

# Effect of medroxyprogesterone and doxycycline on vascular endothelial growth factor (VEGF) expression and corneal neovascularization in corneal alkali trauma



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## ABSTRACT

Ocular chemical trauma is a true emergency in ophthalmology. Chemical trauma on the ocular area may disturb vision, reduce the quality of life, and functional state. Prompt and adequate treatment is needed to prevent further complications, such as a decrease in vision due to corneal neovascularization. Treatment started with immediate eradication of the chemical agent by continuous rinsing and followed by inflammation and epithelial defect control. Medroxyprogesterone and doxycycline are two common treatments used in ocular chemical trauma. We speculate that both medications may influence VEGF expression and corneal neovascularization in corneal alkali trauma. It was demonstrated that medroxyprogesterone and doxycycline reduce the expression of VEGF, hence, reducing corneal neovascularization.

**Keywords:** medroxyprogesterone, doxycycline, VEGF, corneal neovascularization, corneal alkali trauma.

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## INTRODUCTION

An ocular chemical burn is a true emergency in which the ocular surface is in contact with a chemical agent. The incidence is 2.2-13% of all ocular trauma emergencies, often found in children <5 years old and adults due to domestic accidents.<sup>1</sup> The prognosis of the ocular chemical burn depends on the type of agent (acid, base, or irritant) as well as the length of the period of chemical agent contact with the ocular surface. It may reduce patients' vision, quality of life (QoL), functional state, and the well-being of their mental and financial state.<sup>2</sup>

As mentioned previously, chemical ocular burn is divided based on the type of the agent: acid, base, or irritant. The pathophysiology of alkali chemical burn is due to fat saponification property which allows the chemical agent to penetrate deeper into the ocular tissue and cause further deterioration.<sup>3</sup> Based on their severity, ocular chemical burns can be classified based on the Roper-Hall classification or Dua classification. The Roper-Hall classification is based on cornea injury and limbal ischemia

fractional extension. This classification is commonly used in clinical practice. Dua classification is based on limbal involvement duration and bulbar conjunctival involvement percentage. Dua classification is considered to be more accurate in severe cases compared to the Roper-Hall classification.<sup>1</sup>

The essential therapy of ocular chemical burn is prompt ocular irrigation to remove the offending agent.<sup>4</sup> Albeit low osmolarity solution or solution with buffer is preferred in ocular irrigation, when not readily provided, even tap water may become the best solution.<sup>5</sup> After offending agent eradication, management is focused on corneal re-epithelialization, inflammation control, and complications prevention. Agents such as doxycycline, a tetracycline antibiotic, and medroxyprogesterone, progestin type, are recommended as part of ocular chemical burn therapy. Doxycycline is used in epithelial defect control while medroxyprogesterone is used in inflammation control.<sup>1</sup>

Proper medication is needed in terms of complication prevention, such as reduced vision. One of the

causes of reduced vision in the ocular chemical burn is the presence of corneal neovascularization. The cornea is an avascular part of the eye. Vascularization in this area causes the blocking of light and reduced vision.<sup>6</sup> Neovascularization, composed of angiogenesis and vasculogenesis, is mediated by Vascular Endothelial Growth Factor (VEGF, also known as VEGF-A), the major intraocular neovascularization mediator.<sup>7</sup> Hence, it is important to understand the effect of medroxyprogesterone and doxycycline on VEGF expression and corneal neovascularization in corneal alkali trauma.

## Corneal Alkali Trauma

An ocular chemical burn is an ophthalmic emergency due to the contact of the chemical agent with the ocular surface. The chemical agent might be an alkali, acid, or irritant type.<sup>1</sup> Thermal and chemical burns are estimated around 8-18% of ocular trauma, being the most frequently reported ocular injury causes.<sup>8,9</sup> It constitutes 2.2-13% of all ocular trauma emergencies. The highest incidence

was found in children <5 years old due to domestic accidents with household cleaning products. The incidence in the adult population is mostly due to domestic and work accidents.<sup>1</sup> Severe cases of ocular chemical injury have a significant impact. It may affect patients' vision, quality of life, functional status, as well as mental and financial well-being.<sup>2</sup>

