Renal aluminium handling in the rat: a micropuncture assessment

David G. SHIRLEY*, Mary F. WALTER†, Stephen J. WALTER†, Andrew THEWLES‡ and Christopher J. LOTE‡

*Centre for Nephrology and Department of Physiology, Royal Free and University College Medical School, Royal Free Hospital, London NW3 2PF, U.K., †Division of Biomedical Sciences, Imperial College School of Medicine, London SW7 2AZ, U.K., and ‡Division of Medical Sciences, The Medical School, University of Birmingham, Birmingham B15 2TT, U.K.

Uncertainties exist over the glomerular filtration of aluminium and virtually nothing is known about its segmental handling along the nephron. The present study has used micropuncture, combined with electrothermal atomic absorption spectroscopy, to determine directly the aluminium content of glomerular filtrate and of late PCTs (proximal convoluted tubules) and early distal tubules in anaesthetized Munich–Wistar rats infused with three different doses of aluminium citrate (plasma aluminium concentrations, $2.9 \pm 0.1$, $5.2 \pm 0.4$ and $10.0 \pm 0.9 \mu g \cdot ml^{-1}$ respectively). Aluminium filtration into Bowman’s space was found to be considerably greater than that predicted by an in vitro filtration system: in all three groups it was essentially filtered freely. No significant aluminium reabsorption took place along the PCT, but with every dose the FDAl (fractional delivery of aluminium; tubular fluid:plasma aluminium/inulin concentration ratio) was lower at the early distal site than at the late PCT ($P < 0.001$ in each case), indicating net aluminium reabsorption in the loop of Henle. This reabsorption amounted to 19–26% of the filtered aluminium load. In the low- and medium-dose groups, there was no significant difference between FDAl at the early distal site and that in the final urine; however, in the high-dose group, FDAl in the urine ($1.02 \pm 0.06$) exceeded that at the early distal tubule ($0.75 \pm 0.04$; $P < 0.001$), suggesting aluminium secretion in the distal nephron. The results indicate that aluminium loads, when complexed with citrate, are excreted efficiently owing to a combination of glomerular filtration and minimal reabsorption.

INTRODUCTION

Aluminium, the most abundant metal in the Earth’s crust, is ingested from a variety of sources and undergoes partial absorption from the intestine [1]. Prevention of harmful aluminium accumulation in the body relies on aluminium excretion by the kidneys. However, only limited information is available concerning the nature of renal aluminium handling, partly because its filtration characteristics are incompletely understood. In vitro determinations indicate that aluminium is not filtered freely and, moreover, that the degree of filtration varies considerably, depending on its concentration and speciation [2,3].

By combining measurements of ultrafilterable aluminium (as indicated by artificial membranes in vitro) with determinations of GFR (glomerular filtration rate) and urinary excretion of aluminium, some information on overall net tubular reabsorption of aluminium has been gained (for example, see [4]). However, such determinations depend on the accuracy of the in vitro filtration
values and, at present, it is unclear whether these provide a reliable index of actual in vivo ultrafilterability. In addition, clearance measurements only provide, at best, an indication of net aluminium transport in the nephron as a whole; owing to technical difficulties, assessment of aluminium transport in discrete nephron segments has not hitherto been possible.

The aim of the present study, therefore, was two-fold: (i) to collect fluid directly from Bowman’s space in Munich–Wistar rats (the kidneys of which have multiple surface glomeruli accessible to micropuncture) in order to compare in vivo aluminium ultrafilterability with in vitro measurements; and (ii) to determine aluminium deliveries in tubular fluid at specific nephron sites [late PCT (proximal convoluted tubule) and early distal tubule] in order to assess tubular aluminium handling in the PCT, loop of Henle and distal nephron. Aluminium concentrations in Bowman’s space and tubular fluid were measured using electrothermal atomic absorption spectroscopy.

