

Topical oxygen and burn wound healing: a review

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Summary

Oxygen is essential for the epithelialization and contraction of the burn wound as well as for the collagen maturation and synthesis. Inspiration of pure oxygen or employing hyperbaric oxygen might delay wound healing due to an intensive vasoconstriction. The accumulated data suggest a beneficial effect of topical oxygen on the wound healing process of burns.

INTRODUCTION

MICROCLIMATE factors, such as gas composition, relative humidity, pH and temperature, when applied topically, have a significant effect on the healing process of burns. The effect of hyperbaric oxygen on the burn wound is subjected to controversy, possibly due to its severe systemic complications. However, treatment with topical oxygen avoids these untoward effects and promotes epidermal mitosis and migration, as well as collagen synthesis and contraction of the wound. The present paper deals with the aspects of the healing process of the burn wound affected by topical oxygen.

PO₂ in the Burn Tissue

Experimental and clinical data suggest that burn tissue PO₂ is an excellent index of the peripheral perfusion following the burn trauma. Remensnyder (1972) showed a significant diminution of the tissue oxygenation in the unstable zone of the burn oedema in the first 45 minutes following the burn in the rat's cremaster muscle. Steep gradients of O₂ tensions existed over very short distances, and the hypoxic areas corresponded to the zones of vascular stagnation and thrombus formation. Hunt et al., (1975) determined tissue PO₂ levels under unburned skin of

human burned patients, and suggested a micro-circulatory impediment to O₂ diffusion into tissue in these patients. The burn patients managed to exhibit normal arterial responses to O₂ breathing, but the tissue O₂ tensions responded slowly and only marginally to an increment of arterial PO₂. In patients with over 30 per cent burns, it took several weeks before a normal state of tissue oxygenation was re-established. In contrast, the same authors observed that a normal state of peripheral oxygenation was reached in injured but not burned patients as early as 4 to 5 days. Nieminen et al., (1977) studied the early effects of 10 per cent full-thickness skin burns on the subcutaneous tissue PO₂ and PCO₂ in rabbits. Thermal injury resulted in a rapid, progressive decrease of tissue PO₂ both in the burn areas and in the distant tissues, with the greatest decrease in the burn site. The minimum PO₂ levels were observed within 3 to 6 hours following burn injury. The PCO₂ values increased markedly in both areas immediately after the trauma, and gained their maximum within 1 to 3 hours post burn. The highest accumulation of CO₂ occurred, according to this study, at the burn site. Six hours following the burn injury, tissue CO₂ levels were found to be normalized. In another study, Silver (1971) demonstrated that PO₂ levels at the burn site were very low and did not exceed 10 mm/Hg. This hypoxic state persisted for several days.

Burn Wound Oxygen Consumption

Oxygen consumption in the burn wound was demonstrated to be two-fold by the second week following the burn injury (Wilmore and Aulick.

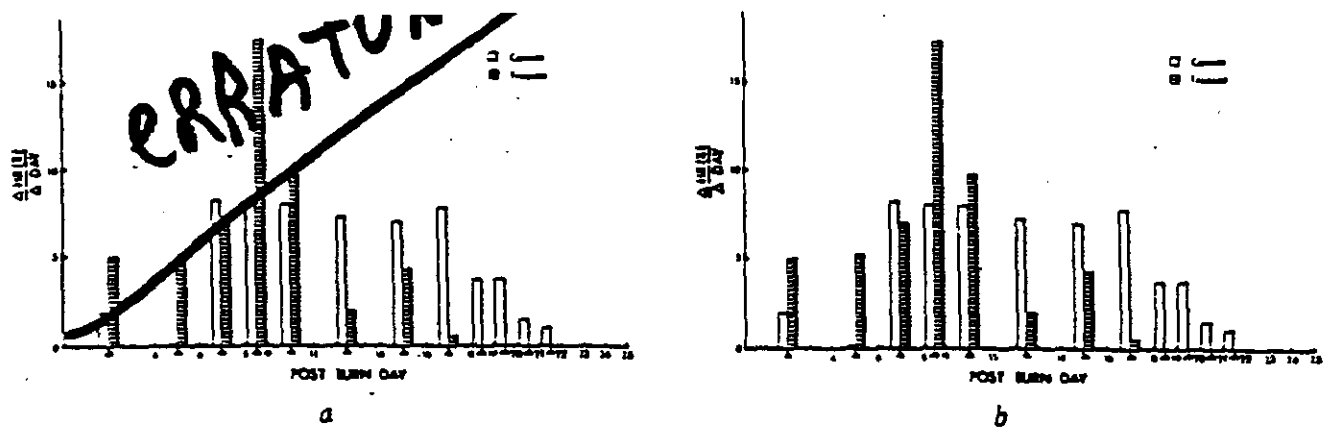


Fig. 1. (a) and (b) The slope of healing rate of experimental third degree burn wounds. ΔHR —represents the difference of healing rate between two sequential measurements. Δday —represents the interval in days between two sequential measurements.

