Effect of Asiatic acid and dexamethasone administration to interleukin (IL)-1 expression and number of mononuclear cells in extraocular muscle post-strabismus surgery

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ABSTRACT

Strabismus surgery aims to improve visual function, binocular vision, and cosmetics by fixing the position of the eyeball. However, inflammation after surgery can lead to fibrous tissue formation. Dexamethasone is routinely used as an anti-inflammatory but has unwanted side effects. On the other hand, Asiatic acid, the most important saponin constituent of Centella asiatica, has anti-inflammatory and anti-fibrous properties. It has been widely used as a traditional medicine plant in China, India, and Indonesia. The findings show that Asiatic acid has been shown to affect wound healing after strabismus surgery positively. Asiatic acid inhibits inflammation, increases cell proliferation, and inhibits fibrosis. It promotes wound healing by increasing the strength of the incision wound, epithelialization, keratinization, and angiogenesis. Overall, using natural compounds like Asiatic acid in wound healing is promising and should be explored further. It can be a safer and more effective alternative to dexamethasone, which has known side effects. However, further studies are required to determine the optimal dosage and administration method of Asiatic acid for post-operative strabismus wound healing. The results of this study are expected to be the basis for selecting alternative therapies to reduce inflammation and fibrosis after strabismus surgery.

Keywords: Asiatic acid, dexamethasone, inflammation, strabismus surgery, wound healing.

INTRODUCTION

Strabismus surgery aims to fix the position of the eyeball in order to improve visual function, binocular vision and cosmetics. The principle of strabismus surgery includes weakening the stronger working muscles and strengthening the weaker muscles. After strabismus surgery, inflammation can occur and form fibrous tissue. One of the responses of inflammation is the emergence of mononuclear cells and various pro-inflammatory cytokines, including interleukin (IL)-1. Dexamethasone sub-conjunctiva is routinely used after strabismus surgery as an anti-inflammatory. Dexamethasone, a synthetic adrenal corticosteroid with strong anti-inflammatory properties, is crucial for suppressing unwanted immune system responses. However, there are some unwanted side effects of using Dexamethasone. A study by Feroze and Khazaeni (2022) stated that steroid-induced ocular hypertension or glaucoma could occur after topical, periocular, or intraocular administration of Dexamethasone. Another study by Zhang et al. (2012) stated that periocular Dexamethasone injection could also cause transient hyperglycemia in diabetic patients after vitreoretinal surgery. The World Health Organization (WHO) states that traditional medicine is widely practiced in developing countries. Centella asiatica, known in Indonesia as pegagan, is a medicinal herb of the Umbelliferae family that is widely used by the public and grows in countries with the tropical climate. Centella asiatica has primary active compounds in the form of triterpenoids, such as Asiatic acid, Asiaticocide, madecassoside, and muriatic acid. Asiatic acid is the most important saponin constituent of Centella asiatica. This compound has a broad spectrum of biological activity, such as anti-inflammatory, anti-fibrous, antioxidant, and neuroprotective. Research on the effectiveness and optimal dose of Asiatic acid for eyes is still limited. The global prevalence of strabismus ranges from 0.14% to 5.65%. Recent studies on postoperative strabismus fibrosis have been carried out. After strabismus surgery, Bani et al. (2022) showed that topical diclofenac could reduce inflammation at the tendon-sclera attachment site. A similar study by Ummah et al. (2018) using Triamcinolone acetonide also showed similar results. Various research has been conducted on the use of Asiatic acid as an anti-fibrous. Tang et al. (2012) showed that oral administration of Asiatic acid as much as 8mg/kgBW in model rats with CC14-induced-fibrosis liver had an anti-fibrous effect by inhibiting Tumor Growth Factor (TGF)-β signaling in vivo and in vitro studies. The study by Bian et al. (2013) showed that Asiatic acid was one of the active components of Centella asiatica sp. responsible for keloid management.
Adtani et al. (2016) found that Asiatic acid significantly downregulated arecoline-induced fibrosis in human buccal fibroblasts by inhibiting TGF-β signaling. The study by Dong et al. (2017) stated that Asiatic acid suppressed inflammatory cell infiltration in bronchoalveolar lavage fluid (BALF) and proinflammatory cytokines in bleomycin-induced lung tissue specimens. Asiatic acid also suppressed TGF-β1 expression in lung tissue, accompanied by a decrease in type I collagen, type III collagen, and matrix metalloproteinase (MMP).1,3–7

