ULTRASTRUCTURAL CHANGES IN UV-IRRADIATED ERYTHROCYTES

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KEY WORDS: UV irradiation of blood; ultrastructure of erythrocytes; renal failure

An intensive study of the mechanisms of the therapeutic action of ultraviolet irradiation (UVI) of blood has been in progress in recent years. Great attention has been paid to the cells, especially erythrocytes, which play the leading role in the many different functions of blood. UVI of blood is known to give rise to structural modification of the erythrocyte surface, and thus to have a definite effect on the course of various processes taking place in the body [1, 4, 5]. Meanwhile ultrastructural changes in UR-irradiated erythrocytes have not been adequately studied [2, 10].

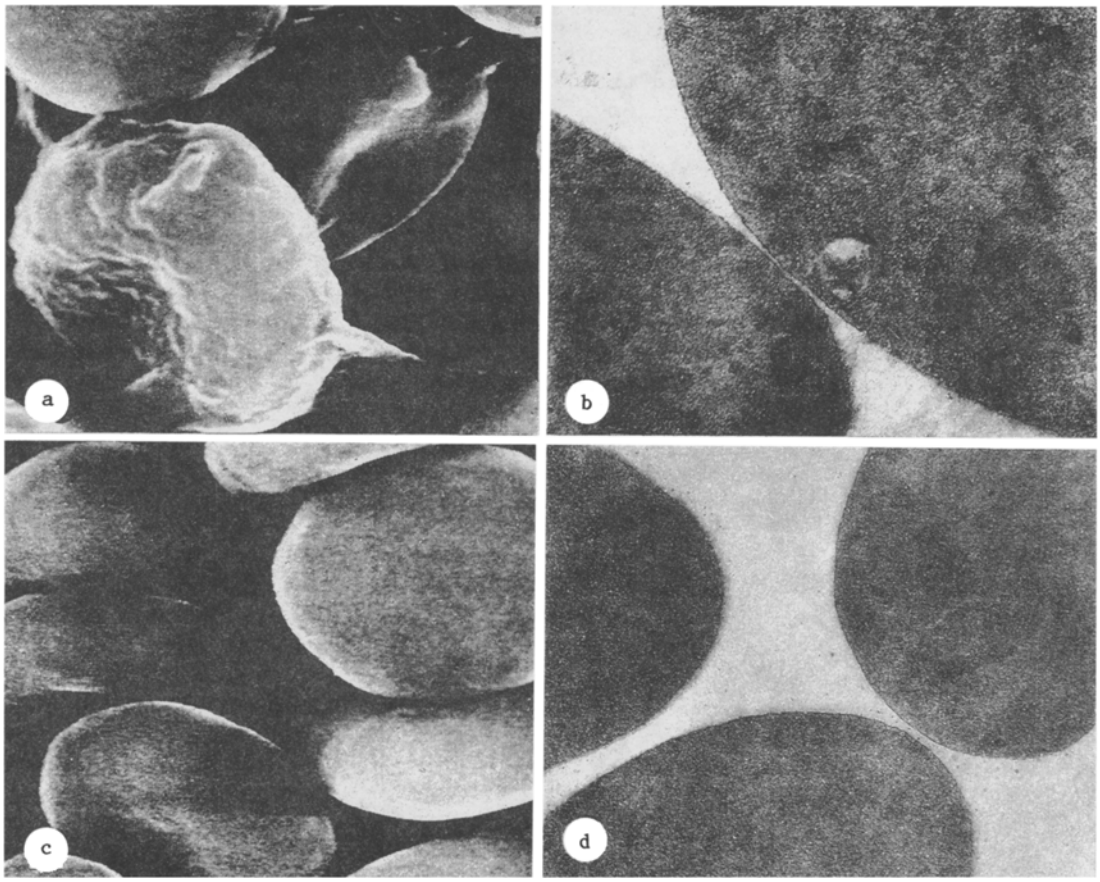

Fig. 1. Electron micrographs of erythrocytes in normal human blood irradiated with UV rays (c, d) and not irradiated (a, b). Magnification: a, c) 5000, b) 24,000, d) 14,000.

