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Short communication

Determination of 1-methylhistamine and 1-methylimidazoleacetic acid in human urine as a tool for the diagnosis of mastocytosis

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Abstract

The determination of the metabolites of histamine, 1-methylhistamine (MHIS) and 1-methylimidazoleacetic acid (MIIA), in human urine is a useful tool for the diagnosis of mastocytosis. MHIS was extracted under basic conditions with chloroform and derivatized with trifluoroacetic acid anhydride, MIIA was derivatized with pentafluorobenzylbromide prior to extraction under basic conditions and the derivative was extracted with chloroform. The samples were assayed by gas chromatography–mass spectrometry. Normal concentrations of MHIS (2.01 μmol l^{-1}) and MIIA (21.3 μmol l^{-1}) in healthy volunteers' urine samples are clearly detectable with coefficients of variation of 7.0 and 8.9%. Pathological concentrations of 10.1 and 113 μmol l^{-1} for MHIS and MIIA, respectively, are quantifiable with coefficients of variation of 6.6 and 4.8%.

Keywords: 1-Methylhistamine; 1-Methylimidazoleacetic acid

1. Introduction

Mastocytosis is a disorder characterized by abnormal proliferation of mast cells which results in most cases in flat or slightly elevated red–brown skin lesions, but all other organs can be involved. It is associated with an above-normal excretion of the histamine metabolites 1-methylhistamine (MHIS) and 1-methylimidazoleacetic acid (MIIA) in urine. Since mastocytosis is sometimes difficult to diagnose, especially if the characteristic skin lesions are not clearly observed, the determination of these two substances in urine can be a useful tool in the detection of this disorder [1].

Some procedures use gas chromatography (GC) for the determination of MIIA [2–4] or MHIS [5,6] in urine with mass-spectrometric or nitrogen–phosphorus detectors. In all cases, the analytes have to be derivatized in order to make the compounds volatile enough for GC. Other procedures include high-performance liquid chromatography (HPLC) to measure MHIS [7–10], MIIA [11] or MHIS together with MIIA [12] in urine with fluorescence or electrochemical detection. For fluorescence detection, a derivatization step is required since the analytes do not show native fluorescence.

The procedure for the determination of MHIS and MIIA in human urine described here utilizes gas

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chromatography with mass spectrometric detection. A simple one-step extraction is used for each of the analytes, which are derivatized by easily accessible and cheap reagents. For MIIA, the derivatization is carried out before the extraction and, therefore, the separation of underivatized MIIA from the matrix by means of ion-exchange solid-phase extraction [2–4,11], which can be a source of problems, is avoided. The procedure is selective and sensitive enough to determine non-pathological concentrations as well as pathological ones for both substances.

2. Experimental

2.1. Apparatus

A gas chromatograph (HP 5890 Series II plus) with an electronic pressure programmer, automatic sampler (HP 7673), split–splitless injector and a mass selective detector (HP 5972) was used. Data were collected and analyzed by a Hewlett-Packard DOS ChemStation version C.01.05 (all Hewlett-Packard, Waldbronn, Germany). The analytes were separated on an RTX-1 capillary column with the dimensions 15 m×0.25 mm I.D. and d_f 0.5 μm (Restek, Bad Soden, Germany).

2.2. Chemicals

1-Methylhistamine dihydrochloride and 1-methylimidazoleacetic acid hydrochloride were purchased from Sigma (Deisenhofen, Germany). 2-(2-Aminoethyl)-pyridine (I.S. for MHIS), 2-pyridylacetic acid hydrochloride (I.S. for MIIA), trifluoroacetic acid anhydride and pentafluorobenzylbromide were purchased from Aldrich (Steinheim, Germany). Chloroform, NaOH (both quality grade p.a.), K_2HPO_4 and Na_2SO_4 (both quality grade anhydrous extra pure) were obtained from Merck (Darmstadt, Germany). Ethyl acetate and acetonitrile (both of reagent grade) were purchased from Baker (Gross-Gerau, Germany). Helium, purity >99.9996, was purchased from Messer-Griessheim (Magdeburg, Germany).

2.3. Sample collection

Urine samples were collected from patients over a period of 24 h. The total amounts were noted down (to compute the total excretion of MHIS and MIIA over 24 h) and, after mixing, about 20 ml of each sample were stored at −20°C until analysis.

