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ORIGINAL ARTICLE

Influence of iodinated contrast media on the activities of histamine inactivating enzymes diamine oxidase and histamine N-methyltransferase in vitro

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Abstract
Background: Iodinated contrast media can cause pseudoallergic reactions associated with histamine release in significant numbers of patients. To clarify whether these adverse reactions may be aggravated by a compromised histamine catabolism we asked if radiographic contrast agents in vitro inhibit the histamine inactivating enzymes diamine oxidase (DAO) and histamine N-methyltransferase (HMT).
Methods: Nine iodinated contrast agents were tested in vitro. Following pre-incubation of purified porcine kidney DAO and recombinant human HMT with 0.1–10 mM of the respective contrast medium (H_2O and specific inhibitors of DAO and HMT as controls) enzyme activities were determined by using radiometric micro assays.
Results: None of the contrast media irrespective of their structure showed significant inhibition of the activities of DAO and HMT. Pre-incubation of the enzymes with specific inhibitors led to complete inhibition of the respective enzymatic activity.
Conclusions: The iodinated contrast media tested in vitro did not exhibit inhibition of histamine converting enzymes at physiologically relevant concentrations. However due to the in vitro character of this study these results do not directly reflect the in vivo situation.

Introduction

Iodinated contrast media (CM) are widely used in computed tomography and angiography. Anaphylactoid reactions have been reported as a side effect after intravenous application of these contrast agents.[1] These are usually characterised by cutaneous symptoms including erythema, pruritus, and urticaria, but gastrointestinal, respiratory, or cardiovascular

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complaints can occur as well.[2] The incidence of these reactions has decreased after introduction of non-ionic substances and has been reported in various studies to be 0.6–3.1%.[3] With an incidence of 0.01–0.04% severe anaphylactoid reactions are rare but unpredictable.[3] Several pre-treatment schemes for their prevention have been discussed, but there is evidence that they are not effective in every case and the risk of adverse reactions can only be reduced rather than completely abolished.[4,5]

The pathophysiology of these anaphylactoid reactions has been discussed controversially and the biochemical pathways are still poorly understood.[1,4] Serious reactions are similar to those of type 1 hypersensitivity according to Coombs and Gell.[1] However, CM molecules are very small and would not likely form haptens that can bind to antibodies.[6]

The biogenic amine histamine acts as an inflammatory mediator, a neurotransmitter, and a regulator of hydrochloric acid secretion from gastric mucosa by binding to and activating four different histamine receptors.[7,8] Histamine has been proposed to be the primary mediator of adverse reactions to contrast media, since symptoms of anaphylaxis can be reproduced by histamine infusions and antihistamines provide effective treatment.[1] In addition, it has been shown that iodinated contrast media can lead to significant histamine release in vitro.[9]

In man, histamine can be inactivated by two alternative pathways catalysed by the enzymes diamine oxidase (DAO, EC 1.4.3.6) and histamine N-methyltransferase (HMT, EC 2.1.1.8), respectively.[10] DAO oxidatively deaminates the primary amino group of histamine forming imidazole acetaldehyde. HMT catalyses the transfer of a methyl group from S-adenosylmethionine (SAM) to the imidazole ring of histamine forming N^{τ}-methylhistamine, which is then further oxidised by monoamine oxidase (MAO, EC 1.4.3.4).[8,10] DAO is expressed in various mammalian tissues including intestine, kidney, and placenta.[11] DAO is a secretory protein that is likely to be responsible for the inactivation of extracellular histamine.[12] HMT is found in most of the human tissues including brain, lung, stomach, intestine, kidney, and liver.[13] It is a cytosolic protein that can convert histamine only inside the cells.[14,15]

A large number of drugs including histamine antagonists, corticosteroids, antibiotics, antimalarials, tranquillisers, antidepressants, neuroleptics, local anaesthetics, dinatriumcromoglycate, and verapamil were reported to inhibit the enzymatic activities of DAO and HMT.[16–18] The effect of iodinated contrast media on the activities of the histamine inactivating enzymes DAO and HMT has not been evaluated so far. Since inadequate histamine inactivation is associated with the development of histamine-related adverse reactions[19] and could thus contribute to and aggravate the side effects reported for CM we analysed if these contrast agents inhibit DAO and HMT activities in vitro.

