

The role of trace elements in uraemic toxicity

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Abstract

Although most research on uraemic toxicity has focused on the retention or removal of organic solutes, subtle changes in the concentration of inorganic compounds are also of importance because these compounds may have significant clinical consequences. Potential clinical implications include increased risk of cancer, cardiovascular disease, immune deficiency, anaemia, renal function impairment and bone disease. In uraemic patients, the most important factor affecting trace element concentration is the degree of renal failure and modality of renal replacement therapy. Accumulation of trace elements in haemodialysis patients has resulted from dialysate contaminated with aluminium and strontium. Several trace elements have been implicated in the decline of renal function. These include arsenic, cadmium, copper, germanium, lead and mercury. In uraemic patients, aluminium, cadmium, chromium, lanthanum, strontium and zinc have been shown to accumulate in bone. In addition to substantial evidence linking aluminium to renal osteodystrophy, studies have also implicated cadmium, iron and strontium in bone disease. Studies using a rat model of chronic renal failure have demonstrated an association between lanthanum accumulation and mineralization defects characteristic of osteomalacia. Investigations of arsenic accumulation in animal models have demonstrated that speciation of trace elements potentially may alter toxicities of trace elements accumulated in uraemic patients. Conversely, the presence of uraemic toxins may also alter the uptake and toxicity of certain trace elements. Although research in uraemic patients has focused primarily on total concentrations of trace elements, the evolution of both inorganic and organic species should be considered separately.

Keywords: accumulation; bone; depletion; toxicity; trace elements; uraemia

Introduction

Uraemia is characterized by functional and biochemical disturbances that result primarily from the diseased kidney's diminished capacity to remove organic solutes from the body. Most research on uraemic toxicity has focused on retention and removal of these organic compounds. However, subtle changes in the concentration of inorganic compounds, including trace elements, may also cause functional or biochemical disturbances.

The term 'trace element' dates back to the 19th century. The term referred to those elements found in the body at concentrations below accurate detection limits of that time. The term persists today, despite new analytical techniques that allow the accurate measurement of most trace elements.

Accurate quantification of trace elements is critical in uraemia research. Because of the very low concentrations of trace elements in the body, extreme care must be taken in the sampling, preparation and measurement of these elements, with particular attention to avoiding contamination. Several analytical techniques are available to measure the concentrations of a variety of trace elements in normal and pathological conditions; these include flame/flameless atomic absorption, electron microprobe analysis, inductively coupled plasma emission spectrometry, inductively coupled mass spectrometry, neutron activation analysis and X-ray fluorescence. Each analytical technique affords specific advantages and disadvantages. Flameless atomic absorption spectrometry is the most commonly available system for single element analysis. For multiple elements, inductively coupled mass spectrometry is preferred. No single method can measure all trace elements accurately [1].

Excessive accumulation or depletion of trace elements may have significant clinical implications,

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including increased risk for cancer, cardiovascular disease, immune deficiency, anaemia, renal function impairment and bone disease. While very little is known about trace element concentration and metabolism in healthy individuals, even less is known about the physiology of trace elements in uraemia.

Factors affecting trace element concentration and toxicity

The concentration and toxicity of trace elements in body fluids can be affected by multiple factors (Table 1). Most factors cause a decrease rather than an increase in trace element concentration. In renal failure, trace element decreases mainly occur through losses to the dialysate and through urinary losses. However, the most important factor affecting trace element concentration in uraemic patients is the degree of renal failure [1].

Decreased concentrations are related mainly to nutritional intake, intestinal uptake and altered distribution. In addition, protein-bound trace elements may be lost more readily in the presence of proteinuria. Increased trace element concentrations can result from excessive homeopathic intake, industrial or environmental exposure, inhalation of cigarette smoke, administration of parenteral fluids or blood contact with contaminated dialysate. Although decreases in trace

element concentrations occur more frequently in end-stage renal disease (ESRD) and in dialysis patients, the greatest pathophysiological impact may actually result from the accumulation of trace elements in these individuals [1].

