PHARMACOLOGY AND TOXICOLOGY

Mechanisms of Histamine-Releasing Action of X-Ray Contrast Media

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It is demonstrated *in vitro* that X-ray contrast media cause the release of histamine from rabbit basophilic leukocytes in a dose-dependent manner at 37°C. They are arranged in the following order of decreasing ability to induce the release of histamine: bilignost~triombrast>peritrast~ultravist>melitrast~omnipaque. The extent to which basophils in whole blood are degranulated during incubation with any of these media is about 40% higher than that upon incubation of isolated leukocytes with these media. The release of histamine under the action of the contrast media is a Ca^{2+}-dependent process.

Key Words: *X-ray contrast media; histamine; calcium*

Most of side effects produced by contrast media are due to degranulation of mast cells and basophilic leukocytes with the release of histamine and other biologically active substances into extracellular fluids [3,7,11]. Free histamine appearing in tissue fluids and blood after administration of a contrast medium contributes to various adverse reactions such as itching eruption, erythema, urticaria, and spasms and edemas of the airways and lungs [3]. However, the mechanisms of these reactions have not been elucidated, and the role of Ca^{2+} is unclear, although calcium is the key second messenger in the transmission of specific transmembrane signals [8].

This study was designed to find out how the histamine-releasing activity of contrast media depends on their structure and to evaluate the significance of Ca^{2+}in this activity.

MATERIALS AND METHODS

The ionic contrast media bilignost and triombrast (manufactured at the Lomonosov Chemical-Pharmaceutical Factory, Ukraine) and peritrast (Dr. Kohler Chemic) and the nonionic media omnipaque (Nycomed), melitrast (Dr. Kohler Chemic), and ultravist (Schering) were incubated for 30 min at room temperature with whole blood or leukocyte suspension at final concentrations of 0.03, 0.3, 3, and 30 mg iodine/ml (mg I/ml). In control tests, the same volumes of isotonic physiological solution were added to the samples.

Basophilic leukocytes were isolated and counted by the standard procedure [12]. Heparin (0.1 ml, 1000 U/ml), dextran glucose (2 ml), and peripheral venous blood (10 ml) were thoroughly mixed in a 20 ml syringe. Erythrocytes were sedimented in a vertically positioned syringe for 50 min at room temperature. Leukocytes from the decanted plasma layer were obtained by centrifugation at 150*g* for 8 min. The cells were washed twice with cold Tris-albumin buffer (150*g*, 8 min) and resuspended in Tris-AMS buffer. Basophils were counted in suspension under a light microscope after staining with 0.1% neutral red in ethanol ($v/v_1=1/50$). Basophil count in a sample incubated with contrast medium was equal to that in whole blood. These procedures were carried out at 4°C.

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The intracellular histamine content was determined spectrofluorimetrically [5]. After incubation with a contrast medium, 0.4 N $HClO_4$ was added to the leukocyte sediment (150*g*, 15 min, 4°C), thoroughly mixed, and histamine was separated from disrupted cells by centrifugation (300*g*, 30 min, 4°C). The samples were neutralized with 1 N NaOH and incubated with 0.1% o-phthalic dialdehyde in methanol for 4 min at room temperature. Then 1 M H_3PO_4 was added. The amount of histamine was measured in a Hitachi-MPF3 spectrofluorimeter (excitation wavelength 365 nm, emission wavelength 450 nm).

Cytosolic free Ca^{2+} was determined spectrofluorimetrically using the fluorescent probe Fura 2-AM [9]. Basophils (15×10^6 cells/ml) were loaded with Fura 2-AM (3 μmol/liter) for 20 min at 37°C. In order to ensure complete saturation of the probe with Ca^{2+}, the plasma membrane of leukocytes was destroyed by digitamine (50 μmol/liter) in the presence of 1 μM calcium. To obtain the minimal intracellular concentration of Ca^{2+}, Ca^{2+} was replaced with Mn^{2+} (3 mM $MnCl_2$).