The pathophysiology of chemical burns shares few common mechanisms despite the type of the agent of cause. The initial phase of chemical ocular trauma is started by the invasion of the inflammation cells. These cells produce detergent enzymes that deteriorate the ocular structures. Afterward, the surrounding healthy tissue will start to regrow (scarring phase). Meanwhile, the area of the destructed vascular network will form an ischaemic lesion. In alkali burns, the anion causes tissue softening by saponification of the fat and lipids. This increase penetration of the chemicals into deeper tissue. Therefore, the rate of penetration in alkali burns is compared to acid burns. At pH >11.5, complete and irreversible are commonly seen.<sup>3</sup>

The symptoms range from pain, tearing, and conjunctival hyperemia, to subconjunctival hemorrhage as well as chemosis. Mild ocular burn is evident by the presence of superficial punctate keratitis. Severe burns can be evident by corneal opacification and edema which decrease the iris and lens. More than 50% epithelium loss and perilimbal ischemia are typical signs of a severe burn.<sup>3</sup> The degree of severity is used to classify the

chemical burn injury, either using the Roper-Hall classification or the Dua classification (Tables 1 and 2).

The ocular damage of alkali burn is proportionate with the penetration rate of the agent. The penetration rate differs depending on the composition of the offending agent. A fast penetration rate of under 3 minutes (ammonium hydroxide) may cause severe ocular chemical burns. A penetration rate of 3-5 minutes can be caused by sodium hydroxide, caustic soda, bleach, and NaClO which can be found in cleaning products, disinfectants, and drain plungers. Penetration rate >5 minutes by potassium hydroxide, caustic potash, and KOH can be found in cleaning products, industrial products, and agricultural fertilizers. Calcium hydroxide, slaked lime, and Ca(OH)<sub>2</sub> which are used in cement, cast, and mortar have slower penetration rates. The toxicity increases when there is a retention of the particles. This kind of composition may produce thermal burns when interacting with water. The slower the penetration rate, the milder the burns is.<sup>1</sup>

Management can be divided into non-operative and operative management. It can be divided into three phases with various objectives and therapeutic measures. In the acute phase (right after the first week), removing the offending agent and normalizing the pH is the main target.<sup>1</sup> It can be achieved by continuous irrigation. Prompt irrigation is essential to eradicate the offending agent.<sup>4</sup> Different solutions have different effectiveness. The recommended rinsing solution is a low

osmolarity solution that can promote corneal edema, diluting chemical agents in the corneal stroma as well as minimizing the agent's penetration. Some of the low osmolarity solutions are tap water, ringer's lactate, and NaCl 0.9%. Another option is the solution with a high buffer capacity, which is able to absorb excess alkali or acid in order to maintain pH. Some good buffering solutions are phosphate-buffered saline, and EDTA.<sup>5</sup> Although studies showing different rinsing solutions showed different effectivity, the solution is the closest to hand.<sup>1</sup>

The next phase is the intermediate/early reparative phase (1-3 weeks). The aim of this phase is promoting corneal re-epithelialization, controlling inflammation as well as preventing complications. Afterward, the management aims at epithelial defects. Several agents have been used such as artificial tears, fibronectin, and epidermal growth factor. Tetracycline is one of the agents used in controlling epithelial defects. In controlling inflammation, corticosteroid, progesterone derivatives, citrate, and non-steroidal anti-inflammatory drugs (NSAID). Complication prevention should be done at any stage of the disease. Complications such as secondary infection, intraocular pressure (IOP) disturbance, and pain might be evident.<sup>4</sup> The last phase is the late reparative phase. In this phase, the aim is to repair the affected ocular area, rehabilitate and preserve visual acuity as well as provide management of long-term complications.<sup>1</sup>

**Table 1. Roper-Hall classification.<sup>1</sup>**

| Grade | Cornea   | Conjunctiva             | Prognosis |
|-------|--|-------------------------|-----------|
| I     | Corneal epithelial damage                                      | No limbal ischemia      | Good      |
| II    | Corneal haze, iris details visible                             | <1/3 limbal ischemia    | Good      |
| III   | Total epithelial loss, stromal haze, and iris details obscured | 1/3-1/2 limbal ischemia | Guarded   |
| IV    | Cornea opaque, iris and pupil obscured                         | >1/2 limbal ischaemia   | Poor      |

