Ultrastructural Study of Pulmonary Bleomycin Toxicity

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SUMMARY

Ultrastructural manifestations of bleomycin A$_2$ toxicity in the human lung were studied in three patients. In addition to the appearance of nucleolar fibrillar centers, an increase in membranous, beaded, and granular nuclear bodies was found in nuclei of type 1, type 2 alveolar epithelial cells, and interstitial fibroblasts in all treated patients. Few such nuclear bodies were found in specimens of untreated patients.

INTRODUCTION

Bleomycin, a glycopeptide complex, has been shown to have antitumor activity (3, 6, 12). A wide variety of studies have been reported on the effects of bleomycin on cell growth (6, 11) as well as on cellular ultrastructure (1, 8). In a detailed ultrastructural study on the effects of bleomycin on Novikoff hepatoma ascites cells (4), it was shown that bleomycin A$_2$ causes an increase in nucleolar fibrillar center formation in vitro and in vivo. In addition, fragmentation of nucleolar fibrillar elements was found, as well as formation of nuclear and cytoplasmic bodies and nucleolar microspherules (4).

Clinically, bleomycin has been used in the treatment of a variety of tumors, particularly squamous cell carcinomas and lymphomas (3). Although it produces some myelotoxicity, bleomycin has produced fatal pulmonary edema associated with alveolar cell hyperplasia and interstitial fibrosis (6).

The present study was undertaken to examine in detail the nuclear ultrastructure of alveolar epithelial cells of lung of patients showing symptoms of bleomycin toxicity and to determine the relationship of these results with previous observations on animal tumors.

MATERIALS AND METHODS

Lung tissue was obtained from 2 patients by open biopsy and from the 3rd patient at autopsy shortly after death. Patient A, a 57-year-old male, had an embryonal cell carcinoma of the testis. He was treated with 30 mg bleomycin (i.v.) every 4 days. After 3 weeks of therapy, the patient developed dyspnea. Pulmonary function studies disclosed a decrease in pulmonary vital capacity. Chemotherapy was interrupted for 2 weeks and then resumed for an additional 10 weeks. Therapy was terminated when the patient received a total of 390 mg bleomycin, at which time lung tissue was obtained at open lung biopsy.

Patient B, a 39-year-old male, had an embryonal cell carcinoma of the testis and received 30 mg bleomycin (i.v.) weekly. Seven weeks after onset of therapy, administration of the drug was stopped because of a sudden onset of dyspnea. Treatment with bleomycin was resumed after 3 weeks for an additional 6 weeks with the same schedule. Patient B received a total of 400 mg of bleomycin.

Patient C, a 66-year-old male, had an embryonal cell carcinoma of the testis. He was started on bleomycin therapy with a dose of 10 mg (i.v.) every 4 days for 2 weeks. The dose was then increased to 30 mg/week, for a total of 360 mg. The lung of Patient C was obtained at autopsy shortly after death.

As controls, surgical specimens were obtained from patients of similar ages who did not receive any therapy. Two specimens were obtained from patients with chondromas with small localized squamous cell carcinomas, and 2 were from patients with localized peripheral adenocarcinomas of the lung.

The samples for electron microscopy were immediately fixed in 2.5% glutaraldehyde in phosphate buffer (pH 7.4) at 0° for 1 hr and postfixed in OsO$_4$ at room temperature for an additional hr. Dehydration was carried out in a graded series of ethanol containing 1% uranyl acetate (pH 4.5). Dehydration was completed in propylene oxide. The blocks were then infiltrated with Epon:Araldite (Shell Chemical Co., New York, N. Y.) (1:1 mixture) and propylene oxide (epoxy:propylene, 1:1) and embedded in fresh epoxy. The blocks were polymerized and thin sections were cut on a Porter-Blum MT-2 ultramicrotome. Sections were stained with uranyl acetate, followed by lead citrate (4, 5), and examined on a Phillips EM 301 electron microscope at 60 kV.

RESULTS

Nuclei of Granular Pneumocytes (Type 2, Alveolar Cells). These nuclei contained nucleoli with well-defined fibrillar centers and were similar in their ultrastructural organization to nucleoli of bleomycin-treated Novikoff hepatoma cells in vitro or in vivo (4) (Figs. 1 and 2a). Although these fibrillar centers were surrounded by nucleolar fibrillar components as in type 1 cells (Fig. 3b), they were of greater electron density and further separated from the main nucleolar body (Fig. 1, c and d). The nucleoplasm of type 2
cells contained a larger number of nuclear bodies than type 1 cells (Table 1). These bodies were of the BNB, MNB, and GNB varieties (Figs. 1 and 2a). Their sizes ranged from 0.3 to 0.7 µm. The core of the GNB contained granules similar in size and morphology to nucleolar granular components, but of higher electron density. All 3 varieties could be found within a single nucleus (Fig. 1a). Nuclei containing as many as 9 nuclear bodies were seen. Frequently, only those nuclear sections containing nucleoli also contained nuclear bodies. GNB’s (Figs. 1, a to c; and 2a) were commonly enclosed by a capsule of low electron density. Morphologically, this capsule was similar to components of the BNB’s or MNB’s. Frequently, the BNB’s contained a highly granular core and a lamellated capsule or cortex (Fig. 1, a to c). Nuclei and nucleoli of septal fibroblasts were similar in their ultrastructure to those of type 2 alveolar cells. However, the number of nuclear bodies was somewhat reduced and consisted mainly of the membranous variety (Table 1; Figs. 2b and 3d). Few nuclear bodies were found in specimens obtained from untreated patients.

