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EC CARDIOLOGY
Review Article

Ischemia and Reperfusion Injury: General Aspects and Mechanisms

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Received: November 12, 2016; **Published:** November 18, 2016

Abstract

Ischemia and reperfusion injury is present in many daily medical situations, as the resolution of thrombolysis, cardiopulmonary bypass, transplantation, circulatory shock and many others. It is defined as the functional and structural changes that occurs after the restoration of the blood flow after a long period of ischemia. The major event that determinates this phenomenon is the oxidative stress caused by the over production of reactive oxygen species and reactive nitrogen species. The Xanthine oxidase enzyme, the leukocytes and the inflammatory mediators plays a key role in this process. The inflammation after this event can be so great that reaches a systemic level, hitting remote organs, leading to the multiple organ dysfunction syndrome. Some techniques were proposed to minimize or even prevent this condition as the ischemic preconditioning, local and at distance, and the ischemic postconditioning, also local and at distance.

Keywords: *Ischemia/Reperfusion Injury; Oxidative Stress; Multiple Organ Dysfunction Syndrome; Ischemic Preconditioning; Ischemic Postconditioning*

General Aspects

Ischemia-reperfusion (I/R) injury, is the term that refers to functional and structural changes that occurs after the restoration of the blood flow after a period of ischemia in a tissue. PARKS and GRANGER have shown that three hours of ischemia followed by one hour of reperfusion determinates greater injury in intestinal mucosa than four hours of exclusive ischemia [1]. The restoration of blood flow after a period of ischemia can lead the ischemic organ to cell necrosis and limit the recovery of its normal function [2]. The mechanism of injury by I/R consists in a complex pathophysiological phenomenon which requires the presence of oxygen for its formation, as well as the activation of vascular, cellular and humoral factors [3], being closely and directly related to the formation of reactive oxygen species (ROS), endothelium injury, increased vascular permeability, leukocyte and platelets activation, as well as pro inflammatory cytokines and activation of the complement system [4]. The exacerbation of the lesion is due to disbalance between the synthesis of vasoconstrictor and vasodilators factors [5], causing deficiency in some segments of the microcirculation, leading to a heterogeneous distribution of blood, with diffuse tissue hypoxia, known as no reflow phenomenon [6]. In addition, the increased production of ROS, in disequilibrium with the endogenous antioxidant system contributes to exacerbation of the inflammatory response, and consequently to the tissue injury [2].

The intensity of inflammation may be so great that reaches systemic level, and these effects are often observed in remote lungs and cardiovascular system, and may lead to systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS), causing a high mortality in intensive care units on tertiary hospitals and also high spending for public health system all over the world [7]. The I/R injury, locally and also at distance has already been described in various organs and happens in many everyday medical situations such as after thrombolytic therapy, transplantation, coronary revascularization, resolution of a pulmonary embolism, and after the use of cardiopulmonary bypass [8].

The oxidative stress and the antioxidant system

The term Free Radical refers to an atom or molecule, whose Valence layer has odd number of electrons, and this unpaired electron gives high reactive potential to the molecule [9]. However free radical is not the ideal term to designate the pathogenic reactive agents, as some do not have unpaired electrons in its valence layer and still has a high instability and reactive power, being more appropriate the designation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [10,11]. In physiological conditions, these molecules are involved in the production of energy, phagocytosis, cell growth regulation, intercellular signaling and production of important cytokines and chemokines. However, the overproduction of these deleterious effects, such as lipoperoxidation of cells membranes, tissue proteins oxidation, such as enzymes, carbohydrates and nucleic acids [12]. The main ROS are the hydroxyl radical ($HO^{\bullet}$), superoxide ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), being the hydroxyl radical ($HO^{\bullet}$) the most deleterious to the body. Among the main ERNs include nitric oxide (NO) and the peroxynitrites ($ONOO^-$) [13].