Houlihan et al., 1967, and Ulvedal et al., 1968). These studies demonstrate that prolonged hyperoxic exposure at sea level and under hyperbaric conditions activates the adrenocortical and medullary function, similar to that observed in the general stress reaction. The ultimate result of this pathologic state is a generalized vasoconstriction of the arterioles in all organs including the skin. Yagi and Ohishi (1977) showed that adult albino rabbits breathing 90 to 95 per cent oxygen for 40 hours induced degeneration of the retina due to circulatory alterations. Other studies also suggest that retinal changes in hyperoxia are caused by an intensive vasoconstriction (Saltzman et al., 1965 and Trokel, 1965)

In view of this data, a delay of wound healing, as well as a marginal response to an increment of arterial PO_2 , might be expected in burned patients when high concentrations of O_2 are inspired for long periods.

Effect of Topical Oxygen on Epithelialization, Contraction and Scar Formation

The topical effect of oxygen has been investigated in part and over very short periods of time, and an adequate methodology for studying this variable has not been available until recently (Kaufman and Hirshowitz, 1982). Hunt and Pai (1972) claimed that Indians who lived in the mountains of Western America believed for centuries that their wounds healed more quickly as they descended into the 'richer air' of the valleys. Moreover, the Dead Sea in Israel, situated 1290 feet below sea level, has been considered for some decades favourable for healing chronic

skin wounds due to the higher oxygen tensions (Gilbert, 1981).

An unrandomized and uncontrolled clinical study showed that topical treatment with pure oxygen improved epithelialization and contraction of chronic bed sores (Fischer, 1969), and Silver (1971) suggested that direct access of pure oxygen to the surface of open wounds would promote epidermal cell mitosis as well as migration. Silver demonstrated that oxygen applied directly on wounds covered by dressings permeable to O_2 , such as Teflon® or polyesthene, raised the epidermal PO_2 , greatly (to 685 and 628 mm/Hg respectively). The local availability of atmospheric oxygen and its capability to penetrate through the surface of open wounds and promote the aerobic metabolism of the epidermal cells resulted in a very rapid rate of healing. In agreement with this assumption, it has been shown that topical oxygen supply is a major factor in determining the rates of both mitotic activity and epithelial migration (Winter, 1963). The additional effect of humidity was proved to be critical for the mitosis and migration of epithelial cells (Winter, 1962). Luccioni (1963), in an uncontrolled clinical study, suggested a beneficial effect of topical oxygen in burn patients. Hypertrophic scars are commonly found at the border of skin grafts and in deep second and third degree burns which heal by contraction and epithelialization over areas of granulation tissue. These areas heal slowly, contain high concentrations of inflammatory cells, and are sites of increased collagen and proteoglycans synthesis. This chronic wound healing environment is continuously exposed to the surrounding ambient oxygen, which diffuses

1978). This extremely high need for oxygen is decreased only by covering the wound with skin grafts. The polymorphonuclear neutrophilic leucocytes necessitate oxygen for the host defence activities, and the fibroblasts need it for synthesis of collagen as well. Moreover, up to 30 per cent of the energetic requirements of the granulation tissue are derived from glucose oxidation pathway. Thus, the increased metabolic demands of the healing burn wound might be met partially by accelerated glucose uptake and oxidation.