**Table 2. Dua classification.<sup>1</sup>**

| Grade | Clinical findings (clock hours of limbal involvement) | Conjunctival involvement | Analogue scale      | Prognosis       |
|-------|---|--------------------------|---------------------|-----------------|
| I     | 0 clock hours   | 0%                       | 0/0%                | Very good       |
| II    | ≤3 clock hours  | ≤30%                     | 0.1-3/1-29.9%       | Good            |
| III   | 3-6 clock hours                                       | 30-50%                   | 3.1-6/31-50%        | Good            |
| IV    | 6-9 clock hours                                       | 50-75%                   | 6.1-9/51-75%        | Good to guarded |
| V     | 9-12 clock hours                                      | 75-99%                   | 9.1-11.9/75.1-99.9% | Guarded to poor |
| VI    | Total limbus (12 clock hours)                         | 100% (total conjunctiva) | 12/100%             | Very poor       |

### The effect of medroxyprogesterone and doxycycline on VEGF expression and corneal neovascularization

To understand the correlation of medroxyprogesterone and doxycycline to the VEGF expression and corneal neovascularization, it is important to understand wound healing and neovascularization in the cornea. Corneal healing is divided into four stages: homeostasis, inflammation, proliferation, and remodelling. The homeostasis phase immediately started after the trauma. In this phase, platelet activation occurs and is followed by Platelet-derived Growth Factor (PDGF), Transforming Growth Factor Beta-1 (TGF- $\beta$ 1), and Vascular Endothelial Growth Factor (VEGF) formation and release. These growth factors will prevent infection, and stimulate cell migration and proliferation of matrix-producing as well as matrix-degrading enzymes. During the first 24-48 hours (inflammatory phase) neutrophils and monocytes will start to migrate to the wound area and bind to the extracellular matrix protein. At 2-10 days post-trauma, the proliferative, there will be increased activity and the number of fibroblasts, angiogenesis, and granulation tissue formation. Afterward, the wound will go into the remodelling phase, which may continue for several years.<sup>10</sup>

In corneal wound healing, one of the matrix-degrading enzymes is a matrix metalloproteinase (MMP), a calcium- and zinc-dependent endopeptidase which function is to degrade several components of ECM. It is found in the process of corneal inflammation, neovascularization, epithelial regeneration as well as corneal wound healing. MMP may be overexpressed due to the IL-1, TGF- $\beta$ , and TNF- $\alpha$ , which lead to stromal scarring and corneal transparency loss. The MMP activity is controlled by Tissue Inhibitor Metalloproteinase (TIMP). Their MMP-TIMP ratio should be balanced to ensure proper wound healing.<sup>10</sup>

Neovascularization is composed of angiogenesis and vasculogenesis. Angiogenesis is the growth of new capillaries from existing capillaries; meanwhile, vasculogenesis is the growth of new vessels from endothelial progenitor cells (EPC).<sup>11</sup> Cornea is an avascular part of the eye in a healthy condition.

Corneal neovascularization is divided into superficial neovascularization, vascular pannus, and deep stromal vascularization. In a damaged cornea, the healing process starts with the corneal and limbal epithelium. The corneal limbus has the capacity to differentiate from the normal epithelium of the cornea. Defects may cause the cells to undergo apoptosis and abnormal repair. This may cause vision deterioration, irregular optical surface, weakened tensile strength as well as incompetent barrier function. Vision deterioration is caused by the presence of vessels in a supposedly avascular area, leading to blocking and diffracting light going into the eye.<sup>6</sup> In chemical ocular injury, neovascularization is related to the treatment of choice as well as patient prognosis. Post-chemical injury, angiogenesis starts from the edge to the center of the cornea. If the corneal edema is severe and extensive angiogenesis is evident in the early phase, it may cause severe vision impairment and increase the need for corneal transplantation.<sup>12</sup>

VEGF or VEGF-A is a major intraocular neovascularization mediator. It is a secreted growth factor peptide that belongs to a gene family which includes VEGF-B, -C, -D, E, and placental growth factor (PlGF).<sup>7</sup> VEGF is known to play a role in physiologic as well as pathologic angiogenesis. It promotes the proliferation, migration, and tube formation of vascular endothelial cells. VEGF plays a key role in inflammation, showed by the increase in vascular leakage and monocyte chemotaxis and B-cells production in mice.<sup>7</sup> It is related to hypoxia and reported to be elevated in several ocular diseases such as age-related macular degeneration (AMD) and diabetic retinopathy.<sup>13</sup>

