Research on Reperfusion Injury

LEUKOCYTE MEDIATED REPERFUSION INJURY: WHAT ROLE HBO?

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What is ischemia reperfusion (I/R) injury?
When a tissue is subjected to ischemia, a sequence of chemical events is initiated that may ultimately lead to cellular dysfunction and necrosis. If ischemia is ended by the restoration of blood flow, a second series of injurious events ensue producing additional injury. Thus, whenever there is a transient decrease or interruption of blood flow the net injury is the sum of two components - the direct injury occurring during the ischemic interval and the indirect or reperfusion injury which follows. When there is a long duration of ischemia, the "direct" damage resulting from hypoxia alone is the predominant mechanism. For shorter duration’s of ischemia, the indirect or reperfusion mediated damage becomes increasingly more important. For example, it has been shown that the intestinal injury induced by 3 hours of ischemia (flow reduced to 20% of normal) and one hour of reperfusion is several times greater than that observed after 4 hours of ischemia alone (Parks and Granger, 1986). These results demonstrate that the injury produced by reperfusion can be more severe than the injury induced by ischemia per se. This same pattern of relative contribution of injury from direct and indirect mechanisms has been shown to occur in all organs.

What is the clinical relevance of reperfusion injury?
Reperfusion injury is relevant to many fields of medicine. For the cardiologist I/R injury occurs following every successfully balloon angioplasty or tPA induced thrombolysis. For the transplant surgeon I/R injury is the sequela of every successful harvest. For the plastic surgeon I/R injury threatens the integrity of every free flap. For the orthopaedist it may take the form of a decompression fasciotomy for a severe compartment syndrome or perhaps the reattachment of a severed extremity. Finally, every physician who has successfully resuscitated critically ill patients knows the frustration of a multiorgan failure syndrome in which reperfusion injury also plays a role.

What is the mechanism for reperfusion injury?
Several studies have demonstrated that anoxic reperfusion of ischemic tissues results in very little damage, and it appears that the reactions initiated at reperfusion involve the formation of cytotoxic oxidants derived from molecular oxygen. Because oxygen radical scavengers afford protection in models of reperfusion injury, a lot of attention has been given to the oxygen radical producing enzyme xanthine oxidase.
During an ischemic episode variable amounts of hypoxanthine are produced. With the restoration of normoxia, the hypoxanthine substrate is combined with oxygen to produce xanthine and an oxygen radical. The xanthine oxidase inhibitors allopurinol and oxypurinol have been shown to dramatically attenuate reperfusion injury in many different tissues. This suggests that xanthine oxidase is an important source of oxidants produced after reperfusion.

Another potential source of reactive oxygen metabolites in post-ischemic tissues is the polymorphonuclear leukocyte (neutrophil). Neutrophils contain an NADPH oxidase that reduces molecular oxygen to the superoxide anion. Grisham, et al. (1986) have examined the influence of ischemia and reperfusion on neutrophil fluxes in the intestinal mucosa. They observed a fivefold increase during the ischemic period, whereas reperfusion produced an 18 fold increase! Neutrophil accumulation initiated by reperfusion is significantly reduced by pretreatment with xanthine oxidase inhibitors, oxygen radical scavengers or iron chelators, suggesting that reactive oxygen metabolites play a role in the recruitment of neutrophils into postischemic tissue. This suggests that xanthine oxidase derived oxidants, produced in epithelial and endothelial cells, initiate the production and release of proinflammatory agents which subsequently attract and activate neutrophils.

An important question that arises from these observations is whether neutrophil accumulation and activation is a cause or an effect of reperfusion injury. Two approaches have been used to define the role of neutrophils in reperfusion injury: neutrophil depletion utilizing antineutrophil serum and prevention of neutrophil adherence with monoclonal antibodies directed against leukocyte adhesion molecules. The use of antineutrophil serum, which depletes circulating neutrophils to less than 5% of control, significantly attenuates the increase in microvascular permeability induced by reperfusion. These results indicate that neutrophils are the primary mediators of reperfusion induced increases in microvascular permeability.