2.4. Sample preparation of 1-methylhistamine

To 5 ml of urine, 50 μl of a solution containing 2-(2-aminoethyl)-pyridine (100 μl in 100 ml of water) and 0.5 ml of 1 *M* NaOH were added. The mixture was extracted with 2 ml of chloroform by shaking it for 15 min. The extraction procedure was carried out twice. The combined organic phases were dried over 100 mg of Na_2SO_4 for about 15 min. The derivatization was carried out by adding 30 μl of trifluoroacetic acid anhydride and allowing the reaction to proceed for 30 min at room temperature. Finally, the mixture was evaporated and the residue was reconstituted with 100 μl of ethyl acetate and subjected to GC–MS analysis.

2.5. Sample preparation of 1-methylimidazoleacetic acid

To 1 ml urine, 100 μl of a solution of the I.S. 2-pyridylacetic acid hydrochloride (1 mg ml^{-1} in water), 1 ml acetonitrile, 100 μl of 1 *M* K_2HPO_4 buffer solution adjusted to pH 11 and 20 μl of pentafluorobenzylbromide were added. The mixture was heated in tightly stoppered vessels to 100°C for 1 h, to allow derivatives of the analytes to form. After cooling, the analytes were extracted twice with 2 ml of chloroform by shaking each time for 15 min. The pooled organic phases were evaporated and the residue was reconstituted with 100 μl of ethyl acetate and subjected to GC–MS analysis.

2.6. Calibration samples

A 1.0-mg quantity of 1-methylhistamine dihydrochloride (M_r=198.2 g mol^{-1}) and 10 mg of 1-methylimidazoleacetic acid hydrochloride (M_r=176.7 g mol^{-1}) were dissolved in 10 ml of water. From this solution, 0.00, 0.01, 0.02, 0.05, 0.10, 0.20, 0.50 and 1.00 ml volumes were added to 10 ml of urine from a healthy volunteer. The spike concentrations of these samples ranged from 0 to 50.45 μmol l^{-1} for MHIS (M_r=125.3 g mol^{-1}) and 0 to 565.9 μmol l^{-1} for MIIA (M_r=140.2 g mol^{-1}), plus the amounts of the two substances that were present

from endogenous sources in the volunteers' urine before spiking (about 1 μmol l^{-1} MHIS and 10 μmol l^{-1} MIIA). The concentration errors introduced by differing amounts of the volunteers urine in the calibration samples represented about 0.2% at the highest spike level and were therefore negligible.

The wide calibration ranges for MHIS and MIIA cover the span of normal and pathological concentrations described in Ref. [1].

2.7. Chromatographic conditions

For both MHIS and MIIA, the same chromatographic conditions were applied. Carrier gas for the chromatographic separation was helium at a flow-rate of 1 ml min^{-1}. This flow-rate was held constant over the run time. The temperature program started at 80°C, was held constant for 1 min, then the temperature was raised by 15°C min^{-1} up to 260°C and held constant for 1 min. The total run time was 14 min. The injector and MS interface temperatures were 300°C. A 1-μl volume was injected in the splitless mode. The splitless time was 1 min, then a split flow of 20 ml min^{-1} was applied. The retention times achieved under these condition were 6.7 min for the derivative of MHIS, 5.2 min for its I.S., 9.6 min for the derivative of MIIA and 8.5 min for its I.S.

The mass spectrometric detector was working in the selected ion monitoring mode and the ions were formed by electron impact ionization with an energy of 70 eV. The relative masses of the observed ions were 95 and 221 m/z for MHIS, 93 and 218 m/z for its I.S., 95 and 320 m/z for MIIA and 93 and 317 m/z for its I.S.

3. Results and discussion

3.1. Extraction and derivatization

Since the amino moiety in MHIS and the carbonic acid in MIIA exhibit totally different behaviors, the two substances cannot be worked up simultaneously. MHIS is extracted under basic conditions and then the amino moiety is derivatized with trifluoroacetic acid anhydride. Conversely, the carbonic acid moiety of MIIA is esterified with pentafluorobenzylbromide prior to the extraction. The two I.S.s exhibit properties that are similar to those of MHIS and MIIA.

