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MASS FRAGMENTOGRAPHIC IDENTIFICATION OF POLYAMINE METABOLITES IN THE URINE OF NORMAL PERSONS AND CANCER PATIENTS, AND ITS RELEVANCE TO THE USE OF POLYAMINES AS TUMOUR MARKERS

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SUMMARY

The mass fragmentographic identification of N-(2-carboxyethyl)-4-amino-*n*-butyric acid, N-(3-aminopropyl)-N^1-(2-carboxyethyl)-1,4-diaminobutane, N,N^1-bis(2-carboxyethyl)-1,4-diaminobutane, and δ-aminovaleric acid in acid-hydrolysed urines of a normal person and two cancer patients is described. A previous study, in which the metabolic fate of intraperitoneally injected polyamines in rats was investigated, revealed that these compounds should be considered as non-α-amino acid metabolites of the naturally occurring polyamines. Quantification of polyamines and their non-α-amino acid metabolites by gas chromatography with nitrogen—phosphorus detection showed that, relative to the parent polyamines, humans normally excrete higher quantities of polyamine catabolites in urine than rats, suggesting that humans catabolize polyamines more efficiently. As illustrated by the follow-up of the concentrations of polyamines and their catabolites in the urine of a patient with high-grade non-Hodgkin lymphoma during chemotherapy, the catabolic pressure on polyamines may be considerably increased during neoplastic diseases, since an even higher proportion of oxidized polyamine metabolites was observed. It is therefore suggested that the additional measurement of the circulating concentrations of polyamine-degrading enzymes is of importance for the correct interpretation of polyamine (metabolite) determinations for oncological purposes.

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INTRODUCTION

The polyamines are polybasic compounds that are fundamentally involved in a variety of cellular processes such as stabilization of nucleic acids and membranes, growth, differentiation and protein synthesis [1—3]. Although polyamine biosynthesis has been extensively examined [1, 3, 4], in man relatively little is known about their catabolism [4—6].

The catabolism of polyamines in rats is complex, comprising the action of a multitude of catabolic enzymes, of which the relative activities may be subject to time-dependent variation caused by enzyme induction [7, 8]. One of the degradative pathways for polyamines includes the oxidative deamination of one or both primary amino groups by amine oxidases, followed by intermediate aldehyde oxidation [6—11]. The products of the combined action of these enzymes are the non-α-amino acid metabolites.

In this study the mass fragmentographic identification of N-(2-carboxyethyl)-4-amino-*n*-butyric acid (a metabolite of spermidine), N-(3-aminopropyl)-N^1-(2-carboxyethyl)-1,4-diaminobutane and N,N^1-bis(2-carboxyethyl)-1,4-diaminobutane (both metabolites of spermine), and δ-aminovaleric acid (a metabolite of cadaverine) is described. Using gas chromatographic (GC) quantification a comparison between the concentrations of polyamines and their non-α-amino acid metabolites in the urine of normal humans and rats was made. The significance of isoputreanine, the quantitatively most important urinary non-α-amino acid metabolite, was investigated in a follow-up study of a patient suffering from non-Hodgkin lymphoma and treated with cytostatic drugs. Finally, the role of oxidative catabolism of polyamines in cancer patients is discussed.

MATERIALS AND METHODS

Standards and reagents

δ-Aminovaleric acid was from Aldrich Europe (Beerse, Belgium), Sep-Pak silica cartridges were from Waters Assoc. (Milford, MA, U.S.A.) and 3 ml aromatic sulphonic acid disposable extraction columns from J.T. Baker (Phillipsburg, NJ, U.S.A.); heptafluorobutyric anhydride (HFBA) was from Pierce (Rockford, IL, U.S.A.); all other reagents were from E. Merck (Darmstadt, F.R.G.).

N,N^1-bis(2-Carboxyethyl)-1,4-diaminobutane (spermic acid 2), and N-(2-carboxyethyl)-4-amino-*n*-butyric acid (spermidic acid 2) were prepared as previously described [8, 12]. N-(3-Aminopropyl)-N^1-(2-carboxyethyl)-1,4-diaminobutane (spermic acid 1) was prepared by cyanoethylation [13] of spermidine with acrylonitrile in alkaline solution, followed by acid hydrolysis in 6 *M* hydrochloric acid at 120°C.