Seven adult male rabbits were used in the study. They were maintained on the standard vivarium diet.

The data were analyzed by the nonparametric Wilcoxon—Mann—Whitney *U* test [1].

RESULTS

The tested contrast media exhibited histamine-releasing activity toward rabbit peripheral blood basophils *in vitro*, as evidenced by a decrease in the intracellular histamine content (Table 1). In experiments with whole blood, this decrease was statistically significant after incubation with 0.03 mg I/ml triombrast and bilignost and 0.3 mg I/ml ultravist, peritrast, triombrast, and bilignost. At 3 and 30 mg I/ml all contrast media caused a significant decrease in the intracellular heparin content. In experiments with suspensions of basophilic leuko-

TABLE 1. Histamine Levels in Rabbit Basophilic Leukocytes After Incubation of Isolated Cells or Whole Blood with Contrast Media

Contrast medium	Concentration, mg I/ml	Histamine levels in basophils, %[1]	
		isolated cells	whole blood
Bilignost	0.03	96.2±2.1	92.3±1.3*
	0.3	99.4±1.9	71.0±1.5*
	3.0	94.5±2.0	63.9±2.0*
	30.0	89.5±1.6*	58.0±1.1*
Triombrast	0.03	97.2±1.6	89.9±1.4*
	0.3	96.3±2.2	75.6±2.3*
	3.0	91.3±2.0*	62.1±1.0*
	30.0	86.9±1.4*	58.6±1.4*
Peritrast	0.03	96.9±1.7	94.7±2.0
	0.3	101.0±2.0	90.1±1.4*
	3.0	95.3±1.6	76.4±2.2*
	30.0	90.4±1.6*	70.4±1.4*
Ultravist	0.03	95.5±2.1	95.6±1.2
	0.3	97.0±1.9	93.0±1.3*
	3.0	97.4±1.9	81.0±1.1*
	30.0	97.4±1.9	72.8±1.4*
Melitrast	0.03	96.1±1.2	97.8±2.3
	0.3	94.9±2.3	98.4±1.7
	3.0	92.1±2.2	88.1±1.4*
	30.0	95.6±1.6	78.1±2.1*
Omnipaque	0.03	99.6±1.0	95.8±1.4
	0.3	102.2±1.7	98.0±1.6
	3.0	96.2±1.3	88.6±1.2*
	30.0	94.5±1.4	79.9±1.2*

Note. Here and in Table 3: [1]100% = control samples (740±30 μg/10^6 cells). $p(H_0)<0.5$ by the *U* test. Each values is the means of at least 15 determinations.

TABLE 2. Threshold Concentrations of Contrast Media for Initiation of Histamine Release from Basophilic Leukocytes After Incubation with Rabbit Blood

Contrast medium	Concentration, mg I/ml
Omnipaque	3.0
Melitrast	3.0
Ultravist	0.3
Peritrast	0.3
Triombrast	0.03
Bilignost	0.03

cytes, the histamine-releasing effect was significant with triombrast at 3 and 30 mg I/ml and peritrast and bilignost at 30 mg I/ml.

Basophil degranulation caused by the contrast media was a dose-dependent process, i.e., the amount of released histamine increased with a rise of the contrast medium concentration (Table 1). This dependence was statistically significant for ultravist, peritrast, triombrast, and bilignost. The release of histamine amounted to 30% at 0.3 mg I/ml bilignost and increased to 45% at 30 mg I/ml. This dose dependence has direct clinical implications, since higher doses of a contrast medium or another histamine-releasing preparation increase both the occurrence and severity of adverse reactions [3,10]. At increased risk of adverse reactions, contrast media cannot be applied in high doses, and X-ray examinations should be conducted at short time periods, bearing in mind the accumulation and dose threshold effects.

