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Angaben zur Veröffentlichung / Publication details:

Keshavarz, Maryam, Ali Mostafaie, Kamran Mansouri, Yadollah Shakiba, and Hamid Reza Mohammadi Motlagh. 2009. "Inhibition of corneal neovascularization with propolis extract." *Archives of Medical Research* 40 (1): 59–61. https://doi.org/10.1016/j.arcmed.2008.10.004.

Inhibition of Corneal Neovascularization with Propolis Extract

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Introduction

Corneal neovascularization is a complication of many entities including infectious keratitis, corneal trauma, alkali injury, and contact lens wear (1). Corneal neovascularization can present a serious problem for some patients who may experience glare, photophobia, or visual loss secondary to corneal scarring and lipid deposition (2). Corneal avascularity requires low levels of angiogenic factors and high levels of anti-angiogenic factors under basal conditions. Rupture of this homeostasis may occur in the pathogenesis of corneal neovascularization (3). Several anti-angiogenic agents have been characterized to inhibit corneal neovascularization in recent years in animal models. Fumagillin analogs (4), thalidomide (5), cyclosporine A (6), and oral shark cartilage (7) all exhibit anti-angiogenic activity in cornea. Tight regulation of corneal neovascularization helps maintain the transparency and immune privilege of the cornea (8).

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Mechanism of Neovascularization

Angiogenesis, the formation of new blood capillaries from pre-existing capillaries, can be separated into several main steps including digestion of basement membrane and extracellular matrix (ECM), migration, proliferation, and rearrangement of endothelial cells to form new blood vessels (9). Currently anti-angiogenic strategies are based on inhibition of endothelial cell adhesion and migration and interference with metalloproteinase (10–12). Matrix metalloproteinases (MMPs) and angiogenic growth factors are the main factors that modulate neovascularization. MMPs can degrade the basal membranes and ECM surrounding the sprouting capillaries. On the other hand, angiogenic growth factors, vascular endothelial growth factor (VEGF) and fibroblast growth factor stimulate proliferation and migration of vascular endothelial cells (13). Many investigations have supported a causal role for VEGF in corneal neovascularization (14–20). VEGF overexpression in cornea stimulates proteolytic activities, proliferation, migration, and tube formation of endothelial cells and initiates formation of new blood vessels (3). Interestingly, requirement of VEGF in corneal angiogenesis was shown by the inhibition of neovascularization after stromal

implantation of an anti-VEGF blocking antibody in a rat model (21,22). At least two VEGF inhibitors are in advanced clinical development for ophthalmic disease, Lucentis (rhuFab V2; Genetech, Inc., San Francisco, CA) and Macugen (pegaptanib sodium; Eyetech Pharmaceuticals, New York, NY) and more are poised to enter to clinical practice (23).

MMPs are a tightly regulated family of zinc-dependent endopeptidases capable of degrading all components of the ECM and basement membrane (24). Several MMPs are believed to be important in angiogenesis, but particular interest has been focused on the MMP-2 and MMP-9 (25). Several studies have shown that MMP-2 expression is upregulated in corneal neovascularization (26−29). Two recent studies investigated corneal neovascularization in MMP-2-deficient mice compared with wild-type mice and showed statistically significant delay of neovascularization in MMP-2 deficient mice (30,31). Another study reported that MMP-2 is required for optimal experimental choroidal neovascularization in a mouse model (32).

Propolis Extract and Angiogenesis

Together these data indicate that proliferation, migration and capillary tube formation are necessary steps for corneal neovascularization. Consequently, any mechanism that interferes with angiogenesis steps or angiogenesis factors like VEGF or MMPs may limit the visual loss associated with corneal neovascularization. Therefore, we propose the hypothesis that local administration of propolis extract may be effective in corneal neovascularization treatment. Propolis, a sticky material that honeybees collect from living plants, has been reported to have multiple biological effects (33−39). The postulated role of propolis extract in treatment of corneal neovascularization rests on its ability for suppressing angiogenesis. Several studies obviously showed that propolis extract and its fractions, especially artepillin C and caffeic acid phenyl ester, are effective in cancer treatment because of their ability to induce apoptosis and reduce secretion of major stimulatory factors involved in cell proliferation and angiogenesis (37−47). Hepşen et al. (39) reported that topical application of a water extract of propolis has an inhibitory effect on corneal neovascularization in rabbit's cornea injured by silver nitrate cauterization. They suggested that the inhibitory effect of propolis is partially due to the activity of both cyclo- and lipo-oxygenase. Another recent study on human umbilical vein endothelial cells showed that propolis extract can 1) inhibit proliferation of endothelial cells, 2) reduce endothelial cell migration and 3) inhibit HUVEC capillary tube formation in a dose-dependent manner (43). Moreover, it has been reported that oral administration of propolis extract suppressed tumor-induced angiogenesis *in vivo* (43). Furthermore artepillin C component alone can suppress angiogenesis in *in vivo* and *in vitro* models, and caffeic acid phenyl ester can inhibit MMP-2, MMP-9 and VEGF activity (38,40,42,43). These data strongly suggest that propolis extract and its components can treat corneal neovascularization by inhibiting critical steps in angiogenesis such as proliferation, migration, tube formation and inhibition of involving factors like VEGF and MMP2, MMP-9 secretion. To test this hypothesis, we propose local use of propolis extract as ophthalmic drops in corneal neovascular states such as alkali burn, infections, stem cell deficiency, stromal keratitis, and corneal graft rejection. Before clinical application, the propolis extract and its responsible components should be tested in appropriate animal models of corneal neovascularization.

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