EFFECT OF ULTRAVIOLET IRRADIATION ON RATE OF Na^+/H^+EXCHANGE IN ERYTHROCYTES OF NORMAL INDIVIDUALS AND PATIENTS WITH ATHEROSCLEROSIS OF LOWER LIMB ARTERIES

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UDC 616.137.8/.9-004.6-085.38.015.2:615.831.4]-036.8-07:616.155.1-008.923.3

KEY WORDS: ultraviolet irradiation; atherosclerosis; erythrocytes; Na^+/H^+ exchange

The method of reinfusion of autologous blood irradiated in vitro with ultraviolet irradiation is used as a therapeutic procedure in many diseases [1, 3, 8, 11]. However, the character of the biological action of ultraviolet irradiation on the various components of the blood has not yet been fully studied. This makes it difficult to analyze the theoretical basis for the use of this technique and, consequently, hinders its more rational introduction into clinical practice. Data are available on the direct involvement of Na^+/H^+ exchange in signal conduction into the cell during the action of several hormones and neurotransmitters and in the regulation of the intracellular sodium and proton concentrations and of proliferation of cells and changes in their volume [6, 9]. In this connection the study of the characteristics of function of this exchanger in the blood cells is of definite interest for the study of the pathogenesis of various diseases and methods of their correction.

The aim of this investigation was to study the effect of UV radiation on ion-transport systems (with particular reference to Na^+/H^+ exchange) of erythrocytes of normal individuals and patients with atherosclerosis of the lower limb arteries (ALLA).

EXPERIMENTAL METHOD

Venous blood from healthy blood donors and patients with ALLA, aged from 40 to 63 years, stabilized by heparin (25-30 U/ml), was used. Ultraviolet irradiation (UVI) of the blood in vitro was carried out on the "Izol'da" MD-73 apparatus. The source of radiation was a DRB-8 miniature quartz lamp (wavelength of radiation 254 nm). During UVI the blood was in a state of flow, and the dose of irradiation was 1500 J/m^2 (2 standard doses). After UVI the blood was centrifuged (1000*g*, 10 min, 2-4°C), the plasma and white blood cells were removed, and the residue of erythrocytes was washed twice with 2-3 volumes of medium containing 140 mM NaCl, 1 mM KCl, 1 mM $MgCl_2$, and 10 mM glucose. The rate of Na^+/H^+ exchange was determined as the amiloride-inhibited component of the rate of outflow of protons with the creation of an electrochemical gradient of this cation, and with values of intracellular and extracellular pH of 6.45 and 8.00 respectively ($\Delta\mu H$-induced Na^+/H^+ exchange). The rate of Na^+/H^+ exchange in the erythrocytes was investigated 1 and 60 min after UVI of the blood. Full details of the technique and of the form of calculation were given previously [4, 8, 11]. All reagents were obtained from "Serva" (Germany) and "Sigma" (USA). The numerical results were subjected to statistical analysis by Student's method.

Department of General Pathology and Pathophysiology, Central Postgraduate Medical Institute, Moscow. (Presented by Academician of the Russian Academy of Medical Sciences T. T. Berezov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 9, pp. 239-241, September, 1992. Original article submitted November 22, 1991.

0007-4888/92/0009-1233$12.50

TABLE 1. Effect of Ultraviolet Irradiation (UVI) on Rate of Na^+/H^+ Exchange in Erythrocytes from Normal Individuals and Patients with Atherosclerosis of the Lower Limb Arteries (ALLA) (M ± m)

Substrate	n	Na^+/H^+ exchange, μmoles H^+/liter cells/min
Healthy human blood before UVI	3	92,8±12,0
1 min after UVI	3	93,4±8,6
1 h after UVI	3	100,0±9,3
Blood from patients with ALLA before UVI	8	64.5±6.8*
1 min after UVI	8	73,7±8,1
1 h after UVI	8	84,9±6,1°

Legend. Asterisk indicates significant differences compared with control, $p < 0.05$ (in healthy individuals before UVI); circles indicate significant differences relative to initial values, $p < 0.05$ (before UVI in patients).

EXPERIMENTAL RESULTS

The results given in Table 1 show that the rate of Na^+/H^+ exchange in the erythrocytes of patients with ALLA was 30-40% lower than in the control. UVI of the blood had no significant effect on this parameter in healthy individuals. By contrast, 1 h after UVI of the blood of patients with ALLA the rate of Na^+/H^+ exchange in the erythrocytes was 30% higher, and was close to values for the control group. In none of the cases compared did we find any difference in the rate of outflow of the proton through the anionic transport system (the amiloride-noninhibited component).

Little information is available on regulation of Na^+/H^+ exchange in erythrocytes. What is known is that in human erythrocytes activity of this exchanger rises by many times in response to the action of protein kinase C activators, and this is accompanied by reduction of the erythrocyte volume and a change in the level of phosphorylation of the cytoskeletal proteins of the membrane [13, 14]. We also know that the mechanisms of the regulatory action on the Na^+/H^+-exchanger of the erythrocytes for some peptides (for example, for atrial natriuretic factor) involves a stage of guanylate cyclase activation, and it is stimulated by activators of its soluble form [9].

Comparatively recently we demonstrated the possibility of modifying activity of Na^+/H^+-exchange by the use of a morphogen isolated from *Hydra* [7]. The molecular mechanism of action of this peptide on function of the exchanger has not yet been established.

At the present time we know that the functional state of the Na^+/H^+-exchanger is involved in the pathogenesis of several diseases. For instance, activation of Na^+/H^+exchange by 60-70% has been found in the erythrocytes of patients with essential hypertension [5]. Incidentally, in this disease protein kinase C activity also is increased and the erythrocyte volume reduced [15]. One of the concepts of the pathogenesis of essential hypertension ascribes a special role to protein kinase hyperfunction due to an internal factor of genetic nature [15].

In our case (patients with ALLA) the velocity of Na^+/H^+-exchange was reduced by 30-40%; the action of UVI, moreover, led to normalization of the activity of this exchanger, and this process developed with time (the maximal response was observed after 1 h). Arising from the aftereffects of hyperfunction of this system, a regulatory type of activation can be postulated after initial hypofunction.

Several investigations have shown that the therapeutic effect of UVI is accompanied by a change in some rheologic characteristics of human erythrocytes: reduction of volume, changes in the degree of deformation (a parameter characterizing the state of the membrane cytoskeleton), reduced capacity for aggregation (in view of the experimental conditions we are inclined to attribute this fact to a decrease in erythrocyte volume) [2]. Changes in the above-mentioned parameters, moreover, develop along a parallel course to the increase in activity of Na^+/H^+-exchange, which we recorded, it is reversible in character, and is observed only during procedures aimed at the whole blood or plasma, but it disappears during exposure of washed erythrocytes to UVI, and is absent also for the hormotensive control. These observations afford further evidence of the regulatory character of the above-mentioned changes and they indicate an external factor (or factors) of exogenous nature, involved in the formation of the spectrum of structural and functional anomalies of the erythrocyte membrane after UVI.

There are indications in the literature that the therapeutic effect of WI is connected with the formation of products of photodestruction of blood plasma proteins (biologically active fragments of protein molecules, polypeptides, low-molecular-weight compounds), which are powerful stimulators of the immunologic reactions of the body [4]. Judging by the rate of their accumulation, and their low molar concentrations, in our view they can reversibly modify activity of the Na^+/H^+-exchanger during the time period with which we are concerned and in accordance with the mechanisms we have described above.

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