SHORT COMMUNICATION

Lanreotide, a somatostatin analogue, reduces insulin-like growth factor I accumulation in proliferating aortic tissue in rabbits in vivo. A preliminary study

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Coronary artery restenosis following percutaneous transluminal angioplasty occurs with a very high incidence. Major efforts have been aimed at revealing the mechanisms and at developing therapies that might prevent or delay the myointimal proliferation. Most clinical trials have been unsuccessful. Endothelial denudation was induced in 30 rabbits using balloon catheterization of iliac arteries and aorta. Immunoreactive insulin-like growth factor I (IGF-I) was measured in arterial tissue samples obtained 1, 2 and 4 days postoperatively using a double extraction procedure to remove IGF-I binding proteins. Half of the animals were treated with subcutaneous injections of lanreotide, 10 µg/kg twice daily, and the other half served as controls. In the latter group, arterial IGF-I was unaltered from baseline at day 1 and increased by 300 and 400% at days 2 and 4. No increase was observed in the rabbits treated with lanreotide. The results indicate that initial local accumulation of IGF-I may be one of the mechanisms involved in myointimal proliferation after experimental arterial injury. The reduction of local IGF-I accumulation by lanreotide may be involved in the previously observed reduction in myointimal proliferation.

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Insulin-like growth factor I (IGF-I) expression is low in vessels in the unstrained, basal condition (1). However, marked rises in blood flow or pressure rapidly induce increasing IGF-I immunostaining, particularly of myo-medial cells (2). During repair and development of arteries and veins a transient increase in tissue IGF-I immunostaining also is seen (1); this can be demonstrated after endothelial denudation, first in invading endothelial cells and later in neointimal muscle cells (3). Recently, Börnfeldt and Armqvist (4) demonstrated an increase in IGF-I mRNA in the intima media of rat aorta, which reached a maximum 2 days after balloon catheter injury concomitantly with a rise in thymidine incorporation into DNA. This report also showed that constant IGF-I infusion further stimulates vascular smooth-muscle proliferation.

Lanreotide (Angiopeptin®), a somatostatin analogue with a plasma half-life of about 2 h, has proved effective in reducing vascular stenosis after experimental balloon or air injury of arteries and veins (5–7). Octreotide, a similar somatostatin octapeptide analogue, has been reported recently (8) to prevent initial renal hypertrophy after unilateral nephrectomy and after induction of diabetes in rats—as well as preventing a preceding transient local accumulation of IGF-I, in this case measured as extractable renal tissue IGF-I.

The aim of the present study therefore was to see if an increase could be demonstrated in extractable immunoreactive IGF-I in the iliac–aortic tissue in the days following balloon injury of the aorta and iliac arteries of rabbits and, if so, whether administration of lanreotide would prevent or reduce the IGF-I accumulation.

Methods and materials

Experimental animals

Thirty-five male New Zealand white rabbits (Hazleton Labs, Vienna, Va, USA) weighing 2.5–2.8 kg were studied. Five served as controls, while the other 30 were subjected to balloon injury of both common and external iliac arteries and aorta. Half of the animals received daily subcutaneous lanreotide injections of 20 µg/kg in two doses, the first injection being given prior to the surgical procedures, while the other half received an identical volume of vehicle subcutaneously.

Balloon injury. The rabbits were anaesthetized with
Zylazine (Mobay Corp., Shawnee, KS, USA) (3.5 mg/kg) and Ketamine (Parker-Davis, Morris Plains NJ, USA) (20 mg/kg) and prepared for balloon injury. A No. 3 French Fogarty embolectomy catheter (American Edwards Laboratory, Añasco, PR, USA) was inserted under aseptic conditions in the aorta through a left femoral arteriotomy. The balloon was advanced 20 cm to the level of the diaphragm, inflated to 3–4 atmospheres and partially withdrawn, still inflated, so that both the abdominal aorta and the left common iliac and external iliac arteries were injured. After such three passages the balloon catheter was removed and the femoral artery closed with a double ligature. The incision was closed with absorbable suture. The animals were allowed to recover under close observation before being placed in their respective cages.

After 1, 2 and 4 days, five rabbits from each of the two groups were anaesthetized and the vessels were prepared for harvesting. The descending thoracic aorta was cannulated and perfused with lactated Ringer solution with 5 mIU heparin (Organon Inc., West Orange, NJ, USA) at a pressure of 80 mmHg for 20 min. The abdominal aorta and the iliac arteries were removed, divided longitudinally and immediately blotted, weighed, snap frozen in liquid nitrogen, shipped to Denmark in this medium and stored at −80°C until extraction of IGF-I. During the transport in liquid nitrogen, tubes containing vessels from five rabbits were crushed, which is why the number (N) is reduced to three or four in three control experiments in Fig. 1.

**Extraction procedure**

Arterial specimens were homogenized in 2 ml of 1 mol/l acetic acid using an Ultra Turrax, followed by further disintegration by means of a Potter–Elvehjem homogenizer, as described previously for renal tissue (8). The homogenate was centrifuged and the supernatant lyophilized.

