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Effects of angiopeptin on transplant arteriosclerosis in the rat

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Abstract The influence of the somatostatin analogue angiopeptin on transplant arteriosclerosis was investigated using two aortic transplantation rat models. One was characterized by ischemia/reperfusion-induced changes in syngeneic transplants while immunologically induced changes dominated in the other allogeneic model. Angiopeptin, 100 μg/kg per day, was administered continuously until the sacrifice of the rats after 8 weeks. No additional immunosuppression was used in either model. An image analysis system was used to quantify the intimal and medial thicknesses of the grafts. In the syngeneic grafts, the intimal thickness was less than 50 % of that of control grafts ($P < 0.05$), but no difference was seen in the allogeneic model. The expression of selected cells, TGF-βs, and PDGF and PDGF α-receptors was detected immunohistochemically and displayed a similar picture in control and angiopeptin-treated grafts in both models. We conclude that angiopeptin has no clear immunosuppressive properties but may counteract ischemia-induced transplant arteriosclerosis.

Key words Arteriosclerosis, angiopeptin, rat · Angiopeptin, arteriosclerosis, rat · Aorta transplantation, arteriosclerosis

Introduction

Chronic vascular rejection (CVR) is characterized by excessive intimal thickening of the vessel wall and is a major cause of diminished long-term survival of transplanted organs [25]. Because of the prominent vascular changes, CVR is often called transplant arteriosclerosis. The factors responsible for the initiation and progression of CVR are not completely understood. However, CVR can be induced and influenced by an immunological response to incompatible antigens [20], by ischemia/reperfusion injury [5, 38, 40], and by infections such as cytomegalovirus [16].

Several therapeutic substances, including peptides, have been used in attempts to inhibit or modify transplant arteriosclerosis. One of these synthetic peptides is angiopeptin. It is a stable and long-acting octapeptide, an analogue to somatostatin, with the amino acid sequence: D-Nal-Cys-Tyr-D-Trp-Lys-Val-Cys-Thr-NH_2. It has been shown that angiopeptin has an inhibitory effect on the proliferation of smooth muscle cells (SMCs) in rat carotid artery explants [36] and that it also inhibits its DNA synthesis in SMCs after balloon angioplasty of the aorta in rabbits [2]. The postulated inhibitory effects of angiopeptin on immune cells are poorly investigated in contrast to the related peptide somatostatin. It is clear that somatostatin inhibits the proliferation of

lymphocytes and their natural killer activity [1, 26]. It also inhibits the growth of malignant cells in both established cell lines [27, 34] and primary cultures [4, 24, 35]. Binding sites for somatostatin have been demonstrated in both lymphocytes and monocytes [3, 31]. Definite information on the immunomodulatory effect of angiopeptin is not available [13]. In accelerated transplant arteriosclerosis, it is clear that angiopeptin has an inhibitory effect on myointimal proliferation [13], but the mechanisms is only partially known. In the development of CVR and spontaneous arteriosclerosis, several growth factors and their receptors, e.g., platelet-derived growth factor (PDGF) and its receptors, may play an important role [9, 28]. It is known that macrophages can produce PDGF and, thus, stimulate the proliferation of intimal cells [29]. Furthermore, the expression of PDGF receptors may be modified or upregulated by a variety of factors, e.g., transforming growth factor-β (TGF-β) and oxidized low-density lipoprotein cholesterol [33]. This suggests an attractive hypothesis, namely, that the inhibitory effect of angiopeptin on myointimal proliferation is caused by the synthesis of growth factors [7] and cytokines secreted by activated lymphocytes and monocytes. Since TGF-β inhibits proliferation of mesenchymal as well as immune cells and stimulates angiogenesis and the production of matrix proteins [30], it may be of particular interest in a pathogenetic mechanism of transplant arteriosclerosis.

