REVIEW OF LITERATURE
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The dose limiting toxicities of anticancer drugs are usually confined to tissues with a high mitotic rate such as bone marrow, gastrointestinal mucosa, and hair follicles. The anthracycline antibiotics, daunomycin and doxorubicin (Adriamycin) are exceptions to this rule. Other antineoplastic agents have been reported to cause mild chest pain and E.C.G. changes (5 FU) or acute mild pericarditis when used in very large single doses (cyclophosphamide). Only the anthracyclines consistently induce alteration in cardiac function which limits the safe cumulative dose of drug which can be given. Both the agents exhibit antineoplastic activity against a wide variety of tumours but unfortunately there is development of acute and chronic cardiac damage often interfering with the full therapeutic potential of the drug. The acute form of cardiac toxicity is generally mild and manifests by arrhythmias and electrocardiographic changes. This contrasts with severe, cumulative dose dependent cardiomyopathy following chronic administration of the drug.

In 1969, Herman et al. at the National Cancer Institute, Bethesda, Maryland carried out experiments on three rodent species, rat, guinea pig and hamsters to study the comparative cardiac toxicity of Daunomycin. The authors
knowing that clinically, total doses greater than 25 mg/kg have been associated with the development of cardiopulmonary symptoms such as tachycardia, with or without arrhythmia, gallop rhythm, congestive heart failure, tachypnea and cases of dyspnea. The studies were initiated to determine whether daunomycin could induce similar or other types of cardiac toxicity in animals. Three male rats (250-300 gms), 5 male guineapigs (450 gms) and 50 male golden hamsters (90-130gms) were given daunomycin in the dose of 50 mg/kg. The cardiovascular system of the rat was relatively resistant to daunomycin during the acute phase of the study. A total of 200-300 mg/kg produced slight decreases in blood pressure and heart rate and some variable changes in the amplitude of E.C.G. waves. In the guineapigs, a total of 75-175 mg/kg daunomycin was given to these animals. After most injections a transient hypotensive effect was seen. Daunomycin had a striking respiratory depressant effect on all animals. Periods of apnoea of 2-4 minutes duration occurred following certain doses. Usually any changes in the E.C.G. were secondary to these periods of apnoea. In case of hamsters, in contrast to the rat and guineapig, a slight to moderate pressor response was usually observed during infusion with daunomycin. In addition, alterations in the E.C.G. were usually seen within 10-40 seconds followed by a definite cardiac arrhythmia 20-90
seconds later. The study concludes that cardiac toxicity induced by daunomycin in the hamsters differs from that observed clinically because it is acute and appears predominately as an arrhythmia.

In the year 1973, Chalcroft et al, studied fine structural changes in rat myocardium induced by daunorubicin. Rats were injected intravenously with daunorubicin in the dose of 25 mg/kg body weight to determine the morphologic basis of its cardiotoxic effects. Although prominent changes were not observed by light microscopy in either atrial or ventricular myocardium, significant fine structural changes were seen by electron microscopy in both. One day after injection, daunorubicin treated rats showed membranous whorls inside the mitochondria and in relation to mitochondrial surfaces. While the nucleoli were of normal shape, the nucleolonema appeared more prominent in same muscle cells. Atrial myocardial cells after the same interval showed sacculation of some mitochondrial cristae. Three days after the treatment mitochondrial swelling, decreased matrix density and loss of cristae were evident in some cardiac muscle cells. Five days after the drug infusion both atrial and ventricular muscle cells contained large numbers of degenerating mitochondria. The study shows that striking abnormalities affected progressively more mitochondria of both atrial
and ventricular muscle cells. In the only comparable study of fine structural changes of daunorubicin on myocardium, Liss and Cotton (1971) briefly describe similar intramitochondrial vacuolation, and also Z line distortion and sarc-comeric disorganization but do not refer to mitochondrial membrane changes or capillary endothelial swelling.

