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Short Communication

Determination of urinary excretion of histamine and 1-methylhistamine by liquid chromatography

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Abstract

Urinary histamine (Him) and 1-methylhistamine (MH) were determined by liquid chromatography (LC) using on-column derivatization coupled with a column-switching technique. An intact urine sample without any purification was applicable to the LC system because all steps for purification and fluorescence derivatization were fully automated. It was observed that the concentrations of Him and MH increased after hydrolysis of the urine, suggesting the presence of conjugated Him and MH. The level of total/free Him in urine was significantly higher in cancer patients than in normal subjects. Further, a significant correlation between Him and MH was observed in the hydrolysed urine of both normal subjects and cancer patients.

1. Introduction

Histamine (Him) is regarded as one of the chemical mediators which are generally known as autacoides, and its physiological activities such as extension of capillaries, contraction of smooth muscles and gastric secretions have been investigated. It has also been reported that in rodents high contents of Him were found in mammary tumour tissue [1,2] and in mast-cell tumours [3]. These data suggest that cell proliferation and differentiation are accompanied by increased amounts of Him in addition to polyamines. Clinical studies [4,5] of the relationship between urinary polyamines and cancer have been reported since the first report by Russell [6] of increased urinary excretion of polyamines by patients with various types of cancer. However, little information has been reported on the relationship between urinary Him or the Him metabolite 1-methylhistamine (MH) and cancer. Hence the determination of these amines in urine is of interest in clinical studies to clarify the exact physiological role of the amines in living systems.

Liquid chromatography (LC) with precolumn [7] or postcolumn [2] derivatization has been used for the determination of Him and MH in urine samples. However, these methods required a tedious procedure for sample purification or a complex eluent system. Recently, we have developed an on-column fluorescence derivatization method [8,9] and coupled it with a column-switching technique for the simultaneous deter-

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mination of Him and MH in mouse tissues [10]; this LC system automatically achieved sample purification and fluorescence derivatization. This approach was claimed to be more useful than a precolumn or postcolumn method, mainly because the proposed method drastically decreased or eliminated the manual sample pretreatment, which shortened the total analytical time considerably.

In this work, we examined the applicability of this method to the determination of Him and MH in urine samples, and also tried to explain the relationship between urinary Him and cancer.

2. Experimental

2.1. Reagents and chromatographic conditions

Him and *o*-phthalaldehyde (OPA) were purchased from Nacalai Tesque (Kyoto, Japan), MH from Sigma (St. Louis, MO, USA), N-acetyl-L-cysteine (NAC) of biochemical grade from Merck (Darmstadt, Germany) and acetonitrile of LC grade from Wako (Osaka, Japan). Water was deionized and passed through a reverse-osmosis membrane. All other chemicals were of analytical-reagent grade and were used as received.

The LC system used and the conditions were identical with those in our previous experiments [10].

2.2. Sample preparation and analysis of urine

A 24-h urine sample from 37 normal humans (age 3–56 years) and occasionally collected urine from 36 cancer patients (4 with gastric cancer, 4 with liver cancer, 4 with lung cancer, 7 with mammary cancer, 7 with uterine cancer, 3 with multiple myeloma, 3 with non-Hodgkin lymphoma, 2 with leukemia, 1 with parotid grand tumour and 1 with cerebral tumour; age 26–82 years) were adjusted to pH 3–4 with concentrated HCl, followed by centrifugation at 1800 *g* for 5 min to remove insoluble materials if necessary. The supernatant was used for the sample of non-hydrolysed urine. An aliquot of the urine was hydrolysed according to the method described by Fujita *et al.* [5]. Briefly, 0.5 ml of urine with 2 *M* HCl is a screw-capped vial was placed in a dry block-bath and refluxed for 3 h at 100°C. After the hydrolysis, the hydrolysate was adjusted to pH 3–4 with a suitable amount of 2 *M* NaOH, diluted to 2 ml with water and filtered through a 0.45-μm membrane filter. A 20-μl sample of each non-hydrolysed and hydrolysed urine was injected on to the LC column. The procedure for Him determination was the same as that described previously [10]. The contents of Him and MH in the non-hydrolysed urine were determined as free Him and free MH, whereas those in hydrolysed urine were determined as total Him and total MH, respectively. The Him and MH concentrations in 24-h urine were expressed as mg/24-h urine or μg/mg of creatinine. Urinary creatinine content was measured using a text kit for creatinine (Wako) according to Jaffe's method [11].

