In Vitro Histamine Release Induced by Magnetic Resonance Imaging and Iodinated Contrast Media

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RATIONALE AND OBJECTIVES. To investigate the mechanism of anaphylactoid reactions to contrast media, in vitro histamine release induced by magnetic resonance imaging, and iodinated contrast agents was examined in a dog mastocytoma cell line.

METHODS. Two gadolinium (Gd)-based magnetic resonance contrast agents, Gd diethylenetriamine pentaacetic acid (Gd-DTPA), dimeglumine, and Gd-bismorpholide, and two iodinated contrast agents, diatrizoate meglumine and iohexol, were incubated with histamine-containing canine mastocytoma cells. Release of histamine into the supernatant was determined at various contrast-medium concentrations after incubation at 37°C for 30 minutes.

RESULTS. Iodinated and Gd-based contrast agents caused release of histamine from mastocytoma cells at similar concentrations (50–150 mM). Mannitol, an osmotic stimulus, caused release of histamine only at concentrations greater than 1,000 mM.

CONCLUSIONS. Histamine release from canine mastocytoma cells does not appear to be solely due to osmotic effects, but results from direct stimulation by contrast media. For all agents examined, the concentration at which in vitro histamine release occurs far exceeds the serum contrast media concentration expected in routine clinical application. Direct release of histamine from mast cells does not completely explain the pathogenesis of idiosyncratic anaphylactoid responses to contrast media.

KEY WORDS. Allergy; histamine; contrast media; mast cell; magnetic resonance imaging; contrast enhancement; gadolinium.

THE PATHOGENESIS OF immediate generalized reactions caused by iodinated radiographic contrast material is controversial. Both immunoglobulin E (IgE)–mediated anaphylaxis and nonimmunologic mechanisms, based on direct release of inflammatory mediators, have been proposed to explain the occurrence of bronchoconstriction, urticaria, and vascular collapse in association with radiocontrast administration.[1-3] Gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA) dimeglumine, a metal-based contrast agent for magnetic resonance imaging (MRI) has been in clinical use since 1988, and adverse responses are rare.[4] Nonetheless, drug reactions necessitating admission to a hospital have occurred, and some have been anaphylactic in character.[5,6]

Mast cells are an important source of inflammatory mediators. They are found in most organs, including the skin, and play a central role in IgE-mediated allergic reactions. In addition, mast cells can be activated and induced to secrete histamine in response to a variety of nonimmunologic stimuli. Two gadolinium (Gd)-based contrast agents, Gd-DTPA dimeglumine and Gd-bismorpholide and two iodinated contrast agents, diatrizoate meglumine and iohexol, were evaluated for their ability to cause in vitro histamine release from a canine mastocytoma cell line. These cells have been well-characterized biochemically and functionally and share important characteristics of normal human mast cells.[7] Histamine release induced by each agent was compared to spontaneous histamine release from cells incubated without contrast media and to histamine release induced by mannitol, a purely osmotic stimulus.[8]

Methods

Contrast Agents

Diatrizoate meglumine (Angiovist 282, Berlex Laboratories, Inc., Wayne, NJ), iohexol (Omnipaque 300, Winthrop, New York, NY), and gadolinium DTPA dimeglumine (Magnevist, Ber-

lex Laboratories) were obtained commercially. Gadolinium bis-morpholide, an experimental nonionic paramagnetic agent, was provided by Schering A.G., Berlin, Germany. All contrast agents were diluted in Tyrode's buffer, pH 7.4, for incubation with cells.

Histamine Release from Mastocytoma Cells

Canine mastocytoma cells were isolated according to the method of Lazarus, et al.[7] Disaggregated cells were washed three times in calcium-magnesium-free Tyrode's buffer and then suspended in complete Tyrode's buffer containing 25 mM N-2-hydroxyethylpiperazine-N[1]-Z-ethanesulforic acid (HEPES) and 0.1% bovine serum albumin. Replicate aliquots of cells were incubated with calcium ionophore A23187 (Calbiochem), and with varying concentrations of Gd-DTPA dimeglumine, Gd-bismorpholide, diatrizoate meglumine, iohexol, and mannitol. Calcium ionophore A23187 was used to determine maximum stimulated release of histamine for each experiment, and mannitol served as a control for the effect of osmolality. The osmolality of selected samples was directly measured by freezing-point depression (model 3DII Osmometer, Advanced Instruments Inc., Needham Heights, MA). Included in each study were tubes that received cells but no releasing agent for calculation of spontaneous histamine release. Preliminary experiments indicate that the major portion of histamine release under the preceding conditions occurs at 1 to 2 minutes and that histamine levels remain stable for more than 45 minutes. Incubations were conducted for 30 minutes at 37°C in polypropylene tubes with a final reaction volume of 1 ml. At the end of the release experiment the reaction was stopped in ice and the cells centrifuged at 450 g for 10 minutes. Supernatant was separated from cell pellet, and perchloric acid was added to both cell and supernatant fractions to a final concentration of 0.2 M to lyse cells. Samples were stored at −70°C until assayed for histamine. Exclusion of the vital dyes erythrosin B and trypan blue established cell viability before and after incubation with contrast media.