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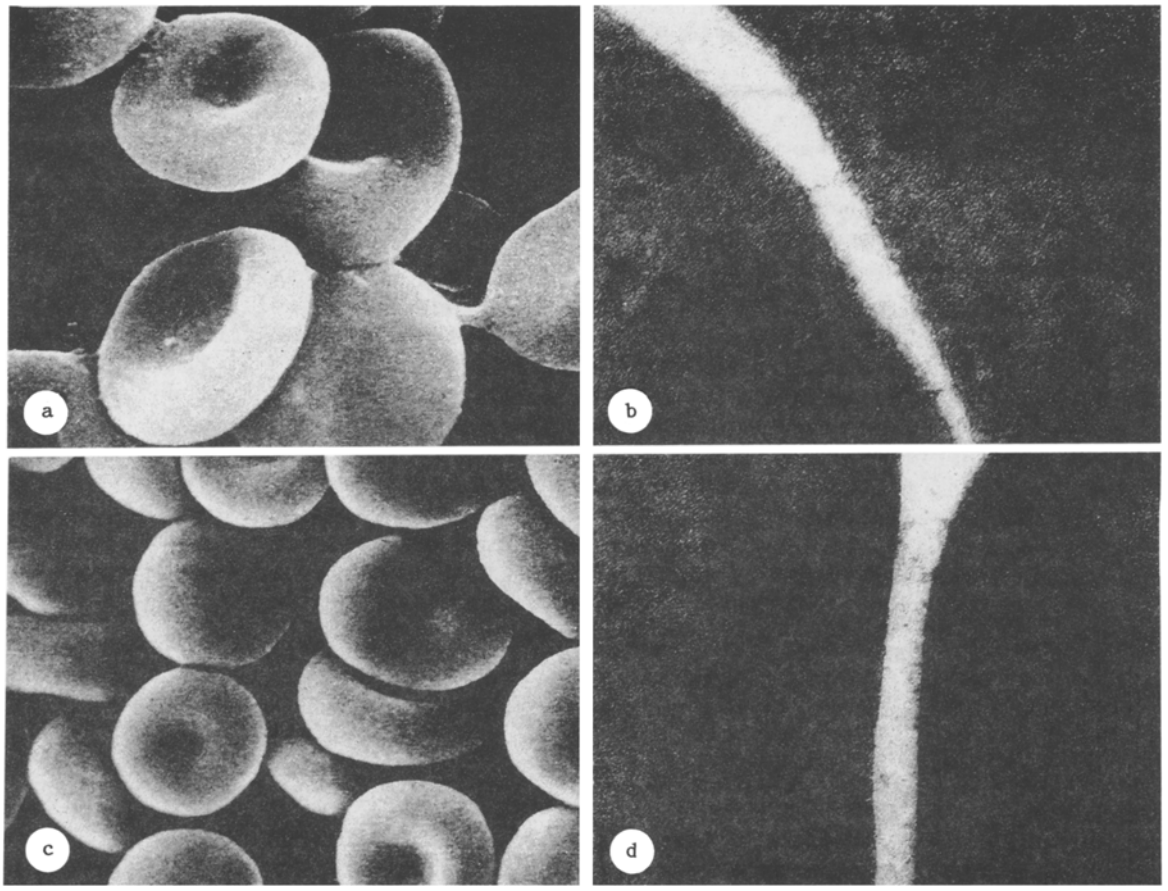

Fig. 2. Electron micrographs of erythrocytes in UV-irradiated blood (c, d) and unirradiated blood (a, b) from patients with renal failure. Magnifications: a) 5000, c) 3000, b, d) 24,000.

We investigated the ultrastructure of erythrocytes from blood obtained from normal healthy donors and also from patients with renal failure after UVI.

EXPERIMENTAL METHOD

Fresh normal blood was used in a volume of 250 ml, stabilized with "Glyugitsir" solution. The blood was UV-irradiated (wavelength 254 nm), using an apparatus of original design (DRB 8-1 lamp) in closed-circuit circulation at the rate of 25-30 ml/min for 10 min (mean exposure dose of irradiation 40-48 J/m^2). Patients with terminal renal failure and with suppurative inflammatory complications underwent UVI of the blood for 40-60 min at the rate of 50 ml/min.

Blood samples were fixed in a 2.4% solution of glutaraldehyde and a 1% solution of osmium tetroxide in cacodylate buffer. Semithin and ultrathin blood sections were obtained on the LKB-8800 Ultrotome. The erythrocytes were studied in a JSM-50 scanning electron microscope (Japan) and a Tesla 2-500 transmission electron microscope (Czechoslovakia).

EXPERIMENTAL RESULTS

Electron-microscopic study fo the intact normal blood revealed the configuration of the erythrocytes and the density of their cytoplasm. Against the background of diskocytes there were many erythrocytes with an altered shape, amounting in some cases to sphereostomatocytes and sphereoechinocytes (Fig. 1a). Relatively coarse bands or isthmuses, connected associatively with other cells, were formed on the surface of individual erythrocytes. The overwhelming majority of erythrocytes had a distinct bilayer membrane, with projection from it in some places. The contents of the cytoplasm were not always homogeneous, but hemoglobin-containing granules were present together with a very small number of sequestra (Fig. 1b).

The ultrastructure of the UV-irradiated erythrocytes showed significant changes compared with the unirradiated cells. For instance, the erythrocytes were more round in shape and resembled stage I-II stomatocytes. Meanwhile an increase in the number of diskocytes was observed (Fig. 1c). The number of pathologically changed erythrocytes was not increased. As associative connection was preserved only between individual cells. Meanwhile, under the influence of UVI, the number of erythrocytes with loosened and condensed cytoplasmic membrane was reduced. Disturbance of integrity of the membrane was not found. As a result of a marked decrease in the number of hemoglobin-containing granules of high electron density the erythrocytes appeared more homogeneous (Fig. 1 d).

