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Local Administration of Estrogen Inhibits Transplant Arteriosclerosis in Rat Aorta Accelerated by Topical Exposure to IGF-I

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MYOINTIMAL hyperplasia is a major pathogenic factor in the development of transplant arteriosclerosis, which is the limiting factor for long-term graft survival. Several growth factors are reported to affect this process in autocrine and paracrine manners. We hypothesized that insulin-like growth factor-I (IGF-I) promotes the development of transplant arteriosclerosis and is inhibited by estrogen. Here, we studied in a rat orthotopic abdominal aortic allotransplantation model whether locally administered IGF-I accelerates transplant arteriosclerosis (acceleration model), and whether this accelerated transplant arteriosclerosis is inhibited by locally administered estrogen (inhibition model).

MATERIALS AND METHODS

Rats were purchased from Harlan (Indianapolis, Ind) with an average weight of 250 g. They were housed in a facility approved by the American Association for Accreditation of Laboratory Animal Care at 20°C with scheduled 12-hour light cycles. The animals were fed a standard rat chow diet and water ad libitum and acclimatized at least for 1 week before use. All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research, and the *Guide for the Care and Use of Laboratory Animals* prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 85-23, revised 1985).

Acceleration Model

Male Brown Norway rats were anesthetized with 80 mg/kg of ketamine (Fort Dodge, Ia, intraperitoneally) and 10 mg/kg of xylazine (Miles, Kan, intraperitoneally). Heparin (200 U/kg; Upjohn, Kalamazoo, Mich) was injected intravenously. The abdominal aorta between the renal arteries and the bifurcation was dissected and harvested. Dulbeco's modified Eagle's medium (DMEM) containing 0, 200, and 500 ng/mL of IGF-I (Upstate Biotechnology Inc., Lake Placid, NY) was infused in the lumen of the harvested aorta. The luminal pressure was maintained at 80 mm Hg. The aorta was then immersed at 37°C for 30 minutes into 400 μL of DMEM containing the same concentration of IGF-I as the luminal solution. Thereafter, the grafts were exposed to 0, 200 or 500 ng/mL IGF-I and implanted end-to-end in the abdominal aorta of male Lewis rats (250 g) using intermittent 10-0 Nylon sutures (Accurate Corp, Westbury, NY). The recipients were heparinized (200 U/kg) prior to implantation. No immunosuppressant was used.

At 7 days, the recipient rats were anesthetized and heparinized for harvesting of graft and native aortas. ^{3}H-thymidine incorporation was determined in the graft and native aorta ex vivo (4 rats for each of the 3 concentration groups: 0, 200, 500 ng/mL of IGF-I). Six aortic rings (3 mm long) were harvested from the aorta graft and the recipient's native aorta. Three of the rings were from the graft, 2 were from the native abdominal aorta adjacent to both suture lines, and 1 was from the native descending thoracic aorta. The labeled thymidine (2 μCi/ml, Amersham Inc, specific activity 160 Ci/mmol/L; Arlington Heights, Ill) incorporated in these rings was determined in a liquid scintillation counter (1600 TR Liquid Scintillation Analyzer, Packard, Downers Grove, Ill). Protein concentrations were measured by Lowrys method.[1] Thymidine incorporation was calculated and expressed as cpm/μg protein.[2]

At 14 days, a further group of recipient rats were sacrificed under deep anesthesia following heparin injection (7 rats for each of the 3 concentration groups: 0, 200, 500 ng/mL of IGF-I). The aorta was pressure fixated (at 80 mm Hg for 30 minutes, HistoChoice (AMRESCO, Solon, Ohio)) after saline perfusion (at 80 mm Hg for 20 minutes). The grafts as well as the native aorta were harvested and each were cut into 3 segments. These 6 specimens were embedded in paraffin, sectioned horizontally, and stained with hematoxylin and eosin. Intimal thickening (IH) was quantitated using the ratio of intimal area over total vessel area (intima + media) [I/(I + M)]. The morphometry of each area was determined by a computerized morphometric analysis system (Woods Hole Educational Associates, Woods Hole, Mass).

Inhibition Model

The abdominal aorta between the renal arteries and the bifurcation was dissected and harvested from male Brown Norway rats in a same manner as the acceleration model. DMEM containing 0, 0.2, 2.0 or 20 μM (Zero, Low, Midium, High; n = 4 for each concentration group) of 17 beta-estradiol (E_2, Sigma Chemical Co, St. Louis, MO) was infused in the lumen of the harvested aorta. The aorta was then immersed at 4°C for 30 minutes into 400 μL of DMEM. Thereafter, the solution was rinsed off, and another DMEM containing 500 ng/mL of IGF-I was infused in the lumen of

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Table 1. Mitogenic Effect of IGF-I on the Orthotopic Rat Aortic Allografts

IGF-I Exposure Before Operation	^{3}H-Thymidine Incorporation at 7 d (cpm/μg prot)	Intimal Thickening at 14 d
0 ng/mL	40.6 ± 7.6	0.18 ± 0.02
200 ng/mL	78.5 ± 12.3*	0.23 ± 0.03
500 ng/mL	66.9 ± 10.1	0.30 ± 0.03**

Mean ± SEM.
*$P < .05$ (n = 4).
**$P < .005$ vs 0 ng/mL (n = 7).

the aorta, and the aorta was immersed into 400 μL of DMEM at 4°C for another 30 minutes. These aorta grafts were implanted orthotopically in male Lewis rats (250 g) as described above. The implanted graft was wrapped with a biodegradable sodium alginate gel (300 cps, Nacalai Tesque, Kyoto, Japan) containing E_2. The biodegradation was designed to result in delivery of 0, 2.6, 26, or 260 pmol/kg/d (Zero, Low, Midium, High, respectively) of E_2 for 14 days. Immunosuppressant drugs were not employed. At 14 days, 6 specimens were harvested from the recipient, and IH was determined as in the acceleration model.