Accumulation of trace elements in dialysis patients may result from exposure to contaminated dialysate. Overt aluminium intoxication as a result of dialysate contamination was first recognized in 1976 in patients receiving chronic dialysis [2]. Dialysate contamination can result from addition of aluminium components to tap water to induce sedimentation of impurities, from the release of aluminium into the river water from industrial waste and/or from contamination of river water by aluminium, which is present as a natural element in the soil of some geographic areas.

Trace element concentrations in uraemia

In uraemic patients, the most important factors for trace element concentration are the degree of renal failure and the modality of renal replacement therapy [1]. In renal failure, elements such as arsenic, cobalt, caesium, chromium, mercury, molybdenum, silicon and strontium tend to increase. Other elements, including bromine, rubidium, selenium and zinc, tend to decrease (Table 2). However, the data from different studies are not entirely consistent. For example, Van Renterghem *et al.* found elevated arsenic levels in the serum of five patients with ESRD undergoing treatment with haemodiafiltration [3], while Mayer *et al.* found decreased arsenic levels in 85 patients suffering from renal failure and undergoing haemodialysis treatment [4].

Although the extent of renal failure can contribute to certain trends in trace element deviations, the data are sometimes inconsistent when groups are compared. For example, bromine was elevated in renal failure patients not yet on renal replacement therapy. However, haemodialysis and continuous ambulatory peritoneal dialysis (CAPD) patients showed a decrease in bromine concentration [1].

Table 1. Factors affecting concentration and toxicity of trace elements

| Inadequate intake | Malabsorption | Altered distribution |
|--|------------------------|--|
| Malnutrition Low income diets Alcoholism Increased requirement (anabolism, etc.) | Intestinal dysfunction | Changes in transport Changes in receptors Inability to store Transient changes: infection myocardial infarction stress |

Table 2. Reported evolution of serum/plasma concentrations of various trace elements in renal failure (range of mean values as reported)

| Element | Out-patient (CRF patients without dialysis) | Haemodialysis | CAPD | References |
|-------------------------------|---|--------------------------------|-----------------------|------------|
| Aluminium ($\mu\text{g/l}$) | \uparrow 50.0–186.3 | \uparrow 50.0–183.6 | \uparrow 105.3 | [5,6] |
| Arsenic ($\mu\text{g/l}$) | – | \uparrow/\downarrow 8.5–79.8 | – | [3,4,7,8] |
| Cadmium ($\mu\text{g/l}$) | – | =/ \uparrow 1.2? | – | [3] |
| Cobalt ($\mu\text{g/l}$) | – | – | \uparrow 0.3 | [9] |
| Copper (mg/l) | =/ \uparrow 0.8–1.3 | =/ \uparrow 0.8–1.5 | = 1.1–1.2 | [3,6,9,10] |
| Iron (mg/l) | = 1.2 | =/ \uparrow 0.9–1.6 | = 0.7 | [3,9,10] |
| Mercury ($\mu\text{g/l}$) | – | \uparrow 2.5 | – | [3] |
| Selenium (mg/l) | = 0.2 | =/ \downarrow 0.05–0.1 | \downarrow 0.06–0.1 | [3,9,11] |
| Vanadium ($\mu\text{g/l}$) | – | \uparrow 18.4 | – | [12] |
| Zinc (mg/l) | \downarrow 0.7–0.9 | \downarrow 0.7–0.9 | \downarrow 0.8 | [6,9,10] |

= defined as no significant difference compared with the reference value; \uparrow and \downarrow defined as significant increase or decrease, respectively, compared with the reference value. Note that reference values may be different from study to study.

Other inconsistencies in trace element profiles stem from a lack of uniformity in the reported results. Values are reported inconsistently from various sources, including whole blood, serum, plasma, packed cells or erythrocytes. In addition, various tissue concentrations are markedly different from blood or plasma concentrations for the majority of trace elements. Finally, concentrations differ from organ to organ. For example, kidneys and skin are known to sequester trace elements, including arsenic and cadmium [1].

