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# Effects of 5-Iminodaunorubicin on Nucleoli of Rats

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***Summary.*** *We have confirmed that doxorubicin induces irreversible changes in the nucleolar ultrastructure of myocardial cells of rats. Similar changes were not caused by an equal dose of the synthetic analogue, 5-iminodaunomycin. These results combined with previous and current comparative tests with this analogue, doxorubicin, and daunomycin suggest that 5-iminodaunomycin may serve as a less cardiotoxic anthracycline derivative.*

## Introduction

Cancer chemotherapy with the anthracyclines doxorubicin (Adriamycin, ADR) and daunorubicin (DRB) is limited by dose-dependent cardiomyopathy and the resultant risk of congestive heart failure as treatment progresses [2, 3]. Consequently, new anthracyclines that combine good antitumor activity with lowered cardiotoxicity are widely sought. 5-Iminodaunorubicin (IDRB) is a synthetic analogue that appears to show a separation of antitumor and cardiotoxic effects [10]. It did not differ significantly from DRB and ADR in potency or efficacy against murine leukemia P388 and melanoma B16 in several dose regimens. However, the cumulative dose of IDRB required to produce significant widening of the QRS complex in the rat electrocardiogram was four to six times, respectively, the cumulative doses of DRB and ADR [10, 11]. The lowered cardiotoxicity thus suggested may be associated with the unique structure of IDRB, which is the only anthracycline modified at the quinone. This modification also resulted in lowered capacity of the compound to catalyze oxygen consumption with the production of oxygen radicals in a rat liver microsomal enzyme system [1, 4]. Also the extent of DNA nicking by the radicals from reductively activated IDRB was considerably less than by those from reduced DRB [7].

As a further test of this new derivative, we compared its effects with those of ADR on the ultrastructure of nucleoli of hepatic and myocardial cells of rats receiving single intravenous doses of the drugs. These studies were designed from those of Merski et al. [8] who observed that ADR caused nucleolar fragmentation and segregation in hepatic and myocardial cells 3 h after intravenous administration of 40 mg ADR/kg to rats. By 27 h after dosing, they found the nucleoli of the hepatic cells had recovered and showed normal ultrastructure. However, myocardial nucleoli failed to recover at this time and had undergone further fragmentation and segregation with conversion to ring-shaped structures. In mice that were treated with ADR, such changes in the nucleolar ultrastructure of myocardial cells have been reported to precede the development of cytoplasmic lesions that are indicative of cardiotoxicity [6]. It has been suggested [9] that the induction of nucleolar segregation in cardiac cells in the rat may have potential as a screening procedure for evaluating anthracycline cardiotoxicity.

## Materials and Methods

ADR was supplied by the National Cancer Institute, and IDRB was synthesized in our laboratories [10]. Solutions for injection were prepared by dissolving the drugs in polyethylene glycol 200: isotonic saline (2 : 1 by volume) to obtain solutions containing 6.7 mg/ml. The rats were injected rapidly via the tail vein with volumes of the drug solutions (6 ml/kg) to obtain doses of 40 mg/kg.

Male Holtzman rats (Holtzman Rat Co., Madison, WI) weighing 200 to 250 g were used. Two rats received the vehicle only and served as control animals. Groups of four rats were given ADR or IDRB between 8:00 and 9:00 a.m. They were maintained on

