Acute Ultrastructural Effects of the Antitumor Antibiotic Carminomycin on Nucleoli of Rat Tissues

Jerome A. Merski, Yerach Daskal, Stanley T. Crooke, and Harris Busch

Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030

ABSTRACT

Male Sprague-Dawley rats were treated with carminomycin i.v. in doses ranging from 1 to 40 mg/kg. Within 1 hr after the administration of carminomycin, 20 mg/kg, nucleoli of cardiac and skeletal muscle cells were segregated, while nucleoli of liver parenchyma cells were unaffected. Three and one-half hr after drug administration, cardiac muscle nucleoli reverted to normal ultrastructure. However, some skeletal muscle cell nucleoli were still segregated. Following treatment with carminomycin, 10 mg/kg, no significant ultrastructural changes were observed.

These results demonstrate that at sufficiently high doses carminomycin induces ultrastructural lesions in nucleoli of both cardiac and muscle cells. The dose of carminomycin required to produce nucleolar segregation in cardiac and skeletal muscle is 6 times greater than the dose of Adriamycin (3.5 mg/kg) required to induce equivalent alterations.

INTRODUCTION

Carminomycin is an anthracycline antibiotic isolated from the mycelium of Actinomadura carminata as one of 7 pigmented components (4). It differs structurally from Adriamycin (Chart 1) by the substitution of a hydroxyl for a methoxy group on C-4 and the absence of the hydroxyl group on C-14 (1, 5). However, like Adriamycin, carminomycin has been suggested to exert cytotoxicity by binding to DNA and inhibiting DNA and RNA synthesis (2, 10).

Carminomycin is effective against a variety of experimental tumors including L1210 leukemia, lymphosarcoma, and Sarcoma 180 (22). Clinical studies have shown that carminomycin has a spectrum of activity similar to that of Adriamycin (8). Toxicological studies in mice have shown that the 50% lethal dose of carminomycin is 3.7 mg/kg (12, 15) while the 50% lethal doses of Adriamycin and daunomycin are 20 and 26 mg/kg (3), respectively. The major toxic effect of carminomycin is myelosuppression (23).

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Studies from this laboratory have demonstrated that in normal rats in vivo treatment with Adriamycin (>3.5 mg/kg) induces nucleolar segregation and fibrillar center formation in cardiac muscle, skeletal muscle, and liver cells (18–21). Similar changes in nucleolar structure have also been observed in Novikoff hepatoma ascites cells following in vitro treatment with Adriamycin or one of several new anthracycline analogs (9). These and other studies suggest that alterations in nucleolar structure may be useful as indicators of anthracycline efficacy as a cytotoxic agent.

In the present study, the acute effects of carminomycin on the nucleolar ultrastructure of cardiac muscle, skeletal muscle, kidney, and liver were evaluated.

MATERIALS AND METHODS

Clinical grade carminomycin was supplied by Bristol Laboratories as a red lyophilized powder in sealed vials. The drug was reconstituted with 0.9% NaCl solution to a concentration of either 2, 4, or 10 mg/ml.

The reconstituted carminomycin was administered i.v. to 2 groups of male Sprague-Dawley rats (180 to 250 g). In the first group, 2 animals were treated with 1.0, 3.3, 10.0 or 20.0 mg/kg. In the second group, 2 animals were each treated with 10.0, 20.0, 30.0, or 40.0 mg/kg. Animals were sacrificed 1 hr after treatment. An additional 2 animals from the first group were sacrificed 3.5 hr after treatment with 20.0 mg/kg. Control animals received equivalent volumes of 0.9% NaCl solution.

Samples of cardiac muscle, skeletal muscle, liver, and kidney were examined from animals in the first treatment group. Only samples of cardiac muscle and liver were evaluated from the animals in the second treatment group.

Tissues were quickly excised and fixed in a solution of 2% glutaraldehyde and 4% paraformaldehyde in cacodylate buffer (pH 7.3). All samples were postfixed in a 2% solution of osmium tetroxide followed by dehydration in a graded series of ethanol solutions. Propylene oxide was used as an intermediate fluid before embedding in a mixture of Epon and Araldite. Silver-gray sections were cut on a Sorvall-MT2 ultramicrotome and mounted on uncoated copper grids. The mounted sections were stained with uranyl acetate followed by lead citrate and photographed on a Philips EM 200 electron microscope.