Clinical implications

Cancer susceptibility, cardiovascular disease, anaemia

Cancer susceptibility is increased in patients with ESRD. Excess concentrations of arsenic and cadmium, as well as selenium deficiency, have been linked to carcinogenicity in non-uraemic populations [1]. Epidemiological studies, reviewed by Bates *et al.*, demonstrated a relationship between arsenic concentration in well water and cancers of the skin, bladder, kidney lung, and liver [13]. It is impossible to compare this exposure with that in uraemic patients, because serum concentrations in the affected subjects were not reported.

Cardiovascular morbidity and mortality is enhanced and accelerated in uraemic patients [1]. Several studies link increased and decreased levels of various trace elements with cardiovascular disease. In individuals without renal disease, high levels of blood lead and plasma aluminium were associated with essential hypertension [14]. Oxidative mechanisms are affected by arsenic, cadmium and copper. Studies in rats demonstrated that arsenic induced lipid peroxidation in the liver, kidney and heart [15], and cadmium produced enhanced lipid peroxidation in the liver, heart and spleen [16]. Studies have related iron excess to lipid oxidation, accelerated arterogenesis and excess risk of acute myocardial infarction [17]. On the other hand, copper deficiency has been associated with cardiovascular disease [18]. *In vitro* experiments showed that Na-K-ATPase was inhibited by mercury, lead and cadmium [19], and increased systolic and diastolic blood pressure was caused by long-term vanadium exposure in rats [20]. In six chronic dialysis patients, Richard *et al.* demonstrated a strong correlation between selenium and plasma glutathione peroxidase and showed that selenium deficiencies could be reversed [21].

Anaemia continues to be problematic for uraemic patients, despite the advent of erythropoietin. Excess arsenic, aluminium and vanadium, as well as copper deficiency, are all related to anaemia. Competition by arsenic for transport on transferrin, together with enhanced uptake of this complex, is thought to lead to high bone marrow concentrations of arsenic that might contribute to renal anaemia [22–24]. A small study of

five non-dialysed patients with chronic renal failure showed a negative correlation between blood haemoglobin and elevated levels of bone marrow arsenic. Haemoglobin levels in affected patients were in the range of 69–105 g/l compared with 142 g/l in controls, while arsenic levels ranged between 36 and 89 ng/g, compared with 19 ng/g in controls. Arsenic may have acted in parallel with or together with other accumulated compounds to inhibit erythropoiesis [24].

Investigators hypothesize that the inhibitory effect of aluminium on erythropoiesis is mediated by the interference of aluminium with iron bioavailability. In a case study of a patient who developed haematological evidence of aluminium accumulation, the inhibitory effect of aluminium was reversed with aluminium chelation therapy [25]. Furthermore, Jain *et al.* demonstrated a relationship between aluminium overload and the accumulation of lipid peroxides and lipofuscin products in red blood cells of haemodialysis patients. The data suggest that aluminium overload may increase membrane peroxidation and reduce red blood cell life span [26].

Although the uraemic state generally causes increased concentrations of copper, a deficiency of this metal has been associated with diminished growth of individual bone marrow cell lines and pancytopenia [27,28]. A study of 80 chronic haemodialysis patients demonstrated an inverse correlation between serum vanadium and red cell count and haemoglobin [12].

Renal failure

Several trace elements have been implicated in the decline of renal function. These include arsenic, cadmium, copper, germanium, lead and mercury (Table 3). In healthy individuals, normal functioning kidneys eliminate trace elements from the body. However, in uraemia, declining kidney function leads to an accumulation of potentially nephrotoxic trace elements, which may contribute to the deterioration of renal function [1].

Tubulointerstitial nephritis is associated with an elevated urinary arsenic concentration. Symptomatic improvement, normalization of abdominal radiographs and stabilization of renal function resulted after removal of the arsenic source, suggesting that this trace element provokes tubulointerstitial nephritis [29]. According to reports by Fowler *et al.*, tubular transport defects leading to the equivalent of the Fanconi syndrome may result from preferential accumulation in kidney tissue of cadmium, copper, lead and mercury [30].

