Effects of Contrast Agents on the Duration of Lag-Period and Rate of Production of Active Oxygen Forms by Neutrophils

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Contrast agents shorten lag-period during the production of active oxygen species. X-ray contrast agents decrease, while magnetic imaging agents increase the rate of production of active oxygen species.

Key Words: *contrast agents; neutrophils; active oxygen forms; lag-period*

Clinical application of contrast agents (CA) sometimes involves nausea, giddiness, hypotone, edemas, etc. Some of these side effects are due to reactions of CA with plasma proteins [5] and circulating immune complexes [4] or inhibition of cellular enzyme systems [5]. Blood cells may play an important role in the development of side effects caused by xenobiotic administration. Administration of exogenous substances is associated with activation of the complement system and neutropenia paralleled by migration of neutrophils to lung capillaries [3]. Therefore, changes of neutrophil function caused by CA are important for predicting side effects of these agents.

Production of active oxygen species (AOS) is a mechanism determining functional activity of neutrophils.

We explored the possibility of direct effect of CA on AOS production in isolated mouse neutrophils. The duration of lag-period (LP) and rate of AOS production were studied.

MATERIALS AND METHODS

Male outbred NMRI SPF mice (30 g) were used. The animals were kept in a vivarium.

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Peritoneal neutrophils were isolated routinely [8]; cell density in a suspension was 10^6 cell/ml. Contrast agent was added to the neutrophil suspension to the final concentrations of 10^{-2}, 10^{-3}, and 10^{-4} M. The following CA were studied: x-ray contrast nonionic Ultravist (Schering) and Omnipaque (Nycomed) and ionic Triombrast (Farmak) and magnetic imaging ionic Magnevist (Schering) and nonionic Omniscan (Nycomed).

Cells were incubated with CA for 1, 5, and 10 min at 37°C in medium 199. AOS production by neutrophils was assessed from the intensity of chemiluminescence in the presence of luminol [1]. Measurements were carried out in an original CL-111 chemilumonometer (Institute of Cell Biophysics). The device records chemiluminescence from 12 cells in succession during 5 sec at 37°C. The records were made for 10 min.

Respiratory explosion of neutrophils was stimulated by 10^{-6} M phorbol-12-myristate-13-acetate (Sigma).

Lag-period was measured as a distance between the point of the beginning of elevation of the chemiluminescence curve and the crossing point of the tangent line drawn toward the middle of the curve and the base line [7].

The rate of AOS production was estimated as the slope angle tangent of the tangent line to the point on the middle of the curve [1].

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Table 1. Effect of CA Concentration on Neutrophil Chemiluminescence LP in Activation of AOS Production by Phorbol-12-Myristate-13-Acetate in a Concentration of 10^{-6} M (*M±m*, *n*=30)

CA	LP (% of control) at CA concentration, M		
	10^{-4}	10^{-3}	10^{-2}
Triombrast	83.0±7.0*	92.0±4.0	74.0±7.0*
Omnipaque	81.0±0.1*	72.0±0.1*	71.0±0.1*
Ultravist	116.0±5.0*	107.0±4.0*	86.0±6.0*
Magnevist	87.0±8.0*	85.0±7.0*	78.0±4.0*
Omniscan	83.0±7.0*	92.0±4.0	83.0±1.0*

Note. Here and in Tables 2-4: *$p<0.05$ *vs.* the control (100%).

Results were routinely processed by mathematical statistics method using Student's *t* curve [2].

RESULTS

The rate of AOS production did not change after addition of CA in concentrations of 10^{-2}-10^{-4} M to the neutrophil suspension. However, CA modified the phorbol myristate-induced chemuliminescence. Similar to diacyl glycerol, this agent activated protein kinase C in neutrophils. Protein kinase C phosphorylation of components of the NADPH-oxidase complex leads to its activation and to an increase in AOS production [6].

In studies of the effect of CA dose on the chemiluminescence parameters neutrophils were preincubated with the agents in concentrations 10^{-4}, 10^{-3}, and 10^{-2} M for 10 min, after which phorbol myristate was added. These doses were selected with consideration for CA concentrations in patient's blood after the agent injection.

All CA altered the duration of LP; in a concentration of 10^{-2} M they significantly ($p<0.05$) decreased LP by 15-30%. Magnevist and Omnipaque significantly decreased LP in all studied concentrations (Table 1).

