Effect of citrate on plasma aluminium concentration and aluminium excretion in the rat

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INTRODUCTION

Aluminium is present in the normal diet, as well as in a number of medications, and there is abundant evidence that the accumulation of aluminium in the tissues of the body is harmful [1-3]. Although only a small proportion (0.1-1%) of dietary aluminium is absorbed [1, 2], tissue accumulation of aluminium would occur if this absorbed aluminium were not excreted.

However, the renal excretion of aluminium is inefficient [4] for a number of reasons. These include protein-binding of aluminium, which limits its ultrafilterability, complexation of aluminium in other non-ultrafilterable chemical forms, and the possibility of renal tubular reabsorption of aluminium (for a review, see [1]). It has been suggested by others that the protein-binding of aluminium in plasma increases with increased plasma aluminium concentration [5, 6]. Our work, in which aluminium chloride was administered intravenously to conscious rats, has shown that while this is true in an absolute sense, at least for plasma aluminium levels up to about 200 ng/ml, nevertheless the fraction of aluminium which is protein-bound decreases with increasing plasma aluminium concentration [4]. At very high plasma aluminium levels (e.g. 20000 ng/ml), the ultrafilterability of aluminium is very low (about 1%), not primarily as a consequence of protein binding, but as a consequence of the insolubility (i.e. colloid formation) of aluminium at these high concentrations. Indeed, the complexity of aluminium speciation in body fluids is a factor which complicates all data on renal aluminium excretion [4, 7], and has received little attention.

Citrate is extremely effective in enhancing the gastrointestinal absorption of aluminium [8, 9], although the mechanism of this effect is still not entirely clear. It is likely that, in the gut, a soluble complex of citrate and aluminium is formed [8, 10], which could be absorbed. In addition, citrate is...
known to open epithelial tight junctions in cultured cells [11] and in intestinal loops in vitro [9].

Since there are similarities between many of the epithelial transport processes in the gut and in the kidney, the effect of citrate on the renal handling of aluminium is of interest. Although there have been previous studies which have shown that when citrate and aluminium are administered orally, aluminium excretion increases (due to increased gastrointestinal absorption of aluminium), there have to date been no investigations in which the effects of citrate on the renal handling of aluminium per se have been studied. This was the question investigated in the present paper, in which we have used intravenous administration of both citrate and aluminium in order to avoid the complications of altered gastrointestinal absorption.

METHODS

Animals and protocol

Experiments were performed on male Sprague-Dawley rats (weight range 330-400 g), which had been previously maintained on a rat cake diet (containing 9.59 ± 0.44 μg of aluminium/g), with free access to water. On the day of the experiment, rats were anaesthetized with ether, and a tail artery and tail vein were cannulated. Seven groups of animals were used.

(1) Control group (n=6). This group received 1 ml of 0.7% NaCl via the venous cannula, while still under ether anaesthesia.

(2) Low dose aluminium chloride group (n=7). This group received 1 ml of 0.7% NaCl containing 25 μg of aluminium (as AlCl₃·6H₂O).

(3) High dose aluminium chloride group (n=6). This group received 1 ml of 0.7% NaCl containing 800 μg of aluminium (as AlCl₃·6H₂O).

(4) Low dose citrate control group (n=5). This group received 1 ml of a solution of 0.09% sodium citrate/0.7% NaCl.

(5) Low dose aluminium citrate group (n=5). This group received 1 ml of the solution as in group 4, but with 25 μg of aluminium (as AlCl₃·6H₂O).

(6) High dose citrate control group (n=6). This group received 1 ml of a solution of 3% sodium citrate/0.7% NaCl.

(7) High dose aluminium citrate group (n=6). This group received 1 ml of the solution as in group 6, but with 800 μg of aluminium (as AlCl₃·6H₂O).

After the above bolus administrations, the animals were placed in individual Perspex restraining cages, and after recovery from the anaesthetic, each rat received a 4.5 h infusion (6 ml/h) of 0.7% NaCl solution via the venous cannula. Urine was collected by spontaneous voiding, and after appropriate dilution was analysed for aluminium by graphite furnace atomic absorption. Details of the diluents and of the furnace conditions are given in [4].