Histamine Assay

Histamine was measured by an o-phthalaldehyde (OPT) spectrophotometric procedure modified for autoanalysis with an automated spectrofluorometric analyzer (Alpkem, Clakamus, OR). Histamine concentrations ≥1 ng/ml were detectable by this method. Histamine release was calculated as the amount of histamine present in the supernatant fraction expressed as a percentage of the total histamine in the supernatant and pellet fractions. The value for spontaneous release was determined for each experiment. Contrast agents were analyzed at each concentration evaluated before incubation with mast cells to exclude direct interference with the spectrophotometric assay.

Data Analysis

Histamine release was determined for each contrast agent at concentrations ranging from approximately 0.8 to 700 mM for diatrizoate meglumine and iohexol, and from 0.05 to 450 mM for Gd-DTPA dimeglumine and Gd-bismorpholide. Each data point represents the measured histamine release averaged from 4 to 10 experiments at each concentration. The mean value for spontaneous release of histamine was determined from tubes containing only mastocytoma cells and Tyrode's buffer. Histamine release in the presence of each contrast agent was compared to that of cells incubated without contrast media and of cells incubated with 1.8 M mannitol. One-way analysis of variance (ANOVA) was used to determine the validity of the data generated. The significance of histamine release at each measured concentration was evaluated by

multiple comparison t test (Student-Newman-Keuls). Values represent mean ± SD.

Results

All contrast agents examined caused significant ($P <$ .001) release of histamine compared with control mastocytoma cell samples incubated without contrast medium and compared with histamine release due to maximal osmotic stimulation. Figure 1 demonstrates the concentration dependence of histamine release due to each contrast agent. To assess the relative propensity of each agent to cause histamine release, an arbitrary threshold of 10% was chosen. This value is approximately two standard deviations above the mean for spontaneous histamine release determined from controls. The mean concentration causing histamine release greater than 10% and maximum level of histamine release were determined for each contrast agent. The results are summarized in Table 1.

Mastocytoma cells incubated with maximal concentrations of each contrast agent fail to absorb the vital dyes erythrosin B and trypan blue. Our finding that cell membranes remain intact indicates that measured release of histamine was due to active secretion from viable cells, rather than leakage from damaged ones.

Discussion

Previous examinations of iodinated contrast medium–induced histamine release have been performed in vitro by stimulation of histamine containing cells extracted from different experimental animals, and, in vivo, by measurement of serum histamine levels after contrast-medium administration.[9–13] This study considers in vitro histamine release due to ionic and nonionic MR contrast agents using a standardized cell line that shares many properties of human mast cells and is known to secrete inflammatory mediators in response to immunologic and nonimmunologic stimuli.[7] Representative ionic and nonionic conventional contrast material were also examined for purposes of comparison with Gd-based contrast agents under identical experimental conditions.

Regarding ionic, iodinated contrast media, our results are similar to those of Salem et al, who detected histamine release from a preparation of human leukocytes at concentrations of approximately 250 mM sodium diatrizoate. Our findings differ in two important respects. The sensitivity of the dog mastocytoma cell appears to be greater than that of the human leukocyte preparation used, as histamine release from diatrizoate meglumine occured at a concentration of 126 ± 50 mM. In addition, we did not detect a difference in histamine release from the ionic and nonionic iodinated agents examined in this study.[11]