The neutrophil membrane glycoprotein CD18 has been shown to play an important role in mediating neutrophil adhesion to microvascular endothelium. Monoclonal antibodies directed against the CD18 receptor inhibit the chemotaxis, aggregation, and adherence of neutrophils to capillary endothelium. Use of this receptor specific antibody has reduced reperfusion injury as effectively as neutropenia induced by radiation, filters, or antineutrophil antibodies. This finding indicates that neutrophil adherence to the microvascular endothelium is an essential step of neutrophil-mediated reperfusion injury.

Reperfusion injury causes neutrophils to adhere to microvascular endothelium and emigrate into the surrounding tissue. Simultaneously there is an activation of the neutrophil which produces a second wave of superoxide radical production. It is
important to emphasize that xanthine oxidase mediated production of free radicals is very short lived (seconds) and comes to a halt as soon as the hypoxanthine substrate is consumed. In contrast, neutrophil mediated production of oxygen free radicals is unlimited. The NADPH mediated production of radicals will continue as long as the cell remains activated and oxygen is present. Therefore, control of neutrophil activity appears to be an important juncture for reducing reperfusion injury.

**The oxygen paradox**

Because oxygen free radicals exert such a damaging effect, it would seem that the provision of hyperbaric oxygen therapy would further fan the flames of oxygen mediated reperfusion injury. Although in numerous experimental designs HBO has been shown to improve the outcome of flaps and extremities subjected to reperfusion injury, it is only recently that mechanisms have been uncovered to explain the paradoxical effects of high dose oxygen.

**What is the effect of HBO on reperfusion injury?**

Although the events that lead to reperfusion injury of skeletal muscle are only partially understood, experiments conducted to evaluate leukocyte endothelial adherence in the presence of HBO showed a striking inhibition of leukocyte adherence. Utilizing intravital microscopy in a pedicle flap model of reperfusion there was very little accumulation of leukocytes within the injured tissue (Zamboni, 1989). In addition there was very little vasoconstriction, which is another deleterious component of reperfusion injury. In this flap model, the net effect of HBO was a significant reduction in reperfusion injury and an overall improvement in flap survival. In a reperfusion model examining the brain of mice, Thom (1993) eloquently showed that HBO selectively inhibits the B2 integrin function of neutrophils. It is the B2 integrin that is responsible for persistent adherence of neutrophils to the endothelium. It also appears that the "other" adherence functions of neutrophils appear to remain intact after exposure to HBO. For example, peritoneal neutrophilia in response to a glycogen challenge is not inhibited by HBO. This selective effect of HBO is, of course, good news, because we are able to blunt neutrophil mediated reperfusion injury without rendering the host functionally neutropenic and open to opportunistic infections.

**What are the clinical implications for HBO?**

The clinical implications of current research findings would argue for early aggressive intervention with HBO whenever significant reperfusion injury is suspected. Once the neutrophil’s B2 integrin mediated endothelial adherence has come into effect, HBO can no longer play an abortive role, where reperfusion mediated tissue injury is concerned. Because a single dose of HBO prevents B2 mediated adherence for as long as 24 hours, it opens up the possibility of using...
HBO in a prophylactic capacity whenever reperfusion injury is anticipated. Such scenarios would include the concurrent use of HBO with thrombolytics such that the "HBO effect" could be induced while waiting for tPA to breakdown the blood clot; for patients scheduled for "high risk" flaps; for transplant situations, and many more.

In summary, the pharmacology of high dose oxygen is quite complex. It transcends the simplistic notion that oxygen is only useful for metabolic needs. In high doses it is capable of bringing about many physiologic changes, that are probably based on peroxidative alteration of enzyme activity or substrate based shifts in equilibrium, such as nitric oxide (NO) production. For the present time HBO stands out as the most promising new "Lazaroid" in the field of reperfusion injury.