Effects of Adriamycin on Ultrastructure of Nucleoli in the Heart and Liver Cells of the Rat

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SUMMARY
Ultrastructural studies of the effects of adriamycin on liver and cardiac cell nucleoli of the rat showed that nucleolar segregation occurred within 1 hr after an i.v. injection of a 40-mg/kg dose.

Between 3 and 27 hr after this single dose, liver cell nucleoli progressively reverted to a normal ultrastructure. Nucleoli of rat myocardial cells did not recover but underwent further fragmentation, segregation, and conversion to ring-shaped structures. The ultrastructural alterations of myocardial cell nucleoli may represent an important aspect of adriamycin toxicity.

INTRODUCTION
Adriamycin is a "broad spectrum" antineoplastic agent found useful in the treatment of a wide variety of cancers including acute leukemia, Hodgkin's and non-Hodgkin's lymphomas, and a number of solid tumors (3-5, 15, 21, 30, 34). This anthracycline antibiotic is derived from the soil fungus Streptomyces peucetius var. caesius. It acts by intercalation into DNA (36, 40) resulting in inhibition of both DNA-dependent DNA and RNA polymerases (14, 35, 39).

A variety of side effects, including reversible myelosuppression, alopecia, nausea, vomiting, phlebitis, and mucositis (4, 21) has been observed with the currently recommended dosage of 60 to 75 mg/sq m (3). Another serious side effect of adriamycin is cardiotoxicity, which may appear as transient changes in the electrocardiogram, development of congestive heart failure, or both (4, 7, 10, 20, 28).

The transient electrocardiogram changes appear to be idiosyncratic, i.e., do not seem to be dose or schedule dependent and are not associated with significant morbidity (3, 20). The development of congestive heart failure, however, is highly dose dependent, occurring with greater frequency at total doses above 550 mg/sq m (10, 23). This condition is usually unresponsive to normal supportive measures and is associated with a high percentage of deaths (3, 20, 23).

Histological and ultrastructural studies (6, 10, 20, 24) have shown degenerative changes including loss of contractile proteins, mitochondrial swelling, and vacuole formation in myocardial cells of patients and animals receiving large doses (>550 mg/sq m) of adriamycin. Although such changes may explain the clinically observed cardiotoxic effects, the particular lesion responsible for these changes has not been elucidated.

In the present report the acute effects of high doses of adriamycin on liver and myocardial cell nucleoli of the rat were studied by transmission electron microscopy.

MATERIALS AND METHODS
Adriamycin (Adria Laboratories, Inc., Wilmington, Del.) in 10-mg vials was a generous gift from Dr. Dan Lehane (Department of Pharmacology, Baylor College of Medicine). The contents of each vial was dissolved in 1 ml of sterile 0.9% NaCl solution and administered iv. (tail vein) to male Holtzman rats (average weight, 215 g) in 1 dose of 40 mg/kg. This dose is comparable to that producing cardiotoxicity in man (3, 20). Control animals received 1 ml of sterile 0.9% NaCl solution.

The animals were sacrificed by decapitation 1, 3, 9, and 27 hr after treatment. Tissue from the left ventricle of the heart and the right lobe of the liver was excised and fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4) at 0° for 1 hr. The tissue was postfixed for 1 hr in 0.50% osmium tetroxide at room temperature. Dehydration was carried out in a graded series of ethanol and 1% aqueous uranyl acetate solutions and was completed by 2 washes in propylene oxide. The samples were infiltrated with Epon:Araldite (1:1), embedded, and sectioned on a Porter-Blum ultramicrotome. Thin sections were stained with uranyl acetate followed by lead citrate and were examined on a Philips 200 electron microscope at 60 kV.

RESULTS
Changes in Nucleoli of Rat Liver Cells. One hr after adriamycin some nucleoli were more compact than those of control cells (Fig. 1) and had segregated into 2 clearly defined areas (Fig. 2). These areas correspond to the granular and fibrillar nucleolar components. Within the fibrillar component, areas of varying electron density were noted (Fig. 2), similar to those discussed by Goldblatt and Sullivan (13).

Three hr after the administration of adriamycin, segregation of the nucleolus was still evident (Fig. 3). In some
nucleoli, fibrillar centers and microspherules (Fig. 3, arrowheads) were observed.

