The Determination of Blood Histamine in Asthmatic Patients with a Simple and Sensitive Method

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IZUMI, H., HOSHI, S., MUE, S., TAKISHIMA, T., SATO, H. and AOKI, T. *The Determination of Blood Histamine in Asthmatic Patients with a Simple and Sensitive Method.* Tohoku J. exp. Med., 1984, **143** (1), 79-85 — The histamine level of whole blood and plasma in asthmatic patients was estimated by means of a simple, sensitive and specific method, which was developed to measure low histamine levels. This method consists of the following procedures; the partial purification of histamine with a small P-cellulose column; its further purification with high performance liquid chromatography (HPLC); and fluorometric detection with precolumn o-phthaldehyde (OPT) reaction. The present assay could detect as little as 0.5 ng of histamine concentration. Blood histamine levels in patient with asthmatic attack, 57 ± 34 ng/ml (mean $\pm$ S.D. $N=14$), were significantly different from those in symptom-free period, 37 ± 15 ng/ml ($N=15$) as well as those in normal subjects, 36 ± 17 ng/ml ($N=12$). However, there were no significant differences among plasma histamine levels in normal subjects, 1.6 ± 1.7 ng/ml ($N=12$) and, in asthmatic patients during attacks, 1.6 ± 1.8 ng/ml ($N=14$) and symptom-free periods, 1.6 ± 1.7 ng/ml ($N=15$). These results indicate that plasma histamine concentration does not increase during asthmatic attacks, even though there was significant increase of blood histamine concentration.—histamine; HPLC; bronchial asthma

Histamine has been suggested to be one of the important chemical mediators for asthmatic attack, which is liberated from mast cells in the lung and triggers specific pathophysiological conditions of bronchial asthma. Recently, Adams and Lichtenstein (1979) indicated that histamine was involved in the early phase of bronchoconstriction of passively sensitized human lung after its release from mast cells by antigen challenge. However, there is some controversy as to whether or not the plasma level of histamine is actually increased in asthmatic patients (Beall 1963; Porter and Mitchell 1970, 1972; McFadden et al. 1980; Atkin et al. 1980). This seems to be in part due to the technical difficulties in plasma histamine measurement because of lack of sensitivity and specificity of

assay (Gleich and Hull 1980).

In this paper, a simple, sensitive and specific method of histamine determination, which combines small P-cellulose column for the partial purification of histamine from other interfering chemical substances, high performance liquid chromatography (HPLC)-system for its further purification, and fluorometric detection with precolumn o-phthalaldehyde (OPT) reaction, has been developed. Using this method, histamine levels in venous blood and plasma from normal volunteers and asthmatic patients both during attacks and in symptom-free periods were determined.

MATERIALS AND METHODS

Subjects

The subjects studied were 1) 12 healthy volunteers (9 men and 3 women), whose ages ranged from 21 to 36 years, having no history of atopic disease, and 2) asthmatic patients (6 men and 11 women), who were seen at the allergy clinic of Tohoku University Hospital, Sendai, and they were diagnosed as having intrinsic type asthma according to Rackeman's criteria.

Determination of histamine concentration in blood and plasma

The procedures are shown in Fig. 1. Blood was drawn from the antecubital vein into a heparinized disposable syringe. Plasma was obtained by centrifugation of aliquot of the

Fig. 1. Procedure of histamine determination in blood and plasma.

blood sample at 3000 rpm for 10 min at 4°C. To each one ml of blood or plasma, an equal volume of 0.4 M HClO_4 was added. After vigorously stirring, the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was then stored in a glass tube at -20°C until assay. Under these conditions of collection and storage, the histamine content remained stable for several months. The partial purification procedure of histamine was carried out by using P-cellulose which was reported by Endo (1981).

Measurement of blood histamine concentration (more than 10 ng). Histamine was eluted with 0.2 M borate buffer (pH 8.5) containing 0.025 M NaCl. The eluate was mixed with 2M NaOH (0.4 ml) and 1% OPT in methanol (0.1 ml) for the OPT reaction. Four minutes later at room temperature, 0.4 ml of 3.5M H_3PO_4 was added to stop the reaction. The fluorescence was measured by a Jasco-FP-550 Spectrofluorometer (Japan Spectroscopic Co. Tokyo) at Ex. 360 nm/Em. 440 nm.

