Vascular Endothelial Growth Factor Potently Induces Human Endoneurial Endothelial Cell Proliferation, Angiogenesis and Wound Healing Ex Vivo.

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INTRODUCTION

The mediators of human blood nerve barrier (BNB) development, differentiation and response to injury are unknown.

Vascular endothelial growth factor (VEGF) has been implicated in restoration of peripheral nerve function following experimental ischemic and diabetic neuropathy in rabbits.

VEGF, growth factors such as basic fibroblast growth factor (bFGF) and hormones such as hydrocortisone have been implicated in mammalian blood vessel growth and repair.

Phenotypic and functional differences between endothelial cells from different species and from different tissues within the same species provides a rationale for studying human BNB angiogenesis during development and in response to injury.

Restoration of BNB structure and function may enhance peripheral nerve recovery following ischemic, toxic or traumatic injury to peripheral nerves.

OBJECTIVE

To elucidate the molecular mediators of human blood-nerve barrier endothelial cell proliferation, angiogenesis and wound healing *ex vivo*.

METHODS

Primary human endoneurial endothelial cells (pHEndECs) were seeded at 5,000 cells/well in 96-well Corning CellBIND® tissue culture plates in basal medium with or without heparin (25 U/mL).

pHEndECs were treated with **VEGF$_{165}$** (0.1-100 ng/mL), **b-FGF** (0.1-500 ng/mL), transforming growth factor-β1 (**TGF-β1**, 0.1-500 ng/mL), glial derived neurotrophic factor (**GDNF**; 0.1-100 ng/mL), and **hydrocortisone** (100-5000 nM).

Proliferation relative to basal conditions without added growth factors was quantified by absorbance after 48 hours using the sensitive non-radioactive tetrazolium salt, WST-1 ((4-[3-(4-Iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate).

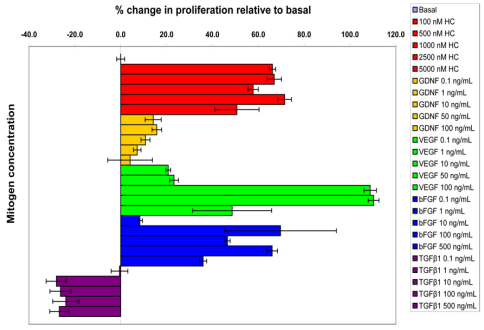
pHEndEC angiogenesis was studied using the 4-hour Matrigel assay. **VEGF** (0.1-100 ng/mL), **bFGF** (0.1-500 ng/mL), **hydrocortisone** (10-5000 nM), **GDNF**(1 ng/ mL) and **TGF-β1** (0.1 ng/mL) were applied in the presence of heparin to determine effect on mean number and total length of microvessels formed, compared to basal conditions.

pHEndECs were grown to >90% confluence on rat tail collagen-coated 12-well CellBIND® tissue culture plates in regular growth media. Sterile micropipette injury (one vertical and three horizontal) was performed. Cellular debris was washed in basal media. The endothelial cells were treated with **VEGF** (1-100 ng/mL), **bFGF** (1-500 ng/mL), **hydrocortisone** (100-1000 nM),1 ng/mL **GDNF** or 0.1 ng/mL **TGF-β1** with and without heparin (25 U/mL).

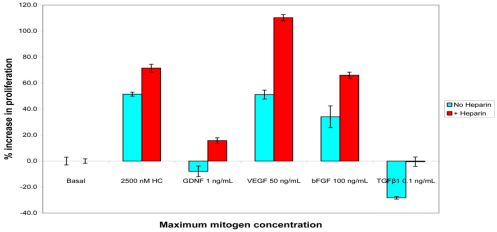
Rate of wound recovery over **30 hours** was quantified using computer assisted measurements of sequential phase contrast photomicrographs of 10 non-overlapping regions per well.

Endoneurial endothelial cell recovery was compared to recovery without added growth factors.

