Summary
SUMMARY AND CONCLUSION

The anthracycline antibiotic ADR is an important antineoplastic agent because of its high antitumor efficacy in both hematological as well as in solid malignancies. Despite its frequent use, the clinical utility of ADR is associated with dose-limiting cardiotoxicity. The mechanisms of ADR induced cardiotoxicity include (a) the formation of free reactive oxygen radicals (b) direct DNA damage and/or interference with DNA repair (c) induction of immune reactions involving antigen presenting cells in the heart.

Any hypothesis that seeks to explain the cardiac toxicity of the anthracycline must also account for the alterations in cardiac biochemistry that occur after doxorubicin exposure. Under normal conditions, the primary antioxidant status is lower in mammalian myocardium cells, when compared to other tissues. Recent studies suggest that ADR induced cardiotoxicity results from a combination of profound oxidative stress and unusual sensitivity of mammalian heart to cytotoxic effect of the oxidative stress.

Several researchers have offered the seemingly paradoxical conclusion that the appropriate administration of antioxidants during chemotherapy may provide relief from the adverse side effects of the latter.

The effect of DL α-lipoic acid on adriamycin induced cardiotoxicity has been studied under the following heads.

1. Chronic studies
2. Acute studies
3. Glutathione depletion studies
4. Macromolecular damages and Apoptotic studies
6.1 Chronic Studies

The activities of cardiac marker enzymes like LDH, CPK, ALT and AST were found to be decreased in heart tissue. Alterations in myocardial sarcolemmal permeability are enough to affect the leakage of these enzymes into blood stream. Thus adriamycin administered rats showed an increase in the above enzymes in the blood. High levels of homocysteine were noted in adriamycin administered animals. Administration of lipoic acid to adriamycin induced animals reverted the activities of these marker enzymes to near normal levels. Histological examination of heart tissue also proved the maintenance, their contractile function and muscle flexibility.

Adriamycin increases oxidative stress on the cell by increasing cardiac tissue lipid peroxidation, by decreasing the status of both enzymic (SOD, CAT, GPX, GST, G6PD and GR) and non-enzymic (GSH, vit C and vit E) antioxidant. The lipoate treatment helped in maintaining the cellular redox status of the animals thereby increasing the efficiency of antioxidant defense status.

Adriamycin causes membrane deterioration in lysosomes resulting in leakage of enzymes from the enclosed sacs, leading to decrease in the activities of NAG, $\beta$-glu, $\beta$-gal and cat-D in myocardial cells. Lipoate supplementation restored the activities of lysosomal enzymes. Lipoate has the capacity of maintaining the membrane integrity replenishing endogenous sulphhydryl groups.

The decline in the activities of mitochondrial enzymes (ICD, MDH, SDH, $\alpha$-KD and aconitase) and mitochondrial complex activities were found to be inhibited in adriamycin induced animals. Mitochondrial respiration was assayed using different substrates namely succinate, malate, glutamate and pyruvate and malate. The substrates levels were found to be significantly altered in adriamycin administered animals. Lipoate supplementation increased
the activities of these enzymes and mitochondrial complex activities. The ATP and RCR were found to be higher in lipoate treated animals. This effect of lipoate may be attributed to its effect on improving the electron transport and subsequent energy production. It is also shown to be involved in maintaining the membrane fluidity and intactness by increasing the reactive –SH groups.

Membrane damage is a basic feature of adriamycin toxicity. The membrane bound ATPases were decreased indicating the severity of the toxicity. The levels of electrolytes were noted to assess the membrane potential. In adriamycin administered animals the cardiac calcium and sodium levels were found to be increased. Magnesium content was found to be decreased and there was no change in potassium levels. The activities were restored to near normal values upon lipoate supplementation, indicating its membrane stabilizing action.

The reactive nitrogen species were significantly increased in adriamycin induced animals. Supplementation of lipoate controlled the above changes.

