Ultrasensitive analysis of the intestinal absorption and compartmentalization of aluminium in uraemic rats: a 26Al tracer study employing accelerator mass spectrometry

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Abstract

Background. Developments in accelerator mass spectrometry (AMS) now permit the determination of femtogram amounts of 26Al in blood and in various tissues with good precision and free of external contamination.

Methods. In the present study we used trace quantities of 26Al to investigate the intestinal absorption and compartmentalization of aluminium in rats with renal failure (Nx, 5/6 nephrectomy) and in pair-fed controls (C). Single oral doses of 20 ng 26Al were administered to six animals in each group and, subsequently, 24-h post-load 26Al was analysed in serum, urine, bone, liver, and spleen by means of AMS.

Results. Serum concentrations of 26Al were significantly lower in uraemic rats compared to controls, whereas urinary excretion was comparable (Nx, 7.11 ± 5.78 pg/day vs C, 9.46 ± 6.10 pg/day), suggesting a higher fraction of ultrafiltrable serum 26Al in uraemia. The target tissues of cellular transferrin-mediated 26Al uptake, liver and spleen, tended to show a larger degree of aluminium accumulation in controls (0.26 ± 0.31 pg/g vs Nx, 0.14 ± 0.10 pg/g and 0.37 ± 0.27 pg/g vs Nx, 0.25 ± 0.27 pg/g respectively). In contrast, in bone, a site of extracellular aluminium deposition, 26Al concentrations were more elevated in uraemia (1.22 ± 0.59 pg/g vs C: 0.68 ± 0.30 pg/g). Estimated total 26Al accumulation in all measured target tissues was significantly higher in uraemic rats (28.15 ± 9.90 pg vs C: 17.03 ± 7.03 pg) and total recovery of 26Al from tissue and urine was 26.58 ± 6.74 pg in controls and 35.75 ± 7.03 pg in uraemic animals, suggesting a fractional absorption of 0.133% and 0.175% respectively.

Conclusions. Our data suggest that fractional absorption from a dietary level dose of 26Al is about 0.13%. Compartmentalization occurs in transferrin-dependent target tissues such as liver and spleen; however, in quantitative terms extracellular deposition in bone is more important. Uraemia has a significant effect on the intestinal absorption and compartmentalization of aluminium. It enhances fractional absorption and increases subsequent extracellular deposition of aluminium in bone. However, at the same time uraemia does not increase transferrin-dependent cellular accumulation of aluminium in liver and spleen.

Key words: aluminium; 26Al tracer; intestinal absorption; compartmentalization; uraemia; accelerator mass spectrometry

Introduction

Although aluminium intoxication in patients with chronic renal failure has been recognized as being involved in the pathogenesis of several clinical disorders including dialysis dementia, vitamin D-resistant adynamic bone disease and hypochromic microcytic anaemia, our knowledge about its toxicokinetics is still limited [1–3]. This lack of a solid data base is largely related to the fact that available methods for the determination of aluminium in serum or tissues, such as flameless atomic absorptiometry, have only limited sensitivity and are hampered by preanalytical errors due to contamination. This problem gains even more importance since despite today’s adequate purification of the water used in the dialysis process, some moderate degree of aluminium accumulation resulting in subtle or overt toxicity may persist in a certain number of dialysis patients. This residual aluminium burden is derived from intestinal absorption from low doses of aluminium-containing phosphate binders and from other dietary sources [3–6]. In addition, several episodes of acute aluminium intoxication have drawn attention to the fact that aluminium toxicity will still have to be considered in the future [7,8].

Recently, measurement of the isotope 26Al by accelerator mass spectrometry (AMS) has been introduced and application of this new technique has the potential
to explore the metabolism of aluminium at physiological levels of exposure [9]. AMS can detect the long-lived isotope $^{26}$Al (half-life about $7 \times 10^5$ years) with extreme sensitivity in the atogram range and with good precision. Most importantly, analysis of samples is not confounded by preanalytical errors due to contamination, and use of this technique may help to resolve many of the conflicting data that have been generated in the past [2,3]. However, access to AMS facilities is limited and AMS analysis is a difficult to perform and expensive technique. Thus, the number of samples that can be analysed is usually restricted and the application of the exquisite sensitivity of this technique has to be focused on carefully selected projects. Despite these limitations several pioneering studies have recently succeeded in studying the kinetics of $^{26}$Al in several experimental animal models and in humans [9–14].