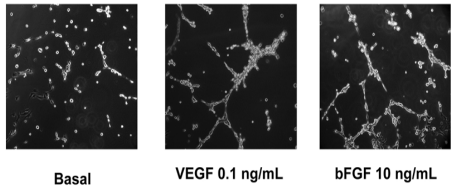
pHEndEC Proliferation Assay (+ Heparin)



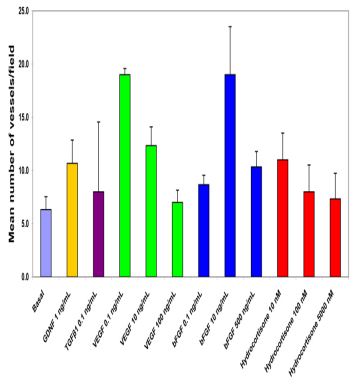
Effect of heparin on mitogen-induced pHEndEC proliferation



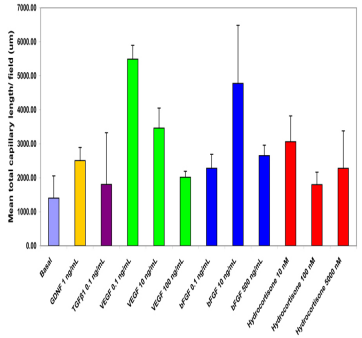
Endoneurial capillary angiogenesis (4h Matrigel assay)



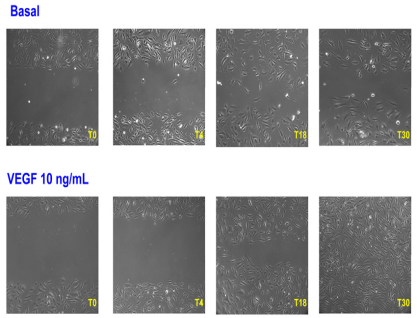
In vitro endoneurial capillary angiogenesis (4hr Matrigel assay)



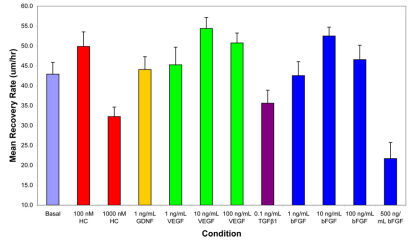
In vitro endoneurial capillary angiogenesis (4hr Matrigel Assay)



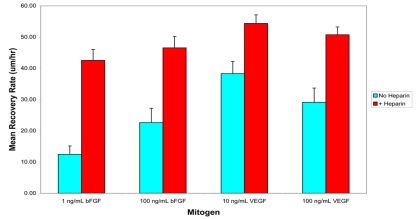
Effect of VEGF on pHEndEC recovery following injury *ex vivo*



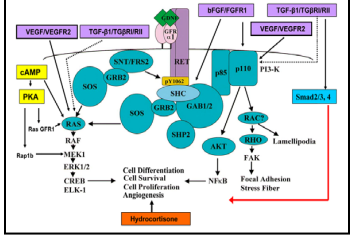
pHEndEC Wound Healing Assay



Effect of heparin on mitogen-induced pHEndEC wound healing



Human IVBNB formation/ repair pathways



CONCLUSIONS

VEGF, in the presence of heparin, is a potent inducer of pHEndEC proliferation and angiogenesis *ex vivo*.

bFGF and hydrocortisone also induce pHEndEC proliferation. GDNF has a minor effect on proliferation, while TGF-β1 inhibits pHEndEC proliferation.

bFGF also induces pHEndEC angiogenesis under the influence of Matrigel at a lower molar concentration than VEGF (2.6 pM vs. 581 pM).

pHEndEC wound healing is a sequential process: 0-4 hours: no change; 4-18 hours: endothelial cell migration; 18-30 hours: endothelial cell proliferation. There is an autocrine effect that contributes to endothelial cell migration.

VEGF enhances rate of pHEndEC wound healing (> bFGF and hydrocortisone) from 4-18 hours, and is the only mitogen capable of inducing complete wound recovery by 30 hours.

VEGF may play an active role in human blood-nerve barrier endothelial cell growth, differentiation to form microvessels and recovery following peripheral nerve injury. Autocrine factors may also contribute to endothelial cell migration following injury.

Redundant effects on BNB cell growth and angiogenesis may be necessary *in vivo*. VEGF seems critical to post-injury endoneurial endothelial cell proliferation required for wound closure.

VEGF could promote restoration of human blood-nerve barrier structure and integrity following peripheral nerve injury.

Further Reading:

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