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Prevention of neointimal proliferation by immunosuppression in synthetic vascular grafts ☆

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Abstract

Objective: Immunosuppressive agents have been proposed to reduce neointimal hyperplasia in synthetic vascular grafts. Thus, the purpose of the present study was to evaluate the safety and efficacy of rapamycins (systemic vs. local vs. oral administration) and mycophenolate mofetil (MMF) to reduce intimal hyperplasia in infrarenal synthetic vascular grafts of the rat. **Methods**: Fifty-four Wistar rats (250 g) completed the study after a synthetic vascular graft (ePTFE, Gore-tex, 2 mm diameter, 10 mm length) was implanted end-to-end in the infrarenal aorta. The animals were divided into three groups: group 1 consisted of 12 control animals, group 2 consisted of 37 rats receiving rapamycins, either per os (RAD, 1.5 or 3 mg/kg), intraperitoneally (RPM, 1.5 or 3 mg/kg) or locally (RPM soaking of the graft); and in group 3 ($n = 5$), MMF (40 mg/kg) was administered orally. The animals were followed weekly with weight controls and signs of toxicity for 30 ($n = 37$) and 60 ($n = 17$) days, respectively. All animals were sacrificed and underwent histological examination at completion of the study. **Results**: All animals survived in groups 1 and 3, but five died in group 2. The weight gain was normal in all groups, except for the subgroup 2a receiving high dose rapamycins orally. All rats in group 3 suffered from diarrhea, whereas animals receiving high dose rapamycins showed toxic signs (hair loss, wound healing problems). Histological examination showed a significant increase in intimal hyperplasia in group 1 (0.03 ± 0.01 and 0.14 ± 0.05 μm after 30 and 60 days, respectively; $P < 0.01$). Rapamycins in either application or dosage had no significant effect on intimal hyperplasia. **Conclusions**: Local or systemic administration of rapamycins has no effect on intimal hyperplasia in synthetic vascular grafts. In contrast, toxic signs with weight loss were observed in animals treated with high dose rapamycins, but not in those treated with MMF. Thus, in the rat model, immunosuppresssion with rapamycins or MMF cannot be recommended for the prevention of intimal hyperplasia in the synthetic vascular graft model.

Keywords: Vascular prosthesis; Intimal hyperplasia; Immunosuppression; Toxicity

1. Introduction

Coronary and peripheral bypass operations are performed routinely for myocardial or peripheral ischemia. The best results are obtained with autologous arterial or venous graft materials, like internal mammary artery and saphenous vein grafts. However, graft material can be limited due to vein disease or previous use of vein material. Therefore, synthetic graft material has been used as a substitute, but vascular grafts below 6 mm diameter have a high occlusion rate, mainly due to graft thrombosis in the early phase and intimal hyperplasia in the late phase [1]. Early graft thrombosis can be minimized with good surgical techniques, and adequate antiplatelet or anticoagulation treatment in patients with a good run-off. More recently, new graft material and the surface (heparin) coating of grafts have been shown to improve graft patency [2]. However, graft occlusion due to intimal hyperplasia remains a major problem. Several methods have been used to improve graft patency in small vascular grafts, such as pharmacological interventions, cell seeding and, more recently, tissue engineering of synthetic grafts [3]. Immunosuppressive agents have been used only rarely due to unwanted side-effects, but the antiproliferative properties may reduce intimal hyperplasia [4–6]. Gregory and co-workers have shown that a

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high dose of rapamycins reduces intimal hyperplasia in the rat carotid artery injury model [7,8]. Others have shown that most immunosuppressive agents have a beneficial effect on intimal hyperplasia in allogenic vascular grafts [9–12]. So far, no study has been carried out showing that rapamycins or mycophenolate mofetil (MMF) have a beneficial effect on intimal proliferation and long-term patency in synthetic vascular grafts.

