Anti-angiogenic/inflammatory Behavior of Mushroom *Ganoderma Lucidum* Extract could be Effective for Treatment of Corneal Neovascularization: A Hypothesis

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A B S T R A C T

Neovascularization of the normally avascular cornea was associated with a notable increase in the expression of the major pro-angiogenic factors, inflammatory cytokines and proteases. The data supporting a causal role for vascular endothelial growth factor (VEGF), inflammatory cytokines and matrix metalloproteinase (MMPs) are extensive. Anti-angiogenic/anti-inflammatory therapy is considered as a possible tool for controlling corneal neovascularization. Mushroom *Ganoderma Lucidum* extracts containing materials that significantly reduced the number of newly formed vessels and expression of inflammatory cytokines and angiogenic factors production from various cells. This study aimed to elucidate anti-angiogenic activity of *Ganoderma Lucidum* extract for inhibiting corneal neovascularization and restoration of corneal neovascularization and restoration.

Introduction

Corneal neovascularization is a serious condition that can lead to a profound decline in vision. The abnormal vessels block light, cause corneal scarring, compromise visual acuity, and may lead to inflammation and edema. Corneal neovascularization occurs when the balance between angiogenic and anti-angiogenic factors is tipped toward angiogenic molecules (Figure 1) [1]. Corneal clarity is the direct result of a very intricate balance between its cellular components and layers [2]. The cornea is also immunoprivileged, which is a major protective feature of the highly organized structure of the eye and also contributes to the high success rates of corneal transplants [1, 3]. The corneal neovascularization can cause a serious problem for some patients who may experience glare vision, photophobia, or visual loss secondary to corneal scarring and lipid deposition [4]. Presence of new vessels in cornea can compromise clarity and thus vision. Corneal vascularity 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 [1, 4, 5]. Angiogenesis was first studied by Judah Folkman in his quest for a cure for cancer. His animal studies demonstrated the dependence of tumors on NV for growth. The corneal neovascularization is defined as new vascular structures derived from angiogenic processes in previously avascular areas [1, 4, 6]. Angiogenesis, the formation of new capillaries from preexisting vessels, is a multistep event involving degradation and remodeling of the underlying basement membrane and the surrounding extracellular matrix (ECM) with subsequent proliferation and migration of vascular endothelial cells into the tissue to be vascularized. Angiogenesis is regulated by matrix metalloproteinase (MMPs) that can degrade the basal membranes and extracellular matrix surrounding the sprouting capillaries and angiogenic growth factors [6], vascular endothelial growth factor (VEGF), and fibroblast growth factor that stimulate proliferation and migration of vascular endothelial cells [1, 2, 4, 6, 7]. Current treatments for corneal neovascularization include topical corticosteroid and non-steroid anti-inflammatory medications, photodynamic therapy, laser photocoagulation, fine needle diathermy, and conjunctival, limbal, and amniotic membrane transplantation [5, 6, 8, 9]. *Ganoderma lucidum* is a medicinal mushroom that has been used as a Chinese traditional folk remedy for centuries. Triterpenoids extracted or isolated from *Ganoderma lucidum* have been reported to be responsible for many of the pharmaceutical activities of *Ganoderma lucidum* [10].

A *Ganoderma lucidum* extract containing a mixture of lanostanoid triterpenes (0.25 mg/mL) was found to induce autophagic cell death in HT-29 cells, while another triterpenoid enriched extract induced apoptosis in SW620 cells at a concentration of 50 μM [11]. Two major types of triterpenoids in *Ganoderma lucidum* are ganoderic acids (C30) and lucidenic acids (C27) and the total triterpenoid content in *Ganoderma lucidum* varies from 0.6 to 11 mg/g dry powder [12, 13]. These triterpenoids were reported to influence metabolic states including exhibiting anti-diabetic properties and regulating inflammatory pathways in cell culture [14, 15].

Fig. 1. (Left) Slit-lamp photographs of patient described corneal neovascularization (Right) *Ganoderma lucidum* ("Lingzhi"), a Chinese medicinal mushroom.

