Speciation of Aluminum in Urine

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A procedure has been developed for the speciation of aluminum in urine samples of four normal subjects fed required diet on two dates. Three fractions of aluminum species, *i.e.*, labile monomeric ions, non-labile monomeric ions along with labile complexes, and non-labile complexes were classified and determined by the combination of ion-exchange (IE), fluorometric spectrometry and graphite-furnace atomic absorption spectrometry (GF-AAS) techniques. The results showed that the aluminum in urine was mainly in non-labile complex forms.

Keywords Aluminum, lumogallion, urine, speciation

The utilization of aluminum (Al) by mankind can be traced back to more than four thousand years. However, its toxicity was not well documented until 1970.¹ The environmental effects of aluminum depend on the form in which it occurs.²⁻⁴ Unfortunately, there is no specific, direct method for measuring these toxic aluminum species. A number of fractionation procedures have been developed to distinguish various aqueous forms of aluminum, based on ion exchange, reactions with colorimetric and fluorometric reagents, dialysis, ultrafiltration and the use of a fluoride ion-selective electrode.⁴⁻⁶ Recently, ion chromatography, size-exclusion chromatography, HPLC coupled with electrothermal atomic absorption spectrometry (ETAAS), flow injection analysis, kinetic studies and the combination of the last two techniques have been developed.⁷⁻¹² The most frequently used method for the fractionation of aluminum in natural water samples is probably the method developed by Driscoll *et al.*¹³

Although aluminum absorption *in vivo* is small, direct investigations are almost impossible in ordinary laboratories due to the extremely high price of radio nuclides, and the requirement of accelerator mass spectrometry for detection. The most credible information has been obtained by determining the urinary excretion rates and regarding them as a minimal amount of absorption. To study the fate of ingested aluminum in the body and subsequent possible health effects, understanding the behavior of aluminum ions in living organisms is essen-

tial. The chemical forms of aluminum in food and beverage and those in body fluids have important implications for the balance between the metal's uptake into tissues and its excretion. However, only aluminum speciation in serum has been investigated.^{14,15} To our knowledge, there have been few reports on the speciation of aluminum in urine samples. Thus, the purpose of present work was to develop a method for classifying aluminum species in urine.

Methodology

The Driscoll methodology is based on a cation-exchange column containing the resin Amberlite IR-120. This scheme directly measures three aluminum fractions in water: acid-reactive aluminum (Al_r), total monomeric aluminum (Al_T) and non-labile monomeric (Al_n). From these three measurements, two more fractions can be calculated: "acid soluble" aluminum (Al_a) from $Al_r - Al_T$, and "labile monomeric" aluminum (Al_l) from $Al_T - Al_n$.¹³

The situation of aluminum in urine is different from that in natural water. However, by combining several analytical techniques, three fractions of urine aluminum can still be determined (Fig. 1). With the ion-exchange (IE) technique, labile monomeric aluminum ions are retained on the column, so that non-labile monomeric ions and labile complexes can be directly determined by fluorometric method.

Labile and non-labile monomeric aluminum ions, as well as labile complexes in urine can be measured using

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Fig. 1 Schematic representation of the aluminum fractionation procedure.

the same analytical method by determining the original urine samples. The difference between the two measurements represents the labile monomeric ions.

Graphite-furnace atomic absorption spectrometry (GF-AAS) can be used to determine the total urine aluminum; the difference between the total and previously mentioned two groups is the fraction of non-labile complexes. All of the compounds which can not be complexed by a fluoromeric reagent (lumogallion) to form an Al-lumogallion complex can be classified into the third group.

Experimental

Reagents

All of the chemicals used were of analytical grade unless otherwise stated. High-purity Milli-Q water (18.2 M Ω) was used throughout.

Stock standard solutions containing 1 g/l of Al(III), Ca(II), Mg(II), Mn(II), Fe(III), Pb(II), Cu(II) and Zn(II) in nitrate forms ("Spectrosol") for atomic absorption spectrometry (AAS) were purchased from BDH (Poole, UK). All of the working standard solutions were freshly prepared daily by appropriate dilution of the stock solution with 0.03 M HNO₃ in volumetric flasks and transferred immediately to polypropylene tubes.