The superoxide radical is formed after the first reduction of O_2 (reaction 1); is generated *in vivo* by phagocytes, lymphocytes and fibroblasts during the inflammatory process, to combat antigens. It's synthetized with the assistance of the enzyme NADPH oxidase, acting as a catalyst for an electron reduction of O_2 (reaction 2).

$$O_2 + e^- \longrightarrow O_2^{\bullet -} \quad \text{Reaction 1}$$

$$2O_2 + NADPH \longrightarrow 2O_2^{\bullet -} + NADP^+ + H^+ \quad \text{Reaction 2}$$

The potential of superoxide as direct oxidant is irrelevant, and is eliminated by the enzyme Superoxide Dismutase (SOD), which is part of the endogenous antioxidant system, that catalyzes the dismutation of two molecules of $O_2^{\bullet -}$ into oxygen and hydrogen peroxide (reaction 3).

$$2O_2^{\bullet -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2 \quad \text{Reaction 3}$$

The hydrogen peroxide, in physiological situations is countered by the endogenous antioxidant system, consisting of the enzymes Glutathione peroxidase (GSH) and catalase (reactions 4 and 5). The hydrogen peroxide does not have high reactivity front organic molecules in the absence of transition metals, however plays a key role in oxidative stress mechanism because its nonpolar molecule configuration allows it to cross the cell membrane easily and can generates the hydroxyl radical.

$$2GSH + H_2O_2 \xrightarrow{GSH\ peroxidase} GSSG + 2H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GSH\ reductase} 2GSH + NADP^+ \quad \text{Reaction 4}$$

$$H_2O_2 + NADPH + H^+ \xrightarrow{GSH} 2H_2O + NADP^+$$

$$2H_2O_2 \xrightarrow{NADPH/\ catalase} O_2 + 2H_2O \quad \text{Reaction 5}$$

Hydroxyl radicals are formed through Fenton's reaction (reaction 6), where the hydrogen peroxide in contact with transition metals, such as iron present in the nuclear membrane, receives one more electron and a hydrogen ion, forming a hydroxyl radical, this being the most reactive and harmful of these intermediaries, as it reacts and changes any cell structure next, damaging enzymes membrane, and nucleic acids [12].

Fenton's reaction

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + HO^{-} \quad \text{Reaction 6}$$

In addition, the superoxide radical reacts with Nitric Oxide producing RNS peroxynitrite ($ONOO^-$), responsible for the nitration, thus amplifying the tissue injury (reaction 7) [12].

$$NO^{\bullet} + O_2^{\bullet -} \longrightarrow ONOO^{-} \quad \text{Reaction 7}$$

Pathological reactive species are generated via several pathways of the organism and include among them the electron carrier chain of mitochondria [14], via enzyme Xanthine oxidase [15], production of ROS by leukocytes, mainly neutrophils and macrophages [16].

Ischemia and reperfusion injury and oxidative stress

Oxidative phosphorylation does not occur in the mitochondria during oxygen deprivation in the ischemic period, because the anaerobic Glycolysis generates energy but is not sufficient for the restoration of ATP. This deficit of ATP affects the transport of ions in the cell membrane, generating an accumulation of sodium in the cell's interior, leading to cell swelling [4]. The ischemia also causes an increase in calcium ion permeability promoting its entry inside the cell. This increase is due to decreased activity of active ATP-dependent calcium transport. This accumulation of intracellular calcium leads to a series of damaging events such as change of the cell by contraction of the cytoskeleton and activation of phospholipase A2, with the consequent release of arachidonic acid metabolites from cellular membranes [17]. The accumulation of intracellular calcium also activates proteases which will potentiate the effects of ROS in the organelles by converting the enzyme Xanthine dehydrogenase in Xanthine oxidase (XOD) in the reperfusion period [18]. The XOD is an abundant enzyme on vascular endothelium by the whole organism, with high ability to generate ROS [19].

During the period of ischemia, due to the expense of energy reserves, the cascade of degradation of ATP in adenosine diphosphate (ADP), adenosine monophosphate, adenosine later, inosine and, finally, hypoxanthine. The accumulation of hypoxanthine allows the mass production of superoxide and hydrogen peroxide by Xanthine oxidase when oxygen is reintroduced in the vascular bed during reperfusion [19].