The activity of lipid metabolizing enzyme CES was found to be increased and the activity of LPL was increased in adriamycin treated animals. The HDLc and PLCAT levels were decreased in adriamycin treated animals. The VLDLc and LDLc were increased in adriamycin treated groups. The plasma and cardiac lipid profiles were found to be increased in adriamycin administered animals. Lipoic acid and lovastatin, lipid lowering drug used in this study had overall beneficial effects on adriamycin induced lipid changes in the plasma as well as the heart. Lipoic acid is better suited for adriamycin cardiotoxicity in that it affords maximum cytoprotection and substantial protection against adriamycin induced secondary hyperlipidemia when compared with lovastatin.
6.2 Acute Studies

During acute adriamycin toxicity, the tissue marker enzymes were decreased due to membrane leakage. The kinetic profile of each myocardial enzyme namely LDH, CPK and AST were analyzed every six hours. The serum marker LDH, CPK and AST showed peak activity at 24\textsuperscript{th}, 12\textsuperscript{th} and 30\textsuperscript{th} hour respectively. The lipid peroxidation and antioxidant enzymes namely SOD, CAT, GPX and GSH were found to be decreased in adriamycin administered animals. Lipoic acid restored the activities of LDH, CPK and AST and also restored the antioxidant status. Thus lipoate supplementation helped in maintaining the redox status of the myocardium during acute toxic conditions.

6.3 Glutathione Depletion Studies

BSO was used to deplete the tissue GSH levels. This was done with an aim to assess the effect of lipoate, both in the presence and absence of glutathione. The salient feature observed was that the 5-membered cyclic disulphide exacerbated the antioxidant defense armory and established its effectiveness in thwarting the lipid peroxidative processes, with and without GSH. This elucidates the fact that high intracellular lipoate concentration even in the absence of GSH synthesis can provide specific protection against adriamycin toxicity.

6.4 Macromolecular Damage and Apoptosis

The levels of protein carbonyls, DNA strand breaks and DNA protein cross-links were found to be elevated in adriamycin induced cardio toxic animals. Supplementation of lipoate brought the levels of this macromolecular damage to control levels suggesting that the macro molecular damages induced by adriamycin is mainly mediated through free radicals and that α-lipoate display a protective role by inhibiting free radical production. Apoptosis in adriamycin toxicity was studied with reference to DNA fragmentation, TNF-α.
activity and NF-κB immuno histochemistry. DNA fragmentation which was seen in adriamycin induced groups, with respect to TNF-α activity and NF-κB induced apoptosis was minimised or prevented on pretreatment with α-lipoate thereby indicating the anti-apoptotic effect of the drug tested.

6.5 Conclusion

The above experimental results obtained in-terms of both biochemical and some molecular changes, were also confirmed by histological and immunohistochemical studies indicating that antioxidant lipoate supplementation could be used in combination with adriamycin to provide protection against oxidative stress without attenuating the clinical efficacy of adriamycin.
Adriamycin toxicity

Free radical production

Semi quinone free radical formation

Macromolecular damage

Proteins
- Enzyme Inactivation
- Loss of membrane stability
- Formation of protein carbonyls

Lipids
- Binds to membrane proteins
- Alters the fluidity and structure
- ↑sed Lipid peroxidation

DNA
- Binds to nucleotides
- Mainly to GC base pairs
- Alters the DNA structure

Subcellular dysfunction

Cytoplasm
- Cytoplasmic vacuolization

Mitochondria
- ↓sed TCA cycle enzymes
- ↓sed ETC activities
- ↓sed RCR
- ↓sed ATP synthesis

Sarcoplasmic reticulam
- Alters Ca²⁺ mobilization

Lysosomes
- ↑sed LPO
- ↓sed enzyme activities

Nucleus
- ↑ DNA topoisomerase II
- Induces endonucleosome
- ↑sed expression NF-kB

Congestive cardiac failure, Left ventricle dysfunction
- Cardiomyopathy and cardiac dilatation

Site of action - LA