Subconjunctival Bevacizumab in corneal neovascularization

Thesis
Submitted for partial fulfillment of MS degree in ophthalmology

By

Sara Maher Al Warraky
M. B., B. Ch. 2009
Resident of Ophthalmology
Cairo University- Kasr El Ainy Hospital

Under supervision of

Prof. Dr. Emad Abd El Aal Sawaby
Professor of Ophthalmology, faculty of medicine, Cairo university

Dr. Ahmed Hassanin Ebaid
Assistant Professor of ophthalmology, faculty of medicine, Cairo University

Dr. Malak Ismail El Shazly
Lecturer of ophthalmology, faculty of medicine, Cairo University
Faculty of medicine
Cairo University
Cairo, Egypt

2014

ABSTRACT

Our patients had deep stromal vessels so we could have achieved better results if we combined intrastromal bevacizumab injection with subconjunctival injection.

Our follow up period was only for 3 months we need more studies with longer follow up periods to see whether the new vessels will continue to regress or the effect is transient

Also we need more studies to evaluate the effects of

Key words

Subconjunctival Bevacizumab in corneal neovascularization

First of all, I wish to express my sincere thanks to ***Allah*** for *his care and generosity throughout of my life.*

I would like to express my sincere appreciation and my deep gratitude to ***Prof. Dr. Emad Abd El Aal Sawaby,*** *Professor of Ophthalmology, Faculty of Medicine Cairo University for his faithful supervision and guidance.*

I am also deeply indebted to ***Dr: Ahmed Hassanin Ebaid,*** *Assist Professor of Ophthalmology, Faculty of Medicine Cairo University for the tremendous effort he has done in the meticulous revision of this work.*

Special thanks to ***Dr. Malak El Shazly*** *lectrure of ophthalmology, Faculty of Medicine, Cairo university for her great helpful support.*

At last, I am indebted for my family

Sara Maher

Contents

Title	Page
List of Abbreviation……	I
List of Tables ……....	II
List of Figures……	III
Introduction……	1
Aim of the Work……..…….……	4
Review of Literature ……...……	
- Angiogenesis and Vasculogenesis of The Eye	5
- Corneal neovascularization & Anti VEGF	18
- Avastin…A Closer View	39
Patients and Methods ……...…...……	52
Results ……..……	55
Discussion ……...……..…	61
Summary & Conclusion ……...…	71
References ……	73
Arabic Summary ……...…	—

List of Abbreviations

Abb.	Meaning
AdPEDF	Adenoviral vector delivered intravitreal PEDF
AMD	Age- related macular degeneration
Ang1,2	Angiopoietins 1,2
bFGF	Basic fibroblast growth factor
CNV	Choroidal neovascularization
CSP	Compound sterile products
ECM	Extra-cellular matrix
EPo	Erythropoietin
FDA	Food and Drug Administration
HIF-1a	Hypoxia inducible factor-1a
IGF	Insulin like growth factor
IL 8	Interleukin 8
MMPs	Matrix metalloproteins
NOS	Nitric oxide synthtase
NV	Neovascularization
PDGF	Placental derived growth factor
PDR	Proliferative diabetic retinopathy
PEDF	Pigment epithelium derived factor
PlGF	Placental growth factor
PTK/ZK	Protein tyrosin kinase inhibitor/ZK
RVO	Retinal vein occlusion
SiRNA	Silencing RNA
SVEGFR-2	Soluble VEGF receptor-2
TGF B	Transforming growth factor B
VEGF	Vascular endothelial growth factor

List of Tables

Table	Title	Page
Tab. (1)	Summary of Anti-VEGF Applications and Properties	26
Tab. (2)	Extent of neovascularization in clock hours in 20 eyes with different corneal disorders before and after a single 2.5-mg subconjunctival bevacizumab injection over a 3-month follow-up.	55
Tab. (3)	mean and SD of study parameters	56

List of figures

Figure	Title	Page
Figure (1)	The receptors for vascular endothelial growth factor	10
Figure (2)	A photo of Avastin vial and package.	39
Figure (3)	Avastin (bevacizumab) molecular structure	40
Figure (4)	Corneal neovascularization caused by long-standing central corneal ulcer (A) before and (B) 3 months after a single 2.5-mg (0.1-ml) bevacizumab injection.	58
Figure (5)	Corneal neovascularization caused by long-standing paracentral corneal ulcer (A) before and (B) 2 weeks after a single 2.5-mg (0.1-ml) bevacizumab injection.	59
Figure (6)	Corneal neovascularization caused by long-standing corneal abcess (A) before and (B) 2 weeks after a single 2.5-mg (0.1-ml) bevacizumab injection.	60

Introduction

Ocular neovascularization (NV) is the primary cause of blindness in a wide range of ocular diseases, such as diabetic retinopathy (DR), agerelated macular degeneration (AMD), retinopathy of prematurity, central and branch retinal vein occlusion (CRVO and BRVO), infectious keratitis, trauma and various inflammatory ocular diseases. The avascular feature of certain ocular compartments, including the cornea, lens, vitreous and outer retina, is a unique anatomical characteristic to meet the requirement for normal visual function. [1]