2. Materials and methods

In 69 Wistar rats, an infrarenal interposition expanded polytetrafluoroethylene (ePTFE; Gore-tex) graft was implanted under general anesthesia using intraperitoneal fluanisone (Janssen-Cilag). The operations were performed through a midline total laparotomy using sterile and microsurgical techniques. After preparation of the infrarenal aorta, clamping, resection and end-to-end single stitch (10.0 monofilament), implantation was performed. The thin-walled, Gore-tex graft had an internal diameter of 2 mm and a length of 10 mm. The graft patency was tested manually and the laparotomy was closed in two layers. Postoperatively, no antiplatelet drugs or heparin therapy were given. All animals had standard care and received normal food.

The animals were divided into three groups: control group 1 ($n = 12$), group 2 treated with rapamycins ($n = 37$), and group 3 treated with MMF ($n = 5$). Group 2 was further subdivided according to the route of administration and the dosage:

2a rapamycins (RAD Novartis 1.5 or 3 mg/kg per os by gavage).
2b rapamycins (RPM Wyeth 1.5 or 3 mg/kg intraperitoneally).
2c rapamycin graft soaking (RPM Wyeth).

In the last group, the grafts were immersed for 24 h in a rapamycin saline solution on an agitator at room temperature. The animals were followed daily for graft occlusion and drug toxicity. Body weight was measured weekly.

Each group was scheduled to comprise six animals. Four animals died perioperatively, of narcosis accident (two) and perioperative bleeding (two), and one animal in the treatment group was inadvertently placed in the long-term control group ($n = 7$). All of those four animals were replaced. In addition, five animals had to be euthanized during the first 2 weeks. Two for graft thrombosis and hind leg ischemia, and three for wound problems (poor healing and secondary dehiscence). These animals were also replaced. Thus, a total of 69 rats were operated.

At the conclusion of the study, either after 30 or 60 days, the animals were anesthetized and euthanized by potassium chloride overdose, and the grafts were dissected free and pressure perfused in situ with glutalaldehyde. The grafts were cut longitudinally, and after hematoxylin and eosin staining, sections were examined at the proximal and distal anastomoses, as well as in the middle of the graft according to the following criteria: graft patency dimensions and intimal proliferation thickness, length and area, as well as a grading score (0, none; 1, mild; 2, moderate; and 3, severe) for intimal proliferation, thrombus formation and cellular ingrowth. The scores were given by an experienced vascular pathologist blinded to the treatment groups.

All animals received human care in compliance with the European Convention on Animal Care. The study was approved by the veterinary ethical committee.

2.1. Statistics

The results are expressed as mean values ± 1 SEM for figures or mean values ± 1 SD for tables, respectively. Differences between proximal and distal anastomoses were calculated using the paired *t*-test. Differences among the three groups were evaluated with a one-way analysis of variance (ANOVA). When the analysis was significant, a Kruskal–Wallis test was performed. Comparisons within the same group were carried out with an ANOVA for repeated measurements (Mann–Whitney Test with Bonferroni correction) using the STATA software (Santa Monica, USA).

3. Results

3.1. Survival, body weight and side-effects

Except for the four animals dying perioperatively and the five animals who were euthanized during the first 2 weeks (see above), all other animals had an uneventful recovery after the intervention.

During the follow-up, no animal died in group 1. Five animals died in group 2, four probably related to drug toxicity (oral RAD) and one from late thrombosis (rapamycin treated graft). In group 3, one animal was euthanized because of severe diarrhea. Thus, 54 animals completed the study.

After 3 weeks of treatment, most animals in group 2, mainly the ones treated with oral or high dose rapamycins, developed signs of toxicity. These included appetite and weight loss, diarrhea, hair loss, aggressivity and wound dehiscences (Table 1).

Rats in the control group showed an increase in body weight of about 150 g during the first month and about an equal amount during the second month. The animals of group 2c (rapamycin graft soaking) and group 3 (MMF) showed a similar weight increase than the control group animals. In contrast, especially the animals of group 2a (RAD) receiving the high dosage, showed a significantly lower weight increase at 30 and 60 days, respectively when compared with controls (Table 1; Fig. 1A,B).

