

Levels of free radical scavengers and antioxidants in post-reperfusion patients of myocardial infarction

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The present study has been undertaken to assess the levels of some free radical scavenging and antioxidant systems of plasma in patients reperfused after myocardial infarction. The study included 30 patients and a control group consisting of 40 age- and sex-matched healthy persons. The findings show a significant decrease in the activity of free radical scavenging enzyme, superoxide dismutase (54%) and levels of ascorbic acid (63%) and total thiols (42%), with significant increase in the levels of malondialdehyde (285%), a marker of oxidative stress, in the patients reperfused after myocardial infarction compared to controls. The findings show that reperfusion of the infarcted myocardium results in a burst of oxygen consumption with enhanced generation of free radicals and free radical-mediated damage. At the same time, there is decrease in the levels both of enzymatic free radical scavenger and antioxidants.

OXYGEN-derived free radicals are known to play a vital role in the genesis of various cardiovascular disorders^{1,2}. Reactive oxygen species (ROS) include superoxide anion, hydroxyl radical, hydrogen peroxide, hypochlorous acid and peroxynitrites. Ischaemia and reperfusion of the ischaemic tissue both lead to the generation of ROS and reactive nitrogen species. Generation of ROS is in delicate balance with antioxidant defences of the cell. Evidence suggests that damage to the myocardial cells induced by the cycle of ischaemia and reperfusion may be due, in part, to the generation of toxic ROS³⁻⁶. These ROS are mainly produced by xanthine oxidase and electron leakage from mitochondria, among others, and are removed by enzymatic free radical scavengers and antioxidants⁷. It has also been recognized that the process of myocardial reperfusion may itself lead to a number of adverse consequences, including myocardial stunning, reperfusion arrhythmias or an increase in the infarct size⁸.

Superoxide radical formed during oxidative stress conditions may react with various molecules and result in either direct damage or generation of potentially harmful products. The deleterious effects of the ROS are nullified by various antioxidants (such as vitamin E, ascorbic acid and thiols) and free radical scavengers such as catalase, glutathione peroxidase and superoxide dismutase (SOD). A significant increase of antioxidant capacity has been reported to occur after

supplementation with ascorbic acid, α -tocopherol or β -carotene⁹.

In the present communication, we have assessed the production of free radicals, damage caused by these radicals and the status of antioxidant system of the plasma in post-reperfusion cases of myocardial infarction.

The study was cleared by Institutional Ethical Committee of the Department and informed consent was taken from each subject. A total of 70 subjects constituting 30 patients and 40 age- and sex-matched healthy persons were selected. All the cases were males, non-smokers, non-alcoholic and non-diabetic. Patients were receiving normal post-infarction medication including aspirin and anti-hypertensives. Reperfusion was carried out by administration of streptokinase. Only those patients with acute myocardial infarction who achieved reperfusion, as evidenced by prompt relief of chest pain, early settling of ST segment elevation, appearance of reperfusion arrhythmias and early peaking of CPK-MB were included in the study.

Venous blood (3.5 ml) was withdrawn within 3 h of reperfusion from the patients and transferred in citrated centrifuge tubes at 4°C. The plasma was prepared by centrifuging the blood at 800 g for 20 min at 4°C. Plasma from healthy persons was simultaneously prepared in similar manner.

SOD activity was assayed by the method of Misra and Fridovich with minor modifications¹⁰. One unit of enzyme activity has been defined to cause 50% inhibition of auto-oxidation of 5 μ mol epinephrine. Protein was estimated by the method of Lowry *et al.*¹¹ using bovine serum albumin as the standard. Specific activity of the enzyme has been expressed as units/mg protein. Plasma ascorbic acid level was estimated by the method of Stanley *et al.*¹². Suitable ascorbic acid solution in 5% (w/v) trichloroacetic acid was used as the standard. Total thiols (T-SH) in plasma were measured by the method of Hu¹³. Reduced glutathione was used as reference. Malondialdehyde (MDA) measurement has been utilized as an index of oxidative stress by the method of Ohkawa, Oshishi and Yagi¹⁴, using thiobarbituric acid reagent. 1,1,3,3 tetraethoxypropane (TEP) was used as the reference.

Statistical analyses were carried out using Student's *t* test. The results have been expressed as mean \pm SD. $P < 0.01$ has been considered significant.

The results obtained are reported in Table 1. There is statistically highly significant decrease in the activity of SOD (54%, $P < 0.0005$) in the patients. Antioxidant levels also show significant decrease (ascorbic acid 63%, $P < 0.0005$ and total thiols 42%, $P < 0.01$). MDA levels in patients were elevated significantly (285%, $P < 0.0005$).

The increased oxidative stress may arise from a variety of factors such as (a) enhanced generation of free radicals, (b) reduced levels of antioxidants available, (c) enhanced consumption, leakage or destruction of antioxidants, (d) decreased protective capacity including antioxidant enzymes, (e) leakage of electrons from the disrupted mitochondrial

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Table 1. Levels of MDA, superoxide dismutase, ascorbic acid and total thiols in human plasma in patients reperfused after myocardial infarction and healthy persons

Case	MDA (nmol/ml)	Superoxide dismutase (unit/mg protein)	Ascorbic acid (mg/dl)	Total thiols (μ M)
Healthy ($n = 40$)	0.281 ± 0.166	0.613 ± 0.158	0.4 ± 0.26	462.62 ± 89.72
Patients ($n = 30$)	1.081 ± 0.962	0.281 ± 0.123	0.15 ± 0.07	269.46 ± 82.63
<i>P</i>	< 0.0005	< 0.0005	< 0.0005	< 0.01

n = the number of cases; the values are mean \pm SD; $P < 0.01$ significant and < 0.0005 highly significant.

electron transport chain, and (f) phagocyte recruitment and activation¹⁵.