3. Results and discussion

3.1. Validation of hydrolysis of urine for Him determination

The determination of amines such as catecholamine or polyamine in urine usually requires acid hydrolysis because most of these amines are conjugated. It has also been reported that Him formed complexes in urine such as acetylhistamine [12], in plasma with metals [13] and proteins [14], and in gastric juices with biliary salt [15]. However, the need for hydrolysis has so far been less pronounced for Him analysis, although Endo [16] reported that the content of Him in urine was increased after hydrolysis. We therefore examined the acid hydrolysis of urine according to the method described by Fujita *et al.* [5]. Fig. 1 shows typical chromatograms of Him and MH from (A) non-hydrolysed urine and (B) hydrolysed urine; the latter sample was diluted fourfold compared with the formed sample by the hydrolysis treatment. It was observed that Him and MH were not subject to interfer-

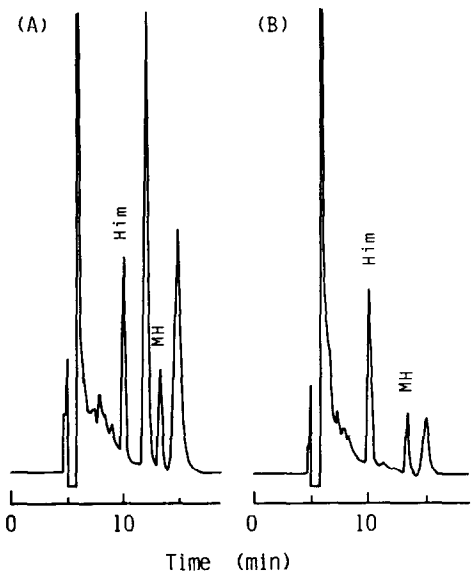

Fig. 1. Typical chromatograms of histamine (Him) and 1-methylhistamine (MH) from (A) non-hydrolysed urine (Him, 0.07 μg/ml; MH, 0.04 μg/ml) and (B) hydrolysed urine (Him, 0.25 μg/ml; MH, 0.11 μg/ml). The latter sample was diluted fourfold compared with the original urine (A) by the hydrolysis treatment.

ence from any impurities in the urine matrices, and moreover not only the Him content but also the MH content increased after hydrolysis. These results indicate that both Him and MH are conjugated in urine. Therefore, acid hydrolysis was thought to be necessary for the determination of urinary Him and MH in order to evaluate Him excretion in urine.

Table 1 summarizes the recoveries of Him and MH from non-hydrolysed and hydrolysed urine added at 0.1 μg/ml each as standards. For hydrolysed urine, the standards were added before hydrolysis. The overall mean recoveries were greater than 94% and the relative standard deviations (R.S.D.s) were less than 3%. Replicate analyses ($n = 5$) of a standard solution (0.5 μg/ml each of Him and MH) yielded corresponding R.S.D.s; all were below 1% for the peak area and below 0.05% for the retention times. The calibration graphs showed excellent linearity over the range 0.01–10 μg/ml, and the detection limit was 0.2 ng (signal-to-noise ratio = 3) for both Him and MH.

Incidentally, an unknown peak appeared between Him and MH with the non-hydrolysed urine, as shown in Fig. 1A. It was considered that this peak was an acetyl derivative of a polyamine because the peak disappeared after hydrolysis (Fig. 1B). By comparing the retention times of each acetylated polyamine and by observing the retention behaviour on the clean-up column, this unknown peak was identified as a mixed peak of N^1-acetylspermidine and N^8-acetylspermidine.