The aim of the present study was to analyse the effect of uraemia on the intestinal absorption, on the compartmentalization, and on the renal excretion of $^{26}$Al as determined by AMS in a fairly large number of samples.

**Methods**

Seventeen 6-week-old, male outbred Sprague–Dawley rats (Han:SPRD, Dept. of Lab. Animal Res., RWTH) weighing 160–180 g were kept on a standard laboratory diet containing 200 mg/kg (3.58 mmol/kg) iron (Fe), 0.80% calcium, 0.75% phosphorus, and 17.3% protein supplemented with 400 units/kg vitamin D$_3$ (Eggersmann, Rinteln, FRG). To compensate for genetic differences all litter mates were distributed equally among the test groups prior to the experiments as described previously [15]. Renal failure rats were rendered uraemic by a two-stage 5/6 nephrectomy (Nx) [16]. Subsequently, all animals had free access to distilled water, but uraemic rats and controls matched for weight were pair-fed.

Four weeks after the second operation residual renal function was determined and all rats were studied by a single oral load of $^{26}$Al in one of two separate experimental series according to a well-established protocol [16–18]. Seventy-two hours prior to aluminium ingestion rats were placed in individual metabolic cages. Forty hours later food was withdrawn but access to deionized drinking water was permitted until 1 h prior to the aluminium load. Each rat received a single oral dose of 20 mg $^{26}$Al together with a small amount of $^{27}$Al (200 µg administered as AlCl$_3$) in 1 ml of deionized water by gastric lavage. For baseline urinary excretion rates of $^{26}$Al 24 h urine collections were obtained twice, and following the aluminium load urine was collected for 24 h. Water was returned 3 h after administration of aluminium. Food was withheld during the subsequent 24 post-load hours to prevent fecal contamination of urine. In the first experimental series six uraemic rats and an equal number of normal rats were sacrificed 24 h after the oral dosing and blood, liver, spleen, and bone were harvested. In the second set of experiments serial blood samples were obtained at hours 2 and 5 in one uraemic rat and in a respective control.

Five millilitres of serum or urine were used for the preparation of samples for analysis by AMS as described previously [12,13]. Briefly, serum was digested with trypsin and 5 mg $^{27}$Al was added as a carrier. For urine samples 50 mg $^{27}$Al as AlCl$_3$ was added. Samples were dried at 90 °C and then ashed (1000 °C) to obtain Al$_2$O$_3$. Tibial bone was prepared as earlier reported [19]. Dried samples (100 °C, 16 h) of liver, spleen, or bone were ground to powder in an agate mill and 10 mg of $^{26}$Al was added to 20 mg aliquots. Samples were redried (90 °C, 2 h), digested with 1 ml concentrated sulphuric acid (100 °C, 16 h), and subsequently ashed (1000 °C, 6 h).

Measurements of $^{26}$Al/$^{27}$Al ratios were performed at the AMS facility of the accelerator laboratory of the University and the Technical University, Munich, FRG. In a high-current ion sputter source, negative Al-ions were generated. After the MP Tandem accelerator a Wien velocity filter was used. The ions were detected in a Bragg ionization chamber. The actual detection limit for the isotope ratio $^{26}$Al/$^{27}$Al was $7 \times 10^{-16}$ [20]. The measured $^{26}$Al/$^{27}$Al ratios of samples of rats where $^{26}$Al was administered were in the range of $10^{-13}$ to $5 \times 10^{-12}$; typically several times $10^{-13}$. $^{26}$Al/$^{27}$Al ratios of blank samples of rats where $^{26}$Al was not administered were in the range of $<5 \times 10^{-14}$. Results were calculated as pg/l or pg/g dry weight and the total tissue burden was estimated according to published normal values of tissue weights in rats [21,22].

Serum analysis of calcium was performed by atomic absorption (Perkin–Elmer 2380, Norwalk, CT, USA), serum phosphate was measured by a colorimetric method (Boehringer Mannheim, FRG), and creatinine was determined by an autoanalyser (Beckman Creatinine Analyzer II, Fullerton, CA, USA). Serum immunoreactive parathyroid hormone (PTH) was determined by a radioimmunnoassay recognizing the mid-molecule sequence (44–68) of PTH employing a reference standard from rat parathyroid homogenates (Immuno Nuclear Corporation, Stillwater, MN, USA). Non-haem liver iron was measured as reported elsewhere [23]. All reported data are expressed as mean value ± 1 standard deviation except where noted otherwise. Non-parametric statistical probabilities are derived from Mann–Whitney U test and Wilcoxon signed rank test. A value of $P<0.05$ was considered significant. Correlation between two parameters was accomplished by the Spearman rank correlation coefficient.