Rogers Jaenke et al (1974) worked for the anthra- cycline antibiotic induced cardiomyopathy in rabbits. The authors found delayed cardiomyopathy similar to that observed in cancer patients during chemotherapy with the anthracycline antibiotics, daunomycin and adriamycin in the rabbit. In chronically treated rabbits, this syndrome was characterized by congestive myocardial failure and focal to disseminated myofibre degeneration and necrosis. Alterations in myocardial cells were characterized by expansion of intracellular membrane bound compartments, the selective isolation and degradation of degenerating mitochondria accompanied by myofibrillar breakdown and eventual complete myolysis and fibrosis. A group of 12 male and 12 female Newzealand white rabbits weighing 2.2 to 3.7 kgs received daunomycin or adriamycin in the doses ranging from 2.4 to 3.0 mg/kg/week. The animals were examined at intervals ranging from 3 to 18 weeks. The distribution of animals and the summary of the relationship of myocardial lesions to total drug received and time administered are briefly given as follows :-
<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of Rabbits</th>
<th>Days treated</th>
<th>Total drug received (mg/mg)</th>
<th>Myocardial alterations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daunomycin</td>
<td>5</td>
<td>28</td>
<td>100-130</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>61-71</td>
<td>200-400</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>96</td>
<td>350-450</td>
<td>2/3 focal myofibre degeneration only.</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>112-126</td>
<td>375-600</td>
<td>1/4 focal myofibre degeneration only.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/2 severe myofibre degeneration and necrosis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1/2 rabbits with degeneration and necrosis, congestive myocardial failure.</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>4</td>
<td>23-30</td>
<td>70-130</td>
<td>Negative</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2/3 rabbits with degeneration and necrosis, congestive myocardial failure.</td>
</tr>
</tbody>
</table>

The ultrastructural study indicates that myocardial degeneration and necrosis were characterized by extensive subcellular alterations following the chronic administration of daunomycin or adriamycin. Although lesions were frequently focal in distribution, some experimental animals developed congestive myocardial failure, indicating that the myocardial pathology could be progressive and fatal in rabbit.
Mettler et al (1977) conducted experiments to study the adriamycin induced cardiotoxicity (Cardiomyopathy and congestive heart failure) in rats. Adriamycin (ADR) was administered to rats at doses of 1 to 2 mg/kg/week for 10 to 14 weeks. The majority of adriamycin treated rats developed cardiomyopathy from 3 to 23 weeks after the last injection. Forty to seventy percent of these rats with cardiomyopathy had gross evidence of congestive heart failure (pleural effusions, ascitis, hepatomegaly, cardiomegaly). Histopathological evaluation of hearts from rats revealed varying degrees of myocardial alterations. The most consistent and predominant finding was marked vacuolization of myocytes, especially in subepicardial and subendocardial regions in addition to perivascular locations. Although vacuolar lesions were observed frequently in ventricles and septa, several rats had severe vacuolation oftrial myocytes. In addition, vacuolar degeneration of ganglion cells was observed in two or three rats where atrial sections were found to include clusters of cardiac neurons. Three rats had focal atrial thrombosis evident histopathologically. Myocytolysis, interstitial edema, and mild fibrosis were evident in occasional areas of more severely affected rat hearts. The incidence and severity of the cardiomyopathy appeared to be dose dependent.
Ultrastructural evaluation of samples of myocardium from adriamycin treated rats revealed varying degrees of sarcoplasmic vacuolation. The vacuoles occurred predominantly in perinuclear position and consisted of distensions of the sarcoplasmic reticulum, the T-tubule system, and Golgi vesicles. In myocytes where vacuolation was severe, there was a concomitant reduction in the quantity of myofibres. A few scattered myocytes were found to contain fragmented sarcomeres with disruption of the characteristic parallel arrays of myofilaments. In general, mitochondria did not appear to be altered markedly, although several were swollen with disruption of cristae. Formation of myelin figures was not a predominant feature of mitochondrial alterations.

Olson et al (1977) at the Department of Veterinary Pathobiology, College of Veterinary Medicine, Theohio State University, Columbus, Ohio carried out an extensive experimental study to observe cardiotoxicity of adriamycin in rats along with biochemical and ultrastructural investigations. The toxicologic and cardiotoxic effects of single and divided high dosages of adriamycin given by intravenous injection were evaluated in 34 day old Newzealand Black Rats. Adriamycin at dosages of 20 mg per kg, 10 mg per kg x 2, 13 mg per kg, and 10 mg per kg produced fatality and
rapid weight loss followed by gradual weight gain in groups of rats that survived the 28 days observation period. Adriamycin at dosages of 5 mg per kg X 2 and 5 mg per kg produced no mortality but rats had attenuated weight gain compared to saline injected controls. The earliest myocardial fine structural alterations included swelling and degeneration of mitochondria and dilatation of sarcoplasmic reticulum at all dosages of adriamycin. More advanced myocardial lesions included separation of myofibrils and the fascial adherens of intercalated discs in rats 4 days after administration of 10 mg per kg of adriamycin.

