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DOXORUBİCİN VE DAUNOBİCİNİN ERİTROSİT VE LÖKOSİTLER ÜZERİNDEKİ SİTOTOKSİK ETKİLERİ

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ÖZET

Doxorubicinin ve daunorubicinin eritrosit ve lökositler üzerindeki sitotoksik etkileri ultrastrüktürel olarak incelendi. Her iki ajan eritrositlerin şeklinde belirgin değişiklikler oluşturdu. Doxorubicin uygulanması ile akantositler başta olmak üzere diskosit, ekinotip, sitomatosit, sferostomatosit, şizosit ve keratositler izlendi. Daunomicin ile stomatositler izlendi. Her iki ajanla eritrositlerin plazma membranları bazen izlenmiyordu ve bu alanlardan eritrositin elektron-yoğun içeriğinin dışarıya taştığı gözlendi. Lökositlerin plazma membranında düzensizlik mevcuttu. Bu hücrelerin plazma membranlarında uzantılar izlendi. İntersellüler alanlarda hücre artıklar gözlendi.

Anahtar Kelimeler: Doxorubicin, Daunorubicin, Eritrosit, Lökosit, Ultrastrüktür

CYTOTOXIC EFFECTS OF DOXORUBICIN AND DAUNORUBICIN ON ERYTHROCYTES AND LEUKOCYTES

SUMMARY

Cytotoxic effects of doxorubicin and daunorubicin on erythrocytes and leukocytes in vitro were investigated ultrastructurally. A remarkable effect induced by both drugs on the shape of erythrocytes was detected. With doxorubicin, predominance of acanthocytes and in addition discocyte, echinotype, stomatocyte, spherostomatocyte, schizocyte and keratocyte shapes were seen. With daunomycin stomatocytes were detected. With both agents, in erythrocytes sometimes plasma membrane was not observed and there electron dense material of erythrocyte was protruded out. Irregularity of plasma membranes of leukocytes was observed. There was projections from plasma membranes of these cells. Debris of cells were observed in intercellular spaces.

Key Words: Doxorubicin, Daunorubicin, Erythrocyte, Leukocyte, Ultrastructure.

INTRODUCTION

Doxorubicin and daunorubicin are closely related anthracyclin antibiotics, and both are highly toxic drugs. The use of daunorubicin has been largely restricted to the treatment of acute leukaemia, in contrast doxorubicin has demonstrated one of the widest spectrums of antitumor activity ever observed, including lymphomas, leukemais, soft tissue sarcomas, and a wide variety of carcinomas.[1] 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.[1,2]

In this study we planned to investigate the probable morphological alterations in normal erytrocytes 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 gluteraldehyte, dehydrated with acetone and postfixed with OsO_4 and embedded in araldide CY 212. Thin sections were stained with lead citrate and uranyl acetate and then examined with JEOL-100 SX electron microscope.

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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 discoyte, echinotype, 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).

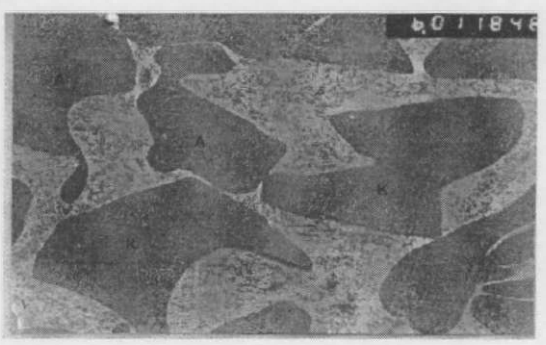

Figure 1. Erytrocytes exposed to 50 mM doxorubicin. A: acanthocyte, K: keratocyte, Lead citrate and uranyl acetate X 6000.

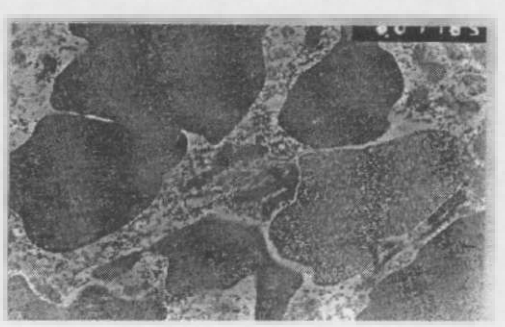

Figure 2. Erytrocytes exposed to 50 mM doxorubicin. , K: keratocyte, S: schizocyte. Note the less electron-dense eyrtrocyte Lead citrate and uranyl acetate X 8000.

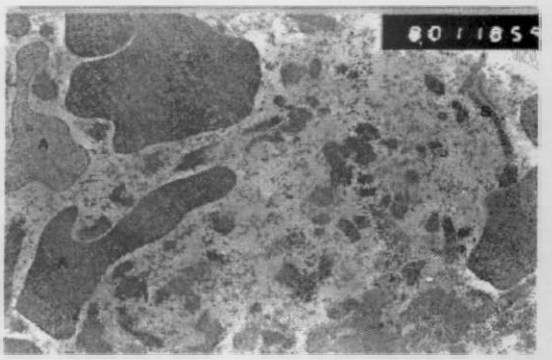

Figure 3. Erytrocytes exposed to 100 mM doxorubicin. A: acanthocyte, S:schizocyte. Debris of cells are seen in the intercellular space. Lead citrate and uranyl acetate X 8000.