Inflammation not handled properly in the wound healing process is an obstacle to the success of postoperative strabismus. Scar remodeling after strabismus surgery contributes 10% to reoperation and 50% to overcorrection. Local complications in strabismus surgery were found in 90% of conjunctival scars with more than 1 muscle in strabismus surgery, resulting in tissue fibrosis that could cause postoperative strabismus muscle restriction. Recurrence of postoperative strabismus is not uncommon and has been described by many researchers to have various associated prognostic factors. Recurring strabismus requires several additional surgeries or long-term therapy. This could cause social issues for patients, such as decreased academic achievement, decreased work performance, and aesthetic dissatisfaction, resulting in insecurity and social stigma from society.6,9

Descriptions above intrigue the authors to conduct a study on the effect of the Asiatic acid extract of Centella asiatica on IL-1 expression and the number of mononuclear cells as an inflammatory response in the extraocular muscles after strabismus. The results of this study are expected to be the basis for selecting alternative therapies to reduce inflammation and fibrosis after strabismus surgery.

Strabismus

Normally, both eyes are fixated in the right position for the fovea to capture the same image. This condition is called orthotropy. Strabismus, also known as crossed eyes, is an abnormality in which the ocular position is not aligned. Strabismus often occurs in children and is idiopathic or associated with refractive disturbances. In most cases of strabismus, the ocular muscles can move freely. However, in some conditions, mechanical restriction of the ocular muscles could occur. This condition is known as restrictive strabismus. Other conditions can also be caused by paresis of the extraocular muscles, such as paralytic strabismus. Ocular misalignment can be horizontal, vertical, torsional or combination. An inward deviation is called esotropia, an outward deviation is called exotropia, and a vertical deviation is called hypertropia. Patients with strabismus have one eye focused on the target, and the other is deviated. The eye deviates inward in esotropia, so the image falls nasally to the fovea. In exotropia, the eye deviates outwards, so the target image is in a position temporal to the fovea.10–12

The prevalence of strabismus reaches 2-5% of the general population, and the number of cases is higher in Caucasians than in non-Caucasians. The number of cases of strabismus in the United States reaches 5 to 15 million people. Exotropia strabismus occurs 3 to 5 times more often in children. In Indonesia, around 2 to 2.5 million people suffer from strabismus; most cases are exotropia. Hereditary or genetic factors can cause strabismus. The study by Lennerstrand et al. showed that the incidence of strabismus increased fourfold in individuals with parents or siblings who had strabismus. Strabismus also often occurs together with other disorders. Strabismus occurs in 50% of cases of Down syndrome and 44% of cases of cerebral palsy. Some risk factors that can increase the incidence of strabismus are congenital ocular abnormalities, head injuries, presence of cataracts, presence of brain or eye tumors such as retinoblastoma, neuromuscular disorders such as myasthenia gravis, encephalitis, meningitis, presence of low birth weight such as in retinopathy of prematurity, and a history of families with strabismus. Strabismus that occurs in adults can be caused by botulism, stroke, Graves‘ disease, Guillain-Barre syndrome and diabetes.10–12

Various factors can cause strabismus. One is refractive disturbances, such as myopia, hypermetropia and astigmatism. In children with refractive disturbance of more than four diopters, 30% develop esotropia by age three. Myopic patients may also suffer from strabismus, especially the esotropia type, due to the eyes constantly converging and nearby punctum remotum. In addition to refractive disturbances, strabismus can also be caused by anatomical abnormalities and nerve disorders. The presence of anatomical abnormalities in the eye can be caused by head trauma or a tumor in the orbital cavity.10–12

Examination of strabismus patients includes evaluation of the eye’s sensory, motor, refractive, and accommodative functions. One of the tests that can be done is the Hirschberg test. This test is performed to assess the degree of abnormal eyeball deviation by examining the light reflex on the cornea. Under normal circumstances, the light reflex should be in the center of the pupil with a symmetrical position between the two eyes. The light is positioned in front of both corneas at a distance of 30 cm. In diverging strabismus, the light reflex will move in a nasal direction. In convergent strabismus, the light reflex will move in a temporal direction. A shift of 1 mm determines a strabismus deviation of 15 PD or 7 degrees. The corneal reflex at the pupil margin about 2 mm from the center of the pupil means there is a deviation of 30 PD or 15 degrees. The corneal reflex between the pupil and limbus, 4 mm from the center of the pupil, means there is a deviation of 60 PD or 30 degrees.10–12