Lead and germanium accumulation are especially significant in renal decline. In the study of a random population sample of 965 men and 1016 women, the creatinine clearance rate was found to be inversely correlated with blood lead and zinc protoporphyrin values. Furthermore, a 10-fold increase in blood lead concentration was associated with a 10–13 ml/min reduction in creatinine clearance. Although the study found that exposure to lead may impair renal

Table 3. Implications: renal failure

| | Arsenic | Cadmium | Copper | Germanium | Lead | Mercury |
|-------------------------------------|---------|---------|--------|-----------|------|---------|
| Impairment of renal function | | | | | X | |
| May cause renal and hepatic failure | | | | X | | |
| Tubular transport defects | | X | X | X | X | X |
| Tubulo-interstitial nephritis | X | | | | | |

function in the general population, the alternative hypothesis that renal impairment results in blood lead accumulation could not be ruled out [31].

In a case study of two young HIV-infected patients, the ingestion of germanium as an immunostimulant for 9 months produced extremely high concentrations of germanium in renal tissue (10–70 times normal) and liver tissue (140 times normal). The male patient continued to have renal dysfunction (creatinine clearance of 43 ml/min/m²) 9 months following cessation of germanium supplements. The female patient presented with severe renal dysfunction (creatinine clearance of 7 ml/min/1.73 m²), which persisted for 2 years after cessation of germanium supplements (14 ml/min/1.73 m²) [32].

Bone disease

The spectrum of renal osteodystrophy covers two general types of bone disease: a low turnover disease characterized by osteomalacia and adynamic bone disease, and a high turnover disease that includes osteitis fibrosa or mild secondary hyperparathyroidism. A mixed or transitional bone disorder may contain histological features of both low and high turnover lesions [33]. Several trace elements, including aluminium, cadmium, iron and strontium, have been implicated in renal osteodystrophy.

In vitro experiments and studies with dialysis patients show an association between aluminium and bone disease. In a study of 48 dialysis patients undergoing bone biopsy, all patients with a positive biopsy for aluminium staining ($n=21$) showed an abnormal morphology. The majority of aluminium-positive biopsies showed osteomalacia ($n=13$). However, in aluminium-negative biopsies ($n=27$), osteomalacia was absent. Furthermore, among aluminium-positive patients, hyperparathyroidism was rare ($n=1$) [34]. *In vitro* studies found that aluminium concentrations of 4 and 40 μM inhibit the affinity of parathyroid hormone (PTH) receptor and suppress PTH-stimulated adenylate cyclase. No PTH-responsive adenylate cyclase or binding to receptor was demonstrated at 200 μM [35].

Cadmium was reported to induce osteomalacia in ovariectomized rats, and cadmium concentrations were increased in bone of ESRD patients [36,37]. D'Haese *et al.* also reported an increased bone strontium and chromium content in patients with ESRD and an association of bone strontium with osteomalacia [33]. Lanthanum carbonate administered to uraemic rats

led to lanthanum accumulation in bone [38,39]. Moreover, studies in a rat model of chronic renal failure demonstrated an association of lanthanum accumulation with a dose-dependent increase in osteoid area and a decreased rate of bone formation—indicative of mineralization defects characteristic of bone histology in osteomalacic patients [38]. In a study of 27 chronic dialysis patients showing either iron or aluminium accumulation in bone, iron overload was associated with an increased frequency of adynamic bone disease [40].

Other functional defects

Alterations of glucose metabolism and insulin resistance have been related to iron overload, as demonstrated by Dmochowski *et al.* in a study of 10 thalassaemic patients [41]. Encephalopathy and coma have been associated with trace element accumulation, especially aluminium. Pre-dialysis patients with chronic renal failure may develop symptoms ranging from mild sensorial clouding to seizures and coma. After administering maintenance dialysis therapy, some patients continue to experience uraemic encephalopathy and coma, possibly as a result of acute trace element intoxications [42]. Furthermore, dialysis dementia has been associated with high level aluminium intoxication, usually in combination with inadequate water treatment at dialysis centres [1].

