Antioxidants and Myocardial Ischemia: Reperfusion Injuries

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Animal studies have demonstrated that restoration of blood flow to severely ischemic myocardium is a prerequisite for myocardial salvage. However, it has been shown that the restoration of blood flow to ischemic myocardium may be associated with deleterious changes of the myocardium, including arrhythmias, enzyme release, and contractile dysfunction. These changes were considered to be additional injuries to the myocardium manifested at the time of reperfusion. The reperfusion was accompanied by a burst of oxygen free radical generation and their role as main mediators of the reperfusion injury have been well accepted. Reactive oxygen species (ROS) and cellular redox status regulate many important cellular activities. The role of antioxidant as a therapy for reperfusion injury has thus been tried with mixed and mostly negative results. Further studies are needed if the antioxidant therapies for ischemia reperfusion injury were to be effective.
(Chang Gung Med J 2005;28:369-77)

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Key words: polyphenolics, ROS, infarction.

It is well known that there is a positive correlation between the levels of dietary saturated fat and the mortality from coronary heart disease (CHD).[1] An interesting statistics however, showed that compared to other developed countries such as the USA, France has a much lower incidence of CHD despite the fact that they consume comparable amounts of dietary fat. This phenomenon, termed the French Paradox,[2] is thought to result from a higher consumption of red wines by the French.[3,4] Although some researchers suggest that the beneficial effects of alcohol are probably due to its hemostatic activity,[5,6] it is now clear that wine, particularly red wine, is rich in phenolic compounds, and some of which have significant cardioprotective activities.[7,8]

The natural phenolic compounds include two major classes, the flavonoids and non-flavonoids. The non-flavonoid compounds in wine include stilbene, hydroxyl cinnamates and hydroxybenzoates. The compound that is responsible for possible cardiovascular benefits is the stilbene resveratrol.[7,9] Resveratrol (RSV, trans-3, 5, 4'-trihydroxystilbene) was first isolated from the roots of the oriental herb and medicinal plant *Polygonum Cuspidatum*.[10] It was also synthesized by leaf tissue from grapevine in response to fungal infection.[11] In a grape, RSV is found mainly in the skin. Among red wines, RSV contents varied from 2 to 10 μg/ml, while white wines had concentrations around 1 to 2 μg/ml.

Many studies have shown that RSV is a potent

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antioxidant.[12-14] It prevents copper ion induced lipid peroxidation of the low density lipoprotein.[12,15] In addition to its antioxidant activity, RSV has also been shown to suppress cell proliferation,[16-18] promote cell differentiation,[19] induce apoptosis,[20-22] inhibit inflammation,[13,23] scavange reactive oxygen species,[24] inhibit platelet aggregation,[25] and have cancer chemopreventive activity.[26] More recently, RSV was found to protect endothelial cells from oxidative damage and ameliorate ischemia-reperfusion injuries in a number of experimental models. In the present article, we review the roles of ROS in ischemia-reperfusion injury (IR injury) of the heart and the effect of some antioxidants in ameriolating the myocardial IR injury.

Redox imbalance and myocardial IR Injury

Inadequate blood supply to a region of the body for a certain period followed by the resumption of blood flow is termed ischemia-reperfusion. Ischemia-reperfusion results in varying degrees of tissue damage depending on the duration and extent of the hypoperfusion.

Myocardial damage induced by ischemia-reperfusion is due, at least in part, to the generation of ROS.[27-31] Evidence supporting ROS as a culprit of myocardial IR injury came from several direct and indirect observations. There have been reports showing a close correlation between the production of ROS and simultaneous consumption of endogenous antioxidants.[32-35] Indirect evidence consistent with this view is the cardioprotective effects of free radical scavengers and antioxidant supplements.[36-38] In addition, direct genetic manipulations to overexpress or underexpress genes participating in the antioxidant defense also exhibit profound influence on the outcome of IR injury.[39-45] It would seem that the link between ROS and IR injury has appeared to have been clear-cut, however, contradicting results have been reported regarding the effects of antioxidants on IR injury.

Inadequate perfusion of a tissue/organ leads to oxygen (O_2) and adenosine triphosphate (ATP) depletion, and the accumulation of toxic metabolites. Another effect of hypoperfusion is the conversion of xanthin dehydrogenase to xanthin oxidase, which upon reperfusion, catalyzes the conversion of hypoxanthine to xanthine with the concomitant production of ROS.