**Results**

Serum chemistry and other parameters of interest in uraemic rats and in pair-fed controls are shown in Table 1. There were no differences with respect to iron concentration in the liver between both groups. Although there was a tendency for serum phosphate and PTH to be elevated and for serum calcium to be lower in uraemic rats as compared to controls these differences did not attain significance. Prior to the oral aluminium load $^{26}$Al could not be detected in either tissues or serum or urine. Twenty-four hours after administration of the aluminium isotope AMS was able to measure femtogram amounts in all tissues and body fluids obtained (Table 2). In the target tissues of cellular transferrin-mediated $^{26}$Al uptake, liver and spleen, aluminium tended to be more elevated in controls although the overall differences were not significant. When both groups were considered together spleen aluminium concentration was significantly higher as compared to liver $^{26}$Al ($0.31 ± 0.26$ pg/g vs $0.20 ± 0.23$ pg/g, $P<0.05$). The increase in bone $^{26}$Al,
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Table 1. Body weight, serum chemistry, and liver iron in uraemic rats and in pair-fed controls studied with a single oral dose of $^{26}$Al

<table>
<thead>
<tr>
<th></th>
<th>$n$</th>
<th>Weight (g)</th>
<th>Creatinine (µmol/l)</th>
<th>Calcium (mmol/l)</th>
<th>Phosphate (mmol/l)</th>
<th>Liver Fe (µmol/l)</th>
<th>PTH (pmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6</td>
<td>226 ± 21</td>
<td>41 ± 6</td>
<td>2.69 ± 0.18</td>
<td>2.08 ± 0.29</td>
<td>7.52 ± 1.39</td>
<td>66 ± 14</td>
</tr>
<tr>
<td>Ureaemic</td>
<td>6</td>
<td>233 ± 23</td>
<td>101* ± 34</td>
<td>2.57 ± 0.27</td>
<td>2.40 ± 0.39</td>
<td>9.67 ± 2.06</td>
<td>89 ± 23</td>
</tr>
</tbody>
</table>

* $P<0.005$ vs control.

Table 2. Serum and tissue of $^{26}$Al and urinary excretion of $^{26}$Al in uraemic rats and in pair-fed controls following a single oral dose $^{26}$Al

<table>
<thead>
<tr>
<th></th>
<th>Serum Al (pg/l)</th>
<th>Urine Al (pg/d)</th>
<th>Liver Al (pg/g)</th>
<th>Spleen Al (pg/g)</th>
<th>Bone Al (pg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.56 ± 11.21</td>
<td>9.46 ± 0.31</td>
<td>0.26 ± 0.14</td>
<td>0.37 ± 0.27</td>
<td>0.68 ± 0.30</td>
</tr>
<tr>
<td>Ureaemic</td>
<td>9.39 ± 7.83</td>
<td>7.11 ± 0.78</td>
<td>0.14 ± 0.10</td>
<td>0.57 ± 0.27</td>
<td>1.22 ± 0.49</td>
</tr>
</tbody>
</table>

* $P<0.05$ vs controls.

A site of extracellular aluminium deposition, was significantly larger in uraemic rats as compared to controls and urinary excretion of aluminium tended to be lower. Surprisingly, serum concentrations of $^{26}$Al were significantly lower in uraemic rats ($9.39±7.83$ pg/l) as compared to controls ($22.56±11.21$ pg/l). The fraction of administered $^{26}$Al recovered from liver was $1.45×10^{-4}$ and $0.76×10^{-4}$ and the fraction detected in bone was $8.01×10^{-4}$ and $14.35×10^{-4}$ in controls and in uraemic rats respectively (Figure 1). There was no correlation between serum $^{26}$Al concentration and the amount of isotope detected in liver tissue in both groups (Figure 2). However, there was a tendency for an inverse correlation between bone aluminium and serum aluminium ($\rho=0.55, P=0.07$) and for almost any given serum $^{26}$Al concentration bone $^{26}$Al was higher in uraemic rats as compared to controls (Figure 3). In uraemic rats there was also a tendency for a correlation between serum creatinine and bone $^{26}$Al suggesting that the more compromised renal function was the more aluminium was deposited in bone ($\rho=0.77, P=0.08$). There was no clear-cut correlation between the amount of aluminium detected in the
Discussion

The extreme sensitivity of AMS for long-lived isotopes such as $^{26}$Al makes it a powerful tool in biomedical research. The present study demonstrates that the $^{26}$Al tracer measured by AMS can be used to address several hitherto conflicting issues: (a) precise quantification of the fractional intestinal absorption of aluminium at physiological levels of exposure; (b) assessment of transferrin-dependent compartmentalization versus extracellular deposition in various target tissues; (c) delineation of the effect of uraemia on both fractional absorption and compartmentalization.

