Intestinal absorption of trace amounts of aluminium in rats studied with $^{26}$aluminium and accelerator mass spectrometry


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1. Until recently studies of intestinal aluminium absorption used pharmacological amounts of stable $^{27}$Al.
2. To examine the intestinal absorption of trace amounts of different chemical compounds of aluminium, in the present study we have employed the long half-life isotope of aluminium, $^{26}$Al, and accelerator mass spectrometry. Trace amounts of $^{26}$Al (2.7–12.1 ng) as the hydroxide, citrate, citrate plus 1 mmol/kg sodium citrate, or maltolate respectively, were administered to four groups of rats ($n = 9$ per group) by gavage. Blood and urine samples were collected for 5 h and the $^{26}$Al content (as a percentage of the administered dose) determined by accelerator mass spectrometry.
3. The 5 h urinary $^{26}$Al excretion amounted to $0.1 \pm 0.02$, $0.7 \pm 0.2$, $5.1 \pm 1.5$ and $0.1 \pm 0.1\%$ of administered dose in the four groups respectively. There was a strong positive correlation between peak plasma $^{26}$Al ($r = 0.98$) and urinary $^{26}$Al excretion in individual animals ($P < 0.001$).
4. We conclude that the fractional intestinal absorption of trace oral doses of aluminium hydroxide is at least 0.1% (compared with the previous estimate of 0.01% using large $^{27}$Al oral loads). Absorption of aluminium citrate given alone is significantly greater (0.7%) and is further increased to 5% by the accompanying sodium citrate, consistent with an enhancing effect of added citrate upon mucosal aluminium permeability. Aluminium maltolate absorption approximates that of aluminium hydroxide (0.1%).

INTRODUCTION

Aluminium is abundant in the Earth's crust but has no known physiological role in mammals. It is inevitably present in the diet but the normal gastrointestinal tract presents a fairly effective barrier to its absorption. In the healthy individual, absorbed aluminium is excreted in the urine. However, aluminium accumulation can occur in patients with renal failure, both before and after initiation of haemodialysis therapy. In such patients, toxic effects from the accumulated aluminium may include encephalopathy [1, 2], osteomalacia [3–5] and microcytic anaemia [6, 7]. Now that appropriate precautions are generally taken to remove aluminium from the water used in dialysis, the major potential source of aluminium overload in these patients is from ingested aluminium. Aluminium derived from intestinal absorption has also been proposed as playing a causative role in Alzheimer’s disease, although this is controversial [8].

Very little is known about the intestinal absorption of normal (trace) amounts of dietary aluminium. Until recently, isotopes of aluminium could not be employed to study absorption and studies were possible only with pharmacological oral doses of salts of stable aluminium ($^{27}$Al). Froment et al. [9], using milligram doses of aluminium in rats, found important differences in absorption of various chemical compounds of aluminium, including aluminium hydroxide, chloride and lactate [9]. They also confirmed the earlier observation [10] that citrate enhanced the absorption of these large loads of aluminium and presented data suggesting that this effect of citrate resulted from opening of 'tight junctions' between intestinal mucosal cells. This occurred most likely as a result of chelation of calcium in the vicinity of these junctions [11]. There are also reports of enhanced gastrointestinal absorption of aluminium in the form of aluminium maltolate [12], a compound formed with maltol, which is a disaccharide found in such foods as bread, jelly and hot chocolate.

The use of $^{27}$Al to study intestinal aluminium absorption is accompanied by a high risk of environmental aluminium ($^{27}$Al) contamination of samples.
as well as the need to use pharmacological rather than physiological doses of aluminium. We have recently demonstrated the feasibility of using the long-lived radioisotope $^{26}$Al, determined with accelerator mass spectrometry (AMS), to study aluminium kinetics in the rat [13]. This technique now permits the measurement of absorption of trace amounts of aluminium, similar to those present in a normal diet, and free from problems of environmental contamination, since $^{26}$Al, unlike $^{27}$Al, is virtually absent from the environment. The extent to which usual (trace) amounts of aluminium, as opposed to pharmacological amounts, are absorbed from the intestine may be of clinical importance in patients with chronic renal failure both before and during dialysis treatment. In such patients the absorption of trace amounts may lead to the accumulation of an important body-burden of aluminium over a long time-period. The purpose of the present study, therefore, was to employ $^{26}$Al and AMS to determine plasma and urinary $^{26}$Al levels in rats after the oral administration of trace (ng) amounts of different chemical forms of aluminium, and to examine the effect of citrate on $^{26}$Al absorption. Another study of gastrointestinal aluminium absorption in rats, employing $^{26}$Al and AMS, first presented simultaneously with our studies [14], has recently been published [15]. Surprisingly, this study reported no effect of citrate upon $^{26}$Al absorption.