RESULTS

Marked alterations in the ultrastructure of cardiac muscle cell nucleoli occurred following 1 hr of treatment with carminomycin, 20 mg/kg or more. Unlike nucleoli of cardiac muscle cells from untreated animals (Fig. 1), the nucleoli of the treated animals were sharply segregated (Fig. 2). The nucleolar organizer components [fibrillar centers (13)], normally present as 2 or 3 distinct regions, formed a single locus at the nucleolar periphery. In addition, a halo-like region of lower electron density than the surrounding nucleoplasm was visible around some nucleoli, indicating separation of the perinucleolar chromatin from the nucleolar surface (Fig. 2, arrows). These changes were not observed in myocardial...
cells 3.5 hr after administration of carminomycin, 20 mg/kg. Alterations in the sarcoplasm, mitochondria, or myofibrils were not observed following carminomycin treatment, 20, 30, or 40 mg/kg.

Nucleoli of skeletal muscle cells were also segregated 1 hr after treatment with carminomycin, 20 mg/kg (Fig. 4). In contrast to nucleoli of skeletal muscle cells from untreated animals (Fig. 3), the skeletal muscle cell nucleoli of carminomycin-treated tissues were compact; only small remnants of vacuoles were present. Perinucleolar chromatin was absent in most cases, and a clear, halo-like region was also present in many instances. Unlike cardiac muscle cells, macrosegregation of nucleoli (6) was still present in skeletal muscle cells 3.5 hr after the 20-mg/kg treatment. No other alterations in the ultrastructure of these were observed at either time.

In contrast to both the cardiac and skeletal muscle cell nucleoli, liver cell nucleoli (Figs. 5 and 6), were not segregated following treatment with carminomycin, 20 mg/kg, for 1 hr. The only alteration observed in these nucleoli was the occasional presence of microspherules (Fig. 5, arrows).

One hr after administration of either a 30- or 40-mg/kg dose of carminomycin, liver cell nucleoli were compact with clearly segregated granular, fibrillar, and nucleolar organizer components (Figs. 7 and 8). In addition, the perinucleolar chromatin of many nucleoli had dissociated from the nucleolar surface. A reduction in the amount of glycogen in some of the liver cells was the only obvious alteration observed in the cytoplasmic compartment following treatment with carminomycin, 30 or 40 mg/kg.

In the kidney (Figs. 9 and 10), no ultrastructural changes in the basement membrane, mitochondria, or nuclei of glomerular or tubular cells were observed 1 hr after treatment with carminomycin, 20 mg/kg (Fig. 9). However, 3.5 hr after treatment, nucleoli of many proximal and distal tubule cells were segregated. Areas of nucleolar organizer component, approximately 0.4 μm in diameter, were present in some of the segregated nucleoli, giving them a characteristic ring-shaped appearance (Fig. 10). The mitochondrial, basement membrane, and other cytoplasmic and nuclear structures appeared unaltered when compared to control cells.

The ultrastructure of cardiac muscle, skeletal muscle, kidney, and liver was unaltered following treatment with carminomycin, 1.0, 3.3, or 10.0 mg/kg.

DISCUSSION

The results of the present study demonstrate that a 20-mg/kg dose of carminomycin caused segregation of the nucleoli in cardiac and skeletal muscle cells, but not of the nucleoli in liver cells. In identical experiments, a similar effect of Adriamycin on cardiac muscle cell nucleoli has also been demonstrated (21). Other studies showed that carminomycin can induce cytoplasmic lesions that are characteristically associated with anthracycline-induced cardiomyopathy (15), such as extensive cytoplasmic vacuolation, and focal myofibrillar fragmentation.

In the present study, a 20-mg/kg dose of carminomycin was needed to induce nucleolar alterations in cardiac muscle cells. The dose of Adriamycin necessary to induce comparable nucleolar alterations in this same cell type, however, was only 3.5 mg/kg (21). This difference in drug action does not appear to be directly related to the toxicity of these 2 agents since in vivo carminomycin is approximately 4 times as toxic (3, 12, 15) and in vitro is 10 times² more toxic than is Adriamycin. The capacity of these drugs to selectively induce nucleolar segregation in cardiac muscle cells may be related to their cardiotoxic potential, inasmuch as preliminary studies have suggested that carminomycin is less cardiotoxic than Adriamycin (8, 14, 21).

