The Effect of Porous Infusion Balloon–Delivered Angiopeptin on Myointimal Hyperplasia After Balloon Injury in the Rabbit

Mun K. Hong, MD; Tahira Bhatti, PhD; Bradford J. Matthews, MD; Karen S. Stark, MD; Seedabarum S. Cathapermal, PhD; Marie L. Foegh, MD, DSc; Peter W. Ramwell, PhD; Kenneth M. Kent, MD, PhD

**Background.** Angiopeptin, a synthetic somatostatin analogue, reduces myointimal hyperplasia after experimental balloon angioplasty when given subcutaneously. The feasibility and efficacy of a single dose of angiopeptin delivered locally via the Wolinsky porous balloon on myointimal hyperplasia were studied.

**Methods and Results.** Three rabbits received 125I-angiopeptin in the mid abdominal aorta via the Wolinsky balloon at 5 atm for 1 minute after balloon injury. Thirty minutes later, autoradiography demonstrated radioactivity in the media and the adventitia. Forty rabbits were divided equally into one control group receiving saline and three angiopeptin groups receiving 1, 10, or 100 μg/mL of angiopeptin delivered locally at 5 atm for 1 minute via the Wolinsky balloon into the mid abdominal aorta after balloon injury of the entire abdominal aorta. On day 21, the abdominal aortas were fixed in situ and harvested. There was no statistical difference in the amount of myointimal hyperplasia in the locally treated aorta in the angiopeptin groups compared with the control group. However, in the lower abdominal aortas, where balloon injury without local delivery was performed, there was a significant reduction of myointimal hyperplasia in the highest-concentration angiopeptin group (P<.001 versus the control group). Electron microscopy showed that the control animals had a pseudointima of smooth muscle cells throughout the aorta, whereas in all the angiopeptin-treated animals, endothelial cells were present at both locations.

**Conclusions.** Angiopeptin can be delivered intramurally via the Wolinsky porous balloon and reduces myointimal hyperplasia only in the area distal to the local drug delivery site (downstream effect), possibly by healing the injured endothelium, by transport via the vasa vasorum, and/or by systemic effect. *(Circulation* 1993;88:638-648)

**KEY WORDS** • angioplasty • restenosis • smooth muscle cell proliferation • local drug delivery

The major limiting factor of percutaneous transluminal coronary angioplasty (PTCA) is the high (30% to 50%) restenosis rate within the first 6 months after a successful procedure.1,2 Effective inhibition of restenosis has not been achieved despite extensive studies of the pathology after experimental and human angioplasty.3-7 Pathogenesis,8-12 time course,13-15 and clinical predictors16,17 of restenosis.

Recently, angiopeptin, a synthetic cyclic octapeptide analogue of somatostatin-14, has been shown to inhibit cellular proliferation after balloon injury of normal arteries in both in vitro and in vivo animal studies when given subcutaneously.18-21 Angiopeptin also reduces myointimal hyperplasia in vein grafts22 and inhibits accelerated coronary artery transplant atherosclerosis,23-25 both of which are a smooth muscle cell proliferative process resembling restenosis after PTCA. This octapeptide analogue differs from somatostatin-14 in binding affinity to the two known somatostatin receptors and has a prolonged plasma half-life compared with somatostatin-14.26 A safer and possibly more effective method of administering drugs is local delivery at the time and the site of PTCA.27 The Wolinsky porous infusion balloon offers this possibility and has been used successfully to deliver various drugs intramurally at the time of balloon injury.28-33 The local delivery of drugs into the arterial wall may eventually enable one-time drug treatment at the time of angioplasty at a higher dose than that tolerated by a prolonged systemic application. The local delivery of angiopeptin may result in a more efficacious inhibition of myointimal hyperplasia, since most of the systemically administered angiopeptin concentrates in the liver, not in vascular walls, as demonstrated in rats,34 and much higher concentrations will be obtained in the vascular wall.

