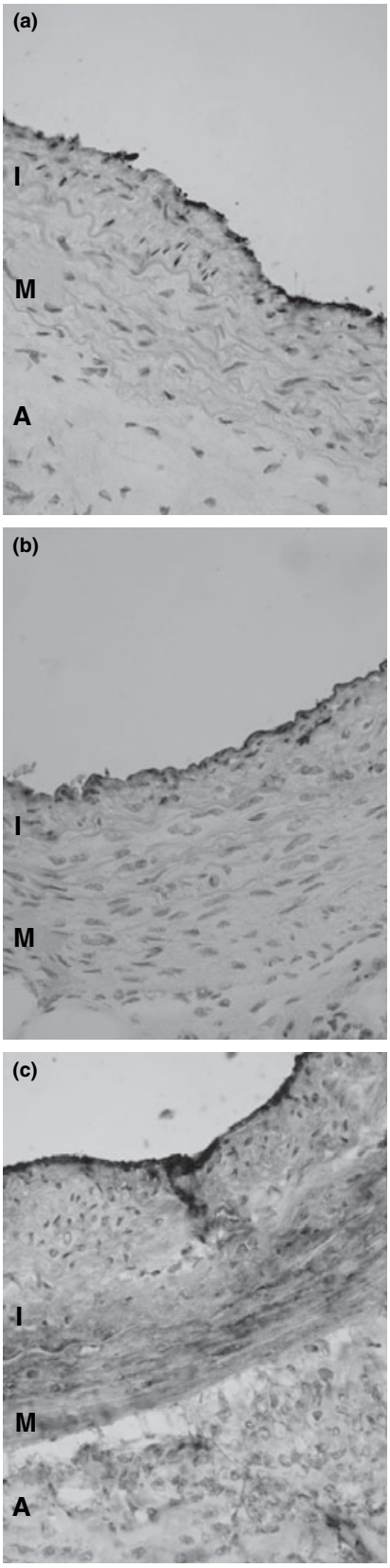
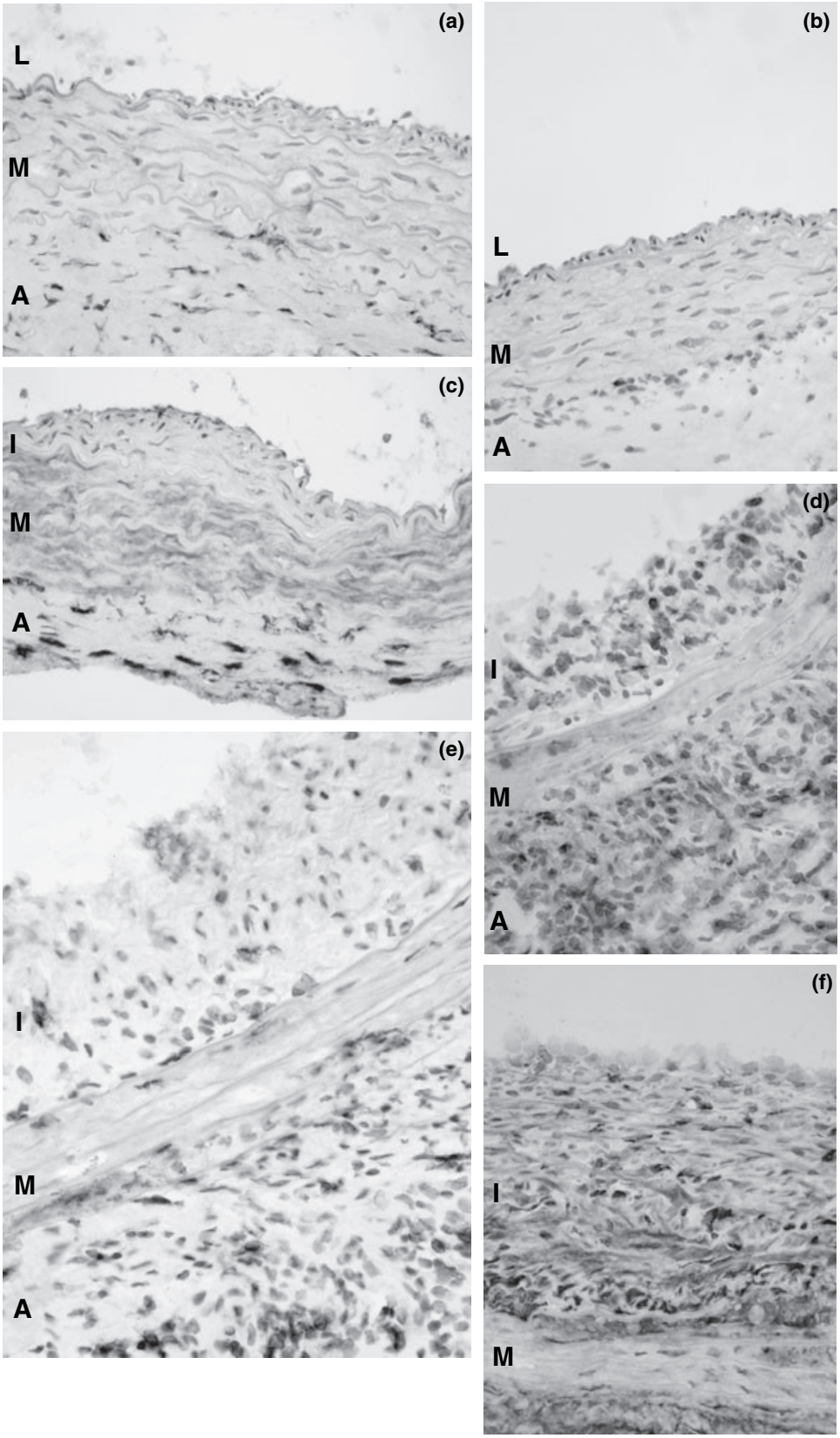