Lumogallion was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) (purity: 94.0%) and a 3 mM solution was prepared by dissolving 0.52 g of the compound in 500 ml of water. A 0.2 M potassium hydrogenphthalate ("Baker Analyzed", purity: 100.02%) (J. T. Baker, Philipsburg, USA) solution was prepared by adding 2 g of the reagent to 50 ml of water. A 1.5 M NaOH ("Suprapur", Merck, Germany) solution was used to adjust the pH values of the reaction mixture.

Containers

All of the reagent and urine samples were stored in high-density polyethylene containers, which were previously leached with 10% nitric acid for 48 h, and filled with high-purity water until use.

Instruments and apparatus

An ion-exchange microcolumn was made using a 3 mm i.d. PTFE tube packed with Amberlite IR-120 Plus from Sigma (St. Louis, USA). The ends of the microcolumn were filled with glass wool and small fragments of Technicon filter in order to avoid any loss of resin from the PTFE tube.

The fluorescence spectra were obtained using a Shimadzu spectrofluorophotometer RF-5000 (Kyoto, Japan). The working conditions were as follows: excitation wavelength, 497 nm; emission wavelength, 574 nm; lamp current, 7 A; and sensitivity, high.

A GF-AAS analysis was performed on a Perkin-Elmer 4100ZL atomic absorption spectrometer equipped with an HGA-500 graphite furnace, a THGA graphite tube and an AS 70 autosampler.

Procedure

Urine sample collection

Urine samples were obtained from four healthy male volunteers who worked at an analytical chemistry laboratory and had no occupational exposure to aluminum. Samples were collected over a period of 24 h on two dates when they were fed the required diet; only deionized water was allowed *ad lib*. Each passage of urine was voided into individual acid-washed containers. The volume and pH values of each sample were measured, and the samples were stored at 4°C in a refrigerator without any treatment. The analyses were

performed within 48 h after collection.

Driscoll ion-exchange method

Ion-exchange procedures have been developed and modified by several research workers, and the optimal conditions were obtained by a comparative study.¹⁶ In the present study, this method was adopted for determining free forms of aluminum in urine (labile monomeric ions). The modifications included that the ionic composition of the analytical column for fractionation be in the Na⁺ form instead of the H⁺ form; small samples were used for the ion-exchange procedure and a later analysis. After a pretreatment of the cation exchange column, about 15 ml of urine was added into the column and 5 ml of the middle fraction was collected for a reaction with lumogallion.

Formation of Al-lumogallion complex

The reaction conditions for forming Al-lumogallion were optimized in our previous study.¹⁷ Briefly, 0.8 ml of 0.2 M potassium hydrogenphthalate was added into 5 ml of the original or ion-exchanged urine samples, and the pH value measured with universal pH indicator of Merck (Darmstadt, Germany). If necessary, 1.5 M NaOH was used to adjust the pH of the mixture to 4.7, 40 µl of 3 mM lumogallion was added to form a fluorescent complex. The mixture was left to stand for 20 min at room temperature before a measurement.

Fluorometric determination

The fluorescence spectrophotometric method was modified from that for measuring the aluminum in serum.¹⁸ All of the instrument-dependent parameters which influence the performance were selected so as to obtain good sensitivity. The excitation wavelength (497 nm), emission wavelength (574 nm) and either 5.0 or 10 nm excitation and emission slits were used. Five-fold replicates of all of the measurements were executed.

The effects of foreign cations on the exchange efficiency of aluminum

The main cations with multiple positive charges found in human urine are listed in Table 1. Seven cations were

spiked into the standard aluminum solution, and their effects on the exchange efficiency of the free forms of aluminum were studied.