In addition there are two more recent preliminary reports of studies in man using $^{26}$Al. In one [16] the amount of $^{26}$Al in the plasma compartment at its peak after oral ingestion is equated with the amount absorbed from the intestine. This study showed approximately 1% absorption of $^{26}$Al (given in an unspecified chemical form) when administered with excess citrate, and approximately 0.01% when given with orange juice. The authors acknowledge that, since $^{26}$Al losses into other body compartments and into the urine are ignored, these values for fractional absorption are underestimates. In the other study [17], after oral administration of $^{26}$Al as aluminium chloride in man, frequent blood and urine samples were analysed for $^{26}$Al for the first 4 days, and less frequent samples thereafter. Compartmental analysis was attempted, ignoring the possible presence of body depots with long retention times. At peak, approximately 0.01% of the ingested dose was in the plasma compartment, and approximately 0.1% had been excreted in the urine.

SUBJECTS AND METHODS

Male Wistar rats (Charles River, St. Constant, Quebec, Canada) weighing approximately 400 g were housed in individual metabolic cages with free access to food (Rodent Laboratory Chow, Ralston Purina Canada Inc., Mississauga, Ontario, Canada) and water. After 1 week of acclimatization, creatinine clearance studies were carried out for determination of glomerular filtration rate. The animals were then randomly divided into four groups of nine.

The $^{26}$Al tracer (half-life, $7.16 \times 10^5$ years) was obtained from the Los Alamos Scientific Laboratory in a solution of 1 mol/l HCl with a $^{26}$Al/$^{27}$Al ratio of 1:16. Aliquots of 2.7 ng of $^{26}$Al as aluminium hydroxide (pH 7.00) (Group A), 5 ng of $^{26}$Al as aluminium citrate (pH 6.03) (Group B), 12.1 ng of $^{26}$Al as aluminium citrate with added sodium citrate (1 mmol/kg) (pH 8.3) (Group C) and 5 ng of $^{26}$Al as aluminium maltolate (pH 6.00) (Group D) were diluted with water to 2 ml and administered by gavage to each of the animals after a 16 h overnight fast with water ad libitum. Since the $^{26}$Al/$^{27}$Al ratio was 1:16, between 40 and 190 ng of $^{27}$Al was administered along with the $^{26}$Al.

The animals were then returned to their metabolic cages and were restrained by a plastic cover which allowed tail vein blood sampling without risk of contamination of urine with blood. Blood samples (0.4–0.5 ml) were taken at 30, 45, 60, 90, 150 and 300 min post-gavage. After removal of the 300 min blood sample, animals were sacrificed with an overdose of anaesthetic, since we wished to avoid potentially heavy contamination of urine by the large amounts of non-absorbed $^{26}$Al that would subsequently have been excreted in the faeces. Urine from each rat collected in its metabolic cage during the 300 min study period was combined with residual urine aspirated from the surgically exposed bladder at the time of sacrifice.

Sample analysis

Small volumes of urine (0.1–1.0 ml) and plasma (0.1 ml) were used for the preparation of samples for analysis by AMS. To each sample, 5 mg of $^{27}$Al was added as a carrier to ensure that sufficient aluminium was in the sample to perform the chemical processing. The $^{27}$Al carrier ensured that the aluminium native to the plasma and urine was quantitatively insignificant and that the measured $^{26}$Al/$^{27}$Al ratio would not be affected by adventitious contamination with $^{27}$Al during sample preparation. Samples were then dried and the resulting residue was ashed as described previously [13]. Measurements of $^{26}$Al/$^{27}$Al ratios were performed at the Zurich AMS facility (Paul Scherrer Institute and Swiss Federal Institute of Technology, Zurich, Switzerland). Plasma and urinary creatinine were measured using standard procedures. Gastrointestinal absorption of aluminium was based on urinary $^{26}$Al excretion, which was also examined in relation to the plasma $^{26}$Al appearance curves.

The error for the measurement of the $^{26}$Al/$^{27}$Al ratio by AMS is approximately 5% for the peak plasma $^{26}$Al value in Group C and approximately 20% for the range of values in Group D. For the urine $^{26}$Al values, the error of measurement of the
$^{26}\text{Al}/^{27}\text{Al}$ ratio is approximately 3–5% in Group C and approximately 5–8% in Group B.