CVR is considered to be caused by both immunological and nonimmunological factors [25]. In the present study, two models were used to induce morphological changes similar to CVR. In a syngeneic graft model, CVR-like changes were induced by ischemia/reperfusion injury, whereas in an allogeneic model, CVR-like changes were induced by a combination of immunological mechanisms and ischemia/reperfusion damage. Hence, it was possible to separate the influence of immunological factors from that of nonimmunological factors. The purpose of this study was to analyze and characterize the effects of angiopeptin on aortic transplant grafts with regard to the intimal and medial changes, infiltration of inflammatory and noninflammatory cells, and the production of TGF-βs and PDGF.

Materials and methods

Animals

Male PVG ($RT1^c$) and DA ($RT1^a$) rats (Møllegaard, Skensved, Denmark) aged 3–4 months and weighing 120–200 g were used. The rats were negative by serological tests for the most common viruses such as Hantaan, Kilham, reo 3, corona-virus, Sendai, and Theiler viruses, and for mycoplasma pulmonis. The allogeneic transplantation were performed from DA donors to PVG recipients, and the syngeneic transplantation from PVG-to-PVG rats. Two weeks prior to surgery, the rats were allowed to get settled and received food and water ad libitum. One week before transplantation they were fed a 0.5 % cholesterol pellet diet (Analy-Cen, Lidköping, Sweden).

Transplantation of aorta

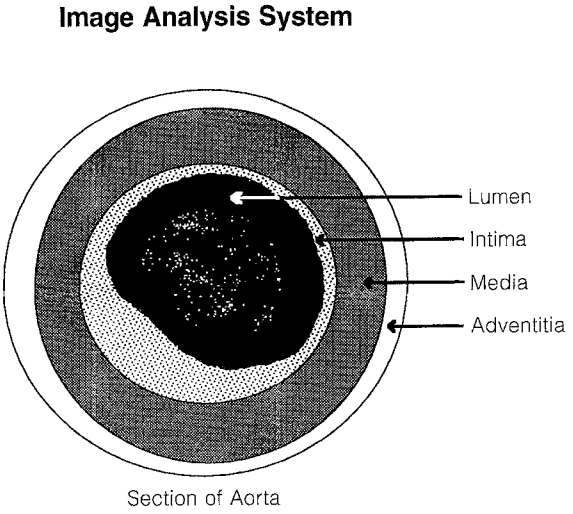
Recipient rats were anesthetized with an intraperitoneal injection of a mixture of chloral hydrate, 195.5 mg/kg, and pentobarbital, 44.6 mg/kg of body weight (Eqviticin, Apoteksbolaget, Umeå, Sweden) and donor rats with thiobarbital, 120 mg/kg body weight (Inactin, Byk Gulden, Konstanz, Germany). The abdominal aorta of each recipient rat was freed just below the renal arteries to the bifurcation of the abdominal aorta. Clamps were then placed on the abdominal aorta at these sites before excising a 10 to 15-mm-long portion for transplantation from each donor rat, as described previously [20]. Portions of allogeneic grafts were stored at 4 °C for 1 h in a histidine-buffered preservation solution (Frödin solution) [14] and those of syngeneic grafts for 4 h. Each graft was immediately implanted orthotopically and was anastomosed from the renal arteries to the caudal abdominal aorta using a 9–0 nylon suture (S & T Neuhausen am Rheinfall, Switzerland). The aortic clamps were then removed and patency of the new vascular connection was confirmed. After 8 weeks, the rats were anesthetized and the grafts excised. From each recipient rat, 10 to 15-mm-long nontransplanted portions of the thoracic and the abdominal aorta were harvested to serve as negative controls for image analysis and immunohistochemical stains. Each transplanted and nontransplanted portion was divided into two parts, one of which was frozen in a mixture of cold isopentane and dry ice and stored at −70 °C until processed for immunohistochemistry. The other part was fixed in 4 % buffered formaldehyde at room temperature and embedded in paraffin. These sections were then stained with van Gieson and Mayer's hematoxylin and eosin for histological and comparative studies.