Under the same experimental conditions, the degree of basophil degranulation is different for different contrast media (Table 1). According to a decrease in histamine-releasing activity, the studied media can be arranged as follows: bilignost~triombrast>peritrast~ultravist>melitrast~omnipaque. Table 2 shows the threshold concentration for each medium, i.e., the minimal concentration at which the release of histamine from basophils was observed in experiments with whole blood. Our findings agree with the observation that nonionic contrast media of low osmolarity induce less pronounced adverse reactions than ionic media do [4]. They also confirm that histamine plays an important role in the pathogenesis of such reactions. Our results are consistent with the data on the effect of contrast media of different osmolarities on human pulmonary mast cells and rat peritoneal mast cells [6,7].

It is noteworthy that the histamine-releasing effect of an ionic contrast medium depends on the structure of both its anionic and cationic moieties. Consequently, the practical significance of peritrast (lysine salt of diatrizoate) is higher than that of triombrast (methylglucamine salt of diatrizoate).

Previously, it was noted that histamine-releasing activity of nonionic contrast media is much lower than that of ionic media. Therefore, we compared calcium-modulating effects of nonionic media (omnipaque, melitrast, and ultravist), which at 30 mg I/ml did not affect basophils upon incubation with the leukocyte suspension, with that of the ionic medium bilignost, which caused a statistically significant degranulation of basophils in the same dose. The calcium content in basophils was determined every 5 min during a 60-min incubation with these media. The Ca^{2+} content in basophils incubated with omnipaque

TABLE 3. Concentrations of Free Intracellular Ca^{2+} in Basophilic Leukocytes After Incubation for Different Times with 30 mg I/ml Bilignost or Omnipaque ($M\pm m$; n=9)

Determination of Ca^{2+} concentration, min	Ca^{2+} concentration, nM		
	control	Omnipaque	Bilignost
0	105.0±0.4	106.7±0.8	109.8±0.5
5	107.7±0.9	105.2±1.4	110.0±2.1
10	106.5±1.4	104.8±0.9	113.7±1.9*
15	101.7±1.3	108.3±0.8	155.0±0.9*
20	106.7±0.5	105.0±1.6	171.7±1.1*
25	104.3±1.8	102.4±0.9	175.3±0.3*
30	113.3±0.9	105.4±0.3*	168.3±0.8*
35	104.0±2.1	106.8±1.0	166.7±1.2*
40	105.8±1.3	104.3±0.9	155.0±1.1*
45	103.5±0.4	105.5±0.4	121.7±0.9*
50	111.7±0.8	108.0±1.1	120.0±2.6*
55	111.0±0.8	108.8±1.1	121.0±0.4*
60	107.3±1.8	111.7±0.6	122.4±0.8*

(107±10 nM) did not differ from the baseline level (108±11 nM); it was not changed after incubation with melitrast and ultravist (Table 3). By contrast, the intracellular Ca^{2+} concentration in basophils incubated with bilignost increased significantly starting from the 10th min of incubation and reached the maximum (175 nM) by the 25th min (the initial concentration was 110 nM). By the 45th min of incubation, the intracellular Ca^{2+} content was slightly higher than the baseline content (120 nM).

Thus, degranulation of isolated basophils induced by contrast media is a calcium-dependent process occurring probably via exocytosis, judging from the study of the relationship between histamine-releasing action and temperature. The six contrast media were incubated with basophils at 4°C (but not at 37°C) in doses of 0.03, 0.3, 3, and 30 mg I/ml. None of them elicited any appreciable histamine-releasing effect at this temperature: $p(H_o)_U > 0.01$ for all media, where H_o is the probability of differences in the distribution of histamine in basophils upon their incubation with the same contrast medium at 37°C and 4°C. Presumably, histamine-releasing activities of the contrast media are due to active processes requiring energy expenditure. Our results provide the basis for the exploration of the possibility of preventing and treating the side effects of contrast media by the calcium-dependent blockers of histamine release Intal and ketotifene.