3.2. Diurnal variation of urinary Him

In general, the collection of 24-h urine is not suitable for a mass screening test because it is troublesome. Therefore, in order to examine the validity of the use of occasionally collected urine, the diurnal variations of the Him and MH contents in the urine of five normal human subjects were examined. Fig. 2 shows a typical graph which represents the diurnal variation of urinary Him. It was observed that the concentration of urinary Him showed large variations during a day. However, compensation of the values with the creatinine content markedly

Table 1
Recoveries of histamine and 1-methylhistamine added to urine and hydrolysed urine

Sample	Added (μg/ml)	Recovery (mean ± R.S.D., $n = 4$) (%)	
		Histamine	1-methylhistamine
Urine	0.1	98.4 ± 1.3	97.9 ± 1.6
Hydrolysed urine[a]	0.1	98.8 ± 1.8	94.8 ± 2.6

[a] Histamine and 1-methylhistamine were added before hydrolysis.

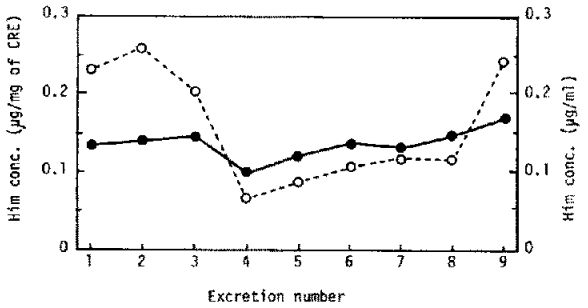

Fig. 2. Typical diurnal variation of histamine content in urine expressed as (●) μg/mg of creatinine and (○) μg/ml. Him = histamine; CRE = creatinine.

decreased the variation. The other four urine samples showed a similar pattern. This result indicated that compensation with the creatinine content was effective for the determination of Him and MH in occasionally collected urine, *i.e.*, fresh morning urine or occasionally collected urine, instead of 24-h urine, is suitable for Him determination.

3.3. Urinary Him and MH in normal subjects and cancer patients

As summarized in Table 2, the values of free Him and free MH were in good agreement with those reported by other investigators [7,17,18]. On the other hand, the levels for both Him and MH contents in urine of cancer patients were nearly equal to those in urine of normal subjects.

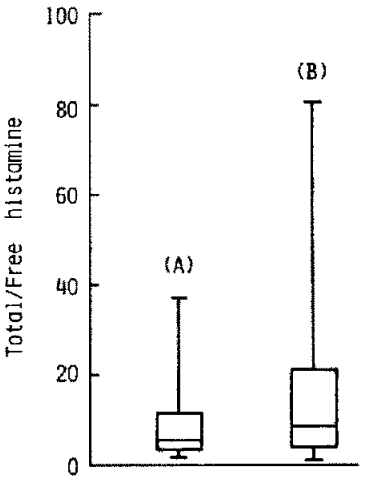

Fig. 3. Box and whisker chart of total/free Him values in urine from (A) normal subjects and (B) cancer patients. Each box represents the values of median and quartile deviation.

When we started this study, we had presumed that the content of Him and/or MH in the urine of a cancer patient was significantly higher than that of a normal subjects. However, the results showed that this was not so.

Incidentally, it was found that the mean level of the total/free Him values in cancer patients was approximately double than that in normal humans. Fig. 3 shows a "box and whisker chart" of the total/free Him values from (A) normal subjects and (B) cancer patients. An *F*-test, an analysis of variance, for the data showed that the

Table 2
Contents of histamine (Him) and 1-methylhistamine (MH) in urine of normal subjects ($n = 37$) and cancer patients ($n = 36$)

Amine	Free amine (μg/mg CRE)		Total amine (μg/mg CRE)		Total/Free	
	Range	Mean	Range	Mean	Range	Mean
Normal humans						
Him	0.01–0.30	0.06	0.05–2.03	0.39	1.6–37.2	8.2
	(0.01–0.44)	(0.07)	(0.03–1.68)	(0.39)		
MH	0.10–0.36	0.18	0.13–1.47	0.39	1.0–5.5	2.2
	(0.04–0.37)	(0.19)	(0.04–1.21)	(0.39)		
Cancer patients						
Him	0.01–0.22	0.05	0.05–1.84	0.45	1.0–81.7	15.3
MH	0.11–0.77	0.27	0.23–1.33	0.53	1.0– 7.3	2.4