Equipment

GC with nitrogen—phosphorus detection was performed with a Hewlett-Packard Model 5880 gas chromatograph equipped with a Model 7672 A automated sampler and interfaced with a Tracor 812 analytical processing data

system. The gas chromatograph was operated under the previously described conditions [12].

Gas chromatography—mass spectrometry was performed using a Varian 3700 gas chromatograph directly coupled to a MAT 212 mass spectrometer, operated under the following conditions: injection temperature 250°C; oven temperature programme 1 min at 50°C, 16°C/min to 250°C, 10 min at 250°C; ion source temperature 200°C; ionization energy 70 eV. The column was a 30 m × 0.32 mm I.D. CP-Sil-5 coated (0.2 μm film thickness), fused-silica capillary from Chrompack (Middelburg, The Netherlands).

Samples

Urine samples (24 h and untimed voidings) from normal persons and patients were collected, acidified to pH 1—2 with hydrochloric acid, and stored at −20°C until analysis.

Prepurification and derivatization

After acid hydrolysis 1,3-diaminopropane, putrescine, cadaverine, isoputreanine, putreanine, spermidine, spermic acid 1, spermic acid 2, and spermine were isolated by silica gel adsorption and converted into their (methyl)heptafluorobutyryl derivatives [12].

From the passage of the silica gel column β-alanine (β-Ala), γ-amino-*n*-butyric acid (GABA), δ-aminovaleric acid, and spermidic acid 2 were isolated by means of cation-exchange chromatography and converted into their isobutylheptafluorobutyryl derivatives [8].

Mass fragmentographic identification

After the on-column injection of derivatized silica gel extracts and cation-exchange extracts, mass fragmentography was performed by monitoring specific electron-impact fragment ions of spermic acid 1 (*m/z* 590, 618, 622, and 650), spermic acid 2 (*m/z* 354, 423, 455, and 483), spermidic acid 2 (*m/z* 368, 410, and 483), and δ-aminovaleric acid (*m/z* 226, 239, 251, 268 and 296). For each compound the peak areas were calculated, using a Finnigan MAT SS-200 data system. Peak areas were expressed as a ratio to the area of the most intense fragment ion.

Identification was performed by comparing peak area data and GC retention times to those of synthetic standards. In some cases the relative peak heights were used.

Quantification of polyamines and their metabolites

Quantification of polyamines and metabolites in untimed voidings of a high-grade non-Hodgkin lymphoma patient were performed by GC with nitrogen–phosphorus detection [12]. Data for the normal 24-h urinary excretion of humans and rats were obtained from previous studies [8, 12].

RESULTS AND DISCUSSION

On the basis of their GC retention times and the peak areas of selected fragment ions spermidic acid 2, spermic acid 1, spermic acid 2 and δ-aminovaleric

acid were identified in the urine of a normal person, a patient with a non-African, Burkitt-type, non-Hodgkin lymphoma, and a patient with metastatic melanoma. In Figs. 1—4 the recordings of the selected ions are depicted together with those obtained from derivatized synthetic standards. The quanti-

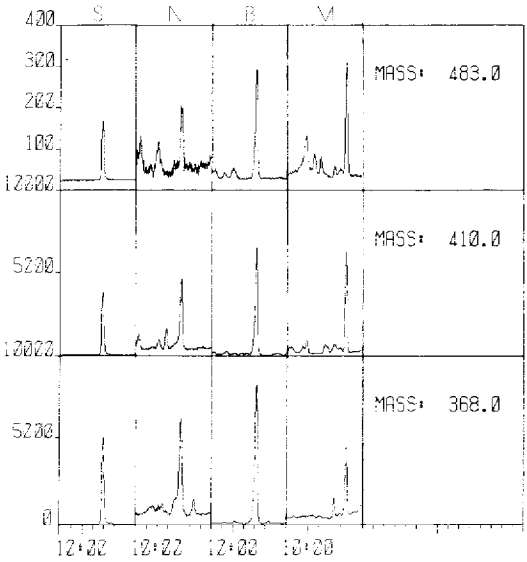

Fig. 1. Mass fragmentograms of spermidic acid 2 in a standard (S) and urine samples of a normal person (N) and patients with non-African, Burkitt-type, non-Hodgkin lymphoma (B) and metastatic melanoma (M). Time scale is in minutes and peak intensity in arbitrary units (counts).