Treatment procedure

Angiopeptin is a nontoxic peptide that can be administered either as a subcutaneous or as an intravenous infusion. It is absorbed quickly into the bloodstream, reaching a peak plasma concentration within 10–15 min after administration [6]. After transplantation of the grafts, immediate treatment with angiopeptin is essential since SMC migration and proliferation occurs within 8 h of vascular injury [11]. For these reasons, infusion of angiopeptin, 100 µg/kg per day, (Henri Beaufour Institute, Washington, D.C., USA), was administered continuously to recipient rats by mini-osmotic pumps (Alzet-model 2ML4, Alza Corporation, Palo Alto, Calif., USA) located subcutaneously in the dorsal region (administration 1). In addition, 8 of each 16 allogeneic and 16 syngeneic grafts were immersed in angiopeptin (50 µg/ml) in the preservation solution before transplantation. Thereafter, rats received angiopeptin treatment by mini-osmotic pumps, as described above (administration 2). The pumps were replaced under anesthesia after 4 weeks.

Image analysis

Paraffin-embedded midgraft sections stained with van Gieson and Mayer's hematoxylin and eosin were used. Image analysis was performed blindly. The areas of the lumen, intima, and media were quantified using the IMTEC image analysis system (Image Technology, Uppsala, Sweden). The sections were analyzed at a magnification × 40 using a conventional microscope. The image of the aorta was transferred to a screen and the vessel examined was cir-



$$Q_{int} = \frac{\text{Intima}}{\text{Lumen} + \text{Intima}} \times 100$$

$$Q_{med} = \frac{\text{Media}}{\text{Lumen} + \text{Intima} + \text{Media}} \times 100$$

Fig. 1 Vascular layers outlined for image analysis system

cumscribed manually. The areas that the vessels occupied were quantified as follows: (i) lumen alone, (ii) lumen + intima, and (iii) lumen + intima + media. From these measurements, the thickness of the intima and media was quantified. The intima quotient (Q_{int}) and media quotient (Q_{med}) were calculated from the image analysis data. Q_{int} indicates the relatives thickness (%) of intima [Q_{int} = intima/(lumen + intima) × 100], and Q_{med} indicates the relative thickness (%) of media [Q_{med} = media/(lumen + intima + media) × 100] (Fig. 1). Therefore, a Q_{int} value of 0.0 % indicates no intimal thickening and a Q_{int} value of 100.0 % indicates a total occlusion of the lumen by thickened intima.

Immunohistochemical analysis

Antibodies

The following monoclonal antibodies (concentration in parentheses) were used: ED2 (1 : 1600) recognizing macrophages, ED1 (1 : 3200) recognizing monocytes, macrophages and dendritic cells (Serotec Lab. Oxford, UK), W3/25 (1 : 300) directed against $CD4^+$ T lymphocytes [39], Ox8 (1 : 1000) directed against $CD8^+$ T lymphocytes [39], Ox6 (1 : 2000) detecting major histocompatibility (MHC) class II antigen [19], antihuman coagulation factor VIII (1 : 12000), a marker of endothelial cells (Immunsystem, Uppsala, Sweden), and anti-α smooth muscle actin (1 : 1400) labelling α-actin containing SMCs (Dakopatts Lab, Copenhagen, Denmark).

Antibodies detecting synthetic peptides corresponding to the C-terminal regions of latency-associated peptides (LAP) of two human TGF-β isoforms, TGF-β_1 and -β_3 precursors were used [37]. Antibodies Ab 96 (1 : 10) and Ab 95 (1 : 10) detect anti-β_1-LAP and anti-β_3-LAP. Anti-β_1-LAP is directed against amino acids 262–272 of β_1-LAP and anti-β_3-LAP against amino acids 282–298 of β_3-LAP. Antibody Ab 39 (1 : 1200) was raised against latent TGF-β-binding protein (LTBP), which was purified from human platelets [23]. Two antibodies, PGF-007 (1 : 1000; Mochida, Tokyo, Japan) and PDGFR-7 (1 : 50) [8], were used to detect PDGF and its α-receptors. The monoclonal antibody PGF-007 is generated against a 25 amino acid peptide derived from a region near the COOH terminus of the PDGF-B chain. The antibody recognized PDGF-AB and -BB but not PDGF-AA in immunoprecipitation experiments [32]. In acetone-fixed cells transfected with PDGF-A or -B chains, the antibody PGF-007 recognizes PDGF-AA and -BB. The antibody designated PDGFR-7 was produced against a synthetic peptide of amino acids 1066–1084 of the C-terminal region of human PDGF α-receptor and binds to PDGF α- but not to the β-receptor. The antibody can be used to detect the corresponding protein in both species since the C-terminal region has 100 % amino acid homology in humans and rats [8].

