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Analysis of polyols in uremic serum by liquid chromatography combined with atmospheric pressure chemical ionization mass spectrometry

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ABSTRACT

Liquid chromatography with atmospheric pressure chemical ionization mass spectrometry in the negative-ion mode was used to analyse polyols in uremic serum obtained from haemodialysis patients. With post-column addition of 1% chloroform methanol as an ionization accelerating solvent, the chloride addition ions, $[M + Cl]^-$, were detected as base peaks, and the molecular masses of the polyols were easily determined by comparing $[M + Cl]^-$ and $[M - H]^-$ ions. Concentrations of erythritol, myoinositol, mannitol and sorbitol were markedly increased, and that of 1,5-anhydroglucitol was markedly decreased in the uremic serum compared with normal serum. After haemodialysis, the serum concentration of these polyols decreased significantly. This method was found to be useful in analysing the profile of polyols.

INTRODUCTION

The concentration of polyols in human physiological fluids has been reported to change in various diseases, such as uremia and diabetes mellitus. Serum levels and urinary excretion of myoinositol increase in uremic patients [1,2]. Accumulation of myoinositol in uremic serum is considered to be responsible for uremic peripheral neuropathy [3–5]. Concentrations of 1,5-anhydroglucitol in serum and cerebrospinal fluid decrease in diabetes mellitus [6–8] and uremia [2]. Serum 1,5-anhydroglucitol was proposed to be a marker of blood glucose control in diabetic patients [9]. The increased production of sorbitol in the lens of the human eye is thought to cause diabetic cataracts [10], and sorbitol also plays an important role in the development of diabetic peripheral neuropathy [11]. Thus, the analysis of polyols in serum and urine is clinically important for detecting the complications of uremia and diabetes mellitus.

Detailed analyses of polyols in serum and urine have been performed by high-resolution gas chromatography–mass spectrometry (HR-GC–MS) [2,12]. However, the method requires derivatization and laborious sample preparation, and cannot be used routinely for the clinical examination of polyols. Liquid chromatography–mass spectrometry (LC–MS) has not yet been applied to the analysis of serum polyols. Since LC–MS can often

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be used to analyse compounds without derivatization, we used liquid chromatography–atmospheric pressure chemical ionization mass spectrometry (LC–APCI-MS) to analyse polyols in uremic serum to determine if it can be used routinely in clinical applications.

EXPERIMENTAL

Materials

Erythritol, xylitol and arabitol were products of Tokyo Kasei Kogyo (Tokyo, Japan). Adonitol (ribitol), mannitol, sorbitol, myoinositol, D-fructose, D-arabinose, D-glucose and D-galactose were the products of Yoneyama Chemical Industries (Osaka, Japan). 1,5-Anhydroglucitol was a gift from Dr. Yutaka Hashimoto (Nippon Kayaku, Tokyo, Japan). All other chemicals used were of analytical grade.

Serum samples were obtained from ten uremic patients before and after haemodialysis (HD) (4 h, three times per week) and six healthy subjects. The serum samples were kept at −20°C until analysis.

Sample preparation

After the addition of 25 μg of adonitol as an internal standard (I.S.), 0.5 ml of serum was deproteinized with 1 ml of ethanol. After centrifugation for 10 min at 1000 *g*, the supernatant was concentrated to 0.5 ml under a nitrogen stream. The solution was applied to a Bond Elut SCX cartridge (cation-exchange, 100 mg/ml, Analytichem International, Harbor City, CA, USA) and eluted with 2 ml of distilled water. The collected eluate was then applied to a Bond Elut SAX cartridge (anion-exchange, 100 mg/ml, Analytichem International) and eluted with 2 ml of distilled water. The eluate was freeze-dried and the residue was dissolved in 100 μl of methanol–distilled water (1:1, v/v). A 20-μl volume of the sample was subjected to LC–APCI-MS.

LC–APCI-MS analysis

A Hitachi L-6200 high-performance liquid chromatographic pump combined with a quadrupole mass spectrometer (Hitachi M-1000S) was equipped with a Gelpack GL-C64Z column (sulphonated styrene–divinylbenzene copolymer with zinc ions, 150 mm × 6 mm I.D., 10 μm particle size, Hitachi-Kasei, Tokyo, Japan). Acetonitrile–distilled water (8:2, v/v) was used as the mobile phase at a flow-rate of 1.0 ml/min. The column temperature was 80°C. To accelerate ionization, 1% chloroform in methanol was added at a flow-rate of 0.5 ml/min to the eluate after it had passed through the column.

