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Dossier: Superoxide dismutases: recent advances and clinical applications

Oxidative stress in organ preservation: a multifaceted approach to cardioplegia

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Abstract

Every transplant is a reperfused organ and, therefore, undergoes some degree of oxidative damage. Postischemic reperfusion injury results in non-specific free radical-mediated acute endothelial damage, cell death and organ failure. The endothelium is a key site of injury from reactive oxygen species (ROS), and the endothelial cell dysfunction is central to the pathogenesis of arteriosclerosis. Accelerated arteriosclerosis, secondary to chronic allograft rejection, is a major long-term complication of heart transplantation. Therefore, preservation methods that would decrease injury during reperfusion are very important. We have developed a unique preservation solution, with a multifaceted approach, which best preserves the organ from ROS for an extended period of time before transplantation. The advantages of extending this period of preservation include an expansion of the donor pool, by permitting more distant procurement, the ability to perform detailed tissue typing, therefore, improves histocompatibility match and a reduction in emergency surgery as a result of graft rejection.

Keywords: Cardioplegia; Ischemia; Reactive oxygen species; Superoxide dismutase

1. Introduction

The production of oxygen-derived free radicals and their detrimental effects to the heart following hypoxia/reoxygenation have been demonstrated at the cellular level, at the isolated organ level, and in vivo [1]. Although it is universally accepted that reperfusion injury involves free radical production, the mechanisms by which free radicals cause tissue injury and cell death are still not well understood. Lipid peroxidation has been frequently implicated in ischemia/reperfusion (I/R) and involves the production of semistable peroxides from free radical intermediates produced by direct or indirect reaction of oxygen-derived reactive metabolites with unsaturated lipids. Lipid peroxidation proceeds by a free radical chain reaction mechanism that may continue for some time after initiation. Evidence for the occurrence of lipid peroxidation in vivo is provided by direct measurements of lipid peroxidation products that accumulate in cells. Reactive oxygen species (ROS) are generated at various points during reperfusion of the organ, and cell membranes are sites for physiological injury due to the free radical attack. Upon reoxygenation, superoxide ($O_2^{\cdot -}$), an ROS, is produced in great abundance within the cell (i.e. from enzymatic sources such as xanthine oxidase [2], membrane-bound NADH oxidases [3] or from ischemically injured mitochondria) or in the interstitial spaces by activated neutrophils or the NADPH oxidase of macrophages [4]. (1) $O_2^{\cdot -}$ can liberate and reduce iron from tissue ferritin [5], as well as cause the secondary formation of hydrogen peroxide and hydroxyl radical [6]. Because iron and hydroxyl radical are both initiators of lipid peroxidation, lipid peroxidation is a prominent component of reperfusion injury [7,8]. Inhibitors of lipid peroxidation [9,10] and superoxide [11], accordingly, protect the myocardium following ischemia and reperfusion; (2) $O_2^{\cdot -}$ is known to inactivate aconitase [12], which is identical with *iron-responsive element binding protein* [13] with an iron–sulfur cluster; (3) $O_2^{\cdot -}$ can also react with nitric oxide (NO) produced from L-arginine abundantly by the endothelium and

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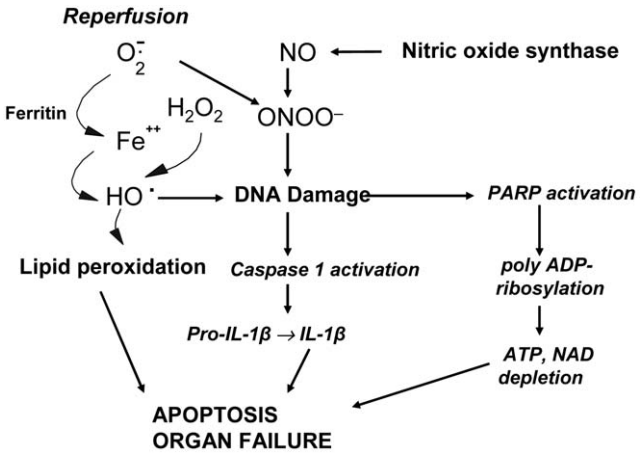

Fig. 1. Molecular oxygen is reduced to superoxide anion during reperfusion that can liberate iron, form H_2O_2 or combine with NO to form peroxynitrite.

form peroxynitrite, a highly reactive oxidant [14]. This would impair the potentially protective actions of NO as a vasodilator [15]. Peroxynitrite ($ONOO^-$) has been proposed to trigger DNA single-strand breakage, induce caspase-1, which is an interleukin-1 converting enzyme, and initiate apoptosis [16,17] (Fig. 1). $ONOO^-$ can also add nitro groups in the ortho position of tyrosine of proteins to form nitrotyrosine [18], which has been shown to be elevated in human atherosclerotic lesions [19], and after I/R [20].