Oxygen and Collagen Synthesis

The effect of oxygen on collagen synthesis has been extensively investigated. Using ^{18}O , Fujimoto et al., (1963), as well as other investigators (Prockop et al. 1963), proved that up to 93 per cent of the oxygen which is incorporated into the OH-groups of newly synthesized hydroxyproline by the prolyl-hydroxylase is derived from the atmosphere. Moreover, another study (Tanzer, 1973) suggested that atmospheric-derived oxygen was also necessary to form the cross-linking bonds in the collagen molecules by the lysyl amine oxidase, as well as for the formation of intermolecular bonds. Kao et al., (1963) observed that *in vitro* synthesis of collagen by the connective tissue was accelerated when atmospheric O_2 was raised from 21 to 95 volumes per cent. In agreement with these results, Peterkofski et al., (1963) demonstrated inhibition of proline hydroxylation in complete anaerobiosis with the accumulation of atypical hydroxyproline-deficient collagen. Similar results have been reported by Hutton et al., (1967) who observed a close correlation between the rate of proline hydroxylation and the O_2 concentration over the range of 0.51 to 14.9 volumes per cent of O_2 using a partially purified chick embryo hydroxylase. Chvapil et al., (1968) showed that maturation and cross-linking of collagen in chick embryo skin slices increased almost linearly when the O_2 concentration of the incubation gas was elevated from 20 to 95 volumes per cent. Studies carried out *in vivo* by Niinikosky et al., (1970) suggested that accumulation of collagen and the amount of RNA produced in cellulose sponges in rats were decreased by hypoxia and increased by hyperoxia, reaching a peak when the inspired O_2 concentration was 70 per cent at 1 Atm. pressure, and decreased again during exposure to higher O_2 tensions. In these studies, as well as the following, modifications of the environment of the whole experimental animal were carried out, and the animal was forced to

breathe in a high PO_2 atmosphere. Hunt and Pai (1972) observed a rough correlation between inspired oxygen and collagen synthesis over the range of 14 to 40 per cent. Breathing 45 per cent of O_2 promoted healing of full-thickness skin wounds in rats, but reduced O_2 tensions led to a slower healing rate (Pai and Hunt, 1972).

Effect of Inspired Oxygen on Wound Healing

Wound tensile strength was found to be dependent on the cross-linking bonds of the collagen, and therefore is directly related to the availability of oxygen. Other investigators (Stephens and Hunt, 1971) reported that improved O_2 supply to experimental incisional wounds in rats breathing high concentrations of O_2 increased tensile strength. In contrast, increased PCO_2 in blood and the wound diminished wound strength. Silver (1971) exhibited a direct correlation between inspired O_2 over the range of 25 to 40 per cent and granulation of tissue formation. On the other hand, when inspired air consisted of higher tensions of O_2 , a delay in granulation tissue formation was observed.

Most of the studies reported regarding the *in vivo* effect of O_2 on healing were carried out by modifying the environment of the whole animal, hence the animal was forced to breathe in high PO_2 tensions. Moreover, most of the investigators would limit the exposure of the tested animal to a maximal O_2 concentration of 40 to 60 per cent. Beyond these levels of oxygen, a delay in wound healing or tensile strength would be expected (Silver, 1971).

One of the pitfalls of the above-mentioned studies is the toxic effects of inspired high oxygen tensions on the pulmonary functions and the central nervous system. At exposure tensions of 1 ATM. or less, the lung is the first vital organ to respond adversely to an increased delivery of oxygen. At exposures of greater than 1 Atm., central nervous system damage occurs concurrently with lung damage (Comroe et al., 1945, Ohlsson, 1947, Caldwell et al., 1966, Puy et al., 1968 and Clark et al., 1971). Pulmonary damage would therefore lead to eventually lowering the PO_2 and hence a decrease of oxygen supply to the wound (Silver, 1971). Another mechanism through which breathing high O_2 concentration inhibits wound healing is the stimulation of the sympathetic autonomic system (Buckingham et al., 1968, Faiman et al., 1966, Haggendal, 1967 and Gerhsenovich et al., 1955), which causes increments of epinephrine secretion and ultimately adrenal hypertrophy (Bean et al., 1953,

through the surface. In contrast, penetration of oxygen through the skin graft and intact skin is precluded (Silver, 1971). Therefore, it is assumed that topical oxygen might be one of the main contributing factors to the formation of a postburn hypertrophic scar.

In a controlled study (Kaufman et al., 1982), topical application of humidified oxygen on experimental third degree burns in unrestrained guinea-pigs breathing room air promoted epithelialization, contraction and collagen synthesis and maturation. Employing a new technology to control the microclimate factors, the effect of continuous application of 96 per cent O₂ and 75 per cent relative humidity on the healing rate of deep burns in unrestrained guinea-pigs was investigated over a healing interval of 25 days. The humidity O₂ treated wounds healed 99 per cent of their initial size by post-burn day 18, and the control wounds required an additional 4 days to close. The slope of healing rate of the O₂-humidity treated wounds showed a more rapid rate of healing immediately following the burn injury, and healing reached a peak by day 8 to 9 (Fig. 1). Then, the rate of healing slowed until complete wound closure was achieved. In contrast, the control wounds healed relatively slowly during the first 6 days following the burn, with a constant rate during the next 10 days. Subsequently, the healing rate of the control wounds also slowed when the wound was nearly completely closed. Healing was mainly from contraction to a lesser extent from epithelialization.