For example, actinomycin D, a potent inhibitor of rRNA synthesis, does not selectively induce nucleolar segregation in cardiac muscle cells (20), but its use has not been associated with significant cardiotoxicity (7). This information suggests that a relationship may exist between the ability of the anthracycline antibiotics to selectively induce nucleolar segregation and the cardiomyopathy that develops as a result of their administration.

Evidence for such a relationship has been reported by Lambertenghi-Deliliers et al. (16), who found that nucleolar segregation preceded the development of cytoplasmic lesions in myocaridial cells. In addition, chronic administration of Adriamycin results in the development of nucleolar aberrations as well as cytoplasmic lesions characteristic of anthracycline-induced cardiomyopathy (11, 18).

The capacity of anthracyclines to induce nucleolar segregation specifically in cardiac and skeletal muscle cells of the rat suggests a potential screening procedure for the evaluation of the cardiotoxic potential of other anthracycline analogs.

REFERENCES

Fig. 1. The nucleolus of this control cardiac muscle cell has intermixed granular (G) and fibrillar (F) components in a loose reticular network typical of a normally active cell. Bar, 0.5 μm. Uranyl acetate-lead citrate, × 31,250.

Fig. 2. The nucleolus of a cardiac muscle cell following 1 hr of treatment with carminomycin, 20 mg/kg. The granular (G) and fibrillar (F) components have clearly segregated. Perinucleolar chromatin has moved away from the nucleolar surface (arrows). Nucleolar organizer component (O) usually present normally active cell. Bar, 1.0 μm. Uranyl acetate-lead citrate, × 31,250.

Fig. 3. The random distribution of granular (G) and fibrillar (F) components of this nucleolus are characteristic of nucleoli in control skeletal muscle cells. Bar, 0.5 μm. Uranyl acetate-lead citrate, × 41,250.

Fig. 4. One hr after treatment with carminomycin, 20 mg/kg, the granular (G) and fibrillar (F) components of this skeletal muscle cell nucleolus have segregated. The nucleolar organizer component (O) appears as a single large area surrounded by fibrillar component. Bar, 1.0 μm. Uranyl acetate-lead citrate, × 26,250.

Fig. 5. A small number of microspherules (arrows) are present in an otherwise normal-appearing nucleolus of a liver cell following 1 hr of treatment with carminomycin, 20 mg/kg. Uranyl acetate-lead citrate, × 25,000.

Fig. 6. The granular (G) and fibrillar (F) components of this nucleolus from a control liver cell are distributed in a random pattern typical of a normally active cell. Uranyl acetate-lead citrate, × 31,250.

Fig. 7. Segregation of the granular and fibrillar components of this liver cell nucleolus is evident 1 hr after treatment with a 30-mg/kg dose of carminomycin. Bar, 1.0 μm. Uranyl acetate-lead citrate, × 28,000.

Fig. 8. Granular, fibrillar, and nucleolar organizer components form distinct regions in this liver cell nucleolus 1 hr after administration of a 40-mg/kg dose of carminomycin. Bar, 1.0 μm. Uranyl acetate-lead citrate, × 23,750.

Fig. 9. The nucleolus (No) of this podocyte appears unaltered 1 hr after treatment with carminomycin, 20 mg/kg. The basement membrane (BM) and other cytoplasmic and nuclear structures also appear unaffected. Bar, 1.0 μm. Uranyl acetate-lead citrate, × 9,450.

Fig. 10. The large circular mass of nucleolar organizer components (arrow) give this proximal tubule cell nucleolus a ring-like appearance. No other alterations were observed in this cell 3.5 hr after treatment with carminomycin, 20 mg/kg. Uranyl acetate-lead citrate, × 20,250.
Acute Ultrastructural Effects of Carminomycin

![Image 5](image5.jpg)
![Image 6](image6.jpg)
![Image 7](image7.jpg)
![Image 8](image8.jpg)
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