Myocardial ischaemia occurs when myocardial oxygen demand exceeds the oxygen supply. If this condition is not reversed, myocardial infarction precipitates. Reperfusion of the ischaemic myocardium can restore oxygen supply but sudden massive increase in oxygen supply causes a burst of oxygen consumption with the consequent generation of free radicals, resulting in an imbalance of oxidative–antioxidative processes. The excess production of ROS may initiate lipid peroxidation in cell membrane, damage membrane proteins or cause DNA fragmentation, etc. These processes may result in a loss of contractile function of the heart and lead to severe myocardial cell damage, collectively termed as reperfusion injury¹⁶.

During ischaemia, energy demand exceeds the capacity of the heart to synthesize ATP. ATP is, therefore, rapidly degraded ultimately to hypoxanthine and xanthine. The failure of the energy-dependent mechanisms lead to deterioration of membrane ion gradients, opening of selective and unselective ion channels and equilibration of most intracellular and extracellular ions. The elevated intracellular calcium ion concentration activates proteases that can convert xanthine dehydrogenase to xanthine oxidase. At this stage, both xanthine oxidase and its substrates hypoxanthine and xanthine are available. Upon reperfusion, when oxygen supply to the tissue is restored, xanthine oxidase uses molecular oxygen as a substrate to produce superoxide anion and uric acid.

The observed highly significant decrease in SOD activity in the patients may be due to the following: (a) Accumulation of superoxide anion to a high level thus causing inhibition of enzyme activity (high substrate inhibition). In post-reperfusion conditions, though SOD activity is decreased, the residual activity is capable of producing H_2O_2 . Our findings (unpublished) and those of Halliwell and Gutteridge¹⁷ show that there is little or no catalase activity in the plasma; thus H_2O_2 is not detoxified and accumulates to high levels and causes inhibition of SOD activity¹⁸. (b) Alterations in the conformation of SOD by free radicals, formed during reperfusion, could result in decreased activity of the enzyme. (c) The lowered SOD activity could result from the reduced synthesis of SOD in the patients, thus rendering them more susceptible to oxidative damage¹⁹. Whatever the cause of the reduced SOD activity, the net result is accumulation of H_2O_2 , one of the most damaging products of

the free radical metabolism. H_2O_2 can readily react with superoxide anion to produce the highly toxic hydroxyl radical and HOCl. Many findings suggest that SOD has relevance to the precipitation of myocardial infarction and these findings are consistent with the notion that increased levels of SOD are protective²⁰. Landmesser *et al.*²¹ have also found a substantially reduced SOD activity and attributed it to the endothelium dysfunction in patients with coronary artery diseases. It has also been suggested that low plasma levels of SOD may directly be related to an increased production of superoxide anion and plasma levels of SOD may be envisaged as a potential marker for risk assessment of coronary artery disease²².

Ascorbic acid acts as the first line of defence against oxidative stress during the ischaemia-reperfusion cycle²³. It is the only antioxidant in plasma capable of completely inhibiting oxidative modification of the low density lipoprotein by aqueous peroxy radicals²⁴. It has been recently demonstrated that during reperfusion of ischaemic heart, there is depletion of endogenous antioxidants and this change depends on the severity of ischaemia-reperfusion. Deficiency of ascorbate also has a direct association with increased atherosclerosis in guinea pigs and its intake has been shown to have an increased relationship to atherosclerosis in quail, rabbit and human. Ascorbate administration exerts a protective role against peroxidative damage of lipids²⁵. Significantly lowered levels of ascorbate observed in the patients, compared to healthy individuals, may be linked to the increased consumption of ascorbic acid due to increased oxidant stress, as evident from enhanced MDA levels or failure of the system to recycle dehydroascorbic acid back to ascorbic acid.

Major part of thiols in plasma is derived from proteins and a smaller part from free –SH group containing substances such as glutathione and certain other coenzymes. Plasma –SH groups are susceptible to oxidation during reperfusion and are thus lowered in patients suffering from coronary artery diseases.

The significantly low level of total thiols observed in the patients appears to be the result of severe damage of functional protein thiol groups, including those of enzymes, thus causing loss of enzyme activity as suggested by Miyagawa *et al.*²⁶.

MDA levels in plasma have been used as an index of free radical-mediated damage, which is significantly elevated

in the patients, clearly denoting extensive damage caused by reperfusion of ischaemic myocardium.

To summarize, the present study shows that during reperfusion of ischaemic myocardium, there is copious generation of ROS, depletion of antioxidants and inhibition of enzymatic free radical scavenging system. These changes are conducive to post-reperfusion injury of myocardial tissues and may perhaps be prevented or minimized by administration of antioxidants such as vitamin C, the safest one, along with routine post-reperfusion therapy.

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Phototoxic effect of some porphyrin derivatives against the larvae of *Aedes aegypti*, a major vector of dengue fever

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Porphyryns when photosensitized, transfer their energy/electron to the triplet ground state oxygen, producing cell-lethal reactive oxygen species. Dengue is a tropical disease and the causative viruses are maintained in a cycle involving humans and the principal urban mosquito vector, *Aedes aegypti*. Infection with dengue virus causes a spectrum of clinical illnesses, ranging from a nonspecific viral syndrome to severe and fatal haemorrhagic disease. In this communication we report on the promising photodynamic effect of porphyrin-generated singlet oxygen on the larvae of *A. aegypti*, with possible applications in controlling the dengue vector.

THERAPEUTIC effects of a chemical agent in the presence of light have long been recognized by mankind. In India, vertigo was treated with plant extracts rich in furanocou-

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