THE ROLE OF OXYGEN METABOLISM IN ISCHEMIA-REPERFUSION

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The implication of free radical (FR) generation in the ischemia-reperfusion phenomenon was definitively demonstrated by electron spin resonance, evidencing direct and spin-adduct signals in myocardium and kidney. The mechanism of FR generation becomes actually more clear: Hypoxia diminishes or abolishes ATP production in mitochondria, with a consequent impairment of the Ca-ATPase pump followed by a major increase in the intracellular calcium concentration. During reoxygenation after a sufficiently long period of oxygen deprivation, the perturbation in the mitochondrial electron transport is maintained, with incomplete oxygen reduction, producing superoxide anion \( \text{O}_2^-\text{hr} \), \( \text{O}_2^-\text{hr} \) itself, can alter the membrane phospholipids by deesterification and, moreover, generates, by a dismutation mechanism, hydrogen peroxide \( \text{H}_2\text{O}_2\text{hr} \). \( \text{H}_2\text{O}_2\text{hr} \), in the presence of iron, is always present in cells and is reduced to \( \text{Fe}^{2+} \) by \( \text{O}_2^-\text{hr} \). It produces the hydroxyl radical \( \cdot \text{OH} \), an active membrane destroyer. By these radical mechanisms, intracellular structures are altered; a release of lysosomal enzymes occurs, particularly of a serine protease, which becomes activated by the elevated \( \text{Ca}^{2+} \) concentration, being thus able to convert xanthine dehydrogenase into xanthine oxidase. Xanthine dehydrogenase produces the ultimate steps of purine oxidation (hypoxanthine \( \rightarrow \text{xanthine} \rightarrow \text{uric acid} \), with \( \text{FAD} \) or \( \text{NAD}^+ \) as the electron acceptor; after conversion into xanthine oxidase, its electron acceptor becomes oxygen, which is reduced to \( \text{O}_2\text{hr} \).

Therefore, a new source of free radicals appears, aggravating the intracellular destruction. These two factors, radical processes and ionic disequilibrium with a dramatic increase in the calcium concentration, lead to cellular death. Several toxic and chemotactic substances are released from the altered cells and can act at a more or less long distance, disturbing the functions of other organs, and attracting and activating granulocytes. Granulocytes, in turn, produce, after activation, several oxidant species (\( \text{H}_2\text{O}_2\text{hr} \), \( \text{O}_2^-\text{hr} \), singlet oxygen, hypochlorous acid, chloramines, etc.) in ischemic reperfused tissues, enhancing the pathologic phenomena by oxidative and radical processes.

It appears, from the above considerations, that the pharmacologic treatment of ischemia-reperfusion will chiefly consist of the use of antiradical and antioxidant agents. These protective substances can be classified into a) stoichiometric agents, inactivating activated oxygen species, molecule by molecule (tocopherol, free radical scavengers such as thiols, mannitol, benzoate, etc.) and b) catalytic agents. These latter consist of a) enzymes: superoxide dismutase, catalase, and glutathion peroxidase, which are able to inactivate a great number of oxidant molecules for each molecule of catalysts; and bi)catalytic inhibitors: allopurinol and oxypurinol, which inhibit xanthine oxidase, and iron chelators, such as desferrioxamine, which inhibit the prooxidant activity of \( \text{Fe}^{2+} \) or copper.

We can conclude that the pharmacology of oxygen, which is actually in continuous development, finds a brilliant application in the important field of transplantation and bypass grafting, where the problems of ischemia-reperfusion are now predominant.

SARCOLEMMAL CHANGES IN REPERFUSION INJURY

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Reperfusion of cardiac muscle after an ischemic episode results in structural changes, including contraction-band formation. After 20-minute ischemia followed by 15-minute reperfusion in the rat model of regional ischemia, we showed, using macromolecular tracer intravascular injection, such as peroxidase or FITC-dextran, that: a) there was a great heterogeneity in the capillary perfusion pattern within ischemic-reperfused myocardium; this is referred to as the no-reflow phenomenon; b) the capillary and sarcolemmal permeability was increased in underperfused areas; c) the ultrastructural damage, i.e., contraction bands, sarcolemmal discontinuities, and swollen mitochondria, was correlated with the permeability changes.