Measurement of plasma histamine concentration (less than 10 ng). Histamine was eluted with 3 ml of 0.1M HCl. The eluate was collected in a centrifuge tube, evaporated to dryness by a centrifugal evaporator, and dissolved in 0.2 ml of 0.1M HCl. Then the solution was mixed with 40 μl of 1M NaOH and 10 μl of 1% OPT reaction. Four minutes later, 20 μl of 0.95M H_2SO_4 was added to stop the reaction. Aliquot (200 μl) of the reaction mixture was injected into the HPLC-fluorescence analysis system, which consisted of a Model 204 Liquid Chromatograph with a 6000A Solvent Delivery System, a U6K Universal Injector, a Waters Radial Pack A column (Waters Associates Inc. Milford, MA, USA), and the Jasco-FP-550 Spectrofluorometer equipped with a flow-cell unit (FA-1030, cell volume; 15 μl). The mobile phase was a mixture of 0.5M phosphate buffer (pH 4.7) (75 ml), triethylamine (0.38 ml), 0.2M HCl (15 ml), and methanol (90 ml). The flow rate was set at 1.0 ml/min. The fluorescence intensity was monitored at Ex. 360 nm/Em. 440 nm.

Materials

Histamine $\cdot$ 2HCl and triethylamine were obtained from Wako Pure Chemicals, Osaka. OPT was purchased from Nakarai Chemicals, Tokyo. All other chemicals of reagent grade were obtained from general commercial sources.

RESULTS

Fig. 2 shows the HPLC chromatograms obtained after OPT reaction with various concentrations of histamine (0–10 ng). Linear relationships between the fluorescence intensity and the amount of histamine concentration (0–10 ng) was definite (Fig. 3), indicating that as little as 0.5 ng of histamine concentration could be detected. The recovery of histamine was checked by adding known amounts of histamine (0–10 ng) to pooled plasma. The recoveries were 96.7%. When the standard samples of various concentrations of histamine (10–100 ng) were added to 1 ml of whole blood from a normal subject (Fig. 4), the linear relationship was also retained. The recovery of histamine in blood were 90.5%. Chemical compounds such as spermine, spermidine, putrescine, agmatine, and cadaverine, which may be eluted from a P-cellulose column as reported by Endo (1981), were examined whether or not they would interfere the measurement of histamine concentration in the present method. Among them, only spermidine formed the fluorophore with OPT, but HPLC system could separate it from histamine. The retention times of histamine-OPT fluorophore and spermidine-OPT fluorophore were 4.8 min and 6 min, respectively.

Fig. 2. HPLC chromatograms of standard histamine after OPT reaction. Various concentrations of histamine (0–10 ng) dissolved in 0.4 N HClO_4 (2 ml) were injected to HPLC after the treatment with same procedure for the measurement of plasma histamine concentration.

Fig. 3. The linear relationship between histamine concentration and fluorescence intensity by HPLC. See detail in Fig. 1.

Blood and plasma histamine levels in normal subjects and asthmatic patients both during attacks and in symptom-free periods were shown in Table 1. The blood histamine levels of asthmatic patients during attacks, 57 ± 34 ng/ml (mean $\pm$ s.d., $N=14$), were significantly higher than both those of normal subjects, 36 ± 17 ng/ml ($N=12$) and those of the same patients in symptom-free periods, 37 ± 15 ng/ml ($N=15$) ($p < 0.5$ in Student's t -test). On the other hand, plasma histamine levels in asthmatic patients during attacks and symptom-free periods are 1.6 ± 1.8 ng/ml ($N=14$) and 1.6 ± 1.7 ng/ml ($N=15$), respectively, being almost similar to those of normal subjects, 1.6 ± 1.7 ng/ml ($N=12$).

Fig. 4. Recovery of histamine standards (0-100 ng) in whole blood. —●—, calibration curve for standard histamine; —○—, calibration curve for standard histamine added to 1 ml of whole blood from normal subject, which apparently contains 35 ng of histamine.

