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Report

The doxorubicin cardioprotective agent dexrazoxane (ICRF-187) induces endopolyploidy in rat neonatal myocytes through inhibition of DNA topoisomerase II

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Dexrazoxane (ICRF-187), which is clinically used to reduce doxorubicin-induced cardiotoxicity, is also a potent catalytic inhibitor of DNA topoisomerase II. In this study we showed that dexrazoxane inhibited the division of neonatal rat ventricular myocytes in culture, and resulted in nuclear multilobulation (demonstrated by three-dimensional reconstruction of confocal images) and marked increases in nuclear size and DNA ploidy levels (as shown by flow cytometry). It was concluded that dexrazoxane interfered with cell division in cardiac myocytes by virtue of its ability to inhibit topoisomerase II. [© 2002 Lippincott Williams & Wilkins.]

Key words: Dexrazoxane, doxorubicin, endopolyploidy, ICRF-187, myocyte.

Introduction

Dexrazoxane (ICRF-187, Zinecard®, Cardioxane®) is used clinically to reduce doxorubicin-induced cardiotoxicity. This effect is likely mediated through the iron-chelating properties of ADR-925, the open-ring hydrolysis product of dexrazoxane. ADR-925 efficiently removes iron from its complex with doxorubicin,[1] thus reducing the iron-based oxygen free radical damage induced by the drug. Dexrazoxane is also a strong catalytic inhibitor of mammalian DNA topoisomerase II (EC 5.99.1.3).[2,3] This enzyme alters DNA topology by catalyzing the passing of an intact DNA double helix through a transient double-stranded break made in a second helix.[3] Topoisomerase II has a critical role in DNA metabolism, including replication, transcription and recombination, and is required for the separation of chromosomes during mitosis.

We previously showed that Chinese hamster ovary (CHO)[4] and human leukemia K562[5] cells continuously exposed to dexrazoxane develop multilobulated nuclei and do not complete cytokinesis, yet continue to increase in size (up to 150-fold greater volume) and ploidy (up to 32*N* DNA). Because dexrazoxane is used clinically in combination with doxorubicin in the treatment of breast cancer and is in clinical trials for various tumors in children,[6] it is important to characterize its effects on cardiac myocytes. In this study, we report on the ability of dexrazoxane to inhibit cell division and induce extensive endopolyploidy and nuclear multilobulations in cultured cardiac myocytes.

Materials and methods

Drugs and chemicals

Dexrazoxane hydrochloride (a gift from Pharmacia & Upjohn, Columbus, OH) was freshly prepared in medium just before use. Trypsin and collagenase were from Worthington (Lakewood, NJ). Fetal bovine serum (FBS) was from Life Technologies (Burlington, Canada).

Cell culture and dexrazoxane treatment

Ventricular myocytes were isolated from 1- to 3-day-old Sprague-Dawley rats.[7] Briefly, minced ventricles

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were serially digested with collagenase and trypsin in phosphate-buffered saline (PBS) at 37°C in the presence of deoxyribonuclease and preplated in large Petri dishes to remove fibroblasts. The myocyte-rich supernatant was plated either on coverslips (for confocal microscopy) or 100-mm plastic culture dishes (6×10^6 cells) in DMEM/F-12 (Dulbecco's modified Eagle medium/Ham's F-12 medium; 1:1) containing 10% FBS. The myocytes were exposed to 90 μM of dexrazoxane 1 h after plating. The medium was replaced daily with fresh medium containing 90 μM of dexrazoxane. Control myocytes were grown without exposure to the drug. The animal protocol was approved by the University of Manitoba Animal Care Committee.

Microscopy and flow cytometry

For laser scanning confocal fluorescence microscopy,[4] cells grown on cover slips were fixed with 10% formalin in PBS. After treatment with ribonuclease and staining with propidium iodide, the cells were examined with a confocal microscope. Changes induced by dexrazoxane in cell cycle progression and DNA ploidy were determined by flow cytometry of cells stained with propidium iodide, as previously described.[4,5] The data are presented as the mean $\pm$ SEM.

Results

Confocal microscopy of cells stained with propidium iodide, followed by tridimensional reconstruction using images obtained by *z*-axis scans, showed that dexrazoxane-treated myocytes displayed multilobulated nuclei that were larger than those of untreated myocytes (Figure 1). In some cells the nuclear multilobulations were interconnected by narrow segments of nuclear membranes (data not shown).

