Hydrogen Peroxide: A Potential Wound Therapeutic Target?

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Significance of the Study

- Currently, effective and practical treatments of chronic wounds are still clinical challenges. The main clinical use of hydrogen peroxide (H_2O_2) is to clean wounds for disinfection in a concentration of 3%. With advances in research, H_2O_2 at μM levels has been reported to act as a signaling molecule which drives redox-sensitive signaling mechanisms to improve dermal wound healing. This review discussed the roles of H_2O_2 in cutaneous wound healing and its future use in treating chronic wounds.

Keywords

Hydrogen peroxide · Wound healing · Therapeutic target

Abstract

Hydrogen peroxide (H_2O_2) is a topical antiseptic used in wound cleaning which kills pathogens through oxidation burst and local oxygen production. H_2O_2 has been reported to be a reactive biochemical molecule synthesized by various cells that influences biological behavior through multiple mechanisms: alterations of membrane potential, generation of new molecules, and changing intracellular redox balance, which results in activation or inactivation of different signaling transduction pathways. Contrary to the traditional viewpoint that H_2O_2 probably impairs tissue through its high oxidative property, a proper level of H_2O_2 is considered an important requirement for normal wound healing. Although the present clinical use of H_2O_2 is still limited to the elimination of microbial contamination and sometimes hemostasis, better understanding towards the sterilization ability and cell behavior regulatory function of H_2O_2 within wounds will enhance the potential to exogenously augment and manipulate healing.

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Introduction

Among various reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) is relatively poorly reactive, which allows it to migrate further from its site of generation to serve as a signaling molecule or second messenger [1]. When a cutaneous injury happens, the concentration of H_2O_2 in surrounding tissue rises immediately and then peaks and fades away [2]. This dynamic change of H_2O_2 level accompanies the wound healing course and the concentration of H_2O_2 in wound tissue influences the outcome to a certain extent.

Wound healing is a tightly controlled process in which H_2O_2 plays multiple functions. Apart from killing microorganisms, H_2O_2 also serves as a signaling molecule or second messenger which delivers a damage message and stimulates effector cells to respond [3]. H_2O_2 regulates gene expression through several ways: synthesis of more transcription factors; inhibiting the ubiquitin E3 ligase complex or decreasing transcription factors associated

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with it to promote stability of the transcription factor; exposing/masking nuclear localization signals; and modulating transcription factor affinity towards deoxyribonucleic acid, coactivators, or repressors [4]. The transcription factors that receive the modulation of H_2O_2 are diverse, including *Escherichia coli* OxyR, NF- κ B, activator protein-1, hypoxia-inducible factor-1, etc. These diverse actions could explain the broad impact brought by H_2O_2 [4].

The biological effect of H_2O_2 is dose dependent during the wound-healing process. For example, in relatively high concentrations, H_2O_2 displays its strong ability of oxidization and proinflammation to disinfect wound tissue; however, in comparatively low concentrations, H_2O_2 assists in removing cell and pathogen debris and promotes secretion of cytokines which help tissue regeneration [5–7]. Hence, in this review, the role of H_2O_2 in cutaneous wound healing and its potential as a chronic wound healing agent are discussed.

The Production of Endogenous Hydrogen Peroxide after Cutaneous Injury

H_2O_2 is produced in aerobic cells as a byproduct of aerobic respiration or an output of enzymatic reactions in mitochondria, peroxisomes, or other cell compartments [8, 9]. The production of H_2O_2 is maintained at a low level under basic conditions because of its reactivity with intracellular antioxidant systems that include ascorbic acid, glutathione, catalase, and other antioxidants [10].

Once a skin wound occurs, based on an experiment performed on zebrafish by mechanically injuring its tail fin, a sustained rise in H_2O_2 concentration was detected at the wound margin immediately after the injury occurred [2]. The H_2O_2 gradient recruited leukocytes to the wound site which peaked about 20 min after occurrence of the injury and then gradually decreased [2]. Hence, the H_2O_2 produced after injury is a chemotactic signal as well as an inflammatory initiator.