The degradation products of arachidonic acid give rise to pro inflammatory mediators such as prostaglandins, Leukotrienes and thromboxane, which lead to adhesion and neutrophilic activation and platelets aggregation [20, 21]. The initial current of neutrophils requires the selectins expression by endothelial cells and interaction with their receivers. The subsequent activation of integrins in neutrophils for chemotactics and upregulation factors of ICAM-1 in endothelial cells leads to neutrophil adhesion on endothelial surface followed by extravasation and migration to the site of inflammation [22]. The neutrophils produce superoxide and hydrogen peroxide and also secrete myeloperoxidase, an enzyme that catalysis the formation of hypochlorous acid, pathological reactive species [19]. Neutrophils also produce proteases able to degrade almost all components of the basement membrane of the endothelium and adhesion proteins that maintain the endothelial barrier functional [23].

The I/R injury induces the deterioration of endothelium-dependent vasodilation in arterioles by changing the balance between the NO and the superoxide in the endothelial cells. Under normal conditions the NO flow exceeds the rate of production of superoxide, this allows the NO to kidnap the low amount effectively intracellular superoxide, reduces the arteriolar tone via activation of guanylyl Cyclase

in smooth muscles, prevent the platelets aggregation and formation of thrombus and minimizes the adhesive interaction between the endothelium and leukocytes. However, after reperfusion for an unbalance between the NO and superoxide, a result of a rise in the production of superoxide by endothelial cells and leukocytes and a reduction in the synthesis of NO by the NO Synthetase (NOS). In addition, the endothelium-dependent vasodilation is committed where NO is not available to serve as second messenger when endogenous vasodilators such as acetylcholine interact with endothelial receptors. The accumulation of superoxide that occurs in the absence of NO after the I/R allows an increase in the generation of hydrogen peroxide, and both the peroxide as superoxide exacerbate inflammatory state in venules [24]. The superoxide reacts faster with NO than with the Superoxide Dismutase (SOD), thus forming the powerful peroxynitrite ($ONOO^-$) [25] (Figure 1).