A highly significant difference was demonstrated between the humidified O₂ treated wounds and the controls during the 25 days of this study. In the above studies, SEM and TEM micrographs of tissue biopsies taken on PBD 25 revealed a marked promotion of collagen maturation in the treated wounds. The humidified-O₂ treated wounds consisted of a high ratio of collagen/ground substance; assembling of distinct fibres to form bundles with individual fibres of 1300 to 1500 Å in diameter, and an organized orientation: a highly aggregated rough endoplasmic reticulum of the fibroblasts, consisting of large cisternae and an abundance of ribosomes. The control burn wounds exposed to ambient air (O₂-21 per cent, temperature: 24°C ± 1 and relative humidity: 30 ± 2) exhibited a low ratio of collagen/ground substance, a loose, rough endoplasmic reticulum: fibres had a diameter of 700-800 Å and a haphazard orientation of the fibres with a poor tendency to form bundles.

CONCLUSIONS

Following burn injury, a persistent hypoxia is established in the burn wound. Epidermal cell mitosis and migration are dependent on local availability of oxygen. Atmospheric-derived oxygen is essential for collagen synthesis and its maturation in the granulation tissue of the burn wound.

Inspiration of pure oxygen or employing hyperbaric oxygen might delay wound healing as a result of an intensive vasoconstriction. The clinical and experimental data suggest a beneficial effect of topical oxygen on the wound healing of burns. However, more studies are necessary to clarify the role of oxygen in this process.

REFERENCES

- Bean J. W., Baker B. L. and Johnson P. (1953) Cytological alterations of adrenal cortex induced by oxygen of high pressure. *Fed. Proc.* 12, 11.
- Buckingham S., Sommers S. C. and McNary W. F. (1968). Experimental respiratory distress syndrome. I. Central autonomic and humoral pathogenetic factors in pulmonary injury of rats induced with hyperbaric oxygen and the protective effects of barbiturates and trasyolol. *Biol. Neonatorum* 12, 261.
- Caldwell P. R. B. et al., (1966). Changes in lung volume, diffusion capacity, and blood gases in men breathing oxygen. *J. Appl. Physiol.* 21, 1477.
- Chvapil M., Hurych J., Ehrlichova E. (1968). The influence of carious oxygen tensions upon proline hydroxylation and the metabolism of collagenous and non-collagenous proteins in skin slices. *Z. Physiol. Chem.* 349, 211.
- Clark J. M., Lambertsen C. J. (1971). Rate of development of pulmonary O₂ toxicity in man during O₂ breathing at 2.0 atm. *Abst. J. Appl. Physiol.* 30, 739.
- Comroe J. H., Dripps R. D., Dumke P. R. et al., (1945). Oxygen toxicity. The effect of inhalation of high concentrations of O₂ for 24 hours on normal men at sea level and at a simulated altitude of 18,000 feet. *JAMA* 128, 710.
- Faiman M. D. and Heble A. R. (1966). The effect of hyperbaric oxygenation on cerebral amine. *Life Sci.* 5, 2225.
- Fischer B. H. (1969). Topical hyperbaric oxygen treatment of Pressure sores and skin ulcers. *Lancet* 2, 405.
- Fujimoto D. and Tamiya N. (1963). Studies on collagen metabolism with ¹⁴O as a tracer. *Biochem Biophys. Acta.* 69, 559.
- Gershenovich Z. S., Kaichevskaya A. A. and Alekseenko L. P. (1955). Adrenaline substance of brain and suprarenals under increased oxygen pressure. *Ukr. Biokhem. Zh.* 27, 3.

