Abstract

Hypoxemia, caused by disrupted vasculature, is a key factor that limits wound healing. Correcting hypoxemia through the administration of supplemental oxygen (O2) can have significant beneficial impact on wound healing in the perioperative and outpatient settings. Beyond its role as a nutrient and antibiotic, O2 may support vital processes such as angiogenesis, cell motility, and extracellular matrix formation. Recent discoveries highlight a novel aspect, addressing the role of O2 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 H2O2) from O2. Defect in these enzymes is associated with impaired healing. Low wound pO2 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 O2 therapy presents potential advantages, as well as risks. There is evidence to suspect that the use of pressure and systemic pure O2 may not be essential in wound care. Elimination of these factors by using sub-pure systemic O2 under normobaric conditions may significantly minimize the risk of O2 toxicity. Furthermore, opportunities to treat dermal wounds using topical O2 therapy warrant further investigation. Given that many growth factors require ROS for their function, it is reasonable to assume that approaches to correct wound pO2 will serve as an effective adjunct in treating chronic wounds. © 2003 Excerpta Medica, Inc. All rights reserved.

Keywords: Redox; Hyperbaric oxygen therapy; Oxygen toxicity; Signal transduction; Hyperoxia
impaired healing in humans [16]. Defects in NADPH oxidase are associated with cyto
cytokine oxidase (renamed as NADPH oxidase) to bacterial control of wound repair have been recently reviewed [5]. This concept sharply departs from the orthodox view of angiogenesis, cell motility, and extracellular matrix formation [5]. These ROS contribute as cellular messengers to pro-
Oxygen: beyond nutritional support

Angiogenesis is a critical early aspect of the wound healing response. While hypoxia can initiate neovascular-
ization, it cannot sustain it. Supplemental O2 administration accelerates vessel growth [21]. It has been established that VEGF is a major long-term angiogenic stimulus at the wound site. O2 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 O2 may trigger the differentiation of fibroblasts to myo-
peroxidase; GF = growth factor.

reactive oxygen species; SOD = superoxide dismutase; GF = growth factor.

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 benefi
catalyze H2O2, which is then converted by catalase to H2O and O2 [5]. These ROS contribute as cellular messengers to pro-
motor responses 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 (O2–) 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 O2 and its derivatives in wound healing [5]. Genetic approaches to bolster NADPH oxidase in nonphagocytic cells promote dermal healing [13]. Thus, O2 has a role in healing beyond its function as a nutrient and antibiotic. Given that growth factors, such as platelet-de-
ned growth factor (PDGF), require ROS for their action on cells [20], it is clear that O2 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.

Among a group of postoperative patients all treated with

reactive oxygen species such as free radicals and H2O2 by which O2 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 O2 to reactive oxygen species (ROS), including oxidizing species such as free radicals and H2O2 [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].

Oxygen: beyond nutritional support

Angiogenesis is a critical early aspect of the wound healing response. While hypoxia can initiate neovascular-
ization, it cannot sustain it. Supplemental O2 administration accelerates vessel growth [21]. It has been established that VEGF is a major long-term angiogenic stimulus at the wound site. O2 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 O2 may trigger the differentiation of fibroblasts to myo-

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 benefi

catalyze H2O2, which is then converted by catalase to H2O and O2 [5]. These ROS contribute as cellular messengers to pro-
motor responses 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 (O2–) 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 O2 and its derivatives in wound healing [5]. Genetic approaches to bolster NADPH oxidase in nonphagocytic cells promote dermal healing [13]. Thus, O2 has a role in healing beyond its function as a nutrient and antibiotic. Given that growth factors, such as platelet-de-
ned growth factor (PDGF), require ROS for their action on cells [20], it is clear that O2 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.

Among a group of postoperative patients all treated with
supplemental O₂ (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, O₂ therapy can optimize collagen deposition and tensile strength.

Oxygen as an antibiotic

Wound tissue pO₂ 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 inoculation of bacteria was inversely proportional to wound oxygenation—hypoxic wounds were large, and the smallest wounds were seen in animals receiving supplemental O₂. The efficacy of supplemental O₂ 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 O₂ availability and the development of wound infection. A subsequent study by Grief et al [38] provided additional clinical evidence that enhancing wound O₂ levels through the administration of supplemental O₂ can improve host immune responses. In that study of 500 patients undergoing abdominal surgery, all of whom received prophylactic antibiotics, administration of O₂ at an 80% FiO₂ during surgery and for 2 hours postoperatively resulted in a 5.2% wound infection rate versus an 11.2% infection rate in patients given O₂ at a 30% FiO₂ [38].