Materials and methods

Contrast agents

The non-ionic monomeric contrast media iomeprol (Imeron®, Bracco Altana, Konstanz, Germany), ioversol (Optiray®, Guerbet, Roissy, France), iopamidol (Solutrast®, Bracco Altana, Konstanz, Germany), iobitridol (Xenetix®, Guerbet, Roissy, France), and iopromid (Ultravist®, Schering, Berlin, Germany), the non-ionic dimeric substances iodixanol (Visipaque®, GE Healthcare AS, Oslo, Norway) and iotrolan (Isovist®, Schering, Berlin, Germany), and the ionic contrast agents iothalamate meglumine (Conray®, Mallinckrodt, St. Louis, USA) and amidotrizoic acid (Peritrast®, Dr. Köhler Chemie, Alsbach-Hähnlein, Germany) were tested. The properties of these CM are summarised in Table 1.

Radiometric diamine oxidase micro assay

DAO activity was determined by using a radiometric micro assay based on the conversion of [^{14}C]putrescine (1,4-diamino-[1,4-^{14}C]butane) to 4-aminobutyraldehyde that is spontaneously converted to Δ_1-pyrroline, which can be extracted into an organic solvent for quantitation by liquid scintillation counting.[20,21] As a source of DAO activity we used homogenously purified porcine kidney DAO, which is highly homologous to human DAO and has almost identical enzymatic properties but is much easier to obtain in pure form in sufficient amounts.[12,22] Briefly, in a total volume of 90 μl, purified pig kidney DAO (81 μU, 54 ng) was pre-incubated in 100 mM sodium phosphate pH 7.2 with 0.1–10 mM of the respective CM for 30 min at 37 °C. Controls included pre-incubation without any additions or in the presence of 0.01–1 mM of the specific DAO inhibitor aminoguanidine.[23] The reaction was then started by addition of 10 μl [^{14}C]putrescine (0.222 Ci/mol, 1 nCi/μl, 4.5 mM; GE Healthcare, Little Chalfront, UK), incubated for 30 min at 37 °C, and stopped by addition of 10 μl 10% perchloric acid followed by alkalisation with 50 μl sodium carbonate pH 12.2. The reaction product [^{14}C]Δ_1-pyrroline was extracted into 1600 μl toluene containing 0.35% 2,5-diphenyloxazole (PPO) as a scintillator. Radioactivity was determined by liquid scintillation counting using a Packard Tri-Carb 2500TR Liquid Scintillation Analyzer. Mean enzymatic activity was determined from duplicate assays and inhibition of DAO activity was calculated relative to the uninhibited control that had a mean activity of 891 dpm corresponding to 81 μU (1 μU = 1 nmol/min).

Radiometric histamine N-methyltransferase micro assay

Measurement of HMT activity is based on the transmethylation of histamine with [^{14}C]SAM (S-adenosyl-L-[methyl-^{14}C]methionine) forming radioactively labelled N^{τ}-methylhistamine that can be extracted at an alkaline pH into an organic solvent for quantitation by liquid scintillation counting.[14,24] As a source of HMT activity we used homogenously purified recombinant human HMT that has identical enzymatic properties as the native enzyme purified from tissues.[15,25] Briefly, in a total volume of 80 μl, purified recombinant human HMT (28 μU, 6 ng) was pre-incubated in 100 mM sodium phosphate pH 7.5 with 0.1–10 mM of the respective CM for 30 min at 37 °C. Controls included pre-incubation without any additions or in the presence of 1–100 μM of the specific HMT inhibitor amodiaquine.[26]

Table 1 Properties of radiographic contrast media used for inhibition studies. M = molecular weight of substance, c_{iodine} = mass concentration of iodine in commercial preparation, c = mass concentration of substance in commercial preparation. n/a = data not available.

