Absorbed aluminium is found with two cytosolic protein fractions, other than ferritin, in the rat duodenum

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Abstract
After in vivo perfusion of the upper intestine of the rat with a range of concentrations of aluminium chloride, entry of the metal into the portal system was only detected when the perfusate exceeded 400 μmol/l, suggesting a mucosal block. Using gel filtration of a mucosal cytosol extract, two consistently appearing aluminium peaks were identified which may represent aluminium binding proteins. Both were heat stable at 60°C and had molecular sizes of about 700 (kilo daltons) (kD) and 17 kD respectively. The larger molecule was distinct from ferritin. Neither molecule associated with 59Fe nor 40Ca. It is suggested that the aluminium peaks are relatively specific aluminium binding proteins that have a scavenging role, reducing entry of the metal from the intestinal contents into the portal blood.

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Aluminium usually enters the gastrointestinal tract in low concentrations in the form of alums found in domestic water, or in higher concentrations after administration of antacids. Gastrointestinal absorption of aluminium has for some years been recognised as important and factors influencing this absorption have recently been the subject of investigation.1–3 To examine the aluminium uptake by the rat duodenum, we initially chose low intraluminal aluminium concentrations at pH 8·5, to mimic physiological circumstances. In the experiments reported here, we noted a discrepancy between uptake from the perfusate and the finding of the metal in portal blood, which only occurred at relatively high perfusate concentrations. We therefore surmised that the mucosa was retarding transport of absorbed aluminium and as there is some evidence that aluminium may interact with ferritin,4 we tested the possibility that cytosolic ferritin might be a scavenger molecule for the metal. Our results failed to show that any binding to ferritin took place, but there was definite association with two other protein fractions, one of high, the other of lower, molecular size.

Methods
Reagents were of laboratory grade and 59Fe in 0·1N HCl and 40Ca were obtained from Amersham International, Amersham, United Kingdom.

DUODENAL PERFUSION
All experiments were carried out on anaesthetised (nembutal) male Porton rats (220–250 g weight), raised on standard food pellets. The duodenum was cannulated proximally and distally and perfused at 0·6 ml/min using a solution of NaCl 100 mmol/l, KCl 10 mmol/l, NaHCO3 30 mmol/l, adjusted to pH 8·5 with NaOH 0·1 mol/l immediately before use, after addition of unbuffered aluminium chloride (AlCl3) 10 mmol/l to achieve the required aluminium concentration. Perfusion was carried out over 50 minutes using two identical peristaltic pumps to deliver and remove the perfusate which was recirculated through a 10 ml reservoir, vigorously stirred. Groups of seven animals were perfused at a particular aluminium concentration, across a range from 0–3·2 mmol/l. Details of the method have been published.4 In experiments to investigate cytosolic aluminium rich fractions, the perfusate aluminium concentration was 1·0 mmol/l and, where required, traces of 59Fe citrate and 40CaCl2 were added. The 59Fe citrate was prepared by adding 10 μl Na citrate 1 mmol/l to 100 μl 59FeCl3 followed by 100 μl NaHCO3 0·1 mmol/l.

PORTAL VEIN
Using a 22 gauge catheter (Jelco, Johnson & Johnson, Tokyo), we cannulated the portal vein by the inferior mesenteric vein which was ligated, as were the splenic vessels. Before the perfusions we collected 500 μl of portal blood for 1 minute for aluminium analysis, replacing the volume with Haemaccel (Behring, Germany), an albumin free plasma substitute. At the end of the perfusions a second portal blood sample was obtained.