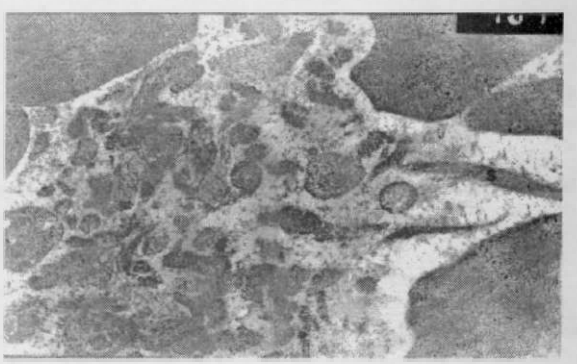

Figure 4. Erytrocytes exposed to 100 mM doxorubicin. S:schizocyte. Debris of cells are seen in the intercellular space. Lead citrate and uranyl acetate X 10000.

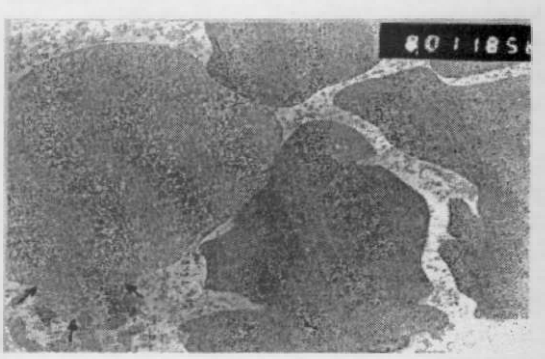

Figure 5. Erytrocytes exposed to 100 mM doxorubicin. Plasma membrane is absent and there electron dense mateficaf of erytrocyte is protrudet out (arrow). This eyrtrocyteis less electron-dense from its neighbours. Lead citrate and uranyl acetate X 8000.

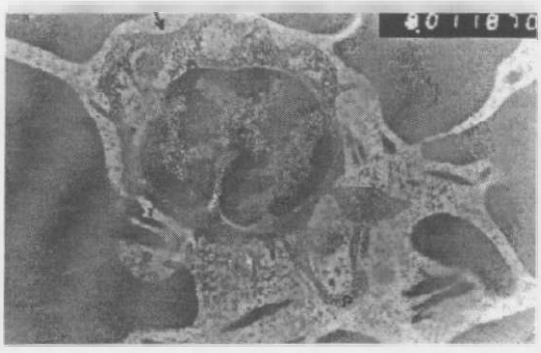

Figure 6. Lymphocyte exposed to 50 mM daunorubicin. P: projections from plasma membrane. Plasma membrane is irregular and electron-dense material is seen in the cytoplasm(arrow). Lead citrate and uranyl acetate X 8000.

DISCUSSION

There has been great interest in trying to establish the mechanisms by which doxorubicin and daunorubicin damage cells. Several hytopheses have been proposed to explain the development of cardiac toxicity including free radical-dependent lipid damage.[3] The predominance of acanthocytes in our doxorubicin exposed sample supports these hypothesis because acanthocytes are generated from normal red blood cells under conditions that alter their membrane lipid content. The mechanism of acanthocyte formation is unknown, once produced, the shape is irreversible.[3]

Environmental stress cause by low pH excess albumin, or cationic phenothiazine derivatives will transform the discocyte into an intermediate form with deeper biconcavities and then into a cupped shaped cell with only a single concavity, a stomatocyte. Thus far the changes are readily reversible, but if the single deep depression on the stomatocyte surface is obliterated by membrane loss, the transformation become irreversible and a spherostomatocyte is the result. In addition to pH and albumin changes there exists a wide array of pharmacological agents that effect stomatocytic-echinocytic changes in red cell shape. These are thought to act by preferentially expanding the outer half of the phospholipid bilayer (echinocytogenic) or the inner half (stomatocytogenic). This exlanation is sometimes referred to as the bilayer-couple hypothesis.[3]

A phospholipid dilution in doxorubicin treated erythrocyte was reported. Arancia et al.[5] 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[6] also reported significant poikilocytosis after doxorubicin administration. Oum'hamed et al[7] 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 appearence 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 circumstance, the rupture of large membrane blebs, with loss of cellular contents, is believed to be the event that finaly causes the death of the injured cell.[8] Doxorubicin binds with high affinity to cellular membranes inflicting multiple lesions which are believed to be important in doxorubicin-mediated neoplastic cell death.[9] We also observed protrusions in some erythrocytes. Intercellular spaces contained debris of fragmented cells. Schizocytes, the fragmented erythrocytes, were observed predominantly in our study.

In the study of el-Mofty et al,[10] 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 erytrocytes as a result of alterations in plasma membrane and cytoplasm.

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