The following experiments were designed to study the feasibility and the efficacy of local delivery via a porous infusion balloon of a single dose of angiopeptin on myointimal hyperplasia after balloon injury in a rabbit model. In all previous published studies, angiopeptin was administered over a period of several days up to 3 weeks. First, radiolabeled angiopeptin was localized in
the vessel after porous infusion balloon delivery. Second, the effect of a single dose of locally delivered angiopeptin on the degree of inhibition of myointimal hyperplasia in the balloon-injured segment and the balloon-injured site distal to the porous infusion balloon site was determined.

Methods

Animals

Adult male New Zealand White rabbits weighing 2.5 to 2.8 kg (Hazleton Farms, Vienna, Va) were housed at constant temperature and a 12-hour cycle of light and darkness in the Research Resources Facility of Georgetown University Medical Center for a period of 1 week for adaptation before the procedures. The rabbits had access to regular rabbit chow (Purina Mills, Inc, Richmond, Ind) and water ad libitum during the entire period. The studies involving the rabbits conformed to the guidelines of the American Physiological Society and were approved by Georgetown University Animal Care and Use Committee.

Autoradiographic Localization of Angiopeptin

Radiolabeled angiopeptin was prepared by iodinating the tyrosine ring of the authentic angiopeptin (10 μg/5 μL) (Henri Beaufour Institute-USA, Inc, Washington, DC) (Fig 1) according to the method described by Greenwood and Hunter35 (with slight modifications) and then was purified by column chromatography. The 125I-angiopeptin was loaded onto a 20×1-cm column packed with carboxyl methyl cellulose (CMC-52) eluted with 0.2 M ammonium acetate at pH 4.6. Elution was performed at 0.5 mL/min. Sixty fractions (1 mL) were collected, and each was cochromatographed with authentic angiopeptin on silica gel TLC plates (Analytech Inc, Newark, Del), then lyophilized and kept at -20°C until use. The purity of the resulting compound was checked by high-pressure liquid chromatography before use. The iodinated angiopeptin has been shown previously to be stable.34

Three rabbits underwent balloon injury of the abdominal aorta with a 3.25-mm angioplasty balloon (USCI, Billerica, Mass) repeated three times under 50 mg/kg of ketamine (Fort Dodge Laboratories, Fort Dodge, Iowa) and 5 mg/kg of xylazine (Mobay Corporation, Shawnee, Kan) anesthesia intramuscularly after midline abdominal incision and isolation of the aorta. 125I-Angiopeptin (specific activity, 0.01 μCi·μg-1·mL-1) in saline was administered to the mid abdominal aorta under direct observation for 1 minute at 5 atm with the 3.25-mm Wolinsky porous infusion balloon (USCI, Billerica, Mass). Details of the Wolinsky balloon were described previously by Wolinsky and Thung.28 The animals were killed 30 minutes later, and the mid abdominal aortas were removed and fixed in formalin overnight. After progressive dehydration in increasing concentrations of ethanol, the fixed tissues were embedded in paraffin. Cross sections of 7 μm were cut and stained with hematoxylin and eosin and were prepared for autoradiography.36 The stained sections were rehydrated overnight and covered with NTB3 emulsion (Eastman Kodak Co, Rochester, NY). After overnight exposure, the sections were developed for 45 seconds with Dektol (Eastman Kodak) and fixed for 5 minutes with GBX (Eastman Kodak). The developed slides were examined under a light microscope for detection of radioactive particles.