CYTOSOLIC PREPARATION
We perfused the duodenum, removed it, and injected intracardiac pentathol. It was rinsed in ice cold deionised water and all subsequent tests were carried out at 0–4°C. The mucosa was scraped off with a firm stroke of a plastic blade, yielding about 0·4 g of cells per animal and the pooled slurry from five rats was suspended in NaCl 150 mmol/l, to wash the cells of residual perfusate. This suspension was centrifuged at 800g for 10 minutes and the pellet was dispersed in more saline. The cells were washed three times in this way. They were homogenised with a 10 ml Teflon glass homogeniser in buffer of composition NaCl 100 mmol/l, Tris HCl 25 mmol/l, pH

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8·0 in the presence of inhibitors (Phenylmethylsulphonylfluoride, benzamidine, chymostatin, 100 μg each per 5 ml homogenate) and centrifuged at 40 000g for 20 minutes. The supernatant was recovered and heated quickly to 60°C for 2 minutes and then stood immediately on ice. After cooling, the mixture was centrifuged as before. The supernatant, about 2 ml, was diluted in 20 vol of the buffer to reduce the amount of loosely bound aluminium and concentrated to about 1·5 ml in an ultrafiltration system using a YM-10 membrane (Amicon, Danvers, Maine). The sample was then mixed with 0·5 ml EDTA 2 mmol/l and 1·0 ml was applied to a calibrated Sepharose 4B column (1·6×90 cm). It was eluted with the NaCl-TRIS buffer at 0·22 ml/min and collected in 2·2 ml fractions. Protein in the eluent was monitored at 280 nm. In some experiments, using the Sepharose 4B, proteoglycans was estimated in the fractions. In pilot experiments, we fractionated the concentrated supernatant, having omitted the heat precipitation step.

In similar experiments, we applied the concentrated heat treated supernatant to a Sephadex G-100 column (1·0×90 cm) and eluted it using the NaCl-TRIS buffer with the flow rate 0·22 ml/min but fraction volumes of 1·4 ml. We estimated the aluminium in the fractions and, when 59Fe or 40Ca had been included in the perfusate, the radioactivity. All specimens for aluminium analysis were treated with a solution of EDTA 1 mmol/l, Triton 1%, of which 20 μl was added to 500 μl of sample. They were estimated by electrothermal atomic absorption spectroscopy using a Varian AA-1475/GTA-95 instrument (Varian, Kilkenny, South Australia). The ferritin was measured by an ELISA technique. The 59Fe was analysed by scintillation counting and 40Ca counted in a gasmspectrometer.

Proteoglycans was identified using periodic acid and Schiff’s reagent and estimated by absorbance at 560 μm. Buffers were prepared with twice distilled deionised water in acid washed glassware and were routinely tested for contamination with aluminium. Between experiments, a 1 ml bolus of EDTA 2 mmol/l was applied to the column which was then washed through with 3 volumes of buffer.

**Results**

Disappearance of aluminium from the perfusate systemically increased with the perfusate aluminium concentration (r=0·99, P<0·001) in a similar way to that previously described. The basal serum aluminium in portal blood ranged from 0 to 0·7 (mean 0·40) μmol/l. The increase in aluminium concentration in the portal blood during the 50 minute perfusion was directly proportional to the perfusate concentration with no intercept on the abscissa. Despite this systematic relation, however, we actually saw no increase in the aluminium in the portal blood up to the perfusate aluminium concentration 400 μmol/l (Fig 1). Moreover, even at higher concentrations, entry of aluminium into portal blood could not be detected in every case.

When fractionated on Sepharose 4B, the heat stable cytosol extract gave a peak of variable size, corresponding to the void volume, followed by two major peaks of ultraviolet absorbance. Ferritin formed a shoulder early in the first peak, where it was present in concentrations up to 5 μmol/l. Compared with these elution pattern, aluminium was found in three zones, excluding that in the void volume, which was not investigated further. The earliest peak, variable both in position and magnitude, appeared in a zone corresponding to a molecular size in the region of 1000 kD. Proteoglycans was detected in this region, but not in any peak that coincided with the aluminium. The first constant peak of aluminium (peak I) corresponded to a molecular size of approximately 700 kD and always eluted just ahead of the ferritin, identified by ELISA assay. The ferritin peak was reliably mimicked by the 40Ca counts, though sometimes a shoulder occurred after the main 40Ca peak and coincided with the elution position of transferrin. A typical experiment is shown (Fig 2). The second constant aluminium peak (peak II) was larger and occurred within the main protein peak. When cytosol that had not been subjected to heat precipitation was used, the protein peaks were
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broader but the elution pattern of the aluminium was the same.