Substance	Brand name	Structure	Osmolarity	Osmolality [mOsm/kg H_2O]	c_{iodine} [g/l]	c [g/l]	M [g/mol]
Iomeprol	Imeron®	Monomeric, non-ionic	Low	618	350	714	777
Ioversol	Optiray®	Monomeric, non-ionic	Low	645	300	636	807
Iopamidol	Solutrast®	Monomeric, non-ionic	Low	616	300	612	777
Iobitridol	Xenetix®	Monomeric, non-ionic	Low	695	300	658	835
Iopromid	Ultravist®	Monomeric, non-ionic	Low	590	300	623	791
Iodixanol	Visipaque®	Dimeric, non-ionic	Iso	290	320	652	1550
Iotrolan	Isovist®	Dimeric, non-ionic	Iso	270	300	641	1626
Iothalamate meglumine	Conray®	Ionic	High	1400	282	600	808
Amidotrizoic acid	Peritrast®	Ionic	High	n/a	300	600	760

The reaction was then started by the addition of 10 μl 500 μM histamine and 10 μl [^{14}C]SAM (2 Ci/mol, 1 nCi/μl, 0.5 mM; GE Healthcare, Little Chalfront, UK), incubated for 30 min at 37 °C, and stopped by addition of 60 μl of a solution of 500 mM boric acid and 1000 mM sodium hydroxide causing a pH shift that facilitates the extraction of the product [^{14}C]N$^{\tau}$-methylhistamine into 1600 μl of toluene/isoamylalcohol (1:1) containing 0.17% PPO as a scintillator. Radioactivity was determined by liquid scintillation counting using a Packard Tri-Carb 2500TR Liquid Scintillation Analyzer. Mean enzymatic activity was determined from duplicate assays and inhibition of HMT activity was calculated relative to the uninhibited control that had a mean activity of 2887 dpm corresponding to 28 μU (1 μU = 1 nmol/min).

Statistical analyses

Statistical analyses were performed by using the SPSS for Windows Version 15.0 software package (SPSS, Chicago, IL, USA). Differences in enzymatic activities of DAO and HMT between samples pre-incubated in the presence of CM and controls were assessed with repeated measures test. Correlation of enzymatic activities with CM concentrations was investigated by Spearman's correlation statistics. A two-tailed p value below 0.05 was considered statistically significant.

Results

The CM concentrations following bolus injection were calculated based on the recommended dosage. In a 70-kg person, a 100-mL injection of iodinated RCM (300 mg I/ml) would yield an extracellular concentration of approximately 2 mg iodine/ml corresponding to 3–8 mmol/l in the equilibrium. Thus, the concentrations used in the assays (0.1–10 mM) covered the whole physiological range.

The results of the determination of the enzymatic activities of DAO and HMT following pre-incubation with physiologically relevant concentrations of the iodinated contrast media tested are shown in Table 2. As it is evident from these data and confirmed by statistical analyses using the repeated measures test, none of the radiographic CM exhibited significant concentration dependent inhibition of DAO ($p = 0.2145$) and HMT ($p = 0.1911$). In contrast, DAO and HMT were efficiently inhibited by the specific inhibitors aminoguanidine and amodiaquine, respectively, at concentrations of 10 μM, showing that the inhibition assays worked as expected.

None of the substances irrespective of their monomeric, dimeric, ionic, or non-ionic structure, their osmolality, or their osmolarity inhibited the activities of DAO and HMT by more than 15% and no concentration dependence was observed for any of the inhibitory effects. Interestingly, pre-incubation with certain substances led to a slightly higher activity than that determined for the control. A slightly higher DAO activity was measured after pre-incubation with iopamidol, iobitridol, iopromid, iodixanol, and amidotrizoic acid whereas HMT activity was slightly higher after pre-incubation with iothalamate meglumine and amidotrizoic acid. However, these small stimulatory effects on DAO or HMT activity were not statistically significant and not significantly concentration dependent (Spearman correlation: DAO: $r = 0.1111$, $p = 0.5812$; HMT: $r = 0.0381$, $p = 0.8503$).

In summary, our results show that at concentrations up to 10 mM the radiographic contrast media tested do not significantly inhibit the activities of the histamine inactivating enzymes DAO and HMT in vitro.