Between the 3rd and 9th hr of treatment, most nucleoli appeared to recover from the drug effects (Fig. 4). The persistence of microspherules (Fig. 4, arrowheads) indicated continued adriamycin toxicity (8).

After 27 hr of drug treatment (Figs. 5 and 6), the liver nucleoli appeared normal with respect to the distribution of both the granular and fibrillar components. No evidence of microspherules was found (Figs. 5 and 6). In addition to the nucleolar changes, randomly distributed chromocenters were observed throughout the nucleoplasm (Fig. 2, arrowheads).

Changes in Nucleoli of Rat Myocardial Cells. The effects of adriamycin on nucleoli of rat myocardial cells (Fig. 7) differed sharply from those on the liver cells. One hr after treatment (Fig. 8), there was extensive fragmentation of nucleoli. This fragmentation did not appear to be random but instead resulted in a physical separation of the granular and fibrillar components of the nucleolus (Fig. 8, large arrows). Portions of the granular fragment retained some characteristics of an intact nucleolus, i.e., “nucleolonema”-like structures were observed (Fig. 8, small arrows).

Three hr after injection of the drug, nucleolar fragmentation was still evident (Figs. 9 and 10). Many myocardial cell nucleoli appeared as atypical nuclear bodies with small amounts of associated granular components (Fig. 9, arrowheads). In some of these nucleolar fragments, the granular component was found mainly at the periphery (Fig. 9, inset, G).

Nine hr after adriamycin was injected, several populations of nucleoli were found. One was characterized by small fragments of nucleolar components that appeared as the isolated structures noted in Fig. 9. The 2nd population appeared as the small segregated structure shown in Figs. 10 and 11. The third (Fig. 12) was typified by hollow-centered spheres, 0.087 to 0.109 μm in diameter, that had a characteristic ring-like appearance in thin sections.

These ring-shaped nucleoli were the predominant form found in the myocardium 27 hr after the administration of adriamycin. The cortex of these ring-shaped nucleoli (Fig. 12, C) consisted mainly of fibrillar components. On numerous occasions a small segment of the cortex was observed to be notably less dense than the remainder of this structure (Fig. 12). Granular particles 130 to 200 Å in diameter were scattered throughout the matrix of the cortex (Fig. 12, arrows). These particles were similar in size and appearance to nucleolar granular component. The central portion of the nucleoli was of low electron density and contained a loosely packed matrix of anastamosing fine fibrils. These fibrils were continuous with the cortex of the nucleolus, where the less electron-dense fibrillar component (Fig. 12) was localized. Foci of condensed fibrils ranging in diameter from 260 to 270 Å were associated with the denser fibrillar component (Fig. 12).

DISCUSSION

The effects of adriamycin on the ultrastructure of cardiac and hepatic cell nucleoli of the rat correlate well with its known inhibition of RNA synthesis (35, 36). Nucleolar segregation patterns similar to those observed in the present study have been found in liver and other cells following exposure to drugs that inhibit RNA synthesis such as actinomycin D and daunomycin (11–13, 18, 25–27, 29, 31–33). Formation of fragmented and ring-shaped nucleoli, however, was not found previously in cardiac cells.

Although adriamycin produced marked ultrastructural changes in the nucleolar populations of both liver and myocardial cells, the extent and severity of these changes differed in these 2 tissues. Nucleoli of the liver, an organ not associated with adriamycin toxicity, regained their normal ultrastructure within 9 hr after administration of the drug (Figs. 5 and 6). This observation is in good agreement with the data that Lambertenghi-Deliliers (19) obtained from experiments carried out on mouse hepatic cell nucleoli. The rapid recovery of hepatic cell nucleoli was suggested to result from metabolism of adriamycin or *de novo* reorganization of nucleoli at different loci within the nucleus (19). The exact mechanisms responsible, however, have not been defined.

Myocardial cell nucleoli, on the other hand, were more severely damaged, as indicated by the presence of fragmented and ring-shaped nucleoli (26). Moreover, myocardial cell nucleoli did not revert to their normal ultrastructure within the experimental period (Figs. 11 and 12). The absence of nucleolar recovery in the myocardial cells may be important in the dose-dependent cardiotoxic effects of adriamycin.