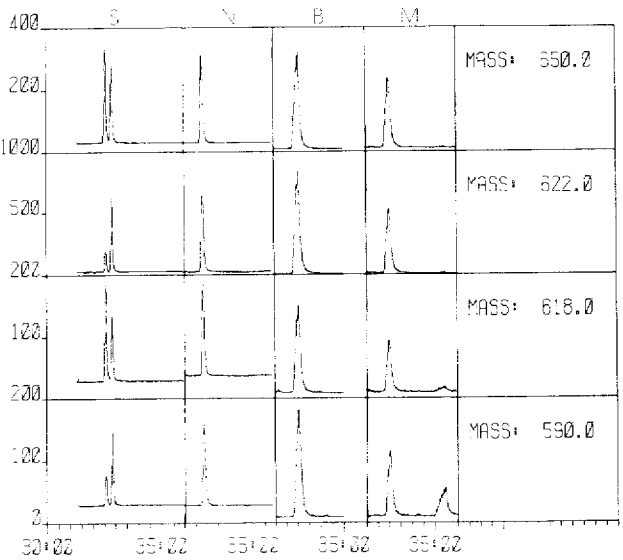

Fig. 2. Mass fragmentograms of spermic acid 1 in a standard (S) and urine samples of a normal person (N) and patients with non-African, Burkitt-type, non-Hodgkin lymphoma (B) and metastatic melanoma (M). Time scale is in minutes and peak intensity in arbitrary units (counts).

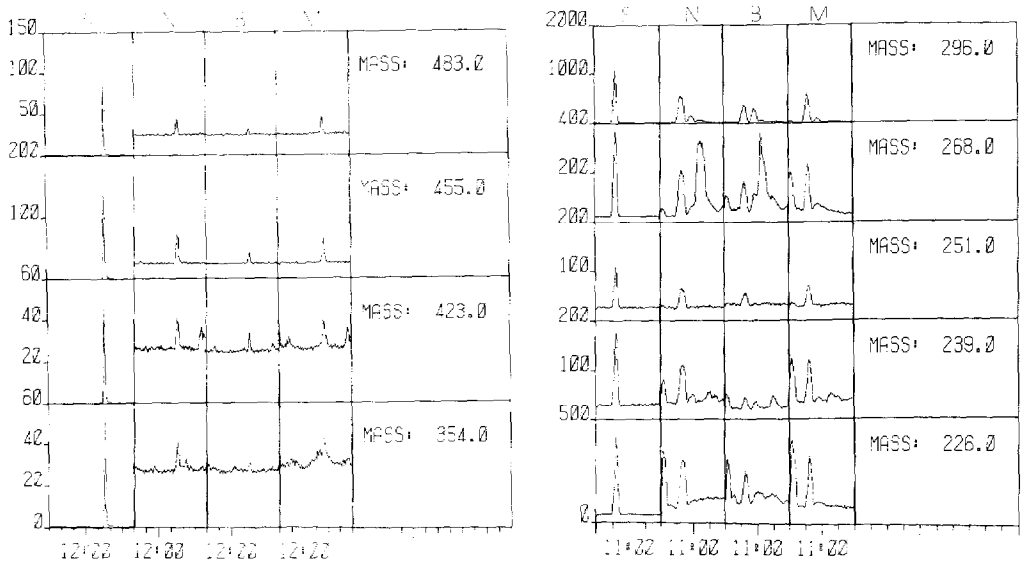

Fig. 3. Mass fragmentograms of spermic acid 2 in a standard (S) and urine samples of a normal person (N) and patients with non-African, Burkitt-type, non-Hodgkin lymphoma (B) and metastatic melanoma (M). Time scale is in minutes and peak intensity in arbitrary units (counts).