Myointimal Hyperplasia at 21 Days

After Balloon Injury

Forty rabbits were randomized into four equal groups. The control group received local delivery of normal saline, the vehicle for angiopeptin, and the other three groups were treated by local delivery of 1, 10, or 100 μg/mL of angiopeptin, respectively. Animals were anesthetized with ketamine and xylazine as described above. The procedure is depicted in Fig 2. The right carotid artery was isolated, and a 3.25-mm USCI angioplasty balloon catheter was inserted via an arteriotomy.
after ligation of the carotid artery distal to the arteriotomy site. The balloon catheter was advanced over a 0.014-in. USCI exchange guide wire to the abdominal aorta, where the injury was performed by inflating the balloon to 5 atm and passing it from the iliac bifurcation to the diaphragm three times under fluoroscopic guidance. No angiogram was performed; however, the mean diameter of the mid abdominal aorta (measured after perfusion fixation) was 2.56 mm, with no significant difference among the four groups; the mean diameter of the lower abdominal aorta was 2.14 mm, with no difference among the groups. The balloon-injured aorta was marked on the shaved abdominal wall with an indelible marker. The angioplasty balloon was removed, and the Wolinsky porous infusion balloon of the same inflated diameter was exchanged over the guide wire. Then, local delivery of angiopeptin (or normal saline) followed with the Wolinsky porous infusion balloon at mid abdominal aorta, defined as the fourth vertebral body above the iliac crests and distal to the renal arteries. This procedure was also done under fluoroscopy. This area was marked on the abdominal wall with indelible ink under fluoroscopy. The angiopeptin or saline was delivered intramurally for 1 minute at 5 atm. The infusion balloon was then removed, the carotid artery proximal to the arteriotomy site was ligated, and the skin was closed with sutures. Serial blood samples were drawn from an indwelling central auricular arterial line from three animals in each group. Each sample was measured three times for plasma angiopeptin concentration by radioimmunoassay. The animals were killed after 3 weeks by intracardiac potassium injection, and thoracic and abdominal aortas were fixed in situ via left thoracotomy by flushing with heparinized Ringer's lactate solution for 20 minutes, followed by Karnovsky's fixative for 20 minutes at 80 mm Hg perfusion pressure. From each animal, 1-cm segments of mid abdominal aorta (local effect) and lower abdominal aorta (downstream effect) separated by a balloon length (2 cm) were removed for light and electron microscopic analyses. To further avoid the potential overlap between these two areas, the 1-cm segments for the local effect were harvested 0.5 cm proximal to and 0.5 cm distal to a central marker of the Wolinsky porous infusion balloon. For morphometric analysis, each 1-cm aortic segment from either the local area or the downstream area was divided into four equal fractions, with each fraction corresponding to 0.25 cm; three 7-μm sections from each 0.25-cm segment then were stained with hematoxylin and eosin; thus, 12 sections from each 1-cm aortic segment were analyzed, and the results were averaged. Animals were excluded from analysis if the entire abdominal aorta had no myointimal hyperplasia; this was possibly related to balloon malfunction (two control animals were thus excluded). In all previous studies in our laboratory using the rabbit balloon injury model, all balloon-injured abdominal aortas have always exhibited myointimal hyperplasia.

**Transmission Electron Microscopy**

Tissue blocks for electron microscopy were fixed in Karnovsky's solution containing 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.3) for an additional 3 to 5 hours. Postfixation was carried out in 1% osmium tetroxide for 1.5 hours, and tissues were dehydrated through graded ethanol and embedded in epon.
were
distribution;

Volume of

betweenthe intima and

Administration of

Parameters

Units

Concentration of
angiopeptin (μg/mL)

1  10 100

\( t_{1/2} \)  ka  Hour  0.228  0.43  0.7
\( t_{1/2} \) α  Hour  0.046  0.20  0.187
\( t_{1/2} \) β  Hour  2.04  2.19  2.4
Clearance  L/h  4.46  5.02  10.85
Volume of distribution  Liter  5.0  4.6  7.7
Area under the curve  ng·mL\(^{-1}·h\(^{-1}\)  0.224  1.99  9.21

\( t_{1/2} \)  ka indicates half-life of absorption; \( t_{1/2} \) α, half-life of distribution; and \( t_{1/2} \) β, half-life of elimination.

Results

There were areas of intense radioactive particles throughout the vessel wall interspersed by adjacent areas devoid of any radioactivity. This suggests a linear or jet intramural penetration of \(^{125}\)I-angiopeptin. Radioactivity was found both in the cytoplasm and in the nuclei of the medial smooth muscle cells as well as in the adventitia (Fig 3).

