Effects of Contrast Media on Production of Active Oxygen Forms by Neutrophils

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Effects of contrast media on the production of active oxygen forms by mouse neutrophils are studied. X-Ray contrast media decrease and magnetic resonance contrast media increase the production of active oxygen forms.

Key Words: *contrast media; neutrophils; active oxygen forms*

Clinical use of contrast media (CM) is often associated with nausea, giddiness, hypotonia, edemas, etc. Some effects can be explained by allergen-like reactions of CM with blood plasma proteins [5] and circulating immune complexes [4], as well as by activation of the complement system. Blood cells, specifically, neutrophils, can play an important role in allergic reactions and edemas associated with administration of xenobiotics [3].

We examined the tentative direct effects of CM on the activity of isolated mouse neutrophils.

MATERIALS AND METHODS

Outbred male NMRI mice weighing 20-25 g were used in the study.

Peritoneal neutrophils were obtained as described previously [8]; the density of cells in suspension was 10^6/ml. Contrast medium was added to the neutrophil suspension to a final concentration of 10^{-2}, 10^{-3}, and 10^{-4} M. The nonionic X-ray contrast media (RCM) ultravist (Schering), omnipaque (Nycomed), the ionic X-ray contrast medium triombrast (Farmak, Ukraine), the magnetic imaging contrast media (MICM): ionic magnevist (Schering), and the nonionic omniscan (Nycomed) were studied.

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Cells were incubated with CM for 1, 5, and 10 min at 37°C in medium 199. The production of active oxygen forms was assessed from the intensity of chemiluminescence in the presence of luminol [1]. Measurements were carried out using chemiluminometer-111 developed at the Department of Nerve Cell Biophysics (Institute of Cell Biophysics). The device permits consecutive recording of chemiluminescence from 12 cells at 37°C. The recording takes 10 min.

The neutrophil respiratory burst was stimulated by phorbol 12-myristate 13-acetate (Sigma) in a concentration of 10^{-6} M and chemotactic peptide (Sigma) in a concentration of 10^{-5} M.

The results were processed by the standard mathematical statistics methods [2].

RESULTS

Phorbol 12-myristate 13-acetate was used to induce the production of active oxygen forms (AOF). This agent induces AOF generation by direct action of protein kinase C localized in cell membrane and phosphorylating membrane proteins, which is followed by activation of NADPH-oxidase [7].

When the effect of CM concentration on AOF production was studied, the preparations in concentrations 10^{-2}, 10^{-3}, and 10^{-4} M were preincubated with neutrophils for 10 min, after which phorbol 12-myristate 13-acetate was added. These doses were selected taking into consideration the probable

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TABLE 1. Effect of CM Concentration on AOF Production by Mouse Neutrophils during 10-Min Incubation at 37°C (*M±m*)

CM	AOF production, % of control		
	CM concentrations, M		
	10^{-2}	10^{-3}	10^{-4}
Triombrast	64.8±3*	88.0±5	89.2±6
Omnipaque	77.8±6*	94.0±9	91.4±9
Ultravist	74.0±5*	89.7±5	102.0±6
Magnevist	100.0±6	129.0±6*	100.0±6
Omniscan	163.0±6*	171.0±9	162.0±9

Note. Here and in Table 2: AOF production is activated by phorbol 12-myristate 13-acetate in a concentration of 10^{-6} M. Here and in Tables 2 and 3: *$p<0.05$ *vs.* the control.

TABLE 2. Relationship between the Duration of CM Incubation in a Concentration of 10^{-2} M with Mouse Neutrophils at 37°C and the Production of AOF (*M±m*)

CM	AOF production, % of control		
	Duration of incubation, min		
	1	5	10
Triombrast	55.7±4*	60.0±3*	64.8±3*
Omnipaque	76.5±4*	85.3±4*	77.8±6*
Ultravist	70.5±4*	83.0±3*	74.0±5*
Magnevist	56.8±5*	66.5±5*	100.0±6
Omniscan	106.0±3	108.0±2	163.0±6*

concentrations of CM in patient's blood. Table 1 shows that all CM change the level of AOF production. Triombrast, omnipaque, and ultravist in a concentration of 10^{-2} M significantly decreased the production of AOF ($p<0.05$). At lower concentrations of RCM this decrease was negligible.

