Chapter - II

REVIEW OF LITERATURE
Ischaemic heart disease is considered as the greatest epidemic of the twentieth century although a critical look does not favour the view that this is an epidemic.\textsuperscript{1} Indeed, in terms of mortality, morbidity, social effects and financial burden, no other disease ranks ahead of coronary heart disease. A number of disorders affect the coronary arteries and cause partial or complete coronary obstruction which in turn leads to an imbalance between myocardial oxygen demand and supply i.e. myocardial ischaemia. Complex dynamic interaction among fixed atherosclerotic narrowing of epicardial coronary arteries\textsuperscript{2}, intraluminal thrombosis over a ruptured or fissured plaque, vasospasm and platelet aggregation account for over 90\% of the cases which suffer from reduction in coronary blood flow.\textsuperscript{3} The link between coronary obstruction, arrhythmia and sudden death was established very early. Heberden first described the clinical syndrome of angina in the 18th century.\textsuperscript{4} and Quain demonstrated damage to heart (Infarction) resulting from obstruction.\textsuperscript{4} The extent of irreversible damage by ischaemic injury depends on the duration of ischaemia. In order to determine the critical time factor for the salvage of ischaemic jeopardized myocardium, animal models have been used since long. In 1840, Erichsen\textsuperscript{5} ligated a coronary artery of a dog. Tennant and Wiggers\textsuperscript{6} demonstrated that following ligation of coronary artery, the contraction of cardiac muscle ceases completely and affected areas appear cyanotic, dilated and bulging.\textsuperscript{6} Myocardial ischaemia leads to an asynergic contraction.\textsuperscript{7} All myocardial cells do not die.
simultaneously in the area of severe myocardial ischaemia. In the dog, irreversible injury occurs as the "wave front" progresses from endocardium towards the epicardium and it depends on the duration of coronary occlusion. An irreversibly damaged myocardial cell fails to perform normal metabolic functions even after removal of the cause. In the injured myocytes, mitochondrial swelling, loss of normal dense mitochondrial granules, incomplete clearing of mitochondrial matrix and absence of granular flocculent densities within the mitochondria can all be demonstrated by electron microscopy. Fifteen minutes of ischaemia results in widening of I-bands, early nuclear clumping and migration of nuclear chromatin, partial loss of glycogen granules and myocyte oedema with intermyofibrillar separation. Fifteen minutes of occlusion followed by 3, 7 and 14 days of reperfusion produces reversal of above changes with focal lipid droplets observed close to mitochondria at 3 days but not at 7 and 14 days. By light microscopy, detectable changes are observed only after 120-240 minutes of ischaemia in the form of focal contraction band necrosis and wavy fiber changes. Haemorrhagic changes are extensive in infarcts of longer duration and are confined to the necrotic areas. Clinical studies have demonstrated that the infarct size is an important determinant of prognosis. The infarct size not only depends on the duration of ischaemia but also on other factors like collateral blood flow which differs in different species. The amount of myocardium supplied by occluded
coronary artery can also alter the infarct size; coronary anatomy moreover varies within species. \(^\text{18}\) Coronary artery occlusion at even identical anatomical site shows significant variation of area at risk in canines. In ischaemic injury, not only myocytes but endothelial cells are also affected. In the canine model, endothelial cell abnormalities are observed only after 60 minutes of myocardial ischaemia.\(^\text{19,20}\) Endothelial cell abnormalities are reflected in form of focal endothelial cell swelling with loss of pinocytotic vesicles.\(^\text{20}\) With the longer duration of ischaemia the greater the endothelial changes, such as clumping and margination of nuclear chromatin, marked swelling of cytoplasm, formation of intraluminal blebs, disruption of endothelial cell border, haemorrhage and fibrin deposition.\(^\text{14, 20}\) Ultrastructural microvascular damage lags behind the myocardial cell injury and is not the primary cause of myocyte injury until reperfusion occurs.\(^\text{20}\)

