Chapter - VI

SUMMARY AND CONCLUSIONS
EXPERIMENTAL MODEL

1. SURVIVAL AND MORTALITY RATE

Incidence, timing and cause of death in the different experimental groups revealed 50% mortality in untreated and acidified NaNO₂ treated groups. The main cause of death was ventricular fibrillation which developed within minutes after reperfusion. Overall survival rate was 87.14%.

2. INFARCT SIZE

a. Area of necrosis percent in left ventricular weight, percent area of necrosis in the area at risk and viability of ischaemic myocardium was significantly less (p < 0.001) in Gr. V of animals which received combined infusion of NAC and SOD in comparison with untreated group (Gr. II). Size of the infarct in Gr III (NAC treated) and Gr. IV (SOD treated) was not significantly different compared to the untreated group.

b. Infusion of N- mercaptopropionyl glycine at the time of reperfusion decreased infarct size significantly when compared with untreated group.

c. Treatment with acidified NaNO₂ had no effect on infarct size. In the presence of acidified NaNO₂, NAC & SOD decreased the infarct size. Marked reduction in infarct size was observed when combination of acidified NaNO₂, SOD & NAC were given at the time of reperfusion.

d. Pre-treatment with α - tocopherol and aspirin decreased infarct size significantly in comparison with the untreated group.
3. **ST ELEVATION, HEART RATE, LEFT VENTRICULAR END DIASTOLIC PRESSURE, LEFT VENTRICULAR SYSTOLIC PRESSURE AND RATE PRESSURE PRODUCT**

   a) Rise in ST segment was observed immediately after coronary artery occlusion. ST elevation was highest after 90 minutes of occlusion. Although reperfusion in untreated group could reduce the ST elevation to some extent, all the other groups except Gr. III & VII showed marked decrease in ST elevation in comparison with untreated group.

   b) The heart rates were not statistically different in treated animals compared to the untreated group following ischaemia and reperfusion. Increase in left ventricular systolic pressure was observed only in Gr. V & VI following reperfusion.

   c) Left ventricular systolic pressure was low in aspirin pre-treated animals before coronary artery occlusion. No effect of ischaemia and reperfusion was observed on left ventricular systolic pressure in Gr. XI & XII.

   d) Left ventricular end diastolic pressure increased after 60 minutes of ischaemia and remained high in untreated group. Reperfusion did decrease the LVEDP but it was not statistically significant in comparison with the LVEDP before reperfusion. In other groups,
LVEDP was significantly low in comparison with their corresponding LVEDP before reperfusion.

e) Rate pressure product remained unchanged in almost all the groups after 90 minutes of ischaemia and 4 hours of reperfusion except in Gr XII where it was less in comparison with untreated control.

f) Reperfusion in untreated control group produced temporary contractile dysfunction. Reperfusion with antioxidants and pre-treatment either with aspirin or α tocopherol reduced the duration of contractile dysfunction.

4. LIPID PEROXIDATION

Lipid peroxidation in RBC, serum and necrosed tissue was high after 90 minutes of ischaemia. In untreated groups reperfusion further increased the lipid peroxidation. Acidified NaNO₂ treatment had no effect on lipid peroxidation in comparison with untreated group after ischaemia and reperfusion. However, the lipid peroxidation following different therapeutic interventions was less in all other groups in comparison with the untreated group.

5. SUPEROXIDE DISMUTASE ACTIVITY

a) In untreated control group SOD activity in RBC decreased after 60 minutes of ischaemia. Reperfusion resulted in further decrease of SOD activity. After one, two, three and four hours of reperfusion, SOD activity
was not significantly different compared to the activity after 1 hour of reperfusion. SOD concentration in Gr. III, IV, V, VI, VII, VIII, IX, X, XI & XII did not show any significant change in comparison with the untreated group after reperfusion.

b) Tissue superoxide dismutase concentration was significantly low after 90 minutes of ischaemia in comparison with SOD activity of normal cardiac tissue. Enzymatic activity further decreased after 4 hours of reperfusion in untreated group. Tissue SOD contents were higher in Gr. V, VI, X, XI & XII in comparison with untreated group following 4 hours of reperfusion.