- view. In: Gilbert D. L. (ed) *Oxygen and Living Processes. Interdisciplinary Approach*. Berlin Springer Verlag, p. 381.
- Haggendal J. (1967). The effect of high pressure air or oxygen with and without carbon dioxide added on the catecholamine levels of the rat brain. *Acta Physiol. Scand.* 69, 147.
- Houlihan R. T., Zavodni J. J. and Cross M. H. (1967). Adaptation to increased oxygen tension at ambient pressure. *Aerosp. Med.* 38, 995.
- Hunt T. K. and Pai M. P. (1972). The effect of ambient oxygen tensions on wound metabolism and collagen synthesis. *Surg. Gynecol. Obstet.* 135, 561.
- Hunt T. K., Sheldon G. and Fuchs R. (1975). Physiological mechanisms in repair of burns. *Burns* 1, 212.
- Hutton J. J. jun., Tappel A. L. and Udenfriend S. (1967) Cofactor and substrate requirements of collagen proline hydroxylase. *Arch. Biochem. Biophysics* 118, 231.
- Kao Ky et al., (1963). Connective tissue. VIII. Factors effecting collagen synthesis by sponge biopsy connective tissue. *Proc. Soc. Exp. Biol. (N. Y.)* 113, 762.
- Kaufman T. Alexander J. W., Nathan P. et al., (1982). The microclimate wound chamber: Topical treatment of experimental deep burns with humidified oxygen. *Surg. Forum.* (In the press).
- Kaufman T. and Hirshowitz B. (1982). The influence of various microclimate conditions on the burn wound. *Burns* 9, 84.
- Luccioni F. (1963) Treatment of burns by flowing oxygen—A film presentation. *Bahama International Conf. on Burns.*
- McWhirter N. (1979). *Guinness Book of World Records*. 17th Ed. New York: Bantam Books.
- Nieminen S. Fraki J. Niinikoski J. et al., (1977). Acute effects of burn injury on tissue gas tensions in the rabbit. *Scand J Plast Reconstr Surg* 11, 69.
- Niinikoski J. (1970). Effect of oxygen supply on wound healing and formation of experimental granulation tissue. *Acta Physiol. Scand.* (Suppl.) 334, 78, 1.
- Ohlsson W. T. L. (1942). Study on oxygen toxicity at atmospheric pressure. *Acta Med. Scand.* (Suppl.) 190.
- Pai M. P. and Hunt T. K. (1972). Effect of varying oxygen tensions on healing of open wounds. *Surg. Gynec. Obstet.* 135, 756.
- Peterkofski B. and Udenfriend S. (1963). Conversion of proline to collagen hydroxyproline in a cell-free system from chick embryo. *J. Biol. Chem.* 238, 3966.
- Prockop D. J., Kaplan A. and Udenfriend S. (1963). Oxygen-18 studies on the conversion of proline to collagen hydroxyproline. *Arch Biochem Biophys.* 101, 499.
- Puy R. J. M., et al., (1968). Alterations in the pulmonary capillary bed during early O₂ toxicity in man. *J. Appl. Physiol.* 24, 537.
- Remensnyder J. P. (1972). Topography of tissue oxygen tension changes in acute burn oedema. *Arch. Surg.* 109, 477.
- Saltzman H. A. et al., (1965). Retinal vascular response to hyperbaric oxygenation. *JAMA* 191, 290.
- Silver I. A. (1971). Wound healing and cellular micro-environment. Final technical report. US Army R and D Command Contract DAJA 37-70-2328.
- Stephens E. O. and Hunt T. K. (1971). Effect of changes in inspired oxygen and carbon dioxide tensions on wound tensile strength. An experimental study. *Ann. Surg.* 173, 515.
- Tanzer M. L. (1973). Crosslinking of collagen. *Science* 180, 561.
- Trockel S. (1965). Effect of respiratory gases upon choroidal haemodynamics. *Arch Ophthalmol.* 73, 838.
- Ulvedal F. and Roberts A. J. (1968). Endocrine functions in an oxygen atmosphere at reduced total pressure. *Aerosp. Med.* 39, 1218.
- Winter G. D. (1962). Formation of the scab and the rate of epithelialization of superficial wounds in the skin of young domestic pigs. *Nature* 193, 293.
- Winter G. D. and Scales J. T. (1963). Effect of air drying and dressings on the surfaces of a wound. *Nature* 197, 91.
- Wilmore D. W. and Auilick L. H. (1978). Metabolic changes in burned patients. *Surg. Clin. North America* 58, 1173.
- Yagi K. and Ohishi N. (1977). Mechanism of degeneration of the retina of animals exposed to a high concentration of oxygen. In: Hayaishi O., Asada, K. (eds) *Biochemical and Medical Aspects of Active Oxygen*. Baltimore University Par. Press. p. 299.

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