The Effects of the Somatostatin Analog Octreotide on Angiogenesis In Vitro

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This study examined the in vitro antiangiogenic effects of the somatostatin analog octreotide on the growth of human HUV-EC-C endothelial cells and vascular cells from explants of rat aorta cultured on fibronectin-coated dishes or included in fibrin gel. A total 10^{-9} mol/L octreotide reduced the mean uptake of ^{3}H-thymidine by HUV-EC-C cells by 37% compared with controls. The 10^{-8} mol/L concentration of octreotide inhibited the proliferation of endothelial and smooth muscle cells growing on fibronectin by 32.6% and reduced the sprouting of cells from the adventitia of aortic rings in fibrin by 33.2% compared with controls, as measured by tetrazolium bioreduction and image analysis, respectively. These results demonstrate that octreotide is an effective inhibitor of vascular cell proliferation in vitro.

SOMATOSTATIN belongs to the expanding family of small regulatory peptides characterized by a wide spectrum of actions in the human body, including regulation of cell growth.[1] After the landmark discovery of octreotide, a synthetic somatostatin analog with a markedly increased half-life in the body and growth hormone (GH)-suppressing activity,[2] several studies have focussed on the possible antiproliferative effect of this peptide, based on the evidence that somatostatin receptors (ssts) are detected in some human tumors.[3] Somatostatin analogs inhibit the biological activity of paracrine and autocrine growth factors, including basic fibroblast growth factor (bFGF) and insulin-like growth factor-1 (IGF-1).[4] Tumor-induced blood vessel growth is the result of the increased secretion of angiogenic factors, including bFGF and IGF-1,[5] and octreotide could be a candidate drug for angiogenesis suppression in view of its growth factor inhibition. The antiangiogenic activity of octreotide has been previously demonstrated in the chick chorioallantoic membrane (CAM)[6]; however, studies testing its in vitro effect on vasculogenesis have been lacking. The availability of in vitro model systems of vascular cell growth would provide screening tools for selecting potentially active molecules for further development. In the present study, the effect of octreotide was tested on human endothelial cells, as well as on a mixed population of vascular cells grown from explants of rat thoracic aorta.

MATERIALS AND METHODS

Cell culture media were obtained from Sigma (St Louis, MO), bFGF from R&D Systems (Minneapolis, MN), and ^{3}H-thymidine (80 mCi/mmol, 1 mCi/mL) from NEN-Dupont (Boston, MA). Octreotide acetate was supplied as a gift by Sandoz Pharma (Basel, Switzerland).

Human endothelial cells HUV-EC-C (ATCC, Rockville, MD) were grown in Ham's F12 containing 20% fetal bovine serum (FBS), 10 ng/mL bFGF, and 100 μg/mL heparin. HUV-EC-C cell proliferation was evaluated on 2×10^{3} cells per well, plated in a microtiter plate, and the cells exposed to octreotide, 10^{-10} to 10^{-6} mol/L for 72 hours. Cells were pulsed with ^{3}H-thymidine, 10 μCi/mL, for 2 hours, and the incorporated label was extracted with ice-cold 20% trichloroacetic acid and 0.25N NaOH.[7] Precipitable counts were measured by liquid scintillation with a Betamatic V scintillation counter (Kontron, Milan, Italy).

Vascular cell proliferation from rat aortic rings on fibronectin was obtained as reported by Diglio et al.[8] Microwell plates were coated with 0.3 μg human fibronectin to improve cell attachment and proliferation. The thoracic aorta was excised from anesthetized rats, and the fibroadipose tissue around the vessel was dissected. Aortic rings (1 mm long) were cut with a scalpel and placed on the flat bottom of a microwell plate containing DMEM supplemented with 10% FBS. Treatment with octreotide, 10^{-10} to 10^{-6} mol/L, was started 5 days later and continued for 48 hours. At the end of the experiment, cell proliferation was evaluated by the Cell Titer 96 cell proliferation kit (Promega, Madison, WI) in accordance with the technical manual.

Microvascular-like sprouts composed of endothelial and smooth muscle cells were obtained from rat aortic rings cultured in fibrin.[9] Briefly, 0.4 mL/well of clotting fibrinogen was transferred to a 24-well plate; the aortic rings were placed on the clot with the margin of section upwards, and additional fibrinogen was added. Fibrin clots were overlayed in a ratio of 1:1 with DMEM and Ham's F12 containing 300 μg/mL ϵ-aminocaproic acid (Bayer, Leverkusen, Germany) to prevent fibrin dissolution, and aorta explants were treated for 6 days with octreotide, 10^{-10} to 10^{-6} mol/L. At the end of the experiment (day 6), the degree of sprouting of aortic rings was measured by a Kontron KS 300 image analyser (Kontron, Eching, Germany).[7]

In all cases, experimental data were analyzed using Student's unpaired *t* test before conversion to percent values, and the level of significance was set at $P < .05$.