6. GLUTATHIONE LEVELS:

a) A reduction in blood glutathione levels was observed after 60 minutes of ischaemia in untreated group. Glutathione levels after 1 hour of reperfusion were low in comparison with glutathione concentration after 90 minutes of ischaemia. Blood glutathione levels in Gr. III, Gr. V, Gr. VI, Gr. VIII, Gr. IX, Gr. X, Gr. XI & Gr. XII were higher than untreated group following reperfusion.

b) Tissue glutathione levels decreased following 90 minutes of ischaemia. Reperfusion further decreased the glutathione concentration. Only in Gr X, tissue glutathione concentration was significantly higher after 4 hours of reperfusion.
7. HISTOPATHOLOGICAL CHANGES:

a) Ninety minutes of ischaemia showed cell oedema, fatty infiltration, haemorrhagic changes without any evidence of necrosis either grossly or microscopically.

b) Reperfusion resulted in contraction band necrosis stretching across the myocardium in untreated as well as in other experimental groups.

RESULTS IN PATIENTS SUFFERING FROM AMI

a) CK-MB level was high on the day of admission. It started decreasing after third day and reached normal on seventh day.

b) SGOT level was high on the day of admission and was normal by seventh day.

c) SGPT levels were high on third day.

d) Blood sugar level was high on the day of admission and blood sugar levels reached to normal values on the fifth day.

e) Lipid peroxidation in RBC and in serum were high on the day of admission in comparison with control. Highest lipid peroxidation was observed on third day following AMI.
f) Superoxide dismutase concentration was low on the day of admission and recovery was observed on fifth day onwards following AMI.

g) Blood glutathione levels were low on the day of admission and showed recovery after six days.

h) Serum T₃ and TSH were not significantly different than normal on the day of admission, 3rd, 5th and on 7th day. Serum T₄ levels were significantly low only on fifth day following AMI.

i) Plasma cortisol and serum prolactin (PRL) levels were significantly high on the day of admission. No significant differences between cortisol and PRL levels of normal individuals and AMI patients were observed on fifth day.

j) Serum testosterone concentrations were low in AMI patients on the day of admission and there was no significant difference between values on the day of admission and discharge. Serum estradiol levels were high on the day of admission in comparison with normal levels. Even after ten days of AMI, the serum estradiol levels were high when compared with the normal estradiol levels.
CONCLUSIONS

There is no doubt that reperfusion can increase the severity of injury. It has been argued that rather than causing injury de novo, reperfusion merely accelerates the injury following ischaemia. Existence of lethal reperfusion injury can be proved by showing that cells were viable before reperfusion. The present investigation shows that 90 minutes of ischaemia in dogs followed by reperfusion results in necrosis which could not be demonstrated before reperfusion.

Combination of antioxidants in contrast to individual antioxidants infused at the time of reperfusion prevented significant amount of ischaemic tissue from becoming necrotic whether assessed as a percentage of the mass at risk or as a percentage of total left ventricular weight. The only exception was N-mercaptopropionyl glycine which was individually effective. These observations strongly suggest the involvement of toxic oxygen free radicals in reperfusion injury. Further, it has also been demonstrated that a "cocktail" of scavengers offer much better protection as it can block the release of oxygen free radicals from multiple sources. Indirect evidences presented in the present work demonstrate the production of oxygen free radicals in man following myocardial infarction.
Therefore, these results highlight the importance of oxidant mediated cell injury following ischaemia and reperfusion in animals. In addition, the present study also demonstrates that in man oxygen free radicals are produced during the process of myocardial infarction.

Oxidative stress can occur in most disease processes (if not all). The precise role of oxygen free radicals in the pathology of disease is not yet fully understood, despite extensive research. Beneficial effects of free radical scavenging agents, when used with thrombolytics as an adjuvant therapy, remain to be tested. Indeed, the future may witness such new strategies for the effective management of oxidant induced myocardial injury. Whether large scale controlled clinical trials for free radical scavengers should be encouraged may be debatable; only then any relevance of antioxidants in the clinical arena could be established.