Discussion

Although iodinated contrast agents are routinely used in diagnostic imaging, relatively little is known about their biological effects in patients. Among the serious complications associated with the application of iodinated CM are the so-called anaphylactoid reactions that resemble type I hypersensitivity reactions but often without a clear involvement of specific IgE.[1] It has been shown that iodinated CM can induce receptor-independent release of inflammatory mediators from basophiles and mast cells in vitro and in vivo, providing a possible explanation for their side effects in patients.[1,27] However – although the pathophysiology of CM hypersensitivity reactions is still discussed controversially – actually its understanding seems to change. Recent studies

Table 2 Enzymatic activities of DAO and HMT after incubation with 0.1–10 mM of iodinated contrast media. Activities were calculated relative to uninhibited controls treated identically but incubated without any additions (100%), which were 891 dpm corresponding to 81 μU for DAO and 2887 dpm corresponding to 28 μU for HMT, respectively. For positive inhibition controls, DAO was incubated with 0.01–1 mM aminoguanidine and HMT with 0.001–0.1 mM amodiaquine, respectively. nd = not determined.

Substance	DAO activity [%]			HMT activity [%]		
Control	100			100		
CM concentrations	0.1 mM	1 mM	10 mM	0.1 mM	1 mM	10 mM
Iomeprol	93	98	97	102	102	101
Ioversol	91	92	92	100	100	99
Iopamidol	109	109	110	100	102	97
Iobitridol	106	110	106	96	97	95
Iopromid	104	105	106	96	100	98
Iodixanol	108	109	108	97	100	98
Iotrolan	96	98	98	96	95	86
Iothalamate meglumine	96	97	98	99	104	107
Amidotrizoic acid	114	119	110	94	105	122
Substance	DAO activity [%]			HMT activity [%]		
Control	100			100		
Inhibitor concentrations	0.01 mM	0.1 mM	1 mM	0.001 mM	0.01 mM	0.1 mM
Aminoguanidine	0	0	0	nd	nd	nd
Amodiaquine	nd	nd	nd	21	3	0

described positive skin tests in at least 50% of the patients, and detected CM specific IgE antibodies in sera of immediate reactors. These observations suggest that severe immediate reactions may be IgE mediated and could therefore be really allergic. In contrast late adverse reactions appear to be T cell mediated.[28–31]

One of the most important of these inflammatory mediators is histamine whose plasma concentration was found to be elevated in patients with early adverse reactions to iodinated CM.[32] Therefore, we asked if in addition to the possible release of histamine these substances might also inhibit histamine inactivation, thus aggravating the effect of this mediator in the observed anaphylactoid reactions.

We tested nine iodinated CM for their possible inhibitory effect on the enzymatic activities of the two histamine inactivating enzymes diamine oxidase and histamine N-methyltransferase in vitro. The radiometric assays used in this study are very sensitive, give highly reproducible results, and provide an accurate estimate of histamine degradation.

Our results clearly showed that at physiologically relevant concentrations, none of the substances tested exhibited significant inhibition of either DAO or HMT, the enzymes catalysing the first steps of histamine catabolism outside and inside cells, respectively. A few of the agents tested even slightly increased DAO and HMT enzymatic activities in vitro. Therefore, we conclude that the iodinated CM tested do not directly affect the inactivation of histamine. However, it is important to note that our observations are based on in vitro experiments which do not consider in vivo conditions of proteins, blood and endothelial cells, which may also play a role in CM hypersensitivity reactions. Therefore, our data do not directly reflect the in vivo situation since the study design only allows us to evaluate the direct effect of CM on histamine degradation.

As stated above, iodinated CM can release histamine from basophiles and mast cells in vitro and in vivo, leading to elevated plasma histamine concentration and severe pseudoallergic reactions.[9,33–35] It is conceivable that this non-immunologic histamine release might overcharge the endogenous histamine degradation capacity, especially in patients with low enzyme levels of DAO and HMT. In fact, there is considerable individual variation in the expression and steady state tissue concentration of histamine inactivating enzymes and recently genetic polymorphisms were described for the DAO and HMT genes that appear to be associated with lower activities of the corresponding enzymes.[36,37] Future studies will have to clarify whether patients with low basal DAO and HMT activities are more prone to develop adverse reactions upon administration of iodinated contrast media. This will help to minimise the risk for these patients and to develop efficient therapeutic strategies to counteract the side effects.

