

Effect of Immunosuppressives on the Zinc Content of Cells

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Recently, the significance of zinc in maintaining the immune status of the organism has been increasingly debated [1,4,6,9,12-14]. Zinc is essential for the activity of many enzymes, including DNA polymerases, DNA-dependent RNA polymerase, and thymidine kinase [11], and is therefore of crucial importance in realizing the specific functions of cells [2]. Its role in lymphocyte functioning has been demonstrated in a number of studies [5,7,8,10]. In this connection studies of the effect of immunosuppressives on zinc metabolism in the cell are of considerable interest. In this study we used cytochemical methods developed by us [2,3] to investigate the internal organs of experimental animals and the blood of human beings administered the said substances.

MATERIALS AND METHODS

The experiments were carried out on 128 mice and 85 human subjects (control individuals and persons administered glucocorticoid as a course of treatment). The animals were administered the following immunosuppressives: cyclosporin A (CSA) in a dose of 7 mg/kg, i.m., and sodium diethyldithiocarbamate (SDEDTC) in a dose of 200 mg/kg, i.p. The duration of the course of treatment was 3 weeks.

Blood was taken from the tail of animals. At the end of the 3 weeks the mice were killed by decapitation, and pieces of brain, pancreas, prostate, and small intestine were taken and fixed in cold acetone to prepare frozen sections. The cytochemical reaction to zinc was reproduced by staining the tissue sections and blood smears with dithizone and 8-(p-toluolsulfonilamino)-quinoline (8-TSQ) according to published methods [2,3]. The intensity of the cytochemical reactions was assessed using a three-point system (a weak positive reaction being assigned one point, a moderate reaction two points, and a pronounced reaction three points).

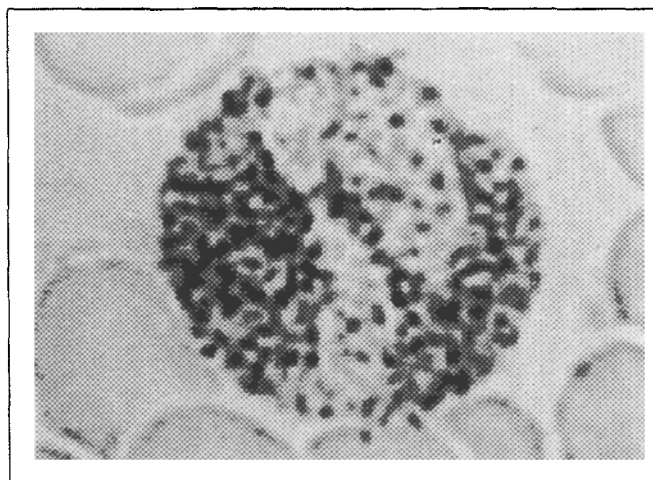


Fig. 1. Cytochemical dithizone reaction to zinc in a human peripheral blood granulocyte. Granules are distinctly seen in the cytoplasm. Fixation with formalin vapor. $\times 900$.

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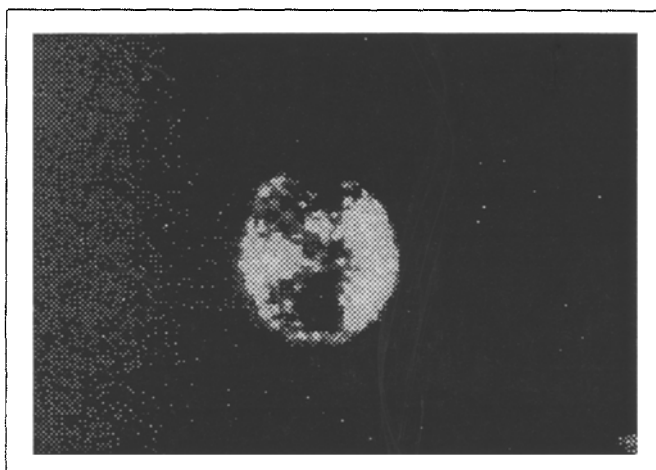


Fig. 2. High-selectivity cytochemical reaction of 8-(p-toluolsulfonilamino)-quinoline to zinc in a human peripheral blood granulocyte. Luminescing granules are seen in the cytoplasm. The specimen was prepared by simultaneous fixing and fluorochroming in an acetone solution of the stain. $\times 900$.

RESULTS

Red granules developed in the cell cytoplasm of the preparations stained with dithizone (Fig. 1), while in the case of their fluorochroming with 8-TSQ granules luminescing with yellowish green light were observed in the same parts of the cells (Fig. 2). A zinc-positive reaction of dithizone and 8-TSQ developed in the granulocytes (Figs. 1, 2), in sections of the brain in the zone of the dentate fascia and the CA2-CA4 fields of the hippocampus, in the pancreas (insulocytes), in the prostate (in the epithelium of the posterior portions), and in the small intestine (in the cells of the basal parts of the crypts).

The numerical data on the zinc content in the cells of different organs are presented in Table 1. We see that the immunodepressants reduced the content of cytochemically responsive zinc. The data of the semiquantitative analysis (the three-point scale) were confirmed by the results of calculations of the number of dithizone-stained granules in the cells. For instance, on average 139 ± 3.1 granules were found in the granular leukocytes of control subjects, whereas in the individuals with immunodeficiency caused by the course of glucocorticoid injections this number was 8-24% lower.

Our findings attest to the possible role of disturbances of zinc metabolism in the cell in bring-

TABLE 1. Intensity of Cytochemical Reaction of 8-(p-toluolsulfonilamino)-quinoline to Zinc in Cells of Different Organs in Mice for Injections of Cyclosporin A (CSA) and Sodium Diethyldithiocarbamate (SDEDTC) ($M \pm m$)

Group of animals	Intensity of reaction, arbitrary units		
	pancreatic islets	hippocampus	small intestine
Control	2.1 ± 0.17	2.0 ± 0.23	1.2 ± 0.13
CSA	$1.4 \pm 0.25^*$	$1.3 \pm 0.19^*$	$0.8 \pm 0.11^*$
SDEDTC	$0.5 \pm 0.12^*$	0.6 ± 0.14	$0.3 \pm 0.08^*$

Note. An asterisk denotes $p < 0.05$ vs. the control.

ing about the immunosuppressive effect of the substances. It is known that some immunodepressants can cause binding and elimination of zinc in the cells [14]. Other mechanisms may underlie the development of the above disturbances. For instance, correction of immunodepressant-disturbed zinc metabolism in the cell was achieved by us by injecting preparations of *Eleuterococcus senticosus* Maxim, licorice (*Glycyrrhiza glabra* L.), and warmot (*Artemisia absinthium*). The effect of immunosuppressives is reduced under the influence of zinc compounds [14].

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