Blumgart et al. in 1947,\(^\text{21}\) confirmed that reperfusion following occlusion lasting for 25 to 45 minutes presents gross evidence of some infarction and the irreversible damage is caused due to 45 minutes of occlusion. This was as extensive as noted, with an occlusion lasting for several weeks. Following prolonged coronary artery occlusion, irreversible myocardial injury refractory to subsequent reperfusion becomes widespread.\(^\text{22, 23}\) In experimental models, following release of coronary artery occlusion "no reflow phenomenon" can be observed because of massive endothelial cell swelling on reperfusion of capillary beds.\(^\text{24}\)
obstruction is caused by voluminous capillary endothelial cells having large intraluminal endothelial protrusions together with neutrophils, red blood cell and platelets. These changes have been described only after 90 minutes of ischaemia followed by reperfusion in dogs. Therefore, Braunwald and Kloner in 1985 described reperfusion as a "double-edged sword". Which could cause death of the salvageable myocardium. However, several other investigators have shown that despite the deleterious effects of 'no-reflow' phenomenon an early reperfusion reduces the extent of injury to the myocardium. In the irreversibly injured myocardium, reperfusion leads to haemorrhage. Views on the importance of myocardial hemorrhage induced by reperfusion are disparate. In zones of ischaemia increased haemorrhage is observed after reperfusion in comparison with zones subjected to ischaemia without reperfusion. The extent of myocardial ischaemia and subsequent development of myocardial injury depends on several factors, viz. depletion of intracellular adenine nucleotide stores, depletion of intracellular substrate, mitochondrial functional impairment, endothelial cell swelling, microvascular damage and finally accumulation of noxious metabolites such free oxygen radicals.

Free radicals are unique chemical species which have an unpaired electron in their structure. Electron in an atom is present in space known as orbit and each orbit can hold a maximum of two electrons.
spinning in opposite directions. Most of the biological molecules are non-radicals containing only a paired electron. In many ways a radical can react with other molecules. Two radicals can combine their unpaired electrons and form covalent bond.

\[ A^\cdot + A^\cdot \longrightarrow A - A \]

A radical might donate its unpaired electron to another molecule or might take an electron from another molecule to form a pair or it may join that molecule. A radical can give one electron or take one electron from or add on to a non-radical to convert it to a radical.

1. Addition
   \[ X^\cdot + Y \longrightarrow (X - Y)^\cdot \]
2. Electron donation
   \[ X^\cdot + Y \longrightarrow Y^- + X^+ \]
3. Electron removal
   \[ X^\cdot + Y \longrightarrow X^\cdot + Y^+ \]

Only when two radicals meet, chain reaction can be terminated:

\[ X^\cdot + X^\cdot \longrightarrow X_2 \]
\[ X^\cdot + Y^\cdot \longrightarrow XY \]

When tissues are exposed to gamma radiation, the most of energy taken up is absorbed by the cell water. The radiation causes one of the oxygen-hydrogen
covalent bond in water to split, leaving a single electron on hydrogen and one on oxygen and two radicals are formed.

\[ \text{H-O-H --- Intermediate --- H}^\cdot + \text{OH} \]

\[ \text{stages} \]

\( \text{H}^\cdot \) is hydrogen radical and \( \cdot \text{OH} \) is hydroxyl radical. The \( \cdot \text{OH} \) or hydroxyl radical can attack and damage every molecule found in the living cells.\(^{42,43} \) \( \cdot \text{OH} \) radical can attack DNA and propagate free radical chain reactions to cause chemical alteration of bases which can lead to mutation as well as strand breakage.\(^{44} \) Best known damage caused by \( \cdot \text{OH} \) radical is its ability to stimulate free radical chain reaction known as lipid peroxidation which is an oxidative deteriation process affecting mainly the fatty acids which contain two or more double bonds.\(^{43} \) The hydroxyl radical causes lipid peroxidation by abstracting a hydrogen atom and combining it to form water. Hydrogen ion has only one electron, this leaves an unpaired electron on the carbon atom from which it was abstracted. Carbon radical undergoes molecular re-arrangement to form a conjugated diene which reacts with water to form peroxyl radical. The peroxyl radical abstracts a hydrogen atom from an adjacent fatty acid side chain to carry on the process by converting itself into lipid peroxide.\(^{41} \)

