Effects of Contrast Agents on Acid Phosphatase Activity in Mouse Neutrophils

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Effects of contrast agents on acid phosphatase activity are studied in mouse neutrophils. Ionic x-ray contrast agents increase and nonionic and magnetic resonance agents decrease specific activity of the enzyme.

Key Words: *contrast agents; neutrophils; acid phosphatase; specific activity*

The ability of contrast agents (CA) to inhibit enzymes is one of the mechanisms of their toxicity [5]. Experiments with direct reactions between CA and enzymes provided data on CA effects on enzyme activity. It is not clear how CA affect enzyme activity in native cells. Neutrophil lysosomal enzymes, which participate in complement activation, trigger blood coagulation and damage the vascular epithelium, are of special interest. Acid phosphatase (AP), which is involved in phagocytosis, is the marker enzyme of lysosomes. Modification of AP activity can lead to changes in phagocytic activity of neutrophils [3].

In this study we examined the effect of CA on AP activity in mouse neutrophils.

MATERIALS AND METHODS

Outbred male NMRI mice (20-25 g) kept under standard conditions were used.

Peritoneal neutrophils were obtained as described elsewhere [6]; cell density in suspension was 10^6 cells/ml. The following CA were studied: nonionic x-ray CA ultravist (Schering) and omnipaque (Nycomed); ionic x-ray CA triombrast (Pharmac); and magnetic resonance CA — ionic magnevist (Schering) and nonionic omniscan (Nycomed) in final concentrations of 10^{-2}, 10^{-3}, and 10^{-4} M.

After 10-min incubation with CA at 37°C in medium 199, cells were transferred on cover slides and fixed for 30 sec in formalin-acetone-citrate buffer. The rate of enzymatic reaction was assessed histochemically by the azocoupling method [1] with standard reagents (Sigma).

After staining, the preparations were scanned in a videodevice consisting of a Carl Zeiss microscope (projective 0.8, objective 40) and a CCD videocamera. The videoimage was analyzed using a program provided by Dr. A. A. Deev from the Institute of Theoretical and Experimental Biophysics.

The effects of CA were estimated using the enzyme specific activity value equal to the ratio of the reaction product weight and to the cell area [5].

Results were processed by standard methods of mathematical statistics [2].

RESULTS

For assessing CA effects on enzyme activity, the cells were incubated with varied concentrations of these CA for 10 min, because side effects of CA develop within the first 10-15 min after administration [7]. The doses of CA were selected on the basis of probable concentrations of CA in patient's blood.

Analysis of histograms of enzyme activity showed that the cells are heterogeneous by this parameter. The presence of a negligible number of cells with

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maximum activity shifted the mean value of the preparation activity to the right. Therefore, for assessing a cell population in general we had to resort to a more stable statistical parameter: the median. It was calculated after transformation of distribution histograms into cumulative curves.

Table 1 presents the time course of median values. Omnipaque, ultravist, and omniscan caused a decrease in specific activity of the enzyme by 10-20% at all concentrations studied. Omnipaque in a concentration of 10^{-4} M caused the maximum decrease in specific activity. At higher concentrations the enzyme specific activity increased monotonously. Similar changes of activity were observed with omniscan. With ultravist, the maximum decrease in specific activity was observed at a concentration of 10^{-3} M.

Magnevist in concentrations 10^{-4} and 10^{-2} M decreased the specific activity of enzyme and in a concentration of 10^{-3} M slightly increased it in comparison with the control.

Only triombrast increased the specific activity of AP at all concentrations tested. An increase in the agent concentration was paralleled by linear growth of the enzyme specific activity.

Our data indicate that CA has a specific effect on AP activity in mouse neutrophils. It is probable that charged molecules of triombrast cannot cross the plasma membrane, and the enzyme activity increases as a result of nonspecific activation of neutrophil. On the other hand, nonionic CA are internalized by vesicular transport [5]. Therefore, after fusion of phagosome with primary lysosome the agents can directly affect lysosomal AP.

Stimulation of lipid peroxidation may be another mechanism of CA action, that can lead to dysfunction of AP, a membrane-bound enzyme. Alteration of AP activity by CA may impair defense properties of neutrophils and efficacy of phagocytosis at the stage of digestion of bacterial cell.

Table 1. Effect of CA Concentration on the Median of Specific Activity of AP in Neutrophils (*M±m*)

CA	Median adjusted to zero, %		
	CA concentration, M		
	10^{-4}	10^{-3}	10^{-2}
Triombrast	103±5	107±5	112±5
Omnipaque	72±5	89±4	91±5
Ultravist	85±6	80±5	89±5
Magnevist	82±6	102±3	96±5
Omniscan	81±4	88±4	98±5

Note. Mean values of 3 tests are presented.

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