Polyol Concentrations in Serum during Hemodialysis

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Polyol concentrations were determined by selected-ion monitoring in 10 sample sets taken before dialysis and hourly for 4 h during dialysis. Predialysis concentrations (μmol/L) exceeding the upper limit of normal (mean ± 2 SD; n = 33) were found for erythritol (25.4–59.0), threitol (11.5–46.7), and arabinitol (21.0–49.3) in all 10 cases; mannitol (2.7–62.0) in nine cases; and xyitol (0.7–1.3) in eight cases. Concentrations of adonitol, galactitol, and sorbitol were all within normal limits. During dialysis, the concentration of six polyols decreased by ~50%, but there were no significant changes for xylitol and adonitol. Erythritol, threitol, and arabinitol concentrations remained more than double the upper limit of normal in all patients after 4 h of dialysis. High residual concentrations were found for mannitol (seven cases) and xyitol (six cases). The results suggest fundamental differences in the homeostasis as well as the dialyzability of different polyols. The potential toxic significance of the high residual concentrations after a 4-h dialysis needs to be investigated.

Additional Keyphrases: selected-ion monitoring mass spectrometry • uremia • homeostasis • renal failure

Uremia in humans, caused by renal failure, results in the accumulation of hundreds of compounds ranging from simple guanidines and phenolic acids to large, complex molecules such as microglobulins and lipoproteins (1, 2). Accumulated quantities of these compounds, ordinarily metabolized or excreted by the kidneys, can act as cell toxins and inhibitors of normal enzyme and membrane transport activities (3). Uremic patients have increased morbidity and mortality, often caused by infections. Although uremia can be treated only by eliminating the underlying causes, hemodialysis provides symptomatic relief. The importance of the specificity of dialysis membranes has long been known (4); however, attempts to make correlations with individual compounds or even compound classes have generally failed, often because of inadequate analytical information.

Polyols (polyhydric alcohols, alditols) are present in almost every living organism, but their metabolism is not well known (5–7). Only a few papers have dealt with (selected) polyol concentrations in uremic serum and urine (5–14). Most of these studies were limited because the methods used were not adequately sensitive for determining certain polyols, e.g., threitol, xylitol, arabinitol, in normal healthy subjects. Our interest in the behavior of polyols in hemodialysis was prompted by difficulties in differentiating between increased arabinitol concentrations in serum due to disseminated visceral candidiasis or to renal dysfunction (15–17). Our objectives were to determine serum concentrations of eight polyols in renal failure and to assess the rate of removal of these polyols during a 4-h conventional hemodialysis. We applied a technique based on selected-ion monitoring that we developed for determining the following eight polyols in sera from normal and cancer patients: erythritol, threitol, adonitol (ribitol), arabinitol, xylitol, galactitol ( dulcitol), sorbitol (glucitol), and mannitol (18).

Materials and Methods

Patients. A set of samples consisted of blood samples taken by venipuncture before conventional hemodialysis and at each hour during the 4-h dialysis period. Ten sets of samples were obtained from eight patients. Two patients, one with chronic polynephritis and one with nephrosclerosis, were sampled more than two months apart, and these sample sets were considered as independent sets. The other six patients had the following underlying diseases: two each with diabetes mellitus with hypertension, and one each with chronic polynephritis, nephrosclerosis, sarcoidosis, and chronic glomerulonephritis (with nephrosclerosis and hypertension).

Hemodialysis. Hemodialysis was performed with 1 m² cellulose acetate, hollow-fiber membrane, with Model Gambro-80M (Hospal-Gambro Corp., Denver, CO) negative-pressure machinery. Blood flow rate was 250 mL/min. The bath was conventional acetate, 41 mmol/L, at a flow rate of 500 mL/min. No mannitol was given to any patient before or during dialysis. Samples were taken by venipuncture on the arterial side of the dialysis line. Heparin was used as anticoagulant. A loading dose of 2000 units (30–50 units/kg of body wt.) of heparin was given to produce systemic anticoagulation, which was maintained by giving a sustaining infusion of 1000 units (10–15 units/kg per hour) during treatment to provide an average of 5000 units administered per total dialysis.