Lipid hydroperoxides disrupt the function of membrane and can cause it to collapse. In addition lipid hydroperoxides can form a range of
highly cytotoxic products like aldehydes. Lipid hydroperoxides are formed by one hydroxyl radical which causes the conversion of many hundred fatty acid side chains into lipid hydroperoxide. Peroxyl radicals and cytotoxic aldehydes damage to membrane proteins, inactivate receptors and membrane bound enzymes. It is well established that free radical (O$_2^-$) and other reactive oxygen species which include not only oxygen centered radicals such as superoxide and hydroxyl radical but also some non-radical derivatives of oxygen like hydrogen peroxide (H$_2$O$_2$), singlet oxygen (1A g) and hypochlorous acid (HOCl). Superoxide radical (O$_2^-$) is less reactive than OH. The enzyme superoxide dismutase removes superoxide radical. Superoxide radical is formed by adding an extra electron onto oxygen.

\[
O_2 + e^- \rightarrow O_2^-
\]

Superoxide dismutase removes O$_2^-$ by catalysing dismutation of reaction.

\[
O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2
\]

Only after discovery of superoxide dismutase by Fridovich in 1974, it was realized that O$_2^-$ is formed in vivo. Hydrogen peroxide(H$_2$O$_2$) is toxic to the cells. Incubation of cells with H$_2$O$_2$ can cause DNA damage, membrane disruption and release of Ca$^{2+}$ ions within the cell to activate calcium
dependent proteolytic enzymes. In presence of iron, H\textsubscript{2}O\textsubscript{2} forms Hydroxyl radicals.

\[
\text{O}_2 + \text{H}_2\text{O}_2 \xrightarrow{\text{Iron Ion}} \cdot \text{OH} + \text{OH} + \text{O}_2
\]

Hydroxyl Radical.

Therefore, removal of H\textsubscript{2}O\textsubscript{2} as well as O\textsubscript{2}\textsuperscript{−} is beneficial. Superoxide dismutase acts along with catalase and glutathione peroxidase to remove H\textsubscript{2}O\textsubscript{2} from the cell. Glutathione peroxidase removes H\textsubscript{2}O\textsubscript{2} by using it to oxidize reduced glutathione (GSH) into oxidised glutathione (GSSG).

\[
2\text{GSH} + \text{H}_2\text{O}_2 \rightarrow \text{GSSG} + 2\text{H}_2\text{O}
\]

Transition metals have variable oxidation numbers e.g. iron as Fe\textsuperscript{2+} or Fe\textsuperscript{3+} and copper as Cu\textsuperscript{+} or Cu\textsuperscript{2+}. Change between oxidation state involves the accepting and donating of a single electron.

\[
\text{Fe}^3 + e^- \rightarrow \text{Fe}^{2+}
\]

\[
\text{Cu}^2 + e^- \rightarrow \text{Cu}^+
\]

Hill in 1981 suggested that transition metal ions are good promoters of free radical reactions. Metal ions can also act by promoting free radical reaction \textit{in vivo}.

Several mechanisms have been described for the formation of free radicals in biological systems. They are Xanthine Oxidase, activated neutrophils, direct donation of electrons from reduced myocardial electron
transport chain. 59 Catecholamine oxidation, 60 cyclo-oxygenase and lipoxygenase enzymes. 61 Ischaemia can result in increased cytosolic calcium which in turn activates a calcium dependent cytosolic proteases that covalently modifies xanthine dehydrogenase to form xanthine oxidase. 62 The enzyme xanthine dehydrogenase catalyses the univalent oxidation of purine substrate with concomitant formation of $O_2^-$ radical, $H_2O_2$ and $^1O_2$. 65 Several investigators have reported the accumulation of these substrates during myocardial ischaemia and conversion of xanthine dehydrogenase to xanthine oxidase. 55,56 Other studies have demonstrated oxygen free radical mediated damage in isolated organ system by exogenous xanthine oxidase along with appropriate purine substrate. 66,68 Inhibitors of xanthine oxidase protect against oxidative damage in ischaemia /reperfusion. 69 Xanthine oxidase can be released from liver following ischaemia to initiate systemic production of free radicals. 70 However, both man and rabbit lack the cardiac xanthine oxidase. 71

The NADPH dependent oxidase system on the membrane surface of neutrophil is the source of $O_2^-$. 72 Neutrophils as a source of oxygen free radical production during myocardial ischaemia and reperfusion was first proposed by Romson et al. 73 Neutrophil depletion reduces infarct size and is comparable to the reduction observed with superoxide dismutase and catalase. 73 Free radicals formed by neutrophils inhibit Na$^+$ - K$^+$. ATPase
activity, and destroy ouabain binding sites and also increase capillary permeability. Superoxide dismutase has been demonstrated to have an anti-inflammatory effect and certain anti-inflammatory drugs can reduce infarct size. Lipoxygenase inhibitors, protect heart from oxidative damage which shows that leukotrienes also have a role in neutrophil mediated cardiovascular injury.