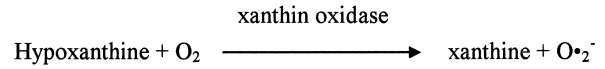

Fig. 1 Production of an oxygen radical by xanthine oxidase

Oxygen radicals ($O\bullet_2^-$) are also produced by the electron transport system of the mitohondria and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase. The highly toxic ROS are converted to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD), and then to H_2O by catalase and/or glutathione oxidase (Fig. 2). However, under ischemic conditions, the endogenous antioxidant system is eroded and the tendency for metal ion assisted conversion of H_2O_2 into the destructive hydroxyl radical ($OH\bullet^-$) is increased.[46]

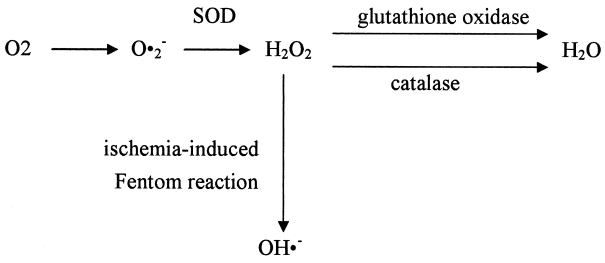

Fig. 2 ROS metabolism under normal and ischemic conditions

In addition to IR injuries, ROS have also been implicated in many clinical conditions including atherosclerosis, autoimmune diseases, alcoholic liver disease, and various inflammation related disorders. Accumulated evidence has shown that ROS production is a key event in reperfusion injury when oxygen is reintroduced to ischemic tissues.[46-49] ROS, especially hydroxyl radical, cause the oxidation of proteins, lipids and nucleic acids, resulting in the structural and functional changes of proteins, disruption of membrane intergrity, and genetic mutations, respectively. ROS also cause severe functional and metabolic disorders, and such effects can be systemic, leading to multi-organ failure. ROS mediated reperfusion injury has been observed in heart, liver, lung, kidney and intestine. The IR injury of the heart is discussed below.

IR injury to vascular endothelium

ROS increase in concentration upon reperfusion

of the ischemic myocardium.[50] The formation of ROS exerts oxidative stress to the myocardium that may cause heart failure. The major ROS that are responsible for the oxidative stress are superoxide anion ($O\bullet_2^-$), hydroxyl radical ($OH\bullet^-$) and H_2O_2 In the vascular walls, the enzyme systems involved in the production of these radicals including xanthine oxidase, NADPH oxidase and the endothelial nitric oxide (NO) synthase (eNOS). Because of its location, the endothelium is probably the prime target for ROS damage.

The endothelium-dependent vasorelaxation activity is highly sensitive to IR injury. Elevated levels of ROS reduce the bioavailability of NO through reacting with NO to form peroxynitrite.[51] The reduced NO availability aggravates local oxidative stress by the formation of peroxynitrite and a reduced blood flow due to decreased NO availability. ROS have also shown to disrupt the integrity of the endothelial cell junctions leading to increased endothelial permeability, tissue edema and protein leakage.[52] Additional endothelial dysfunction related to ROS include production of proinflammatory cytoicines, activation of complement system, decreased production of prostacyclin (PGI_2), increased production of platelet activation factor (PAF), and thromboxane A_2 (TXA_2) and increased expression of adhesion molecules.[53]

The decreased PGI_2 and increased PAF and TXA_2 productions by endothelium would certainly compromise the nonthrombogenic nature of the vascular surface. In an in vitro study, we showed that oxidized low density lipoprotein (oxLDL) dose-dependently reduced the ability of endothelial cells (EC) to stabilize platelets from adenosine diphosphate-induced (ADP-induced) aggregation and platelet $[Ca^{+2}]i$ rise (Fig. 3). Treatment of EC with RSV (from 5 μM to 20 μM) prior to oxLDL exposure effectively preserved the anti-platelet aggregation activity of the EC. The exposure time required for the maximal effect of RSV appeared to be short; 30 min incubation and 2 hr incubation were equally effective.