Antibody E11 (1 : 100), directed against the calcium-binding receptor of human parathyroid cells, was used as an irrelevant control [15].

Immunohistochemical procedure

Five-micron cryostat sections of the midgraft were fixed in 100 % cold acetone rinsed in 0.1 M phosphate-buffered saline solution (PBS) and immunohistochemically stained as follows. Sections of the grafts were incubated at +4 °C overnight with the primary antibody diluted in PBS containing 0.1 % bovine serum albumin (BSA). Biotinylated anti-mouse IgG (1 : 80; Vector Lab, Burlinghame, Calif., USA) in PBS containing 0.1 % BSA, 2 % rat serum, and 2 % horse serum was subsequently applied. The biotin avidin amplification system was used as recommended by the manufacturer. The sections were counterstained with Mayer's hematoxylin, mounted, and examined for antigen expressions.

Evaluation of immunohistochemistry

The staining intensity of the intima and media was scored on a scale ranging from 0, indicating no expression, to 3, indicating the most intense expression of antigens. Scoring was done independently by two investigators. In the event of disagreement, the sections were re-examined and a common view obtained. The final semiquantitative evaluation is presented as an average [mean ± standard error of the mean (SEM)] of immunohistochemical staining (Table 2). The staining intensity was evaluated regardless of the thickness of the intima and media.

Statistical analysis

The allogeneic and syngeneic control and angiopeptin-treated transplants were compared using the nonparametric Mann-Whitney U-test. Its primary use is to compare irregularly distributed and independent data. The unpaired *t*-test was used to compare the thickness of the media in allogeneic and syngeneic control groups and in the corresponding nontransplanted portions of the abdominal aorta of recipient rats.

The Mann-Whitney U-test was also used to compare the semiquantitative immunohistochemical stainings of control versus angiopeptin-treated syngeneic and allogeneic grafts.

Results

Six angiopeptin-treated rats and two control rats died due to surgical complications.

Table 1 Intimal and medial thickness of aortic grafts in controls and rats treated with angiopeptin, 100 μg/kg per day

		n	% Intimal thickness (median and range values)	% Medial thickness (median and range values)
Allogeneic	Control	15	32.0 (r = 1.2–54.5)** [c]	22.0 (r = 18.0–43.3)** [d]
	Angiopeptin	16	40.0 (r = 3.9–61.2)	24.0 (r = 14.7–51.0)
	Administration 1[a]	8	38.1 (r = 5.1–61.0)	23.8 (r = 14.7–29.1)
	Administration 2[b]	8	41.5 (r = 3.9–61.2)	26.1 (r = 20.0–51.0)
Syngeneic	Control	10	20.0 (r = 0.0–38.2)* [e]	36.0 (r = 30.4–42.5)
	Angiopeptin	16	5.3 (r = 0.0–32.3)	41.3 (r = 26.7–60.9)
	Administration 1[a]	8	5.1 (r = 0.0–20.0)	44.0 (r = 26.7–60.9)
	Administration 2[b]	8	6.5 (r = 0.0–32.3)	38.9 (r = 28.0–50.8)

[a] Angiopeptin treatment by mini-osmotic pumps only
[b] Angiopeptin treatment in preservation solution and by mini-osmotic pumps
[c] significantly greater than in the syngeneic control grafts
[d] significantly less than in the syngeneic control grafts
[e] significantly greater than in the syngeneic treated grafts with angiopeptin

Morphometric measurements

The median values and ranges of the intimal and medial thickness of the syngeneic and allogeneic control and angiopeptin-treated grafts are shown in Table 1. Both administration 1 and 2 of angiopeptin in the allogeneic and syngeneic combinations were considered and presented as one group since they have a similar effect on the intimal and medial thickness.