METHODS

Animals and surgical procedures
All experiments were performed in accordance with UK legislation. Adult male Munich–Wistar rats were anaesthetized with sodium thiopentone (100 mg⋅kg\(^{-1}\) of body weight, intraperitoneally; May & Baker, Dagenham, Essex, U.K.) and prepared surgically for micropuncture of the left kidney [5]. After equilibration for 1 h, i.v. (intravenous) infusion of [\(^3\)H]inulin (60 \(\mu\)Ci primer, 1 \(\mu\)Ci⋅min\(^{-1}\); Amersham Biosciences, Little Chalfont, Bucks., U.K.) and 0.9 % NaCl solution (3 ml⋅h\(^{-1}\)) containing aluminium chloride in 3 % (v/v) sodium citrate solution into the rats was initiated. Aluminium was administered at one of three doses: (i) 200 \(\mu\)g bolus, followed by continuous infusion of 5 \(\mu\)g⋅min\(^{-1}\) (low dose; \(n = 9\) rats); (ii) 400 \(\mu\)g bolus, followed by 10 \(\mu\)g⋅min\(^{-1}\) infusion (medium dose; \(n = 9\) rats); or (iii) 800 \(\mu\)g bolus, followed by 20 \(\mu\)g⋅min\(^{-1}\) infusion (high dose; \(n = 9\) rats). At 1 h after the bolus doses, an arterial blood sample (approx. 70 \(\mu\)l) was taken for measurement of plasma [\(^3\)H]inulin and aluminium, and micropuncture collections were begun. Timed collections, using sharpened glass micropipettes (tip diameter 8–12 \(\mu\)m) filled with Sudan black-stained oil, were made from Bowman’s space (16–24 min), late proximal convolutions (20–30 min) and early distal tubules (30–50 min), using methods described previously [6]. Initial identification of Bowman’s space was made on the basis of its proximity to a surface glomerulus, its characteristic shape when partially filled with stained oil and the large number of proximal tubular segments seen to succeed it when a small oil droplet was allowed to move downstream; confirmation was based on its [\(^3\)H]inulin concentration relative to that of plasma. Late proximal segments were identified on the basis of the movement of a small droplet of oil injected at the puncture site, and early distal segments were identified following i.v. injection of Lissamine green (30 \(\mu\)l of a 5 % solution). In each of the latter cases, confirmation of the collection site was achieved by intratubular injection of silicone rubber solution (Flow Tech, Carver, MA, U.S.A.) and subsequent microdissection [7]. At least two collections from each site were made in each rat. Throughout the period of micropuncture, urine was collected from both kidneys and arterial blood pressure was monitored using a SensoNor transducer (Horten, Norway) connected to a MacLab 8 recording system (AD Instruments, Palo Alto, CA, U.S.A.). Blood samples (approx. 70 \(\mu\)l) were taken at approx. 1 h intervals for measurement of plasma [\(^3\)H]inulin and aluminium. Immediately after the final micropuncture collection, a further sample was taken for measurement of plasma [\(^3\)H]inulin and aluminium, followed by a 2 ml terminal blood sample for assessment of in vitro filtration of aluminium. At the end of each experiment, the rat was killed with an overdose of sodium thiopentone.

Effect of frusemide
In separate experiments, the effect of frusemide on aluminium handling in the loop of Henle was investigated. Rats (\(n = 14\)) were anaesthetized and prepared for micropuncture as described above. After equilibration for 1 h, rats were infused with [\(^3\)H]inulin and high-dose aluminium. At the same time, an i.v. infusion of frusemide (3 mg⋅h\(^{-1}⋅\)kg\(^{-1}\) of body weight; Hoechst, Frankfurt, Germany) was initiated in half of the animals, fluid balance being maintained by i.v. infusion of a replacement solution (135 mmol NaCl and 15 mmol KCl) at a rate that matched frusemide-induced urinary losses [8]. The other seven rats acted as controls. After a further 1 h, tubular fluid collections were made from late proximal convolutions and early distal tubules; urine was collected and blood samples were taken as described above.