Both iodinated and Gd-based contrast media caused release of histamine from cultured dog mastocytoma cells. The maximum level of histamine release when cells were
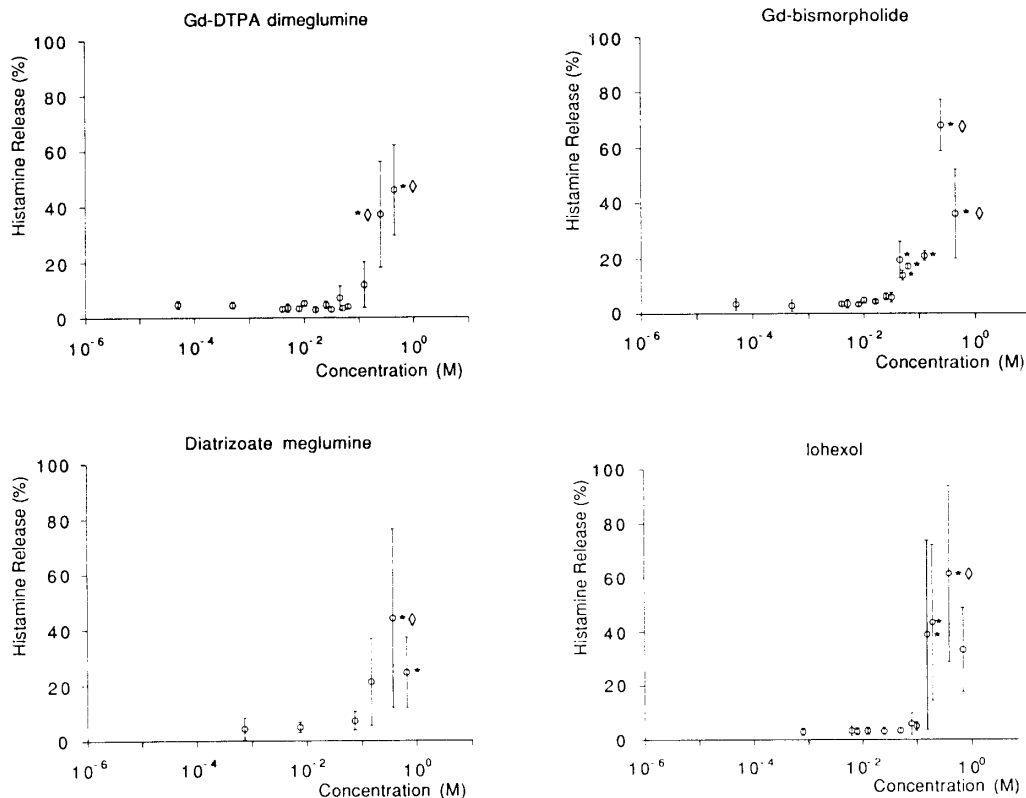
Fig. 1. Release of histamine from mastocytoma cells was determined after incubation with canine mastocytoma cells for each contrast agent evaluated. One-way analysis of variance (ANOVA) was used to determine the significance of the data generated ($P < .001$ for all experiments compared with control). Histamine release at each measured concentration was compared with spontaneous histamine release and to release induced by 1.8 M mannitol. Data were evaluated by multiple comparison t test (Student–Newman–Keuls) with $P < .05$ considered significant. Values represent mean ± SE. $^*P < 0.05$, compared with control. $^\diamond P < .05$, compared with mannitol.

incubated with selected iodinated and MR contrast agents was greater than both spontaneous histamine release and that induced by mannitol (Figs. 1 and 2). Ionic and nonionic contrast media caused release of histamine at similar molar concentrations. The effect of diatrizoate meglumine, iohexol, Gd-DTPA dimeglumine, and Gd-bismorpholide on dog mastocytoma cells is not due to osmotic induction of histamine release, but rather reflects a direct stimulatory action on the cells that depends on absolute concentration of contrast material (Fig. 2).

We examined the clinical implications of in vitro histamine release due to gadolinium-based and iodinated contrast agents. The mean concentration of contrast media

causing greater than 10% histamine release was compared with an estimate for the equilibrium serum concentration of each contrast agent as administered in typical imaging studies. A 10% threshold was arbitrarily chosen and represents a value approximately two standard deviations above mean in vitro spontaneous histamine release from mastocytoma

TABLE 1. Characteristics of Histamine Release due to Iodinated and Magnetic Resonance Contrast Agents

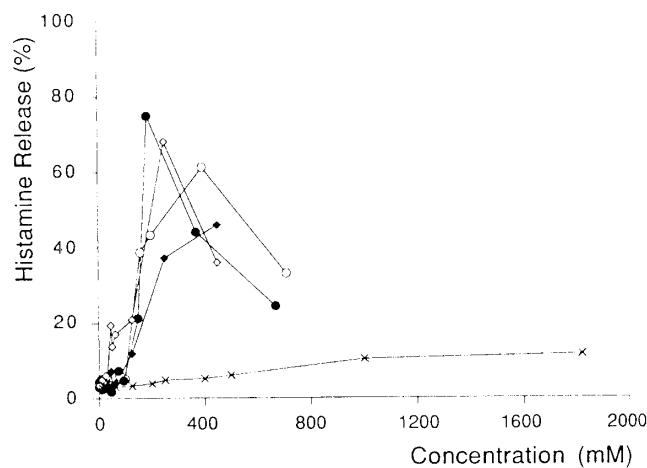| Agent	Concentration causing >10% histamine release in vitro (mM)	Maximum histamine release (%)
Diatrizoate	126 ± 50	44 ± 32
Iohexol	184 ± 78	61 ± 33
Gd-DTPA	153 ± 81	46 ± 16
Gd-bismorpholide	48 ± 26	68 ± 9
Mannitol	1000 (1286 mosm)	11.4 ± 1.6
Ca^{++} ionophore A23187	—	68 ± 13
Spontaneous release	—	4.6 ± 2.3