The thickness of the intima in the allogeneic control grafts was significantly greater than in the syngeneic control grafts ($P < 0.001$). The medial thickness in the allogeneic control grafts was significantly less than in the syngeneic control grafts ($P < 0.001$).

The intima was not thickened in nontransplanted portions of the abdominal and thoracic aorta in the syngeneic and allogeneic recipient rats.

Allogeneic grafts

Q_{int} was 32.0 % in control grafts ($n = 15$) and 40.0 % in angiopeptin-treated grafts ($n = 16$). They did not differ statistically ($P = 0.36$). In administration 1 and 2 of angiopeptin, Q_{int} was 38.1 % and 41.5 %, respectively ($P = 0.76$).

Q_{med} was 22.0 % in control allogeneic grafts ($n = 15$), while Q_{med} in the nontransplanted abdominal aortic portions ($n = 6$) was 46.3 % ($P = 0.001$). The Q_{med} value was 24.0 % in angiopeptin-treated grafts. The thickness of the media were similar in control and angiopeptin-treated grafts ($P = 0.22$). In administration 1 and 2 of angiopeptin, Q_{med} was 23.8 % and 26.1 %, respectively ($P = 0.62$).

Syngeneic grafts

The thickness of the intima was less in the syngeneic grafts of rats treated with angiopeptin ($n = 16$) with a Q_{int} of 5.3 % compared with control grafts ($n = 10$) with a Q_{int} of 20.0 % (Fig. 2). In the treated syngeneic grafts, the thickness of the intima was reduced by over 50 % ($P < 0.05$). Q_{int}, in administration 1 and 2 of angiopeptin, was 5.1 % and 6.5 %, respectively. They did not differ statistically ($P = 0.76$). Thickening of the intima did not occur at all in 5 of the 16 treated syngeneic grafts nor in 1 of the 9 control syngeneic grafts.

The Q_{med} values were 36.0 % and 43 %, respectively, in the syngeneic control grafts ($n = 10$) and nontransplanted portions of abdominal aorta of recipient rats ($n = 14$). Statistically, the values were similar ($P = 0.36$). The Q_{med} value was 41.3 % after angiopeptin treatment. The thickness of the media of these grafts was not different from that of control grafts ($P = 0.41$). In administration 1 and 2 of angiopeptin, Q_{med} was 44.0 % and 38.9 %, respectively ($P = 0.62$).

Immunohistochemical observations

The results of the immunohistochemical investigations of the intima and media in control and angiopeptin-treated grafts are shown in Table 2. The values of the semiquantitative evaluations of all immunohistochemical stainings were statistically similar in control versus angiopeptin-treated allogeneic and syngeneic grafts.

Allogeneic grafts

The entire aortic wall of control and angiopeptin-treated allogeneic grafts was infiltrated by macrophages and monocytes and fewer numbers of $CD4^+$ and $CD8^+$ T lymphocytes. The expression of MHC class II antigen, in control and allogeneic grafts treated with angiopeptin, was pronounced in the adventitia (data not shown), less in the media, and least in the intima. The intima in control and angiopeptin-treated grafts contained many α-actin-positive SMCs. The media of treated and control grafts consisted mainly of α-actin containing SMCs.