Mitochondria are largest intracellular source of oxygen free radical (O_2^-) and H_2O_2 via electron transport system. Leakage of electron carriers out of chain reduces, the oxygen in mitochondria and O_2^- is formed. It can occur by breakdown of ubisemiquinone and NADH dehydrogenation. Significant contribution of O_2^- formed by these sources on ischaemia and reperfusion is unknown.

The auto-oxidation of catecholamines results in formation of O-quinone, 2H^+ and 2e^- These electron are then taken up by molecular oxygen to produce O_2^-, inducing the further chain reactions leading to the formation of OH and causing membrane lipid peroxidation.

Reperfusion is one of the stimuli for the synthesis of prostaglandins. Konots et al. have demonstrated that many free radical species are formed when cyclo-oxygenase catalyses the oxygenation of arachidonic acid to PGG_2 and hydroperoxidase converts PGG_2 to PGH_2.
Purified PGH₂ synthase forms O₂⁻ when NADH or NADPH is available in presence of substrates (arachidonic acid, linoleic acid or PGG₂). An antioxidant, nafazatrom blocks cyclo-oxygenase activity and reduces myocardial infarct size. Further, this drug also blocks lipoxygenase activity which is a potential source of singlet oxygen.

Many investigators have suggested that an influx of calcium can initiate free radical formation. Calcium influx is responsible for oxidation in isolated perfused hearts following low oxygen conditions. Calcium overload damages cardiac mitochondria which affects their ability to reduce oxygen tertravalently to water. This in turn probably diverts oxygen to univalent pathways and aids in formation of free radical.

Therefore, the protective enzyme systems present in the myocardial tissues like superoxide dismutase, catalase and glutathione peroxidase can prevent the damage induced by oxygen free radicals.

Glutathione is an endogenous sulfhydryl containing peptide and it plays important role in prevention of free radical formation. Glutathione protects the tissues from O₂⁻ injury by acting as a substrate for glutathione peroxidase during reduction of H₂O₂ and organic peroxides. Oxidized glutathione is either reduced via glutathione reductase - catalysed reaction with NADPH or transported out of the cell. Driving out of
oxidized glutathione (glutathione disulphide [GSSG]) by cell has been used as an indicator of oxidant stress.\textsuperscript{98,99}

Catalase can eliminate $\text{H}_2\text{O}_2$ produced in the cell during the metabolic reactions. It has been reported that catalase is a less effective scavenger of $\text{H}_2\text{O}_2$ at low concentrations.\textsuperscript{99} Reduced glutathione is present in all cells and is responsible for maintaining intracellular reducing conditions. Glutathione plays a major role in drug detoxification, peroxide metabolism and amino acid transport.\textsuperscript{100} Naturally occurring antioxidants like α-tocopherol can also be useful in oxidative stress. The α-tocopherol is a fat-soluble vitamin present in the cell membrane and acts as a free radical scavenger by breaking peroxyl radical chain reaction of membrane lipids.\textsuperscript{101} An hydroxyl (-OH) group is attached to the hydrophobic structure of α-tocopherol whose hydrogen atom can be removed. Therefore, when peroxyl and alkoxyl radicals are generated during lipid peroxidation they combine with α-tocopherol instead of fatty acids and this action terminates the chain reaction.

\[
\begin{align*}
\text{O}_2^- \\
\text{t} & \text{ocopherol} \quad \text{OH}^+ \quad \text{C}^- \quad \rightarrow \quad \text{O}_2\text{H} \\
\text{t} & \text{ocopherol} \quad \text{O}^- \quad \text{+} \quad \text{C}^- 
\end{align*}
\]