Table 2. Relationship between Incubation Time and LP of Neutrophil Chemuliminescence in the Presence of 10^{-2} M CA and AOS Production Activation by 10^{-6} M Phorbol-12-Myristate-13-Acetate (*M±m*, *n*=30)

CA	LP (% of control) at incubation, min		
	1	5	10
Triombrast	65.0±5.0*	42.0±6.0*	74.0±7.0*
Omnipaque	64.0±5.0*	85.0±1.0*	71.0±1.0*
Ultravist	35.0±5.0*	56.0±6.0*	86.0±6.0*
Magnevist	66.0±1.0*	80.0±6.0*	78.0±4.0*
Omniscan	63.0±5.0*	81.0±6.0*	83.0±1.0*

Ultravist (10^{-3}-10^{-4} M) prolonged LP by 7 and 16%, respectively, *vs.* the control.

According to clinical reports, the majority of side effects are observed during the first 10 min after injection of CA [9]. Therefore, we studied the relationship between the time of neutrophil incubation with CA in a concentration of 10^{-2} M and the duration of LP.

All studied agents decreased LP (Table 2). Except triombrast, the maximum decrease of LP was observed during the first minute of exposure. In the presence of omnipaque and magnevist LP decreased, then increased by the fifth minute, after which decreased again. Triombrast maximally decreased LP by the fifth minute of incubation. The duration of LP virtually did not change between the first and tenth minutes.

The effects of CA on LP duration did not correlate with osmotic activities and hydrophobic properties of the agents.

One more important parameter characterizing the neutrophil function is the rate of AOS production by the NADPH-oxidase complex.

All x-ray CA decreased the rate of oxygen radical production: triombrast in all studied concentrations and omnipaque and ultravist only in the maximum concentrations (Table 3).

The highest effect of triombrast is apparently due to its ionic nature.

Unlike x-ray CA, magnetic imaging CA increased the rate of AOS production. Omniscan increased it by 20-22% in all concentrations studied, while Magnevist only in a concentration of 10^{-3} M (by 12%). The rates did not depend on the duration of cell incubation with CA (Table 4).

It is noteworthy that CA do not stimulate neutrophils, but modulate activating effect of the classical activator phorbol myristate, which triggers a series of biochemical reactions leading to activation of the NADPH-oxidase complex.

The decrease of the rate of AOS production caused by x-ray CA may be due to osmotic properties of these agents. The coefficients of correlation between osmotic activity, toxicity of CA, and the rate of AOS production are high: -0.96 and 0.92, respectively, indicating that neutrophils can serve as a model for assessing the cytotoxicity of x-ray CA and contribute to their side effects, specifically, modify immune reactions involving neutrophils. These results confirm the higher safety of nonionic x-ray CA in comparison with ionic CA.

Magnetic imaging x-ray CA increase the rate of AOS production. This process does not depend on osmotic activity or hydrophobic properties of the agent and is apparently determined by its chemical

Table 3. Effect of CA Concentration on the Rate of AOS Production by Mouse Neutrophils Activated with 10^{-6} M Phorbol-12-Myristate-13-Acetate ($M\pm m$, n=30)

CA	Rate of AOS production (% of control) at CA concentration, M		
	10^{-4}	10^{-3}	10^{-2}
Triombrast	87.0±6.0*	85.0±4.0*	66.0±8.0*
Omnipaque	110.0±10.0	96.0±10.0	80.0±5.0*
Ultravist	90.0±8.0	76.0±6.0*	81.0±6.0*
Magnevist	100.0±7.0	112.0±5.0*	87.0±8.0
Omniscan	118.0±6.0*	124.0±12.0*	122.0±10.0*

Table 4. Effect of Duration of Incubation on the Rate of AOS Production in Neutrophils in the Presence of 10^{-2} M CA during Activation with Phorbol-12-Myristate-13-Acetate ($M\pm m$, n=30)

CA	Rate of AOS production (% of control) after incubation, min		
	1	5	10
Triombrast	57.0±10.0*	64.0±6.0*	66.0±8.0*
Omnipaque	82.0±6.0*	79.0±3.0*	80.0±5.0*
Ultravist	73.0±5.0*	84.0±6.0*	81.0±6.0*
Magnevist	70.0±10.0*	70.0±10.0*	87.0±8.0
Omniscan	128±12.0*	120±10.0*	122±10.0*

structure. Magnevist is the safest agent for neutrophils.

Generally, the rate of neutrophil AOS production is a more informative parameter for assessing CA effect on these cells than the duration of LP. Other aspects of CA-neutrophil interactions will be investigated for a better understanding of the role of these cells in realization of biological activity of CA.

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