All data are expressed as mean ± SEM, unless otherwise specified. Differences among the groups were compared by a two-way factorial ANOVA followed by multiple comparison analysis. A P value lower than .05 was considered significant.

RESULTS

Acceleration Model

At 7 days the incorporation of ^{3}H-thymidine in the allograft from the 3 concentration groups (0, 200, 500 ng/mL of IGF-I) was 40.6 ± 7.6, 78.5 ± 12.3, and 66.9 ± 10.1 cpm/μg protein, respectively (Table 1). The thymidine incorporation in the grafts exposed to IGF-I, 200 ng/mL were significantly greater than the thymidine incorporation in the grafts exposed to IGF-I, 0 ng/mL, and the thymidine incorporation in the grafts exposed to IGF-I 500 ng/mL were also greater than that in the grafts exposed to no IGF-I (0 ng/mL).

The degree of intimal hyperplasia at 14 days was dose-dependently increased with the exposure to IGF-I (Table 1). The intimal thickening in the grafts exposed to 500 ng/mL of IGF-I was significantly greater than that in the grafts exposed to no IGF-I.

Inhibition Model

The intimal hyperplasia in the grafts from the four zero, low (Lo), midium (Mid), and high (Hi) dose estrogen groups at 14 days was 0.43 ± 0.03, 0.41 ± 0.09, 0.37 ± 0.10, and 0.29 ± 0.05, respectively (Table 2). The degree of intimal hyperplasia in the high dose estrogen group (Hi) was significantly lower than that in Zero group ($P < .005$).

DISCUSSION

In this study, we demonstrated that orthotopic rat aortic allografts develop significant myointimal hyperplasia within 14 days and a significant cell proliferation in the graft vascular wall at 7 days posttransplantation. Further, locally administered IGF-I accelerated the transplant arteriosclerosis. In the inhibition model, we demonstrated that this IGF-I-induced acceleration of transplant arteriosclerosis was abolished by locally delivered estrogen.

IGF-I increases the proliferative ratio of cultured smooth muscle cells (SMCs) dose-dependently,[3,4] and antisense IGF-I receptor oligonucleotide suppresses cell proliferation and DNA synthesis of rat aortic SMCs and leaves SMC less responsive to PDGF and FGF.[4] IGF-I also increases vascular wall cell proliferation in allograft explants ex vivo.[5] Here, in vivo, IGF-I accelerated proliferation of SMCs and intimal thickening in rat aortic allografts. These findings suggest that IGF-I is also crucial for smooth muscle cell proliferation in transplant arteriosclerosis.

Transplantation of a vascular graft with a high tissue concentration of IGF-I accelerated both cell proliferation and myointimal thickening. Increased expression of IGF-I in the vascular wall is seen in aortic coarctation-induced hypertension[6] and following balloon injury.[7] In the latter model, inhibition of this increase in IGF-I protein is associated with inhibition of myointimal proliferation.[8] Our data implies that surgical and immunologic injury mediated expression of IGF-I, leading to smooth muscle cell proliferation and myointimal thickening is further augmented by exogenous IGF-I.

Estrogen exposure prevented the exogenous IGF-I-induced myointimal thickening. We and others have reported that estrogen suppresses proliferation of vascular smooth muscle cells both in vitro[9,10] and in vivo.[2] The attenuation of transplant arteriosclerosis by locally administered estrogen is probably a locally exerted effect. The adverse effect of systemically administered estrogen may be prevented by using estrogen locally at the vessel site.

CONCLUSION

In orthotopic rat aortic allotransplantation, locally administered IGF-I accelerates proliferation of smooth muscle

Table 2. Attenuation of IGF-I-Induced Transplant Arteriosclerosis by Locally Administered Estrogen

Group	Estrogen Exposure Before Operation	IGF-I Exposure Before Operation	Estrogen Wrapping Following Operation	Intimal Thickening at 14 days
Zero	0 μmol/L	500 ng/mL	0 pmol/kg/d	0.43 ± 0.03
Lo	0.2 μmol/L	500 ng/mL	2.6 pmol/kg/d	0.41 ± 0.09
Mid	2.0 μmol/L	500 ng/mL	26 pmol/kg/d	0.37 ± 0.10
Hi	20 μmol/L	500 ng/mL	260 pmol/kg/d	0.29 ± 0.05**

Mean ± SEM.
**$P < .005$ vs zero, n = 4.

cells at 7 days and myointimal thickening at 14 days. This IGF-I-induced acceleration of transplant arteriosclerosis is abolished by locally administered estrogen. IGF-I may play an important role in the development of transplant arteriosclerosis, which is attenuated by locally applied estrogen.

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