DISCUSSION
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The anthracycline antibiotics are important antitumour agents. These drugs are sufficiently efficacious to allow for the expression of chronic toxicity. The chronic toxicity that appears to be unique to this class of compounds is a dose related cardiomyopathy (Bristow et al, 1978, Lefark et al, 1973) which limits the use of doxorubicin (Adriamycin) and other anthracyclines.

In addition to chronic toxicity, the anthracycline may also produce acute and subacute cardiovascular effects (Bristow et al, 1978). The acute effects were originally considered to be unrelated to the chronic, as they seemed to have no relationship to the subsequent development of heart failure. However, work carried out by Bristow et al, (1981) in which a dose of doxorubicin, that was associated with the development of a cardiomyopathy, produced acute haemodynamic effects that were entirely related to the release of vasoactive substances, and no acute cardiac effects of doxorubicin were noted in the presence of agents that antagonize histamine and catecholamines. Taylor (1982) in order to assess acute adriamycin cardiotoxicity perfused rabbit hearts with oxygenated krebs-Ringer-bicarbonate buffer at 39°C containing adriamycin for periods of 30 to 150 minutes to determine the very early changes in subcellular structures.
The study done by Olson et al (1977) revealed the subacute cardiotoxicity of adriamycin in rats. The cardiotoxicity was precipitated by single or divided high doses of adriamycin. 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, rapid weight loss, fine structural alterations compatible with severe myocardial damage, and significant increases of myocardial tissue calcium and serum enzymes (CPK, LDH).

Clinical and experimental investigations of adriamycin cardiotoxicity has been the delayed dose dependent degenerative cardiomyopathy. Bristow et al (1978); Singer et al (1978); Chalcroft et al (1973) and Herman et al (1969) have worked on acute toxicity, and have described fibrinous pericarditis myocarditis, left ventricular failure and arrhythmias as an early manifestation of adriamycin administration.

The purpose of the present investigation was to study the acute effects of adriamycin on rat heart and also to assess the possible mechanism of its cardiotoxicity.

In the present study, myocardial degenerative and necrotic changes were observed in rats following administration of doxorubicin. The lesions commonly consisted of focal areas of myofibre necrosis of variable grades and
inflammatory reaction with lymphocytes and occasional polymorphonuclear leucocytes around areas of myofibre necrosis and small blood vessels. When the cardiac lesions were more pronounced, larger number of myofibres were necrosed, occasionally in confluent areas. Pericarditis and valvulitis with or without involvement of the valve ring were seen in many animals. Cellular oedema with separation of myofibres were observed in the heart of few rats. In most of the cases blood vessels were dilated, few of them were congested and few ruptured with extravasation of blood. Interstitial haemorrhages were a consistent feature; there were numerous small multiple foci of interstitial haemorrhages in most of the animals with large confluent foci of haemorrhage seen at places in few of them.

It was of interest to observe the involvement of papillary muscle in some of the animals which attains special significance as this type of injury may account for early onset of cardiototoxic manifestations resulting in various types of cardiac dysfunctions viz arrhythmias, electrocardiographic changes and the haemodynamic derangements leading to heart failure.

In animals of Group A and B, who received doxorubicin either as a single i.p. i. of 10 mg/kg or as two i.p. i. on two consecutive days of 5 mg/kg each, the histopathological
changes were most marked at day 3 and 7 after the injection
and the lesions tended to regress in animals sacrificed on
day fourteen. Whereas in animals of Group C who received 10
i.p.i. of 1 mg/kg doxorubicin on 10 consecutive days, the
extent and severity of cardiac lesions was almost similar at
different period of sacrifice, indicating thereby that re-
peated doses of doxorubicin, even though small are sufficient
to cause persistent myocardial damage in these animals. Obvi-
ously these findings have direct significance in clinical
practice.

Maral et al (1967) using doxorubicin in doses of
1 mg/kg/day have also reported myocardial lesions consisting
of interstitial oedema, degeneration of myocardial fibres and
of 8-100 mg/kg single i.p.i. or multiple i.p.i. of 1-10 mg/kg
for 4 days have also observed the cardiac changes in the form
of dilated and congested myocardial capillaries, ischaemic
necrosis of cardiac fibres and slight neutrophilic infiltrations.
Lefark et al (1973) studied the cardiotoxic effects
of adriamycin in 399 patients treated for advanced carcinoma,
the postmortem examination of the hearts in two cases who
expired during the treatment with doxorubicin revealed de-
crease in the number of cardiac muscle cell and degeneration
of the myocardial cells.
Although the present study is restricted to the light microscopic changes. Chalcrot (1973) performed studies on fine structural effects of daunorubicin in both atrial and ventricular myocardium. He demonstrated that on 5th day after the single dose of 25 mg daunorubicin/kg i.v.i., along with degenerating mitochondria and membranous whorls resembling myelin figures the myocardium also showed many endothelial cells which were pale and swollen and they frequently bulged into the capillary lumen. These observations support the occurrence of vascular dilatation, congestion and rupture of small myocardial capillaries with extravasation of blood as observed in the present study.