In reperfusion injury, a putative alteration of the sarcolemmal membrane is linked to the generation of oxygen free radicals and massive calcium influx, as opposed to permanent...
ischemia, in which membrane alteration is a relatively late event.

REPERFUSION INJURY AND ITS MECHANISM

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Reperfusion injury, a controversial concept, includes reperfusion arrhythmias, vascular damage, and myocardial functional stunning. In animal models, there are two main proposed mechanisms: calcium overload (Opie, Int J Cardiol 1989; 23:159) and formation of oxygen free radicals (Guarnieri et al., J Mol Cell Cardiol 1989; 20:131). An intervention given at the time of reperfusion should be able to diminish the injury. In the case of arrhythmias thought to be mediated by excess calcium recycling, early reperfusion ventricular arrhythmias in the coronary-ligated isolated rat heart have not been prevented by free radical scavengers nor by spin-trap agents, nor has the incidence of reoxygenation automaticity in the isolated papillary muscle preparation been decreased by scavengers (Coetzee et al., Cardiorense Res, 1989, submitted). In the case of myocardial stunning, experimental evidence suggests a role for a burst of free radicals formed within the first minute of reperfusion and improved by free radical scavengers given at the time of reperfusion. An alternate hypothesis is that cytosolic calcium overload damages intracellular organelles to explain why the stunned myocardium responds to beta-receptor agonists. However, the abrupt onset of reoxygenation in animal models may not correspond to the slower onset with thrombolytic reperfusion. The basis of stunning in patients is still not known. A further component of reperfusion injury is microvascular damage with alterations at the level of platelets, leukocytes, and endothelial integrity. From the therapeutic point of view, the best method currently available to limit reperfusion injury in patients is by early reperfusion to minimize the severity of ischemic damage and to optimize the metabolic status of the ischemic myocardium at the end of the ischemic period (Opie, Circulation 1989; 80:1).

REPERFUSION OF THE ISCHEMIC BRAIN

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Many problems posed by reperfusion are shared by all organs and tissues, however, the ischemic brain reveals certain specificities and implies certain difficulties, which explains the relatively limited development of technical advances in reperfusion in cerebral ischemia, in contrast to the evolution of treatment used for coronary circulation or lower limbs. Surgical relief of obstruction has been used since the early 1960s. Two risks predominate: first, fragmentation of the clot and distal embolic scattering during relief of obstruction; second, hemorrhagic transformation of a developing infarct, in which reperfusion occurs in ischemic tissue with alteration of the blood-brain barrier. The risk is proportional to the duration of the occlusion.

The use of fibrinolytics is not well accepted in cases of cerebral infarct. This poor reputation is based on results of seven studies carried out between 1961 and 1981 on 260 patients treated by intravenous fibrinolytics (notably urokinase or streptokinase), which reported either no beneficial action, or in some studies, a high number of deaths of hemorrhagic transformation of infarct.

Fibrinolytic injection into intracranial arterial occlusion in the early hours of stroke remains, nevertheless, a most delicate technique, demanding a highly specialized, immediately available, infrastructure. Use of this technique will most probably remain limited to a small number of carefully selected patients. However, another avenue of research, theoretically more promising, is that of second-generation fibrinolytics in the form of plasminogen endogenous activators, rt-PA and scu-PA. These substances, apparently acting more selectively on the blood clot, could be used intravenously with less risk of systemic fibrinolysis. Successfully used in several animal models of cerebral ischemia, these products—particularly rt-PA—are currently being tested in humans in the early phase of stroke.

It is reasonable to hope, in the near future, that the recanalization of an occluded cerebral artery may be possible pharmacologically under sufficiently safe conditions. The time factor will remain essential and will necessitate a modification of the medical mentality, which too often adopts a "wait-and-see" attitude in stroke.

NORADRENALINE RELEASE AND MYOCARDIAL REPERFUSION

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Tennant and Wiggers (1935) first demonstrated in experimental models that reperfusion of acutely ischemic myocardium produced malignant ventricular arrhythmias. Later Di-dier et al. (1986) showed in the isolated rat heart that