Figure 2 Light microscopic findings of arterial allografts obtained 30 days after the transplantation. Immunostaining with anti-Von Willebrand factor antibody. Positive endothelial cells (EC) are stained brown. The monolayer of ECs covering the neointima of aortal grafts was observed in the syngeneic group A (a), from day 7 immunosuppressed allogeneic group D (b) as well as in nonimmunosuppressed group B (c). The immune reaction in the field of no suppression led to a significantly higher formation of neointima in group B (c) when compared both with the isogeneic group A (a) and from day 7 immunosuppressed group D (b). Original magnification, ×400. I, tunica intima; M, tunica media; A, tunica adventitia.



surrounding smooth muscle cells only in the allogeneic control group B. The regions of IgG deposition correlated well with the regions of maximum medial degenerative changes (Fig. 4).

Tunica adventitia

The interstitial infiltration by mononuclear cells was the main feature in the adventitial layer observed in all the groups on day 7. The highest degree of $CD4^+$ cellular infiltration was observed in the allogeneic nonimmunosuppressed group B. In group C, the $CD4^+$ cellular infiltration was even lower when compared with the isogeneic control group A. The proportion of $CD8^+$ adventitial infiltration on day 7 was almost identical to that of $CD4^+$ cells. However, the difference between the syngeneic and the immunosuppressed grafts observed in $CD4^+$ was not noticed in $CD8^+$ cells infiltration.

Both regimens of immunosuppression resulted in a lower adventitial infiltration rate by both $CD4^+$ and $CD8^+$ cells when compared with the isogeneic group A on day 30 (Fig. 5). The adventitial layer of allogeneic nonimmunosuppressed grafts was infiltrated by a high amount of $CD4^+$ and $CD8^+$ cells (Fig. 3). Most of them were confined beneath the medial layer. The regions of $CD4^+$ and $CD8^+$ infiltrations correlated well with the regions of maximum medial degenerative changes.

