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The Effectiveness of Depigmentation, Interleukin-1β, and Transforming Growth Factor-β Antibodies in Activating and Increasing Collagenase in Keloid as Adjuvant Therapy After Scar Excision

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ABSTRACT

Background: Keloid is an abnormal scar in previously traumatic skin after going through the wound healing process. One hundred million cases have been found in developing countries with the main complaint of scar appearances. To overcome this problem, 24 literatures from various journals and textbooks are reviewed. **Reviews:** Keloid formation is based on high melanin amount which inhibits the collagenase enzyme. Moreover, the high melanin amount would block interleukin (IL)-1β work resulting in collagen synthesis and collagenase reduction. Depigmentation effort with 4% hydroquinone is implemented to reduce the amount of melanin presented in the skin. With melanin reduction, IL-1β can work optimally by inhibiting fibroblast growth in keloid tissue without affecting on normal skin. It also induces Matrix Metalloproteinase (MMP)-1 which is an interstitial collagenase. IL-1β has an opposing effect compared to Transforming Growth Factor (TGF)-β, thus TGF-β antibody is needed to potentiate IL-1β therapeutic effect. TGF-β antibody can neutralize TGF-β ligand and αvß6 integrin resulting in blocking of COL1A1 gene expression which is responsible for MMP-1 production and type-I collagen synthesis. These three components are combined in cream with liposome as a drug carrier. This combination is applicated for adjuvant therapy after scar excision. Liposomes are chosen because of their high biocompatibility, low toxicity, and low biodegradability. Liposomes also can release slowly in the extravascular area such as skin. This advantage may carry drug components effectively to the target location.
Summary: The combination of depigmentation, IL-1β, and TGF-β antibodies has a potency to be developed as a future adjuvant therapy of keloid.

Keywords: Keloid, Depigmentation, IL-1β, TGF-β Antibodies

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INTRODUCTION

Keloids are abnormal scar tissue on the skin that has previously been traumatized and undergoes a wound healing process.[1–4] The appearance of keloids disturbs sufferers, especially in aesthetics. Keloids are also still a nightmare for surgeons after the surgery. Keloid occurs due to uncontrolled accumulation of collagen in the skin.[5–7] Accumulation occurs by an imbalance between the synthesis and degradation of collagen.[4] This is due to three possibilities, i.e.:

1. Synthesis of collagen increases, but is not followed by an increase in collagen degradation
2. Synthesis of normal collagen, but the degradation process is decreased
3. Synthesis and degradation processes are increased, but the percentage increase is greater in the synthesis process of collagen.[8]

Every year there are 100 million patients in developing countries who complain of scarring, where 55 million cases result from elective surgery and another 25 million cases result from surgery for trauma cases.[4] There are several risk factors for keloid including age. Keloids are common in the 10 – 30 years old range.[4,6,9] Apart from age, risk factors that affect keloids include skin tension, anatomical location, trauma, pregnancy, and genetics.[6,10] The incidence of keloids by sex tends to be the same.[4–6] However, more women are found in clinics because women prioritize aesthetics from their body aspects. Women will immediately go to the clinic or doctor to remove the keloid, especially if the location of the keloid is on the face.[11] In addition, there are reports that the incidence of keloids occurs in the range of 4.5% - 16%, that is, 6 – 16% of them are experienced by black African races.[4] The highest incidence is found among blacks and Hispanics.[12] From these data, it can be interpreted that the darkness of skin determines the tendency to experience keloids.

There are various kinds of therapy to overcome this keloid, such as using corticosteroid injection after mass reduction by excision. However, corticosteroid therapy mostly causes many side effects such as immune suppression. Therefore, in the following literature review, we offer therapy in the form of a combination of depigmentation cream, IL-1β, and TGF-β antibodies to activate and increase collagenase in keloid therapy.

METHODS

The literature search procedure was carried out through online searches using ScienceDirect, PubMed, Google Scholar, and WHO instruments. In addition, searches are also carried out through journals and textbooks in the library. The keywords used were Keloid, TGF-β, TGF-β antibodies, IL-1β, depigmentation, collagenase, MMP, and keloid therapy. With a publication limit of 10 years, 24 literatures were obtained that could be considered relevant and reliable.

REVIEW

Depigmentation

Keloid is abnormal scar tissue caused by the accumulation of collagen and extracellular matrix excess.[1–4] The important thing in the formation of scar tissue is the activation of fibroblasts into myofibroblasts. Myofibroblasts are responsible for the deposition of the extracellular matrix in scar tissue. The highest incidence of keloids occurs in the black race, which means that the amount of melanin affects

the formation of keloids.[4] The darkness of a person's skin is determined by the amount of melanin in his skin. Melanin is a complex biopolymer produced by melanosomes which is a product of melanocytes.[2,9] The process of melanin formation in melanosomes is acidic due to activity of acid phosphatase in the melanosome walls which plays a role in melanosome degradation and transfer. Indirectly, acid phosphatase has a role in the melanization process. In addition, in the process of melanization, the enzyme tyrosinase plays a role for tyrosine to become dopa and then to dopakuionone before becoming eumelanin and pheomelanin. In another experiment, a carboxylic acid product was obtained in the melanin granule. Carboxylic acids are weak acids with the formula RCOOH. The high concentration of melanin in the skin makes the pH condition more acidic, around 6.8 ± 0.20. Other evidence shows that an increase in melanin by 1.7 will increase collagen density 2.3 times.[8]