The importance of VEGF in corneal neovascularization has been demonstrated. This is related to the mechanism of treatment of choice, the medroxyprogesterone and doxycycline. Medroxyprogesterone acetate (MPA) is a hormonal drug of progestin type, derived from 17- $\alpha$  hydroxyprogesterone. It works by binding to the progesterone receptor, which is found in corneal epithelium, stroma, and endothelial cells.<sup>10</sup> Progesterone mechanism of action in chemical ocular trauma is by

inhibiting collagenase activity as well as being an anti-inflammation.<sup>4</sup> The anti-inflammatory properties are due to its affinity to glucocorticoid receptors. In the case of corneal ulcers, the collagenase releases dependent- and independent-polymorphonuclear (PMN). MPA inhibits dependent PMN, therefore promoting collagen synthesis which is needed in corneal healing. MPA was also found to inhibit IL-1 $\beta$  which was followed by suppression of MMP-1, MMP-2, MMP-3, MMP-9, and p38 MAPK expression. The increase in MMP-3 induces ECM degradation, leading to the release of growth factors, for example, the VEGF, which then stimulates angiogenesis. The suppression of the previously mentioned MMP and p38 expression gives advantages in alkali ocular burn.<sup>10</sup> MPA was found to be as effective as prednisolone in depressing the corneal wound strength and collagen accumulation. It also reduces deep ulceration and perforation incidence.<sup>14</sup> Reduce corneal neovascularization and promote wound healing in the use of topical 1% MPA in the ocular chemical burn was demonstrated in previous studies.<sup>15,16</sup> The usage of tetracycline in ocular chemical injury had been quite established. Dan et al. demonstrate that oral doxycycline in alkali ocular burn rats may inhibit corneal neovascularization without dexamethasone-related side effects. It also showed that doxycycline prevents corneal neovascularization growth to the center of the cornea. This leads to corneal turbidity reduction and reduces the post-corneal transplantation rejection rates.<sup>12</sup>

Tetracycline is another medication that is often used in alkali ocular burns. The likelihood of ulceration in alkali ocular burn in rabbits decreases in proportion to the increased level of tetracycline.<sup>17</sup> Tetracycline may prevent collagenolytic corneal degeneration post-ocular chemical injury. Tetracycline mechanism of action in MMP inhibition is through several mechanisms such as gene expression restriction of the neutrophil collagenase and epithelial gelatinase, alpha 1-antitrypsin degeneration suppression, and scavenging of reactive oxygen species (ROS).<sup>17</sup> Doxycycline, one of the tetracycline antibiotics, exerts matrix

metalloproteinase (MMP) inhibition, independent of its bactericidal properties.<sup>4</sup> Su et al. in their study demonstrated that doxycycline inhibits corneal neovascularization induced by VEGF in vivo, in terms of the length and area of the vessels. The study showed that an MMP-independent mechanism through the PI3K/Akt-eNOS pathway is implicated in angiogenesis inhibition mediated by doxycycline.<sup>18</sup> Similarly, a study by He et al., showed that doxycycline downregulates MMP-9, MMP-2, and VEGF which leads to the inhibition of proliferation, migration, invasion, and vascular mimicry. VEGF, an angiogenic factor, may activate PI3K and stimulate the MMP precursor to form MMP.<sup>19</sup>

## CONCLUSION

Based on the aforementioned studies, it is demonstrated that medroxyprogesterone and doxycycline have effects on VEGF expression and corneal neovascularization in corneal alkali trauma. Medroxyprogesterone was found to suppress the expression of MMP-3, which led to reduced EMC degradation. This event decreases the release of growth factors such as VEGF. Doxycycline was also found to downregulate VEGF. VEGF is known to be the major angiogenesis mediator in the ocular. When VEGF is suppressed neovascularization in the post-trauma ocular, neovascularization will decrease as well. In short, the usage of doxycycline and medroxyprogesterone may help in reducing VEGF expression and corneal neovascularization.

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## CONFLICT OF INTEREST

In this review article there is no potential conflict of interest.

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## AUTHOR'S CONTRIBUTION

All authors contributed equally to this review article

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