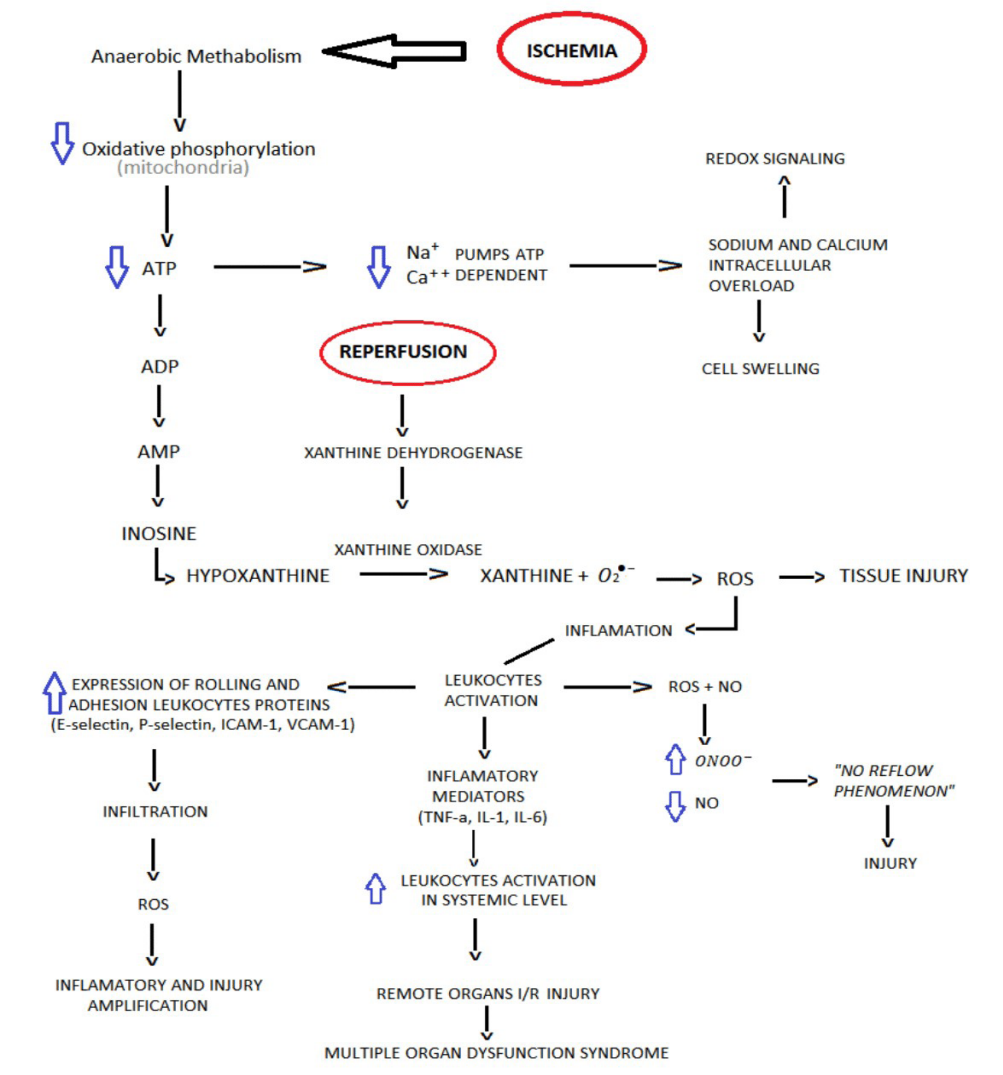

Figure 1: *Physiopathology of ischemia and reperfusion injury.*

I/R injury in remote tissue

During reperfusion of an organ, the release of inflammatory mediators can activate endothelial cells of organs that were not subjected to direct ischemia, but who will be injured as a result of reperfusion injury [26]. The I/R causes systemic release of inflammatory mediators, thus promoting the Neutrophilic activation, inducing the widespread increase of the expression of endothelial adhesion molecules and leukocytes, and increasing the possibility of Leukocyte-endothelium interaction [27]. The inflammatory signaling activates a cascade of events, such as TNF receptor phosphorylation leading to activation of TAK1, which phosphorylate the IKK complex, leading to ubiquitination of the inhibitor of Nuclear Factor KB, then the Nuclear Factor KB (NfKb) moves to the nucleus and binds to specific DNA regions beginning the transcription of several genes, which will express Chemokines, cytokines and other pro inflammatory mediators [28]. The TNF-alpha, which after being released from a post ischemic tissue, enters the bloodstream and configures a phase of increase of Neutrophilic infiltration, thus amplifying the neutrophil-mediated damage in remote organs [29]. Has already been demonstrated that the plasma levels of TNF-alpha increase after aortic clamping, and this associated with pulmonary vascular dysfunction [30].

The Platelet activating factor (PAF) is formed primarily by endothelial cells in experimental models and humans during ischemia and reperfusion by increasing superoxide generation by neutrophils. In addition, the PAF is a potent activator of the Integrin Beta-2 Mac-1 and the formation of ROS adhesion dependent [31].

Complement activation is a critical event during reperfusion in animal and human experiments. The complement cascade can be quickly activated via the big release of cellular proteins during the initial period of reperfusion. The complement system factors such as C5a, upregulates the Mac-1 receiver in circulating neutrophils and cause the recruitment of neutrophils into the capillaries. C5a prioritizes and activates neutrophils and macrophages to form reactive oxygen species. However, the complement activation has no effect on the NF-kappa B activation and expression of adhesion molecules on endothelial cells. In addition, for pro inflammatory effect, membrane attack complex can cause direct damage to the cell. The evidence for the deposition of the complement was found in livers of mice and humans during reperfusion [32].

Systemic inflammatory reaction after I/R injury is responsible for the development of multiple organ Dysfunction syndrome (SDMO), this being the main cause of mortality in critical patients [2] (Figure 1).

The lung damage associated with is represented by progressive injury MODS, from moderate injury known as acute lung injury (Ali) until the severe injury called acute respiratory distress syndrome (ARDS) [33]. The respiratory failure is followed by liver failure, renal and gastrointestinal dysfunction, and later involvement of the central nervous system and myocardial infarction [34]. The I/R injury in these tissues is characterized by the increase of microvascular permeability, cell swelling and tissue edema, an accumulation of neutrophils in tissue, destruction of the parenchyma and hemorrhagic areas disseminated [35-38].