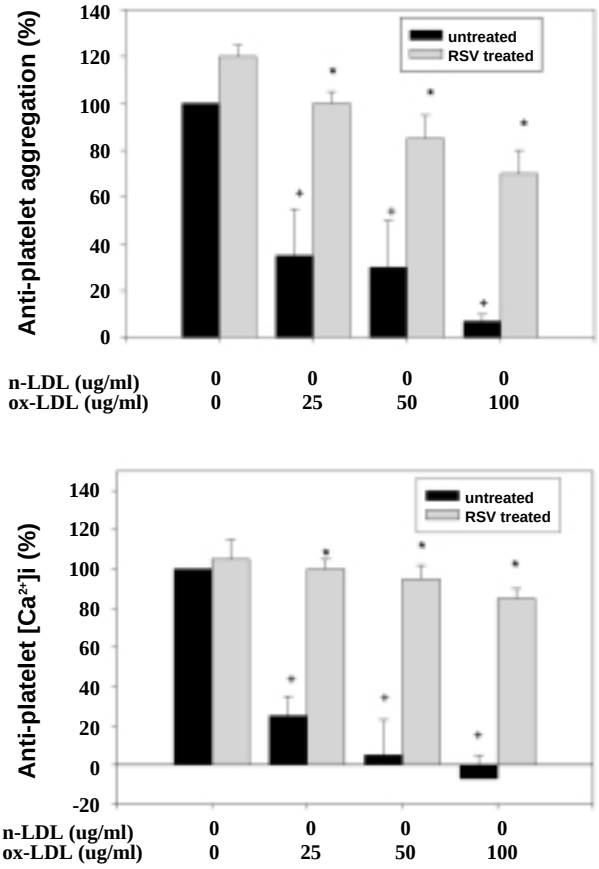

Fig. 3 RSV attenuates oxLDL-induced reduction of the anti-platelet aggregation activity of the endothelial cells. EC were treated with oxLDL as indicated for 1 hr with or without a 30 min prexposure to RSV before they were coincubated with platelets and ADP (4 μM). Platelet activation was measured by aggregation (Panel A) and intraplatelet Ca^{+2} rise (Panel B).

IR injury to myocardium

In the myocardium, O_2 is reduced via (1) the mitochondrial electron transport system (cytochrome oxidase system), which reduces 95% of O_2 to H_2O by tetravalent reduction without the production of ROS,[54] (2) the univalent pathway in which ROS are produced (Fig. 2). The endogenous antioxidant systems endow tissue with substantial ability to balance the ROS effect under normoxic conditions. However, under ischemia followed by reperfusion, the antioxidant defense is undermined and the oxidative damage to the tissue is ensured.

• Metabolic disorder

A shortage of oxygen supply at the mitochondria level results in intracellular acidosis and an increased concentration of inorganic phosphate due

to the breakdown of high energy phosphates. These early metabolic changes weaken the contractility of the ischemic zone.[55] The onset of anaerobic respiration and lactate release is another metabolic alteration which allows production of a small amount of ATP without the consumption of oxygen. However, prolonged ischemia results in a decrease in lactate production because anaerobic glycolysis is inhibited by further intracellular acidosis, and thus, a further drop in the intracellular ATP and creatine phosphate concentrations. At this stage of ischemia, the ionic conditions of the myocytes are altered, with an increase of the intracellular Na^{+1} and a decrease of K^{+1} and Mg^{+2}. Sodium ion extrusion through sarcolemmal Na^{+1}/K^{+1} - ATPase is inhibited by a lack of ATP. Na^{+1} influx leads to Ca^{+2} influx since the Na^{+1}/Ca^{+2} exchanger operates in reverse mode in Na^{+1} –overloaded, depolarized cells. As a result, ischemic cells develop cytosolic Ca^{+2} –overload. A Ca^{+2} –overloaded ischemic cardiomyocyte may develop uncontrolled activation of the contractile machinery (contracture) upon reoxygenation.[56,57]

It has been shown that ischemic, Ca^{+2} –overloaded cardiomyocytes develop hypercontracture immediately upon reperfusion.[56,58] Reperfusion brings about the rapid recovery of the oxidative ATP production if the cytochrome oxidase system of the mitochondria was not damaged during the ischemic period. ATP provides energy for cardiomyocytes to recover from cytosolic ion imbalance and reactivate the contractile function. However, the contractile activation is usually faster than Ca^{+2} recovery and it leads to a Ca^{+2} - dependent hypercontracture.

ROS may damage sarcoplasmic reticulum causing Ca^{+2} release and an increase of the cytosolic Ca^{+2} levels.[59] This suggests that ROS formation and Ca^{+2} surge might be involved in the contractile dysfunction of the ischemic myocardium (Fig. 4).