Analyses
Collections from Bowman’s space and from proximal and distal tubules were deposited under oil and their volumes measured using calibrated constriction pipettes. Samples (usually single but, where possible, in duplicate) were taken for measurement of aluminium concentration (300–900 nl each) and duplicate samples were taken for measurement of [\(^3\)H]inulin activity (20–60 nl each). Urine and plasma samples (in triplicate) were treated similarly. [\(^3\)H]Inulin activities in tubular fluid, urine and plasma were determined by \(\beta\)-scintillation counting (Canberra Packard 2000CA; Pangbourne, Berkswell, U.K.) after dispersal in Aquasol 2 scintillation.
fluid (PerkinElmer Life Sciences, Cambridge, U.K.). Aluminium concentrations were determined, after 150- to 300-fold dilution with aluminium-free water (Elga UHQ 2 purification unit; High Wycombe, Bucks., U.K.), using electrothermal atomic absorption spectrophotometry (Varian Spectra A360; Palo Alto, CA, U.S.A.). Furnace programme details have been reported previously [4]. For the frusemide experiments, urine osmolality was measured by freezing point depression (Roebling; Camlab, Cambridge, U.K.).

For determination of ultrafilterable aluminium using the in vitro method, 0.5 ml of plasma was pipetted into an Amicon Micropartition System (MPS-1; Stonehouse, Gloucestershire, U.K.) filtration cell and centrifuged at 2000 g for 20 min [4].

Calculations

GFR was calculated as the renal clearance of [3H]inulin. SNGFR (single-nephron GFR) was calculated, using distal tubular fluid collections, as:

\[ \text{SNGFR} = \frac{V_T F \cdot (T F / P_{in})}{C_{\text{inulin}}} \]

where \( V_T F \) is the tubular fluid flow rate at the collection site and TF/P\(_{in}\) is the tubular fluid/plasma [3H]inulin concentration ratio. FD\(_{Al}\) (fractional delivery of aluminium) to each puncture site was calculated as (TF/P\(_{Al}\))/(TF/P\(_{in}\)), where TF/P\(_{Al}\) is the tubular fluid/plasma aluminium concentration ratio. (This ignores the observed minor deviations from 100% filterability.) Similarly, in order to allow comparison of early distal tubular FD\(_{Al}\) with excreted aluminium, FD\(_{Al}\) in the urine was calculated as \( (U/P_{Al})/(U/P_{in}) \), where U/P\(_{Al}\) and U/P\(_{in}\) are the urinary/plasma aluminium and [3H]inulin concentration ratios respectively.

Statistics

A single value for each variable (GFR, mean arterial blood pressure, plasma aluminium, ultrafilterable aluminium and micropuncture data for each nephron site) was calculated for each rat. Values are presented as the means ± S.E.M. calculated from these individual values. Statistical comparisons between groups with respect to arterial blood pressure, GFR, SNGFR and TF/P\(_{in}\) at a given puncture site were made using one-way ANOVA. Statistical comparisons between puncture sites and between FD\(_{Al}\) at the early distal tubule and FD\(_{Al}\) in the urine were made using one-way ANOVA with repeated measures and Student’s paired \( t \) test, as appropriate.

RESULTS

Plasma concentrations of aluminium achieved with the low, medium and high doses of aluminium infusion were 2.9 ± 0.1, 5.2 ± 0.4 and 10.0 ± 0.9 \( \mu \text{g} \cdot \text{ml}^{-1} \) respectively. There was no consistent change in plasma aluminium during the course of the experiment.

Confirming previous reports in Munich–Wistar rats (for example, [9]), arterial blood pressure was higher than seen in the parent strain (mean arterial pressure was 128 ± 2, 129 ± 3 and 131 ± 3 mmHg in the low-, medium- and high-dose groups respectively). There was no significant difference between the groups with regard to total GFR (respective values were 1.12 ± 0.06, 1.10 ± 0.07 and 1.13 ± 0.09 ml·min\(^{-1} \) respectively). Confirming previous reports in Munich–Wistar rats, arterial blood pressure was higher than seen in the parent strain (mean arterial pressure was 128 ± 2, 129 ± 3 and 131 ± 3 mmHg in the low-, medium- and high-dose groups respectively). There was no significant difference between the groups with regard to total GFR (respective values were 1.12 ± 0.06, 1.10 ± 0.07 and 1.13 ± 0.09 ml·min\(^{-1} \)) or SNGFR (46.8 ± 2.5, 44.9 ± 3.3 and 46.2 ± 2.2 ml·min\(^{-1} \)).