TABLE 4. Significance of Differences (*U* Test) Between the Histamine Contents of Basophils After Incubation of Whole Blood and Basophil Suspension with Contrast Media

Contrast medium	Concentration, mg I/ml			
	0.03	0.3	3	30
Omnipaque	-	-	0.05	0.01
Melitrast	-	-	0.05	0.01
Ultravist	-	0.05	0.01	0.005
Peritrast	-	0.05	0.01	0.005
Triombrast	0.05	0.01	0.01	0.005
Bilignost	0.05	0.01	0.01	0.005

It was demonstrated that the release of histamine from basophilic leukocytes is significantly higher (by 40%) upon incubation of the contrast media with

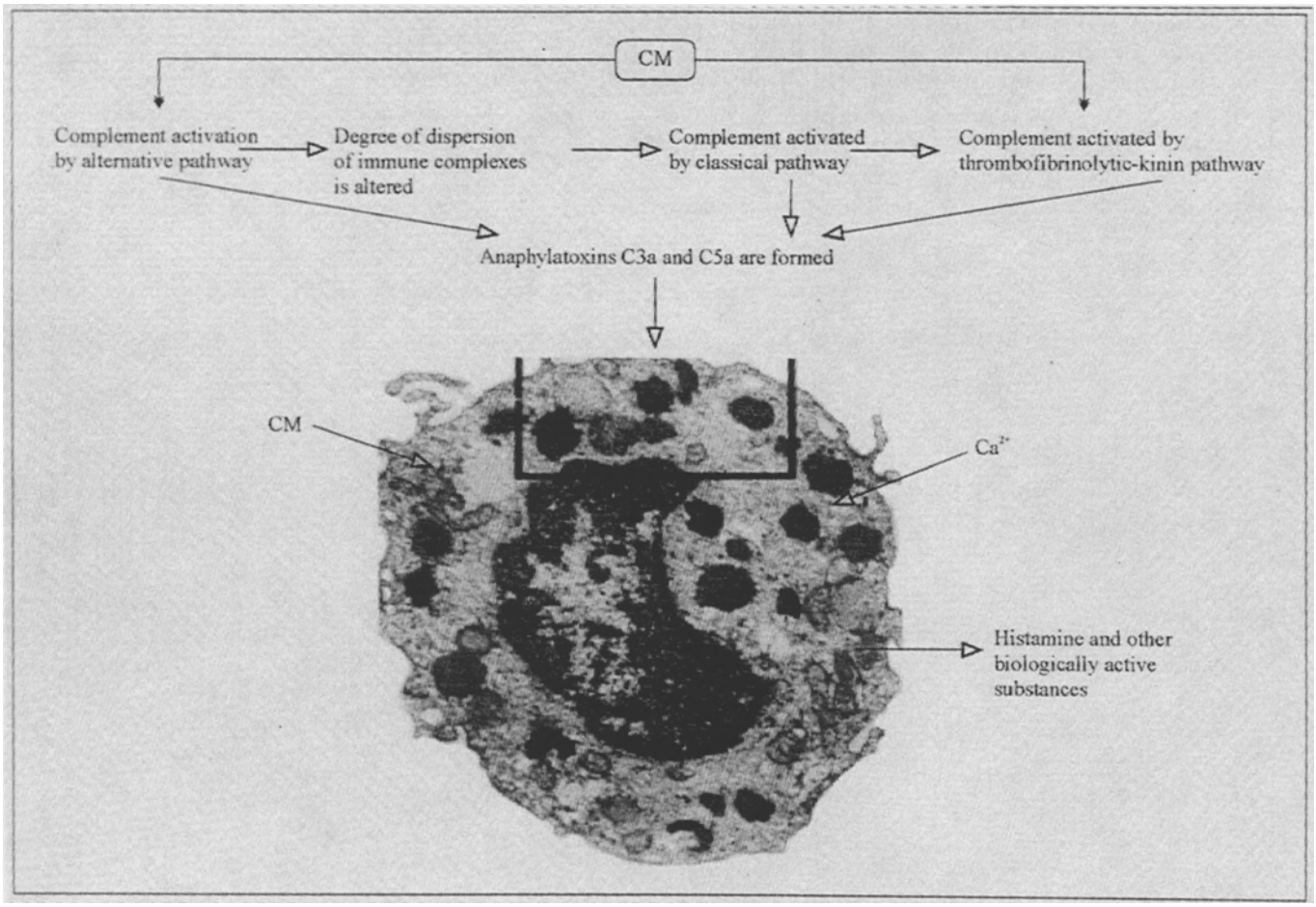

Fig. 1. A scheme illustrating the role of complement (C) in histamine release from mast cells and peripheral blood basophilic leukocytes induced by contrast media (CM).