The most severe lesions were observed in rats 8 days after receiving 10 mg per kg X 2 of adriamycin and included focal myocyte degeneration with increased electron density and contraction of the sarcomeres, nuclear pyknosis, and mitochondrial degeneration. Toxic dosages of adriamycin produced an acute, reversible hypocalcemia in rats. Significant increases of serum creatine phosphokinase and lactic dehydrogenase preceded the onset of highest mortality following adriamycin administration. Ventricular tissue calcium concentration was significantly increased in groups of rats receiving toxic dosages of adriamycin. This investigation demonstrates that the rat is sensitive to the cardiotoxic effects of single or divided high dosages of adriamycin.
The study shows that the rat is sensitive to the cardiotoxic effects of single or divided high dosages of adriamycin given by intravenous injection. Fatal dosages of adriamycin either produced acute weight loss or attenuation of weight gain, acute reversible hypocalcemia, and fine structural alterations compatible with severe myocardial damage. Significant increase of myocardial tissue calcium and serum enzymes (CPK, LDH) in association with myocardial damage were found to precede the onset of highest mortality rate. The decrease in mortality and concomitant increase in body weight gains with decreasing dosages of adriamycin suggest that the pathogenic mechanisms related to morbidity and mortality are both dose and time dependent. These effects also are dependent upon scheduling of adriamycin treatment, since dividing the total dosage into two boluses given at 48 hours intervals consistently results in prolongation of the interval from dosing until death. The debilitation and death associated with high dose adriamycin treatment suggests a multifactorial process including anorexia and wasting associated with gastrointestinal toxicity and bone marrow suppression. The rat therefore also appears to be sensitive, short term predictor for cardiotoxicity of these drugs in man.

Bristow et al (1978) collected data on all patients hospitalized at Stanford University Hospital between June, 30, 1976 and July 1, 1977 who had recently received their first
or second course of an anthracycline antibiotic (doxorubicin or daunorubicin). From these data eight patients were selected in whom (1) other precipitating causes of myocardial dysfunction could not be identified and (2) a similar type of cardiotoxic manifestations has been reported in animal models. Clinical observations revealed that of the eight patients identified as having early anthracycline cardiotoxicity, the first four patients presented with pericarditis and three patients had objective evidence of severe cardiac dysfunction. These patients were all relatively young and had no known history of cardiac disease, with the exception of one patient in whom pericarditis developed 24 hours after a single dose (60 mg/m²) of daunorubicin. In contrast four patients were elderly and three of them had evidence of previous myocardial dysfunction. All these four patients presented with left sided heart failure. In one patient electrocardiographic evidence of anterior wall myocardial infarction developed.

Histopathology was available in two of these patients which showed evidence of acute inflammatory process involving the pericardium and epimyocardium. Four patients had clinical and chest X-ray evidence of heart failure developing shortly (one to 10 days) after the administration of anthracycline. The most obvious finding was that in the
absence of risk factors. Anthracycline associated heart failure is extremely uncommon at doses less than 550 mg/m² and myocyte damage is usually minimal at doses less than 300 mg/m². The mean total dose given to their patients was 137 mg/m².

Starkebaum (1975) and Harrison (1976) have independently described a 41 year old woman in whom pericarditis, myocarditis, fatal pump failure and cardiac arrest developed nine days after treatment with daunorubicin given as three daily 70 mg/m² doses.

Ippoliti (1976) reported the development of cardiogenic shock refractory to therapy in a 56 year old man one week after he received 80 mg total dose of daunorubicin, the same investigators reported a case of apparent myocardial infarction 48 hours after the patient received 50 mg dose of daunorubicin. Ainger et al (1971) reported a case of fatal pump failure in a 12 year old child 12 days after completion of a 250 mg/m² course of daunorubicin therapy given over five days. Shortly before death, the patients electrocardiogram showed an anterior injury pattern.