DISCUSSION

In the last decade, the enzymatic isotope procedure (Beaven et al. 1972) has been commonly used to measure low histamine levels in human plasma. This method needs to use a radioisotope and to purify a histamine-N-methyltransferase enzyme, since it is based upon the conversion of histamine to ^{14}C methylhistamine by incubation with histamine-N-methyltransferase and S-adenosylmethionine- ^{14}C -methyl. Previously, Tsuruta et al. (1978) applied HPLC for the measurement of plasma histamine levels after extraction of histamine with butanol and chroloform. When we measured histamine concentration using this method, we found that the extraction procedure gave variable and low recovery of histamine. The recovery of standard histamine added to the plasma and blood in the present method was 96.7% and 90.5%, respectively. However, it should be taken into consideration that the quantitative relationship between the amount of P-cellulose in a column and that of blood or plasma applied on it is critical in the partial purification procedure with a P-cellulose column, because the capacity of P-cellulose varies with lot number of products even from the same company. Although Endo (1981) reported that spermine, spermidine, putrescine, agmatine and cadaverine were eluted from a P-cellulose column, these chemicals except spermidine in a dose up to $1\text{ }\mu\text{g}$ showed no fluorescence in the OPT reaction. In addition, the HPLC system used in the present method could clearly separate spermidine from histamine. Thus, the procedure described in this paper has a number of advantages such as simplicity, specificity, and reproducibility, as well as sensitivity over many other methods previously reported (Beaven et al. 1972; Siraganian 1974; Tsuruta et al. 1978; Endo 1981).

The present assay of histamine which detects as little as 0.5 ng of histamine concentration (Fig. 2) introduces new possibility in the investigation of the role of

TABLE 1. *Histamine levels in venous blood and plasma from normal subjects and patients with bronchial asthma*

			Histamine concentration (ng/ml)			
			Blood		Plasma	
Normal subjects (<i>N</i> = 12)			36 ± 17 (mean ± s.d.) (range, 6–56)		1.6 ± 1.7 (mean ± s.d.) (range, <0.5–4.5)	
Asthmatic patients			During attacks	In symptom free periods	During attacks	In symptom free periods
No	Age	Sex				
1	52	M	40	25	1.5	0.5
2	47	M	55	43	<0.5	3.5
3	28	M	101	47	0.5	0.5
4	19	M	52	40	<0.5	3.0
5	56	M	35	21	<0.5	<0.5
6	25	M	68	NT	4.0	NT
7	60	F	23	55	<0.5	<0.5
8	61	F	NT	53	1.0	5.5
9	39	F	29	NT	5.5	NT
10	59	F	81	64	1.0	1.5
11	40	F	136	39	4.0	1.5
12	34	F	47	21	3.0	1.0
13	50	F	85	36	3.0	3.5
14	31	F	32	29	0.5	<0.5
15	32	F	13	9	0.5	<0.5
16	53	F	NT	37	0.5	2.5
17	44	F	NT	40	NT	0.5
Mean ± s.d.			57 ± 34*	37 ± 15	1.6 ± 1.8	1.6 ± 1.7

M, male; F, female; NT, not tested. * $p < 0.05$ compared with symptom free period and normal subjects (Student's *t*-test).

histamine in pathological conditions in man. The concentration of histamine in the plasma of 12 normal subjects determined by the present method varied from less than 0.5 ng/ml to 4.5 ng/ml (1.6 ± 1.7 ng/ml, mean $\pm$ s.d.), which indicates great fluctuation among normal individuals. This value was a little higher than those estimated by Beaven et al. (less than 0.5 ng/ml) (1972) and Tsuruta et al. (0.61 ± 0.16 ng/ml) (1978). As shown in Table 1, blood histamine levels in asthmatic patients during attacks were significantly higher than those in symptom-free periods as well as those of normal subjects. In contrast, plasma histamine levels in asthmatic patients during attacks were not different from those in symptom-free periods as well as those in normal subjects, despite the usage of the present sensitive and reliable assay of histamine. Thus, it seems clear that histamine increase in blood is not due to the increase of histamine in plasma. It may be also

possible that failure to find the elevation of plasma histamine level is in part due to the time at which we collected the blood, because the time of blood collection is very critical to assay histamine in antigen provocation test. Furthermore, it is well known that histamine is rapidly metabolized when administered intravenously (Waalkes et al. 1957). So far, we could not find any explanations for an increase in whole blood histamine levels in asthmatic attacks.

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