Cell cycle analysis, using flow cytometry, was carried out on control myocytes (Figure 2A) and dexrazoxane-treated myocytes (Figure 2B). Aggregated cells detected by light scattering were gated out of the analysis. The data obtained were expressed as the logarithm of the propidium iodide fluorescence, rather than as the more conventional linear fluorescence measure, because on a logarithmic scale there is an equidistant separation between peaks that differ in ploidy by a constant factor of two. Such peaks correspond to cells with 2*N*, 4*N* and 8*N* ploidy (where *N* is the haploid DNA content), and can be easily distinguished in Figure 2(A and B). Study of the back correlation between the light scatter data on cell size (not shown) and the DNA fluorescence showed that the high ploidy peaks were associated with larger cell sizes. The data in Figure 2(B) showed that dexrazoxane treatment did not cause a cell cycle blockage at G_2/M but, rather, that the myocytes continued to cycle to a higher ploidy level. The percentages of myocytes in each of 2*N*, 4*N* and 8*N* ploidy levels were determined by measuring the total number of cells from one peak value to the next (Figure 2A and B). While this analysis does not take into account cells that were in the S phase, it should, nonetheless, give an approximate measure of the

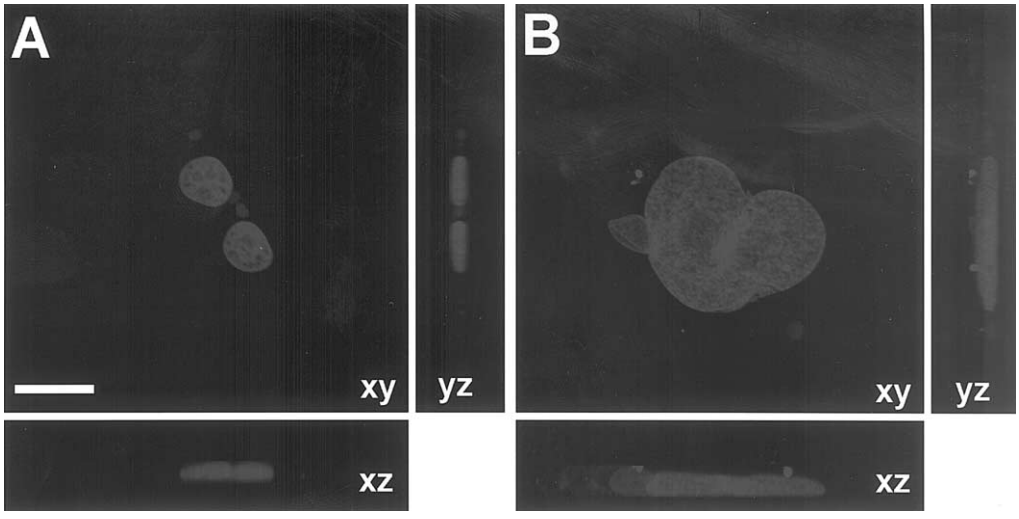

Figure 1. Confocal microscopic images showing three-dimensional reconstructions of serial scans (*xy*-, *yz*- and *xz*-axes) of cultured neonatal rat ventricular myocytes stained for DNA with propidium iodide after no exposure (A) or 4 days (B) of continuous exposure to 90 μM dexrazoxane. The nuclei of control myocytes appear normal (A). The dexrazoxane-treated cells typically display larger multilobulated nuclei. Scale bar = 10 μm for all panels.