Plasma Angiopeptin Levels

The mean plasma concentrations of angiopeptin versus time after local administration of angiopeptin as determined by radioimmunoassay are shown in Fig 4. The plasma concentrations reached the peak at the time of the first blood samples, approximately 15 minutes after local delivery.

The kinetic parameters and distribution of angiopeptin determined from data in Fig 4 are shown in the Table. The absorption half-life of locally delivered angiopeptin varies according to the dose of drug administered. Although direct comparisons cannot be made, the absorption half-life, the distribution half-life, and the elimination half-life of angiopeptin when administered locally are less than when administered subcutaneously in rats but greater than when administered intravenously in rats. Thus, it is most likely that when angiopeptin is administered locally, its kinetics are between those associated with subcutaneous and intravenous administration.

In one animal receiving the angiopeptin at 100 μg/mL concentration and another receiving the angiopeptin at 10 μg/mL, angiopeptin was extracted from the locally treated aortic tissue (638 pg/0.2 g of tissue from the 100 μg/mL animal and 439 pg/0.2 g of tissue from the 10 μg/mL animal), suggesting that the locally delivered angiopeptin may stay in the vessel wall for at least 24 hours.

Statistics

The data for the morphometric analysis are presented as mean±SEM. The degree of myointimal hyperplasia among the four groups was analyzed by ANOVA. The planned comparisons of the degree of myointimal hyperplasia among the control animals versus the different combinations of the angiopeptin groups were done using the Student’s \( t \) test. Statistical significance is represented by probability values <.05.
hours. (These animals were killed 24 hours after local delivery of angiopeptin.)

**Morphometric Study**

*Local delivery area.* The degree of myointimal hyperplasia for the study groups is shown in Fig 5. The values of percent myointimal hyperplasia (mean±SEM) were 8.2±1.0 for the control group; 7.5±1.5 for the angiopeptin group receiving 1 μg/mL; 6.5±1.7 for the angiopeptin group receiving 10 μg/mL; and 6.2±1.4 for the highest-dose angiopeptin group. The morphometry done by the two independent readers had a correlation coefficient of .9 and P<.001. The readings were averaged for data analysis. In the control group, the myointimal hyperplasia consisted of circumferentially uniform, multiple layers of smooth muscle cells (Fig 6, A). In some of the angiopeptin-treated groups, there was a substantial decrease in the myointimal hyperplasia and the myointimal thickening was patchy, varying within regions of a given vessel section (Fig 6, B). In the angiopeptin-treated animals, there was a dose-dependent reduction of percent myointimal hyperplasia. However, by ANOVA, there was no statistical difference among the angiopeptin groups compared with the control group.

There were no differences in the amount of volume delivered via the porous infusion balloon among the groups (with volume of 6.1±1.8 mL/min, mean±SD) and no correlation between the volume delivered (or the total amount of angiopeptin locally delivered in the angiopeptin groups) and the percent myointimal hyperplasia in any of the groups.

*Downstream area.* The percent myointimal hyperplasia is shown in Fig 7. The values of percent myointimal hyperplasia (mean±SEM) were 11.4±0.6 for the control group; 7.9±2.0 for the lowest-dose angiopeptin group; 7.9±1.9 for the angiopeptin group receiving 10 μg/mL; and 5.4±1.2 for the highest angiopeptin concentration group. The correlation of the two independent readings was excellent (r= .9, P<.001), and mean values of the readings were used for data analysis. Again

![Graph showing morphometric analysis of myointimal hyperplasia: downstream effect](image)

**Fig 7.** Bar graph shows morphometric analysis of myointimal hyperplasia: downstream effect. Values are mean±SEM. *P<.001 vs the control group.
in the control animals, there was a circumferentially uniform myointimal hyperplasia. In the angiopeptin-treated animals, the myointimal hyperplasia was markedly reduced and, contrary to the local delivery area, was also uniform circumferentially (Fig 6, C). The degrees of myointimal hyperplasia were different among the four groups (P<.05 by ANOVA). When the pairwise t test was performed for the different combinations of comparison groups, the significant difference was found only in the highest angiopeptin dose group compared with the control group (P<.001).