3.2. Histological evaluation (Fig. 2A,B)

Intimal hyperplasia thickness was present at both anasto-

Table 1
Results after 3 weeks

Group		$n = 54$	Application (mg/kg BW)	Survival (days)	Weight change (g)[a]	Side-effects (n)	P[b]
Group 1 (controls)		5	None	30 ± 0	166 ± 3	0/5	–
		7	None	61 ± 0	349 ± 12	0/7	–
Group 2 (rapamycins)	2a	4	p.o./(1.5)[c]	31 ± 0	48 ± 6	2/4	0.073
		6	p.o./(3.0)[c]	27 ± 5	−15 ± 20	2/6	0.154
		4	p.o./(3.0)[c]	56 ± 1	67 ± 16	4/4	0.001
	2b	6	i.p./(1.5)[d]	30 ± 0	176 ± 13	2/6	0.154
		6	i.p./(3.0)[d]	28 ± 1	12 ± 6	3/6	0.064
	2c	5	Soaking[d]	32 ± 1	146 ± 14	0/5	–
		6	Soaking[d]	62 ± 2	267 ± 5	0/6	–
Group 3 (MMF)		5	p.o./(40)[e]	31 ± 0	171 ± 17	5/5[f]	0.053

[a] Significances are shown on Fig. 1.
[b] Side-effects (χ^2 to controls).
[c] RAD Novartis.
[d] Rapamycin Wyeth.
[e] MMF.
[f] Diarrhea.

moses and similar in groups 1 and 2 after 30 days. The animals of group 3 showed a higher intimal proliferation compared with the animals treated systemically with rapamycins (0.06 ± 0.02 vs. 0.02 ± 0.01; $P < 0.01$). There was a significant increase in the controls from 30 to 60 days (0.03 ± 0.01 vs. 0.14 ± 0.05 μm; $P < 0.01$). In group 2, there were some differences with regard to the mode of rapamycin application and dosage, i.e. there was a trend towards a lower intimal proliferation in animals receiving systemic RAD per os, especially in the high dosage. Animals treated with the high dosage RAD per os showed a significantly lower intimal thickening when compared with control animals at 2 months (0.04 ± 0.02 vs. 0.1 ± 0.08; $P < 0.05$). In contrast, the animals of group 2c (RPM graft soaking) showed no beneficial effect on intimal proliferation. After 2 months, the animals had a similar intimal proliferation to the controls. Intimal hyperplasia was more pronounced at the proximal anastomosis, followed by the distal anastomosis and the middle of the prosthesis (Table 2; Fig. 3A,B).

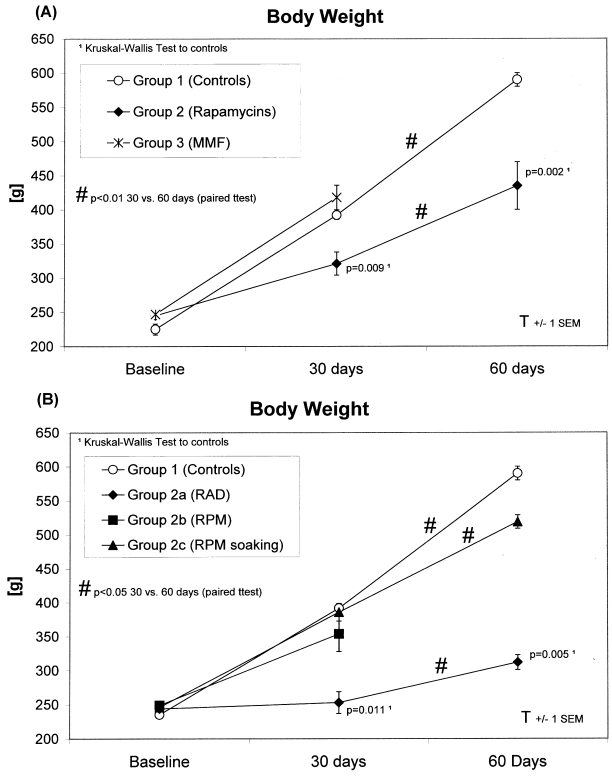

Fig. 1. Body weight changes. (A) Body weight at baseline after 30 and 60 days: animals of group 1 (controls) and group 3 (MMF) show a similar normal weight increase during the first and second months, respectively. Animals of group 2 (rapamycins) show a significantly lower weight increase at 1 and 2 months compared with controls. (B) Body weight after 30 and 60 days for group 2 (rapamycins) compared with controls. Group 2c (RPM soaking) shows a similar weight increase as the controls up to 1 month. Group 2b (RPM) has a normal weight increase at 1 month, whereas group 2a (RAD) shows a significantly reduced weight increase at 30 ($P = 0.01$) and 60 days ($P = 0.005$).

