Special article

Revisiting the essential role of oxygen in wound healing

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Abstract

Hypoxemia, caused by disrupted vasculature, is a key factor that limits wound healing. Correcting hypoxemia through the administration of supplemental oxygen (O_2) can have significant beneficial impact on wound healing in the perioperative and outpatient settings. Beyond its role as a nutrient and antibiotic, O_2 may support vital processes such as angiogenesis, cell motility, and extracellular matrix formation. Recent discoveries highlight a novel aspect, addressing the role of O_2 in wound healing via the production of reactive oxygen species (ROS). Almost all wound-related cells possess specialized enzymes that generate ROS (including free radicals and H_2O_2) from O_2. Defect in these enzymes is associated with impaired healing. Low wound pO_2 is expected to compromise the function of these enzymes. At low concentrations, ROS serve as cellular messengers to support wound healing. The use of systemic hyperbaric O_2 therapy presents potential advantages, as well as risks. There is evidence to suspect that the use of pressure and systemic pure O_2 may not be essential in wound care. Elimination of these factors by using sub-pure systemic O_2 under normobaric conditions may significantly minimize the risk of O_2 toxicity. Furthermore, opportunities to treat dermal wounds using topical O_2 therapy warrant further investigation. Given that many growth factors require ROS for their function, it is reasonable to assume that approaches to correct wound pO_2 will serve as an effective adjunct in treating chronic wounds. © 2003 Excerpta Medica, Inc. All rights reserved.

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Wound healing represents a well-orchestrated reparative response that occurs after all surgical procedures or traumatic injury. As such, the principles of wound healing contribute substantially to the foundations of surgical care across all specialties. Since wound healing is a ubiquitous aspect of surgical practice, it is also inevitable that every surgeon will care for patients with problem wounds. A compromised wound frequently represents the convergence of multiple factors that disrupt the normal wound healing sequence [1]. Determining the impediments to wound healing and developing an appropriate treatment plan to overcome these obstacles is the first step toward achieving a healed wound.

Hypoxemia, caused by disrupted vasculature, is a key factor that limits wound healing [2]. The central area of the wound is most hypoxic, with a progressive increase in the oxygen (O_2) gradient toward the uninjured tissue at the periphery. The pO_2 of dermal wounds ranges from 0 to 10 mm Hg centrally to 60 mm Hg at the periphery, while the pO_2 in the arterial blood is approximately 100 mm Hg. Clinical use of O_2 to promote wound healing began in the 1960s, with administration of systemic hyperbaric O_2 to treat wounds. While the conditions (eg, pressure, O_2 concentration, frequency, and duration of administration) for systemic hyperbaric O_2 therapy (HBOT) have not been optimized on the basis of randomized clinical trials, HBOT is an FDA-approved therapeutic modality used in wound clinics with an encouraging success rate. Reliance on empiricism and a paucity of data that meets the highest criteria for evidence-based medicine has hindered the general acceptance of O_2 therapy as a standard modality in wound care. Embracing the concept of O_2 therapy depends not only on favorable clinical outcome, but also on detailed mechanistic insight that explains those outcome results. It is generally accepted that correction of wound-hypoxia is required to provide enough O_2 that would support growth of regenerating tissues. This article will summarize findings regarding the mechanisms through which O_2 promotes wound healing, address issues related to modes of O_2 therapy in the

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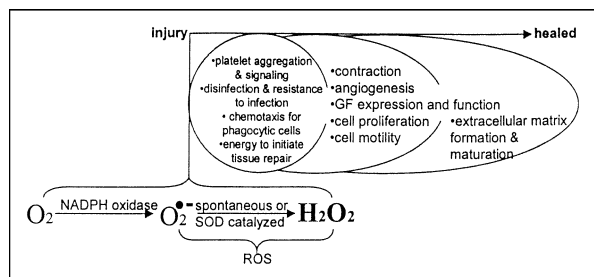*E-mail address:* sen-1@medctr.osu.edu

Fig. 1. Molecular oxygen and its reactive derivatives support numerous key processes associated with wound healing. ROS-driven redox-sensitive mechanisms in healing have been recently reviewed (Sen, 2003). While ROS may be beneficial at low concentrations, excess ROS (eg, 3% H_2O_2 commonly used clinically for wound disinfection) may be detrimental for overall healing. ROS = reactive oxygen species; SOD = superoxide dismutase; GF = growth factor.

perioperative and dermal wound setting, and discuss the limitations and current concepts relevant to this topic.