Mass spectra were recorded using negative-ion (NI) APCI. The drift voltage was −25 V, the vaporizer temperature 250°C, and the desolvator temperature 399°C.

To quantify the polyols in the serum samples, calibration curves were obtained with selected-ion monitoring (SIM) chromatograms using standard solutions. Amounts of polyols ranging from 0.1 to 50 μg were added to 0.5 ml of distilled water. After addition of 25 μg of adonitol as the I.S., these solutions were processed as described and analysed by LC–APCI-MS. Calibration lines relating the concentration of polyols to the peak-height ratios of the polyols at *m/z* 157 (erythritol), *m/z* 185 (arabinose), *m/z* 187 (xylitol), *m/z* 199 (1,5-anhydroglucitol), *m/z* 215 (myoinositol) and *m/z* 217 (mannitol and sorbitol), representing their $[M + Cl]^-$ ions, to the I.S. at *m/z* 187 were obtained from the SIM chromatograms. Correlation coefficients of the calibration lines ranged from 0.9996 to 0.9999.

Statistical analysis was performed by the Welch test [13] or paired *t*-test [14].

RESULTS

Figs. 1 and 2 show the NI-APCI mass spectra of polyols and monosaccharides. With post-column addition of 1% chloroform in methanol to accelerate ionization, the $[M + Cl]^-$ ions were detected as base peaks in the mass spectra. By comparing $[M + Cl]^-$ ions to $[M - H]^-$ ions, the molecular masses of the polyols and monosaccharides were easily determined.

Fig. 3 shows the SIM chromatograms of the standards, an extract from pre-HD uremic serum and an extract from normal serum. The peaks of

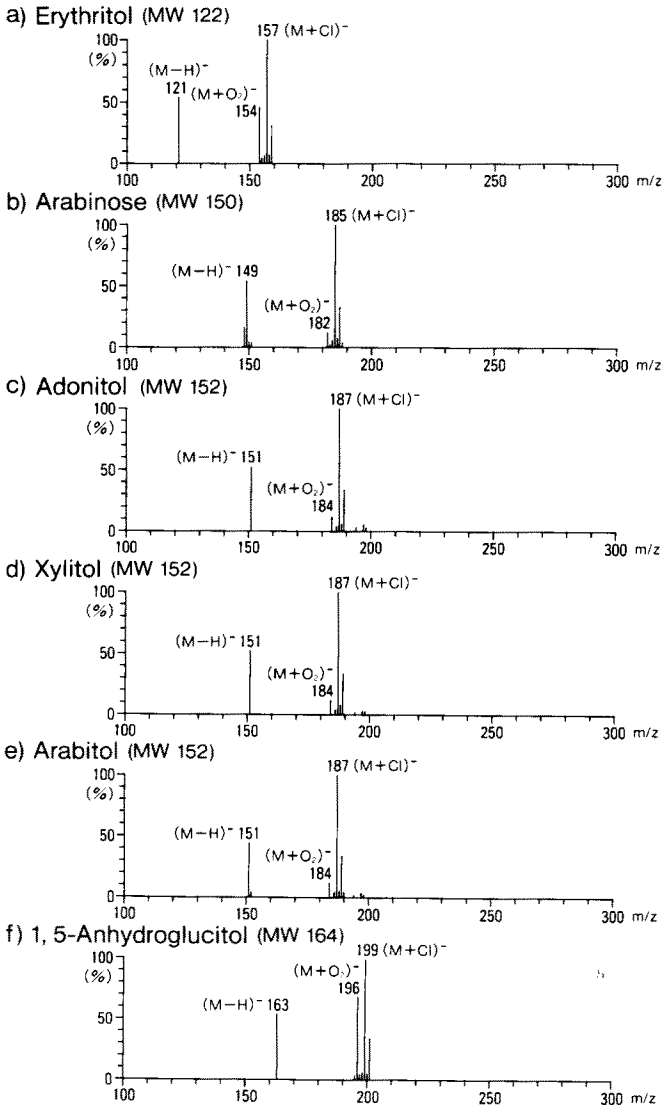

Fig. 1. NI-APCI mass spectra of erythritol, arabinose, adonitol, xylitol, arabitol and 1,5-anhydroglucitol.

the polyols and monosaccharides were well separated in the SIM chromatograms. The mass spectra and the retention times of the peaks were identical with those of the authentic compounds. Erythritol, mannitol, sorbitol and myoinositol were markedly elevated in uremic compared with normal serum. However, 1,5-anhydroglucitol was markedly decreased in uremic compared with normal serum.