Intimal hyperplasia, thrombus formation and cellular ingrowth scores were assessed by using a score from 0 to 3 (0, none; 3, severe). The results of the intimal hyperplasia score were similar to the measured intimal hyperplasia thicknesses (Table 2). There was a trend towards more thrombus formation on the inner surface of the graft in the animals of group 2a which were treated with oral RAD. In addition, grafts treated with rapamycins induced more thrombus formation (group 2c; Fig. 4A,B). Cellular ingrowth was similar to intimal hyperplasia formation and showed some trend towards a lesser cellular ingrowth in animals with high dose immunosuppression.

4. Discussion

Intimal hyperplasia and graft occlusion are the major limitations for small caliber synthetic vascular grafts. Therefore, veins and, more recently, arteries have been

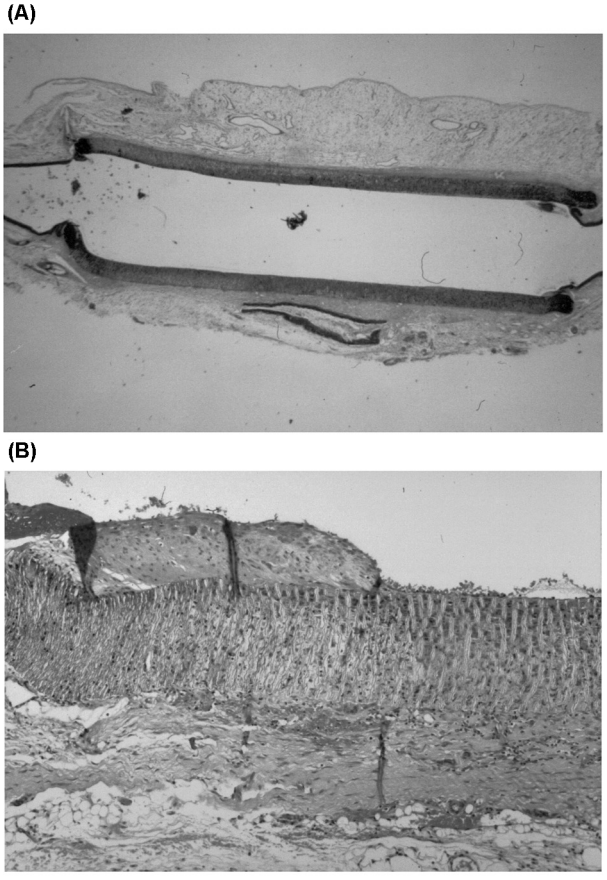

Fig. 2. Histological sections of the ePTFE graft anastomosed to the infrarenal aorta (hematoxylin and eosin). (A) Shows very little reaction and a 2–3 layer thick intimal hyperplasia proliferation in the vicinity of the proximal anastomosis. The rest of the prosthesis is free of pseudo-intima (group 2A at 1 month). (B) Proximal anastomosis with severe intimal hyperplasia reaction on the endoluminal side (group 1, 60 days).