GF-AAS determination of total aluminum

The total aluminum in urine samples was measured by GF-AAS without a sample pretreatment. Magnesium nitrate was used as a modifier and other relevant instrumental conditions were the same as that in our previous work.²¹

Contamination control

The same precautions as those mentioned in our previous paper¹⁷ were taken against background aluminum contamination. However, blank values were ubiquitous. Since the background was stable, signals superimposed on the blank value could be measured with sufficient precision. Since the pH values of our samples were around 7, they could be adjusted to 4.7 using only a buffer solution, thus preventing a large aluminum contamination from the addition of an acid or a base.

Results and Discussion

Effects of the flow rate in the ion-exchange method

The accuracy of the labile aluminum species somewhat depended on the flow rate of the solution through the column: the longer was the retention time of the aluminum species, the greater was the disruption of the non-labile aluminum species. Since the aqua aluminum was stripped from inorganic ligands by a polar cation exchange resin, inorganic forms of aluminum were readily removed from the solution. Organic ligands, however, form strong complexes with aluminum, and therefore more effectively compete with the cation-exchange resin. In order to prevent an over-estimation of the free forms of the aluminum fraction, a quick flow rate was used (4 ml/min).

Cation interference to the retention of labile monomeric aluminum by an ion-exchange resin

The main constituents of urine are water, urea, uric acid, creatinine, sodium, potassium, chloride, calcium, magnesium, phosphates, sulfates, and ammonia. Urine can also contain structures, such as casts, crystals, blood cells, and epithelial cells.²² Normal urinary aluminum excretion has been assumed to be 20 to 50 µg/d²⁰, i.e., the normal aluminum concentration is about 10 to 40 µg/l.^{23,24} The coexisting cations were at similar or slightly higher levels as aluminum, much lower than those spiked in interference tests. In addition, just as complicated as aluminum, since these metal ions are also in different forms or species, the competition for an interaction on the cation exchange resin from the positively charged particles in urine are less. As an example, studying iron in urine is not practical since the quantity is extremely small unless the urine contains hemoglobin or myoglobin.²² The cation-exchange resin has a strong

Table 1 Metals in urine from healthy subjects¹⁹

Metal	Quantity mg d ⁻¹	Metal/Al ^a
Sodium	4200	120000
Potassium	2500	71429
Magnesium	100	2857
Calcium	23	650
Zinc	1.4	40
Iron	0.5	14
Manganese	0.05	1
Copper	0.04	1
Lead	0.03	1

a. Normal urinary aluminum excretion is assumed to be 20 to 50 µg/d.²⁰

Table 2 Levels of three aluminum species in urine samples of four subjects (A, B, C and D) on two dates ($\mu\text{g/l}$) ($n=3$)

Subjects	Species			
	Labile monomeric	Non-labile monomeric & labile complex	Non-labile complex	Total
A ₁	0.7 $\pm$ 1.4 (3.9%)	4.2 $\pm$ 1.3 (23.3%)	13.1 (72.8%)	18.0 $\pm$ 3.2
B ₁	2.2 $\pm$ 1.1 (13.0%)	1.7 $\pm$ 1.5 (10.1%)	13.0 (76.9%)	16.9 $\pm$ 2.8
C ₁	0.8 $\pm$ 1.2 (3.6%)	4.6 $\pm$ 1.7 (20.8%)	16.7 (75.6%)	22.1 $\pm$ 2.6
D ₁	4.7 $\pm$ 1.8 (11.6%)	7.9 $\pm$ 2.6 (19.6%)	27.8 (68.8%)	40.4 $\pm$ 2.5
A ₂	1.9 $\pm$ 1.3 (5.5%)	4.9 $\pm$ 1.0 (14.2%)	27.7 (80.3%)	34.5 $\pm$ 2.9
B ₂	1.7 $\pm$ 1.4 (1.8%)	5.7 $\pm$ 1.0 (6.0%)	87.7 (92.2%)	95.1 $\pm$ 1.4
C ₂	1.3 $\pm$ 2.1 (4.3%)	5.4 $\pm$ 1.4 (17.5%)	24.1 (78.2%)	30.8 $\pm$ 1.8
D ₂	1.2 $\pm$ 1.5 (2.4%)	9.5 $\pm$ 1.2 (18.8%)	39.9 (78.9%)	50.6 $\pm$ 2.3

The figure in the parenthesis indicates the percentage of the concentration of the species to the total concentration.

affinity for aluminum, and thus preferentially adsorbs the positively charged inorganic monomeric aluminum rather than the negatively charged organic fraction.