Standards and reagents. All polyols were from Sigma Chemical Co., St. Louis, MO. The internal standard, 2-deoxygalactitol, was synthesized from 2-deoxygalactose (15). Silylation-grade pyridine was from Pierce Chemical Co., Rockford, IL. Acetic anhydride and HPLC-grade solvents were from Fisher Scientific Co., Pittsburgh, PA. “High purity” isobutane and helium were from the Linde Division, Union Carbide Corp., Somerset, NJ.

Sample preparation and analysis. We used the sample preparation technique detailed in our previous paper (18), with calibration conditions appropriate for the present objectives. In brief, to 0.5 mL of serum or filtration fluid we added 1.0 μg of the internal standard and precipitated the serum proteins with methanol. After centrifuging and evaporating the supernate, we acetylated the residue with a mixture of acetic acid/pyridine at 80 °C for 15 min. The reaction was stopped with water, the acetates were extracted with hexane/chloroform, and the organic phase was washed with water and evaporated. This residue was dissolved in 100 μL of hexane, and 4-μL aliquots were injected into the gas chromatograph—mass spectrometer system (Model 3300; Finnigan Corp., San Jose, CA). The gas chromatographic column (glass, 1.5 m × 2.0 mm (i.d.), filled with 3% SP-3340; Supelco Inc., Bellefonte, PA) was temperature-programmed from 230 to 260 °C at a rate of

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8 °C/min. Isobutane was used as both the carrier gas and the reagent gas in the chemical-ionization source.

The following masses were monitored: \( m/z = 231 \) for the tetritols, \( m/z = 303 \) for the pentitols, \( m/z = 317 \) for 2-deoxygalactitol, and \( m/z = 375 \) for the hexitols. Calibration curves were prepared by using serum samples supplemented with polyols to yield the following concentrations (\( \mu \text{mol/L} \)): erythritol—0, 4.91, 9.83, 19.65, 29.48, 39.31, 58.96, and 78.61; threitol—0, 3.28, 6.52, 13.10, 19.65, 26.21, 39.31, and 52.42; arabinitol—0, 3.94, 7.89, 5.77, 23.66, 31.55, 47.32, and 63.09; adonitol and xylitol—0, 1.31, 2.63, 5.26, 7.89, 10.52, 15.77, and 21.03; mannitol—0, 3.29, 6.59, 13.17, 19.76, 26.35, 39.52, and 52.69; sorbitol—0, 2.20, 4.39, 8.78, 13.17, 17.57, 26.35, and 35.13; galactitol—0, 1.10, 2.20, 4.39, 6.59, 8.78, 13.17, and 17.57. (In mg/L units the concentrations were as follows: erythritol, arabinitol, mannitol—0, 0.6, 1.2, 2.4, 3.6, 4.8, 7.2, and 9.6; threitol and sorbitol—0, 0.4, 0.8, 1.6, 2.4, 3.2, 4.8, and 6.4; adonitol, xylitol, and galactitol—0, 0.2, 0.4, 0.8, 1.2, 1.6, 2.4, and 3.2.) We included a set of calibration runs with every set of samples analyzed.

**Results and Discussion**

Figure 1 illustrates the polyol patterns in a normal serum and in a typical patient’s serum (patient with nephrosclerosis) before and after 4 h of dialysis. The peaks monitored correspond to the (M—59)⁺ ions, resulting from the loss of a CH₃COO⁻ group from the molecular ion. These ions carried virtually all of the ion current (J8). The presence of heparin did not interfere with the analysis, as determined by quantifying polyols in control blood samples without and with heparin present. In the sample shown in Figure 1, the predialysis concentrations of threitol, erythritol, arabinitol, and mannitol were substantially above normal. Although the concentrations of these polyols decreased steadily during dialysis, they remained considerably above normal at the end of the dialysis period. The concentrations of the other polyols were either normal or only marginally increased in the predialysis sample.

Our first objective was to determine the serum concentrations of the polyols in renal failure. The zero-time points in Figure 2 show the polyol concentrations of the 10

**Fig. 1.** Polyol patterns in a normal serum sample and in a typical patient’s serum (patient with nephrosclerosis) before, during, and after dialysis

The following masses were monitored: \( m/z = 231 \) for erythritol and threitol; \( m/z = 303 \) for adonitol, arabinitol, and xylitol; \( m/z = 317 \) for mannitol, galactitol, and sorbitol. We also monitored \( m/z = 317 \) for 2-deoxygalactitol, the internal standard (not shown)

**Fig. 2.** Means (and SD; n = 10) of the polyol concentrations before and during dialysis

The broken lines indicate the upper limits of endogenous concentrations for the particular polyol, defined as the mean plus 2 SD in normal subjects (n = 33)
samples before dialysis. Numerical data for the predialysis means and ranges are given in Table 1, which also includes the upper limits of normals, taken as the mean (n = 33) plus 2 SD, and the corresponding normal ranges taken from our previous work (18); the endogenous concentrations of all polyols obtained in frequent calibration runs fell within those ranges.