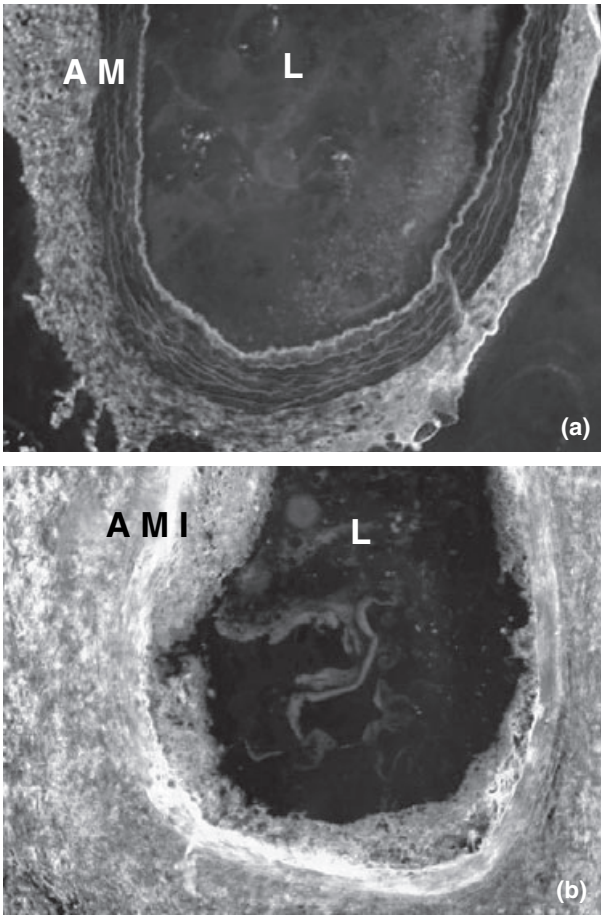


Figure 4 Light microscopic findings of arterial allografts 30 days after the transplantation. Immunofluorescent staining with anti-IgG antibody. No IgG granulations was observed in the medial layer of aortal grafts in group D (a). The rejection of aortal grafts observed in group B was characterized by a focal deposition of immunoglobulins into the tunica media, accompanied by a massive neointimal reaction (b). Original magnification, ×100. L, aortal lumen; I, tunica intima; M, tunica media; A, tunica adventitia.

Discussion

The results of our experimental study concerning the morphological changes in all three layers of the arterial wall revealed a similar morphological picture, irrespective of whether the immunosuppression with a low-dose of FK 506 was administered immediately after, or from day 7 after the operation. While the process of rejection in the field of no immunosuppression resulted in intimal hyperplasia, medial necrosis and adventitial infiltration of aortal allografts, doses of FK 506 given from day 7 suppressed and reversed the immune response against arterial wall antigens.

Histological signs of endothelial denudation and light leukocyte adhesion on day 7 were comparable between the syngeneic and the allogeneic groups, no matter whether immunosuppressives were given, or not. The intimal reaction is believed to be triggered by alloantigen-independent ECs damage and an activation caused by ischemic and reperfusion (I/R) injury [24]. The intensity of the intimal reaction and subsequent rejection is proportional to the value of the I/R damage and increases proportionally to the time of ischemia [25].

Figure 3 Light microscopic findings of aortal grafts obtained from animals of group D (a, b, c) and group B (d, e, f) 30 days after the transplantation. Immunostaining revealed a marked suppressive effect of the delayed FK 506 administration in group D on the neointimal and advential infiltration, both by $CD4^+$ cells (a) and $CD8^+$ cells (b) when compared with the nonimmunosuppressed group B (d, e). The anti Lewis MHC class II antibody was used to identify the origin of the invading cells (c, f). The massive neointimal and adventitial infiltration by the recipient's immunocompetent cells were observed in nonimmunosuppressed animals in group B (f), in contrast to animals of group D (c) immunosuppressed from day 7. The positive cells are stained brown. Original magnification, ×400. L, aortal lumen; I, tunica intima; M, tunica media; A, tunica adventitia.

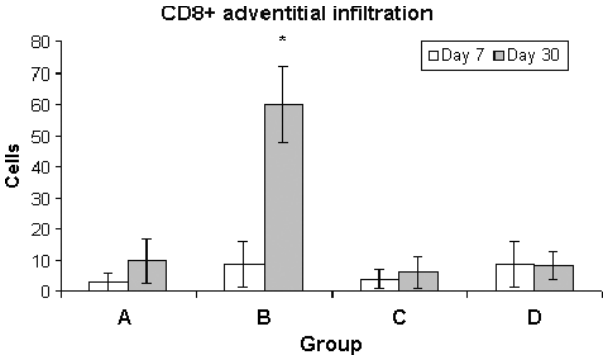


Figure 5 The extent of the $CD8^+$ infiltration on day 30 in both immunosuppressed groups (C,D) was comparable with that of the isogeneic group (A). The $CD8^+$ cells infiltration in the allogeneic nonimmunosuppressed group B on day 30 was significantly higher ($P < 0.05$) when compared with groups A, C and D.