**Statistical methods**

The extremely high analytical effort and cost for AMS $^{26}\text{Al}$ determinations permitted the measurement of only a small number of samples. Therefore the plasma samples from the nine rats at each time point were pooled in each group to obtain the mean plasma $^{26}\text{Al}$ concentration. The 0–300 min urine samples from the nine rats in each group were pooled in an analogous way, to obtain mean urinary $^{26}\text{Al}$ excretion. However, to obtain an index of between-rat variability, we were able to include individual plasma samples from at least three randomly selected animals at four time points (45, 60, 150 and 300 min) in each group, with some samples from additional animals at the most significant time points (a total of 67 individual plasma $^{26}\text{Al}$ samples) and randomly-selected individual urine samples (0–300 min) from four to six animals in each group (a total of 19 individual urine $^{26}\text{Al}$ samples). The plasma data from pooled samples (means) were fitted as a function of time using the exponential difference model ($k\exp(at-\exp bt)$), to obtain more precise estimates of the area under the plasma $^{26}\text{Al}$ appearance curve (AUC), the peak plasma $^{26}\text{Al}$ content and the time to peak plasma $^{26}\text{Al}$ content [18–20]. Ninety-five percent confidence intervals for the estimated means were also calculated. Results are expressed as percentages of orally-administered doses (% dose) in urine and plasma (assuming a plasma volume of 5% body weight). Comparisons of time to peak plasma $^{26}\text{Al}$ content, AUC and urinary $^{26}\text{Al}$ excretion were made between the four groups using the analysis of variance with Bonferroni adjustments for multiple comparisons [21]. The time to peak was transformed logarithmically to satisfy statistical assumptions for the purposes of analysis.

**RESULTS**

Table 1 shows the data derived from the pooled and individual plasma and urine samples from each group with respect to peak plasma $^{26}\text{Al}$ level, time to peak, AUC and total (300 min) urinary $^{26}\text{Al}$ excretion. Figure 1 shows the fitted mean plasma $^{26}\text{Al}$ content (% dose) in each of the four groups of rats as a function of time post-gavage. As described above, these values were determined from pooled plasma samples from the nine rats in each group. In all four groups, the peak plasma $^{26}\text{Al}$ concentration occurred between 30 and 90 min post-gavage, and the time to peak was not statistically different in the four groups of rats. The lowest peak plasma $^{26}\text{Al}$ content was found in Groups A and D which received $^{26}\text{Al}$-aluminium hydroxide and $^{26}\text{Al}$-aluminium maltolate respectively. In these groups, values were not significantly different. In group B rats given $^{26}\text{Al}$-aluminium citrate alone, both peak plasma $^{26}\text{Al}$ levels and AUC were at least two-fold higher than those after administration of $^{26}\text{Al}$-aluminium hydroxide or maltolate ($P<0.01$). When $^{26}\text{Al}$-aluminium citrate was combined with sodium citrate (1 mmol/kg) in group C animals, both the peak plasma $^{26}\text{Al}$ concentration and AUC were more than 2.5-fold higher than those in Group B ($P<0.001$).

Figure 2 shows total urinary $^{26}\text{Al}$ content (% dose) for the 300 min period in the pooled samples from each group of rats. The addition of sodium citrate (1 mmol/kg) to $^{26}\text{Al}$-aluminium citrate in Group C animals enhanced urinary $^{26}\text{Al}$ excretion 7-fold relative to that of Group B, and a further 6-fold relative to Groups A and D ($P<0.001$). Based on urinary $^{26}\text{Al}$ excretion, the percentage of gastrointestinal absorption of aluminium was calculated as 0.1% in Groups A and D, 1% in Group B and 5% in Group C rats. These values for fractional absorption may be somewhat underestimated, since they ignore the quantity of $^{26}\text{Al}$ retained in body pools at 5 h. However, our previous study [13] showed that 50–60% of an intravenously administered trace dose of $^{26}\text{Al}$ had been excreted in the urine within 5 h.

Figure 3 shows the very strong positive correlation between urinary $^{26}\text{Al}$ excretion (% dose) and the peak plasma $^{26}\text{Al}$ in randomly selected individual rats in the four groups of animals ($r=0.98$, $P<0.001$). A strong correlation was also found between urinary $^{26}\text{Al}$ excretion and plasma $^{26}\text{Al}$ AUC in individual animals ($r=0.95$, $P<0.001$).

Table 1. Percentage of administered dose of $^{26}\text{Al}$ (% dose) in plasma and urine samples after oral administration of $^{26}\text{Al}$ in various aluminium compounds. Values shown are pooled means ± 95% confidence intervals.

<table>
<thead>
<tr>
<th>Group</th>
<th>Plasma</th>
<th>Urine</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Area under curve (% dose at 300 min)</td>
<td>Peak level (% dose)</td>
</tr>
<tr>
<td>A: Aluminium hydroxide</td>
<td>10.9 ± 3.7</td>
<td>0.1 ± 0.03</td>
</tr>
<tr>
<td>B: Aluminium citrate</td>
<td>24.5 ± 7.8</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>C: Aluminium citrate + added citrate</td>
<td>62.1 ± 5.3</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>D: Aluminium maltolate</td>
<td>4.0 ± 1.8</td>
<td>0.02 ± 0.01</td>
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Fig. 1. Percentage of administered dose of $^{26}$Al in the plasma compartment of groups of rats as a function of time post-gavage after administration of different aluminium compounds. Values shown are mean values based on pooled plasma samples from nine rats in each group (Group A: $^{26}$aluminium hydroxide; Group B: $^{26}$aluminium citrate; Group C: $^{26}$aluminium citrate plus added citrate (sodium citrate, 1 mmol/kg body weight); Group D: $^{26}$aluminium maltolate). The error for the measurement of $^{26}$Al/$^{57}$Al by AMS is approximately 5% for the peak value in Group C and approximately 20% for the range of values in Group D.