Collagenase enzymes are a family of matrix metalloproteinases (MMP). Collagenase is divided into 3 types, namely interstitial collagenase commonly called MMP-1, collagenase PMN commonly called MMP-8, and collagenase-3 called MMP-13. Collagenase has a role in collagen degradation and can only work optimally at pH 7.5. In dark skin with a high melanin composition, the work of the collagenase enzyme will be lost and there is an accumulation of collagen that causes keloids. In addition, melanin also inhibits the performance of IL-1β which works by inhibiting collagen synthesis and encouraging collagenase production.[5]

Depigmentation is used to suppress the melanin in the skin. This results in pH control of the wound during the healing phase to optimize the collagenase enzyme's work. With the optimal work of the collagenase enzyme, it is expected that the degradation function will return to normal and be balanced with the synthesis process of collagen so that the collagen density decreases and the keloid will decrease.[2,13]

Depigmentation efforts are carried out using hydroquinone. Hydroquinone (benzene-1,4-diol/$C_6H_2(OH)_2$) or quinol is an organic aromatic compound that is solid, crystalline, powder, odorless, colorless to white, sweet taste, has 7% water solubility at temperature 25°C, also soluble in ether, alcohol, acetone, dimethyl sulfoxide, carbon tetrachloride and slightly soluble in benzene. In natural compounds, hydroquinone is found in coffee and tea. Usually we find it as a whitening cream that is used at concentrations of 2-4%.[14] The oxidation of hydroquinone causes damage to membrane lipids and proteins such as tyrosinase and depletion of glutathione. This will inhibit the performance of the tyrosinase enzyme and the degradation of melanosomes which results in inhibition of the conversion of DOPA to melanin. The first step of pigment formation has been inhibited so that all pigment production lines are also blocked. Hydroquinone can decrease tyrosinase enzyme activity by up to 90% by affecting DNA and RNA synthesis which causes reversible inhibition of cellular metabolism.[8]

In dealing with keloid problems, 4% hydroquinone is used with an alkaline solvent in order to reduce the amount of melanin so that the pH of the skin will increase. The administration of hydroquinone and alkaline solvents covered with liposomes can reduce skin sensitivity due to acid-base changes and reduce side effects caused by hydroquinone,

such as temporary skin irritation ranging from mild itching to electric shocks.[15]

Interleukin 1-Beta (IL-1β) in Keloid Therapy

Interleukin 1 (IL-1) is a cytokine associated with the inflammatory response. [12] IL-1 has 3 proteins, there are IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1ra). IL-1α is bound on membrane while 1IL-1β, the most common form of IL-1, can be secreted and found in the circulation. Most IL-1β is secreted by monocytes and some by macrophages, endothelial cells, fibroblasts, and other cells via stimuli. [13] Stimulus from monocytes occurs in 2 stages, through inflammatory signals and exogenous ATP. The type of inflammatory signals such as lipopolysaccharide (LPS) triggers the synthesis and cytoplasmic accumulation of inactive precursors (pro-IL-1β). The second signal, exogenous ATP, which is secreted autocrine or paracrine by endotoxin-activated monocytes, is further amplified by ATP released by cells associated with inflammation, such as platelets. [14] The IL-1β protein is released in the form of a zymogen which is further activated by IL-1β converting enzyme (ICE).[16]

IL-1 has receptors, which acts on fibroblasts, called type I receptors. IL-1 is associated with cyclooxygenase and lipoxygenase, MMP synthesis, collagenase, osteoblasts, and osteoclast activation.[16] Research on the response of fibroblasts with normal skin to IL-1β and IL-6 states that IL-1β can inhibit fibroblast growth in keloid tissue without being followed by inhibition in normal skin tissue, whereas IL-6 can inhibit fibroblast growth in both keloid tissue and normal skin.[8] Besides, IL-1β can also promote the formation of MMP-1. Perdanakusuma[8] stated that in human dermal fibroblasts given IL-1β was able to stimulate the formation of MMP-1 mRNA 10-20 times. However, the action of IL-1β can be inhibited by the presence of melanin so that depigmentation efforts are needed and then IL-1β can work optimally.