Clearly, polyols are affected differently by renal failure, which suggests fundamental differences in their homeostasis. Erythritol, threitol, and arabinitol concentrations were significantly above the upper limit of normal in all patients. The concentration of erythritol in serum is known to increase in uremia (8); however, increased serum threitol is, to our knowledge, reported here for the first time. Arabinitol is an interesting case because endogenous serum arabinitol contains both the D- and L-enantiomers, the former probably originating from resident Candida species (which are known to produce only D-arabinitol). We have shown (19), using a new method to separate the enantiomers, that in severe renal dysfunction but without the presence of disseminated candidiasis the D/L-arabinitol ratio remains the same as in normal subjects, despite significantly increased concentrations of total arabinitol (such as are shown in Figure 1).

Mannitol concentrations significantly exceeded the upper limit of normal in nine of 10 cases, in agreement with previous findings (11). Although the endogenous concentrations of xylitol and adonitol are virtually identical (Table 1), serum xylitol was increased in eight of the 10 predialysis samples, and adonitol remained below the upper limit of normal in all cases. The two normal xylitol concentrations were from the patients with diabetes mellitus, contradicting some indirect evidence cited for possible changes in xylitol metabolism in diabetes mellitus (7). Galactitol and sorbitol were not influenced by renal failure, as evidenced by their concentrations remaining within the normal range in all patients. The normal sorbitol concentrations found in the sera of the two diabetic subjects, in agreement with earlier observations (11), are of interest in light of the significantly increased concentrations reported in erythrocytes in insulin-dependent diabetes (20) and suggest in vivo synthesis of sorbitol (probably from glucose) in erythrocytes in diabetes.

Our second objective was to assess the rate of removal of these polyols during a 4-h conventional hemodialysis. After the 4-h dialysis, there was no residual renal function in any patient, as measured by urine output. The average creatinine values at the beginning and at the end of dialysis were 900 (range 620–1000) and 480 (range 360–650) μmol/L, respectively. The decrease during the 4-h dialysis period was linear.

The polyol concentrations observed during dialysis (Figure 1 and Table 1) can be grouped into three categories: (a) above normal at all times in all samples: erythritol, threitol, and arabinitol; (b) varying from normal to above normal: mannitol and xylitol; and (c) always normal: adonitol, galactitol, and sorbitol.

Among the tetrots, the mean concentration of erythritol steadily decreased to 76%, 68%, and 58% of the predialysis value during the first, second, and third hours of dialysis, respectively, before reaching the final value of 49%. Threitol behaved similarly. The concentrations of both remained well above the upper limit of normal in all samples at the end of dialysis.

There was considerable variation in the behavior of the pentitols. Arabinitol concentrations declined steadily (1 h, 76%; 2 h, 68%; 3 h, 56%); however, the mean concentration at the end of dialysis, 48% of the predialysis value, remained significantly higher than the upper limit of normal in all patients. This is in reasonable agreement with previous data (before and after dialysis) for four uninfected patients with chronic renal failure (21). In another study, increased (19.72–38.12 μmol/L, 3.0–5.8 mg/L) arabinitol concentrations were found in serum of three patients with confirmed Candida peritonitis while on continuous ambulatory peritoneal dialysis; in the single case where both pre- and postdialysis data were available, arabinitol concentration declined by ~20% by the end of dialysis (22).

It has been recommended that arabinitol/creatinine ratios, rather than just arabinitol concentrations, should be considered when serum arabinitol is used for the diagnosis of disseminated candidiasis (23, 24). In the present work, the ranges of arabinitol and creatinine concentrations in predialysis serum were 23.66–49.29 μmol/L (3.6–7.5 mg/L) and 690–1300 μmol/L (70.2–140.9 mg/L), respectively. We were unable to find a statistically significant correlation between individual arabinitol and creatinine concentrations (r = 0.40), in agreement with the suggestion that the arabinitol/creatinine ratios may not apply to patients on dialysis (22). We also found no correlation between those polyols with higher than normal concentrations and the predialysis creatinine concentrations (r = 0.30, 0.27, and 0.47 for erythritol, threitol, and mannitol, respectively).