Complications and healing process after strabismus surgery

Surgery for strabismus aims to correct ocular misalignment. It involves several mechanisms, such as relaxing the muscles, tightening the muscles, reducing the length of the moment arm, and changing the vector of the muscle force by changing the insertion site of the extraocular muscles.1,3,13–15 After strabismus surgery, restriction and formation of normal scar tissue normally occur in a not excessive manner. However, an incomplete wound-healing process in some postoperative strabismus patients can lead to excessive fibroproliferation and scarring of the extraocular muscles, tendon capsule, conjunctiva, sclera and orbital fat.
tissue. Several antifibrosis agents and antimetabolites have been used to prevent this process from occurring. Adhesion as a postoperative complication of strabismus can cause eyeball movement disorders and recurring strabismus. Excessive inflammatory processes, as well as excessive production of extracellular matrix, can lead to fibrovascular proliferation and excessive scar tissue formation. The occurrence of adhesion often requires re-surgery. However, therapeutic adhesiolysis and repositioning of the extraocular muscles are neither easy nor effective because new adhesions can reappear. Recurrent strabismus can change the structure of the extraocular muscles and cause muscle degeneration, making it difficult for subsequent surgical treatment. (Figure 1)\textsuperscript{16–20}

**Interleukin (IL)-1 and mononuclear cells**

IL-1 has various biological functions as the main regulator of inflammation by controlling the innate immune system. The IL-1 cytokine family consists of a total of 11 members with same or different biological effects, including IL-1α, IL-1β, IL-1Ra, IL-18, IL-33, IL-36α, IL-36β, IL-36γ, IL-36Ra IL-37, and IL-38. Among the various cytokines, IL-1α, IL-1β, IL-18, IL-33, and IL-36 are receptor agonists, while IL-1Ra, IL-36Ra, and IL-38 are receptor antagonists. IL-1α and IL-1β are expressed in various tissues and cells, especially in macrophages in lymphoid organs, including the thymus, spleen, lymph nodes, Peyer’s patches, and bone marrow. In non-lymphoid organs, IL-1α and IL-1β are expressed in tissue macrophages in the lung, gastrointestinal tract, and liver. They are also expressed in the cellular subepithelial endometrial tissue of the uterus, glomeruli, outer cortical areas of the kidney, and a variety of specific cell types, including neutrophils, keratinocytes, epithelial and endothelial cells, lymphocytes, smooth muscle cells, and fibroblasts. Various studies state that the IL-1 cytokine family stimulates fibroblast proliferation in the human eye.\textsuperscript{21–23}

IL-1 is produced by monocytes and lymphocytes, which are mononuclear cells. IL-1 is also known as a mononuclear cell factor. Mononuclear cells originate from hematopoietic stem cells residing in the bone marrow. Mononuclear cells then migrate to areas of injury and participate in angiogenesis. When mononuclear cells migrate to the periphery, monocytes differentiate into two types of cells: macrophages that play a role in inflammation (macrophages M-1) and macrophages that play a role in anti-inflammation (macrophages M-2). M-1 macrophages, in inflammation, express toll-like receptors (TLRs) that regulate pathogen patterns and cell damage. Mononuclear cells and macrophages play an important role in cell regulation to activate keratinocytes, fibroblasts and endothelial cells. Lack of macrophages can lead to poor wound healing due to lack of wound debridement, delayed proliferation and maturation of fibroblasts, and delayed angiogenesis resulting in an inadequate wound healing process. Based on Lin et al. (2015), inflammation is classified based on the number of mononuclear cells present using the following grading: mild inflammation with the number of mononuclear inflammatory cells less than 5, moderate inflammation with mononuclear inflammatory cells scattered...
throughout the tissue but the connective tissue stroma in the background still clearly visible, and severe inflammation with abundant mononuclear inflammatory cells infiltrating the tissue.\textsuperscript{2–21,24–26}

**Asiatic acid**

*Centella asiatica*, commonly known as *Pegagan* in Indonesia, has been widely used as traditional medicine, especially in tropical countries such as China and India. *Centella asiatica* has been known to have several benefits such as antipyretic, diuretic, antibacterial, antiviral and cognitive enhancer. The benefits of using *Centella asiatica* have been widely studied, and its utilization has been increasing in Eastern and Western countries. The active compounds found in *Centella asiatica* include Asiaticoside, termolonic acid, madecassid acid, Asiatic acid, batulinic acid, and madAsiatic acid. The Asiatic acid in this study has shown the potential to benefit biological activities, such as anticancer, antidiabetic, anti-inflammatory, wound healing, antioxidant, hepatoprotective and neuroprotective. The chemical structure of Asiatic acid is C\textsubscript{30}H\textsubscript{48}O\textsubscript{5} and has a molecular weight of 488.7 kD (Figure 2).\textsuperscript{2–27}