The production of H_2O_2 after damage is mainly mediated by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, an enzyme which has at least 7 isomers (NOX₁, NOX₂, NOX₃, NOX₄, NOX₅, DUOX₁, and DUOX₂) [3, 11]. It is expressed mainly on the plasma membrane and subcellular membranes such as the mitochondrial and endoplasmic reticulum membrane [12, 13]. Multiple factors can induce the activation of NADPH oxidase such as mechanical injury, pathogen attack, and inflammatory cytokines [11, 14]. After activa-

tion, NADPH oxidase will convert one oxygen molecule into a superoxide anion (O_2^-) which quickly transforms into H_2O_2 under the effect of superoxide dismutase [9].

Regulatory Role of Hydrogen Peroxide in Wound Healing

Hemostasis Stage

Vascular destruction often appears in cutaneous wounds, resulting in blood loss and evasion of pathogens. Hence, hemostasis is the first step to restore blood volume and reduce infection. H_2O_2 facilitates hemostasis with several plausible mechanisms that include activating latent cell surface tissue factor, platelet aggregation, stimulating platelet-derived growth factor activation and regulating the contractility and barrier function of endothelial cells [15].

Inflammatory Reaction Stage

Inflammation disinfects wound tissue to prepare a suitable environment for cell proliferation. H_2O_2 in wound tissue increases significantly during the inflammatory reaction stage to act as a potent inflammatory initiator and promoter [16].

The earliest immune cells arriving at the wound site are neutrophils and macrophages. They possess powerful abilities of engulfing evading microorganisms and killing them with proteases and elastase in granules [17]. Both ROS and protease are important for a phagocyte's killing efficacy [18]. The generation of ROS causes an influx of potassium ions (K^+) into the phagocytic vacuole with an attendant rise in pH to the optimal level for the activity of the granule proteases [19]. H_2O_2 also induces mRNA expression of macrophage inflammatory protein-1 α , macrophage inflammatory protein-2, and macrophage chemokine protein-1, which works as chemoattractant to recruit phagocytes [20–22]. Cellular adhesion molecules, such as intercellular adhesion molecule-1 and leukocyte function-associated antigen-1, can promote leukocyte endothelial adhesion and assist in leukocytopoiesis. Their expressions are also elevated in the presence of H_2O_2 [23, 24]. The recruitment of phagocytes is an essential step to initiate inflammation while insufficient phagocyte assembling often results in infection that hinders the wound-healing course [25].

H_2O_2 helps with the production of some molecules with higher oxidative potential and stronger bactericidal ability. For example, H_2O_2 oxidizes pseudohalide thiocyanate (SCN^-) to generate hypothiocyanite (HOSCN)

under the catalysis of lactoperoxidase [26]. It also reacts with chloride ions to produce hypochloric acid (HOCl) in the presence of myeloperoxidase [27]. Both HOSCN and HOCl are quite cytotoxic. The H_2O_2 oxidizes a ferrous ion (Fe^{2+}) to generate a ferric ion (Fe^{3+}), a hydroxyl radical, and a hydroxyl anion in the Fenton reaction [28]. Hydroxyl radicals are highly aggressive and able to cause oxidation of cellular macromolecules [29, 30].

Neutrophil extracellular trap (NET) is an effective bactericidal mechanism whose first step depends on the ROS that are derived from NADPH oxidase activation [31, 32]. Neutrophil cytosolic factor 1 (an essential component of the NOX₂ complex)-mutated mice lacked formation of NETs when they developed arthritis [33]. The priming step of NACHT, LRR, and PYD domains containing protein 3 (NLRP3) inflammasome expression requires ROS as well [34]. NETs and NLRP3 inflammasome are 2 effective mechanisms of neutrophil host defense. As a most abundant ROS, H_2O_2 may be a participator.