It is intriguing that the mean xylitol concentrations were above normal while adonitol remained below normal, and also that these pentitols were removed only very slowly by

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* To convert μg/L, divide by 9.19 for the tetrots, 6.57 for the pentitols, and 5.48 for the hexitols.

* Mean of normal plus 2 SD (n = 33).
dialysis. Eight of the 10 samples had predialysis xylitol concentrations above normal; six of these remained above normal after dialysis. Although xylitol was present in relatively small amounts, 0.39–1.31 μmol/L (0.06–0.2 mg/L), other polyols present in comparable concentrations did decrease during dialysis. For example, one patient had a predialysis sorbitol concentration of 0.55 μmol/L (0.1 mg/L), which decreased to 0.22 μmol/L (0.04 mg/L) after the 4-h dialysis. The fact that only small quantities were present therefore does not explain why xylitol did not decrease with dialysis.

Among the heptitols, galactitol and sorbitol were present in normal concentrations before dialysis, yet both decreased by about 50% during dialysis. There was a wide variation in the mannitol concentrations. Of the nine samples with high predialysis concentrations, seven remained above normal at the end of dialysis and two decreased to normal. One patient had a normal predialysis concentration, 4.50 μmol/L (0.82 mg/L), which decreased to 1.81 μmol/L (0.33 mg/L). We have previously noticed similar wide variations in mannitol concentrations in normal subjects as well as in cancer patients (18).

The basic principle of hemodialysis is simple: Blood (anticoagulated) and the dialysate (a balanced salt solution) are perfused to opposite sides of a semipermeable membrane, and solutes from blood diffuse down a concentration gradient into the dialysate. Solute is removed by diffusion, and extracorporeal fluid is removed by ultrafiltration. The effects of the area of the membrane surface and the flow rates of dialysate and blood on the rate of solute removal are linear. It is generally believed that the molecular removal (clearance) of molecules with molecular masses <300 Da is high and nonspecific.

The tetritols, pentitols, and heptitols studied have molecular masses of 122, 162, and 182 Da, respectively; thus, nearly identical dialyzability behavior would be expected for all of them. As Figure 2 shows, the concentrations of six of the eight polyols decreased consistently, nearly linearly during dialysis. The mean concentrations of these polyols were ~50% (range 40%–55%) less by the end of the 4-h dialysis (Table 1), despite a 20-fold difference in their predialysis values. In contrast, mean xylitol and adonitol concentrations decreased by only 8% and 20%, respectively, during dialysis; as mentioned already, the low serum concentrations cannot alone explain why xylitol concentrations remained nearly constant during dialysis.

One need not be concerned about adonitol, galactitol, and sorbitol because their concentrations were normal in all patients at all times. Xylitol is more interesting because it was above normal in eight of the 10 cases, six of which remained high at the end of dialysis. Conceivably, xylitol may diffuse from erythrocytes during dialysis, so that in serum its concentration might appear to be unchanged. Despite numerous studies on the metabolism of xylitol (see 25), we could find no information on its concentration in erythrocytes or on its binding properties. We attempted to quantify xylitol (and also the other polyols) in aliquots of the dialysis fluid, but the enormous dilution of already very low initial concentrations prevented our doing so, even after repeated preconcentrations.

At the end of the 4-h dialysis, concentrations of erythritol, threitol, and arabinitol remained significantly above the upper limit of normal in all samples, and that of mannitol in seven of the samples. Of these polyols, only mannitol is known to be present in erythrocytes in appreciable quantities. According to Pitkanen et al. (11), the concentration of mannitol in erythrocytes remains essentially unchanged during dialysis, suggesting that mannitol moves slowly from the intracellular space to the extracellular space through the erythrocyte membrane.

The potential toxic significance of the high residual concentrations of these polyols after 4 h of dialysis needs to be investigated. Because abnormal polyol metabolism in tissues as well as in body fluids has been associated with disease complications (e.g., in diabetes mellitus), monitoring polyol concentrations in sera of patients with renal failure may contribute to a better understanding of this complex and inadequately understood condition.

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References