Our data could not elucidate the real effects of these ions on the aluminum-ion exchange efficiency. This was possibly due to the following factors: firstly, there was inevitably aluminum contamination in these standard solutions even though high-purity chemicals were used, which resulted in more than the original aluminum being found in the ion exchange effluents; secondly, spiked metal ions were mostly in the free forms instead of the complex forms in urine. The complicity requires further study.

Performance characteristics

Each analytical step inevitably introduced errors in the determination of aluminum species in urine; by controlling the quality of each analytical technique, the combined method could be carried out with both good accuracy and precision. A triplicate determination of the standard solutions and urine sample was carried out.

In the fluorometric detection method, the fluorescent intensity increased linearly from 1 to 30 $\mu\text{g/l}$ of aluminum in standard solutions. The correlation coefficients (r) were over 0.99 (mean of 4 replicate measuring). The background aluminum contamination was estimated to be around 0.5 to 1 $\mu\text{g/l}$ and the detection limit, calculated as the amounts equivalent to twice the standard deviation of the blank, was 1–2 $\mu\text{g/l}$.

In the GF-AAS procedure, triplicate measuring for all of the standard solution and real samples were brought about. The calibration graph was linear up to 100 $\mu\text{g/l}$ of aluminum in standard solutions. The correlation coefficients (r) were also over 0.99 for the standards. The background aluminum contamination due to the reagents and vessels was about 5 $\mu\text{g/l}$. The detection limits (for the injection of 20 μl of sample) were calculated to be twice the standard deviation of the blank signal at 2 $\mu\text{g/l}$ levels.

The species and levels of aluminum in urine

The distribution of species in all of the samples and the total levels of Al in urine samples are given in Table 2.

As mentioned in the Methodology section, the aluminum species in the urine were classified into four groups of three fractions. In the first fraction, labile monomeric aluminum ions accounted for less than 13% of the total aluminum.

About 6–24% of the total aluminum was of the second fraction, including non-labile monomeric and labile complex aluminum species. This fraction might have included amorphous $\text{Al}(\text{OH})_x(\text{H}_2\text{O})_{y(3-x)+}$, aluminum citrate, lactate, oxalate and fulvic acetate complexes. The last fraction (>68.8% of aluminum), that of non-labile complexes, represented colloidal, polymeric and very strongly bound alumino-organic macromolecular complexes. Phosphate, bicarbonate, carbohydrates, silicates, organic acids, amino acids, nucleotides are often used as chelating agents for aluminum, and some of the complexes may be very stable. As small quantity of Al-protein complexes may also be present in urine.

In biological systems, it is likely that Al^{3+} associates with oxygen-donor ligands, at least some of which should be anionic to help counter the 3+ charge on the cation. Carboxylate and phosphate groups, inorganic phosphate, nucleotides and polynucleotides meet this requirement. The ionic milieu and speciation of aluminum in various body fluids are important factors which influence the nature and rate of aluminum elimination by various routes. Urine excretion is a main path of aluminum excretion. It is fortunate that most of the aluminum in urine is in the form of a non-labile complex. Although it is still not known whether small aluminum complexes or protein-bound forms contribute more to aluminum's toxicity, the absorption of aluminum by tissue could be minimized, due to the formation of non-labile complexes.

As a part of systematic studies of Al metabolism in humans, the present work was only the introduction of an analytical technique for Al speciation in urine samples, and thus further study using a sufficiently large population to allow good statistical power for a stratified analysis or randomization is needed; also the effects of beverages and diets on speciation requires a consecutive investigation.

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