Reactive derivatives of oxygen support healing: a new horizon

In the words of Thomas Hunt, a pioneer in the field of oxygen and wound healing, the search for the mechanisms by which O_2 exerts its vital functions in wound healing has evolved another major step [3] making room for a new paradigm [4]. Recent discoveries have illuminated that not only phagocytes, but almost each and every cell in the wound microenvironment is fitted with a specialized enzyme to convert O_2 to reactive oxygen species (ROS), including oxidizing species such as free radicals and H_2O_2 [5]. These ROS contribute as cellular messengers to promote processes that support wound healing (Fig. 1). These redox-sensitive processes include cytokine action, angiogenesis, cell motility, and extracellular matrix formation [5]. This concept sharply departs from the orthodox view that ROS are inherently damaging in nature. A more refined view now postulates that, in low concentrations, ROS may act as a signaling mediator that modulates a wide variety of cellular responses [6–13]. Details addressing the redox control of wound repair have been recently reviewed [5].

Sbarra and Karnovsky's 1959 discovery of the leukocyte oxidase [14] in phagocytes came into the limelight during the late 1970s, when the pioneering works of Babior linked the explosive production of superoxide ions ($O_2^{·−}$) by leukocyte oxidase (renamed as NADPH oxidase) to bacterial killing [15]. Defects in NADPH oxidase are associated with impaired healing in humans [16–18]. Four decades later, the discovery of specific NADPH oxidases in nonphagocytes [19] unveils a new dimension that has the potential to explain the role of O_2 and its derivatives in wound healing [5]. Genetic approaches to bolster NADPH oxidase in nonphagocytic cells promote dermal healing [13]. Thus, O_2 has a role in healing beyond its function as a nutrient and antibiotic. Given that growth factors, such as platelet-de-

rived growth factor (PDGF), require ROS for their action on cells [20], it is clear that O_2 therapy may act as an effective adjunct. Finally, there is clinical validation of this concept. Patients with chronic granulomatous disease have defects in genes that encode NADPH oxidase, and the manifestations of this defect are increased susceptibility to infection and impaired wound healing.

Oxygen: beyond nutritional support

Angiogenesis is a critical early aspect of the wound healing response. While hypoxia can initiate neovascularization, it cannot sustain it. Supplemental O_2 administration accelerates vessel growth [21]. It has been established that VEGF is a major long-term angiogenic stimulus at the wound site. O_2 treatment induces VEGF mRNA levels in endothelial cells and macrophages [22–24] and increases VEGF protein expression in wounds in vivo [25]. Recently it has been shown that O_2 may trigger the differentiation of fibroblasts to myofibroblasts [26], cells responsible for wound contraction.

Collagen deposition is a fundamental step in wound healing that provides the matrix for angiogenesis and tissue remodeling. There are several posttranslational steps in collagen synthesis that are O_2 dependent. The enzymes prolyl hydroxylase, lysyl hydroxylase and lysyl oxidase all require molecular O_2 as a cofactor. Prolyl hydroxylase is required to convert proline residues to hydroxyproline, which allows the procollagen peptide chains to assume their triple helix configuration. Without this triple helix configuration, the synthesized procollagen chains accumulate in the rough endoplasmic reticulum and are eventually excreted as nonfunctional gelatinous protein [27,28]. Once the procollagen has assumed the triple helix conformation and been excreted, the individual collagen fibers are arranged into linear fibrils via cross-linking of lysyl hydroxylase, and finally cross-linking between large fibrils is performed by lysyl oxidase. These extracellular cross-linkages are ultimately responsible for the tensile strength achieved in healed wounds. Of the O_2-dependent enzymatic processes, the rate of collagen synthesis is reflected by the rate at which prolyl hydroxylation occurs [27,28]. The amount of O_2 at which collagen synthesis is half-maximal (K_m using Micahelis-Menton equation) has been determined to occur at a pO_2 of 20 to 25 mm Hg [29,30], with V_{max} occurring at levels approaching 250 mm Hg. This represents levels of O_2 availability that exceed the pO_2 normally present in wounds, and suggests that supplemental O_2 may enhance collagen synthesis. This has, in fact, been shown to be true, both in in vivo models and in human subjects. Increasing wound oxygenation results in increased collagen deposition and tensile strength, with maximal effects seen at levels in which wound oxygenation is increased above normal physiologic conditions by the addition of supplemental O_2 [31–33]. Among a group of postoperative patients all treated with

supplemental O_2 (4 L/min through nasal cannula for 12 hours for 3 days), three times as much collagen was deposited in wound cylinders in patients with well-perfused and oxygenated wounds compared with those with lower oxygenation and perfusion scores [34]. Thus, O_2 therapy can optimize collagen deposition and tensile strength.