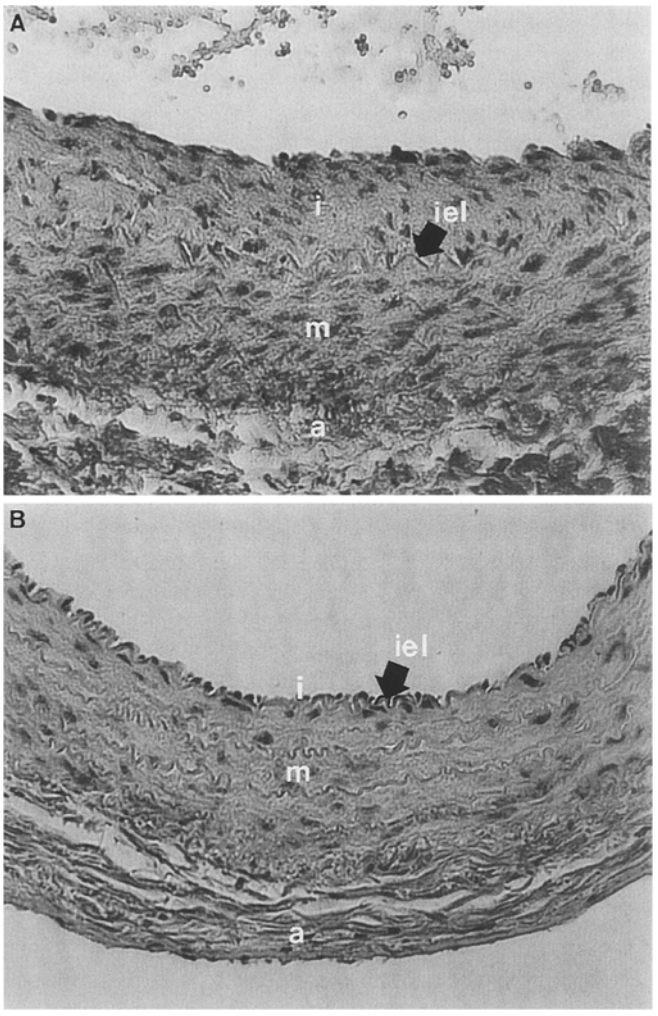


Fig. 2 A, B Photomicrographic sections of syngeneic grafts ischemic for 4 h seen 8 weeks after transplantation, van Gieson's stain, ×250: **A** control graft with pronounced intimal thickness; **B** reduced intimal thickness after angiopeptin treatment (*i* intima, *m* media, *a* adventitia, *iel* internal elastic lamina)

Occasionally, T lymphocytes, monocytes, and macrophages occurred in the media of allogeneic grafts. Monocytes, macrophages, and T lymphocytes predominated in the adventitia of control and treated allogeneic grafts.

Syngeneic grafts

In the intimal thickening of the control and angiopeptin-treated syngeneic grafts, an infiltration of α-actin containing SMCs, monocytes, and macrophages was seen. In some angiopeptin-treated grafts with less marked intimal thickening, fewer SMCs were present, but the staining intensity of α-actin was similar to that in those control rats with increased intimal thickening. Expression of MHC class II antigen was not detected in the media of angiopeptin-treated syngeneic grafts; however, some expression was seen in the intima and adventitia. Most SMCs in control and angiopeptin-treated grafts were seen in the media, but a few were also present within the intima. Few macrophages, monocytes, and $CD4^+$ and $CD8^+$ T lymphocytes existed in the media of control and treated grafts, and in some cases they were absent. Interruption of the internal elastic lamina was unusual in treated grafts but frequent in control grafts. In the adventitia of the control and treated grafts, macrophages, monocytes, and T lymphocytes predominated.

The expression of PDGF-B chain, PDGF-α receptors, TGF-β_3, and LTBP was similar in both controls and angiopeptin-treated syngeneic grafts. Only in the case of TGF-β_1 was there a nonsignificant, slight reduction in the staining intensity of intima and media of angiopeptin-treated syngeneic grafts.

Discussion

The modulating effect of angiopeptin on accelerated transplant arteriosclerosis was studied in allogeneic and syngeneic aortic transplants in the rat. Furthermore, the expression of various inflammatory and noninflammatory cells and their mediators were investigated. We found that angiopeptin partially inhibited intimal proliferation in the syngeneic grafts but not in the corresponding allogeneic model. Secondly, immunohistochemical stainings of selected inflammatory and noninflammatory cells and their mediators remained nearly unaltered after treatment with angiopeptin in both models.