2. Functional consequences of the earlier-mentioned biochemical reactions

2.1. Programed cell death via apoptosis

Myocardial damage determines the morbidity and outcomes in transplant rejection. Myocyte apoptosis has been shown to contribute to cardiac allograft rejection [21–24]. ROS have been implicated in the initiation of apoptosis in bovine aortic endothelial cells [25]. The term apoptosis was first used by Kerr et al. [26] to describe the death of certain hepatocytes in the ischemic liver. In the central zone of complete ischemia, it was clear that cells died of necrosis, with swelling and bursting. At the periphery of the ischemic zone, however, the morphology was quite different; the cells died via apoptosis characterized by cell shrinkage with nuclear condensation and fragmentation [26,27]. In retrospect, of course, these are the cells exposed to the greatest oxidative stress, where injured cells are still oxygenated and superoxide production is elevated.

Even more recently, Gottlieb et al. [28] demonstrated apoptosis following regional I/R in the rabbit heart in vivo by measuring DNA fragmentation. Interestingly, the phenomenon was reperfusion dependent. Other work has indicated a role for apoptotic cell death in cerebral ischemia and reperfusion [29,30]. While a number of metabolic conditions may trigger apoptosis, it is clear that an oxidative shift in cellular redox status is one such condition [31–33]. Cell culture studies have shown oxidative stress results in apoptosis and can be blocked by vitamin E and, consistent with a role for peroxynitrite generation, by NO synthase inhibitors [34,35]. One of the mechanisms by which oxidative stress could result in apoptosis is by the increased production of interleukin-1β (IL-1β) as a result of the upregulation of ICE family proteases (specifically caspase-1) [36,37]. Inhibition of caspase 1 has been shown to reduce the processing of endogenous precursors of IL-18 and IL-1β that play a significant role in I/R-induced myocardial contractile dysfunction [38]. The end result of apoptosis is the subsequent organ failure.

2.2. Immunological and inflammatory responses

It seems increasingly clear that initial dysfunction with an early acute rejection interacts to substantially reduce long-term graft success. The superoxide produced after a heart is subjected to ischemia and reperfusion can activate a latent plasma factor that becomes a potent chemoattractant for neutrophils. This recruitment of neutrophils produces an inflammatory component. Postischemic reperfusion injury results in non-specific free radical-mediated acute endothelial damage. Endothelial cells are in close proximity to neutrophils that undergo vascular endothelial margination upon activation. Following endothelial margination of activated inflammatory cells, cell injury can occur by superoxide or other ROS being released into the extracellular milieu and react with cell surface targets. The events surrounding I/R could trigger upregulation of major histocompatibility (MHC) antigens, and the potentially increased graft immunogenicity may promote host cellular infiltration and elicit a non-specific inflammatory response [39]. MHC antigens function in the presentation of antigens to T lymphocytes. T cells are pivotal in transplant rejection. MHC expression on cells is controlled by cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) that have been shown to be powerful inducers of MHC-antigen expression on many cell types. ROS can activate macrophages to release these cytokines [40]. I/R injury presumably causes elevation of P-selectin, which is rapidly translocated to endothelial surfaces within 5 nm of revascularization of the organ leading to the tethering of polymorphonuclear leukocytes (PMN) to the vascular intima [41]. P-selectin is the key adhesion molecule involved in the earliest events in the adherence of circulating leukocytes in rolling to tissues in inflammatory states [42]. It mediates the adhesion of PMNs or monocytes to activated platelets or endothelial cells [43,44]. Local production of monocyte chemotactic protein-1 [45], IL-1β and/or TNF-α by these leukocytes induces P- and E-selectin expression on endothelium that continues the cascade of events increasing cell adherence and infiltration of the injured tissue [46–49] and ultimately contribute to host alloresponsiveness. TNF-α also has been shown to activate p38 mitogen-activating protein kinase to produce interleukin-8 by vascular endothelial cells [50]. The release of inflammatory mediators due to I/R injury play a role in the mechanism of I/R-induced host's immune