Ischemic Preconditioning and ischemic postconditioning

The ischemic preconditioning (IPC) consists in an important defense mechanism gained when the tissue is submitted to intermittent short periods of ischemia and reperfusion before being exposed to a prolonged period of ischemia for more than ten minutes, in order to prevent cases of acute ischemia [39]. The first description of this procedure was made by Murry., *et al*. in 1986, first described in myocardial I/R model [40]. However, this mechanism has been demonstrated in other organs with satisfactory results, preserving endothelial function in arterioles, capillaries and venules of the entire vascular tree [41,42].

The IPC protection mechanism occurs by the G Protein activation by sensitive to pertussis, triggered by alpha1 adrenergic stimulation and adenosine. This seems to be the critical point of the beginning of ICP's response through the stimulation of Phospholipase C and D. The humoral hypothesis is that the hydrolysis of Phospholipase C, stimulated by the adenosine receptor, increases the intracellular concentration of diacylglycerol and Protein Kinase C activation (PKC) induces translocation of specific isoforms of the same protein, an inac-

tive isoform in the cytosol to the membrane cell [2,43,44]. Oxidants produced during the brief initial period of ischemia also contribute to the phenomenon of ICP, activating PKC directly [2,45]. During the subsequent periods of prolonged ischemia, adenosine receptors are stimulated again, by clicking G proteins sensitive to Pertussis, as well as the activation of Phospholipase C and diacylglycerol production. However, after a ICP, the stimulus activates PKC diacylglycerol which is now dispersed, and coupled the membrane [2,43]. This effect of the PKC translocation to the cell surface increases the production of cellular adenosine during prolonged ischemia and can confer protection to increase cellular energy reserves and inhibit the adhesion of leukocytes, however this mechanism remains speculative [2,46].

The ICP is not always applicable in many clinical situations, another technique called remote ischemic preconditioning (rICP) has demonstrated to be a promising procedure to prevent I/R injury [47]. It consists in inducing briefs cycles of ischemia and reperfusion in a tissue at a distance from the target organ. The main hypothesis that justify this phenomenon is the neural pathway hypothesis [48]. Many studies confirm this theory. In a experimental study of I/R, using hindlimb ischemia and reperfusion cycles, demonstrating the rICP protection needs a complete innervation of the lower limb [49]. Another evidence is that the pre-ischemic efferent vagal stimulation increases acetylcholine release and minimize the I/R injury [50]. The remote preconditioned organ releases endogenous substances that stimulates afferent nerve fibers, such as adenosine, bradykinin, acetylcholine and calcitonin gene-related peptide, that confers protection to target organ.

However, there are several clinical situations in which the prolonged ischemia is already established, and it is not possible the use of ICP. In 2003 at work published by Zhao., *et al*. proposed the Postconditioning technique (IPoC), which consists of short cycles of ischemia and reperfusion immediately after prolonged ischemia, and before the reperfusion permanently [51]. In 2009 Santos., *et al*. demonstrated the similarity of efficacy of IPoC and ICP, in preventing I/R injury in the intestine of rats subjected to mesenteric ischemia, there is no statistically significant difference between the two methods in preventing local I/R injury [52]. In 2015 Dorsa., *et al*. demonstrated the remote effect of IPoC in minimizing the I/R injury on distance, evaluating the protective effect on Lung parenchyma in rats submitted to clamp the abdominal aortic artery, followed by reperfusion, there was no significant statistic difference between remote IPoC and the rIPC [53].

Conclusion

Ischemia and reperfusion injury is a common medical situation, and the better understanding of its pathophysiology has received increasing attention in the last decades. Mitochondrial dysfunction, xanthine oxidase enzyme, leukocytes activation and the release of inflammatory mediators are the link between the ischemia and reperfusion events and the injury by oxidative stress. Ischemic preconditioning and ischemic postconditioning are successful techniques the can be used to minimize both local and remote injury after ischemia and reperfusion.

Acknowledgements

The authors thanks the International Academy of Cardiovascular Sciences (IACS) for the inspiration. Thanks also to the Academic League of Cardiovascular Surgery of the Regional Hospital of Mato Grosso do Sul (LACCV-HRMS). The authors are greatfull to Leticia Dias, Murillo de Almeida, João Victor Duraes, Ian Chaves, Gisele Cruz, Maria Helena of the laboratory and Felisma for their help.

Conflict of Interest

The authors declare no conflict of interest.

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Volume 2 Issue 5 November 2016