DOXORUBICIN VE DAUNORUBICININ ERİTROSİTE VE LÖKOSİTLER ÜZERİNDEKİ SİTOKSKİK ETKİLERİ

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ÖZET


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INTRODUCTION

Doxorubicin and daunorubicin are closely related anthracycin antibiotics, and both are highly toxic drugs. The use of daunorubicin has been largely restricted to the treatment of acute leukemia. In contrast doxorubicin has demonstrated one of the widest spectrums of antitumor activity ever observed, including lymphomas, leukemias, soft tissue sarcomas, and a wide variety of carcinomas. These drugs may damage DNA by the formation of free radicals, they may chelate important metal ions and may be cytotoxic without entering cells by a direct action on cell membranes.

In this study we planned to investigate the probable morphological alterations in normal erythrocytes and leukocytes following doxorubicin and daunorubicin exposure in vitro.

MATERIALS AND METHODS

Whole blood obtained from five healthy children aged 8-12 were exposed to 50 μM and 100 μM doxorubicin and daunorubicin for two hours at 37°C in vitro before processing for transmission electron microscope. These samples were centrifuged for five minutes at 5000 rpm. Then fixed with 2% gluteraldehyde, dehydrated with acetone and postfixed with OsO₄ and embedded in araldite CY 212. Thin sections were stained with lead citrate and uranyl acetate and then examined with JEOL-100 SX electron microscopes.

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RESULTS

Our observations revealed a remarkable effect induced by both drugs on the shape of erythrocytes. With doxorubicin, predominance of acanthocytes, irregularly spiculated erythrocytes with projections of varying length and position, was observed. In addition discocyte, echinocyte, stomatocyte, spherostomatocyte, schizocyte and keratocyte shapes were seen (Figure 1-4). With daunomycin stomatocytes were detected. Some of the doxorubicin exposed erythrocytes were less electron-dense from others (Figure 2,3,5) In erythrocytes sometimes plasma membrane was not observed and there electron dense material of erythrocyte was protruded out (Figure 5). With both agent, irregularity of plasma membranes of leukocytes was observed. There was an electron-dense material in the cytoplasm under this irregular membrane. Projections from plasma membranes of leukocytes were observed (Figure 6). Debris of cells were observed in intercellular spaces (Figure 2,4).

Figure 1. Erythrocytes exposed to 50 mM doxorubicin. A: acanthocyte, K: keratocyte, Lead citrate and uranyl acetate X 6000.

Figure 2. Erythrocytes exposed to 50 mM doxorubicin. K: keratocyte, S: schizocyte. Note the less electron-dense erythrocyte Lead citrate and uranyl acetate X 8000.

Figure 3. Erythrocytes exposed to 100 mM doxorubicin. A: acanthocyte, S: schizocyte. Debris of cells are seen in the intercellular space. Lead citrate and uranyl acetate X 8000.

Figure 4. Erythrocytes exposed to 100 mM doxorubicin. S: schizocyte. Debris of cells are seen in the intercellular space. Lead citrate and uranyl acetate X10000.

Figure 5. Erythrocytes exposed to 100 mM doxorubicin. Plasma membrane is absent and there electron dense material of erythrocyte is protruded out (arrow). This erythrocytes less electron-dense from its neighbours. Lead citrate and uranyl acetate X 8000.
A phospholipid dilution in doxorubicin treated erythrocyte was reported. Arancia et al. observed discoid to stomatocyte transition in doxorubicin treated erythrocytes and they attributed these alterations to the lipid bilayer changes. They also observed transformed discocytic cells to stomatocytes only at the higher concentration of daunorubicin, in contrast we observed stomatocytes a low and high concentration. O’Keefe et al. also reported significant poikilocytosis after doxorubicin administration. Oum‘hamed et al. revealed that only the induction by doxorubicin causes membrane phenotypical changes which are erythroid differentiation specific.

One of the earliest events following injury to many cell types is the appearance of protrusions at the surface of the plasma membrane termed “blebs”. Blebbing of the cell surface occurs before any change in membrane permeability is observed and initially is reversible. At some point the injury becomes irreversible, although the specific biochemical event that converts reversible injury to irreversible injury is not known. In some circumstances, the rupture of large membrane blebs, with loss of cellular contents, is believed to be the event that finally causes the death of the injured cell. Doxorubicin binds with high affinity to cellular membranes inflicting multiple lesions which are believed to be important in doxorubicin-mediated neoplastic cell death. We also observed protrusions in some erythrocytes. Inter cellular spaces contained debris of fragmented cells. Schizocytes, the fragmented erythrocytes, were observed predominantly in our study.

In the study of el-Mofty et al., doxorubicin abnormalities in the cytoplasm of erythrocytes was reported. The less electron-lucent erythrocytes we observed may be due to these cytoplasmic alterations.

We concluded that the administration of particularly doxorubicin may cause anemia due to fragmentation of erythrocytes as a result of alterations in plasma membrane and cytoplasm.

REFERENCES


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