In healthy adults, the fully developed ocular vascular system is in a quiescent status (also known as homeostasis), which is tightly controlled by the balance between the angiogenic stimulating factors, such as vascular endothelial growth factor (VEGF) and angiogenic inhibitors, such as pigment epithelium-derived factor (PEDF) .[2]

The homeostasis in the eye is important to keep the structural and functional integrity of the ocular vascular system. In a variety of pathological conditions, such as

hypoxia, ischemia, inflammation, infection and trauma, the balance between angiogenic stimulators and angiogenic inhibitors is disturbed, leading to the formation of new vessels.[2]

The abnormal growth of new vessels into the avascular compartments will cause disturbance of light transmission. Moreover, these new vessels have abnormal cellular components and lack basement membrane and pericytes, and thus, are very fragile, leaking, and susceptible to hemorrhage. The resultant hemorrhage or accumulation of blood in ocular cavities, such as anterior chamber and vitreous, leads to the further blockage of light transmission and decrease of visual acuity.

In addition, the leakage and accumulation of fluid, protein and lipid result in edema and exudation, which will cause visual damage by decreasing corneal transparency and impairment of the structure and function of the retinal neurons. [1]

Among the different tissues in the eye, the retina, choroid and cornea are the most frequent sites of ocular neovascularization (NV). Iris NV is also seen in severe

ischemic ocular diseases and neovascular glaucoma. The exact mechanism underlying the pathogenesis of ocular NV is not yet well understood, and as a consequence, no specific and satisfactory therapy is available for ocular NV at present, although laser treatment does show some effects on the control of retinal and choroidal neovascularization (CNV). Although , a number of studies provided increasing evidence demonstrating that the imbalance between the angiogenic stimulating factors and angiogenic inhibitors is the major causative contributor to the angiogenesis induced by various insults, such as hypoxia, ischemia, inflammations or tumors. [2]

Angiogenic inhibitors alone or in combination with other existing therapies are, therefore, promising in the treatment of ocular NV in the near future. The recent progress in the studies on the mechanisms and treatment of ocular NV are focusing on the implication and therapeutic potential of endogenous angiogenic inhibitors in ocular NV. [2]

Aim of the work

To evaluate the therapeutic effect of subconjunctival Bevacizumab on corneal neovascularization.

Angiogenesis and Vasculogenesis of The Eye

Generally, there are two types of new blood vessel formation during the embryonic development and postnatal vascular remodeling.

One is vasculogenesis, which is referred to as the process of de novo new vessel formation by the aggregation and differentiation of vascular endothelial precursor cells (EPC), known as angioblasts, which occurs during the very early stage of embryogenesis.

Another type is angiogenesis, which is referred to as the formation of new blood vessels from pre-existing ones, responsible for the extension and remodeling of the preexisting capillary network. Although these two forms are different in many aspects, such as the source of endothelial cells (EC), they also share some similarities, e.g., they are both dependent on the activation of VEGF receptor-2, and involved in both embryonic vascular development and postembryonic vascular remodeling. [3]

During the development of the embryonic vascular system, vasculogenesis is believed to be the major form

responsible for the formation of new vessels in most tissues.[3] However, angiogenesis has also been shown to play an important role in the embryonic blood vessel formation. For example, a study suggests that in human fetal retina, the formation of primordial vessels in the central retina is mediated by vasculogenesis, whereas, angiogenesis is responsible for increasing vascular density and peripheral vascularization in the inner retina. In contrast, the outer plexus and the radial peripapillary capillaries are formed by angiogenesis only. [4]

In postembryonic life, angiogenesis is the primary form responsible for the vascular remodeling during physiological events, such as reproduction, wound healing, and bone repair.

Angiogenesis is a complex, step-wise process characterized by a combination of sprouting of new vessels from the sides and ends of preexisting ones. The earliest step initiating angiogenesis is the vasodilation of existing vessels accompanied by increased vascular permeability [5,6] .The increase of vascular permeability leads to the extravasations of the plasma proteins, such as fibrin, growth factors and inflammatory factors into the surrounding area. The accumulated plasma proteins form a

supporting structure for the subsequent endothelial cell migration.

The growth factors and inflammatory factors further activate the enzymes to degrade extracellular matrix (ECM). The degradation of ECM not only makes room for EC to migrate, but also releases the angiogenic factors which anchor in the matrix, such as VEGF, basic fibroblast growth factor (bFGF), and insulin-like growth factor (IGF-1). These growth factors further promote the activation of EC, which migrate from the preexisting vessels and form sprouting tube.[7]

Vascular Endothelial Growth Factor

VEGF is a 45 kD glycoprotein whose mRNA is alternatively spliced. A long mRNA for VEGF is produced by the nucleus of the cell then modified by the cell with production of one of the several different length proteins that represent splice variants of VEGF.

The four best characterized splice variants of VEGF have different properties. The two smaller isoforms, which are defined by the number of amino acids, are soluble and