whole blood than with isolated leukocytes (Table 4). This difference indicates that there exist at least two mechanisms of histamine release from these cells: with (anaphylactoid or allergic mechanism) and without (chemotoxic mechanism) participation of plasma factors. The complement is one of the main blood factors leading to degranulation of histamine-containing cells by a contrast medium [2,3,13].

A tentative scheme showing the role of the complement system in the release of histamine under the action of contrast media is shown in Fig. 1. It is based on our results and those reported by other researchers. It is hypothesized that contrast media induce degranulation of mast cells and basophilic leukocytes by three pathways: 1) activation of the complement system by the alternative pathway, 2) activation of the complement system by the classical pathway via circulating immune complexes. The interaction of immune complexes with the complement components formed as a result of activation by the alternative pathway increases dispersion of these complexes and their ability to activate the complement by classical pathway. The third mechanism consists in the activation of the complement by the thrombofibrinolytic-kinin pathway via the Hageman factor and plasmin. Plasmin activates the complement system by cleaving C1 component. These three mechanisms are responsible for the formation of C3a and C5a anaphylatoxins that induce degranulation of histamine-containing cells by interacting with their plasma membranes. In addition, histamine-releasing activity of a contrast medium may result from its chemotoxic effect on histamine-containing cells; in this case histamine is released by exocytosis.

Since histamine-releasing activity of the nonionic contrast media melitrast, omnipaque, and ultravist and the ionic medium peritrast is lower, they are safer than triombrast and bilignost.

REFERENCES

1. E. V. Gubler, *Computing Methods for Analysis and Recognition of Pathological Processes* [in Russian], Leningrad (1978).
2. P. V. Sergeev, A. Ya. Kul'berg, N. L. Shimanovskii, *et al.*, *Byull. Eksp. Biol. Med.*, **107**, No. 6, 716-718 (1989).
3. P. V. Sergeev, N. K. Sviridov, and N. L. Shimanovskii, *Contrast Media* [in Russian], Moscow (1993).
4. N. L. Shimanovskii, "Molecular pharmacology of contrast media," Author's synopsis of Doct. Med. Sci. Dissertation [in Russian], Moscow (1990).
5. S. Udenfriend, *Fluorescence Analysis in Biology and Medicine* [Russian translation from German], Moscow (1965).
6. V. V. Akagim and K. Tasak, *Methods Find. Exp. Clin. Pharmacol.*, **12**, 337-384 (1991).
7. E. U. Amon, M. Ennis, W. Lorenz, *et al.*, *Int. Arch. Allergy Appl. Immunol.*, **92**, 203-208 (1990).
8. P. D. Bourdillon, M. A. Bettmann, S. McGracken, *et al.*, *J. Am. Coll. Cardiol.*, No. 6, 845-853 (1985).
9. G. R. Grinkiewic, M. Ponie, and R. Y. Tisen, *J. Biol. Chem.*, **260**, 3340-3350 (1983).
10. C. M. Herd, A. R. Robertson, D. B. Frewin, *et al.*, *Br. J. Radiol.*, **61**, 5-11 (1988).
11. E. C. Lasser, *Invest. Radiol.*, **20**, 579-583 (1985).
12. D. S. May, M. Lyman, R. Alberto, and J. Chendy, *J. Allergy*, **46**, 12-16 (1970).
13. T. W. Morris and H. W. Fischer, *Annu. Rev. Pharmacol. Toxicol.*, **26**, 143-160 (1986).