**Electron Microscopic Studies**

**Local delivery area.** The control group had markedly abnormal intima and showed loss of endothelial integrity. In many areas, the endothelial cell layer was missing and was replaced by a pseudointima of smooth muscle cells, which were either modified smooth muscle cells with the presence of vesicles and microtubules or typical secretory smooth muscle cells with many mitochondria, dilated endoplasmic reticulum, and large Golgi complexes (Fig 8, left). These smooth muscle cells also exhibited fingerlike projections into the lumen (Fig 8, left). Furthermore, there was a significant difference in the structure and frequency of a number of cellular organelles of the smooth muscle cells in the control and the angiopeptin-treated groups. In the control group, the rough endoplasmic reticulum was most prominent (Fig 8, left). The number of plasmalemma vesicles also appeared to be increased, especially in smooth muscle cells in the control group, where they were occasionally shown to occupy the entire cytoplasm. The subendothelial space was consistently wider in the control group than in the angiopeptin-treated groups. In the control group, the subendothelial space consisted of numerous layers of smooth muscle cells (Fig 8, right).

In the angiopeptin-treated groups, the intima was covered by a morphologically intact endothelial cell layer, although there were occasional gaps between endothelial cells (Fig 9, left). In the angiopeptin 10 µg/mL group, the endothelial cells were normal in that they were flattened and elongated with close proximity with the adjacent cells, indicating tight junctions. The cytoplasm contained moderate amounts of mitochondria, endoplasmic reticulum, ribosomes, and pinocytic vesicles along the cell membrane (Fig 9, right). The endothelial cells of the angiopeptin 1 µg/mL and 100 µg/mL groups appeared to have fewer vacuoles and occasionally breaks between the endothelial cells. Many of the endothelial cells appeared to be similar to regenerating cells, with prominent nuclei and the cells bulging into the lumen (Fig 9, left). Even in areas with underlying myointimal hyperplasia, the endothelial cells appeared ultrastructurally normal. The subendothelial space in the angiopeptin-treated groups varied from none to a few layers of smooth muscle cells.

**Downstream area.** Similar ultrastructural differences between the control and the angiopeptin-treated animals in the local delivery area were also found in the downstream area. In the control animals, the myointimal hyperplasia was highly cellular and the lumen was lined with cells consisting of dense bodies observed among a few peripherally situated bundles of filaments; thus, these cells can be identified as smooth muscle cells (Fig 10, left). In the angiopeptin-treated animals, the endothelial cells were flattened and normal-appearing, especially in the highest-concentration angiopeptin group, whether there was no myointimal hyperplasia (Fig 10, right) or a small amount of myointimal hyperplasia.

**Discussion**

This study shows that (1) it is feasible to deliver angiopeptin intramura1vially via the Wolinsky porous infusion balloon; (2) angiopeptin delivered locally does not reduce myointimal hyperplasia at the local delivery site but reduces myointimal hyperplasia significantly only in the downstream area; and (3) angiopeptin may facilitate reendothelialization.

Our original hypothesis was that the locally delivered angiopeptin would reduce myointimal hyperplasia at the local delivery site because of its antiproliferative effect compared with the control group. Furthermore,
FIG 9. **Left**, transmission electron micrograph from an angiopeptin-treated (1 μg/mL) rabbit. The endothelial cells (E) appear cuboidal in shape, with moderate amounts of intracellular organelles. The cells are attached by tight junctions. Discontinuities and fraying of the internal elastic lamina (IEL) are evident. Collagen fibers (arrowheads) are abundant in the intercellular space. Fibrous and granular material is observed in the subendothelial space (magnification ×10 000). **Right**, transmission electron micrograph from an angiopeptin-treated (10 μg/mL) rabbit. The endothelial cells are flat, elongated, and closely applied to the IEL; tight junctions are visible (small arrowheads) (magnification ×14 500). L indicates luminal side in both figures.