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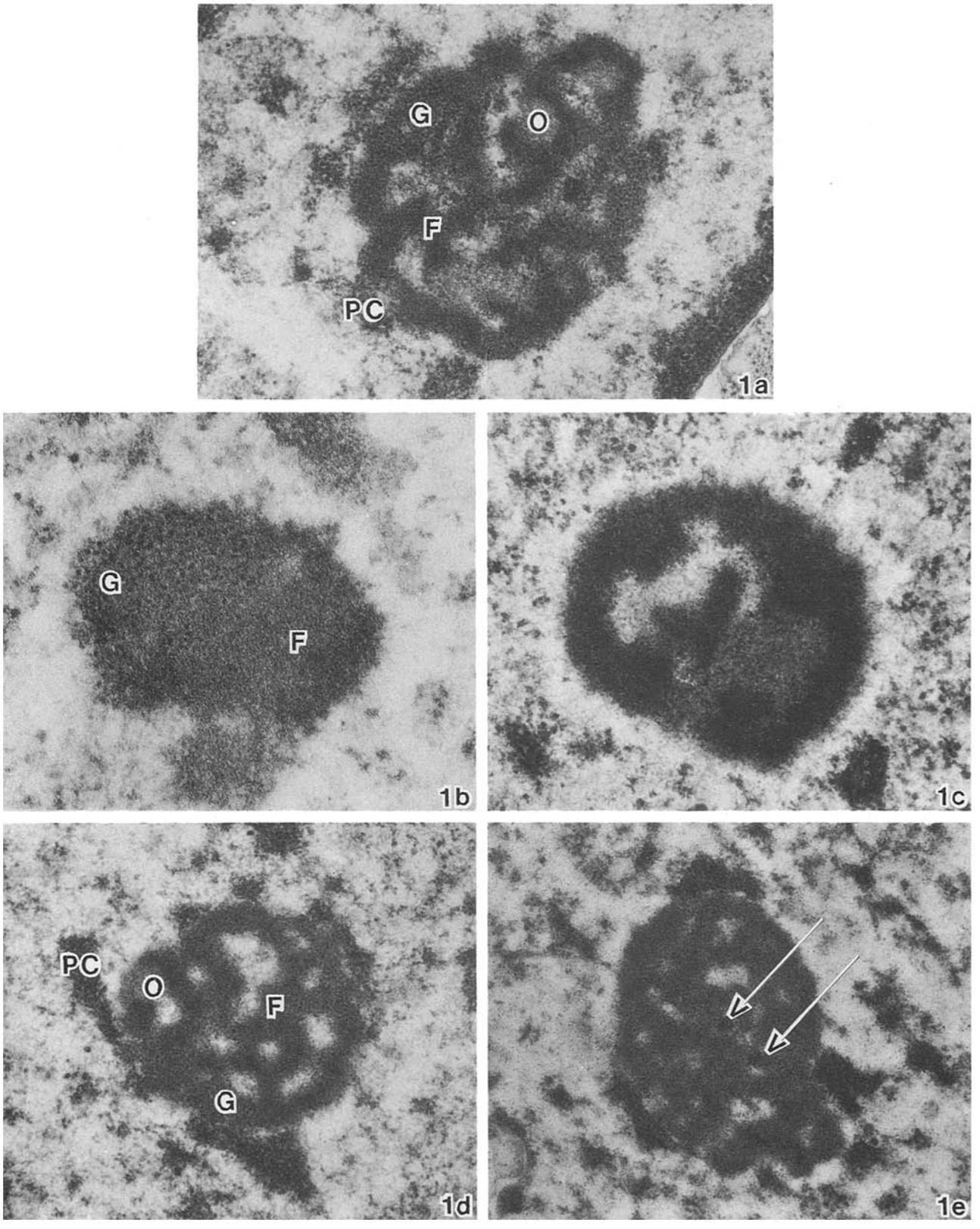


**Fig. 1. a** The nucleolus of this control cardiac muscle cell has intermixed granular (G) and fibrillar (F) components in a loose reticular network. The nucleolar organizer component (0) is present as small, distinct regions. Perinucleolar chromatin (PC) is near the nucleolar surface. (× 33,800) **b** The nucleolus of a cardiac muscle cell 3 h after treatment with ADR. The granular (G) and fibrillar (F) components are segregated. (× 53,800) **c** The nucleolus of a cardiac muscle cell 27 h after treatment with ADR. The nucleolus has assumed a ring shape, and perinucleolar chromatin is not present. Fewer granules are present. (× 47,700). **d** The nucleolus of a cardiac musle cell 3 h after IDRB. No segregation of the fibrillar and granular components is observed. The structure is similar to that of the control (Fig. 1a). (× 29,600) **e** The nucleolus of a cardiac muscle cell 27 h after IDRB. The only abnormality appears to be condensation of the fibrillar component and occasional microspherules (**arrows**). (× 30,400)