**Nuclei of Type 1 Alveolar Epithelial Cells.** Type 1 alveolar epithelial cells were less abundant in specimens from treated patients. Their nuclei often contained 2 or more nucleoli. Distinct large fibrillar centers (9) were distributed throughout the nucleolus (Fig. 3b, arrows). These fibrillar centers were surrounded by well-defined fibrillar components and microspheres (Fig. 3b, pointers), as in nucleoli of type 2 pneumocytes. Some localized segregation appeared between the granular and fibrillar components of the nucleolus, but this differed from the typical nucleolar segregation induced by actinomycin D (7).

As was the case with type 2 cells, the most conspicuous ultrastructural change in these nuclei was the increase in nuclear bodies (5) (Fig. 3, a to c, black pointers; Table 1); which were frequently found in the proximity of the nucleolus (Fig. 3, a to c) and were of 2 main varieties, MNB and BNB (Fig. 3, a to c). Frequently, these nuclear bodies were surrounded by a “halo” of nucleoplasmic matrix where fine fibers were anastomosing with the nucleolar body proper and other nuclear components (Fig. 3, b and c, white arrows). Cytoplasmic fibrillar bodies (4) or perinuclear tufts were not detected in these cells.

**DISCUSSION**

The present study extends previous observations in tumor cells on the alterations in nucleolar ultrastructure induced by bleomycin (4). In both the experimental tumors and in human lung there was evidence for nucleolar fragmentation and nucleolar microspherule formation. In both instances, these changes are accompanied by a general increase in fibrillar center formation (4). However, no evidence was found for the formation of cytoplasmic fibrillar bodies. The perinuclear tufts (4) found in the rat after bleomycin treatment were not found in human alveolar epithelial cells.

This study demonstrates formation of nuclear bodies (2) in pulmonary epithelial cells in patients with bleomycin toxicity. Bleomycin may be more selectively toxic to type 1 alveolar epithelial cells because their number is reduced in lungs in bleomycin-treated patients (1). The possibility exists that the intracellular concentration and the effect of the drug are different in the 3 cell types (1) and these differences may be reflected by both the reduction in the type 1 cells and the higher number of nuclear bodies found in type 2 cells (Table 1).

Nuclear bodies have been reported in a variety of normal and pathological conditions. Buttner and Horstmann (2) have concluded that nuclear bodies (sphareides) should be considered as a functional differentiation of the nucleoplasm of normal cells. Dupuy-Coin and Bouteille (5) suggested that the MNB’s, BNB’s, and GNB’s are differentiation stages of the same organelle that originates from the nucleolus. They view the presence of nuclear bodies as a pathological effect.

Smetana et al. (10) suggested a relationship between the formation of the fibrillar centers of the nucleolus and the nuclear bodies. In the present study, an increase in the number of nuclear bodies (Table 1) also occurred under conditions where fibrillar center formation was induced (4, 10). Since the presence of fibrillar centers with fragmented nucleolar fibrillar components is suggestive of ultrastructural evidence for aberration in nucleolar RNA synthesis, the increase in nuclear body formation may represent an additional structural marker for the interference of bleomycin A2 with nucleolar RNA synthesis.

The presence of all 3 varieties of nuclear bodies within 1 nucleus, as well as the structural similarities between the components of these organelles, suggests the possibility of a common origin of these bodies. These 3 types may represent differentiation stages of the same structure.

**REFERENCES**

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Fig. 1. Type 2 alveolar cell nuclei from Patient A containing characteristic nuclear bodies. In this nucleus, all 3 varieties of nuclear bodies are in the proximity of the nucleolus. Note the lamellated nature of the cortex of these bodies. Arrows, nucleolar fibrillar centers. Lead citrate-uranyl acetate staining. a, X 39,000; b, X 32,500; c, X 34,500; d, X 38,250.

Fig. 2. a, Type 2 alveolar epithelial cell from Patient B at low magnification. White pointers indicate the presence of nuclear bodies. b, Nucleolus of an alveolar interstitial fibroblast (from Patient C) with fibrillar centers (arrow) and 5 nuclear bodies (pointers), most of them of the membranous variety. Lead citrate-uranyl acetate. 2a, X 13,275; 2b, X 38,500.

Fig. 3. Type 1 alveolar epithelial cells from Patient A. The BNB may be differentiated into a membranous cortex and a granular core (1a, pointers). Nucleoli contain fibrillar centers (3b, arrows), condensed fibrillar components, and microspherules (3b, white pointers). At higher magnifications, nuclear bodies can be seen to be surrounded by a halo of low electron density (3c, white arrows). In this halo, fine filaments interconnect the nuclear body and nucleoplasmic components. This particular nuclear body appears to be an intermediate between the membranous and beaded variety. 3d, alveolar interstitial fibroblast from Patient C, with a characteristic MNB. Lead citrate-uranyl acetate. a, X 30,000; b, X 35,250; c, X 45,000; d, X 45,600.
Y. Daskal et al.
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