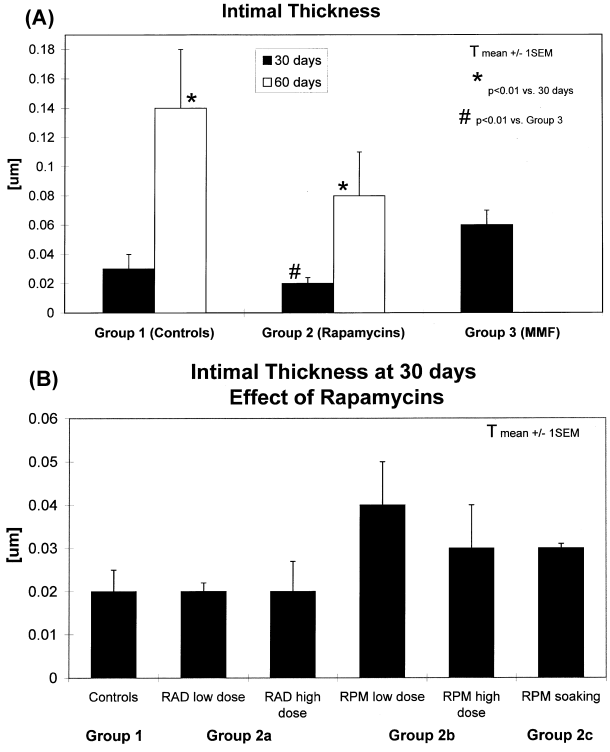

Fig. 3. Intimal thickness. (A) Results of intimal thickness at 30 and 60 days for groups 1, 2 and 3. There is a significant increase in intimal thickness between animals surviving 30 and 60 days (groups 1 and 2; $P < 0.01$). Group 3 shows a significantly higher ($P < 0.01$) value than the animals of group 2 after 30 days ($P < 0.01$). (B) The subgroups of rapamycin treated animals after 1 month in comparison with controls at 1 month are shown. Group 2a (RAD) shows a trend towards less intimal thickness than 2b or 2c.

used for cardiovascular bypass surgery, but a limited availability of graft material has led to the use of complex anastomoses in bypass techniques, such as the use of gastroepiploic, radial and mammary arteries.

The need for synthetic graft material for small bypass grafts is increasing dramatically, especially for the second

Table 2
Results after 2 months

Group		$n = 54$	Application (mg/kg BW)	Survival (days)	Intimal hyperplasia (0–3)			P[d]
					Proximal anastomosis	Mid-prosthesis	Distal anastomosis	
Group 1 (controls)		5	None	30 ± 0	1 ± 0	0 ± 0	0 ± 0	0.000
		7	None	61 ± 0	2 ± 0.4	0.4 ± 0.2	0.8 ± 0.4	0.089
Group 2 (rapamycins)	2a	4	p.o./(1.5)[a]	31 ± 0	1 ± 0	0 ± 0	0.3 ± 0.3	0.029
		6	p.o./(3.0)[a]	27 ± 5	0.5 ± 0.3	0 ± 0	0.3 ± 0.2	0.371
		4	p.o./(3.0)[a]	56 ± 1	1.3 ± 0.5	0 ± 0	0.8 ± 0.3	0.196
	2b	6	i.p./(1.5)[b]	30 ± 0	1.5 ± 0.3	0 ± 0	0.2 ± 0.2	0.001
		6	i.p./(3.0)[b]	28 ± 1	0.8 ± 0.3	0.2 ± 0.2	0.3 ± 0.2	0.102
	2c	5	Soaking[b]	32 ± 1	1 ± 0	0 ± 0	0.4 ± 0.2	0.035
		6	Soaking[b]	62 ± 2	1.5 ± 0.3	0.7 ± 0.2	1.2 ± 0.2	0.233
Group 3 (MMF)		5	p.o./(40)[c]	31 ± 0	1.4 ± 0.4	0 ± 0	0.4 ± 0.2	0.071

[a] RAD Novartis.
[b] Rapamycin Wyeth.
[c] MMF.
[d] P, paired t-test proximal vs. distal anastomosis.

or third bypass operation or for patients with inadequate autologous graft material. The purpose of the present study was to assess the effect of immunosuppressive drugs for the prevention of neointimal hyperplasia in small vascular synthetic grafts.

The results were assessed at 30 and 60 days, since it has been shown in rat models that the strongest mediator and growth-factor activity happens during the first 2–3 weeks [13].

