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Short Communication

Measurement of histamine in rat bronchoalveolar lavage fluid by high-performance cation-exchange chromatography coupled with post-column derivatization to *o*-phthaldialdehyde

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ABSTRACT

To better define involvement of mast cell derived mediators in the pulmonary response to fibrogenic dusts, a rapid and accurate method was required to analyze samples of bronchoalveolar lavage fluid for histamine. Samples of rat lung lavage were analyzed for histamine via high-performance cation-exchange chromatography coupled with post-column derivatization with *o*-phthaldialdehyde. The fluorescent derivative could be detected to *ca.* 1 ng/ml of lavage. Recoveries averaged 94.2% with an average relative standard deviation of ± 5.3%. There were no correlations between amount or fibrogenicity of inhaled dust and subsequent release of histamine into lavage fluid.

INTRODUCTION

There is increasing evidence that mast cell degranulation products (*e.g.* histamine) are involved in the pathogenesis of pulmonary fibrosis [1,2]. To better understand the role of histamine release by mast cells in chronic lung disease, we sought to monitor changes in lung histamine levels in an animal model of pulmonary fibrosis [3]. This required a histamine analysis method which was rapid, sensitive and could be routinely used for large numbers of bronchoalveolar lavage (BAL) samples. Many histamine methods [4–6] require extensive sample preparation and analysis time. To avoid these limitations we adopted the straightforward method of Yamotodani *et al.* [7] and modified it for analysis of rat lung lavage fluid. It combines strong cation-exchange high-performance liquid chromatography (HPSCX) and post-column derivatization with *o*-phthaldialdehyde (OPA) for the rapid and selective measurement of histamine with minimal sample clean up.

EXPERIMENTAL

Materials

OPA, histamine diphosphate, cadaverine, putrescine, spermine and spermidine were from Sigma (St. Louis, MO, USA), phosphate-buffered saline (PBS) was from Gibco (Grand Island, NY, USA). All other compounds were reagent grade or better. Stock solutions of OPA and histamine were prepared daily.

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Chromatography

The liquid chromatograph was a Waters 600 multisolvent delivery system equipped with a 700 Satellite WISP auto-injector (with refrigeration unit) and a Kratos fluorescence detector (Model FS970). Separations were done isocratically on a 100 mm × 4.6 mm I.D., 5 μm particle size, Partisil 5 SCX RAC11 column (Whatman, Clifton, NJ, USA) with 0.17 *M* KH_2PO_4 at a flow-rate of 1.0 ml/min. A 250 mm × 4.6 mm I.D. column filled with 30–40 μm silica had been installed between the pump system and the WISP to saturate the solvent with silicon. Silicon in the solvent inhibited destruction of the separation column. Column eluate was mixed with alkaline OPA solution in a turbulent mixer. The OPA derivatizing solution was prepared as described [7] and delivered to the mixer via an Eldex pump (Model A-30-S) at *ca.* 0.5 ml/min. The reaction mixture then flowed into a reaction coil (PTFE tubing 305 cm × 0.5 mm I.D.) wound around an aluminum heating block maintained at 65°C (Flatron Heater FH-30 and Flatron Controller TC-50). Optimum sensitivity was obtained when the post-column reaction pH was adjusted to between 11.7 and 12.0. Effluent pH was monitored via a VWR pH meter (Model 2000). Contrary to Yamotodani *et al.* [7], the effluent was not acidified because endogenous histamine exceeded 1 ng/ml. The OPA derivative was detected using an excitation wavelength of 230 nm and a 418 nm Kratos filter.

Preparation of samples

Animals were instilled with saline, a fibrogenic dust silicon dioxide (SiO_2), or a relatively innocuous dust titanium dioxide (TiO_2) and BAL collection done as previously described with PBS [3]. Samples were immediately frozen until analysis. Lung BAL samples were thawed, mixed and filtered (Millex 0.22 μm unit). The samples were kept at 5°C until injection. As recommended [8] only polyethylene plastic ware was used in preparing the samples and standards. An injection volume of 100 μl was applied.

Coelution of polyamines

Standards were prepared in PBS. Interference with histamine detection was assessed by determining retention time values of individual polyamines in PBS or spiked into a control lavage sample.

Preparation of calibration standards

Histamine standards equivalent to 3–65 ng/ml of lavage were prepared in PBS and analyzed on the system as described above.

RESULTS AND DISCUSSION

Chromatography

The combination of SCX and post-column derivatization with OPA provided sufficient selectivity to detect histamine in crude BAL samples, where histamine eluted at *ca.* 4.6 min. Fig. 1 shows a chromatogram of control lung lavage with endogenous histamine. Peak identity was confirmed by co-injection of histamine standards with lavage samples. Other polyamines had respective retention times in PBS of cadaverine (void volume), spermine (void volume), putrescine (void volume) and spermidine (9.90 and 10.3 min). They were not detectable when spiked into lavage fluid at concentrations equivalent to 30–40 ng injected on column (data not shown).