Earlier studies in uraemic patients already suggested that bone, spleen, and liver are major sites of aluminium deposition when the body is exposed parenterally to high aluminium concentrations [24]. However, little was known about whether this pattern of tissue deposition would also apply to physiological aluminium metabolism, since in individuals with normal renal function, tissue aluminium levels do not increase with age [1,24]. Furthermore, increases in tissue aluminium following a single oral load are far too small to be reliably detected by conventional analytical technology. Subsequent experimental studies suggested that in rats similar to humans the highest concentrations of aluminium are found in bone, spleen, and liver [2]. However, again these data were generated under somewhat artificial conditions, or interpretation was difficult because of methodological problems. Following intraperitoneal administration of aluminium lactate, tissue aluminium concentrations were highest in spleen followed by liver tissue [25]. Surprisingly, aluminium concentrations in bone were considerably lower, which was at odds with previous findings in patients receiving an undesired aluminium load during haemodialysis [1,24]. In a similar study employing intraperitoneal injections of aluminium chloride, liver aluminium was again twice as high compared with bone aluminium concentrations; however, it remains an open question whether the route of administration favoured an excessive increase in portal blood aluminium concentrations that may not reflect usual toxicokinetics [26]. In contrast, rats dosed with aluminium orally were reported to accumulate aluminium predominantly in bone, but the absolute increases in tissue aluminium were in a low range and are therefore subject to many analytical errors [27,28]. Finally, surrogate isotopes for aluminium kinetics such as $^{67}$Ga were found to be of limited value to assess the compartmentalization of aluminium [28].

The data of the present study provide some clarifying insights into compartmentalization and tissue retention of aluminium in the dose range of a dietary level. Prior to administration of the $^{26}$Al tracer we were unable to detect any $^{26}$Al in all biomaterials analysed, suggesting that our results were not confounded by contamination. Twenty-four hours after ingestion of the tracer, significant amounts of $^{26}$Al could be detected in both liver and spleen tissue, but spleen aluminium concentrations were significantly more elevated when uraemic rats and controls were considered together. In both target tissues cellular accumulation of aluminium has been shown to occur via receptor-mediated internalization of transferrin-bound aluminium [29]. Transferrin-mediated aluminium uptake has been demonstrated in hepatocytes and in lymphocytes and it is also likely to occur in macrophages [30–32]. In liver cells aluminium could be demonstrated in cytoplasm and various organelles following intravenous administration [33]. The present study did not assess the respective roles of these cells for tissue uptake of aluminium; however, our data indicate possible preferential uptake by lymphocytes or macrophages, since spleen aluminium concentration was 1.3-fold higher.
compared to liver. This finding is of particular interest since aluminium overload has been implicated in the pathogenesis of impaired cellular immune response in uraemia and aluminium mobilization following renal allografting may reduce the incidence of graft rejection [34,35].