Omniscan in all the studied concentrations and magnevist in a concentration of 10^{-3} M significantly increased the production of AOF ($p<0.05$); a increase in the magnevist concentration to 10^{-2} M led to a decrease in AOF production to the baseline level.

According to clinical reports, side effects of CM develop within 10 min after administration [9]. Therefore, we studied the relationship between the duration of neutrophil incubation with CM in a concentration of 10^{-2} M and AOF production.

The duration of incubation with RCM slightly changed the production of AOF (Table 2). Studies of MICM showed that magnevist significantly decreased the production of AOF after 1- and 5-min incubation, but by the tenth min the production increased to control values. Omniscan did not change the production of AOF during incubation for 1 and 5 min, but by the 10th min the production of free oxygen radicals drastically increased.

In order to detect the biochemical reaction leading to CM-induced generation of AOF after neutrophil activation, we performed experiments using a chemotactic peptide as activator. This agent induces the production of AOF in response to activation of the receptor located on the neutrophil membrane [3].

Nonionic RCM and MICM changed the production of AOF induced by chemotactic peptide, the changes coinciding with those caused by phorbol 12-myristate 13-acetate (Table 3). Only the ionic RCM triombrast suppressed the production of AOF by 95% of the control after activation with the chemotactic peptide and by 32% after activation with phorbol 12-myristate 13-acetate. This suggests that triombrast binds to the receptor groups charged for chemotactic peptide, predominantly due to electrostatic force. This hypothesis is supported by the data on the probable transformation of electrostatic bonds between triombrast and charged blood protein groups [6].

RCM may change the production of AOF due to their osmotic properties. The correlation between changed level of AOF production by neutrophils and osmotic pressure in the studied RCM [6] is statistically significant ($r=-0.96$), which indicates a virtually linear relationship between osmotic pressure in RCM and suppression of AOF production in neutrophils. Presumably, cell volume changes during this process, which affects the neutrophil plasma membrane and function of enzymes responsible for the production of AOF.

MICM increase the production of AOF; this process depends on the duration of incubation. It can be suggested that MICM enter the cell by vesicular transport, therefore, the effect of MICM on AOF production is observed later than that of RCM.

Thus, all the tested CM modify AOF production by mouse neutrophils; the effects of MICM and RCM are contralateral.

Concerning the clinical significance of our results, it should be noted that AOF are the most

TABLE 3. AOF Production during Incubation of Mouse Neutrophils with CM in a Concentration of 10^{-2} M for 10 Min at 37°C (*M±m*)

CM	AOF production, % of control
Triombrast	5.0±3*
Omnipaque	87.0±2*
Ultravist	58.5±7*
Magnevist	93.0±8
Omniscan	175.0±9*

Note. AOF production is activated by chemotactic peptide in concentration 10^{-5} M.

important factor of phagocyte cytopathogenicity. The following destructive effects of AOF are known: lipid peroxidation, structural protein damage, enzyme inactivation, and DNA injury [3]. Neutrophils of patients with chronic granulomatosis defective for oxygen metabolism poorly lyse tumor cells [3]. Therefore, the increase in AOF production by neutrophils induced by MICM can lead to side effects involving injury to vascular endothelium and developing by the allergic mechanism.

Our findings indicate that nonionic RCM should be preferred in clinical practice and that further studies of the molecular mechanisms of CM interactions with neutrophils and other blood cells are necessary.

From our results it can be concluded that:

1) Triombrast, omnipaque, and ultravist in a concentration of 10^{-2} M significantly decrease and omniscan in concentrations 10^{-4}-10^{-2} M and magnevist (less than omniscan) in a concentration of 10^{-3} M increase the production of AOF ($p<0.05$). The intensity of RCM effects on AOF production increases as follows: omnipaque<ultravist<triombrast.

2) There is a strong correlation ($r=-0.96$) between changes in AOF level in neutrophils and osmotic activity of RCM.

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