H_2O_2 is able to enhance the expression of inflammation-related genes and the synthesis of proinflammatory cytokines. TNF- α mRNA expression in human middle ear epithelial cells was significantly increased by treating with H_2O_2 at concentrations over 100 μM [35]. The intragastric administration of 5% H_2O_2 significantly increased the expression of TNF- α , IL-1 β , and IL-5 mRNA [36]. It also induces secretion of proinflammatory molecules TNF- α , macrophage chemokine protein-1, IL-8, and IFN- α in epithelial cells in a dose-dependent manner [37].

Patients with chronic granulomatous disease are hypersensitive to various bacterial and fungal infections due to defective NADPH oxidase activity. The inability of phagocytes to kill ingested pathogens or undergo apoptosis for the absence of H_2O_2 results in accumulation of bacteria-containing phagocytes and development of granulomas [38, 39]. Defective H_2O_2 generation contributes to lasting inflammation and suggests that H_2O_2 plays an essential role in inflammation regulation.

Cell Proliferation Stage

Once infectious sources and cell fragments are removed, restoring the absent tissue becomes the subsequent task comprised mainly in 2 forms: reepithelialization and formation of granulating tissue. For reepithelialization to begin, keratinocytes need to change their ability of adhesion and mobility to migrate from surrounding tissue to the wound site and then proliferate. A scratch-wound model made up of keratinocyte culture showed that H_2O_2 promoted keratinocytes' mobility at a low concentration of about 500 μM without any loss of the

cells' viability [40]. The keratinocytes treated with H_2O_2 at a low concentration have enhanced epidermal growth factor receptor activation and ERK1/2 phosphorylation, which explains its higher potential of migration [6, 40].

Angiogenesis is a key step in formation of granulation tissue. By topical application of 10 mM H_2O_2 to rat excisional wounds, the wound closure rate was significantly increased by a strong promotion of angiogenesis and connective tissue regeneration [5]. Cyclooxygenase-derived products, particularly prostaglandin E₂, play an important role in endothelial cell migration [41, 42], while H_2O_2 augmented cyclooxygenase-2 protein synthesis in human endothelial cells [43]. In vitro, H_2O_2 can stimulate macrophages [44], retinal keratinocytes [45], and vascular smooth muscle cells [46] to release vascular endothelial growth factor which possesses a strong ability of promoting angiogenesis.

In zebrafish, H_2O_2 derived from wounded skin cells strengthened injury-induced peripheral sensory axon regeneration that helps to innervate healing skin [47]. Similarly, H_2O_2 in concentrations less than 500 μM enhanced the release of heat shock protein (HSP70, HSP90) and fibroblast growth factor from cultured rat astrocytes, which contributes to neuron survival, neurite outgrowth, and angiogenesis [7]. Hence, H_2O_2 is probably favorable in both the structural and functional recovery of cutaneous wound.

Tissue Remodeling Phase

Early gestational fetal skin can undergo scarless repair for a lack of inflammation phase [48]. Therefore, the influence exerted by H_2O_2 on the inflammation phase may have a carryover effect to influence tissue remodeling.

H_2O_2 disturbs the balance between matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases [49]. A study using a murine fetal wound repair model showed that H_2O_2 elevated the expression of transforming growth factor (TGF)-1 and enhanced proliferation of fibroblasts [50]. NOX₂ is revealed to involve in the differentiation of human dermal fibroblast into myofibroblasts in response to TGF-1 [51]. NOX₄ is also reported to be involved in collagen deposition for its stimulatory effect of TGF- β ₁ [52] (Fig. 1).

Current Clinical Use of Hydrogen Peroxide

For clinical irrigation, H_2O_2 is usually 3% (975 μM), which oxidizes protein, nucleic acid, lipids of normal healthy cells, and microorganisms at the same time [53].

Fig. 1. The roles of hydrogen peroxide (H_2O_2) in wound healing course. TF, tissue factor; VEGF, vascular endothelial growth factor; Cox-2, cyclooxygenase-2; EFGR, epidermal growth factor receptor; TGF- β_1 , transforming growth factor- β_1 .