Billingham, et al (1970) obtained percutaneous transvenous endomycardial biopsy from 60 patients aged (19-78 years) receiving adriamycin. The tissue obtained by endomycardial biopsy was usually not more than 3 mm in maximum dimensions. The rapidly fixed tissue provides excellent artifact free myocardium for conventional microscopy as well as optimal tissue for ultrastructural examination. In the earliest lesions, single cells with advanced degenerative changes could be seen isolated against a background of morphologically intact myocardium. The subendocardium and trabeculae represented a particularly prominent site of involvement in the early stages. Inflammatory infiltrate was not seen even in very severe lesions. Light microscopic changes included an increase in interstitial fibrosis from normal; however this change is dose dependent and not always present. There are two main types of myocyte injury which occur. The first is myofibrillar loss which is partial to begin with but which may become total. Myofibrillar lysis can also be recognized by light microscopy as smaller, shrunken cells with homogenous pale cytoplasm. In the second type of myocyte damage, the cell undergoes vacuolar degeneration. The earliest manifestation is distension of the sarcoplasmic reticulum which eventually swells and coalesce to form large membrane bound clear spaces in the cytoplasm. This change can also occur with preservation of the mitochondria and nucleus.
These lesions eventually progress until the death of the myocyte at which time the mitochondria do degenerate by swelling and cristolysis, myelin figures appear, and the nuclei become pyknotic and disintegrate. In their study, the endothelial cells of the capillaries and small arterioles, as well as the nerve endings, were found to be unaffected when compared with normal control biopsies, even if they were from patients who had received maximal dose of anthra-cycline. In addition their data showed neither depletion nor increase in intramyocyte glycogen in severe, anthracycline induced cardiotoxicity.

Bertazoll et al (1979) worked for quantitative experimental evaluation of adriamycin cardiotoxicity in the mouse. Histologic patterns of control and treated mice, showed different degrees of degenerative lesions. Both severity and extension of the lesions were dose related. At the lowest dose 2 mg/kg given twice a week for 5 week, the microscopic lesions were represented by characteristic microvacuolization of fibres, the injury involved few myocytes. When a total I/V dose of 40 mg/kg of adriamycin, as a single I/V dose of 4 mg/kg was given twice a week for 5 weeks, vacuolization, myolysis, myofiber atrophy and focal interstitial fibrosis were evident. These lesions were generally accompanied by a more extensive involvement of the myocardium.
In addition, swelling of single endocardial cells and thickening of the endocardium were observed in atria.

Taylor et al. (1982) tried to determine the very early changes in subcellular structure caused by adriamycin in an isolated, perfused, working heart. Eighteen rabbit hearts were perfused with oxygenated Krebs Ringer Bicarbonate buffer at 39°C containing either 1, 2, 4 or 8 μg per litre of adriamycin for periods of 30 to 150 minutes, in all twenty eight hearts were studied, there were 10 control hearts and 18 hearts exposed to adriamycin. In all hearts contractile function persisted throughout the experimental period, but anthracycline exposed hearts showed greater variability in function with time than did control hearts. Light microscopic findings were as follows (1) Separation of myofibres consistent with interstitial edema (2) Focal contraction band necrosis was present (3) Cytoplasmic and particularly perinuclear vacuolization with 17 of 18 hearts showing scattered vacuolization of myocardial cells and nine of 18 (50 percent) showing marked alterations with some vacuolization evident in virtually every low power (x10) field studied (4) No nuclear alterations were seen by light microscopy. Electron microscopic findings of hearts exposed to adriamycin consisted of disruption of sarcomeres with myocytolysis, cytoplasmic vacuolization,
and swelling of mitochondria with occasional presence of flocculent densities. A distinctive, central clumping of the nuclear material with clearing of chromatin along the nuclear membrane was evident in the nuclei of all cells from adriamycin exposed hearts. Other findings noted in small numbers of treated hearts were myelin bodies in one heart and prominent lipid granules in four hearts. In no instance was the central nuclear clumping with clearing of chromatin from the nuclear membrane occurring in the adriamycin hearts seen in the control hearts.

Van Vleet et al (1986) reported that gross lesions of cardiotoxicity in pigs, rabbits and dogs were (1) hydropericardium (2) hydrothorax (3) Ascitis. In occasional pigs, fibrinous pericarditis was present. The myocardium was pale, and hearts were dilated when compared with control hearts.

The three major lesions observed in myocytes were (1) Sarcoplasmic vacuolization (2) Myocytolysis (3) Hyaline necrosis.