Upon reconstitution in standard buffer the residue was analysed for IGF-I immunoreactivity (see below); however, determination analysis in serial dilutions failed to exhibit parallelism to the human recombinant standard solutions. Following a further extraction of the residue using ethanol/HCl, the putative interfering substances (probably IGF binding proteins) were removed and full cross-reactivity was indicated by identical results in serial dilutions. Western ligand blotting followed by densitometry showed that while the acetic acid extracts contained four bands of 125I-labelled IGF-I binding proteins, no significant binding protein activity was found after the second extraction with ethanol/HCl (Table 1).

### Immunoassay

An in-house immunofluorimetric assay (DELFIA principle, Wallac, Turku, Finland) was utilized, with two monoclonal antibodies directed at different sites on human recombinant IGF-I. The detection limit was 2 ng/l, and the standard dose response was linear up to 2 μg/l. Intra-assay and interassay coefficients of variation were less than 5 and 10%, respectively. However, all determinations were run in one assay.

### Statistics

The IGF-I contents in aortic samples from the groups were compared using an unpaired non-parametric test (Mann–Whitney). In Fig. 1, means ± SEM are shown.

### Results

Figure 1 shows the results of measurements of IGF-I immunoreactivity expressed in μg/kg arterial tissue. The contents on day 1 are unchanged from pretreatment levels in both placebo and lanreotide-treated animals and stay at about this level in the latter throughout, while placebo-treated rabbit arteries attained an increase of 300% on day 2 (p = 0.1) and 400% on day 4 (p < 0.025).

### Discussion

The present study demonstrates that arterial endothelial denudation obtained after balloon catheterization in rabbits is followed by tissue accumulation of extractable IGF-I immunoreactivity, reaching 300–400% over pretreatment levels 2–4 days postoperatively. No increase was found 24 h after arterial balloon injury, which is in accord with analyses of IGF-I mRNA.
in rats similarly treated, in which a rise was demonstrated on day 2 that still persisted on days 7 and 14 (4). This study also demonstrated that thymidine incorporation peaked on day 2 and had declined to almost basal levels 7 and 14 days after. In addition, IGF-I receptor mRNA had increased already on day 1 and stayed high during the first week. The IGF binding proteins also are secreted from cultured vascular smooth-muscle cells (9); this may serve to entrap circulating IGF-I and possibly may enhance cellular binding (10), although this effect is controversial. Furthermore, other cells present in the injured areas, such as macrophages and endothelial cells, also can express IGF-I (11, 12). The accumulation of IGF-I in the cells of the vascular wall thus may derive from local synthesis as well as from uptake from the circulation. These observations, as well as a great number of other data referred to in the introduction, support the concept that IGF-I is involved in generation, regeneration and repair of vascular tissue after injury.

Evidence has appeared that IGF-I is expressed also in proliferating vascular cells in autoimmune and inflammatory diseases (13).

There is thus support for the concept that expression of local IGF-I may participate in normal repair processes in vessels as well as in inexpedient and harmful excessive vascular proliferation. An example of the latter may be the frequently occurring restenosis after coronary artery balloon angioplasty used in treatment of coronary atherosclerosis. Several pharmacological intervention trials have had little or no success, some of them based on the idea of inhibiting the formation and action of various growth factors locally or in the circulation (14–16).

Very recently, Bornfeldt et al. (17) demonstrated that IGF-I in high concentrations stimulates directed migration of smooth-muscle cells from human aortic tissue with an efficacy similar to that of platelet-derived growth factor BB, the strongest known chemoattractant for vascular smooth-muscle cells.

The effect of IGF-I may be mediated via circulating growth hormone; Ledet (18, 19) demonstrated that the addition of growth hormone in physiological concentrations to cultures of rabbit aortic myomedial cells stimulated their growth, and that the addition of growth hormone antiserum reduced the stimulatory growth effect of normal and diabetic serum.

As mentioned above, octreotide prevents acute renal

\[ \text{Aorta IGF-I content (\( \mu g/kg \) tissue)} \]

\[ \text{(human IGF-I equivalents)} \]

\[ \begin{array}{c|c|c|c|c}
\text{Days in study} & 0 & 1 & 2 & 3 & 4 \\
\hline
n=4 & n=5 & n=5 & n=5 & n=3 \\
\end{array} \]

* \( p = 0.025 \)

Fig. 1. Immunoreactive insulin-like growth factor I (IGF-I) in rabbit aortic tissue (\( \mu g/kg \)) (mean ± sem) before (day 0) and 1, 2 and 4 days following balloon catheterization. Half of the rabbits received lanreotide, 10 \( \mu g/kg \) subcutaneously twice daily (○), starting just before surgery. The control group received the same volume of placebo (●).
hypertrophy occurring after unilateral nephrectomy and induction of diabetes in rats, hypertrophies that include vascular (glomerular) growth and are preceded by local accumulation of IGF-I, which also is abolished by the administration of octreotide (8).

It is encouraging that lanreotide in the present study was capable of reducing/preventing the increase in vascular contents of extractable IGF-I seen in the placebo rabbits. Encouraging for the ongoing therapy trials of the possible antagonistic effect of lanreotide on the restenosis incidence after balloon catheterization of human coronary arteries. The mechanisms behind the inhibitory effects have not been clarified. However, in the kidney hypertrophy studies (8) the inhibition was observed before any detectable reduction took place in total extractable serum IGF-I.

References

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