The use of H_2O_2 to disinfect wounds continues today, but no beneficial effect of 3% H_2O_2 in promoting wound healing has been seen in the literature [16, 54]. In addition, the killing ability of H_2O_2 on pathogenic bacteria like *Pseudomonas aeruginosa* is doubtful because catalases are reported to exist in their bodies [55]. H_2O_2 is also used regularly to prepare the bony bed in cemented arthroplasties as well as to achieve hemostasis in neurosurgery [56, 57]. It is also an adjunct hemostatic to topical epinephrine in patients with known platelet dysfunction after burn excision [58]. Equally important, it has an inherent risk of fatal oxygen embolism formation [59, 60].

Some drugs that contain H_2O_2 to treat cutaneous infection have been developed. In a cream formula, LHP[®], 1% H_2O_2 is included in a stabilized form that allows a slow degradation and a prolonged effect [61]. H_2O_2 cream (Crystacide; Mipharm, Milan, Italy) is another formulation of H_2O_2 1% in stabilized cream that has shown good antimicrobial effect and skin tolerability [62]. A prospective clinical trial demonstrated that wound cleansing with 2% H_2O_2 on chronic-colonized burn wounds for 5 min followed by normal saline irrigation and grafting elevated the success rate of graft take when compared with the conventional method of debridement and skin grafting [63].

Hydrogen Peroxide: Implications for Chronic Wound Treatment

Chronic wounds are characterized by chronic inflammation which also appears in many chronic inflammatory diseases, such as diabetes mellitus, rheumatoid arthritis, periodontal disease, cardiovascular disease, and inflammatory bowel disease. A correct balance between H_2O_2 generation and detoxification mechanism must be properly maintained to avoid oxidative damages [64]. Defective leukocyte apoptosis and subsequent removal of apoptotic cells by phagocytes is thought to be important for the initiation and propagation of chronic inflammation. The role of NADPH oxidase-derived H_2O_2 to induce apoptosis of phagocytes and resolution of inflammation has been reported in a model of antigen-induced arthritis [65]. It is possible to take advantage of this function of H_2O_2 to regulate pathogenic inflammation in chronic wounds.

The H_2O_2 concentrations change in wound tissue influences the healing rate. In a murine model of wound healing, topical application of 50 mM H_2O_2 promoted wound closure while 3% H_2O_2 (980 mM) delayed healing [16]. In a mouse model of excisional wounds, 10 mM H_2O_2 promoted wound closure but 166 mM retarded it when compared with control mice [5]. H_2O_2 can pass through the plasma membrane through specific aquapo-

rin expressed on cells' membranes [8]. Pentafluorobenzenesulfonyl-fluorescein (HPF), a H_2O_2 -selective chemical sensor showed an elevated intracellular redox level after exogenous H_2O_2 treatment [66]. By treating wild-type zebrafish larvae in the absence of injury with 3 mM H_2O_2 and then comparing their mRNA with an untreated group, 414 transcripts were found to be significantly up-regulated while 256 were significantly downregulated [66]. Therefore, the application of exogenous H_2O_2 can lead to cellular behavior change. Apparently, H_2O_2 wound healing might be mainly based on acute injury models. There are few articles [37, 67] about the behavior of H_2O_2 in chronic wounds. The abnormal inflammation underlying a chronic wound may disturb the dynamic generation and clearance of H_2O_2 at the wound site.

Hypoxia is a key feature of many chronic wounds. The partial pressure of oxygen (PO_2) in nonspecified chronic wounds has been reported to be in the range of 5–20 mm Hg while typical values in healthy tissue are 30–50 mm Hg [68]. The production of ROS mediated by NADPH-linked oxygenase is a highly oxygen-dependent process: the half maximal velocity (km) for NADPH-linked oxygenase with oxygen as a substrate is a PO_2 value of 40–80 mm Hg [67]. The level of ROS is highly relevant with neutrophil antibacterial activity because it is responsible for neutrophil respiratory burst. Neutrophils were shown in vitro to lose their bacterial killing capacity at a PO_2 level below 40 mm Hg [67]. This loss could be attributed to the reduction of ROS. The decrease in neutrophil antibacterial activity contributes to infection and this may partly explain the significant bacterial colonization in hypoxic chronic wounds. Therefore, long-time hypoxia could lead to ROS reduction. As a most abundant ROS, the reduction of H_2O_2 will impact negatively on wound healing, such as aggravated infection, decreased cytokines secretion, and abnormal inflammation.