In renal failure complicated by suppurative infection, marked cell polymorphism was observed (Fig. 2a, b). A very small number of typical diskocytes was found, but most cells resembled the normocyte only partially in their configuration and size. The surface of the erythrocytes was nodular and wide bands and isthmuses, associatively connected with other cells, were formed (Fig. 2a). The internal contents of the erythrocytes appeared homogeneous, but in some cells foci of osmiphilia could be seen, probably due to heme. The cytoplasmic membranes of the erythrocytes were heterogeneous, with areas of loosening and condensation (Fig. 2b). Predominance of deformed cell types in patients with renal failure was due, perhaps, to several causes but mainly to hyperosmolarity of the plasma and its abnormal electrolyte composition [3, 9].

UVI of autologous blood has a positive action on erythrocyte ultrastructure, manifested as an increase in the number of diskocytes and a decrease in the number of deformed erythrocytes (Fig. 2c). Associative connections between the cells become more delicate and the contents of the cytoplasm more homogeneous. Meanwhile some enlargement of the osmiophilic granules in the cytoplasm of the erythrocytes is observed, evidently due to an increase in their oxyhemoglobin content (Fig. 2d). This hypothesis is conclusively confirmed by the results of our previous investigations [6, 8], which demonstrated an oxygenating action of UVI of patients' blood.

The electron-microscopic investigations show that exposure of blood to UVI gives rise to ultrastructural changes in the circulating erythrocytes, expressed as diminution of the associative connections between the cells, tendency toward normalization of their configuration, and stabilization of the cytoplasmic membrane and contents of the erythrocytes, i.e., the changes affect mainly the surface membrane of the erythrocytes. This conclusion is in agreement with data obtained by the study of UV-irradiated erythrocytes by intravital cytochemistry [1, 7].

Considering that the erythrocyte membrane possesses barrier, transport, receptor, immunologic, and other vitally important functions, it can be tentatively suggested that ultrastructural photomodification of the erythrocyte membrane under the influence of UVI involves a change in functional activity of the erythrocytes and in the functions of the blood. It is this phenomenon which can explain to a certain degree the effects of UVI of the blood that are already known: increased rigidity of the erythrocytes, reduced permeability of their membranes for gases, reduced aggregative capacity of the erythrocytes, increased expression of erythrocytic antigens, facilitating correction of hemorheologic distrubances and the microcirculation, and improvement of the oxygen-transporting function of the blood.

It can be accordingly be concluded from the data described above that close correlation exists between the character of the ultrastructural modification of erythrocytes induced by UV radiation and the therapeutic effect of UVI of blood.

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ULTRASTRUCTURAL SIGNS OF HEART FAILURE AND ITS CORRECTION AFTER ASPHYXIA

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Restoration of adequate cardiac activity after clinical death is largely responsible for the final outcome of resuscitation [4]. Functional heart failure developing in the recovering period leads to a disturbance of the systematic circulation and calls for professional measures to protect the myocardium. Accordingly, in this study on a model of clinical death from mechanical asphyxia, our aim was an experimental study of the ulrastructural signs of heart failure and the effectiveness of drugs with antihypoxic, membrane-stabilizing, and protease-inhibiting action during resuscitation.

EXPERIMENTAL METHOD

Experiments were carried out on 60 albino rats weighing 170-200 g, six of which served as the control. All the experiments were carried out under ether anesthesia. Clinical death was induced by compressing the intubation tube, and subsequent resuscitation effected by indirect cardiac massage and artificial ventilation of the lungs. The drugs were injected intraperitoneally during the resuscitation measures in the following doses: gutimin (guanylthiourea) 100 mg/kg, sodium hydroxybutyate 150 mg/kg, prednisolone 0.3 mg/kg, and contrykal 2000 antitrypsin units (ATU)/kg. Nine rats, subjected to asphyxia, were not treated with these drugs. The myocardium was taken for electron-microscopic study 1, 5, 6, and 24 h after resuscitation. Fixation was carried out in a 4% solution of praformaldehyde and the material was embedded in Epon-Araldite. Ultrathin sections were studied in the EVM-100 AK electron microscope.

EXPERIMENTAL RESULTS

Widespread destructive changes were found in the myocardium of rats not receiving the drugs in the postresuscitation period. Toward 1.5 h after resuscitation, profuse intracullar edema developed, leading to disintegration of organelles and isolation of the sarcotubular system. Denudation of the cytoplasmic matrix was observed, due to the almost total disappearance of glycogen granules. The greatest changes affected the contractile apparatus of the cardiomyocytes, as we observed also on a different model of clinical death [2], and was expressed as widespread microlysis and relaxation of the myofibrils. The active lytic process led to a reduction in thickness and fragmentation of the myofibrils and was accompanied by the appearance of morphologically intact mitochondria and a few polysomes in the areas of lysis. A uniform widening of the pace of many intercalated disks and moderate dystrophic changes in the vascular endothelium were observed.

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