Mechanism of neovascularization

Corneal neovascularization has been one of the most extensively investigated external eye disorders [6, 16]. Corneal neovascularization is a main cause of loss of vision and blindness in a wide range of corneal diseases worldwide. It is estimated that 4% of the US population has corneal neovascularization and every year 1.4 million patients in the US may develop corneal neovascularization [1, 16, 17]. Corneal neovascularization can affect the prognosis of corneal transplantation [4, 6, 16, 17]. It has been reported that 20% of corneal specimens obtained during corneal transplantation show the histopathological evidences of neovascularization [16, 18]. During corneal injury, angiogenic factors are released from corneal epithelial and stromal cells as well as infiltrating immune cells like macrophages. In fact, the balance between angiogenic and anti-angiogenic factors is shifted towards angiogenic molecules in the corneal neovascularization [17]. Angiogenesis is the growth of blood vessels from the existing vasculature. The field of angiogenesis has grown enormously in the past 30 years, with only 40 papers published in 1980 and nearly 6000 in 2010 [19]. There are several evidences that support a casual role for VEGF in corneal neovascularization [4, 20]. It has been shown that VEGF expression is significantly higher in vascularized corneas than in normal corneas. VEGF over expression in the cornea promotes several steps of angiogenesis, including proteolytic activities, proliferation, migration, and tube formation of endothelial cells [4, 6, 16, 17, 21].). The critical role of VEGF in the pathogenesis of neovascularization confirmed by studies that showed anti-VEGF agents can inhibit corneal neovascularization [2-4, 6, 9, 10, 14, 15, 17]. Matrix metalloproteinase (MMPs) are considered as other key mediators of angiogenesis. They are a family of zinc-dependent enzyme essential for degradation of extravascular matrix (ECM) and vascular basement membrane during angiogenesis [16, 22, 23]. Several MMPs are believed tube important in angiogenesis, but particular interest has been focused on MMP-2 and MMP-9. MMP-2 is one of the earliest and its sustained activity require for successful corneal vascularization [16, 20, 22, 24, 25]. The overexpression of MMP-2 during corneal neovascularization has been confirmed by numerous investigations [17, 22-25]. MMp-9 is overexpressed in vascularized cornea and plays a role in wound healing. There is high level of MMP-9 in the tear of patient with recurrent corneal ulcers [16, 23]. Taken together, these data indicated that both MMP-2 and MMP-9 have roles in the disruption of basement membrane, integrity of the corneal epithelium and corneal neovascularization; thus targeting them may be a useful therapeutic option for inhibition of angiogenesis in the cornea. Since inflammatory and infectious diseases are major etiologies of corneal neovascularization [26], corneal NV is almost always accompanied by inflammation [17, 23, 26]. However, pervious investigations showed that the corneal cell secret various inflammatory cytokines such as that are involved in ocular surface disease. Inflammatory cytokines such as IL-1, IL-6, IL-8, TNF-α, TGF-β, inflamm- atory mediator nitric oxide (NO), prostaglandin E2 (PGE2) and cyclooxygenase 2 (COX 2) [4, 10, 11, 16, 21, 23, 26, 27].

Evaluation of hypothesis

Corneal neovascularization was associated with angiogenic steps and inflammatory cytokines [16]. Inflammation and angiogenesis are two tightly linked processes [16, 19]. In fact many inflammatory mediators have a significant pro-angiogenic effect while pro-angiogenic factor, as VEGF or angio-protein up-regulates several pro-inflammatory pathways leading to leukocytes recruitment, infiltration and secretion of inflammatory mediators. Major pro-inflammatory cytokines like TNF-α, IL-6, IL-17, IL-1 have direct or indirect (mostly via VEGF) pro-angiogenic activity. Under inflammatory stimuli, increased angiogenesis provides wider endothelial surface that permits cellular trafficking from blood stream to cornea [16, 19, 28] consequently, any mechanism that could strongly depress angiogenesis steps on angiogenic factors and inflammatory factors may limit the visual loss

associated with corneal neovascularization [10, 11, 15-17, 19, 20, 27, 28]. Therefore, we propose the hypothesis that local administration of mushroom *Ganoderma Lucidum* extract may be effective in corneal neovascularization treatment. *Ganoderma Lucidum* is a well-known and important medicinal mushroom, widely used in traditional Chinese medicine [12]. The *G. Lucidum* has been used in various human diseases such as hepatitis, hypertension, arthritis, bronchitis, and tumorigenic disease[12, 14]. Indeed, pervious pre-clinical studies have shown *G. Lucidum* have anti-inflammatory and analgesic effects [14]. Furthermore, triterpenes isolated from the mushroom *G. Lucidum* suppressed inflammatory response (TNF-α, IL-6, NO, prostaglandin E2 (PE2), NF-κβ and cyclooxygenase2 (cox-2) [10, 12]. These data strongly suggest that *G. Lucidum* extract and its components can tear corneal neovascularization by inhibiting critical steps in angiogenesis such as proliferation, migration, and tube formation via inhibiting the secretion of involving factors like VEGF, bFGF, anderogen, and MMP-2, MMP-9 [10, 12]. Another mechanism was that the extract could interference with inflammatory cytokine as noted above. These data collectively indicate that *G. Lucidum* extract is a very good choice for inhibition of corneal neovascularization due to its strong anti-angiogenic and anti-inflammatory activities. However before clinical application, *G. Lucidum* should be tested in appropriate animal models of corneal neovascularization.

Acknowledgement

Conflict of interest statement

Authors certify that no actual or potential conflict of interest in relation to this article exists.

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