Asiatic acid has several roles, including anti-inflammatory, antioxidant, anti-tumor and wound healing. Asiatic acid has shown benefits in activating several enzymes and receptors, such as PPAR-gamma and GABA receptors. Furthermore, Asiatic acid can inhibit receptors, such as angiotensin, endothelin 1, and toll-like receptors. Asiatic acid also could inhibit several enzymes, such as alpha-glucosidase, leukotriene synthase C4. Moreover, Asiatic acid could stimulate matrix metalloproteinase (MMP) production and the synthesis of collagen 1, plasminogen 1, and acetylcholine. Asiatic acid also shows effects in the antioxidant process. In addition, Asiatic acid also shows benefits as anti-inflammatory. Asiatic acid could regulate proinflammatory cytokines and prevent further development of immune diseases. In vitro study showed that Asiatic acid had the effect of inhibiting NF-κB. Asiatic acid also shows benefits in inhibiting inflammatory processes triggered by inflammatory cytokines, such as IL-6, IL-8, and TNF-α. Asiatic acid also benefits anti-complementary activity by inhibiting hemolytic activity against erythrocytes. Asiatic acid also inhibits the activation of toll-like receptors, which have crucial roles in the expression of proinflammatory cytokines.\textsuperscript{2–27}

From the molecular point of view, Asiatic acid exhibits various pharmacological characteristics which have been demonstrated in in vitro, in vivo and silico studies. Asiatic acid can modify molecular targets by altering gene expression and signaling pathways. Asiatic acid also regulates the expression of cytokines such as TNF-α, IL-1, IL-4, IL-5, IL-10, IL-6, chemokines, growth factors, signaling molecules, adhesion molecules, proteins related to the process of apoptosis, cell cycle proteins, and receptors. In addition, Asiatic acid also regulates several transcription factors and their signaling pathways. Asiatic acid shows benefits in conditions of metabolic disorders such as diabetes, hyperlipidemia and obesity. The mechanisms of action that Asiatic acid exhibits include inhibiting alpha-glucosidase, inhibiting glycogen phosphorylation, inhibiting HMG CoA-reductase and inhibiting the action of the lipase enzyme. Asiatic acid also has neuroprotective properties that are beneficial in several conditions, such as Alzheimer’s, dopamine neurotoxicity, memory impairment in dementia, and epilepsy. The mechanisms involved are inhibition of amyloid beta protein, PAPPR modulation, increased synthesis of acetylcholine, and activation of the glutamine synthetase enzyme. Asiatic acid has also shown several benefits in cancer pathogenesis, especially gastric cancer. The processes involved are the inhibition of NOS, COX-2, PARP, MMP-2, and MMP-9.\textsuperscript{2,27}

**Role of Asiatic Acid in Wound Healing After Strabismus Surgery**

Asiatic acid plays a role in wound healing in various processes, such as inhibiting inflammation, increasing cell proliferation and inhibiting fibrosis formation. In addition, Asiatic acid also increases the strength of the incision wound, epithelialization, keratinization and angiogenesis. A study by Chen et al. showed that Asiatic acid reduced the formation of inflammatory factors, such as IL-8, IL-6, IL-1β, TNF-α, and TGF-β. Asiatic acid also significantly inhibited intracellular ROS concentrations in the inflammatory phase. Studies have shown that Asiatic acid plays a role in inhibiting the production of collagen matrix from hepatic stellate cells and keloid fibroblasts by inhibiting the autocrine effect of TGF-β. A study showed that Asiatic acid suppressed the phosphorylation of Smad2/3, which was triggered by TGF-β, thereby inhibiting the expression of type I collagen in keloid fibroblasts. Studies also have found that Asiatic acid is a Smad7 agonist. Asiatic acid and naringenin can maximize the inhibitory effect of TGF-β/Smad signaling in renal fibrosis. Asiatic acid can inhibit Smad3 phosphorylation and renal fibrosis by increasing Smad7. Another study also found that Asiatic acid inhibited liver fibrosis by stimulating Smad7. Smad7 can inhibit Smad3 and protect against tissue fibrosis. In an in vivo study regarding the anti-liver fibrosis effect of Asiatic acid, the group treated with Asiatic acid showed lower liver fibrosis and accumulation of collagen fibers than the control group. Asiatic acid showed increased expression of Smad7 in hepatic stellate cells and inhibition of TGF-β/Smad signaling by inhibiting the regulation of TGF-β. The study by Dong et al. showed that Asiatic acid could inhibit fibrosis and lung injury stimulated by bleomycin. Asiatic acid also could inhibit TGF-β expression in lung tissue. Various effects of Asiatic acid on inhibiting fibrosis indicate its potential to prevent the formation of excessive fibrous tissue in postoperative strabismus wound healing.\textsuperscript{2,25,28–31}

