

Seasonal variations of total histamine in patients with seasonal allergic rhinitis

MAKOTO HASEGAWA*, YOZO SAITO*, FUJI NAKA† and MASAYOSHI KIYOI†

* *Department of Otorhinolaryngology, Tokyo Medical and Dental University, Tokyo,*
and † *The Nipponzoki Laboratory, Osaka, Japan*

(Received 21 July 1981; resubmitted 31 December 1981; accepted for publication 31 December 1981)

Summary

Seasonal variations of total blood histamine were examined in twenty patients with seasonal allergic rhinitis (Japanese cedar pollinosis). In blood specimens taken in the pollen season of 1978, the symptom-free season of 1978, and the pollen season of 1979, the mean values of blood histamine were 61.6, 50.0 and 84.2 ng/ml respectively. The differences in blood histamine levels between the pollen season of 1978 and the symptom-free season of 1978 and between the symptom-free season of 1978 and the pollen season of 1979 were statistically significant. In the symptom-free season of 1978, the blood histamine levels of twenty normal subjects were also examined; the mean value was 58.2 ng/ml. The blood histamine levels of the patients in the symptom-free season were not statistically different from those of the normal subjects.

Introduction

Histamine is one of the important chemical mediators that produce bronchoconstriction in bronchial asthma and sneezing, watery rhinorrhea, and nasal obstruction in allergic rhinitis. This chemical mediator is released mainly from mast cells or basophilic leucocytes and produces an immediate hypersensitivity type I reaction (Ishizaka *et al.*, 1972). During an attack of bronchial asthma or allergic rhinitis, the offending allergen combines with reaginic antibody (IgE), which is fixed on the surface of mast cells in the tissue and basophilic leucocytes in the blood; histamine and other biochemical mediators are then liberated.

Several articles have been published concerning blood histamine levels in asthma. Some authors reported that levels of histamine in the blood were high during acute asthmatic symptoms (Haworth & MacDonald, 1937; DeGara, 1951). Other authors reported that levels were increased during attacks and were normal or slightly elevated

in symptom-free periods (Konoshita, 1963; Jimenez-Diaz, Arjona & Perianes, 1955). Still others noted no increased level of blood histamine in asthmatic and allergic subjects (Eggels & Nelemans, 1966).

So far as we know, no one has reported in detail on blood histamine levels in allergic rhinitis. Japanese cedar pollinosis is the dominant pollinosis in Japan. It is seen in February, March, April, and sometimes May. It causes allergic rhinitis mainly and bronchial asthma rarely.

The aim of this study was to compare the blood histamine levels of patients with Japanese cedar pollinosis in the pollen season with those in the symptom-free season without pollen to find out whether a seasonal difference in blood histamine levels exists. Blood histamine levels of normal subjects in the symptom-free season were also determined in this study.

Materials and methods

Subjects

Blood histamine levels were examined in the pollen season and in the symptom-free season without pollen in twenty patients (sixteen males, four females) with Japanese cedar pollinosis. Their ages ranged from 6 to 50 years. They did not have a history of bronchial asthma. Blood specimens from eleven of the twenty patients were obtained once in March of the pollen season of 1978, once in September–December of the symptom-free season of 1978, and once in March of 1979. Blood specimens from the other nine patients were obtained similarly in the pollen season of 1978 and in the symptom-free season of 1978. No subject had any medication, before the blood specimens were taken. Blood specimens were also obtained, in the symptom-free season of 1978, from normal subjects who did not have a history of allergic rhinitis and bronchial asthma. This group consisted of ten men and ten women aged 19 to 46 years.