Concentrated cytosol extract fractionated on the Sephadex G-100 column yielded the aluminium of peak I in the void volume. The aluminium of peak II corresponded to a molecular size of approximately 17 kD. The absorbed 59Fe was associated most strongly with the ferritin zone but there was also 59Fe coinciding with the transferrin region. It was not found to coincide with the eluted aluminium peak (Fig 3). Occasionally, traces of 59Fe were found within the very low molecular weight region. The 54Ca was detected as a single peak, after peak II, and corresponded to a molecular size 11 kD. Aluminium was not found in the buffers at concentrations >0.4 umol/l, our limit of sensitivity being 0.2 umol/l. Following the fractionation, the EDTA bolus carried through a peak of aluminium that had been retained with the gel estimated in three experiments with Sepharose 4B at between 12% and 25% of the total applied load. The retention was not quantitated with the Sephadex G-100 column.

Discussion

Aluminium is now known to be a toxic metal and there is increasing evidence that it may interfere with cell regulatory mechanisms,1-6 presumably as a result of its high charge density. At the same time, it is widely distributed in nature and it is therefore reasonable to expect that there will be a number of barriers to its absorption from the gastrointestinal tract. The first of these is its well known amphoteric behaviour as pH changes in dilute aqueous solutions. Thus, in the small bowel where an alkaline pH is maintained, the species of aluminium will typically be in the sparingly soluble state of Al(OH)₃ or Al(OH)₄⁻. None the less, we have made the empirical finding that aluminium in this form seemed to be systemically taken up by the mucosa though there was no evidence of entry of the metal into the portal blood until the perfusate aluminium concentration had been raised beyond 400 umol/l, well above the values expected with a normal diet.15 16 Only when this concentration had been exceeded, was the value of aluminium in portal blood a function of the perfusate concentration, over the range tested. This discrepancy, between the aluminium taken up by the mucosa and its occurrence in the blood, suggested some intracellular blockade. There is evidence that ferritin can bind small amounts of aluminium.9 Our heat stable fraction of mucosal cytosol contained ferritin that had been able to interact with 59Fe taken up from the perfusate. We found, however, no association between this ferritin and aluminium. The peak I was persistently in a zone which just preceded the ferritin, where the 59Fe was carried, and corresponded to a molecular mass of about 700 kD.

The peak II contained more abundant aluminium and corresponded to a molecular mass of 17 kD and again carried no 59Fe. The 54Ca was found in the appropriate position to represent binding to calbindin, the vitamin D dependent protein, but was not found with aluminium.

The nature of the aluminium rich zones remains to be determined. It is possible that mucus, or its components, accounted for the variable, very high molecular mass material that was associated with aluminium, as proteoglycans was readily detected. There was no strict association, however, with aluminium and a proteoglycans peak. The other possibility is that the aluminium was being carried with portions of other structural components of the cell, such as membrane fragments. These considerations do not apply to peaks I and II which had constant elution patterns. Furthermore, they had significant affinity for the aluminium as it was not removed by washing procedures or transient mixing with EDTA when applied to the gel filtration column.

It seems likely that these zones represent aluminium binding proteins whose purpose, however, remains speculative. The starting point for our experiments was the finding that the duodenum seemed to block uptake of perfused aluminium, until the luminal concentration exceeded 400 umol/l. This is consistent with the idea of duodenal proteins forming a sink, tending to reduce absorption of the metal. In view of the rapid turnover of duodenal mucosal cells, such a sink would provide an effective second line of defence against entry of aluminium into the plasma.

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7 Fleming J, Joshi KG. Ferritin: isolation of aluminium-ferritin

![Figure 3: The distribution aluminium and 59Fe peaks after elution of cytosol extract on Sephadex G-100.](http://gut.bmj.com/ Gut: first published as 10.1136/gut.34.5.643 on 1 May 1993. Downloaded from http://gut.bmj.com/ on September 17, 2023 by guest. Protected by copyright.)