Oxygen as an antibiotic

Wound tissue pO_2 levels are a major determinant of susceptibility to infection, and this has been shown both in experimental models and in human subjects. In a guinea pig model, the amount of skin loss seen after subcutaneous innoculation of bacteria was inversely proportional to wound oxygenation—hypoxic wounds were large, and the smallest wounds were seen in animals receiving supplemental O_2. The efficacy of supplemental O_2 in preventing skin loss was similar to antibiotic administration, and combining both modalities had additive beneficial effects [35,36]. These experimental observations are supported by clinical studies. Wound tissue oxygenation is an extremely sensitive indicator for the risk of infection in surgical patients [37]. This study established a clear clinical correlation between O_2 availability and the development of wound infection. A subsequent study by Grief et al [38] provided additional clinical evidence that enhancing wound O_2 levels through the administration of supplemental O_2 can improve host immune responses. In that study of 500 patients undergoing abdominal surgery, all of whom received prophylactic antibiotics, administration of O_2 at an 80% FiO_2 during surgery and for 2 hours postoperatively resulted in a 5.2% wound infection rate versus an 11.2% infection rate in patients given O_2 at a 30% FiO_2 [38].

The ability of supplemental O_2 to reduce infection is mediated by ROS generated by NADPH oxidases in wound neutrophils and macrophages. The concentration of O_2 necessary to achieve half maximal ROS production (the K_m) is in the range of 45 to 80 mm Hg, with maximal ROS production seen at pO_2 at >300 mm Hg [39]. Thus, just as with the enzymes regulating collagen synthesis, the maximal effects of this biologic process can be achieved only through the administration of supplemental O_2 to attain wound pO_2 levels beyond those encountered when breathing room air. In fact, approximately 98% of the O_2 consumed by wound neutrophils and macrophages is utilized for respiratory burst [39]. At the wound site, ROS are generated by almost all wound-related cells. The biological significance of such ROS has been recently reviewed [5].

Oxygen therapy: diagnostic, preventive and therapeutic

The availability of respired O_2 to wound tissues depends upon vascular supply, vasomotor tone, arterial pO_2, and the diffusion distance for molecular O_2. Edema and necrotic debris both increase the diffusion distance for O_2 to reach the wound, so debridement is an important step to diminish obstruction to wound oxygenation. Peripheral vasoconstriction can also significantly limit wound perfusion and oxygenation, so that little to no enhancement of wound pO_2 levels are achieved despite breathing supplemental O_2 [37,40,41]. Furthermore, correction of hypoxemia and vasoconstriction can yield a 10-fold rise in collagen deposition [33,34,37,42]. Therefore, for optimal wound perfusion and oxygenation, patients must be warm and have adequate intravascular volume and adequate control of pain and anxiety. In estimating intravascular volume, tissue oxygenation is extremely sensitive, but is not practical at this time. Urine output is not a reliable indicator of intravascular volume, and the standard maintenance fluids given after surgery are usually insufficient [43,44]. For practical purposes, capillary refill (<1.5 seconds at the forehead) or eye turgor are more sensitive indicators of intravascular volume status. Many surgeons take these concepts for granted, but when properly addressed, all have been shown to have a significant impact on wound healing in clinical settings. Clinical trials have shown that keeping patients normothermic and administering supplemental O_2, both of which enhance wound oxygenation, decreases the rate of wound infection in surgical patients and shortens the average length of hospital stay [38,45].

The clinical application of O_2 to wound healing occurs at many levels: diagnostic, preventive and therapeutic. From a diagnostic standpoint, many surgeons already use measurements of wound oxygenation to guide their treatment planning when they obtain transcutaneous O_2 measurements (TcO_2) with noninvasive vascular studies. TcO_2 measurements provide reliable prognostic information regarding the ability of wounds to heal, and this has been used to determine amputation levels [46,47]. It is important to note, though, that TcO_2 measurements do not reflect wound-site pO_2. They overestimate pO_2 in the intact tissue at the wound perimeter. Standard TcO_2 measurements are conducted under conditions where the skin is warmed to 42°C. This warmth factor contributes to overestimation of pO_2, especially because O_2 therapy to the wound typically is not accompanied by warming of the wound site. Advancement of technology to directly estimate pO_2 in the wound core is warranted. It is important to note that there is a fundamental difference between the intact skin in the perimeter of the wound compared with the wound core. Whereas the former is well vascularized, wound cores are typically characterized by disrupted vasculature, and are unlikely to benefit from respired O_2 carried to tissues by blood vessels.

