Role of Iron and Oxygen Radicals in Hemorrhage and Shock

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Summary. This contribution focuses on the role of iron as a critical component in the genesis of oxygen radical mediated tissue injury occurring after global ischemia associated with severe hypovolemic shock. Conventional colloid or crystalloid fluid resuscitation does not adequately protect organs susceptible to reperfusion injury. One approach aimed at attenuating such post-trauma reperfusion injury is systemic, high dose, iron chelation used in combination with colloid fluid replacement.

Key words: Hemorrhage - Resuscitation fluids - Deferoxamine

Hypovolemic shock represents a clinical indication well suited for anti-oxidant intervention. Microvascular blood flow in several organs is dramatically decreased leading to metabolic changes associated with hypoxia. At the time of resuscitation/reflow, these organs become susceptible to cellular and molecular damage caused by oxygen and lipid derived radicals (Reilly et al. 1991). Pharmacological intervention aimed at attenuating this type of global reperfusion injury will, within the near future, become standard medical practice.

Normally, initial therapeutic intervention during resuscitation of a victim of severe hypovolemic shock/trauma involves fluid therapy using crystalloid or colloid solutions. Whenever blood is available, either whole blood or red cells suspended in crystalloid are administered to provide oxygen carrying capacity. By initially providing crystalloid or colloid solutions to a hypovolemic patient some degree of tissue oxygenation is achieved, but the opportunity for providing anti-oxidant therapy is lost. These solutions have no anti-oxidant capacity and therefore do not significantly attenuate tissue injury occurring during reperfusion.

The concept of the “Golden Hour”, as applied to the initial resuscitative period by the pioneering shock/trauma researcher, R. Adams Cowley, is probably a misnomer in the context of reperfusion injury. The term “Golden Minute” may be a better description of the time period during which most radical mediated tissue injury occurs following reoxygenation. Studies involving the ischemic myocardium suggest that a significant component of reperfusion injury occurs within the first few minutes of reflow (Bolli et al. 1989, 1990; Tsao and Lefer 1990). It will be difficult to prove that a similar time course applies to all organs susceptible to reperfusion injury, but it seems clear that the practice of conventional fluid resuscitation, using crystalloid or colloid solutions, followed by anti-oxidant therapy at a later time, represents a suboptimal therapeutic approach.

Role of Iron in Reperfusion Injury

The appearance of increased levels of plasma iron subsequent to global hypoperfusion has been described in several papers published in the mid-1950s by Mazur and coworkers (Mazur et al. 1955; Green and Mazur 1957; Mazur et al. 1958). These investigators demonstrated that iron, in quantities sufficient to completely saturate the available transferrin binding capacity, was released during hemorrhagic shock. At the time of these studies, superoxide and superoxide dismutase were unknown entities. Mazur proposed that uric acid could directly reduce and liberate iron from ferri-
tin. A few years after Mazur's studies, Janoff and coworkers (1960) observed similar increases in plasma iron following hypovolemic, but not septic, shock.

Our knowledge of the biochemical mechanisms leading to microvascular dysfunction following severe ischemic insults has gradually expanded during the thirty-five years since Mazur's studies of the role of xanthine oxidase, ferritin and iron in hypoxic tissue. The concept of reperfusion injury is now well defined and the critical role of reactive, oxygen derived, radicals in the genesis of this type of vascular injury is fully documented. In addition, there is a general recognition that iron may be an important component of this process. Ferrous iron, released from ferritin by superoxide (Thomas et al. 1985; Biemond et al. 1986) or other reducing agents, acts as a catalyst for decomposition of hydrogen peroxide (the Fenton reaction) leading to the formation of the highly reactive hydroxyl radical. “Anti-oxidant therapy” of many types has proven efficacious in attenuating “oxidant” injury in a variety of preclinical models. This discussion will focus on the role of iron in the genesis of reperfusion injury and pharmacological approaches aimed at ameliorating such injury.

Additional evidence pointing to the importance of “free”, catalytically active iron, in contributing to tissue injury or organ dysfunction secondary to ischemia and reperfusion derives from preclinical studies in which iron chelators have afforded protection. In most of these studies, the evidence for the involvement of iron is indirect. Thus, if a compound that binds and neutralizes iron also protects against tissue injury, then iron is presumably involved in the reactions leading to the observed injury. Other explanations for this protection, such as direct scavenging of free radicals, have been proposed but are unlikely. Halliwell (1989) concluded that the protective effect of deferoxamine (DFO, Desferal®, CIBA-Geigy), in animal models of human disease, is due largely to its ability to inhibit iron-dependent free radical reactions. Furthermore, in a number of studies it has been demonstrated that the iron-saturated form of DFO, ferrooxamine, does not afford the protection observed by DFO, thereby demonstrating that iron sequestration is the protective mechanism.

In addition to the studies discussed above in which systemic iron release was observed following periods of global ischemia (Mazur et al. 1955; Janoff et al. 1960), there are a number of recent studies suggesting that ischemic insults to isolated organs also result in release of iron. Krause et al. (1985) observed delocalization of low molecular weight iron stores in the the brain following cardiac arrest in dogs. Holt et al. (1987) have implicated iron in the pathophysiology of the severely ischemic myocardium by demonstrating increased levels of low molecular weight iron compounds in homogenates of myocardial tissue exposed to two hours of ischemia “in vivo”. In a rat model involving 60 min of renal ischemia Paller and Hedlund (1988) demonstrated that urinary iron increases ten-fold during reperfusion compared to pre-ischemic values. Similarly, Robinson and Hedlund (1989) noted a 60% increase in the plasma iron concentration following reperfusion in rats exposed to 90 min of total intestinal ischemia. These observations suggest that iron release may be a general phenomenon due to altered metabolic and/or redox status of cells exposed to a hypoxic insult.

In this short report it is not possible to provide a comprehensive review of applications of iron chelation in preclinical models of ischemia and reperfusion. Only a few representative studies will be mentioned. In studies of the ischemic stomach (Smith et al. 1987), intestine (Hernandez et al. 1987) and kidney (Paller and Hedlund 1988) DFO significantly attenuated ischemia/reperfusion induced organ injury. Early experiments in isolated heart preparations demonstrated that both superoxide dismutase and DFO attenuate the metabolic and functional alterations occurring at reperfusion (Ambrosio et al. 1987a, b). More recently, the same group demonstrated that DFO, given during the ischemic insult, significantly reduces reperfusion-induced oxygen radical generation (Williams et al. 1991). Intravenous (Bolli et al. 1987) and intra-atrial infusion of DFO (Farber et al. 1988) significantly attenuated post-ischemic ventricular dysfunction following moderate cardiac ischemia in the dog (the stunned myocardium). In more severe models of myocardial ischemia, two groups have documented reduction in infarct size when DFO was given prior to the ischemic insult (Reddy et al. 1989; Lesniewsky et al. 1990).

As noted in the introduction, “in vivo” studies of myocardial reperfusion injury suggest that if the radical scavenger, N-2-mercaptopropionyl-glycine (Bolli et al. 1989), or the iron chelator, DFO (Bolli et al. 1990), is administered only one minute after reperfusion, the protective effect is largely lost. These investigators measured a burst of radicals in the coronary circulation within the first five minutes of reperfusion. Similarly, in “ex vivo” studies on the isolated rat heart, a burst of radical formation, as measured by chemiluminescence, was observed within 15 s of reperfusion (Tsao and Lefer 1990).