The use of pentafluorobenzylbromide as the derivatization reagent for MIIA offers some advantages over the previously reported methods [2–4]. MIIA is derivatized prior to the extraction and, therefore, solid-phase extraction with ion-exchange resins is replaced by the one step liquid–liquid extraction. A second derivatization step, which is required in refs. [2–4], is not necessary since the derivative yielded in this procedure has good gas chromatographic properties. The derivatization product of MHIS with trifluoroacetic acid anhydride is also suitable for gas chromatography, and it is produced in a much easier way than the derivatives described in reference [5] (two step derivatization) or [6] (long reaction time overnight with the expensive reagent heptafluorobutyric acid anhydride).

Values for the extraction yields of the analytes from urine cannot be provided. MIIA is derivatized prior to the extraction, so the extraction would have to be referred to the derivative of MIIA, which is not easy available as a pure substance. Furthermore, it is impossible to obtain urine free of MHIS and MIIA. Extraction yields that are obtained by the addition of the analytes to urine have to be corrected by subtracting the endogenous concentrations of the analytes from the amounts added. This operation would lead to high statistical uncertainties of the found values.

3.2. Mass spectrometry and gas chromatography

In Fig. 1 the mass spectra of the derivatives of MHIS and MIIA are depicted. In both substances the molecular ions show high relative abundances, facilitating selective and sensitive detection. The fragmentation patterns of the molecules are essentially described by the cleavage of the alkyl chain–functional group bonds and by the cleavage of the functional group–derivatizing moiety bonds of MHIS and MIIA. The I.S.s for both analytes behave in a similar manner.

In Fig. 2 some typical chromatograms of urine extracts can be seen: (a) shows the I.S. of MHIS (peak group 1) and the derivative of MHIS at different concentration levels. Peak 3 refers to spike level 5, resulting in a concentration of 10.5 μmol l^{-1} and peak 4 corresponds to the endogenous con-

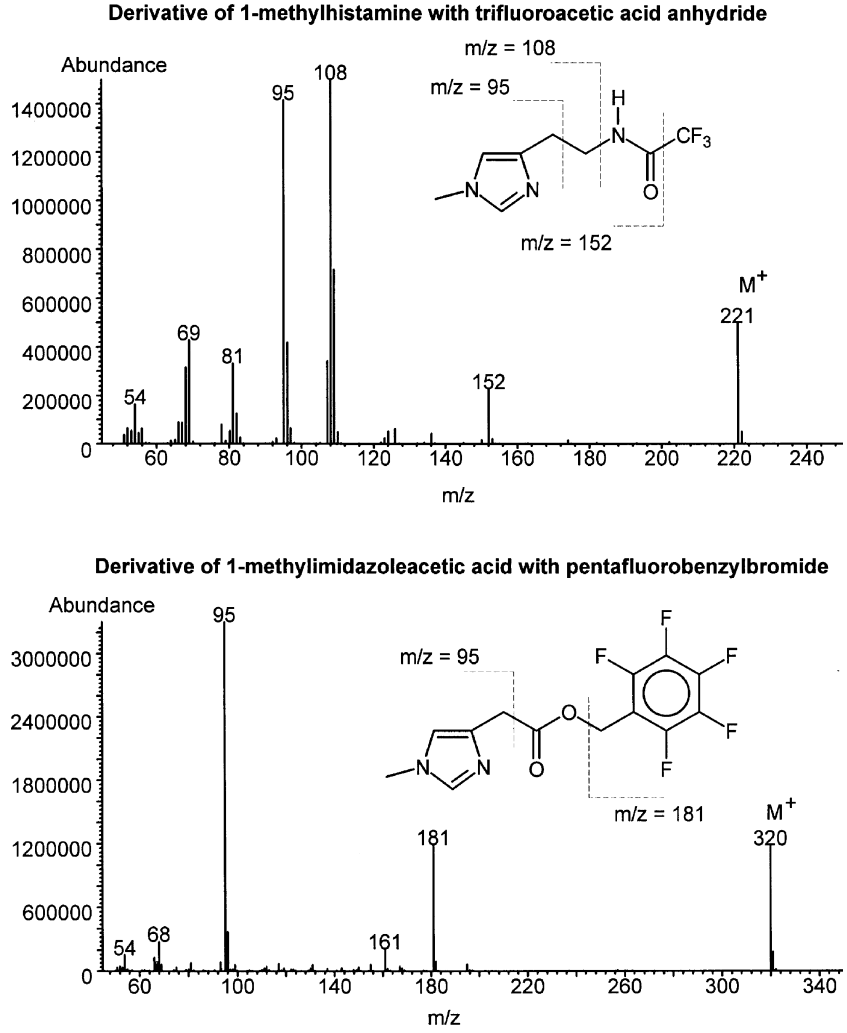

Fig. 1. Mass spectra of the derivatives of MHIS (above) and MIIA (below).