Van Vleet et al (1980) studied cardiac disease induced by chronic adriamycin administration in dogs and an evaluation of Vitamin E and selenium as cardioprotectants. Chronic adriamycin intoxication was produced in three groups of beagle dogs by weekly intravenous injections (1 mg/kg body
weight) for 20 weeks (cumulative dose 400 mg/sq m). Group A (6 dogs) received adriamycin only; Group B (6 dogs) were given adriamycin and weekly doses of Vitamin E (17 mg/kg body weight) as alpha tochopherol acetate, and Group C (6 dogs) received adriamycin and weekly doses of Vitamin E as did Group B and selenium (0.06 mg/kg body weight as selenite). Each of the 18 dogs developed adriamycin induced cardiomyopathy, and death occurred in 11 dogs during weeks 17-20. Mortality was lowest in Group B (2 of 6), but no differences between groups were seen either in survival time of the dogs that died or in severity of cardiomyopathy. Cardiomyopathy was more severe in dogs that died than in survivors. Congestive heart failure with tranudation was present in 4 of 11 dogs that died. Cardiac histopathology was characterized by vacuolar degeneration of myocytes. Myocardial damage was most severe in the left ventricle and the ventricular septum, intermediate in the right ventricle and the left atrium, and least in the right atrium. Ultrastructural study showed that an early alteration in damaged myocytes was distension of sarcoplasmic reticulum to form sarcoplasmic vacuoles. Occasional damaged fibres had myofibrillar lysis and focal proliferation of sarcoplasmic reticulum. The lack of cardioprotection from Vitamin E and selenium supplementation fails to support the proposed role of lipoperoxidative damage in the development of chronic adriamycin induced cardiomyopathy.
Bristow et al (1981) studied anthracycline associated cardiac and renal damage in rabbits. The authors tested the hypothesis that anthracycline induced cardiac and renal damage is mediated by vaso active substances. A 1 minute exposure to 5 ug per ml of doxorubicin produced cardiac histamine release in isolated rabbit hearts. Under conditions in which histamine uptake and metabolism were impaired, the administration of doxorubicin, 2 mg/kg, over 1 minute was associated with elevations in arterial histamine and catecholamines. The chronic weekly administration of doxorubicin produced severe cardiac and renal damage. The administration of combined histamine and adrenergic blockade with diphenhydramine, cimetidine, phentolamine and propranolol pre and immediately post doxorubicin resulted in near total protection against doxorubicin mediated cardiac damage and prevented the majority of the renal lesions. The observations that (1) doxorubicin in a dose that produces a cardiomyopathy and causes cardiac histamine release and elevations in systemic levels of histamine and catecholamines and (2) pretreatment with agents that block histamine and catecholamine receptors prevents doxorubicin related chronic cardiac damage strongly suggests that at least part of anthracycline associated cardiac toxicity is mediated by vasoactive substances. Therefore, the evidence supports the original hypothesis that the acute and chronic cardiovascular effects of anthracyclines may be related to a common mechanism, the release and increased
Jackson et al (1981) carried out experiments to evaluate free radical effects and catecholamine alterations in Adriamycin cardiotoxicity. In acute studies, Adriamycin treated rabbits exhibited significantly increased levels (upto 50%) of total and reduced glutathione, unchanged levels of oxidized glutathione, and a slight decrease in the percentage of oxidized glutathione. In the chronic study (dose 1.1 mg/kg twice weekly per 10 weeks) levels of total and reduced glutathione were increased significantly by 23-36% after 9-12 and 16-20 injections without change in the percentage of oxidized glutathione peroxidase activity was not reduced significantly in any group of acute or chronic Adriamycin treated animals. Tests for lipid peroxidation (malondialdehyde and ethane production) were negative in acute studies. Myocardial catecholamine levels were unchanged in acute and chronic Adriamycin treated animals. Thus (1) the cardiac glutathione glutathione peroxidase system in activated with Adriamycin treatment at the onset of cellular damage, and (2) cellular damage progresses without further alteration of this system, loss of glutathione peroxidase activity, or reduction in myocardial catecholamines in rabbit models of Adriamycin cardiotoxicity. These findings suggest that free radical generation in the heart may contribute to Adriamycin cardiotoxicity, but that other factors probably play a more important role in pathogenesis of the myocardial damage.