Precautions were taken at all stages of the experiments to prevent aluminium contamination of infusion fluids or samples. Aluminium-free water was prepared by passing deionized water through an Elga UHQ2 water purification unit (Elga, High Wycombe, Bucks, U.K.). All containers were washed in this aluminium-free water, and all infusates were prepared using it.

For the determination of plasma aluminium concentration blood (0.3 ml) was taken from the arterial line at the beginning of the experiment (before administration of the bolus), then immediately after administration of the bolus, and subsequently at hourly intervals. The blood withdrawn was replaced by an equal volume of heparinized (50 units/ml) saline (150 mmol/l NaCl). The blood was immediately centrifuged and the plasma was separated. At the end of the experiment (4.5 h) a 2 ml terminal blood sample was taken.

Ultrafilterability of aluminium citrate for plasma and aqueous solutions

An in vitro ultrafiltration technique (Amicon Micropartition System MPS 1; Amicon, Danvers, MA, U.S.A.) was used to determine the ultrafilterability of aluminium chloride with sodium citrate, in the way we have already described for aluminium chloride alone [4]. Determinations were made in aqueous solution (19 mmol/l NaH₂PO₄·2H₂O; 81 mmol/l Na₂HPO₄, pH 7.4) and also in plasma obtained from rats immediately after the administration of the aluminium chloride/sodium citrate bolus.

Statistics

Differences (in corresponding time periods) between experimental groups were assessed using the Wilcoxon rank sum test (for unpaired samples).

RESULTS

Animal experiments

The low dose of aluminium administered in this study (in groups 2 and 5), 25 μg, is 0.94 μmol. For group 5, this dose of aluminium was administered together with 3.1 μmol of sodium citrate. The measured osmolality of that 1 ml bolus of aluminium chloride and sodium citrate was 236 mosmol/kg H₂O. The high dose of aluminium used in groups 3 and 7, 800 μg, is 29.6 μmol. For group 7, this dose was administered with 102 μmol of sodium citrate (measured osmolality of administered bolus, 572 mosmol/kg H₂O). Thus for both groups (5 and 7) that received aluminium and citrate, there was at least a threefold molar excess of citrate over administered aluminium.

Fig. 1 shows the aluminium excretion in the seven groups over the 4.5 h infusion period, and Table 1 shows the total aluminium excretion for the groups over the 4.5 h period. There were no consistent
Effect of citrate on aluminium excretion

4.500 -
4.000 -
3.500 -
3.000 -
2.500 -
2.000 -
1.500 -
1.000 -
0.500 -
0.000 -

Time (h)

Fig. 1. Urinary aluminium excretion over the duration of the experiment. Values are means ±SEM. Note the different scales in (a) and (b). (a) ■ Group 1 (control); ○ group 2 (low dose aluminium chloride); △ group 4 (low dose citrate control); ◇ group 5 (low dose aluminium citrate); □ group 6 (high dose citrate control). P values are for differences between groups 2 and 5. (b) ■ Group 3 (high dose aluminium chloride); ○ group 7 (high dose aluminium citrate). P values are for differences between the two groups.

Table I. Total urinary aluminium excretion (0-4.5 h) in the seven groups. Values are means ± SEM. For both doses of aluminium, the aluminium excretion when citrate was administered was significantly higher (P < 0.01) than when aluminium chloride only was administered.

<table>
<thead>
<tr>
<th>Urinary aluminium excretion (µg)</th>
<th>Control (vehicle only)</th>
<th>25 µg of aluminium</th>
<th>800 µg of aluminium</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.7% NaCl</td>
<td>1.18 ± 0.31</td>
<td>13.39 ± 0.87</td>
<td>160.03 ± 18.49</td>
</tr>
<tr>
<td>0.0% sodium citrate in 0.7% NaCl</td>
<td>0.68 ± 0.15</td>
<td>21.85 ± 3.05</td>
<td>—</td>
</tr>
<tr>
<td>3.0% sodium citrate in 0.7% NaCl</td>
<td>1.50 ± 0.33</td>
<td>—</td>
<td>451.16 ± 36.76</td>
</tr>
</tbody>
</table>

difference was significant for both the low dose and the high dose of aluminium (P < 0.01). Fig. 2 shows the plasma aluminium concentrations during the time course of the experiment. It is obvious that aluminium administered as the chloride (AlCl₃·6H₂O) produced higher plasma concentrations than did the same dose of aluminium administered as AlCl₃·6H₂O plus sodium citrate. Nevertheless, in the citrate groups the urinary excretion from the lower plasma level was greater than when AlCl₃·6H₂O alone was administered. This indicates greater ultrafilterability of the plasma aluminium in the presence of citrate, since we have already shown that glomerular filtration rate is not affected by the presence of aluminium in the plasma in the concentrations achieved in the present study [4].
Ultrafilterability of aluminium