The downstream area, where balloon injury alone without local delivery was performed, could act as a control site within each animal so that this site would have a similar amount of myointimal hyperplasia among the four groups.

The unexpected observation that myointimal hyperplasia was significantly reduced only in the downstream area and not in the local delivery area may have plausible explanations. In the treatment groups, the myointimal hyperplasia in the local delivery area was patchy with noncircumferential distribution, whereas in the downstream area, the reduction was concentric with uniform circumferential myointimal hyperplasia. We have observed in our previous angiopeptin studies using subcutaneous administration that the inhibition of the myointimal hyperplasia tended to be concentric.20-24 The patchy inhibition in this study may be due to the noncircumferential arrangement of pores on the balloon. Furthermore, since heparin was avoided in our study because of its reported inhibition of myointimal hyperplasia,38 plugging of the pores with thrombus and fibrin clots was seen. When the Wolinsky porous infusion balloon was removed after local delivery, it was noted that extremely high pressure (at times greater than 10 atm) was needed to open up even a small fraction of the pores. This would indicate the possibility that in vivo, there may have been only a small number of pores open at the time of local treatment, infusing volume into the vessel wall at higher than intended pressure, possibly resulting in vascular injury. Likewise, local delivery of any drug via the porous infusion balloon may cause additional local injury regardless of its efficacy even when all the pores are patent. The jet streams under pressure may result in vascular injury and be deleterious, as already shown by others.39,40

The downstream area is a smaller vessel (diameter of 2.14

FIG 10. **Left**, electron micrograph from a control rabbit shows a modified smooth muscle cell (Msmc) at the lumen (L) (pseudointima), with myofilaments identified by asterisks. A smooth muscle cell (arrows) is seen adjacent to it (magnification ×50 000). **Right**, transmission electron micrograph of the lower abdominal aorta from an angiopeptin-treated (100 μg/mL) rabbit. A flattened endothelial cell (E) is seen at the lumen. Smooth muscle cells (SMC) of the contractile type are seen in the subendothelial space closely adjacent to the internal elastic lamina (IEL) (magnification ×10 000).
mm versus 2.56 mm, respectively). There may be greater initial vascular injury from the balloon denudation and a larger degree of myointimal hyperplasia (11.4% versus 8.2% in the control group, respectively), thus facilitating a greater difference between control and angiopeptin-treated groups. It is difficult to determine what fraction of the locally delivered drug actually stays at the local delivery site and has local effect. It is possible that some of the drug may be transported distally via the vasa vasora or by diffusing into the adventitia. The presence of radioactivity in the adventitia also suggests the possibility of localization of angiopeptin in the vasa vasora and possible transport to the peri–local delivery area. The average volume delivered is high compared with previous studies conducted in the brachial arteries in dogs,28 and because of the many branch vessels in the abdominal aorta where local delivery was performed, some of the agent may have been delivered systemically via these vessels. A more convincing evidence of systemic delivery from the local infusion is that the volume of the aorta receiving local delivery is approximately 0.1 mL (using the conventional formula for volume calculation and assuming that the wall thickness is 500 μm). Thus, even if the wall could retain volume equivalent to its own, only 1.7% of the mean volume infused would have stayed at the local delivery site. Also, during the feasibility study in which the local delivery was performed under direct observation, the vessel wall was stretched paper-thin, making possible the extravasation of some of the volume into the adventitia and surrounding tissue.