Fig. 4. Mass fragmentograms of δ-aminovaleric acid in a standard (S) and urine samples of a normal person (N) and patients with non-African, Burkitt-type, non-Hodgkin lymphoma (B) and metastatic melanoma (M). Time scale is in minutes and peak intensity in arbitrary units (counts).

tative results relevant for the identification are shown in Table I. The intensities of the characteristic ions are presented as a percentage of the most intense fragment ion of each compound. To avoid day-to-day variation in mass spectrometric performance the measurements for each individual metabolite were collected within one day. In Fig. 2 the mass fragmentographic peaks in the standard, with a retention time of 32 min and 35 sec, were caused by N-(3-aminopropyl)-N^1-(3-carboxypropyl)-1,3-diaminopropane. The relative peak areas of this isomer, which was formed in an amount comparable to that of spermic acid 1 during its synthesis, were found to be markedly different from those of spermic acid 1. The relatively high amounts of compounds that were co-extracted during the clean-up procedure led to peak broadening in the analysis of urine samples.

Table II shows a comparison between the levels of polyamines and their non-α-amino acid metabolites in the urine of normal three-month-old rats [8] and human adults [12]. Previous studies in rats [8] revealed that isoputreanine, putreanine and spermidic acid 2 should be considered as unique metabolites of spermidine, while spermic acid 1 and δ-aminovaleric acid are unique metabolites of spermine and cadaverine, respectively. A comparison between the ratios of these unique metabolites and their respective parent polyamines in normal rats and humans shows that, except for δ-aminovaleric acid/cadaverine, all metabolite/parent polyamine ratios are higher in human urine than in rat urine. A similar picture is obtained when GABA/putrescine ratios are

TABLE I

RELATIVE INTENSITIES AND GAS CHROMATOGRAPHIC RETENTION TIMES FOR SELECTED IONS OF SPERMIDIC ACID 2, SPERMIC ACID 1, SPERMIC ACID 2 AND δ-AMINOVALERIC ACID IN STANDARDS AND URINE SAMPLES

Relative intensities are expressed as a percentage of the most intense fragment ion (m/z = 368 for spermidic acid 2, m/z = 622 for spermic acid 1, m/z = 455 for spermic acid 2 and m/z = 296 for δ-aminovaleric acid). t_R = Gas chromatographic retention time; S = standard; N = urine sample of a normal person; B = urine sample of a patient with non-African, Burkitt-type, non-Hodgkin lymphoma; M = urine sample of a patient with metastatic melanoma.

Compound	S	N	B	M
Spermidic acid 2				
m/z 410	74	74	77	71
m/z 483	3	3	3	3
t_R	10 min 26 sec	10 min 32 sec	10 min 32 sec	10 min 36 sec
Spermic acid 1				
m/z 650	44	45	45	44
m/z 590	19	21	20	19
m/z 618	17	17	16	16
t_R	32 min 51 sec	32 min 53 sec	33 min 01 sec	33 min 01 sec
Spermic acid 2				
m/z 483	56	50	53	54
m/z 423	32	33	38	28
m/z 354	29	28	26	26
t_R	12 min 23 sec	12 min 20 sec	12 min 23 sec	12 min 24 sec
δ-Aminovaleric acid				
m/z 226	39	35	37	36
m/z 268	33	35	32	32
m/z 239	14	14	14	14
m/z 251	8	8	7	7
t_R	10 min 52 sec	10 min 53 sec	10 min 52 sec	10 min 52 sec

considered. However, GABA is not a unique metabolite of putrescine and may also be formed by decarboxylation of glutamic acid [14]. As β-alanine at least partly originates from the catabolism of uracil, and no precursor–metabolite relationship between 1,3-diaminopropane and β-alanine seems to exist in rats [8], this ratio has not been considered. The relatively higher concentrations of oxidized metabolites of polyamines is suggestive for the existence of a higher catabolic pressure on polyamines in humans. Other, less probable, possibilities are that humans salvage polyamines more efficiently, or catabolize the non-α-amino acid metabolites less efficiently. The δ-aminovaleric acid/cadaverine ratio seems to be an exception to the rule, which may be connected with the, contrary to human beings, normal physiological role of cadaverine in rats, such as is established during pregnancy [15].

