Trace elements and micropollutant anions in the dialysis and reinfusion fluid prepared on-line for haemodiafiltration

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Abstract. Exposure to the trace elements and micropollutants of tap water may be very considerable in dialysis patients. As few data on trace elements in reinfusion fluid prepared on-line for haemodiafiltration (HDF) have been reported, we studied nine trace elements (µg/l; Al, As, Cd, Cr, Cu, Hg, Pb, Se, Zn) and five anions (mg/l; F⁻, NO₂⁻, NO₃⁻, PO₄³⁻, SO₄²⁻) in tap water, in water after two passages of reverse osmosis (2RO), in dialysate and on-line prepared reinfusate. NO₃⁻ and SO₄²⁻ were somewhat elevated in our tap water (22.2 ± 7.6 and 21.8 ± 11.3 mg/l) but decreased (P<0.001) after 2RO (1.4 ± 1.5 and 0.9 ± 1.1 mg/l); the other anions, which were at a very low level, remained unchanged. All trace elements decreased, with statistical significance only for Al, Cr and Zn from 14.9 ± 19.9, 2.6 ± 0.6 and 35.1 ± 41.1 µg/l to 3.2 ± 2.1, 0.2 ± 0.2 and 3.5 ± 4.8 µg/l, respectively. Due to impurities in concentrate salts for Al (5.4 ± 3.1), Cr (0.5 ± 0.4) and SO₄²⁻ (2.4 ± 1.8), greater concentrations were found in dialysate and reinfusate than in tap water after 2RO (P<0.03). For all measurements, trace elements and anions were at acceptable levels according to international standards. Simultaneous determinations of trace elements at inflow (Din) and outflow (Dout) of the dialysate as well as in plasma or in whole blood at the beginning of on-line HDF documented Dout/Din > 1 for Al, Cu and Zn and a positive gradient between the concentration in blood and dialysate inlet. In conclusion, our dialysate and reinfusate can be considered safe regarding trace elements and micropollution: two passages through reverse osmosis reduces the concentrations of trace elements and anions. The impurities of concentrates are acceptable. Accumulation or depletion of trace elements should be evaluated after longitudinal studies of plasma concentrations.

Key words: dialysis fluids; micropollutants; on-line haemodiafiltration; reinfusion fluids; trace elements

Introduction

Water and salts used for preparing dialysate have been recognized as potential sources of contamination with trace elements or micropollutants [1]. The clinical and toxicological significance of the imbalance of aluminium has been demonstrated [2]; however, we cannot exclude that even low concentrations of trace elements in dialysate could result in a significantly increased exposure of dialysis patients [3–6]. The abnormal overload does not necessarily mean toxicity, although a more elevated exposure than routine dietary intestinal absorption can be assumed as an index for potential adverse effects [3]. Reverse osmosis (RO) has improved the quality of the water to give acceptable levels of trace elements, but we cannot reduce the quantity of some trace elements in the commercial salts used for dialysate [7].

In haemodialysis, a large volume (360 l/week) of dialysate comes into contact with the blood through the membrane, so that the exposure may be greatly elevated [3]. However, some factors can influence the transfer [3,8–11]: not only the concentration gradient between dialysate and blood, but also the chemical form of trace elements or the ability of these elements to bind carriers as well as the type of haemodialysis treatment must be taken into account. In haemodiafiltration (HDF), the reinfusion fluid (infusate) and large membrane pores which allow the passage of large molecules such as albumin carrying some trace elements may also be additional factors influencing the balance of trace elements [12]. Accumulation or depletion of trace elements may be more relevant if large quantities of reinfusate are used or with on-line preparation [13–15].

For these reasons, we studied some trace elements and micropollutant anions in tap water in an industrial metropolitan area of Milan, in water after two passages of RO (2RO) and in dialysate or reinfusate prepared on-line for HDF.

Materials and methods

Nine trace elements (Al, As, Cd, Cr, Cu, Hg, Pb, Se and Zn) and five anions (F⁻, NO₂⁻, NO₃⁻, PO₄³⁻ and SO₄²⁻) were
Table 1. Trace metals (µg/l) in tap water (T), in water after two passages through reverse osmosis (2RO) and in dialysis/reinfusion fluid (D/R) determined by atomic absorption spectroscopy

<table>
<thead>
<tr>
<th>Element</th>
<th>Tap Water (T)</th>
<th>Water After 2RO (2RO)</th>
<th>Dialysis/Reinfusion Fluid (D/R)</th>
<th>Lipid Ratio (D/R vs 2RO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>14.96 ± 19.91</td>
<td>3.29 ± 2.18</td>
<td>0.03</td>
<td>5.43 ± 3.16</td>
</tr>
<tr>
<td>As</td>
<td>2.59 ± 1.1</td>
<td>2.06 ± 2.03</td>
<td>ns</td>
<td>3.26 ± 2.61</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt; 0.05</td>
<td>&gt; 0.05</td>
<td>ns</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Cr</td>
<td>2.61 ± 0.64</td>
<td>0.24 ± 0.26</td>
<td>0.001</td>
<td>0.55 ± 0.43</td>
</tr>
<tr>
<td>Cu</td>
<td>3.67 ± 4.39</td>
<td>2.19 ± 2.78</td>
<td>ns</td>
<td>4.06 ± 5.32</td>
</tr>
<tr>
<td>Hg</td>
<td>0.93 ± 0.19</td>
<td>0.9 ± 0.18</td>
<td>ns</td>
<td>0.92 ± 0.22</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt; 1</td>
<td>&lt; 1</td>
<td>ns</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Se</td>
<td>1.92 ± 0.28</td>
<td>1.88 ± 0.35</td>
<td>ns</td>
<td>2.15 ± 0.78</td>
</tr>
<tr>
<td>Zn</td>
<td>35.15 ± 41.14</td>
<td>3.54 ± 4.88</td>
<td>0.03</td>
<td>7.19 ± 7.12</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation, with minimum and maximum values (in parentheses); * = no. of determinations.

evaluated in tap water, in water after 2RO and in dialysate which is identical to reinfusate in on-line HDF. The composition of dialysate/reinfusate was Na⁺ 138, K⁺ 2, Ca²⁺ 1.5, Mg²⁺ 0.5, Cl⁻ 109, bicarbonate 32 and acetate 3 mol/l.