The ultrafilterability of aluminium chloride with sodium citrate was determined in an aqueous buffered solution, and in rat plasma after the administration of 800 μg or 25 μg of aluminium as a 1 ml bolus in 0.7% NaCl with either 3% sodium citrate (for the 800 μg dose) or 0.09% sodium citrate (for the 25 μg dose). The results are shown in Table 2, together with previously published data for aluminium chloride alone.

DISCUSSION

The findings of the present study indicate that aluminium administered with citrate is more efficiently excreted than aluminium given as the chloride. It is clear both from the present work and from our previous observations [4] that there are several reasons for this.

The initial concentration of aluminium in plasma after aluminium chloride (25 μg) administration (1427±233 ng/ml) indicates a volume of distribution of 17.5 ml, i.e. the aluminium is confined to the vascular space. (The calculated plasma volume for a 350 g rat is 4.7% of body weight, i.e. 15 ml). When the 25 μg of aluminium is administered with citrate, the significantly lower (P<0.05) initial concentration (594±56.7 ng/ml) indicates a volume of distribution of 42.1 ml; clearly, the citrate form of aluminium is able to leave the plasma and enter other body fluid compartments.

The differences in volume of distribution are consistent with differences in ultrafilterability. We have previously shown [4] that 41.9±7.8% of the 25 μg dose of aluminium as the chloride is ultrafilterable from the plasma. The non-ultrafilterability of the remaining approximately 60% is due to binding; this is likely to be mainly to the iron-binding protein transferrin [11], but combination with plasma phosphate as AlPO₄·2H₂O [which could also be written as Al(OH₂)H₂PO₄] to form insoluble 'variscite' could also be involved [11]. In contrast, aluminium (25 μg) administered together with citrate is 84.2±8.4% ultrafilterable from plasma, indicating much less binding to substances which render the aluminium non-ultrafilterable than is the case with the administration of aluminium chloride alone.

With the 800 μg dose of aluminium, only 1.06±0.13% was ultrafilterable from the plasma if the aluminium was administered as chloride [4], whereas 79.8±7.1% was ultrafilterable when the aluminium chloride was administered with sodium citrate. Clearly, the citrate is maintaining the aluminium in an ultrafilterable form.

The plasma aluminium concentration immediately after the high dose aluminium bolus was not significantly different between the group given chloride and that given citrate (Fig. 2). This may be due to insufficient time for such a large dose of aluminium citrate to diffuse from the plasma into other body fluid compartments. In all subsequent periods, however, the plasma level of aluminium in the group given chloride was higher than that in the group that received citrate, and this mainly reflects the higher rate of aluminium excretion when the citrate form is administered.

Do the findings imply that intravenous administration of citrate would have beneficial effects for patients with high plasma aluminium concentrations? It is at present difficult to give an unequivocal answer to this question. Certainly renal aluminium excretion is more efficient if citrate is administered, but this is because the aluminium–citrate complexes formed are more ultrafilterable than the forms in which aluminium is bound in the absence of citrate. However, because the increased ultrafilterability of aluminium–citrate complexes is reflected in a larger volume of distribution, this could make the aluminium more accessible to tissues where it could be deposited. Nevertheless, after low dose aluminium administration, almost twice as much was excreted in 4.5 h in the presence of excess citrate than in its absence, and after high dose aluminium administration, the enhancement of excretion in the presence of excess citrate was almost threefold. Clearly, more information is still required about the longer term excretion and longer term tissue deposition of aluminium in the presence of excess citrate, but it nevertheless does appear that intravenous administration of citrate could have therapeutic applications in patients who have been exposed to aluminium.

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REFERENCES