Ethical disclosures

Protection of human and animal subjects in research. The authors declare that no experiments were performed on humans or animals for this investigation.

Patients' data protection. Confidentiality of data. The authors declare that no patient data appears in this article.

Right to privacy and informed consent. The authors declare that no patient data appears in this article.

Conflict of interest

The authors have no conflict of interest to declare.

References

1. Morcos SK. Review article: acute serious and fatal reactions to contrast media: our current understanding. Br J Radiol. 2005;78:686–93.
2. Ring J, Behrendt H. Anaphylaxis and anaphylactoid reactions. Classification and pathophysiology. Clin Rev Allergy Immunol. 1999;17:387–99.
3. Christiansen C. X-ray contrast media—an overview. Toxicology. 2005;209:185–7.
4. Idee JM, Pines E, Prigent P, Corot C. Allergy-like reactions to iodinated contrast agents. A critical analysis. Fundam Clin Pharmacol. 2005;19:263–81.
5. Lasser EC, Berry CC, Talner LB, Santini LC, Lang EK, Gerber FH, et al. Pretreatment with corticosteroids to alleviate reactions to intravenous contrast material. N Engl J Med. 1987;317:845–9.
6. Eloy R, Corot C, Belleville J. Contrast media for angiography: physicochemical properties, pharmacokinetics and biocompatibility. Clin Mater. 1991;7:89–197.
7. Hough LB. Genomics meets histamine receptors: new subtypes, new receptors. Mol Pharmacol. 2001;59:415–9.
8. Pearce FL. Biological effects of histamine: an overview. Agents Actions. 1991;33:4–7.
9. Baxter AB, Lazarus SC, Brasch RC. In vitro histamine release induced by magnetic resonance imaging and iodinated contrast media. Invest Radiol. 1993;28:308–12.
10. Maslinski C, Fogel WA. Catabolism of histamine. In: Uvnäs B, editor. Handbook of experimental pharmacology. Histamine and histamine antagonists, vol. 97. Berlin, Heidelberg: Springer; 1991. p. 165–89.
11. Schwelberger HG, Hittmair A, Kohlwein SD. Analysis of tissue and subcellular localization of mammalian diamine oxidase by confocal laser scanning fluorescence microscopy. Inflamm Res. 1998;47 Suppl. 1:S60–1.
12. Schwelberger HG. Diamine oxidase (DAO) enzyme and gene. In: Falus A, editor. Histamine: Biology and Medical Aspects. Budapest: SpringMed Publishing; 2004. p. 43–52.
13. Hesterberg R, Sattler J, Lorenz W, Stahlknecht CD, Barth H, Crombach M, et al. Histamine content, diamine oxidase activity and histamine methyltransferase activity in human tissues: fact or fictions. Agents Actions. 1984;14:325–34.
14. Brown DD, Tomchick R, Axelrod J. The distribution and properties of a histamine-methylating enzyme. J Biol Chem. 1959;234:2948–50.
15. Schwelberger HG. Histamine N-methyltransferase (HNMT) enzyme and gene. In: Falus A, editor. Histamine: biology and medical aspects. Budapest: SpringMed Publishing; 2004. p. 53–9.
16. Pacifici GM, Donatelli P, Giuliani L. Histamine N-methyl transferase: inhibition by drugs. Br J Clin Pharmacol. 1992;34:322–7.
17. Sattler J, Hesterberg R, Lorenz W, Schmidt U, Crombach M, Stahlknecht CD. Inhibition of human and canine diamine oxidase by drugs used in an intensive care unit: relevance for clinical side effects. Agents Actions. 1985;16:91–4.
18. Tachibana T, Taniguchi S, Imamura S, Fujiwara M, Hayashi H. Effects of drugs on the activity of histamine-N-methyltransferase from guinea pig skin. Biochem Pharmacol. 1988;37:2872–6.