Table 1 shows TF/P\(_{in}\) values at each micropuncture site in the three groups. Again, there were no significant differences between the groups. In the medium and high-dose groups respectively).

Aluminium filterability

Table 2 compares measurements of aluminium filterability using the Amicon Micropartition System (in vitro) and direct measurements of aluminium concentration in Bowman’s space (in vivo). At each plasma aluminium concentration, the in vitro system indicated incomplete filtration. In contrast, direct micropuncture collections showed that TF/P\(_{Al}\) was close to unity in each group.
Aluminium handling in the PCT and loop of Henle

Figure 1 compares values for $FD_{Al}$ at Bowman’s space, the end of the PCT and the start of the distal tubule. No significant change in $FD_{Al}$ was discernible between Bowman’s space and late PCT for any of the doses used. In contrast, in every rat in all three groups, $FD_{Al}$ fell between the late PCT and early distal tubule, indicating net aluminium reabsorption in the anatomical loop of Henle; this reabsorption ranged from 19% of the filtered load in the medium- and high-dose groups to 26% in the low-dose group.

The effect of the loop diuretic frusemide (3 mg $\cdot$ h$^{-1}$ $\cdot$ kg$^{-1}$ of body weight) on aluminium transport in the loop of Henle was assessed in a separate group of animals infused with high-dose aluminium. Frusemide-treated rats ($n = 7$) had a urine flow rate of $210 \pm 13$ µl $\cdot$ min$^{-1}$ (left kidney only) compared with $12 \pm 3$ µl $\cdot$ min$^{-1}$ in control rats ($n = 7$), and urine osmolality was $338 \pm 20$ mosm/kg of water compared with $1847 \pm 100$ mosm/kg of water. These data argue for the efficacy of the dose employed. Comparison of $FD_{Al}$ at the late PCT and early distal tubule sites showed that frusemide inhibited aluminium reabsorption in the loop. In control rats, $FD_{Al}$ was $0.92 \pm 0.05$ at the late PCT and $0.66 \pm 0.05$ at the early distal tubule ($P < 0.001$). Corresponding values in frusemide-treated rats were $0.89 \pm 0.04$ and $0.79 \pm 0.03$ (not significant).

Aluminium handling beyond the loop of Henle

Figure 2 compares values for $FD_{Al}$ at the early distal tubule with corresponding values for $FD_{Al}$ in the urine in each rat (together with means $\pm$ S.E.M.). With the low and medium doses of aluminium, there was no significant difference between early distal tubular $FD_{Al}$ and urinary $FD_{Al}$. However, with the high dose, $FD_{Al}$ in the urine was higher in each rat than the corresponding $FD_{Al}$ at the early distal tubule.

DISCUSSION

The present study is the first to assess both aluminium filterability at the glomerulus and segmental aluminium handling along the nephron. In order to allow the determination of aluminium concentrations in tubular fluid, it was necessary to use supraphysiological doses of aluminium: even the lowest infusion rate raised...
plasma aluminium concentration to a value considerably higher than the normal endogenous level in the rat [4,10]. Although we attempted to mitigate this problem by using three different doses of aluminium (to test for dose-dependency), it is nevertheless a limitation of our investigation and must be borne in mind when interpreting the findings.

The first objective was to assess previous in vitro estimates of aluminium filterability by measuring aluminium concentrations in fluid collected directly from Bowman’s space, made feasible by the use of Munich-Wistar rats whose kidneys have a number of surface glomeruli. Unlike proximal and distal tubular collections, it was not possible to confirm the Bowman’s space puncture site by injection of silicone rubber compound, since the natural rate of fluid filtration forced puncture site by injection of silicone rubber compound. Therefore, we relied on initial anatomical clues and on the TF/Pin ratio. The latter would be expected to be close to unity, therefore reliance on initial anatomical clues and on the TF/Pin ratio downstream before it could set. We pound, since the natural rate of fluid filtration forced puncture site by injection of silicone rubber compound.