Method

Since Shore and associates, in 1959, reported on a method to determine blood

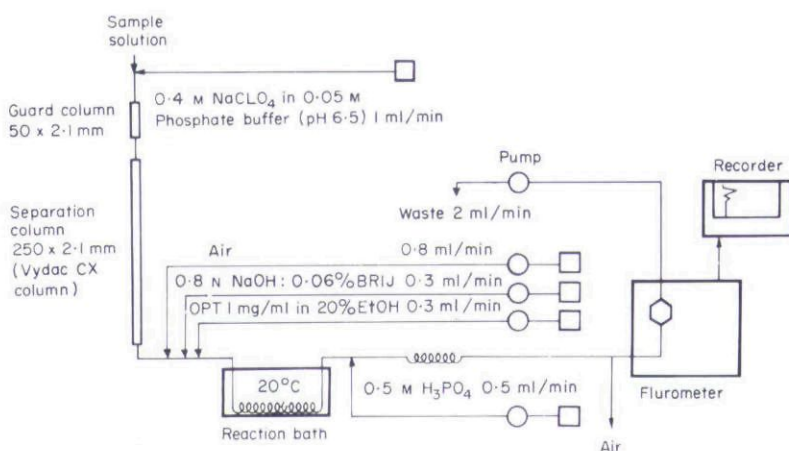


Fig. 1. Flow diagram for histamine analysis.

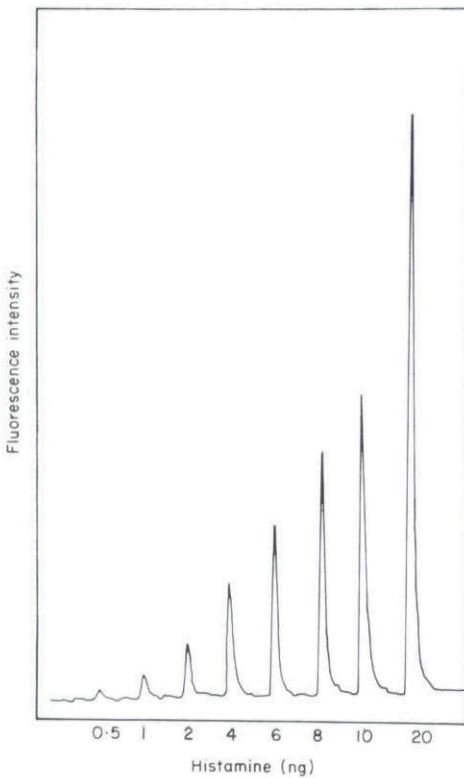


Fig. 2. Chromatogram of histamine standard applied to high-performance liquid chromatography. Standard solutions prepared to contain indicated amounts of histamine in 50 μ l were applied to the chromatograph.

histamine by use of *o*-phthalaldehyde (OPT), several modified techniques have been introduced (Ruff *et al.*, 1967; Beaven, Jacobson & Horakova, 1972; Siraganian & Brodsky, 1976; Tsuruta, Kohashi & Ohkura, 1978; Onda *et al.*, 1978). Our technique, uses an auto-analyser and high-performance liquid chromatography (HPLC).

The details of this technique are as follows: The blood sample was homolyzed by the addition of an equal volume of distilled water. Two millilitres of this solution was transferred into a 15-ml test tube, 1 ml of 1.5 M perchloric acid solution was added, and the combination was mixed. After standing for 1 h in the cold, the mixture was centrifuged at 3300 rev/min for 10 min (Hitachi 05PR centrifugal separator). One and a half millilitres of the supernatant fluid was transferred to a 12-ml glass-stoppered shaking tube containing 1 g NaCl; 3.5 ml *n*-butanol-chloroform mixture (3:2) was added, followed by 0.5 ml 5 N NaOH solution. Without delay, the tube was agitated for 5 min on a shaking machine and centrifuged at 3000 rev/min for 5 min. Three millilitres of the upper layer (organic phase) was transferred into another shaking tube, which contained 1 ml 0.1 N HCl and 2 ml *n*-heptane. To the residual solution in the first shaking tube another 3 ml *n*-butanol-chloroform mixture (3:2) was added, and a second extraction was performed. Three millilitres of the upper layer was pipetted and added to the previous 3 ml. The tube containing 1 ml 0.1 N HCl and 8 ml organic