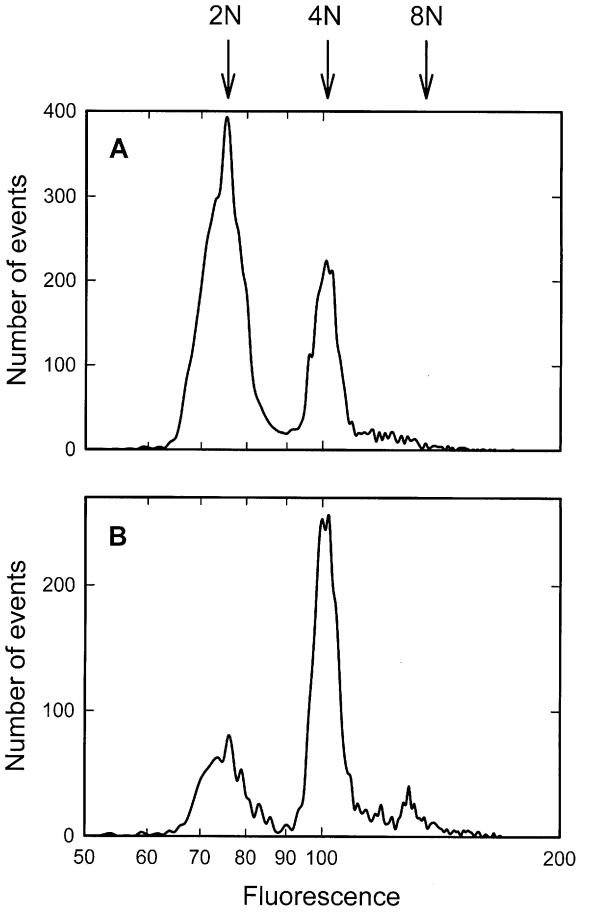

Figure 2. Cell cycle analysis of cardiac myocytes grown for 4 days after no exposure (A) or 4 days (B) of continuous exposure to 90 μM dexrazoxane. After staining with propidium iodide, 10 000 cells were analyzed by flow cytometry. The results shown are representative of two separate experiments. The cell counts are plotted on the vertical axis and the integrated red fluorescence is plotted on a logarithmic scale. The fluorescence of the 2*N*, 4*N* and 8*N* cells, which differ by a factor of 2, are equally spaced on a logarithmic scale and are indicated by the vertical arrows.

dexrazoxane-treated myocytes that were present in each ploidy level. Given the complex nature of the cell cycle distribution curves at longer times, a more detailed analysis taking into account what was presumably up to two different S phases was not attempted. The average percentage of cells in the 2*N* ploidy level in control myocytes (60 ± 3) was significantly larger ($p < 0.001$ by unpaired *t*-test) than that of the dexrazoxane-treated myocytes (25 ± 4). The average percentage of cells in the 4*N* ploidy level in control myocytes (36 ± 3) was significantly smaller ($p < 0.0006$) than that of the dexrazoxane-treated myocytes (60 ± 2). Similarly, the average percentage (4.1 ± 0.7) of control myocytes in the 8*N* ploidy level was significantly smaller ($p < 0.003$) than that of dexrazoxane-treated myocytes (12.7 ± 1.3).

Discussion

The present study showed that neonatal rat ventricular myocytes exposed to dexrazoxane in culture developed cellular enlargement, nuclear multilobulations and increased ploidy levels, changes which were similar to those observed in CHO[4] or K562[5] cells treated with this agent. We are not aware of other drugs capable of inducing nuclear multilobulations or such a rapid increase in ploidy in cardiac myocytes and we have no reason to believe that these changes are related to chelation of iron by dexrazoxane. This agent is a strong catalytic inhibitor (K_i of 13 μM) of topoisomerase II[2,3] and interferes with the separation of chromosomes during mitosis. Nevertheless, the exact mechanism of induction by dexrazoxane of the changes found in the present study remain to be determined. When given clinically in a dose of 600 mg/m^2, dexrazoxane yields a peak plasma concentration of 340 μM, with an elimination $t_{1/2}$ of 4.2 ± 2.9 h.[8] Thus, the 90 μM concentration of dexrazoxane used in this study is comparable to that attained during the clinical use of the drug. However, dexrazoxane is used clinically only intermittently (once/week or once every 3 weeks).

Cardiac myocytes isolated from neonatal rats[9] and from pediatric and adult humans[10] have a limited ability to replicate (doubling time of 2–4 days) after isolation. The large increase in 2*N* and 8*N* ploidy levels observed in myocytes that were cultured for 4 days in the presence of dexrazoxane is consistent with a blockage of both karyokinesis and cytokinesis. This blockage may have resulted from failure of the chromosomes to separate. The nuclear multilobulations may be a consequence of failure of the nuclear membranes to become completely disrupted, as is normally the case during karyokinesis. The high level of polyploidization seen in the dexrazoxane-treated cells indicated that at least one and possibly two cycles of DNA reduplication had occurred without cytokinesis. The distinct ploidy levels seen indicated that DNA reduplication only occurred once per cell cycle. Thus, dexrazoxane may not induce any changes in the replication of postnatal myocytes *in vivo,* since these cells are no longer capable of undergoing mitosis,[11] although it may prevent the division of other cell types that do maintain their ability to replicate.

Conclusion

In conclusion, this study showed that dexrazoxane, an inhibitor of DNA topoisomerase II, interfered with the division of newborn rat ventricular myocytes in culture, while allowing these cells to increase in size and DNA content, and to develop multilobulated nuclei. Thus, dexrazoxane-treated myocytes constitute a unique model for the study of DNA synthesis, karyokinesis and cytokinesis.

Acknowledgments

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