Studies on the development of the myocardium have shown that fully differentiated ventricular cardiac muscle cells of the adult mammal lack the ability to replicate DNA, undergo mitosis, or proliferate (9, 38). *In vitro* studies on the rates of protein synthesis of myocardial cells have shown that the half-life of these proteins is about 7 days (22). Interference with an adequate supply of ribosomes, as a result of decreased rRNA synthesis, would severely decrease the protein synthetic activity of these cells (17) and eventually their functional capability.

The extent of nucleolar damage observed in the present study and the extended half-life of adriamycin demonstrated in pharmacokinetic studies (1, 2, 16, 37) suggest that adriamycin could inhibit protein synthesis sufficiently to induce damage in myocardial cells. This process could result in generalized deterioration and eventual failure of the heart.

REFERENCES

Fig. 1. Nucleolus of untreated rat liver cell. Areas containing granular (G) and fibrillar (F) components are distributed randomly throughout the nucleolus. Cytoplasm. Lead citrate-uranyl acetate, × 25,000.

Fig. 2. Nucleolus of rat liver cell after adriamycin treatment (40 mg/kg) for 1 hr. Clear segregation of the granular (G) and fibrillar components has occurred. Areas of relatively high (Fa) and low (Fb) electron density are evident in the fibrillar component. Dense chromocenters (ChC) are distributed throughout the nucleolus. Lead citrate-uranyl acetate, × 18,000.

Fig. 3. Nucleolus of rat liver cell after treatment with adriamycin for 3 hr. Nucleoli appear compact with the granular components (G) restricted to the central portion of the nucleolus. Areas containing granular (G) and fibrillar (F) components are distributed randomly throughout the nucleolus. Cytoplasm. Lead citrate-uranyl acetate, × 41,400.

Fig. 4. Rat liver cells treated with adriamycin for 9 hr. No evidence for segregation of the nucleolar components is seen. Numerous microspHERes (arrowheads) are distributed throughout the nucleolus. Lead citrate-uranyl acetate, × 18,000.

Figs. 5 and 6. Rat liver cell nucleoli treated with adriamycin for 27 hr. The nucleoli appear to have recovered from the effects of the drug. The distribution of the granular (G) and fibrillar (F) components in the nucleoli does not differ from untreated liver cell nucleoli (Fig. 1). Lead citrate-uranyl acetate. Fig. 5, × 30,000; Fig. 6, × 21,900.

Fig. 7. Nucleolus of untreated rat myocardial cell. Distribution of all components is random. M, myofibril. Lead citrate-uranyl acetate, × 44,850.

Fig. 8. Rat myocardial cell nucleoli treated with adriamycin for 1 hr. Macrosegregation and fragmentation of the nucleoli appear to have taken place. The granular component (G) retains some nucleoloneima-like structure and the fibrillar component shows dense fibrillar density (Fa, high density; Fb, low density). M, myofibril. Lead citrate-uranyl acetate, × 44,850.

Fig. 9. Portion of rat myocardial cell nuclei after 3 hr of adriamycin treatment. Fibrillar fragments, with associated granular component (inset, G), appear as isolated atypical nuclear bodies (arrowheads). M, myofibril. Lead citrate-uranyl acetate, × 25,000; inset, × 50,000.

Fig. 10. Nucleolus of rat myocardial cell after treatment with adriamycin for 3 hr. Nucleoli appear compact with the granular components (G) restricted to the central portion of the nucleolus. Lead citrate-uranyl acetate, × 18,000.

Fig. 11. Rat myocardial cell nucleoli treated with adriamycin for 9 hr. Differences in electron density are clearly evident in this fibrillar fragment (Fa, high density; Fb, low density), while granular component is virtually absent. Lead citrate-uranyl acetate, × 46,250.

Fig. 12. High-powered nucleus of rat myocardial cell after 27 hr of adriamycin treatment. The cortex (C) is mainly fibrillar in composition with a small number of granular particles scattered throughout the matrix (arrow). A distinctive area of low electron density is present within the cortex (Fb). Small electron-dense granules (arrowheads) are found among the anastomosing fibers in the central portion of the ring. Lead citrate-uranyl acetate, × 48,750.
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