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Oxygen: At the Foundation of Wound Healing—Introduction

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Abstract. "Wound Healing: Oxygen & Emerging Therapeutics" Columbus, Ohio, September 12–15, 2002. Sponsored by the National Institutes of Health (R13AR049171), International Union of Biochemistry & Molecular Biology and UNESCO-Global Network of Molecular & Cell Biology. Conference co-chairs: Chandan K. Sen, the Ohio State University Medical Center and Thomas K. Hunt, University of California- San Francisco. This congress was conceived for two reasons: to consolidate what is known about oxygen in the repair process and to stimulate discussion about new developments of control of healing by redox regulated signaling processes. A historical and evolutionary perspective on the role of oxygen in wound healing—from the classical physiology of oxygen in the wound to the refined concept of redox signaling— is presented.

This congress [1] was conceived for two reasons: to consolidate what is known about oxygen in the repair process and to stimulate discussion about new developments in the control of healing by redox regulation.

The history of discovery of the multiple roles of oxygen in wound healing makes interesting reading. In 1964, one of us (T.K.H.) was asked by the National Science Foundation (NSF) to visit Glasgow, Scotland, to investigate claims being made for hyperbaric oxygen (HBO) (as things were then, the application was proforma!). Jacques Cousteau's divers had reported that they healed their work wounds significantly better when they lived in an undersea habitat about 35 feet under the surface of the Red Sea, and the NSF was interested in pursuing the observation. An application through the usual routes probably would have gone no farther than the wastebasket, but Cousteau held the day. The Glasgow unit at that time was controversial even in the Western Infirmary where it was located. Most of the medical staff thought HBO was nonsense because they were committed to a wrong but profoundly tenacious belief that only the oxygen that reached tissue via being bound to hemoglobin had any biological or clinical value. And that was all there was. They were not timid about making their views known.

As silly as it is, this resistance still lives despite the fact that "oxygenases,"—that is, enzymes whose substrate is oxygen—have long since been discovered. The laws of enzyme kinetics rule that like any other enzyme, their rate of product formation is controlled by the concentration of substrate and by oxygen concentration. Therefore, the rate of the product formation is proportional to PO_2. Though "oxygenase" is proper terminology, the word is rarely seen and rarely survives the editors' desks. Better use would lead to better understanding because "oxidase," which is usually substituted for it in this case, no longer distinguishes these enzymes from those that transfer hydroxyl groups from water and are at maximum rate at almost all times. This argument would be just academic if it were not true that many oxygenases exist, and that some of them are critical to healing [2–5].

In wounds, the PO_2 may vary from almost zero to as high as a few hundred tor. Because they are sensitive to PO_2 and not to the amount of oxygen delivered, and because the Kms for oxygen are as high as 50, their rate may vary by a factor of 10 or more in the known concentration found in wounds. One of them, usually called "prolyl hydroxylase," a dioxygenase, governs the rate of collagen deposition. Another, also a prolyl hydroxylase, governs (represses) the activity of HIF-1α and is considered highly likely to influence the rate of angiogenesis. Another highly critical oxygenase is the NAD(P)H-linked oxygenase of leukocytes, a complex monooxygenase that releases lactate and oxidants. Raising or lowering PO_2 is destined to enhance or decrease their functions, and these functions are important to wound healing.

The pioneers in polarography, the field that made this conference possible, were literally trapped in wound healing because their detractors objected to the injury that the electrodes made. Ian Silver, of Cambridge University, for one, had used very small surfaces to measure PO_2 in wounds, and showed that PO_2 in wounds is dependent on arterial PO_2. That work was ignored, in large part because shortsighted readers, focusing on discovering "normal" oxygen tensions, thought that the values reported were both too low and flawed because of the artifact of the electrode injury that was sustained in the measurement. Small electrodes were less reliable in those times. Eventually, these values were confirmed by aspirating fluid from wounds and measuring it in Clark electrodes, which could be calibrated. This broke the tyranny of the belief that dissolved oxygen in blood could carry no useful oxygen to the tissue. Silver went on the produce an oxygen profile of wounds that almost solved the whole issue; it is reported and embellished below.

Juha Niinikoski of Turku, Finland, a participant in this symposium, also stimulated by Cousteau's divers, had already showed in about 1964 that added oxygen increases collagen and DNA deposition in animal wounds. The Hunt laboratory then confirmed this process in human wounds.

To generalize on these findings, raising the PO_2 of blood even above full saturation of hemoglobin will raise the PO_2 of any tissue in which the oxygen extraction fraction is low. The rate of production of any oxygenases will then be raised. The wound oxygen extraction fraction, later to be determined, is about 0.7 to 1 vol%, higher with oxygen breathing. In other words, about 1 ml of oxygen is extracted from every 100 ml of oxygen delivered to most wounds. This fraction is low, far below cardiac muscle, for example, which uses several percent even while resting. The fact that the oxygen

extraction fraction rises with increased arterial PO_2 proves that oxygen consumption rises; that is, oxygenases are at work and their rates (functions) are changing.

Given the low oxygen concentrations, we thought that hypoxia and energy deficit would be facts of life for wound angiogenesis. We proposed that hypoxia was the instigator of angiogenesis, and one of us (T.K.H.) apologizes for popularizing that idea. He dropped it when the Niinikoski and Hunt laboratories confirmed that hypoxia slows wound healing and hyperoxia hastens it. In another study, Niinikoski also found more hemoglobin in wounds when 50% oxygen was breathed for 3 weeks. This led us to suspect that oxygen also increases angiogenesis, a fact only recently confirmed, and still resisted by critics. There is truth in hypoxia instigating angiogenesis factors, but angiogenesis, like collagen, requires oxygen and the more the better up to pulmonary toxicity.