Intimal proliferation increased with time and was more pronounced at the anastomoses than in the center of the synthetic vascular grafts (Table 2). Rapamycin treated grafts showed less intimal proliferation than control grafts (>0.05), but due to the large variations, this difference did not reach statistical significance. No effect was seen with MFF treated rats when compared with controls (Fig. 3A). Due to the high dose, rapamycins were associated with weight loss and signs of toxicity (diarrhea, loss of hair and wound healing problems). However, no clear effect on patency was found. There was an increased thrombus formation, especially in the high dose rapamycin groups ($P < 0.01$; Fig. 4B). This is probably due to the diminished endothelialization provoked by the high dose of immunosuppression [14–16].

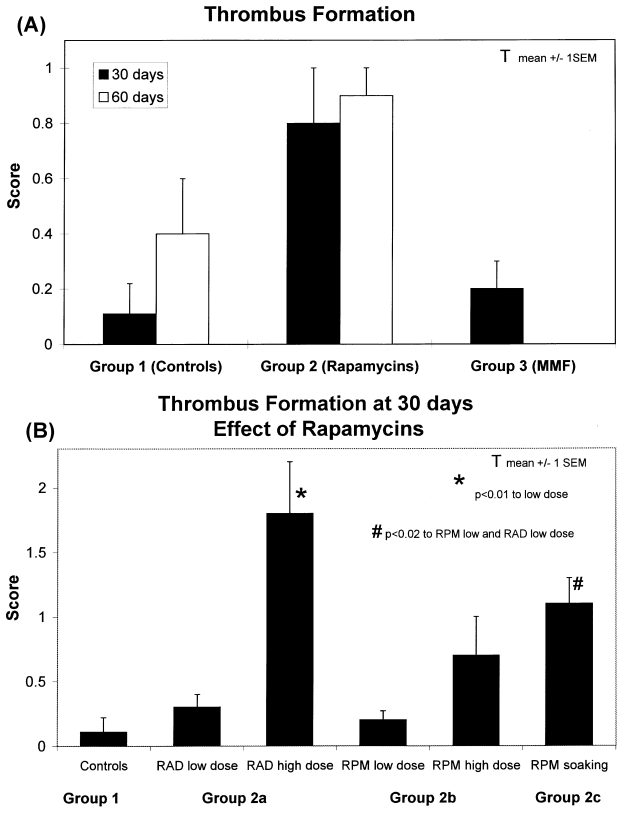

Fig. 4. Thrombus formation score (0–3: 0, none; 3, severe). (A) Animals of group 2 show a markedly higher thrombus formation score than the control animals. (B) There is a significant increase of thrombus formation on rapamycin treated grafts (RPM soaking; group 2c) compared with low dose rapamycins, and a significant increase in animals treated with high dose rapamycins (group 2a; $P = 0.01$).

In conclusion, high dose immunosuppression reduces mildly intimal proliferation, especially at the anastomosis in synthetic vascular grafts in the rat. This beneficial effect is linked with increased drug toxicity. Thus, these side-effects and the lack of a significant reduction in intimal hyperplasia limit the use of systemically administered immunosuppressive drugs for the prevention of intimal hyperplasia and graft occlusion in small caliber synthetic vascular grafts.

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Appendix A. Conference discussion

Dr M. Murtra (*Barcelona, Spain*): Could I ask if all animals survived the experiment, and if you have any controls after performing surgery to make sure that all grafts were patent? Did you have 100% survival of all animals, because it's a pretty tough operation?

Dr Walpoth: The randomization was done after the surgery. So the animals who would have a technical problem would be replaced. So we started off with six animals in each group, and during the course, some animals have been euthanized, especially the animal with severe side-effects. That's why the groups had different numbers of animals.

Dr J.R.L. Hamilton (*Newcastle-upon-Tyne, UK*): Why did you choose these drugs particularly?

Dr Walpoth: Because it has been shown that mainly rapamycin has the strongest inhibition on smooth muscle cell and endothelial cell proliferation.

Dr Murtra: Do you think in the future there will be any clinical application? And if so, for how long would you recommend to keep the treatment – 3 months, 6 months?

Dr Walpoth: As I said in my conclusion, I don't think it's the way to go clinically. Most reports in the literature have been using allotransplanted grafts requiring immunosuppression anyhow, and then they have shown that there is a significant reduction of intimal hyperplasia.