centration of MHIS in the volunteers' urine (i.e. 0.9 μmol l^{-1}). The analogous chromatograms of the I.S. of MIIA (peak group 5) and the derivative of MIIA (peaks 6 to 8) are shown in (b). Peak 7 corresponds to spike level 5, resulting in a concentration of 141 μmol l^{-1}, and peak 8 refers to the endogenous concentration of MIIA in the volunteers' urine (i.e. 21 μmol l^{-1}). Peak 2 in chromatogram (a) and peak 6 in chromatogram (b) refer to a urine sample from a patient with concentrations of 11.1 μmol l^{-1} of MHIS and 157 μmol l^{-1} of MIIA. As can be seen, all analyte peaks are sufficiently separated from peaks of endogenous substances and the endogenous concentrations of MHIS and MIIA in the urine from healthy volunteers can be detected without difficulty.

3.3. Calibration functions, precision and accuracy

The calibration functions of MHIS and MIIA are linear (slope=4.74E−6±6.22E−8 for MHIS and 7.79E−2±8.05E−4 for MIIA) in the calibration range (0–50.5 μmol l^{-1} for MHIS and 0–566 μmol l^{-1} for MIIA) and show very good correlations (r=0.9995 for MHIS and for MIIA). The intercepts of the calibration functions (1.19E−3±2.51E−4 for MHIS and 2.33E−1±3.25E−2 for MIIA) are above