CRE = creatinine. The values in parentheses are expressed as mg/24-h urine.

rate of variance was 5.275 [$>F$ (1, 71; 0.05) = 3.976], indicating the higher level of total/free Him in the cancer patients was significant. In this study, because the patients were selected at random, the kind and stage of the cancer disease and the conditions of medical treatment were not defined. If the background conditions of the cancer patients were defined, more significant data could be obtained. Although increases in Him content after hydrolysis of urine were reported by Endo [16], the significant elevation of the total/free Him level in cancer patients was more pronounced in our study. Further, we found that there is a significant correlation between the values of total Him and those of total MH both in normal subjects ($r = 0.921$) and in cancer patients ($r = 0.740$), as shown in Fig. 4. It is known that for Him metabolism two major pathways exist: ring N^1-methylation by the enzyme histamine-N-methyltransferase to form MH or deamination by diamine oxidase (histaminase) to form imidazoleacetic acid. These two enzymes were said to work alternatively as a compensatory enzyme with each other in order to maintain a constant level of Him concentration *in vivo*. Accordingly, we thought that the MH concentration is also regulated for maintaining the homeostasis of the MH/Him level and that the regulatory function could not be influenced, even if the value of Him *in vivo* was increased or decreased by such a cancer disease. Hence, an MH/Him level should also be taken into consideration for the determination of Him in biological materials.

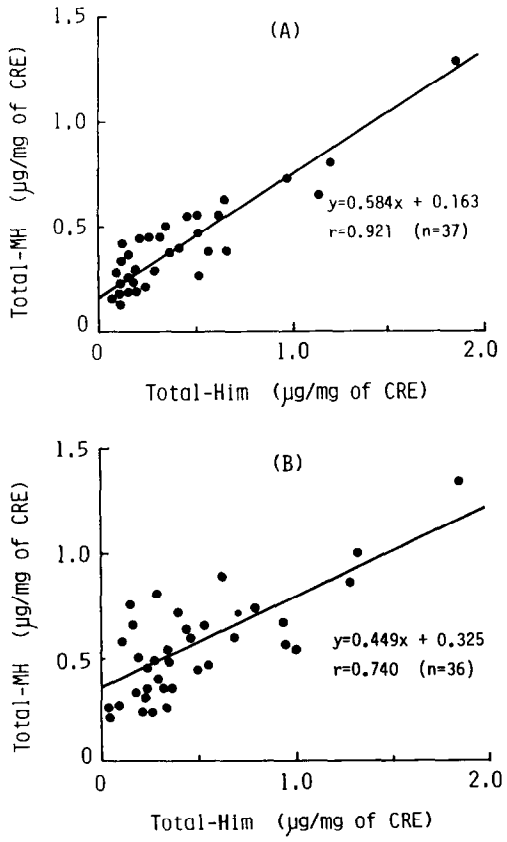

Fig. 4. Correlation between total Him and total MH in urine of (A) normal subjects and (B) cancer patients.

On the basis of these experiments, our results have demonstrated that the proposed LC method is applicable for the determination of urinary excretion of Him and MH. As there is no need for tedious sample purification and fluorescence derivatization by manual operations, direct injection of urine samples without any purification was possible by using the proposed LC method. An important observation in this work is a significant elevation of total/free Him value of urinary Him in cancer patients. Another observation is the presence of a significant correlation between total Him and total MH in the urine of both normal subjects and cancer patients, suggesting the presence of homeostasis of urinary MH/Him level. These observations were clarified in this study. Although the intact value of urinary Him or MH content could not be used for the diagnosis of cancer, the information on the total/free Him value might be a useful aid in the diagnosis of cancer or for following the clinical efficacy of cancer treatment.

4. Acknowledgements

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