In rats considerable catabolic shifts were encountered between the first and second intraperitoneal injection of labelled polyamines. These shifts were explained to be indicative of the induction of degradative enzymes by the first

TABLE II

CONCENTRATIONS OF TOTAL POLYAMINES AND METABOLITES IN THE URINE OF RATS OF THE WISTAR STRAIN AND NORMAL HUMAN ADULTS

Concentrations are expressed as mmol/mol of creatinine.

	Rats (n = 5)		Humans (n = 52)	
	Mean	C.V.* (%)	Mean	C.V. (%)
1,3-Diaminopropane	2.10	32.9	0.33	30.5
β-Alanine	9.96	35.5	6.33**	27.3
Putrescine	10.93	19.9	1.44	32.9
γ-Aminobutyric acid	6.12	33.5	3.20**	34.7
Cadaverine	3.04	27.6	0.50	128.1
δ-Aminovaleric acid	10.90	52.3	1.10**	50.5
Spermidine	4.74	19.0	0.58	27.5
Isoputreanine	3.48	30.7	1.31	32.9
Putreanine	0.91	25.3	0.27***	23.8
Spermidic acid 2	1.51	67.5	0.39***	76.9
Spermine	0.45	33.3	0.13	85.2
Spermic acid 1	0.20	25.0	0.10	21.4
Spermic acid 2	1.03	57.3	—	—
γ-Aminobutyric acid/putrescine	0.59	40.7	2.22§	—
δ-Aminovaleric acid/cadaverine	4.21	72.7	2.20§	—
Isoputreanine/spermidine	0.78	47.4	2.34	25.1
Putreanine/spermidine	0.20	35.0	0.54	30.8
Spermidic acid 2/spermidine	0.36	91.7	0.67§	—
Spermic acid 1/spermine	0.47	31.9	1.17	52.5

*C.V. = coefficient of variation.
**Mean of five individuals.
***Population containing 32 individuals.
§Calculated by dividing the respective mean values.

injection [8]. In man, catabolic enzyme induction is perceived when relatively high amounts of polyamines are liberated by spontaneous or therapeutically induced (tumour) cell loss [16]. It has been demonstrated that a gestational age dependent increase in the serum levels of diamine oxidase [17] and polyamine oxidase [18] takes place during normal pregnancy. Increased diamine oxidase levels have been associated with several types of cancer [19].

In the urine samples of an adult patient with non-Hodgkin lymphoma (Fig. 5) we observed a highly significant increase of isoputreanine during successful chemotherapy but not of its parent polyamine, spermidine. The ratio between the areas under the curve of isoputreanine and spermidine was calculated to be 6.02 (normal mean ± S.D. for adults = 2.34 ± 0.59 [12]). These data suggest that at least some cases of cancer are characterized by an increased degradative metabolic pressure on polyamines and endorse the use of simultaneous determination of polyamines and their non-α-amino acid metabolites as general markers for neoplastic diseases. The additional measurements of the circulating concentrations of polyamine-degrading enzymes (e.g. diamine oxidase, polyamine oxidase and possibly monoamine oxidase) may become of use for both the detection and follow-up of cancer, as their increased concentration reflects the body's natural defense against increased polyamine liberation.