Table I shows the serum concentrations of polyols in uremic and normal serum as determined by SIM of LC–APCI-MS. The concentrations of erythritol, myoinositol, mannitol and sorbitol were significantly increased in the pre-HD uremic compared with normal serum. The concentration of 1,5-anhydroglucitol was significantly decreased in uremic compared with normal serum. The serum concentrations of 1,5-anhydroglucitol, myoinositol and mannitol decreased significantly after HD.

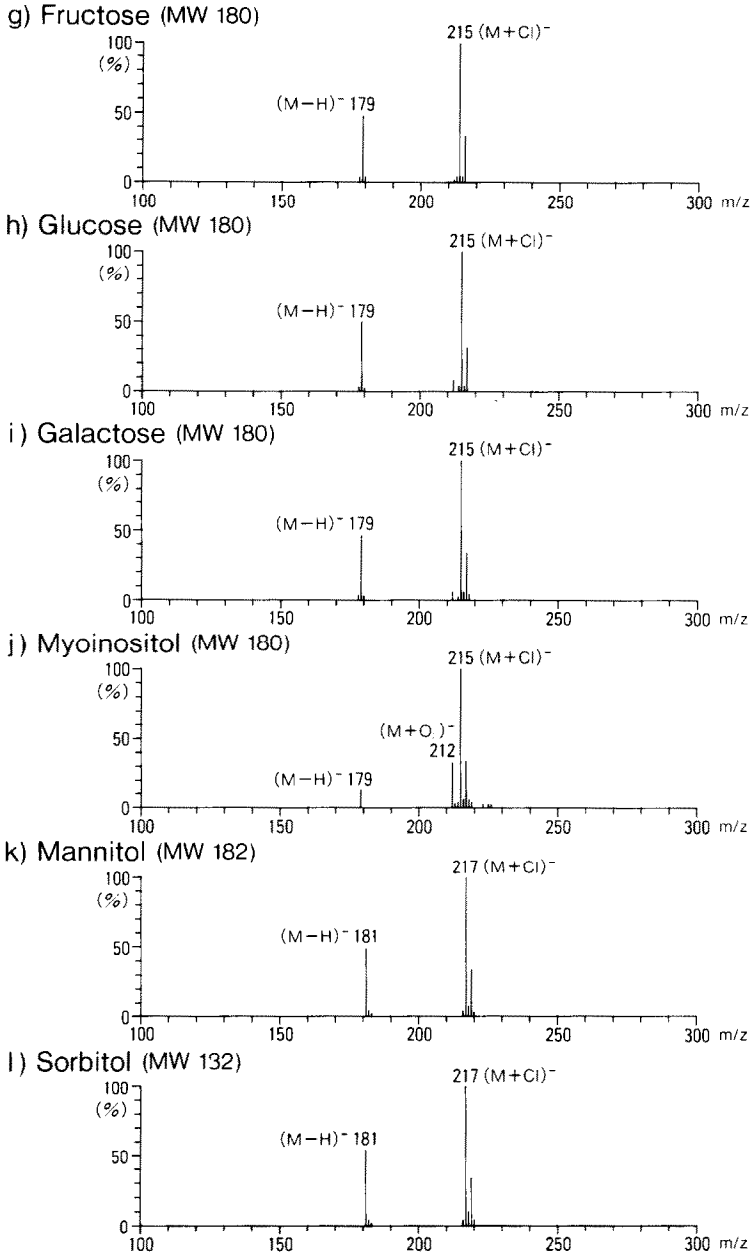

Fig. 2. NI-APCI mass spectra of fructose, glucose, galactose, myoinositol, mannitol and sorbitol.

DISCUSSION

Our analysis of serum polyols by LC–APCI-MS showed the concentrations of erythritol, mannitol, sorbitol and myoinositol to be elevated in uremic serum, as found previously using GC–MS [2]. However, serum 1,5-anhydroglucitol was decreased markedly in uremic serum, although by what mechanism is as yet unknown. Serum 1,5-anhydroglucitol was also been reported to be decreased in diabetes mellitus patients [6–8]. In diabetes mellitus, reabsorption of 1,5-anhydroglucitol in renal proximal tubules is impaired because of competitive inhibition by the high