food and water ad libitum. Three hours after treatment, the two vehicle-treated rats and two of the drug-treated rats were sacrificed. The remaining two drug-treated rats of each group were sacrificed 27 h after treatment. Immediately after sacrifice, tissue samples were excised from the left ventricle of the heart and the right lobe of the liver. They were fixed in 2.0% glutaraldehyde in cacodylate buffer (pH 7.4) at 0° C for 1 h. The tissues were then washed in the same buffer and post-fixed for 1 h in 1% $OsO_4$ in veronal acetate buffer (pH 7.4) containing 7.8% sucrose. The tissues were dehydrated in a graded series of ethanol solutions, infiltrated with resin (Araldite), and embedded in Beam capsules. Thin sections were cut with a diamond knife on a Porter Blum 2 ultramicrotome, picked up on 150-mesh nickel grids, stained with lead citrate and uranyl acetate, and viewed on a Philips 200 electron microscope.

## Results

*Changes in the Nucleoli of Rat Liver Cells.* Three hours after treatment with ADR, we found that some nucleoli were more compact than control cells and had segregated into granular and fibrillar components. Twenty-seven hours after treatment, however, the nucleoli appeared normal with respect to the reticular network and the distribution of fibrillar and granular components[1]. These results are similar to those reported by Merski et al. [8].

Three and 27 h after administration of IDRB, we observed no changes in nucleoli structure. The reticular network and the distribution of fibrillar and granular components were the same as in controls[1].

*Changes in Nucleoli of Rat Myocardial Cells.* Three hours after ADR, we detected fragmentation of the nucleolar reticular network and segregation of the granular and fibrillar components (Figs. 1a and 1b). Twenty-seven hours after ADR, the nucleoli had not returned to normal, and the predominant features were ring-shaped structures (Fig. 1c), as reported by Merski et al. [8].

Three hours after treatment with IDRB, we found that the nucleoli were the same as those in controls (Fig. 1d). Twenty-seven hours after treatment, the nucleoli were still similar to those observed in controls except that the fibrillar component appeared condensed and microspherules were occasionally observed (Fig. 1e).

## Discussion

The results of this study demonstrate that a 40 mg/kg dose of IDRB caused no change in nucleoli of liver cells and only minimal changes in cardiac muscle cells at 27 h after injection of the drug. In contrast, a 40 mg/kg dose of ADR caused segregation of fibrillar and granular components in nucleoli from liver and cardiac muscle and formation of abnormal ring-shaped nucleoli in cardiac muscle cells 27 h after injection of the drug. The changes observed after ADR are similar to those reported by Merski et al. [8] and may be related to the cardiotoxic potential of ADR. With another anthracycline, carminomycin, nucleolar alterations in rat myocardial cells have also been reported [9] that may be related to the cardiotoxic potential of this derivative. Our observation that IDRB exhibited no ability to cause nucleolar changes compared to ADR parallels earlier observations that IDRB is four to six times less capable than ADR for inducing electrocardiographic changes (i.e., QRS widening) in intact rats [11] and is less active than ADR for catalyzing the redox cycle in rat liver microsomes that produces free radicals [1, 4]. Either or both of these two processes may be precursors to overt cardiotoxicity. In other studies (to be published), we have confirmed both these observations of other groups. Thus, rats receiving a total of 80 to 90 mg IDRB/kg (in multiple doses of either 4 or 10 mg/kg, IP) exhibited QRS widening similar to rats receiving a total of 16 mg ADR/kg (in multiple doses of 4 mg/kg, IP). In addition, IDRB was markedly less active than ADR in the in vitro tests on oxygen cycling using rat liver microsomes. We observed that IDRB exhibited approximately twice the $K_m$ value and one-tenth the $V_{max}$ value found for ADR in this system [5]. The current results reported herein on effects on nucleolar structure add another observation to the comparative pattern of various activities of IDRB and ADR. Such multiple indirect studies are required because, thus far, no single method has been developed and validated for predicting cardiotoxicity in the clinic.

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1 Electronmicrographs are available from the authors on request

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