When all this happened, it had been known for several years that collagen requires post-translational hydroxylation to be deposited, and that the hydroxylation step was insertion of an oxygen atom derived from dissolved oxygen via the oxygenase prolyl hydroxylase. With this in mind, connecting PO_2 to collagen deposition was a logical step, and in the process, Karli Kiviriko's estimate of the Km for that step of about 25 mmHg was confirmed. Thus, with wound PO_2 at that level, we could expect that collagen deposition will rise with arterial hyperoxia and will fall with arterial hypoxia.

A few years later, the NADP-linked oxygenase of leukocytes came to attention, and a postulate that the resistance to infection would also be highly sensitive to oxygen with a Km of about 50 mmHg or so was demonstrated, first in animals and later in patients. This finding led to major changes in wound care. As a result acute, postoperative wound infections have been reduced by more than 60% in large series of surgical patients in whom wound perfusion and oxygenation have been increased by providing pain relief, by maintaining normal body temperature, and by administering high PO_2 breathing mixtures.

Redox signaling has now emerged as a key element in numerous facets of medicine, warranting publication of a dedicated journal (www.liebertpub.com/ars). Just after oxidative killing was worked out, redox signaling became a viable concept [6–12], and a hypothesis that oxygen influences healing through oxidants—i.e., redox signaling, as well as a source of energy or a nutrient—became defensible. The same oxidants that kill bacteria are the currency of redox signaling!

At this point, however, a major source of confusion arose. The question of hypoxia versus hyperoxia versus redox signaling was raised again, and in a more advanced form: Both vascular endothelial growth factor (VEGF) and hypoxia-inducible factor (HIF)-1α are instigated by hypoxia—well, it is not that simple. Expression of most of the growth factors has by now been related to hypoxia, hyperoxia, and/or redox signaling. It is a bit confusing yet as to which—or whether all—might be important. For instance, VEGF has been firmly related to all of them. Peroxide exists in wounds at redox signaling levels, and a VEGF promoter that responds only to peroxide has been found (C.K.S./T.K.H.). Oxidants stimulate angiogenic behavior in endothelial cells and VEGF in macrophages. Consider that conversion of oxygen to oxidants contributes to hypoxia—the two are reciprocals! Instead of just hypoxia, we have to consider hypoxia and oxidants. Platelets and neutrophils apparently produce VEGF, but not in response to hypoxia.

We now need to rationalize the data. To this end, we offer the following depiction of the action at the wound edge via a look at a single "module" of granulation tissue as done by Silver of Bristol, England, and filled in as we see it. We view NADP(H)-linked oxygenase as a key factor. This enzyme, when activated, elevates leukocytic oxygen consumption by as much as 50-fold, uses most of the oxygen that is delivered to wounds, and produces (probably) most of the oxidants and lactate found in wounds.

Keep in mind that high lactate (8 to 15 mM) characterizes wounds. Is it a signal? Keep in mind that oxygen and oxidant concentrations are reciprocals. Conversion of oxygen to oxidants contributes to hypoxia. Does a low oxygen level or a high oxidant level stimulate wound functions like VEGF expression, or are both necessary? We know that exposing macrophages leads to VEGF production, but what is the role of the lactate that also stimulates VEGF in macrophages? Keep in mind also that according to the above expression, raising the oxygen supply will raise lactate production. Is it true that this one enzyme produces four significant signals to angiogenesis and collagen deposition, i.e., hypoxia, lactate, oxidants, and low glucose? Probably, we cannot place the whole burden on this one class of enzymes, but the old adage is true: "no inflammation, no healing." But "no inflammation, fewer oxidants" is also true. We cannot agree yet on the point at which oxidants, inevitably, become harmful, but we are sure that wounds have a competent antioxidant defense system. Also consider that the Km for oxygen in phox [2] is about 50 mmHg, set so that its affinity for oxygen is not great enough to disturb more important life functions. This is our synthesis of the data to this point.

Résumé. Un congrès intitulé "Wound Healing: Oxygen & Emerging Therapeutics" a eu lieu à Columbus, Ohio, le 12–15 Septembre 2002, sponsorisé par les National Institutes of Health (R13AR049171), International Union of Biochemistry & Molecular Biology et l'UNESCO-Global Network of Molecular & Cell Biology, dont les deux co-modérateurs étaient Chandan K. Sen du Centre Médical de l'université Ohio State et Thomas K. Hunt, de l'université de Californie- San Francisco. Ce congrès a été conçu pour deux raisons: revoir ce qui était déjà connu sur l'oxygène, et stimuler la discussion sur les nouveaux développements dans le contrôle du processus de cicatrisation par des procédés de signalement de redox régulé. On présente ici une perspective historique et évolutive sur le rôle de l'oxygène dans la cicatrisation, depuis la physiologie classique de l'oxygène dans la plaie jusqu'au concepte plus raffiné de la signalisation redox.

Resumen. Este congreso fue concebido por dos razones: consolidar el conocimiento sobre el papel del oxígeno en el proceso de reparación y estimular la discusión sobre nuevos desarrollos en el control de la cicatrización por procesos regulados por señales redox. Se presenta una perspectiva histórica y evolutiva del rol del oxígeno en la cicatrización de herida, desde la fisiología clásica del oxígeno en la herida hasta el concepto de la señalización redox.

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