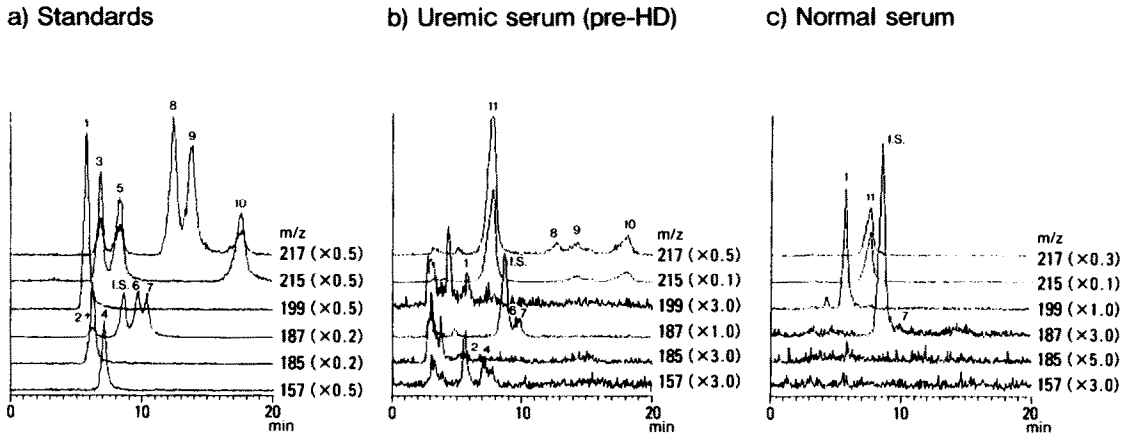

Fig. 3. SIM chromatograms of (a) standards, (b) the extract from pre-HD uremic serum, (c) the extract from normal serum, obtained using LC–NI-APCI-MS. Peaks: 1 = 1,5-anhydroglucitol; 2 = arabinose; 3 = fructose; 4 = erythritol; 5 = galactose; 6 = xylitol; 7 = arabitol; 8 = mannitol; 9 = sorbitol; 10 = myoinositol; 11 = glucose; I.S. = adonitol (internal standard).

TABLE I

CONCENTRATION OF POLYOLS IN UREMIC AND NORMAL SERUM

Compound	Concentration (mean ± S.D.) (μg/ml)		
	Normal ($n = 6$)	Uremic ($n = 10$)	
		Before HD	After HD
Erythritol	1.2 ± 0.3	11.0 ± 2.2[a]	11.2 ± 3.8
1,5-Anhydroglucitol	36.1 ± 9.4	3.2 ± 0.6[a]	2.0 ± 1.0[c]
Myoinositol	5.8 ± 1.7	82.6 ± 38.1[a]	39.6 ± 9.2[c]
Mannitol	1.5 ± 0.5	29.0 ± 32.9[b]	10.8 ± 6.9[d]
Sorbitol	1.6 ± 0.4	10.8 ± 7.1[a]	9.4 ± 3.2

[a] $p < 0.01$ compared with normal serum by the Welch test.
[b] $p < 0.05$ compared with normal serum by the Welch test.
[c] $p < 0.01$ compared with that before HD by the paired t-test.
[d] $p < 0.05$ compared with that before HD by the paired t-test.

concentration of glucose in the urine [15], and thus urinary excretion of 1,5-anhydroglucitol increases so that the serum level decreases.

Myoinositol of dietary or endogenous origin from glucose is normally metabolized to D-glucuronic acid in the renal cortex [16] and then oxidized to CO_2 through the glucuronate–xylose–pentose phosphate pathway [17]. Thus, the impaired renal oxidation of myoinositol in uremic patients leads to the accumulation of myoinositol in uremic serum and increased urinary excretion in undialysed uremic patients [2]. The increased levels of mannitol and sorbitol in uremic serum and urine [2] suggest that the production of mannitol and sorbitol is increased in uremia. Since the urinary excretion of erythritol is decreased in uremic patients [2], erythritol accumulates in uremic serum because renal function is impaired.

Serum polyols were detected by SIM of LC–APCI-MS as their chloride addition ions, $[M + Cl]^-$, with high sensitivity and high specificity. By using 1% chloroform in methanol to accelerate ionization, serum polyols could be quantified with high sensitivity by LC–APCI-MS. The detection limit of polyols in serum was 0.1 μg/ml, which was sufficient to measure the serum levels of polyols. By comparing $[M - H]^-$ ions with $[M + Cl]^-$ ions, the molecular masses of the polyols and monosaccharides can be easily recognized. When methanol was used as the ionization accelerating solution, instead of 1% chloroform in methanol, only $[M - H]^-$ ions were detected and the $[M + Cl]^-$ ions were not observed. Since analysis by LC–APCI-MS does not require derivatization, a step that is essential for GC or GC-MS analysis, it may be of interest for the routine measurement of serum polyols in clinical applications.

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