The ability of supplemental O₂ to reduce infection is mediated by ROS generated by NADPH oxidases in wound neutrophils and macrophages. The concentration of O₂ necessary to achieve half maximal ROS production (the Kₘ) is in the range of 45 to 80 mm Hg, with maximal ROS production seen at pO₂ 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 O₂ to attain wound pO₂ levels beyond those encountered when breathing room air. In fact, approximately 98% of the O₂ 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 O₂ to wound tissues depends upon vascular supply, vasomotor tone, arterial pO₂, and the diffusion distance for molecular O₂. Edema and necrotic debris both increase the diffusion distance for O₂ 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 pO₂ levels are achieved despite breathing supplemental O₂ [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 O₂, 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 O₂ 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 O₂ measurements (TcO₂) with noninvasive vascular studies. TcO₂ 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 TcO₂ measurements do not reflect wound-site pO₂. They overestimate pO₂ in the intact tissue at the wound perimeter. Standard TcO₂ measurements are conducted under conditions where the skin is warmed to 42°C. This warmth factor contributes to overestimation of pO₂ especially because O₂ therapy to the wound typically is not accompanied by warming of the wound site. Advancement of technology to directly estimate pO₂ 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 O₂ carried to tissues by blood vessels.

In preventive applications, optimizing wound perfusion and providing supplemental O₂ in the perioperative period have been shown clinically to reduce the incidence of postoperative infections [38,45]. For therapeutic applications to wounds, O₂ can be given to the patient systemically, using pure O₂ (either pressurized or not), or can be delivered
Table 1
Contrasting hyperbaric oxygen therapy with topical oxygen delivery modalities for wound care

<table>
<thead>
<tr>
<th>Systemic hyperbaric oxygenation</th>
<th>Topical delivery of oxygen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Requires specialized facilities and personnel</td>
<td>Topically oxygenates would tissue at 1 atmosphere</td>
</tr>
<tr>
<td>Relatively expensive</td>
<td>Portable devices: available bedside and in field</td>
</tr>
<tr>
<td>Relies on vascular system to deliver O₂ to wound</td>
<td>Inexpensive</td>
</tr>
<tr>
<td>Poor vascularity of wound tissue limits O₂ diffusion</td>
<td>Can deliver oxygen directly to superficial wounded tissue severed from circulation</td>
</tr>
<tr>
<td>Risk of multiorgan oxygen toxicity</td>
<td>Oxygenation not dependent on vascular bed</td>
</tr>
<tr>
<td>Relatively well-studied for outcome, limited studies addressing</td>
<td>No risk of multi-organ oxygen toxicity</td>
</tr>
<tr>
<td>underlying mechanisms</td>
<td>More limited research literature on outcome and mechanism</td>
</tr>
</tbody>
</table>

locally to the wound using a topical device. Hyperbaric O₂ therapy (HBOT) delivers 100% O₂ 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 pO₂ as high as 1200 mm Hg. As discussed above, systemically administered O₂ relies on the vasculature to be delivered to tissues. Thus, while such a form of therapy may efficiently improve pO₂ 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 O₂ as well. This additional route of O₂ delivery to the wound is frequently overlooked, with the tendency to explain all benefits on the basis of O₂ administered systemically. While topical O₂ 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 O₂ may correct pO₂ 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 O₂ versus topical O₂ is the risk of systemic pure O₂ 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 O₂ may result in oxidative stress and genotoxicity [48]. There is no question that exposure to pure O₂ presents risks and that it is prudent to avoid unnecessary exposure to a risk factor. Favorable outcomes in studies using sub-pure O₂ under normobaric conditions [38] lead us to question the use of pure O₂ under pressure for wound therapy. Furthermore, encouraging outcomes obtained from the use of topical O₂ alone [47] warrant a more detailed investigation comparing the systemic and topical O₂ modalities (Table 1) under normobaric and hyperbaric conditions. Such fine-tuning of conditions for O₂ therapy should result in more cost-effective and efficient care, minimizing barotraumas and other risks associated with use of pressurized pure O₂. If proven to be efficient, topical O₂ 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 pO₂ is a fundamental issue that by itself may trigger wound healing. More importantly, approaches to correct wound pO₂ 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 O₂ therapy to the mainstream of medicine.

Acknowledgments

Supported by GM27345 and DE013749 (seed) to CKS.

References