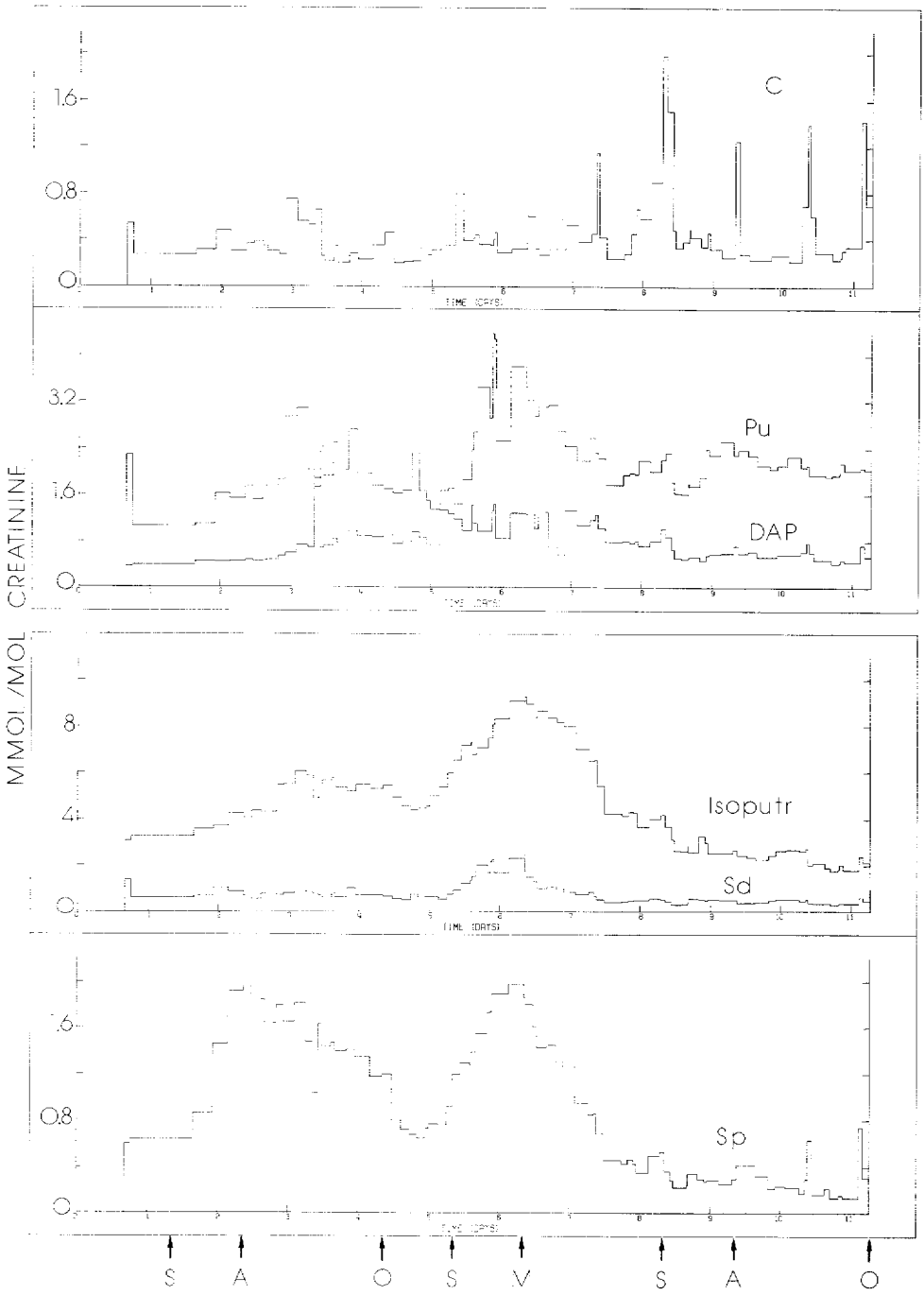

Fig. 5. Follow-up curve of polyamines and metabolite concentrations in untimed urine voidings of a 64-year-old man with stage IV non-Hodgkin lymphoma during treatment with a new chemotherapeutic regimen. S = Solumedrol® (methylprednisolone); A = cytosine arabinoside; O = Oncovin® (vincristine); M = methotrexate; DAP = 1,3-diaminopropane; Pu = putrescine; C = cadaverine; Isoputr = isoputreanine; Sd = spermidine; Sp = spermine. For normal urinary concentrations see Table II.

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