In the present study, a continuous administration of angiopeptin at a dose of 100 μg/kg per day was selected. The rationale behind this was based upon findings of previous investigations. Continuously administered doses ranging between 20 and 100 μg/kg per day have proven to be effective in inhibiting vascular myointimal proliferation, either following endothelial injury [17] or in the development of transplant atherosclerosis [10, 12, 22]. However, doses below 100 μg/kg per day did not inhibit the incorporation of thymidine at the site of carotid artery injury [17]. In addition, we tested another mode of angiopeptin administration. Angiopeptin was added to the preservation solution, thus allowing angiopeptin to affect grafts immediately after their excision and also during the ischemic period. This was tested in half of the grafts. However, this procedure had no superior effect on intimal proliferation in comparison to rats receiving angiopeptin by mini-osmotic pumps alone. Therefore, all angiopeptin-treated rats were considered as one group.

In our study, angiopeptin did not inhibit intimal thickening in the allogeneic grafts. This lack of effect could be explained by the fact that angiopeptin may have no immunomodulatory effect on immune cells as

Table 2 Immunohistochemical stainings of aortic grafts

A. Intima

• Antibodies	Syngeneic		Allogeneic	
	Control $n = 15$	Angiopeptin $n = 16$	Control $n = 10$	Angiopeptin $n = 16$
Macrophages • ED2	0.13 ± 0.13	0.00 ± 0.00	0.08 ± 0.08	0.14 ± 0.09
Monocytes, macrophages • ED1	0.38 ± 0.24	0.38 ± 0.08	1.25 ± 0.18	2.00 ± 0.34
$CD4^+$ T lymphocytes • W3/25	0.25 ± 0.25	0.25 ± 0.13	0.33 ± 0.21	0.67 ± 0.21
$CD8^+$ T lymphocytes • Ox8	0.25 ± 0.25	0.62 ± 0.62	0.42 ± 0.20	0.93 ± 0.20
MHC class II antigen • Ox6	0.75 ± 0.43	0.31 ± 0.09	1.75 ± 0.21	1.50 ± 0.50
SMCs • α-actin	1.75 ± 0.75	2.13 ± 0.30	2.90 ± 0.10	2.20 ± 0.46
TGF-β_1 • Ab96	0.62 ± 0.24	0.17 ± 0.17		
TGF-β_3 • Ab95	0.00 ± 0.00	0.00 ± 0.00		
LTBP • Ab39	0.13 ± 0.13	0.28 ± 0.18		
PDGF-B chain • PGF-007	0.38 ± 0.18	0.30 ± 0.20		
PDGF-α receptors • PDGFR-7	0.00 ± 0.00	0.13 ± 0.13		

Mean ± SEM values of semiquantitative evaluations, scale; 0 ➧ 3

B. Media

• Antibodies	Syngeneic		Allogeneic	
	Control $n = 15$	Angiopeptin $n = 16$	Control $n = 10$	Angiopeptin $n = 16$
Macrophages • ED2	0.25 ± 0.14	0.00 ± 0.00	0.08 ± 0.08	0.00 ± 0.00
Monocytes, macrophages • ED1	0.63 ± 0.32	0.31 ± 0.09	1.08 ± 0.42	1.83 ± 0.54
$CD4^+$ T lymphocytes • W3/25	0.25 ± 0.25	0.25 ± 0.94	0.83 ± 0.17	0.67 ± 0.17
$CD8^+$ T lymphocytes • Ox8	0.38 ± 0.24	0.06 ± 0.06	0.25 ± 0.11	0.93 ± 0.20
MHC class II antigen • Ox6	0.50 ± 0.50	0.06 ± 0.06	2.30 ± 0.20	2.00 ± 0.32
SMCs • α-actin	2.38 ± 0.47	2.38 ± 0.18	0.30 ± 0.12	0.67 ± 0.30
TGF-β_1 • Ab96	0.94 ± 0.20	0.58 ± 0.12		
TGF-β_3 • Ab95	0.00 ± 0.00	0.00 ± 0.00		
LTBP • Ab39	0.56 ± 0.13	0.62 ± 0.18		
PDGF-B chain • PGF-007	0.50 ± 0.19	0.25 ± 0.17		
PDGF-α receptors • PDGFR-7	0.21 ± 0.15	0.31 ± 0.09		