19. Maintz L, Novak N. Histamine and histamine intolerance. Am J Clin Nutr. 2007;85:1185–96.
20. Kusche J, Richter H, Hesterberg R, Schmidt J, Lorenz W. Comparison of the 14-C-putrescine assay with the NADH test for the determination of diamine oxidase: description of a standard procedure with a high precision and an improved accuracy. Agents Actions. 1973;3:148–56.
21. Schwelberger HG, Klocker J, Sattler J, Bodner E. Determination of the activity of diamine oxidase in extremely small tissue samples. Inflamm Res. 1995;44 Suppl. 1:S94–5.
22. Wilflingseder D, Schwelberger HG. Highly efficient purification of porcine diamine oxidase. J Chromatogr B Biomed Sci Appl. 2000;737:161–6.
23. Tamura H, Horiike K, Fukuda H, Watanabe T. Kinetic studies on the inhibition mechanism of diamine oxidase from porcine kidney by aminoguanidine. J Biochem. 1989;105:299–306.
24. Kufner MA, Ulrich P, Raithel M, Schwelberger HG. Determination of histamine degradation capacity in extremely small human colon samples. Inflamm Res. 2001;50 Suppl. 2:S96–7.
25. Huetz GN, Schwelberger HG. Simultaneous purification of the histamine degrading enzymes diamine oxidase and histamine N-methyltransferase from the same tissue. Inflamm Res. 2003;52 Suppl. 1:S65–6.
26. Barth H, Lorenz W, Troidl H. Effect of amodiaquine on gastric histamine methyl-transferase and on histamine-stimulated gastric secretion. Br J Pharmacol. 1975;55:321–7.
27. Peachell PT, Morcos SK. Effect of radiographic contrast media on histamine release from human mast cells and basophils. Br J Radiol. 1998;71:24–30.
28. Brockow K, Christiansen C, Kanny G, Clément O, Barbaud A, Bircher A, et al. Management of hypersensitivity reactions to iodinated contrast media. Allergy. 2005;60:150–8.
29. Kvedariene V, Martins P, Rouanet L, Demoly P. Diagnosis of iodinated contrast media hypersensitivity: results of a 6-year period. Clin Exp Allergy. 2006;36:1072–7.
30. Brockow K, Romano A, Aberer W, Bircher AJ, Barbaud A, Bonadonna P, et al. Skin testing in patients with hypersensitivity reactions to iodinated contrast media—a European multicenter study. Allergy. 2009;64:234–41.
31. Dewachter P, Laroche D, Mouton-Faivre C, Bloch-Morot E, Cercueil JP, Metge L, et al. Immediate reactions following iodinated contrast media injection: a study of 38 cases. Eur J Radiol. 2011;77:495–501.
32. Laroche D, Aimone-Gastin I, Dubois F, Huet H, Gérard P, Vergnaud MC, et al. Mechanisms of severe, immediate reactions to iodinated contrast material. Radiology. 1998;209:183–90.
33. Ennis M, Lorenz W, Nehring E, Schneider C. In vitro and in vivo studies of radiographic contrast media-induced histamine release in pigs. Agents Actions. 1991;33:26–9.
34. Ennis M, Nehring E, Schneider C. Adverse reactions to drugs: in vitro studies with isolated cells. Inflamm Res. 2004;53 Suppl. 2:S105–8.
35. Laroche D. Immediate reactions to contrast media: mediator release and value of diagnostic testing. Toxicology. 2005;209:193–4.
36. Petersen J, Raithel M, Schwelberger HG. Characterisation of functional polymorphisms of the human diamine oxidase gene. Inflamm Res. 2005;54 Suppl. 1:S58–9.
37. Preuss CV, Wood TC, Szumlanski CL, Raftogianis RB, Otterness DM, Girard B, et al. Human histamine N-methyltransferase pharmacogenetics: common genetic polymorphisms that alter activity. Mol Pharmacol. 1998;53:708–17.