Fig. 2. Release of histamine from mastocytoma cells was examined with respect to the molar concentration of contrast agent with which cells were incubated. (●) diatrizoate meglumine; (○) iohexol; (♦) Gd-GTPA dimeglumine; (◊) Gd-bismorpholide; (×) mannitol.

cells. An estimate of serum concentration was determined by the following formula:

$$\text{Serum concentration} = (CM \times \text{dose})/(V_d \times \text{weight})$$

where, CM = concentration of administered contrast agent (mM); dose = the dose administered (ml); and V_d = volume of distribution of contrast medium (ml/kg).

A body CT scan typically requires 150 ml of iodinated contrast media. For diatrizoate meglumine and iohexol the concentration of undiluted contrast medium is approximately 700 mM. The volume of distribution for both iodinated contrast media and Gd-DTPA corresponds to that of the extracellular space (~270 ml/kg for man).[14,15] Assuming uniform distribution in extracellular fluid, the estimated concentration of diatrizoate meglumine and iohexol immediately after administration in a 70-kg adult is given by:

$$\text{Serum concentration} = (700 \text{ mM} \times 150 \text{ ml})/$$
$$(270 \text{ ml/kg})(70 \text{ kg}) = 5.6 \text{ mM}$$

The concentration of diatrizoate meglumine causing 10% histamine release in vitro is 126 ± 50 mM. The concentration of iohexol causing 10% histamine release in vitro is 184 ± 78 mM. These values are respectively 23 and 33 times greater than the estimated serum concentration of the iodinated contrast media examined (Table 2). It is possible, under certain circumstances, for the local concentration of iodinated contrast medium to approach a level that causes 10% histamine release in vitro. For example, transiently elevated local concentrations of contrast material occur during rapid-bolus arteriography. Alternatively, in susceptible individuals, high local concentrations of contrast media could cause release of histamine that might then initiate a cascade of inflammatory mediators, independent of contrast medium concentration.

The same analysis performed for Gd-DTPA, assuming a dose of 14 ml and undiluted concentration of 500 mM, yields an estimated equilibrium serum concentration of 0.4 mM. The concentration of Gd-DTPA causing 10% histamine release in vitro is 153 ± 81 mM. The concentration of Gd-bismorpholide causing 10% histamine release in vitro is

47 ± 26 mM. These values are 383 and 118 times the estimated equilibrium serum concentrations of these gadolinium chelates respectively (Table 2).

Concerning contrast media related anaphylactoid reactions, these data must be interpreted with caution. While the dog mastocytoma cell model permits comparison of different contrast agents under defined experimental conditions, the use of nonhuman cell lines in the evaluation of clinical phenomena is inherently limited. Furthermore, we chose the incubation time of 30 minutes to permit comparison of maximal histamine release at given contrast media concentrations. As there is a rapid decrease in local and systemic contrast media concentration in humans following injection, we obtained our data under conditions designed to be more sensitive to contrast induced histamine release than would be expected to occur in vivo.

We conclude that direct release of histamine from tissues exposed to contrast media is an unlikely explanation for the rare anaphylactoid reactions to Gd-DTPA observed in clinical MRI practice. The concentration of Gd-based contrast medium necessary to induce in vitro release of histamine is 100 to 400 times the estimated equilibrium serum concentrations following a standard administration. Upon exposure to iodinated contrast agents, mastocytoma cells require concentrations approximately 20 to 30 times that of estimated serum levels to release histamine in vitro. While direct histamine release probably cannot explain the majority of anaphylactoid reactions to iodinated agents, concentrations sufficient to cause histamine release might be achieved transiently during high-dose, rapid bolus administration studies such as pulmonary angiography. Experiments designed to more closely approximate in vivo conditions would be necessary to evaluate this possibility.

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TABLE 2. Comparison of in vitro Histamine Release to Estimated Serum Contrast Media Concentration

Agent	Estimated serum concentration for body CT or MRI examination (mM)	Concentration causing >10% histamine release in vitro (mM) Estimated serum concentration for body CT or MRI examination (mM)
Diatrizoate	5.6	23
Iohexol	5.6	33
Gd-DTPA	0.4	383
Gd-bismorpholide	0.4	118

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