RESULTS AND CONCLUSIONS

HUV-EC-C endothelial cell proliferation was evaluated on the basis of the amount of DNA labeled with ^{3}H-thymidine. Treatment with octreotide produced a maximum 37% ± 4.9% inhibition of DNA synthesis at 10^{-9} mol/L (Fig 1). A linear, dose-dependent inhibition of growth was not observed; on the contrary, the effect of octreotide on ^{3}H-thymidine uptake was reduced at 10^{-10} mol/L and below 10^{-8} mol/L (Fig 1). Indeed, this effect is not uncommon with octreotide, and has also been reported in previous studies of cell growth inhibition in vitro.[4]

A mixed population of endothelial and smooth muscle cells originated from the margins of sections of aortic rings and formed a monolayer on the surface of the fibronectin-coated microwells. Cell proliferation entered the log phase of growth 4 days after the explant, and reached its plateau

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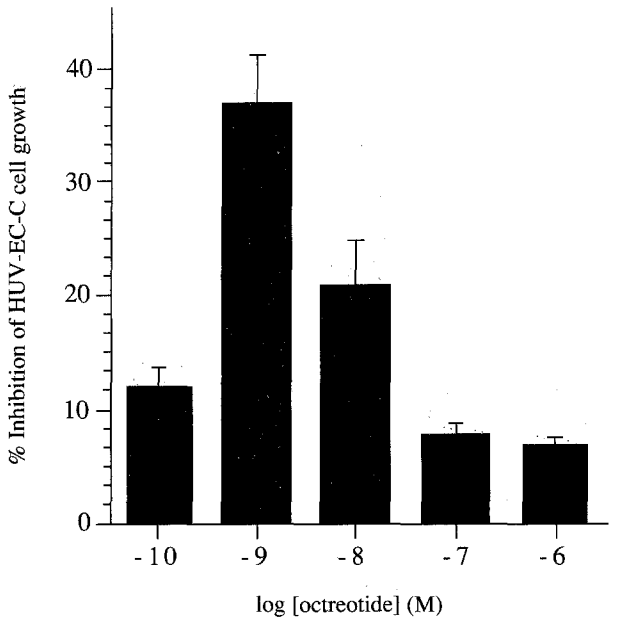

Fig 1. Inhibition of HUV-EC-C endothelial cell proliferation by octreotide. Data are expressed as the percentage of control values obtained in vehicle-treated cells and are the mean ± SE of 3 separate experiments.

by 8 days. The evaluation of cell proliferation was made by absorbance reading of the blue formazan, derived from the tetrazolium salt, in proportion to the amount of dehydrogenase activity present in living cells. Treatment with octreotide produced a significant inhibition of cell proliferation, with a maximum effect of −32.6% ± 5.8% at 10^{-8} mol/L ($P < .05$ *v* control values). The profile of drug-induced inhibition of cell growth was bell-shaped, with a reduction of the antiproliferative effect at concentrations less than 10^{-9} mol/L and greater than 10^{-7} mol/L.

A large number of microvessel-like structures formed within the fibrin gel from the adventitial surface of explants. Phase-contrast microscopy showed that microvascular sprouts were present after 5 days in culture and formed a rich network around the aortic rings. The sprouts originated from the margins of sections, as well as from the portion of the small aortic branches remaining after stripping off the adventitial fibroadipose tissue. The proliferation, migration, and enzymatic activity of cells are demonstrated by the elongation of microchannels that actively lyse fibrin by producing plasmin. After 10 days in culture, the area of microvessels of control explants was 1.7 ± 0.3 mm^2. Aortic rings treated with octreotide, 10^{-8} mol/L, produced a thin crown of microvessel sprouts and rapidly reached the plateau phase; significant reductions in the vascular area (0.8 ± 0.1 mm^2, $P < .05$ *v* controls) and optical density (−33.2% ± 4.6%, $P < .05$ *v* controls) were observed.

Taken together, the results of the present study provide evidence that octreotide is an effective inhibitor of angiogenesis in vitro. This activity might be mediated by the presence of specific receptors on vascular cells, particularly the sst_2 and sst_5 subtypes, of which octreotide is a selective agonist.[10] However, the mechanism of action of this agent on vascular cells is still unclear, and for this reason further studies on the cellular mechanisms of the antiangiogenic activity of the somatostatin analog octreotide are needed.

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