Apart from the ischemic time, the next important factor affecting the endothelial damage of arterial grafts is represented by properties of the solution used for conservation [26]. The HTK solution used in our experiment is routinely used in clinical as well as experimental organ transplantations with good results [27].

The process of a massive leukocyte adhesion and strong neointima formation after day 7 continued only in the allogeneic group with no immunosuppression. When given from day 1 or even from day 7 after the transplantation, FK 506 was sufficient to suppress secondary alloantigen-dependent ECs damage which commonly occurred between day 14 and day 21 after the transplantation, as described previously by Gohra *et al.* [28] and Azuma *et al.* [22].

In all the experimental groups, the monolayer of ECs covered the neointima on day 30. Our method did not permit to determine the origin of the ECs, i.e. from the donor or from the recipient. Nevertheless, we suppose that the suppression of rejection by the delayed administration of FK 506 was even sufficient to allow the regeneration of ECs of donor origin, as observed by other research teams [22,24].

The adequate host immune system suppression by the delayed FK 506 administration was also confirmed by the absence of any signs of the recipients' LEW MHC class II positive cells infiltration into the neointima of the donors' BN aortal grafts on day 30.

The immunosuppressive protocol involving FK 506 being administered from day 7 after the transplantation succeeded in suppressing the IgG deposition in the tunica media, as well as the necrosis of aortal smooth muscle cells. This layer was considered to be the delayed target of recipient's immune response. In our experiment, the first signs of rejection appeared after day 8 and subsequently led to a necrosis of medial layer.

A specific characteristic feature of the tunica media rejection process is the absence of inflammatory cells infiltration [21]. Several mechanisms involved in smooth muscle cell necrosis have been mentioned in the literature: a severe nutritional deficiency caused by an extensive inflammation of adventitia and lack of ingrowth of the new recipient-derived vasa vasorum into the media [24], apoptotic cell death caused by up-regulation of $CD8^+$ T-cell derived inducers of apoptosis [29], and IgG-dependent smooth muscle cells injuries related to the mechanism other than complement-induced cell necrosis [21].

FK 506 given from day 7 suppressed even the process of ongoing inflammation and adventitial infiltration by $CD4^+$ and $CD8^+$ positive cells. The characteristic feature of adventitial layer acute rejection observed in other studies occurred only in the condition of no immunosuppression [29].

The main effectors of the adventitial reaction seem to be $CD8^+$ T lymphocytes. The process of infiltration starts on day 5 and reaches its peak on day 18 after the transplantation [21]. After that day, the medial smooth muscle cells start to disappear as a result of apoptosis [29]. Azuma *et al.* [22] observed a massive cellular infiltration of the tunica adventitia 2 weeks after the cessation of FK 506. Some findings indicate that the medial smooth muscle cells destruction and intimal proliferation are regulated independently [29,30].

FK 506 inhibits the mixed lymphocyte reaction assay, the formation of IL-2 by T lymphocytes, and formation of other soluble mediators, including IL-3, IL-4, IL-5, tumor necrosis factor alpha and granulocyte–macrophage colony-stimulating factor [31]. Almawi *et al.* have confirmed that, in exerting its antiproliferative effect, FK 506 acts on two levels: by inhibiting the cytokine availability and by suppressing the cytokine effect on target cells [32]. This finding can explain the beneficial effect of FK 506 in attenuating ongoing immune responses. The effect of FK 506 on the augmentation of T-cell apoptosis was observed in experiments as well [31].

The predicted effect of FK 506 observed in our study was the suppression of $CD8^+$ cells as well as the growth factor formation. The first effect resulted in a suppression of smooth medial cells necrosis; the second one in a suppression of intimal hyperplasia of aortal allografts.