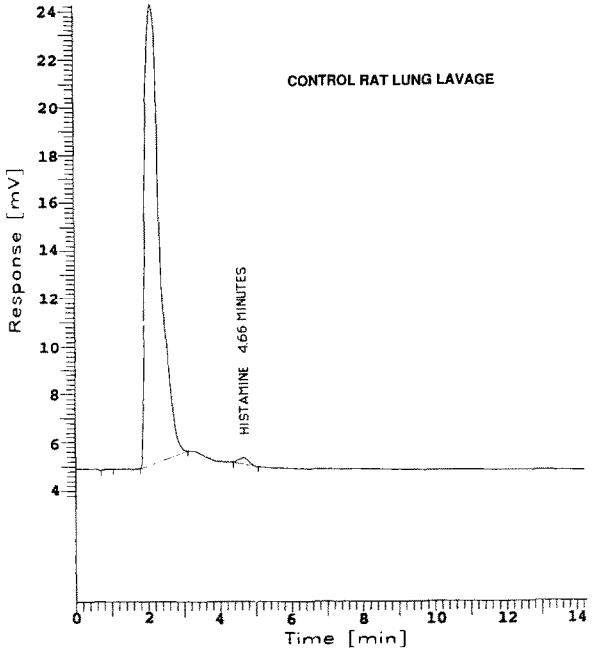

Fig. 1. Representative chromatogram of a lavage sample from control rats showing endogenous histamine. Histamine eluted at *ca.* 4.6 min. Samples were prepared as described in the text.

TABLE I

REPRODUCIBILITY OF SAME-DAY AND DAY-TO-DAY HISTAMINE ANALYSES

Amount injected (ng)	Amount detected, mean ± S.D. (ng)	
	Same day[a]	Day to day[b]
3.28	3.04 ± 0.05	3.35 ± 0.18
6.56	6.34 ± 0.19	6.63 ± 0.12
9.84	9.34 ± 0.27	10.01 ± 0.58
16.40	15.24 ± 0.91	16.78 ± 0.86

[a] Four replicates of each sample were analyzed.
[b] Replicates were analyzed on three consecutive days.

Method accuracy and reproducibility

Data on the precision and accuracy of the analysis are shown in Table I. Multiple injections of histamine standards ($n = 4$) had R.S.D. values from 1.8 to 6.0% in the range 3.28–16.4 ng/ml, with an average value of 3.4%. Day-to-day variation for the same concentration range ran from 2.7 to 6.4% R.S.D., with an average value of 5.3%. Accuracy was assessed by measuring total histamine in spiked samples. The average recovery from three sets of spiked samples ($n = 4$) was 94.2% (Table II).

Linearity and detection limit

The results from the procedure were linear between 1 and 65 ng/ml with a correlation coefficient (R) of 0.997. Most BAL histamine concentrations were between 7 and 20 ng/ml and thus in the linear range of the assay. Histamine was detected to at least 1 ng/ml with a signal-to-noise ratio of 3:1.

TABLE II

RECOVERY OF HISTAMINE FROM SPIKED SAMPLES

Amount added (ng)[a]	Amount recovered, mean ± S.D. (ng)	R.S.D. (%)	Recovery (%)[b]
3.33	2.95 ± 0.146	4.95	88.5
5.49	5.37 ± 0.158	2.94	97.8
11.11	10.29 ± 0.225	2.19	96.2

[a] Replicates of lung lavage ($n = 4$) were spiked with histamine stock solution.
[b] Percentage recovery values were averaged for the four replicates.

TABLE III

HISTAMINE VALUES FROM BAL SAMPLES OF SALINE- AND DUST-TREATED RATS

Day	Concentration of histamine in lavage (ng/ml)[a]		
	Saline[b]	Titanium dioxide[b,c]	Silicon dioxide[b,c]
1	15	21	7
7	25	10	21
14	21	11	14
28	13	8	12
60	9	4	7

[a] BAL was collected and analyzed as described in Experimental.
[b] Each value represents an average from the five animals in each group.
[c] Dusts were instilled at a ratio of 1 mg/100 g of body weight.

Sample values

Table III contains data from control rats and those treated with SiO_2 or TiO_2. Each data point was within the linear range of the assay. There were no correlations between BAL histamine concentrations and the fibrogenicity of the dusts. Further evidence that histamine concentration was not correlated to the toxicity of the dusts was evident from the wide range in values (±30–80%) for the samples. Each value in the table represents an average from five different animals.

DISCUSSION

Other methods have been described for measuring histamine in lung lavage which provide comparable [4,5,9] or better [6] analytical accuracy and precision to ours. However, these methods require substantial sample preparation and so are not as rapid [9] or suitable for automation as our method. The reduced sample preparation facilitated by our approach lessens the potential of analytical artefacts. Further, as discussed by Allenmark *et al.* [10], post-column derviatization is desirable for reasons of column selectivity and flexibility in reaction conditions. The non-interference of selected polyamines is a good example

of this point. Precolumn derivatization [9] may not always provide the necessary selectivity for complex matrices such as BAL.

Our results with HPSCX of lung lavage fluid were consistent with previous reports on other sample matrices [7,10,11] in terms of precision, linearity and percent recovery. Because the histamine/OPA conjugate was not acidified, the limit of detection was *ca.* 1 ng/ml, *versus* 5–10 pg/ml reported by other workers [7,10–12]. Lavage fluid from these studies contained enough histamine that the extra sensitivity was not required.

Previous studies have demonstrated increased BAL histamine levels in patients with idiopathic pulmonary fibrosis and increased histamine in lung tissue from rats developing fibrosis after exposure to bleomycin [1,2]. The lack of correlation between BAL histamine and fibrosis in the present study suggests that mast cells and mast cell-derived products may not be involved in silica-induced fibrosis. Alternatively, the possibility can not be excluded that increased levels of mast cell products are released after dust exposure, but into a lung compartment not accessible by BAL (*e.g.*, pulmonary interstitium).

In conclusion, histamine can be quickly and accurately measured at 1 ng/ml levels in rat lung lavage by SCX followed by post-column derivatization with OPA and subsequent fluorescence detection. This method should be applicable to other complex sample matrices.

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