In the only other attempt to compare in vitro estimates of aluminium filterability by measuring aluminium concentrations in fluid collected directly from Bowman’s space, made feasible by the use of Munich-Wistar rats whose kidneys have a number of surface glomeruli. Unlike proximal and distal tubular collections, it was not possible to confirm the Bowman’s space puncture site by injection of silicone rubber compound, since the natural rate of fluid filtration forced puncture site by injection of silicone rubber compound. Therefore, we relied on initial anatomical clues and on the TF/Pin ratio. The latter would be expected to be close to unity, therefore reliance on initial anatomical clues and on the TF/Pin ratio downstream before it could set. We pound, since the natural rate of fluid filtration forced puncture site by injection of silicone rubber compound.

Average TF/Pin of approx. 1.02. If aluminium was not high enough to overwhelm protein precipitation, it was also found that filtration of aluminium, as assessed by artificial filtration membranes, has been shown to be incomplete and highly dependent on both its concentration and speciation [3,12]. This is partly due to aluminium binding to plasma proteins (principally transferrin) [13], but also to the formation, in certain circumstances, of other non-filterable complexes [14]. In the present study, the high exogenous concentrations of aluminium should have largely overwhelmed the capacity of transferrin to bind it. Nevertheless, the filterability of aluminium, according to the Amicon filter system, was only 60–76%, approximately in line with previously documented values for aluminium citrate [3]. In contrast, direct collection from Bowman’s space indicated a degree of filtration approaching 100%, even when corrected for plasma water: FDis in Bowman’s space was 90 ± 5%, 94 ± 6% and 98 ± 5% in low-, medium- and high-dose groups respectively. The lower mean value for the low-dose group (albeit not statistically significant) probably indicates that in these rats the concentration of endogenous aluminium was not high enough to overwhelm protein binding completely. The marked difference between in vitro and in vivo measurements of aluminium filterability underlines the importance of treating the results of in vitro systems with caution and has implications for previous clearance studies employing the Amicon system.

In the only other attempt to compare in vitro and in vivo aluminium filterability, it was also found that direct measurements in Bowman’s space correlated badly with data using artificial membranes [2], although in that study the aluminium was administered as AlCl3, in which form it is very poorly filtered [3,12].

Despite this clear evidence that artificial filtration systems cannot be used to predict in vivo glomerular filtration characteristics, our present findings cannot be taken to indicate that endogenous aluminium is filtered freely. The high plasma concentrations of exogenous aluminium used in the present study preclude this extrapolation. However, under normal circumstances, most non-protein-bound endogenous aluminium is complexed with citrate [14], and aluminium citrate does appear to be filtered freely. On this basis, we suggest that our data concerning segmental aluminium handling along the nephron should shed some light on what normally happens to the proportion of aluminium that is filtered.

The first observation was the absence, in all three groups, of convincing evidence of net aluminium reabsorption (or secretion) in the PCT. Although in each case the end-proximal FDis was on average slightly lower than the FDis in glomerular filtrate, the difference was not statistically significant. Given the usual limitations to precision of measurement and taking into consideration the fact that the filtered load of aluminium in the present study greatly exceeded normal values, we cannot discount the possibility of some aluminium reabsorption in the PCT under normal circumstances. However, it is clear that any such reabsorption must be subject to a transport maximum. Moreover, given that approximately half the filtered water was reabsorbed in the PCT, the absence of quantitatively significant aluminium reabsorption indicates that the proximal tubular epithelial wall has a low passive permeability to aluminium: the increase in proximal tubular fluid aluminium concentration was comparable with that for inulin.

There is a dearth of information on renal transport systems for aluminium. In mammalian brain, there is some evidence that aluminium citrate can be transported using the H+-monocarboxylate transporter [15]. However, although isoforms of this transporter are found in a number of nephron segments [16–18], they are generally expressed in the basolateral membrane and, moreover, it is unknown whether they can carry aluminium citrate. The citrate ion itself is taken up into proximal tubular cells using a recently cloned Na+-dicarboxylate cotransporter (NaDC-1) [19,20]. However, only free citrate (in the form of the divalent ion) can use this; citrate complexes are not carried. Thus, assuming that some of the filtered citrate was taken into the cells using this transporter in the present study, at least some of the filtered aluminium would either have to become complexed with alternative species or be left as free Al3+ ions.