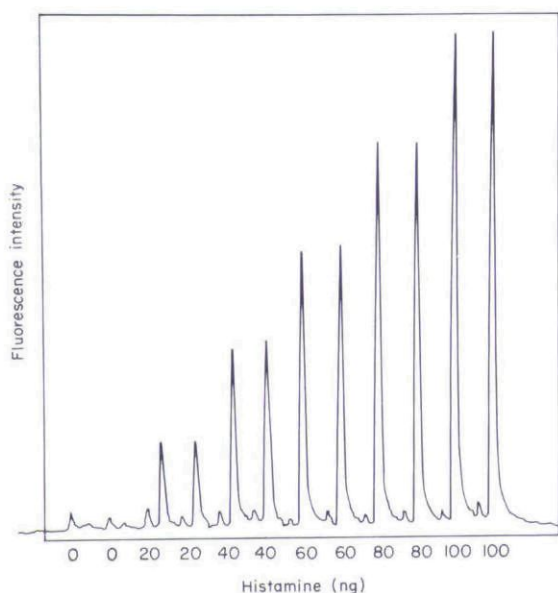


Fig. 3. Chromatogram of histamine standard applied to high-performance liquid chromatography after extraction process with organic solvent mixture. Standard solutions containing indicated amounts of histamine in 1 ml were extracted and determined as described in text.

mixture was shaken and centrifuged. After the organic phase was removed by aspiration, the residual acidic aqueous phase was evaporated to dryness under reduced pressure in a test tube evaporator (Tokyo Rikakikai Vapour Mix.).

The residue was taken up in 100 μ l of distilled water, and then 50 μ l of the solution was injected into a column (2.1 mm diam. and 300 mm long) packed with strong cation exchanger (Separations Group Vydac CX) attached to a high-performance liquid chromatograph (Gasukurokogyo Model 500). The column was eluted with a solution of 0.4 M NaClO₄ in 0.05 M phosphate buffer (pH 6.5) at a flow rate of 1 ml/min under a pressure of 30–40 kg/cm².

To the elute was added NaOH and OPT solution; This mixture was introduced into a reaction coil kept at 20°C. After 5 min of reaction, phosphoric acid was added and the reaction mixture was introduced to a spectrofluorometer (Hitachi Model 203), which continuously recorded the fluorescence intensity at 440 nm at 365 nm excitation. The histamine content was calculated by the ratio of the peak height of the sample to that of a standard solution. The flow diagram of the automated histamine analysis is shown in Fig. 1.

Results

Standard solutions of histamine resolved in 50 μ l of 0.001 N HCl were applied to HPLC at 5-min intervals one by one. The recorder chart, after the reaction with OPT is shown in Fig. 2. One-millilitre aliquots of the histamine solution and 0.001 N HCl in duplicate were extracted, and the fluorescence intensities were determined by the method described above. The results are shown in Fig. 3. There was a linear relationship

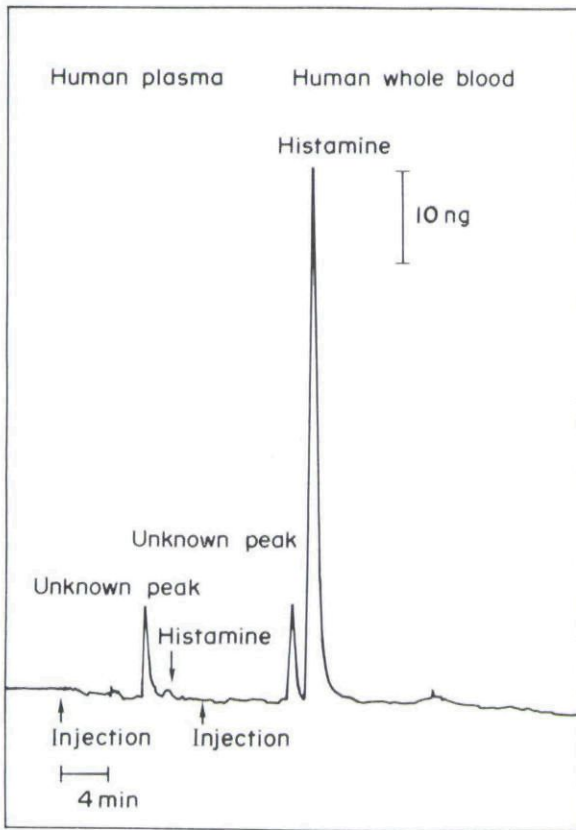


Fig. 4. Histamine content in human plasma and human whole blood. Unknown peak, probably, of histidine, is about ten times higher than that of histamine in human plasma (by high-performance liquid chromatography).

between the content of histamine and the intensity of fluorescence. So far as the highest sensitivity was concerned, 0.5 ng/ml histamine was the smallest amount that could be measured by this technique. Figure 4 shows the comparison of histamine content between in human plasma and human whole blood. Human plasma contains a very small amount of histamine, and it is difficult to determine histamine in human plasma accurately by this technique.