DISCUSSION

Use of $^{26}$Al and AMS makes it possible to study the access of trace quantities of ingested $^{26}$Al to the bloodstream and, subsequently, to the urine. Previous studies [9], using milligram quantities of different chemical compounds of aluminium, have suggested that absorption is dependent on the solubility and chemical species of the compound at intestinal pH (pH 6–7) and have shown that citrate enhanced the absorption of these large intestinal loads of aluminium, probably by opening 'tight junctions' and allowing increased passive absorption [11].

These previous studies by Froment et al. [9, 11] have led to an estimated fractional absorption of aluminium (estimated from the plasma aluminium levels achieved and the calculated volume of distribution) of 0.015% for insoluble salts such as sucral-fate and aluminium hydroxide. When citrate was ingested together with the poorly absorbed aluminium salts, absorption, estimated by change in urinary excretion, was increased to 0.80–2.27% [9].

We have obtained data on the absorption of trace (ng) amounts of aluminium, based on the analysis of the plasma AUC as well as urinary excretion. In the normal rat, the fraction of the ingested dose of $^{26}$aluminium hydroxide (or $^{26}$aluminium maltolate) excreted in the urine was approximately 0.1% and for $^{26}$aluminium citrate approximately 1%. The very strong correlation between urinary $^{26}$Al and both peak plasma $^{26}$Al (Fig. 3) and AUC attest to the precision of the method. Absorption based on urinary $^{26}$Al excretion may represent an underestimation, since Jouhanneau et al. [15] have reported that after oral administration of $^{26}$Al, a comparable amount to that excreted in the urine was retained in bone. However, we have reported in a previous
study [13] that after intravenous administration of $^{26}\text{Al}$, 75% was excreted in the urine within 24 h, and Priest et al. [22] have recently estimated that 65% of an injected dose of $^{26}\text{Al}$umium citrate was lost in the urine in the first 24 h.

In the present study, when $^{26}\text{Al}$umium citrate was administered in the presence of mmol amounts of sodium citrate, net intestinal absorption was at least 5%. Since the solubility of aluminium citrate is little affected by pH [9], the presence of 1 mmol/kg sodium citrate would not be expected to influence the solubility of $^{26}\text{Al}$umium citrate [11]. Our data indicate therefore, a specific enhancing effect of the citrate ion on the absorption of trace amounts of aluminium, similar to that recently reported in man in experiments in which plasma $^{26}\text{Al}$ levels were measured after oral $^{26}\text{Al}$ administration [16] and similar to what has previously been shown with large aluminium loads. We presume that the nanogram amounts of citrate present in the administered $^{26}\text{Al}$umium citrate would be insufficient to have this effect. There are several differences in the experimental design that may account for the failure of Jouhanneau et al. [15] to observe an enhancing effect of citrate on $^{26}\text{Al}$ absorption. These authors do not indicate the chemical form in which $^{26}\text{Al}$ was given, and their animals were not fasted before the oral administration of $^{26}\text{Al}$. Differences in other dietary constituents, such as silicon, might also contribute to the different observed fractional aluminium absorptions [23].

The approximately 10-fold greater fractional absorptions that we have observed with trace quantities of aluminium, compared with previous experiments using large amounts of $^{27}\text{Al}$ (0.1% compared with 0.01% found by Froment et al. [9, 11]) could be of major practical importance. It is unlikely that there is saturable active transport of aluminium to account for this observation. It is possible, however, that the large doses of $^{27}\text{Al}$ given in previous experiments might greatly exceed the passive transport capacity of the tight junctions.

In summary, the present studies confirm the usefulness of $^{26}\text{Al}$ and AMS for the study of aluminium kinetics, and indicate that the fractional intestinal absorption of trace amounts of aluminium may be substantially greater (at least 0.1% for $^{26}\text{Al}$umium hydroxide and 1% for $^{26}\text{Al}$umium citrate) than that reported for large aluminium loads (0.01%). Furthermore, we have found that the addition of excess citrate as sodium citrate to a trace dose of $^{26}\text{Al}$umium citrate results in a further enhancement of aluminium absorption to at least 5%, which could potentially lead to important aluminium accumulation if citrate-containing medications are taken together with normal trace dietary aluminium intakes, in patients with chronic renal failure, even in the absence of pharmacological doses of aluminium hydroxide.

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REFERENCES