In this reaction the α-tocopherol is converted into new radical, tocopherol-O which is very poorly reactive, being unable to attack adjacent fatty acid side chains. MacKay suggested that the tocopherol radical can migrate to the...
membrane surface and is converted back to \( \alpha \) tocopherol by reaction with ascorbic acid.\(^{102}\) It is known since long that vitamin E deficiency causes myocardial lesions in livestock.\(^{103,104}\) In vitamin E deficient rats, the free radical production increases during postischaemic reoxygenation.\(^{105}\) No adverse effects have been demonstrated due to chronic hypervitaminosis. Hypervitaminosis is not associated with mutagenic, carcinogenic or teratogenic effects.\(^{106}\) In porcine model of coronary artery ligation, intravenous or intra-arterial infusion of vitamin E along with vitamin C reduces the infarct size.\(^{107}\) Further, vitamin E can alter arachidonic acid metabolism by inhibiting phospholipase A\(_1\), A\(_2\) and thromboxane B\(_2\) synthesis and it also enhances prostaglandin I\(_2\) synthesis.\(^{108 -110}\) Many investigators have shown the role of vitamin E as a cardio protective agent\(^{111 -115}\) while others question its effect.\(^{116,117}\)

Antioxidant, N-2-mercapto propionyl glycine scavenges the hydroxyl radical and significantly reduces the infarct size in animals subjected to 90 minutes of ischaemia and 6 hours of reperfusion.\(^{118}\) N-acetylcysteine (NAC) is another antioxidant which is has low molecular weight and is precursor of glutathione.\(^{119}\) It has been postulated that N-acetylcysteine acts on many sites in production and propagation of free radicals.\(^{120}\) N-acetylcysteine replenishes glutathione stores, increases superoxide dismutase activity, scavenges hydroxyl free radicals and interferes with autocatalytic lipid peroxidation.\(^{121}\) Therefore, use of anti-free radical
compounds like superoxide dismutase, catalase, N-2-mercaptopropionyl glycine, dimethyl thiourea, N-acetylcysteine and xanthine oxidase inhibitors like allopurinol, oxypurinol have consistently demonstrated improved contractile function in the animal models where duration of ischaemia was less than two hours. 119,121-126

Burst of oxygen free radicals within first few minutes of reperfusion have been demonstrated in isolated hearts subjected to global ischaemia by several investigators. 127-130 Oxygen free radical burst peaks at 2 minutes after reflow and its production continues upto 3 hours following reperfusion in the dogs subjected to 15 minutes of coronary artery ligation and reperfusion. 131 These above studies have provided direct evidence of oxygen free radical generation in the model of ischaemia followed by reperfusion. But the techniques of electron paramagnetic resonance spectroscopy has some limitations. Certain electron paramagnetic resonance spectra which were thought to be due to oxygen free radicals were infact due to freezing and mechanical manipulation of the tissue. 132 However, use of spin traps and chemiluminescence have confirmed the generation of oxygen free radical after ischaemia and reperfusion. 133 Are oxygen free radicals directly toxic to the heart? The answer has come mainly from the studies carried out to assess the damage to the myocardium both in vitro and in vivo. Xanthine oxidase plus a purine base plus a transition metal, catalyst, auto oxidation of epinephrine to adrenochrome or stimulation of
neutrophils \textsuperscript{134,135} generate free radicals \textit{in vitro}. Oxygen free radical generated by a combination of purine plus xanthine oxidase causes depression of the function of isolated papillary muscles. \textsuperscript{136,137} In isolated perfused rat heart preparation, superoxide radicals depress left ventricular pressure, deplete high energy phosphate levels and causes cellular oedema. \textsuperscript{138} Superoxide anions produced by activated neutrophils significantly depress calcium uptake and reduce Ca\textsuperscript{2+} stimulated Mg\textsuperscript{2+} dependent ATPase activity of isolated sarcoplasmic reticulum. \textsuperscript{139}

In anesthetized canine models, infusion of xanthine oxidase and purine with iron loaded transferin administered through coronary veins results in left ventricular wall motion abnormalities. \textsuperscript{140} Higher doses of oxygen free radicals in solution when injected into the aortic roots in rats resulted in contraction band and oedema. \textsuperscript{141} Oxygen free radicals damage myocardial cells both \textit{in vitro} and \textit{in vivo}. Indirect evidences from \textit{in vitro} studies suggest that free radical scavengers, antioxidants, quenching agents and iron chelators can blunt myocardial injury and dysfunction caused by ischaemia or hypoxia and reoxygenation. \textsuperscript{142,143} Some investigators have implicated superoxide anion as the main injury causing radical. \textsuperscript{144,145} While others are of opinion that hydroxyl radical or H\textsubscript{2}O\textsubscript{2} may be main source \textsuperscript{136-139} yet others feel that all these collectively cause of tissue injury. \textsuperscript{143,145,146}
Oxygen free radical have been implicated in reperfusion injt.