Mean ± SEM values of semiquantitative evaluations, scale; 0 ➧ 3

no additional immunosuppressive regimen was used. Thus, angiopeptin could not reverse the intimal thickening caused by the combination of immunological and inflammatory mechanisms on the driving proliferative forces in the allogeneic grafts. In other models of allogeneic transplantation, angiopeptin was reported to be beneficial. Angiopeptin treatment reduced the development of transplant atherosclerosis in coronary arteries in an allogeneic heart transplantation model both in rabbits [12] and in rats [10]. In both of these models, however, the animals were treated with cyclosporin A immunosuppression. In contrast, Mennander et al. found that angiopeptin reduced both the number of SMCs and the intimal thickening without immunosuppression in an allogeneic aortic graft model. Compared with our results this difference may be explained by the different inbred rat strains used [from WF (AGB^2-$RT1^u$) donors to DA (AGB^4-$RT1^a$) recipients]. However, in their model, the beneficial effect of angiopeptin did not last longer than 3 months in spite of continuous medication, thus leading to final results comparable to ours [22]. We have observed that the media is thinner in the allogeneic grafts than in the syngeneic grafts. The reason for this, as observed by other investigators as well, is probably migration of SMCs [11, 17, 25] and/or medial necrosis due to cytotoxic effects of cell mediators in the allogeneic immunological reaction [20, 21].

The CVR-like changes in the syngeneic grafts were induced by the ischemia/reperfusion injury in our model. We were able to demonstrate a beneficial effect of angiopeptin in this syngeneic group. Ischemia/reperfusion injury in grafts has been recognized as crucial for graft outcome [5]. The relevance of this type of injury to the long-term survival of the transplant has recently been studied and the results are still controversial [18, 40]. Previously, with our model, however, a positive correlation between the duration of ischemia and intimal thickening was noted in the syngeneic grafts but not in the allogeneic grafts [38]. Based on our earlier results, the syngeneic grafts were exposed to a prolonged ischemia of 4 h. This syngeneic model represents non-immunologically induced transplant arteriosclerosis. Angiopeptin may have its main inhibitory effect on cells other than immune cells, such as SMCs and/or endothelial cells. It has been proposed that endothelial cells secrete factors that inhibit SMC proliferation in a paracrine fashion. In the absence of these factors, the SMCs may respond to chemotactic and mitogenic signals. It has been suggested that angiopeptin is functionally related to this "paracrine inhibitor" [17]. Several mechanisms have been proposed to explain how angiopeptin inhibits accelerated arteriosclerosis following vascular injury. These include inhibition of growth factors such as PDGF, insulin-like growth factor-1, and epidermal growth factor, and inhibition of the proliferation and migration of SMCs [17]. In the same aortic transplant model, it was found that the expression of PDGF and TGF-β increases after transplantation (J. Waltenberger et al., unpublished data). The expression of PDGF and TGF-βs was only studied in the syngeneic grafts, since the inhibitory effects of angiopeptin were morphometrically seen only in the syngeneic grafts. We did not observe any differences in the expression of PDGF-B chain, PDGF α-receptors, TGF-β_1, TGF-β_3, or LTBP in control or angiopeptin-treated syngeneic grafts after 8 weeks. However, one cannot exclude the possibility that the expression of these growth factors increased in an earlier phase of transplant arteriosclerosis.

How angiopeptin interferes with myointimal proliferation is not entirely understood. In the ischemia/reperfusion-induced syngeneic transplant model, intimal thickening is reduced by angiopeptin treatment whereas no inhibition was evident in allografts when no immunosuppression was used. Immunosuppressive treatment may be required in allograft recipients for angiopeptin to have an inhibitory effect on intimal thickening. Angiopeptin may counteract ischemia/reperfusion-induced chronic vascular rejection and, thus, benefit the long-term survival of transplanted organs.

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