The retention times of histamine, histidine, and spermidine were determined by injecting the mixture into the high-performance liquid chromatograph and recording the fluorescence. As shown in Fig. 5, unknown substance, probably, histidine was eluted from 90 to 100 sec later, and spermidine was eluted from 15 to 20 min later than histidine. Histidine and spermidine had much worse sensitivities than histamine. In Fig. 6, the addition of diamine oxidase (Sigma, U.S.A.) that acts as histaminase significantly reduces the elevated peak of histamine fraction. In Fig. 7, the fluorescence intensities of 10 ng histamine without the extraction process, 50 ng histamine with the extraction process, and 1 ml blood of a patient with the extraction process are recorded. Adding the extraction process resulted in a peak eluting ahead of histamine.

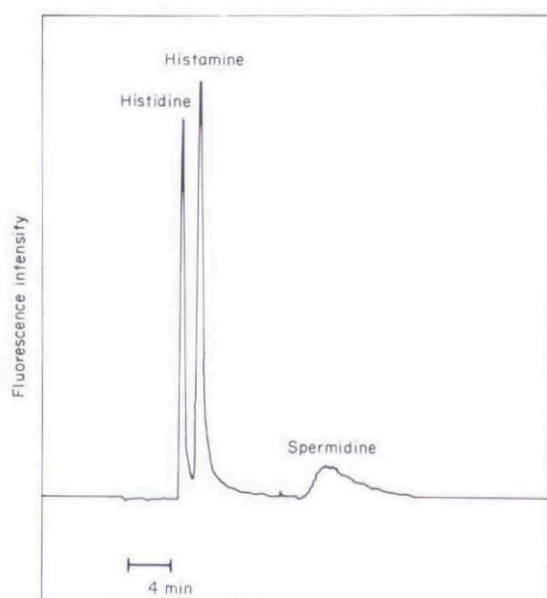


Fig. 5. Chromatogram of histamine, histidine, and spermidine. Mixture of histamine (10 ng), histidine (2 μ g), and spermidine (2 μ g) was applied to high-performance liquid chromatography, and fluorescence intensity was recorded.

Table 1. Seasonal variations of blood histamine levels in allergic rhinitis

Patient	Sex	Age (years)	Total blood histamine (ng/ml)		
			Pollen season of 1978	Symptom-free season of 1978	Pollen season of 1979
1	M	40	79	63	102
2	M	40	39	21	76
3	M	36	108	97	106
4	M	36	117	77	154
5	M	41	56	40	72
6	M	50	37	38	69
7	F	27	26	36	46
8	F	38	49	15	56
9	M	36	62	52	104
10	M	49	54	37	86
11	M	37	26	23	55
12	F	47	30	39	
13	M	35	96	91	
14	M	42	77	63	
15	M	36	61	49	
16	F	52	34	27	
17	M	32	75	104	
18	M	6	75	63	
19	M	39	68	40	
20	M	42	62	25	
Mean			61.6	50.0	84.2
s.e. mean			5.9	5.8	9.3