Whereas transferrin-mediated accumulation of aluminium represents an interesting mechanism of toxicity, our 26Al tracer data clearly show that even at a low level of exposure the major compartment of tissue retention is bone. Our findings are in line with recent 26Al tracer studies in rats suggesting that approximately 50% of the absorbed aluminium is excreted in the urine over 5 days and the remainder is largely retained in bone [10,36]. In the present study 60% of the absorbed fraction was detected in tibial bone and 35% in urine after just 24 h. Since bone is a site of predominant extracellular deposition of aluminium and since renal excretion, too, stems from ultrafiltrable non-protein-bound aluminium, these data indicate that excretion and compartmentalization are mainly determined by transferrin-independent pathways. Reported data regarding the size of the ultrafiltrable fraction of aluminium in serum vary considerably but it may be around 5–20% and there are several possible constituents of the small-molecule complexes in serum such as citrate or silicate [2,37–41]. Furthermore, it is unknown whether aluminium is absorbed paracellularly from the intestine as a small-molecule complex or whether absorption occurs as a transcellular process and protein-bound aluminium in the portal blood equilibrates thereafter with complexing ligands [3,38]. Recent experiments from our laboratory would suggest that apart from the presence of complexing constituents in the diet, the uraemic state is an important determinant of either possible pathway [23]. In rats with normal renal function, blockade of the paracellular pathway did not reduce fractional absorption of aluminium in the absence of dietary citrate, whereas in uraemia a substantial amount of aluminium was absorbed via the paracellular pathway. The data of the present study are in line with this notion since uraemia had a significant effect on both the absorbed fraction as well as on the compartmentalization of aluminium. The target tissues of cellular transferrin-mediated 26Al uptake, liver and spleen, tended to show a lower degree of aluminium accumulation in uraemic rats. However, the overall differences were not significant and the relationship between serum aluminium concentration and the concentration of 26Al deposited in liver tissue was similar in uraemic rats and in controls. In contrast, in bone, a site of extracellular aluminium deposition, 26Al concentrations were significantly more elevated in uraemia. Moreover, for any given serum aluminium concentration bone aluminium tended to be larger in uraemic animals as compared to controls. Considering our recent finding of increased paracellular intestinal permeability in uraemia these data are compatible with the hypothesis that enhanced absorption of small-molecule complex-bound aluminium resulted in increased extracellular deposition in bone. This would also explain the fact that urinary aluminium excretion rates were similar in both groups although uraemic rats had a compromised renal function [37] and it would explain earlier preliminary 26Al data suggesting a greater ‘affinity for aluminium’ of uraemic bone [42]. Alternatively, it cannot be excluded that the uraemic state might have decreased aluminium binding to serum proteins. This phenomenon has been suggested by several investigators but has never been convincingly confirmed by others [2,43]. Although serum aluminium concentrations were more elevated in uraemia 2 h after the tracer load, this does not explain higher bone aluminium deposition, since post-load serum aluminium after 5 and 24 h was actually significantly lower compared to controls, again suggesting increased avidity for bone deposition. Rather, this early larger increase in serum 26Al in uraemia is related to enhanced paracellular absorption, and this finding compares well with the effect of dietary citrate, which has also been shown to increase the absorption of aluminium via the paracellular pathway [36,44].

Interpreting the present data it has to be acknowledged that the small sample size and the moderate degree of renal failure impose some limitations. Thus, based on previously published data [16], it seems possible that a longer duration and a more severe degree of uraemia could have revealed a more profound effect on aluminium metabolism.

Fractional absorption of aluminium from the intestine has been difficult to determine in the past [3]. The best estimates using conventional techniques were derived from the post-load measurement of urinary aluminium excretion over several days, suggesting an absorbed fraction of 0.06% in the presence of normal renal function and enhanced fractional absorption of 0.2% or more in uraemia [15–18]. However, it was likely that these estimates were confounded by tissue retention of aluminium and these approaches did not allow the determination of the degree of aluminium absorption at low levels of exposure with certainty. The present study clarifies this issue. Total recovery of 26Al from tissue and urine was 26.58 ± 6.74 pg in controls and 35.75 ± 7.03 pg in uraemic animals, suggesting a fractional absorption from intestine of 0.133% and 0.175% respectively. These figures confirm earlier estimates of aluminium absorption and are in line with recent 26Al studies addressing the effect of citrate [15–18,36]. In addition, these figures confirm enhanced absorption of aluminium in uraemia which has been a consistent phenomenon in earlier studies using pharmacological doses of aluminium and extend this finding to low levels of exposure [15–18]. The present study makes also clear that investigations that restrict measurements to serum and urine aluminium will underestimate the absorbed aluminium fraction, since uraemia favours deposition of aluminium in bone. For the same reason data of 26Al studies in humans that rely on just one or a few measurements of serum aluminium should be interpreted with some caution [11,45]. In contrast, safe estimates of the fractional absorption can be obtained by summing up 26Al contents of urine, serum,
References

7. Simoes J, Barata JD, D’Haese PC, De Broe ME. Cela n’arrive qu’aux autres (Aluminium intoxication only happens in the other). Medical assistance. This work was presented in part at the 33rd EDTA-ERA Congress, Amsterdam, The Netherlands, 18–21 June 1996. This work was supported by the Deutsche Forschungsgemeinschaft grants It 3/5–1, It 3/5–2, and No 198/6–1

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