The antigenicity of arterial allografts has been documented frequently in experiments, as well as in clinical practice [9,21]. The process of rejection was adjusted using various immunosuppressive drugs, including calcineurin inhibitors, in many experimental studies [22,23,33,34]. However, the use of immunosuppression after an alloarterial or allovenous transplantation to increase the patency rate of reconstructions in humans is not widely accepted. Moreover, the suppression of the

immune system in patients after the removal of an infected prosthesis, followed by an arterial transplantation, has not yielded a definite solution yet [1,35,36].

The present study considered a possibility of delaying the administration of FK 506 after an arterial transplantation for 1 week without any influence on morphological signs of an acute arterial wall rejection.

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References

1. Kieffer E, Gomes D, Chiche L, Fleron MH, Koskas F, Bahnini A. Allograft replacement for infrarenal aortic graft infection: early and late results in 179 patients. *J Vasc Surg* 2004; **39**: 1009.
2. Gabriel M, Pukacki F, Dzieciuchowicz Ł, Oszkinis G, Checinski P. Cryopreserved arterial allografts in the treatment of prosthetic graft infections. *Eur J Vasc Endovasc Surg* 2004; **27**: 590.
3. Teebken OE, Pichlmaier MA, Brand S, Haverich A. Cryopreserved arterial allografts for in situ reconstruction of infected arterial vessels. *Eur J Vasc Endovasc Surg* 2004; **27**: 597.
4. Schmitz-Rixen T, Megerman J, Colvin RB, Williams AM, Abbott WM. Immunosuppressive treatment of aortic allografts. *J Vasc Surg* 1988; **7**: 82.
5. Marois Y, Wagner E, Pâris E, Roy R, Douville Y, Guidoin R. Comparison of healing in fresh and preserved arterial allografts in the dog. *Ann Vasc Surg* 1999; **13**: 130.
6. Pirrelli S, Arici V, Bozzani A, Odero A. Aortic graft infections: treatment with arterial allograft. *Transplant Proc* 2005; **37**: 2694.
7. Vogt PR, Stallmach T, Niederhäuser U *et al.* Explanted cryopreserved allografts: a morphological and immunohistochemical comparison between arterial allografts and allograft heart valves from infants and adults. *Eur J Cardiothorac Surg* 1999; **15**: 639.
8. Prager M, Hölzenbein T, Aslim E, Domenig Ch, Mühlbacher F, Kretschmer G. Fresh arterial homograft transplantation: a novel concept for critical limb ischaemia. *Eur J Vasc Endovasc Surg* 2002; **24**: 314.
9. Mirelli M, Stella A, Faggioli GL *et al.* Immune response following fresh arterial homograft replacement for aortoiliac graft infection. *Eur J Vasc Endovasc Surg* 1999; **18**: 424.
10. Stadler P, Sebesta P, Klika T, Sedivy P, Slizova D, Krs O. Allografts in the vascular surgery. *Rozhl Chir* 2005; **84**: 350.
11. Luther B, Balzer KM, Reinecke P, Wassmuth R, Sandmann W. Homologous vein transplantation in cruropedal arterial reconstruction. *Chirurg* 2004; **75**: 153.
12. Pupka A, Skora J, Janczak D *et al.* Immunosuppression in the treatment of vascular prosthesis infection with arterial transplantation. *Pol Mer Lek* 2004; **16**: 8.
13. Matia I, Adamec M, Janousek L, Lipar K, Viklicky O. Fresh arterial grafts as conduits for vascular reconstructions in transplanted patients. *Eur J Vasc Endovasc Surg* 2006; **32**: 549.
14. Zoja C, Furci L, Ghilardi F, Zilio P, Benigni A, Remuzzi G. Cyclosporin-induced endothelial cell injury. *Lab Invest* 1986; **55**: 455.
15. Khanna A, Kapur S, Sharma V, Li B, Suthanthiran M. In vivo hyperexpression of transforming growth factor-beta 1 in mice: stimulation by cyclosporine. *Transplantation* 1997; **63**: 1037.
16. Vincenti F, Jensik SC, Filo RS, Miller J, Pirsch J. A long-term comparison of tacrolimus (FK506) and cyclosporine in kidney transplantation: evidence for improved allograft survival at five years. *Transplantation* 2002; **73**: 775.
17. Faivre L, Saoudi S, Astier A *et al.* FK 506 dose in transplantation: from theory to practice. *Transplant Proc* 2001; **33**: 2594.
18. Gjertson DW, Cecka JM, Terasaki PI. The relative effects of FK506 and cyclosporine on short- and long-term kidney graft survival. *Transplantation* 1995; **60**: 1384.
19. Wang T, Donahoe PK, Zervos AS. Specific interaction of type I receptors of the TGF-beta family with the immunophilin FKBP-12. *Science* 1994; **265**: 674.
20. Mennander A, Tiisala S, Halttunen J, Yilmaz S, Paavonen T, Häyry P. Chronic rejection in rat aortic allografts. An experimental model for transplant arteriosclerosis. *Arterioscler Thromb* 1991; **11**: 671.
21. Plissonnier D, Nochy D, Poncet P *et al.* Sequential immunological targeting of chronic experimental arterial allograft. *Transplantation* 1995; **60**: 414.
22. Azuma N, Sasajima T, Kubo Y. Immunosuppression with FK506 in rat arterial allografts: fate of allogeneic endothelial cells. *J Vasc Surg* 1999; **29**: 694.
23. Waller JR, Murphy GJ, Bicknell GR, Toomey D, Nicholson ML. Effects of the combination of rapamycin with tacrolimus or cyclosporin on experimental intimal hyperplasia. *Br J Surg* 2002; **89**: 1390.