**Role of Dexamethasone in Wound Healing After Strabismus Surgery**

The wound healing process highly depends on the collagen composition in the tissue. One of the phases of wound healing is the inflammatory phase, in which increased vascular permeability, cell chemotaxis


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from the blood circulation to the wound site, production of cytokines and growth factors, and cell migration occur. Corticosteroids suppress inflammation, which affects cell migration, proliferation and angiogenesis. High corticosteroid levels delay the emergence of inflammatory cells and fibroblasts, collagen deposition, and epithelial migration. The effect of corticosteroids on fibroblasts shows the potential of corticosteroids in preventing excessive scar tissue formation in the recovery process after strabismus surgery. One of the important components of wound healing is IL-1. IL-1 is a key regulator of inflammation by controlling various innate immune processes. Many studies show that Dexamethasone is important in IL-1 regulation. A study with Escherichia coli lipopolysaccharide found that Dexamethasone could inhibit IL-1 production. In a study on the effects of intraperitoneal corticosteroids on wound healing in rats, the group treated with dexamethasone showed significantly lower collagenization, epithelialization and fibroblast levels than the control group with p-values of 0.002, 0.041 and 0.023. Corticosteroids have been used to treat corrosive esophageal burns to prevent stricture formation. Corticosteroids trigger a decrease in type 1 collagen synthesis by suppressing procollagen type 1 mRNA levels. Corticosteroids, such as keloids, are also used to treat diseases with excessive fibrotic conditions. Corticosteroids play a role in inhibiting collagen synthesis. In a study on the effect of Dexamethasone on gingival fibroblasts, corticosteroids showed an effect in reducing MMP-2 expression in a culture medium which was calculated using zymography. This shows that corticosteroids reduce the activity of type IV collagenase or gelatinase in fibroblasts.32-39

This study used Dexamethasone subconjunctival injection as a comparison. Dexamethasone inhibits the activation of the NF-κB transcription factor, suppressing pro-inflammatory cytokines that act as anti-fibrosis. However, some studies stated that long-term use of corticosteroids could cause cataracts and secondary glaucoma. Even though Dexamethasone has good anti-inflammatory and anti-fibrotic effects, some studies found that long-term use could inhibit wound healing. If this study shows that Asiatic acid has an anti-fibrotic effect that is as good as Dexamethasone’s, hopefully, Asiatic acid can be an additional therapy or a substitute for Dexamethasone as an anti-fibrotic in post-strabismus surgery.5,29,33,40-42

CONCLUSION

Strabismus surgery can result in normal scar tissue formation, but incomplete wound healing can cause excessive scarring and complications like adhesion and recurring strabismus. Both Asiatic acid and Dexamethasone have potential roles in wound healing after strabismus surgery. Asiatic acid plays a role in inhibiting inflammation, increasing cell proliferation, and inhibiting fibrosis formation. It inhibits inflammation by reducing the levels of pro-inflammatory cytokines such as IL-8, IL-6, IL-1β, TNF-α, and TGF-β. It also increases cell proliferation, inhibits fibrosis, and promotes wound healing by increasing the strength of the incision wound, epithelialization, keratinization, and angiogenesis. It is also particularly effective in promoting wound healing by inhibiting the production of collagen matrix from hepatic stellate cells and keloid fibroblasts by inhibiting the autocrine effect of TGF-β.

On the other hand, Dexamethasone suppresses inflammation and affects cell migration, proliferation, and angiogenesis. It also has anti-fibrotic effects by inhibiting collagen synthesis. While Dexamethasone has been commonly used as an anti-fibrotic in post-operative strabismus wound healing, there are concerns about its long-term use, causing cataracts and secondary glaucoma. Therefore, the potential use of Asiatic acid as an additional therapy or a substitute for Dexamethasone could be explored. It has the potential to be a safer and more effective alternative to traditional treatments, like Dexamethasone, which has known side effects. However, further studies are required to confirm the effectiveness of Asiatic acid in post-operative strabismus wound healing and to determine its optimal dosage and administration method.

CONFLICT OF INTEREST

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All authors contribute to the design, intelligent content description, literature quest, data collection, data processing, manuscript writing, manuscript editing, and manuscript review. The corresponding author is the guarantor and constructs the concept of the manuscript.

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