In preventive applications, optimizing wound perfusion and providing supplemental O_2 in the perioperative period have been shown clinically to reduce the incidence of postoperative infections [38,45]. For therapeutic applications to wounds, O_2 can be given to the patient systemically, using pure O_2 (either pressurized or not), or can be delivered

Table 1
Contrasting hyperbaric oxygen therapy with topical oxygen delivery modalities for wound care

Systemic hyperbaric oxygenation	Topical delivery of oxygen
Systemically oxygenates blood at 2–3 atmospheres	Topically oxygenates would tissue at 1 atmosphere
Requires specialized facilities and personnel	Portable devices: available bedside and in field
Relatively expensive	Inexpensive
Relies on vascular system to deliver O_2 to wound	Can deliver oxygen directly to superficial wounded tissue severed from circulation
Poor vascularity of wound tissue limits O_2 diffusion	Oxygenation not dependent on vascular bed
Risk of multiorgan oxygen toxicity	No risk of multi-organ oxygen toxicity
Relatively well-studied for outcome, limited studies addressing underlying mechanisms	More limited research literature on outcome and mechanism

locally to the wound using a topical device. Hyperbaric O_2 therapy (HBOT) delivers 100% O_2 at 2 to 3 atmospheres (atm) of pressure and patients typically receive 10 to 30 treatments, depending upon the diagnosis. These treatments are usually 60 to 120 minutes long, given 5 days a week, and performed in specialized chambers at facilities with physician supervision. HBOT is capable of elevating arterial pO_2 as high as 1200 mm Hg. As discussed above, systemically administered O_2 relies on the vasculature to be delivered to tissues. Thus, while such a form of therapy may efficiently improve pO_2 of skin in the wound perimeter, it is reasonable to assume that areas of the wound not supported by blood vessels will not benefit as much. Note that when HBOT is applied in a monoplace chamber, exposed dermal wound receives topical O_2 as well. This additional route of O_2 delivery to the wound is frequently overlooked, with the tendency to explain all benefits on the basis of O_2 administered systemically. While topical O_2 is not likely to diffuse into deeper tissues, it does have the advantageous potential to oxygenate superficial areas of the wound not supported by intact vasculature. In this way, topical O_2 may correct pO_2 of cells at the wound core, thus correcting hypoxia-induced impairment of NADPH oxidase function in those cells. NADPH oxidase function in wound-related cells contributes to favorable processes such as cell motility, angiogenesis, and extracellular matrix formation [5].

Another key issue that warrants a careful dissection in comparing the effects of systemic O_2 versus topical O_2 is the risk of systemic pure O_2 toxicity to vital organs. Like many other risk factors, including cigarette smoking, HBOT does not result in immediate manifestation of clinical abnormalities in most cases. This line of evidence cannot be accepted as proof of safety unless detailed biochemical and molecular investigation is conducted to test markers of oxidative damage in the blood and urine of treated subjects. It is general knowledge that exposure of biological cells and tissues to pure O_2 may result in oxidative stress and genotoxicity [48]. There is no question that exposure to pure O_2 presents risks and that it is prudent to avoid unnecessary exposure to a risk factor. Favorable outcomes in studies using sub-pure O_2 under normobaric conditions [38] lead us to question the use of pure O_2 under pressure for wound therapy. Furthermore, encouraging outcomes obtained from the use of topical O_2 alone [47] warrant a more detailed

investigation comparing the systemic and topical O_2 modalities (Table 1) under normobaric and hyperbaric conditions. Such fine-tuning of conditions for O_2 therapy should result in more cost-effective and efficient care, minimizing barotraumas and other risks associated with use of pressurized pure O_2. If proven to be efficient, topical O_2 therapy has the added advantage of caring for a much larger potential patient population, especially under conditions of public disaster and in a field-setting where HBOT is simply not applicable.

Correction of wound pO_2 is a fundamental issue that by itself may trigger wound healing. More importantly, approaches to correct wound pO_2 are expected to have a profoundly favorable influence on other therapies, such as responsiveness to growth factors and acceptance of grafts [5]. Investigative efforts that focus on mechanisms and rigorous clinical evaluation on the basis of randomized controlled trials will assist in elevating O_2 therapy to the mainstream of medicine.

Acknowledgments

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