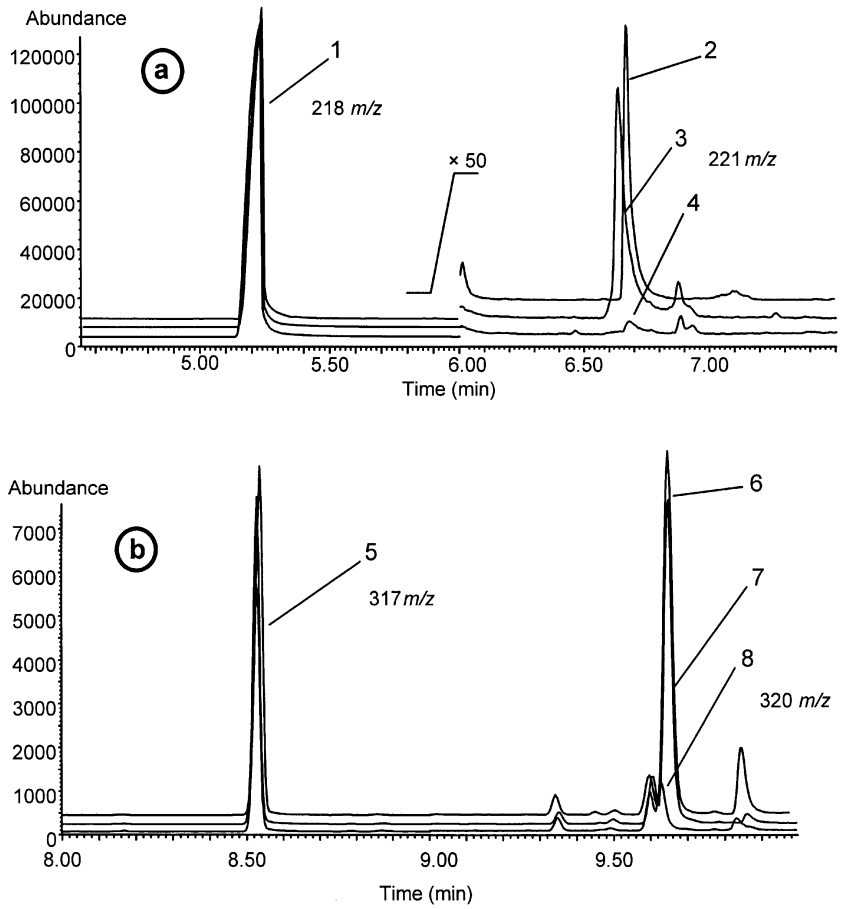

Fig. 2. Typical chromatograms of the derivatives of MHIS (a) and MIIA (b). The signed peaks are (1) I.S. of MHIS (83.5 μmol l^{-1}), (2) derivative of MHIS (11.1 μmol l^{-1}, sample from a patient), (3) derivative of MHIS (10.5 μmol l^{-1}), (4) derivative of MHIS (0.9 μmol l^{-1}, i.e. endogenous concentration), (5) I.S. of MIIA (576 μmol l^{-1}), (6) derivative of MIIA (157 μmol l^{-1}, sample from a patient), (7) derivative of MIIA (141 μmol l^{-1}) and (8) derivative of MIIA (21 μmol l^{-1}, i.e. endogenous concentration).

zero, indicating the endogenous concentrations of the analytes in the urine of the healthy volunteer.

The inter-day precision and accuracy tests are listed in Table 1. The endogenous concentrations plus the spike concentrations result in the total concentrations of the analytes in the urine samples. The quite high deviation of the found concentrations at the low levels from the expected concentrations

Table 1
Inter-day precision and accuracy

Test series	Endogenous concentrations (μmol l^{-1})	Spike concentrations (μmol l^{-1})	Found (μmol l^{-1})	*n*	RSD (%)	Accuracy (%)
MHIS low	2.01	0.00	1.74	8	6.98	−13.6
MHIS high	2.01	10.10	12.07	8	6.56	−0.03
MIIA low	21.3	0.0	23.3	7	8.94	+9.2
MIIA high	21.3	113.3	132.0	7	4.81	−1.9

results from the very wide calibration range, due to which the accuracy in the lower concentration ranges is impaired.

4. Measurements in healthy volunteers and patients

The determination of MHIS and MIIA in urine samples from nine healthy volunteers (five male, four female) results in concentrations of 1.0 ± 0.5 μmol l^{-1} for MHIS and 24 ± 11 μmol l^{-1} for MIIA. These concentrations are in the range of non-pathological levels published in Ref. [1]. In contrast, a sample from a patient (Fig. 2) with a diagnosed mastocytosis contains 11.1 μmol l^{-1} MHIS and 157 μmol l^{-1} MIIA. These levels are far above the normal values and, therefore, provide additional evidence for the diagnosis of mastocytosis.

5. Conclusion

The described method is capable of determining pathological as well as non-pathological concentrations of MHIS and MIIA in human urine. The I.S.s and the derivatization reagents are relatively cheap and readily accessible, the sample preparation process is easy and not prone to errors and the technical equipment needed is nowadays standard in many clinical laboratories. To conclude, this method can be a valuable tool for the diagnosis of mastocytosis.

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References

[1] J.J. Keyzer, J.G.R. De Monchy, J.J. van Doormaal, P.C. van Voorst, New Engl. J. Med. 309 (1983) 1603.
[2] S. Murray, G. O'Malley, I.K. Taylor, A.I. Mallet, G.W. Taylor, J. Chromatogr. 491 (1989) 15.
[3] A.I. Mallet, N.B. Rendell, Biomed. Environ. Mass Spectrom. 17 (1988) 275.
[4] J.J. Keyzer, B.G. Wolthers, H. Breukelman, H.F. Kauffmann, J.G.R. de Monchy, Clin. Chim. Acta 121 (1982) 379.
[5] A. Wollin, H. Navert, Anal. Biochem. 145 (1985) 73.
[6] J.J. Keyzer, B.G. Wolthers, H. Breukelman, W. van der Slik, D. de Vries, J. Chromatogr. 275 (1983) 261.
[7] K. Saito, M. Horie, H. Nakazawa, J. Chromatogr. B 654 (1994) 270.
[8] A.A. Houdi, P.A. Crooks, G.R. Van Loon, C.A.W. Schubert, J. Pharm. Sci. 76 (1987) 398.
[9] K. Mine, K.A. Jacobson, K.L. Kirk, Y. Kitajima, M. Linnoila, Anal. Biochem. 152 (1986) 127.
[10] J.L. Devalia, B.D. Sheinman, R.J. Davies, J. Chromatogr. 343 (1985) 407.
[11] Y. Tsuruta, H. Tomida, K Kohashi, Y. Ohkura, J. Chromatogr. 416 (1987) 63.
[12] G. Granerus, U. Wass, Agents Actions 14 (1984) 341.