In contrast with events in the PCT, approx. 20–25% of filtered aluminium was reabsorbed in the nephron...
segment between the late PCT and the early distal tubule: the anatomical loop of Henle. This comprises (in superficial nephrons) the proximal straight tubule (pars recta), thin descending limb of Henle, TALH (thick ascending limb of Henle) and a very short segment of distal convoluted tubule. Although the precise subsegment(s) involved cannot be ascertained from the present study, the absence of significant aluminium reabsorption in the pars convoluta argues against a role for the pars recta. In our view, the TALH is the most likely site. Aluminium citrate complexes in biological fluids are thought to be in two forms, one neutral and one anionic, but at the pH of tubular fluid the anionic form predominates [21]. It is difficult to envisage how the anionic complex could be reabsorbed in the loop without invoking a specific transporter. It must be presumed, however, that aluminium citrate will be in equilibrium with free Al\textsuperscript{3+} ions, and it seems likely that Al\textsuperscript{3+} ions would be subject to paracellular reabsorption, driven by the lumen-positive transepithelial potential difference in the TALH [22]. In this context, it is worth noting that the effective ionic radius of Al\textsuperscript{3+} in the TALH [22]. In this context, it is worth noting that aluminium reabsorption in the loop is blocked by i.v. frusemide, which abolishes the transepithelial potential difference in the TALH [24]. The finding that aluminium reabsorption in the loop is blocked by i.v. frusemide, which abolishes the transepithelial potential difference in the TALH, is consistent with the hypothesis that aluminium is normally reabsorbed passively at this site, although frusemide-induced disruption of the medullary osmotic gradient would also inhibit water reabsorption (and therefore any putative passive aluminium reabsorption) in the thin descending limb.

Concerning aluminium transport beyond the loop of Henle, inconsistent results were obtained between the three groups. In the low- and medium-dose groups, no evidence was obtained for significant net aluminium transport between the early distal tubule and the final urine, whereas in the high-dose group FD\textsubscript{Al} in the urine significantly exceeded that in the early distal tubule and, in fact, approximated the original filtered load. Unless there is significant inter-nephron heterogeneity with respect to aluminium handling, the latter observation indicates net aluminium secretion in the distal nephron. This is consistent with indirect evidence from a stop-flow study in pigs [25]. The precise subsegment of the distal nephron responsible is unknown. However, in some animals (n = 5), we obtained samples from late distal tubular segments and found no evidence for secretion of aluminium in the accessible distal tubule [FD\textsubscript{Al} = 0.66 ± 0.06 and 0.61 ± 0.06 (not significant) in early and late distal tubular sites respectively]. In our view, the most likely source of aluminium addition to the tubule is passive diffusion into collecting ducts from the medullary interstitium, where aluminium presumably accumulates as a consequence of its reabsorption in the loop; however, the transepithelial secretory pathway remains unknown. In any event, we regard this particular aspect of aluminium transport as being confined to extremely high aluminium doses; it is most unlikely to be a feature of normal renal aluminium handling.

In conclusion, our findings indicate that, when present as aluminium citrate, aluminium is essentially filtered freely at the glomerulus and undergoes net reabsorption only in the loop of Henle. Under conditions of extremely high plasma aluminium concentrations, it may additionally be secreted in the distal nephron. The data reinforce an earlier recommendation [26] that an effective means of ridding the body of potentially toxic doses of aluminium is to raise plasma citrate concentrations, thereby enhancing aluminium filtration and, consequently, its elimination in the urine.

ACKNOWLEDGMENTS

We thank the National Kidney Research Fund for financial support, and Mr John Skinner for technical assistance.

REFERENCES

9 Le Grimelec, C., Poujoul, P. and de Rouffignac, C. (1975) \textsuperscript{3}H-inulin and electrolyte concentrations in Bowman’s capsule in rat kidney. Pfuiigers Arch. 354, 117–131
Ackley, D. C. and Yokel, R. A. (1997) Aluminum citrate is transported from brain into blood via the monocarboxylic acid transporter located at the blood-brain barrier. Toxicol. 120, 89–97


Received 17 February 2004; accepted 15 March 2004
Published as Immediate Publication 31 March 2004. DOI 10.1042/CS20040052