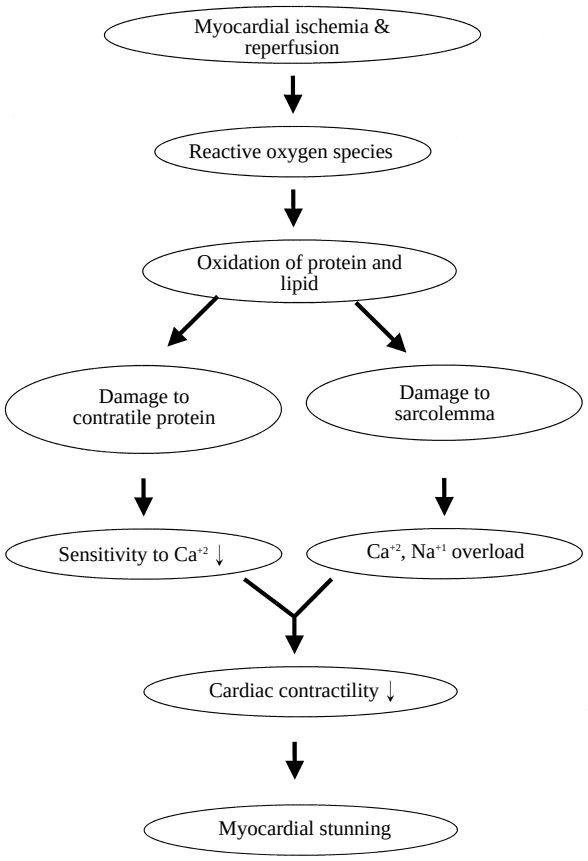

Fig. 4 Possible mechanism underlying ischemia reperfusion induced myocardial contractile stunning.

• Antioxidants and myocardial IR injury

It has become clear that redox balance is implicated in cell metabolism, signal transduction and gene expression.[60,61] Cellular redox imbalance may compromise cell function and even cause cell death. Oxidative damage to protein, lipid and nucleic acid by ROS is well recognized. These oxidation reactions are believed to be implicated in numerous diseases in many organ systems including the cardiovascular system. However, antioxidants have also been shown to cause apoptosis of both normal and transformed cells. These observations strongly suggest that normal cellular function requires an optimal redox environment. The endogenous antioxidants in an ischemic tissue are believed to be eroded along the duration of the ischemia. To justify the use of antioxidants to prevent or ameriolate IR injury of a tissue or organ, it is necessary to establish a time-dependent change of the antioxidant profile of the tissue following ischemia-reperfusion. Animal studies have consistently shown a depletion of myocardial nonenzymatic antioxidant levels. However, changes in the enzymatic antioxidants have been controversial,[34,62-64] a decrease, increase, or maintaining no change in activities have been reported. In humans, the formation and release of oxidized glutathione (GSSG) in the coronary sinus following myocardial IR has been reported.[65] The release of

GSSG was positively correlated with the duration of the ischemic period suggesting the consumption of glutathione (GSH) during cardiac ischemia. Work by De Vecchi et al., (1998)[66] showed massive reduction of glutathione in myocardium during bypass surgery and such glutathionine loss might be related to left ventricle dysfunction in ischemic human heart.

• Antioxidants as therapeutics

Numerous studies have evaluated the effects of antioxidants on IR injury in animals or in patients undergoing bypass surgery. Treatment of intestinal IR injury by antioxidants (Vitamins C and E, manitol and methyl prednisolone) in an animal model has been reported.[67] They showed that treatment with vit. C and manitol attenuated the IR injury, while treatment with vit. E and methyl prednisolone had no significant effect. Vitamins C, E, and thiol compounds, either alone or in combinations, have been used to evaluate the therapeutic effect on IR injury in patients undergoing cardiopulmonary bypass.[68-71] The results were mixed, as follows: protection by a high dose of vit. C (250 mg/kg bw) was observed in one study,[71] other studies showed that vitamin supplement was correlated with reduction in IR injury-related biochemical parameters, however, they were not consistently correlated with a more functional recovery, or clinical improvement.[68-70]

• Resveratrol ameriolates cardiac IR injury-Animal study

The effects of RSV administration on IR-induced cardiac injury have been studied in Sprague-Dawley rats subjected to myocardial ischemia by a temporary occlusion of the left main coronary artery.[72] In studies by Hung and colleagues, animals were infused with a boulus of RSV, at the desired doses, from the jugular vein 15 min before coronary occlusion.[14] Adminstration of RSV was found to have no effect on the hemodynamic parameters of the sham operated animals.[15,73,74] Occlusion of the coronary artery induced severe ventricular arrhythmias in animals of the vehicle group, which began after 6-7 min of occlusion, peaked after 8-12 min, and usually subsided at approximately 15 min. In the vehicle group, 100% of the animals developed ventricular tarchycardia (VT) and from 63% to 73% of the animals developed ventricular fibrillation (VF). Administration of RSV at 2.3×10^{-5} g/kg had no effect on ischemia-induced arrhythmias, or on mortality.[14]