These were detected at the time of reperfusion by using spin traps and chemiluminescence assay. According to Jenning et al. "the reperfusion injury refers to cell death induced by reperfusion in contradistinction to cell death induced by preceeding episode of ischaemia". Question of lethal reperfusion injuries has been mainly addressed by use of antioxidants infused at the time of reperfusion. Jolly et. al. in 1984 were the first to examine the issue of oxygen free radical induced reperfusion injury in canine models following 90 minutes of ischaemia and 24 hours of reperfusion. Superoxide dismutase and catalase were started 15 minutes before reflow and continued for 45 minutes. Reduction in infarct size in comparison to control group was observed showing that without intervention, reperfusion might have resulted in larger infarcts.

A number of studies have been carried out to investigate the effect of free radical scavengers on myocardial infarct size following ischaemia and reperfusion. The important ones are summarized below in tabular form.

<table>
<thead>
<tr>
<th>Author</th>
<th>Duration of ischaemia and reperfusion</th>
<th>Scavenger used</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambrosio et. al.</td>
<td>90 min occlusion followed by 48h reperfusion</td>
<td>SOD at reperfusion then infused</td>
<td>Infarct size reduced</td>
</tr>
</tbody>
</table>

138, 140
<table>
<thead>
<tr>
<th>Study</th>
<th>Occlusion Time</th>
<th>Reperfusion Time</th>
<th>Treatment Details</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chambers et. al.</td>
<td>60 min.</td>
<td>4 h.</td>
<td>Occlusion or SOD on occlusion and reperfusion.</td>
<td>Infarct size reduced</td>
</tr>
<tr>
<td>Jolly et. al.</td>
<td>90 min.</td>
<td>Gradual release</td>
<td>24 h reperfusion</td>
<td>Infarct size reduced</td>
</tr>
<tr>
<td>Mitsos et. al.</td>
<td>90 min.</td>
<td>6 h.</td>
<td>Reperfusion MPG 15 min before ligation &amp; neutrophil antiserum</td>
<td>MPG as well as neutrophil antiserum reduced infarct size</td>
</tr>
<tr>
<td>Werns et. al.</td>
<td>90 min.</td>
<td>6 h.</td>
<td>SOD 15 min. before &amp; 15 min. after coronary occlusion.</td>
<td>Infarct size reduced</td>
</tr>
<tr>
<td>Werns et. al.</td>
<td>90 min.</td>
<td>6 h.</td>
<td>Allopurinol before occlusion, Allopurinol and SOD 15 min. before reperfusion</td>
<td>Allopurinol reduced infarct size. No effect of SOD</td>
</tr>
<tr>
<td>Uraizee et. al.</td>
<td>40 min.</td>
<td>4 days</td>
<td>Reperfusion. SOD, 25 min. before reperfusion</td>
<td>Infarct size unchanged</td>
</tr>
<tr>
<td>Richard et. al.</td>
<td>90 min.</td>
<td>4 days</td>
<td>SOD + catalase 15 min. before occlusion and SOD, 35 min. thereafter oxypurinol</td>
<td>Infarct size unchanged</td>
</tr>
<tr>
<td>Reimer and Jennings</td>
<td>40 min.</td>
<td>4 days</td>
<td>Allopurinol 35 min. before occlusion continued for 40 min. reperfusion</td>
<td>No effect on histological infarct size</td>
</tr>
<tr>
<td>Przyklenk and Kloner</td>
<td>2 hours</td>
<td>4 h.</td>
<td>Reperfusion SOD and Catalase at reperfusion thereafter 4 hours</td>
<td>No effect on infarct size</td>
</tr>
<tr>
<td>Patel et. al.</td>
<td>2 hour</td>
<td>4 or 48 hours</td>
<td>Human SOD, 2 min before reflow and 45 min. thereafter</td>
<td>No effect on infarct size</td>
</tr>
<tr>
<td>Gallagher et. al.</td>
<td>Closed chest</td>
<td>3 h.</td>
<td>Conscious dogs, 1 day reperfusion. SOD, 15 min. before reflow.</td>
<td>No effect on infarct size</td>
</tr>
</tbody>
</table>
Superoxide radicals (\(O_2^-\)) can also cause vasoconstriction which may produce deleterious effects in some clinical conditions. \(O_2^-\) radical reacts with nitric oxide (NO), and a free radical produced by vascular endothelial cells forms peroxynitrite.\(^{158}\) Nitric Oxide acts on smooth muscle cells in the vessel wall to produce relaxation and is the active component of amyl nitrite, glyceryl trinitrite and other nitrovasodilators.\(^{159}\) Nitric Oxide is synthesised from L-arginine by vascular endothelium and has biological properties of endothelium derived relaxing factor (EDRF).\(^{160-162}\) It acts by stimulating guanlylate cyclase and has a half life of few seconds.\(^{163}\) Endothelium derived nitric oxide causes vasodilation and inhibits platelet aggregation. In experimental conditions, acidified sodium nitrite (NaNO\(_2\)) forms nitric oxide at a pH of 2.\(^{157,164}\) In open chest anesthлизed cats,
acidified sodium nitrite in combination with human superoxide dismutase exerted synergistic action in cardio- protection following ischaemia.  