Some treatments which generate low concentration H_2O_2 accelerate wound healing to a certain extent. Non-thermal atmospheric plasma (NAP) has been used in the clinical setting to accelerate wound healing [69]. Some changes exerted by NAP were abolished by catalase and the cells' responses to NAP treatment are similar to incubating in H_2O_2 in a similar concentration [69, 70], as exemplified by the plasma-induced profound extracellular trap formation (NET), which can be inhibited by the presence of catalase. However, adding an equivalent concentration of H_2O_2 cannot induce NET [71]. The NET formation may involve other constituents induced by plasma, but H_2O_2 is indispensable. In clinical practice, the application of NAP can achieve a significant reduction in

bacterial load on chronic wounds and successfully remove the biofilm [72, 73]. Its sterilization effect does not depend on the pathogen species and can even resist multidrug-resistant bacteria [74]. Some reports indicate that NAP can enhance the proliferation rate of basal keratinocytes and endothelial cells [75, 76]. The 350 downregulated and 400 upregulated transcripts of keratinocytes after NAP treatment highlighted its powerful ability to influence gene expression [77].

Modern licensed dressings containing medical-grade honey like Surgihoney[®] and Revamil[®] have earned renewed interest in its clinical potential for conventional wound care [78, 79]. Laboratory investigations have shown that low concentrations of H_2O_2 are normally generated in these honeys when they are diluted. Glucose oxidase (an enzyme secreted into honey by worker bees) oxidizes glucose to gluconic acid with the release of H_2O_2 [78]. The antimicrobial ability of honey is partly contributed to H_2O_2 . In a study testing the antimicrobial activity and the maximum output of H_2O_2 among 3 honey prototypes, there was a linear relationship between them. The more H_2O_2 the honey produces, the stronger antimicrobial ability it has [79]. Some biologically modified honey has also been reported to stimulate monocytes to secrete cytokines like TNF- α , IL-1 β , and IL-6 and it may be attributed to H_2O_2 [80].

Hydrogen Peroxide Could Be a Target for Wound Treatment

One of the priorities of chronic wound treatment is to form a favorable microenvironment that is receptive to therapies. Therapies that correct H_2O_2 to an appropriate level may help wound healing through ameliorating wound redox environment.

However, more basic experiments and clinical trials are needed to testify this hypothesis. First, it should be explored whether there are abnormalities in the distribution and concentration of H_2O_2 in chronic wounds. Second, new methods to regulate H_2O_2 more stably and precisely should be further researched to make treatment more standardized.

Potential Future Use

Uncontrolled production or decomposition of H_2O_2 is likely to result in tissue injury and has been associated with increased susceptibility to diseases due to the unbal-

anced redox homeostasis. Further study about the critical role of H_2O_2 in inflammation initiation, development, and resolution could help precise regulation of inflammation progression. The therapeutic effect of H_2O_2 might not be limited to only chronic wound, but also applied to other diseases characterized by abnormal inflammation.

Conclusion

Normal wound healing is a carefully controlled balance of destructive processes necessary to remove damaged tissue and repair processes which lead to new tissue formation. The dynamic change of H_2O_2 in wound tissue helps to keep the balance during the wound-healing course. H_2O_2 promotes oxidative stress as well as resolves inflammation, which makes it a bidirectional inflammation

regulator. Uncontrolled H_2O_2 generation will result in chronic inflammation which contributes to delayed wound healing. Through further research upon its immune regulatory function, some therapies taking H_2O_2 as a target can be invented to promote chronic wound healing.

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Disclosure Statement

The authors report no conflicting interests.

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