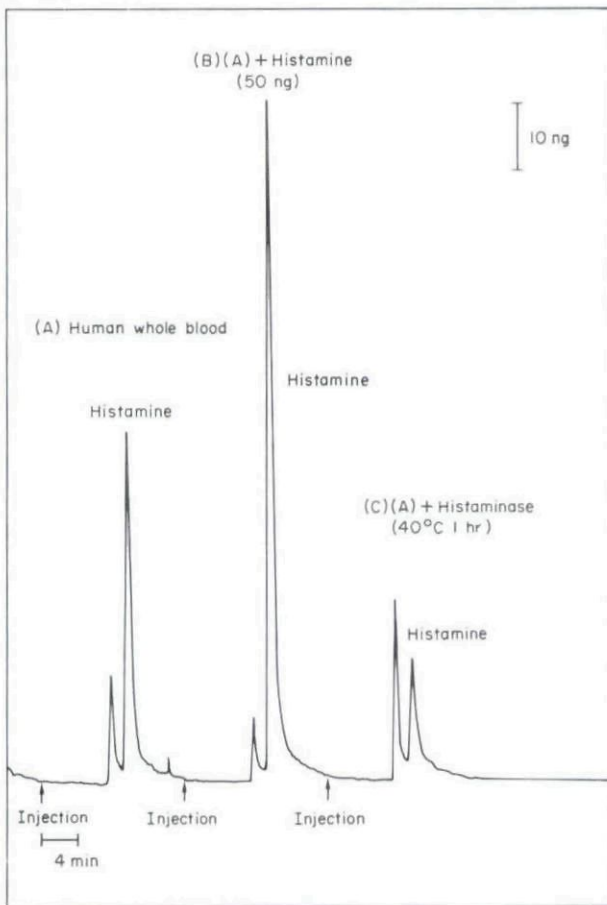


Fig. 6. The variations of the peak of histamine fraction are induced by adding histamine and histaminase in human whole blood.

This seemed to be due to the reagents used, and (Fig. 5) was to contamination by histidine.

The seasonal variations of the blood histamine levels in the twenty patients with Japanese cedar pollinosis are as follows: The mean values of the blood histamine levels in the pollen season of 1978, the symptom-free season of 1978, and the pollen season of 1979 were 61.6, 50.0 and 84.2 ng/ml respectively (Table 1).

The difference in blood histamine levels of the patients between the pollen season of 1978 and the symptom-free season of 1978 was statistically significant ($n = 20$; $t = 3.24$, $P < 0.01$, by paired t -test). The difference between results in the symptom-free season of 1978 and those in the pollen season of 1979 also was statistically significant ($n = 11$; $t = 6.54$, $P < 0.01$, by paired t -test).

The mean value of the blood histamine levels in normal subjects in the symptom-free season of 1978 was 58.2 ng/ml. In this season, blood histamine values in the patients were not significantly different from those in the normal subjects (Table 2).

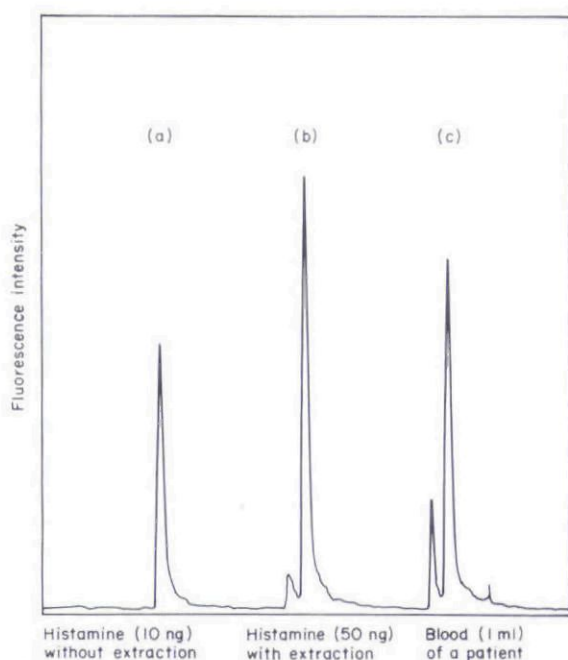


Fig. 7. Chromatograms of histamine standard and blood of patient. Three samples were applied to high-performance liquid chromatography. (a) 10 ng of histamine without extraction process; (b) 50 ng of histamine with extraction process; and (c) 1 ml of blood of patient with extraction process.

Table 2. Blood histamine levels of normal subjects in the symptom-free season without pollen

Subject	Sex	Age (years)	Blood histamine (ng/ml) in the symptom-free season of 1978
1	F	22	64
2	M	24	59
3	F	23	59
4	M	25	41
5	M	25	44
6	M	24	60
7	M	25	54
8	M	35	22
9	F	21	129
10	F	24	96
11	F	36	60
12	F	30	57
13	M	23	87
14	M	23	54
15	F	25	51
16	M	25	59
17	F	28	34
18	M	23	50
19	F	46	56
20	F	19	28
Mean			58.2
s.e. mean			5.3

Discussion

Japanese cedar pollinosis mainly causes allergic rhinitis. The symptoms are generally severe, and administration of steroids is the only effective treatment in some cases. However, the noses of the patients seem to be completely normal in the symptom-free season.