There may be several mechanisms by which angiopeptin, a somatostatin analogue, inhibits smooth muscle cell proliferation. First of all, somatostatin itself is known to inhibit secretion of many hormones from different cell types41-45; thus, it is very likely but not yet shown conclusively that angiopeptin inhibits the secretion of growth factors such as platelet-derived growth factor, insulin-like growth factor-1, epidermal growth factor, fibroblast growth factor, and transforming growth factor-α from the different cells such as endothelial cells, monocytes/macrophages, and the smooth muscle cells themselves46 (Fig 11). The growth factors, insulin-like growth factor-1, platelet-derived growth factor, insulin, epidermal growth factor, and fibroblast growth factor are all ligands for receptors, where the intracellular domain is a tyrosine kinase. Binding of these growth factor ligands to their receptors causes autophosphorylation of the tyrosine kinase domain. This initiates the signal transduction chain of events, which results in cell proliferation. It has been shown that somatostatin and some somatostatin analogues, including angiopeptin, activate a membrane-bound phosphatase, which dephosphorylates the activated tyrosine kinase.47 This latter mechanism would explain why angiopeptin seems so successful in inhibiting smooth muscle cell proliferation in comparison to, for example, monoclonal antibodies directed against a specific growth factor–like fibroblast growth factor or platelet-derived growth factor. Dephosphorylation of the activated tyrosine kinase receptors means that angiopeptin will exert inhibitory activity toward a series of growth factors, namely, at least the tyrosine kinase receptor-dependent ligands.46

The autoradiography studies show that angiopeptin localizes in the smooth muscle cells and inhibits proliferation of smooth muscle cells.18,19 There may be receptors for somatostatin analogues on smooth muscle cells. Somatostatin receptors have been found in various cells, including nonendocrine and tumor cells.48 Furthermore, in vitro binding studies with somatostatin and angiopeptin on cultured rat cardiac smooth muscle cells suggest the presence of specific binding sites (personal communication, Dr Dariusz Leszcynski). The presence of radiolabeled angiopeptin in the adventitia would suggest transport of angiopeptin from the site of local delivery via the vasa vasora. Although there is a small chance that intramural radioactivity may represent biologically inactive peptide, our previous study showed that [35S]-angiopeptin retains its biological activity up to 30 days. Extraction of radiolabeled angiopeptin from biological samples followed by high-pressure liquid chromatography revealed the presence of only one peak that coeluted with authentic radiolabeled angiopeptin, thus confirming our proposal that we are dealing with biologically active angiopeptin, not inactive peptides.44

Recently, an angiotensin converting enzyme inhibitor has been shown to reduce myointimal hyperplasia in an animal model.49 Angiotensin II receptors occur on vascular smooth muscle cells.50 Furthermore, angiotensin II stimulates not only protein synthesis and hypertrophy of the smooth muscle cells51,52 but also migration53 and proliferation54 of vascular smooth muscle cells. Angiopeptin has been shown to significantly reduce angiotensin II receptor expression in smooth muscle cells in culture.55 Thus, some of the inhibitory effects of angiopeptin may be through attenuating angiotensin II–mediated proliferative effects.

Cell growth requires competence factors that induce dormant cells to enter the replication cycle and progression factors that enable the competent cells to convert from G0 to G1 phases and undergo S phase.56 Thus, angiopeptin could act at several growth arrest points or

FIG 11. Illustration shows proposed mechanism of inhibition by angiopeptin of myointimal hyperplasia. EC indicates endothelial cell; MO, monocytes; SMC, smooth muscle cells; IGF-1, insulin-like growth factor-1; EGF, epidermal growth factor; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; TGFA, transforming growth factor-α, and IEL, internal elastic lamina.
potentiate other growth inhibitors, such as transforming growth factor-β. Delaying angiopetin treatment in vivo showed that if the treatment was delayed for 8 hours, the inhibition of myointimal hyperplasia was not significant, and an 18-hour delay abolished any inhibitory effects of angiopetin, suggesting that the mechanisms of action may be on the competence factors rather than on the progression factors. Angiopetin is less likely to affect the migration of the smooth muscle cells from the media to the intima, as this migration is a late event, and the delay of treatment for 18 hours abolished any inhibitory effects of angiopetin.