24. Hillebrands JL, Klatter FA, van den Hurk BM, Popa ER, Nieuwenhuis P, Rozing J. Origin of neointimal endothelium and alpha-actin-positive smooth muscle cells in transplant arteriosclerosis. *J Clin Invest* 2001; **107**: 1411.
25. Knight RJ, Dikman S, Liu H, Martinelli GP. Cold ischemic injury accelerates the progression to chronic rejection in a rat cardiac allograft model. *Transplantation* 1997; **64**: 1102.
26. Vischjager M, Van Gulik TM, Pfaffendorf M *et al.* Morphology and function of dog arterial grafts preserved in UW-solution. *Eur J Vasc Endovasc Surg* 1995; **10**: 431.
27. Balaz P, Matia I, Jackanin S *et al.* Preservation injury of jejunal grafts and its modulation by custodiol and university of wisconsin perfusion solutions in wistar rats. *Eur Surg Res* 2004; **36**: 192.
28. Gohra H, McDonald TO, Verrier ED, Aziz S. Endothelial loss and regeneration in a model of transplant arteriosclerosis. *Transplantation* 1995; **60**: 96.
29. Légaré JF, Issekutz T, Lee TD, Hirsch G. $CD8^+$ T lymphocytes mediate destruction of the vascular media in a model of chronic rejection. *Am J Path* 2000; **157**: 859.
30. Mennander A, Paavonen T, Hayry P. Intimal thickening and medial necrosis in allograft arteriosclerosis (chronic rejection) are independently regulated. *Arterioscler Thromb Vasc Biol* 1993; **13**: 1019.
31. Hashimoto Y, Matsuoka N, Kawakami A *et al.* Novel immunosuppressive effect of FK506 by augmentation of T-cell apoptosis. *Clin Exp Immunol* 2001; **125**: 19.
32. Almawi WY, Assi JW, Chudzik DM, Jaoude MM, Rieder MJ. Inhibition of cytokine production and cytokine-stimulated T-cell activation by FK506 (tacrolimus)1. *Cell Transplant* 2001; **10**: 615.
33. Vischjager M, Van Gulik TM, De Kleine RH *et al.* Experimental arterial allografting under low and therapeutic dosages of cyclosporine for immunosuppression. *Transplantation* 1996; **61**: 1138.
34. Lacha J, Rossmann P, Lodererova A, Havlickova J, Vitko S. LF 08–0299 in the prophylaxis and treatment of chronic rejection in a rat aortic allograft model. *Transpl Int* 2000; **13**: S565.
35. Lesèche G, Castier Y, Petit MD *et al.* Long-term results of cryopreserved arterial allograft reconstruction in infected prosthetic grafts and mycotic aneurysms of the abdominal aorta. *J Vasc Surg* 2001; **34**: 616.
36. Adamec M, Matia I, Janousek L *et al.* Renal transplantation in patients with abdominal aortic aneurysm – a new surgical approach. *Transpl Int* 2004; **17**: 647.