Several authors have reported that blood histamine values are elevated during the acute stage of bronchial asthma. We wanted to know whether blood histamine levels in allergic rhinitis were higher during the pollen season than during the symptom-free season. Despite the fact that the surface area of the nose is much smaller than that of the lung, our data revealed that allergic rhinitis influenced the blood histamine levels.

In this study, HPLC step was added to the conventional Shore technique, because it could exclude other fluorescent compounds with OPT in the blood very effectively. Figure 5 shows the HPLC step for the determination of histamine in human whole blood. If the extraction process only was used, without HPLC, histidine, histamine and spermidine, it might be measured at one time. This HPLC step is very helpful in distinguishing histamine from other substances. Methyl histamine is eluted at the same peak as histamine. However, the fluorescence intensity of the compound with OPT is very weak. Shore technique with HPLC step added is a very sensitive and specific technique for determination of histamine in human whole blood.

Basophilic leucocytes and tissue mast cells are the main sources of blood histamine. A significant correlation exists between total blood histamine levels and counts of basophilic leucocytes in the circulatory blood (Porter & Mitchell, 1970). During an acute episode of bronchial asthma and allergic rhinitis, histamine is released from mast cells or basophilic leucocytes in the tissue. The increased levels of histamine in the blood during the pollen season probably reflects the continuous replacement of basophilic leucocytes to the nasal mucosa from the blood.

References

- BEAVEN, M.A., JACOBSON, S. & HORAKOVA, Z. (1972) Modification of the enzymatic isotopic assay of histamine and its application to measurement of histamine in tissues, serum and urine. *Clinical Chimica Acta*, **37**, 91.
- DEGARA, P.F. (1963) Chemical determination of histamine in blood in health and disease. *Journal of Allergy*, **22**, 429.
- EGGELS, P.H. & NELEMANS, F.A. (1966) The histamine content of blood in non-allergic and allergic patients. *Allergie und Asthma (Leipzig)*, **12**, 38.
- HAWORTH, E. & MACDONALD, A.D. (1937) On histamine in cotton dust, and in the blood of cotton workers. *Journal of Hygiene*, **37**, 234.
- ISHIAZAKA, T., DEBERNARDO, R., TOMIOKA, H., LICHTENSTEIN, L.M. & ISHIZAKA, K. (1972) Identification of basophil granulocytes as a site of allergic histamine release. *Journal of Immunology*, **108**, 1000.
- JIMENEZ-DIAZ, C., ARJONA, E. & PERIANES, J. (1955) Studies on histamine in allergic patients. *International Archives of Allergy and Applied Immunology*, **6**, 243.
- KONOSHITA, K. (1963) Studies on blood histamine in infancy and childhood (abstract). *Acta Paediatrica Japonica (overseas)*, **5**, 28.
- ONDA, M., NOGUCHI, A., OHE, K., MIYOSHI, A., YOSHIDA, H., UENO, Y., KOIKE, N. & NAKAJIMA, T. (1978) Determination of tissue histamine and spermidine by means of high-performance liquid chromatography. *Hiroshima Journal of Medical Science*, **27**, 93.
- PORTER, J.F. & MITCHELL, R.G. (1970) The distribution of histamine in the blood of healthy and asthmatic children. *Clinical Science*, **38**, 135.
- RUFF, F., SAINDELLE, A., DUTRIPON, E., & PARROT, J.-L. (1967) Continuous automatic fluorometric evaluation of total blood histamine (letter to the editor). *Nature*, **214**, 279.

- SHORE, P.A., BURKHALTER, A. & COHN V.H., JR (1959) A method for the fluorometric assay of histamine in tissues. *Journal of Pharmacology and Experimental Therapeutics*, **127**, 182.
- SIRAGANIAN, R.P., & BRODSKY, M.J. (1976) Automated histamine analysis for in vitro allergy testing. I. A method utilizing allergen-induced histamine release from whole blood. *Journal of Allergy and Clinical Immunology*, **57**, 525.
- TSURUTA, Y., KOHASHI, K., & OHKURA, Y. (1978) Determination of histamine in plasma by high-speed liquid chromatography. *Journal of Chromatography*, **146**, 490.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.