The while there are many positive studies there are as many negative findings which have failed to document significant beneficial effects of oxygen free radicals. How can the discrepancies among these various studies be explained? The cause for these diversities are unknown. Therefore, at present there is no clear consensus on whether oxygen free radicals given at the time of re-flow are capable of reducing myocardial infarct size. There is need to develop a "cocktail" of scavengers which can eliminate all the species of toxic free radicals.

Possibility of a drug which can alter platelet reactivity to be useful as an antithrombotic agent was reported initially in 1967 by Weiss and Aledort. Subsequent controlled clinical studies demonstrated that aspirin (Acetylsalicylic Acid) can reduce morbidity and mortality in a variety of thrombosis mediated clinical syndromes.

The most important determinant of short and long-term morbidity and mortality after acute myocardial infarction is the extent of myocardium irreversibly damaged by ischaemic injury. Occlusive coronary artery thrombus is often present in the patients. Pioneering studies were performed by Fletcher, Sherry and their colleagues in 1957 and 1959 to induce thrombolysis in man. Since then, coronary reperfusion by thrombolytic
therapy, percutaneous transluminal angioplasty and bypass surgery have emerged as most fundamental strategies in the management of acute ischaemic myocardial infarction. The thrombolytic drugs like streptokinase, urokinase, tissue type plasminogen activator and anisoylated plasminogen streptokinase activator complex (APSAL) have all been used by intravenous route to facilitate reperfusion in the patients. Streptokinase (SK) and APSAC reduce circulating fibrinogen level which in turn reduces plasma viscosity and blood pressure. In addition, circulation of anticoagulant fibrin degradation products may prevent re-thrombosis. During the last two decades thirty one randomized trials involving 41,000 patients with AMI have compared the effect of steptokinase with standard treatment. These analyses revealed that the treatment with streptokinase reduced mortality by 24%. Two other very large trials GISSI and ISIS-2 demonstrated significant reduction in mortality. The long term followup of the patients of above two trials demonstrated that the short term reduction in mortality was maintained for atleast one to two years. The anglo-Scandinavian study of Early thrombolysis (ASSET) studied the effect intravenous t-pA or placebo given within 5 hours of chest pain. All patients received intravenous heparin for 24 hours. In this study, t-PA reduced mortality by 26%. The available results from many large trials must be interpreted cautiously and should not be used to assess the relative efficacy of the agent because the results of the trials might be
influenced by differences in patient selection, timing of treatment and co-interventions.

Therefore, it is evident from the literature that even thrombolytic trial studies might also be influenced by many other factors. Low doses of antiplatelet drugs have shown promising results. The concept of reperfusion injury still remains to be proven.

Here you should state the specific hypotheses you are studying and what your primary and secondary endpoints are.
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