Ultraviolet also plays a role in collagen degradation by stimulating IL-1β to release MMP to the extracellular matrix to suppress fibroblasts and cause collagen breakdown.[8] IL-1β has the opposite action with TGF-β1 by reversing the pro-fibrotic effect produced by TGF-β1. Besides, IL-1β works to inhibit the process of fibroblasts into myofibroblasts. Thus, IL-1β has the potential to reduce the severity of keloid tissue.[17]

IL-1β that interacts with angiotensin II receptor 1 (AT1-R) can increase disease progression in heart failure.[18] In addition, IL-1B can cause chronic inflammation that leads to cancer and autoimmune diseases.[19]

TGF-β Antibody as Keloid Therapy

Transforming Growth Factor Beta (TGF-b) is a fibrotic process mediator on the skin. The main thing is that the TGF-β is produced by the macrophage, platelet, fibroblast, and keratinocytes. There are three types of TGF-β, which are TGF-β1, TGF-β2, and TGF-β3, that have different roles in wound healing.[20]

In the inflammation phase, TGF-β1 is released by the platelet which is aggregated in the injured area of blood vessels. This TGF-β1 mediates leucocytes migration along with the cytokines and another growth factor called IL-1 (interleukin-1) and PDGF (platelet-derived growth factor). Besides, TGF-β1 will also mediate negative feedback from superoxide release in macrophages. In the proliferation

phase, TGF-β1 has a role in expressing extracellular matrix's main component, which are fibronectin, type I and III collagen, and VEGF (vascular endothelial growth factor). The presence of these factors induces the proliferation of endotel and new blood vessels to restore vascularization in an injured blood vessel (angiogenesis). In the tissue remodeling phase, type III collagen will be formed into type I collagen. This process is preceded by TGF-β1 and PDGF which cause the regression of granulated tissue to make new collagen. There is an enzyme that inhibits collagen synthesis called matrix metalloproteinase (MMP). However, the enzyme production is inhibited by TGF-β1. This causes an excessive accumulation of collagen in the tissue.[21]

TGF-β2 has a quite similar role to TGF-β1, which is to express collagen as an extracellular matrix. Thus, these two growth factors work synergistically in forming wound fibrous tissue.[20] However, TGF-β3 has a slightly different effect than TGF-β1 and TGF-β2. In a small wound that left no scar, the ratio of TGF-β3/TGF-β1 amount is substantial and the forming of collagen is minimal. TGF-β3 can stimulate the work of MMP-9 to degrade formed collagens. Therefore, exogenous TGF-β3 injection is known for reducing scar formation in wound healing.[21] However, the use of TGF-β3 is still under research because the injection does not provide any wound area reduction. Nevertheless, the wound will become larger and deeper without the presence of TGF-β3. This shows that TGF-β3 is indeed needed for wound healing, even though the effect is still unknown.[22] Overall, even though these three types of TGF-β have an antagonistic role, they work altogether to balance the fibrotic process. Besides inhibiting the MMP-1 gene expression, TGF-β also stimulates the expression of the COL1A1 gene which affects type I collagen synthesis.[23] TIMP-1 gene (Tissue Inhibitor of Metalloproteinase-1) which inhibits the work of MMP is also activated that causes the deposition of type I collagen and fibrous tissue formation.[21]

Currently, it is known that too much TGF-β activity can lead to scar hypertrophy and keloids. Therefore, the development of antibodies against the action of TGF-β has the potential to be therapeutic in cases of hypertrophic scars and keloids. There are various ways to reduce TGF-β levels in the blood, such as (1) neutralizing antibody works at the TGFR2, (2) receptor kinase inhibitor works at an activated TGFR1, (3) Smad7 agonist and Smad3 inhibitor interfere with an activated Smad3, (4) histone deacetylase (HDAC) reduces transcription, and (5) locked nucleic acid (LNA) anti-sense miRNA downregulates profibrotic miRNA and long-coding RNA (lncRNA). One of them is the neutralizing antibodies can act in TGF-β receptor 2 (TGFR2) so that TGF-β cannot attach to the receptor. The use of the TGF-β antibody for a long time is considered quite safe and well-tolerated in experimental animals such as mice.[24]

These three components, combined in cream with liposome as a drug carrier should be studied. This combination is applicated for future adjuvant therapy after scar excision. Liposomes have double lipid layers to cover the components. Liposomes make the alkaline properties of these substances only arise in the dermis layer. Liposomes are drug carriers with high biocompatibility, low toxicity, and low biodegradability. Also, liposomes are slow-released in extravascular areas such as skin so that they can be applied directly to target locations.[14]

SUMMARY

Depigmentation using 4% hydroquinone and alkaline solvents is used to suppress the melanin in the skin. Melanin plays a role in inhibiting the performance of IL-β1 which functions as an inhibitor of collagen synthesis and encourages collagenase production. With a reduction in melanin levels, IL-1β can work optimally. Administration of IL-1β inhibits fibroblast growth in keloid tissue, prevents the conversion of fibroblasts into myofibroblasts, and promotes the formation of Matrix Metalloproteinase (MMP) -1. IL-1β has an opposite action with TGF-β1 so that the TGF-β antibody is needed. TGF-β antibody aims to neutralize the TGF-β or αvß6 integrin ligand so that TGF-β cannot stimulate the expression of the COL1A1 gene which is responsible for MMP-1 production and collagen type I synthesis. Liposomes were chosen because they have high biocompatibility, low toxicity, and low biodegradability. Based on these findings, depigmentation efforts, administration of IL-1β, and TGF- β antibodies have the potential to activate and increase collagenase so that it can be used as keloid therapy. Perspectives and further clinical trials are needed to obtain more scientific evidence for this subject.

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