Billingham et al (1978) making use of the percutaneous transvenous endomyocardial biopsy found histopathological lesions to be both focal and disseminated; their findings of advanced degenerative changes seen isolated against a background of morphologically intact myocardium and the subendocardium representing a particularly prominent site of involvement. These authors also reported vacuolar degeneration of myocytes. Similar observations have also been made in rats treated with doxorubicin in the present study. The electron microscopic studies carried out by these authors have revealed that the earliest manifestation of doxorubicin cardiotoxicity is distension of sarcoplasmic
reticulum which eventually swells and coalesces to form large membrane bound clear spaces in the cytoplasm, these lesions eventually progress until the death of the myocyte at which time the mitochondria do degenerate by swelling and crystalysis, myelin figures appear and the nuclei become pyknotic and disintegrate. However in this study, the endothelial cells of the capillaries and small arterioles were found to be unaffected while in the present study damage to the endothelium resulting into extravasation of the blood was more or less a uniform feature. Bertazzoli et al (1979) reported vacuolization, myolysis myofibertrophy, thickening of the endocardium, swollen endothelial cells and inflammatory reaction.

There is paucity of work on acute histological changes of cardiotoxicity in animals treated with doxorubicin. However, the histological findings of acute cardiotoxicity of this drug as seen by Taylor et al (1982) are quite similar to those observed in the present study. These authors perfused rabbit hearts with the perfusate having adriamycin at concentrations of either 1, 2 or 8 mg/litre over a period of 30 to 150 minutes. Hearts treated with doxorubicin revealed separation of myofibres consistent with interstitial edema, frequent and marked cytoplasmic vacuolization particularly perinuclear vacuolization in 17/18 hearts showing scattered vacuolization
of myocardial cells and nine of 18 (50 percent) showing marked alteration with some vacuolization evident in virtually every low power field studied. These findings are almost similar to those reported in the present study. Taylor et al (1982) needs emphasis because it gives a clue to the histological findings seen by us on light microscopy after giving doxorubicin and taking sections on 1st, 3rd, 7th and 14th day of the drug administration. Taylor et al (1982) studied electron microscopy findings in the myocardial of these animals and observed that the morphologic changes in 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. 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. These findings indicate that in acute studies the nucleus is the first organelle to be affected with the cytoplasm frequently minimally altered. Nuclear alterations have also been reported by Jaenke et al (1974); Lambertenghi Deliliers et al (1976); Buja et al (1974).
Acute cardiotoxic manifestations of this drug have also been reported in man. Starkebaum (1975) and Harrison (1976) have independently described a patient in whom pericarditis, myocarditis, fatal pump failure and cardiac arrest developed nine days after treatment with daunorubicin given as three daily doses of 70 mg/m².

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 years old child 12 days after the drug administration, shortly before death, the patients electrocardiogram showed an anterior injury pattern. Rinehart et al. (1974), Greco et al. (1976) and Singer et al. (1978) have also described transient deterioration in left ventricular function following doses of doxorubicin.

None of the studies made so far on acute adriamycin cardiotoxicity have mentioned about valvulitis and papillary muscle involvement as observed in the present study in the doxorubicin administered rats. These observation are of considerable clinical interest in view of various
types of acute cardiac dysfunctions reported by various workers in patients receiving doxorubicin even for short periods (Ippoliti, 1976; Ainger et al., 1971; Rinehart et al., 1974; Greco et al., 1976; Singer et al., 1978).

Bristow et al. (1978) observed early clinically significant cardiotoxic manifestations developed within one to twenty three days of anthracycline administration. They concluded that single doses or initial courses of anthracycline may be associated with atleast three types of cardiac effects. The first is a pericarditis/myocarditis syndrome, which appears to be associated with high mortality and significant impairment of myocardial performance. The second type of early cardiotoxicity is a transient, reversible myocardial dysfunction that may result in reversible heart failure in patients with borderline cardiac reserve. Rhythm disturbances are a third type of early cardiotoxic effect.