Immune depression in ESRD has been associated with zinc [43] and selenium deficiency [44], as well as iron overload [45,46]. Enzyme dysfunctions have been associated with trace elements in uraemia. In a study by Emenaker *et al.*, copper-erythrocyte superoxide dismutase activities were high in haemodialysis patients and showed a non-significant trend toward high values in CAPD patients [47]. In another study of patients with chronic renal failure, decreased plasma zinc and selenium correlated with erythrocyte superoxide dismutase [48]. A strong correlation was also observed between selenium and plasma glutathione peroxidase [48].

Research interests: arsenic as a special case

In recent years, several questions have gained importance among uraemic research interests. Which trace metals show the most spectacular changes? Can trace metal concentrations be influenced by the dialysate?

These questions have led some research groups to focus on the behaviour of arsenic, which has shown important changes in uraemia and potential for toxic side effects, not yet reported in the literature.

In a study of five uraemic patients on haemodiafiltration, deviations of trace element concentrations from normal reference values showed increasing or decreasing trends (Table 4). However, large inter-individual differences were observed and arsenic showed the most marked increase, exceeding the reference value by a factor of 50 in one patient [3].

Although caesium, iron, rubidium, selenium and zinc showed trends for decreased concentrations, the trends were not consistent throughout the five patients. While four patients showed a decrease in iron and caesium concentration, one patient unexpectedly had an increased concentration of these trace metals. Patient 5 showed a normal zinc concentration, compared with the other four patients with decreased zinc concentrations [3].

Consecutively, arsenic concentrations were studied in seven chronic haemodialysis patients, and showed a correlation between intracellular arsenic and serum arsenic concentrations. Arsenic accumulations of >10-fold were determined in serum and packed cells of chronic haemodialysis patients. However, arsenic concentrations remained unaltered, before and after a single haemodialysis treatment, with no arsenic detectable in the dialysate or heparin solution [8].

The magnitude of arsenic accumulation may be related to the degree of chronic renal insufficiency. Arsenic is already increased for moderate degrees of renal failure as demonstrated in pre-dialysis outpatients [49]. Zhang *et al.* determined serum arsenic to be 5.8 ± 3.3 $\mu\text{g/l}$ compared with a normal value of 0.382 $\mu\text{g/l}$ for a mean serum creatinine of 4.4 ± 3.3 mg/dl . In addition, higher arsenic concentrations were found in serum and in packed cells of patients having a greater degree of chronic renal insufficiency [49].

Arsenic species and uraemic toxicity

Too often, the metabolism of inorganics has been neglected in trace element research. In a study of

arsenic metabolites in Flemish giant rabbits, De Kimpe *et al.* observed disparate behaviour between organic and inorganic species that resulted from arsenate metabolism. Following the administration of a bolus of inorganic arsenic, high concentrations of inorganic species, such as arsenate and arsenite, peaked very early and then gradually decreased thereafter. These inorganic species are thought to exert the highest toxicity [50].

The organic species monomethylarsenic acid and dimethylarsenic acid were registered soon after the inorganic peaks. For dimethylarsenic acid, the gradual decrease was less dramatic than either of the inorganic species. Protein-bound arsenic appeared only after a few hours and showed no decline. When the same amount of arsenic was administered to uraemic rabbits, inorganic species tended to peak higher and to disappear more slowly, whereas the appearance of organic species was postponed. Arsenic distribution in tissues varied widely, with the highest concentrations in kidneys, liver and lungs. Arsenic accumulated in bone, compared with rapid clearance rates in other tissues and blood [50].

In humans with renal failure, the main detectable species were the relatively innocuous arsenobetaine (3.6 ± 4.6 $\mu\text{g/l}$) and dimethylarsenic acid (0.8 ± 1.1 $\mu\text{g/l}$) [51]. Toxic inorganic species such as arsenite and arsenate were below the detection limit, making a comparison with healthy controls impossible. In animals, more inorganic species were accumulated in those with renal failure than in those with normal renal function [52,53].