The transmission electron microscopy of the neointima in the present study not only provides supportive evidence of the efficacy of angiopetin at the ultrastructural level but also suggests a possible new mechanism of angiopetin, namely, the facilitation of healing of the injured endothelium. The angiopetin-treated animals had endothelial cells that were either elongated endothelial cells or large, plump endothelial cells bulging into the lumen with prominent nuclei, morphologically resembling regener-ated endothelial cells. In the control group, modified smooth muscle cells rather than true endothelial cells were lining the vessel lumen. No other compounds in vivo have been shown to facilitate endothelialization, but in a rabbit aorta allograft transplant model, we have found that estradiol preserves the ultrastructural integrity of endothelial cells. The presence of endothelial cells in all the arteries from angiopetin-treated animals suggests that this drug may directly affect endothelial cell regrowth or inhibit endothelial cell antiproliferative factors. The latter mechanism may allow fibroblast growth factor to express its stimulatory effect on endothelial cell proliferation. Rapid regrowth of the endothelium after vessel wall injury may prevent the migration of smooth muscle cells to the intima and may inhibit the growth of smooth muscle cells by secreting a heparin-like molecule, thus preventing myointimal hyperplasia. The transmission electron microscopy also shows that in angiopetin-treated animals, the internal elastic laminae may not appear as injured as in the control animals. However, it was shown that even intact internal elastic laminae in rabbit aorta allow the migration of medial smooth muscle cells. Thus, the internal elastic laminae may not play as important a role as the endothelial layer in inhibiting myointimal hyperplasia in this animal model.

Study Limitations

There are several limitations to our study. This rabbit model of balloon injury is not analogous to that of human restenosis because there is no preexisting atheromatous plaque and neither heparin nor aspirin was used. Furthermore, the small amount of myointimal hyperplasia (with much variability even in the same treatment group in this model) may limit the ability to distinguish the true effect of angiopetin. However, restenosis after PTCA is likely to involve the same mechanism of myointimal hyperplasia as that seen in our experimental model. No angiogram was performed to guide the balloon size, although we felt that the 3.25-mm balloon size selected was adequate to cause sufficient barotrauma from our observation during the feasibility study. We intentionally selected a much larger balloon to ensure endothelial and medial damage for maximal myointimal hyperplasia. Also, several anatomic markers were used to ensure correct localization of local delivery, such as the fourth vertebral body in each animal. The methods of defining the area of balloon injury and distinguishing the local area from the downstream area are not precise and remain limitations of the study. To minimize the potential overlap, the harvested 1-cm aortic segments from the local effect and the downstream effect were separated by at least a balloon length (2 cm). In this preliminary study, duration of local delivery and not the volume delivered was controlled, resulting in varying amounts of volume and drug delivered even in the same treatment group. However, there was no correlation between volume delivered and inhibition of myointimal hyperplasia, again supporting the possibility that most of the locally infused volume may actually have been systemically administered. The avoidance of heparin may have caused the plugging of the pores in the balloon and noncircumferential delivery of the solution. No physiological study was performed to evaluate the degree of reendothelialization among the different groups, and the functional importance of reendothelialization is unknown.

Although the observations from this preliminary study are promising, further studies are needed to define the role of local delivery of angiopetin and to elucidate the mechanism of angiopetin. Other routes of administration of angiopetin need to be examined, as neither subcutaneous nor local administration alone completely abolishes myointimal hyperplasia. Combination of these two routes or continuous infusion with a subcutaneous pump may further reduce the amount of myointimal hyperplasia. Most likely, a pretreatment may be required. It is also unknown how much of the volume (thus, the drug) delivered remains in the vessel wall. Also unknown is how long the drug should remain in the vessel wall to have an effect. It is possible that the duration may not be as important as the timing of the local delivery in relation to the vessel injury.

Conclusions

In summary, in this preliminary study, we have shown that at the time of balloon injury, it is feasible to infuse angiopetin intramurally via the Wolinsky porous infusion balloon and that locally administered angiopetin reduces myointimal hyperplasia not at the local delivery site but distal to the local delivery site, possibly by a downstream transport via the vasa vasora, by facilitating the healing of the injured endothelial cells and/or by a systemic effect. Additional studies are required to ascertain the role and the optimal method of local delivery, especially in atherosclerotic arteries.

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