The involvement of papillary muscle and valves with cusp damage as seen in the present study, to some extent may explain the early clinically significant cardiotoxic manifestations. In order to know the type of valvular damage, valve ring involvement and lesions affecting the papillary muscles, their anatomical considerations shall help in assessment of possible relationship between structural cardiac damage and cardiac dysfunction which has a direct bearing in clinical assessment of patients on doxorubicin therapy.
ANATOMY OF VALVULAR CUSP CHORDAE TENDINEA
AND PAPILLARY MUSCLE
The cardiac valves are made up of a fibrous ring to which the cusps are attached; the cusps are flat and project into the ventricular cavity. Each cusp has an attached and a free margin, and an atrial and ventricular surface. The atrial surface is smooth, the free margins and ventricular surfaces are rough and irregular due to attachment of chordae tendinae. The valves are closed during ventricular systole by apposition of the atrial surfaces near the serrated margins. The chordae tendinea connect the free margin and ventricular surfaces of the cusps to the apices of the papillary muscle. They prevent eversion of the free margins and limit the amount of balloning of the cusps towards the cavity of the atrium. The atrioventricular valves are kept competent by active contraction of the papillary muscle, which pull on the chordae tendinae during ventricular systole. The involvement of papillary muscles along with valve cusps and valve ring cardiotoxicity may cause incompetent atrioventricular valve leading to accumulation of blood in the heart and venous circulation at the expense of arterial volume and ultimately leads to heart failure.

The rhythmic contraction of the heart is called the heart beat. The impulse to contract is generated in specialized, Nodal tissue in the wall of the right atrium.
Impulses are discharged rhythmically from this Sino-Atrial-Node (The "Pacemaker"). The wave of excitation spreads throughout the muscle of both atria which are excited to contract. The impulse is picked up by another mass of Nodal tissue—the atrioventricular node and relayed by Purkinje Tissue (in Bundle of His and its branches) lying beneath the endocardium on the interventricular septum. This relays the impulse to contract to the muscle of both ventricles.

Not infrequent involvement of papillary muscles and valve ring close to A.V. junction as observed in the present study, may hamper the A.V. node function resulting in the causation of arrhythmias and other cardiac dysfunctions, so common in the early anthracycline cardiotoxicity.

Another interesting observation encountered in this study was the demonstrating of contraction band necrosis in the heart of some of the animals. These lesions constitute some what translucent intensely eosinophilic transverse bands within a myocyte or group of adjoining myocytes. Electron microscopic studies have revealed that these lesions are usually located close to an intercalated disc accompanied by shortening and scalloping of the sarcomeres, fragmentation of the Z band, distortion of the myofilaments, displacement of the mitochondria away from the intercalated disc. Such a
phenomenon is supposed to have been produced by hyper-
contraction of myofibrils in the dying cells consequent
to acute transient myocardial ischaemia. Vascular damage
as observed in the present study supports such a concept.
Whether contrature band myocardial necrosis could play any
significant functional cardiac alterations is not known.

In the present study, in order to have some
quantitative assessment of myocardial injury in different
rats, the attempt was made for numerical scoring of the
injury. For this purpose the points awarded to a lesion
on half point scale were added and their sum total repre sen-
ted the injury score for the particular animal, based on
which a injury score for a batch or a group were calculated
which reflected the total injury occurring to myocardium
after doxorubicin administration in various doses, and it
appeared relatively a better procedure to assess the cardio-
toxic damage produced in these animals. Bertazzoli et al
(1979) to access the median cumulative cardiotoxic doses
have also used similar quantification.

Despite extensive investigations the precise
pathogenetic mechanisms of adriamycin induced cardiotoxic-
city remain to be defined. Several theories regarding the
genesis of adriamycin cardiotoxicity have been advanced.
These include release of histamine and catecholamines with
resultant myocardial damage; free radical generation and
subsequent lipid peroxidation; effects on various membrane systems, including Na\(^+\) - K\(^+\) ATPase pump; binding of adriamycin to cell membrane lipids; damage to mitochondria; excess calcium influx; and effects on nucleic acids and on protein synthesis. Whatever be the exact mechanism at subcellular level, the histologic picture observed after the drug administration strongly suggest that doxorubicin cardiotoxicity may be initiated by toxic endothelial damage. The endothelial damage leads to capillary microthrombosis and increased permeability of endothelium with leakage of the plasma proteins and erythrocytes into the myocardial interstitium and into myocardial muscle cells themselves. The direct endothelial damage is followed by extravasation of blood containing high levels of doxorubicin with resultant toxic damage of muscle cells. Such a hypothesis of toxic damage of muscle cells has also been reported by D'Agostino (1963).