Uraemic compounds may alter toxicity and kinetics of trace elements

Certain uraemic compounds may influence the cellular accumulation of trace elements. *In vitro* experiments demonstrated that uraemic solutes present in ultrafiltrate fractions were related to an increased cellular uptake and toxicity of aluminium. Dose-response curves confirmed that aluminium uptake and cell toxicity were proportional to *p*-cresol concentrations in culture medium. *p*-Cresol and other uraemic compounds from these ultrafiltrate fractions may

Table 4. Trace elements in haemodiafiltration

| Trend | Element | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Reference |
|---|------------------------|-----------|-----------|-----------|-----------|-----------|-----------|
| Increased trace element concentrations compared with normal reference | As ($\mu\text{g/l}$) | 43.1 | 3.8 | 7.5 | 12.5 | 9.6 | 0.9 |
| | Cd ($\mu\text{g/l}$) | 3.1 | 3.7 | <0.4 | <0.5 | <0.3 | <0.2 |
| | Cu (mg/l) | 1.4 | 1.6 | 1.5 | 1.4 | 1.8 | 0.7 |
| | Hg ($\mu\text{g/l}$) | 4.0 | 4.8 | 1.1 | 1.4 | 1.4 | 0.5 |
| | Mo ($\mu\text{g/l}$) | 2.0 | 1.8 | 4.0 | 2.7 | 1.8 | 0.6 |
| Decreased trace element concentrations compared with normal reference | Cs ($\mu\text{g/l}$) | 0.3 | 0.5 | 0.7 | 1.1 | 0.6 | 0.7 |
| | Fe (mg/l) | 1.5 | 0.8 | 0.6 | 4.1 | 1.0 | 1.6 |
| | Rb ($\mu\text{g/l}$) | 56 | 100 | 102 | 104 | 95 | 170 |
| | Se (mg/l) | 0.06 | 0.07 | 0.09 | 0.07 | 0.06 | 0.13 |
| | Zn (mg/l) | 0.8 | 0.8 | 0.8 | 0.7 | 1.1 | 1.1 |

Adapted from [3].

play a role in the accumulation and toxicity of aluminium in the liver of ESRD patients [54].

Detoxification of arsenite can be altered by uraemic toxins. *p*-Cresol and other uraemic toxins, including oxalate, hypoxanthine, homocysteine and myo-inositol, were found to inhibit arsenic methylation [55].

Conclusions

Plasma concentrations of several trace elements are altered in uraemia and may play an important role in mediating a variety of pathophysiological events affecting the general condition of uraemic patients. Clinical implications from uraemic trace elements include increased cancer susceptibility, enhanced cardiovascular morbidity/mortality, anaemia, renal failure and bone disease. Several trace metals have been shown to accumulate in bone of uraemic patients, including aluminium, cadmium, chromium, lanthanum, strontium and zinc. The potential use of certain metal-containing therapeutics requires further health risk analysis. Although bone disease resulting from aluminium intoxication has declined, renal osteodystrophy still persists. Further research is necessary to determine accurately the effect of trace metal accumulation in relation to bone disease.

Among the various factors influencing trace element accumulation, the most important factors are the stage of renal failure and the type of renal replacement therapy. The metabolism of inorganics has been neglected in trace element research. Trace element accumulation and toxicity may differ depending on the retained inorganic species. Arsenic behaviour in uraemia illustrates the importance of inorganic metabolism. The inorganic species, arsenate and arsenite, are thought to exert the highest toxicity. The uraemic syndrome may also partially alter the uptake and toxicity of trace elements. Uraemic compounds have been related to increased cellular uptake and toxicity of aluminium and arsenic.

While little of the behaviour of trace elements is understood in healthy individuals, even less is known about trace element disturbances in uraemic patients. Although research has focused on total concentrations of trace elements, the evolution of both inorganic and organic species should be considered separately.

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