A 5-min period of left main coronary artery occlusion, followed by 30-min reperfusion induced rhythm disturbances, and the severity of the disturbances is positively correlated with the ischemic duration. IR protocol-induced arrhythmias have been reported as relative to superoxide anion production.[75,76] Administration of RSV at 15 min before IR, effectively reduced IR induced VT, VF, and the mortality rate of the experimental animals.[14]

In the same series of studies, Hung and colleagues evaluated myocardial damage by measuring plasma lactate dehydrogenase (LDH) level and the infarct size of the occluded zone. The left main coronary artery was occluded for 5-min followed by a 30-min reperfusion period. Blood samples were taken at the end of the reperfusion. They found that RSV pretreatment reduced plasma LDH activity by more than 50% compared to the control animals.

To evaluate the effect of RSV on the infarct size, the left main coronary artery was occluded for 1 hr and reperfused for 3 hrs, or occluded for 4 hrs without reperfusion, and the infarct area was identified by staining with triphenyl tetrazolium chloride and –Evans blue.[77] Hung and colleagues found that RSV pretreatment reduced the infarct size, again, by more than 50%[74] compared to the control animals. These results clearly indicated that RSV possesses cardioprotective effect against IR induced injury of the myocardium.

Conclusions

Despite the fact that the *in vivo* measurement of ROS is rather difficult, the elevated production of ROS during IR is generally believed to be associated with myocardial tissue damage. Multiple enzymes and cell types are responsible for the accelerated ROS formation. However, because cells contain numerous antioxidant activities, it is therefore, uncertain whether an increase in ROS production is an accurate indication of oxidative damage. In addition, although oxidative damage of protein, lipids and nucleic acid by ROS have been well established, it has not been clear whether ROS produced at the time of reperfusion directly damage the myocardium.

The incomplete understanding of the roles of ROS in the IR injury plus the seemingly inconsistent results regarding the antioxidant effects in IR have hampered their therapeutic applications. Although

vit. C, vit. E, and several thio compounds have been evaluated for alleviating IR injury in patients undergoing cardiopulmonary bypass, either alone or in combinations,[68-71] their beneficial effects still await further establishement.

Reports by Hung and colleagues[14,15,73,74] clearly demonstrated that RSV administered 15 min before occlusion effectively alleviates ischemia-reperfusion induced rhythm disturbances and mortality. They found that the protective effects of RSV against ventricular arrhymia and mortality rate are NO-independent, while the protective effect against cardiomyocyte damage (LDTT and creatine kinase release) and infarction size are NO-dependent.[74] However, the mechanism underlying the protective effects of RSV against ischemia-reperfusion injury to date has not been fully elucidated, or their possible cytotoxic effect been evaluated. In addition, the possible existence of interspecies heterogeneity of the response to IR between human and rat also needs to be clarified. Before we can address all these issues, RSV and its derivatives will not be able to find their way into clinical applications in order to prevent or treat myocardial IR injury.

Acknowledgements

We are indebt to Dr. L-M Hung for part of the literature search during the preparation of this manuscript, and to Ms. H-W Huang for the typing work.

Supported by NSC grant 93-2320-B-182-002 from National Science Council, Taiwan.

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抗氧化劑與心肌缺血再灌流傷害

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動物實驗明顯指出，恢復血流是降低缺氧心臟心肌損傷的先決要件。但是各種缺血再灌流的研究指出，缺氧的心臟恢復血流時，往往伴隨著明顯的心肌功能上和結構上的損傷；包括心律不整，細胞傷害致胞內蛋白釋出，及收縮功能異常等。這些變化被認爲是心臟再灌流時引起的二度傷害。由於再灌流時會伴隨著激烈的自由基產生，故一般認爲再灌流時的心肌二度傷害，是因爲氧化壓力造成。細胞內許多重要的生化活動受到自由基及細胞氧化還原狀態的調節，氧化還原狀態的失衡，是引起細胞功能異常的重要原因之一。唯利用抗氧化劑治療缺血再灌流傷害的研究，各方的結果並不一致，且以負面的結果居多。故抗氧化劑治療的臨床利用，仍有待進一步研究。(長庚醫誌 2005;28:369-77)

關鍵字：自由基，缺血再灌流，心肌損傷，抗氧化劑。

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受文日期：民國94年3月30日；接受刊載：民國94年4月12日。
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