Reinfusate was obtained by two passages through a synthetic Fresenius Polysulfone membrane with a wall thickness of 40 µm (Diasafe®). The monitor which prepared dialysate and reinfusate (post-dilution reinfusion of 20.5 ± 0.4 l/run) was Fresenius 4008 B. Clinical data regarding the study or type of run are reported in the accompanying paper [15].

The samples of tap water, 2RO and dialysate/reinfusate were taken at the beginning of the session into polythene containers and stored at −20°C until the analysis (not more than 15 days). Each container was washed previously with diluted HCl and rinsed with double-distilled water. Trace elements were determined by atomic absorption spectroscopy (AAS Perkin Elmer 3030 Zeeman or 1100 B). Al, Cd, Cr and Pb were determined by electrothermal AAS; Cu and Zn by flame AAS; As, Hg and Se by hydride generation AAS [16]. Samples were analysed unchanged or simply added with matrix modified for each element. Standard solutions of each element and all reagents used were ultrapure grade (Suprapure Merck, Germany). Samples were analysed in duplicate for each trace element. Quantitative determination was obtained by the method of standard addition. The analytical performance was checked every day by standard solutions with known concentrations of each anion (Merck, Germany).

We also analysed seven trace elements after 10–60 min of on-line HDF, taken simultaneously in the dialysate at the inflow site (Din), and at the outflow site after the infusion of reinfusate (Dout) and in plasma (Al, Cu, Se) or whole blood (Cd, Cr, Pb, Zn). The methods of analysis of the plasma or whole blood are reported in the accompanying manuscript [15]. A positive ratio of Dout/Din and a positive gradient plasma or blood/Din was considered as indicative of a possible passage from the patient to dialysate.

All results are presented as mean ± standard deviation. Statistical analysis used the appropriate Student’s t-test for paired and unpaired data. The limit of significance was set at P < 0.05.

Results

The data regarding trace elements and micropollutant anions are reported in Tables 1 and 2. Cd, Pb, F⁻, NO₃⁻ and PO₄³⁻ showed values lower than the detection limits of the analytical methods in all samples. In our tap water, the values of NO₃⁻ and SO₄²⁻ were rather elevated but they decreased significantly after 2RO. There was also a decrease in all trace elements after 2RO, with significance only for Al, Cr and Zn. The concentrate salts added small quantities of trace elements as documented by greater values in dialysate/reinfusate. Only for Al, Cr and SO₄²⁻ was the increase in dialysate/reinfusate significant. The analysis of the
single data (not reported in the tables) showed only for As, with 17.4 µg/l, a value more than five times greater than other. Comparing individual data with maximum blood values of normal subjects of our region, reported by others [16] or by us [15], in 9/47 (19%) samples for Al, 1/40 (2%) for As and 11/19 (58%) for Cr there were values greater than the normal range. In no case did a patient receive a dialysate/reinfusate with trace element and micropollutant concentrations greater than recommended by international concentrations of trace elements in dialysate and reinfusate, with trace element and micropollutant concentrations greater than recommended by international associations (Table 3).

In Table 4, the simultaneous determinations of Din, Dout and plasma or blood are reported. For all determinations, there was a positive Dout/Din ratio and plasma or blood/Din gradient.

### Discussion

In this work, we studied nine trace elements and five micropollutants in tap water, after 2RO and in dialysate and reinfusate prepared on-line for HDF. To our knowledge, it is the only study on trace elements of a reinfusate prepared for HDF in the dialysis centre.

On-line HDF has the advantage of using, without storage, large quantities of a cheap reinfusate and of producing an ultrapure dialysate with the same composition as reinfusate [13,14]. As large quantities of dialysate (160 l/run) and reinfusate (20.5±0.4 l) are in contact with the blood of the on-line HDF patients, we evaluated the possibility of a loss of or a transfer to the patient of some trace elements. Acute as well as chronic intoxication or depletion of trace elements have been described in haemodialysis patients [18,19], and we cannot exclude that, in the presence of elevated concentrations of trace elements in dialysate/reinfusate, such disturbances might also occur in on-line HDF patients.

Tap water must be considered as the main source of contamination with trace elements. 'Acid rain' increases the concentration of Al, Cr, Hg, Mn, Ni and Pb in tap water [20]. Moreover, Al frequently is added as flocculate in the water purification process. The repeated measurements of trace elements and anions in our tap water do not document elevated levels, with the exception of NO₃ and SO₄ which were out of the range admitted by international standards. On the other hand, the composition of tap water may vary in different geographical areas or seasonally in some areas. Fe and Mn were found to be particularly high in tap water in a centre close to ours [8]. At the present time, the trace elements analysed in different samples of our tap water do not represent a risk to our patients undergoing haemodialysis or on-line HDF.

RO adequately removes all the contaminants. However, the possibility of dialysate contamination due to bad working devices (such as the heating tank or inadequate connection of the RO system) has been reported [19]. Salvadeo et al. [8] found a significant reduction of trace elements, except for Pb and Zn, after deionization of tap water. In the present study, 2RO resulted in a decrease of trace elements which was significant only for Al, Cr and Zn, whose concentrations were, as for the other trace elements, in the range suggested by international standards (Table 3). NO₃ and SO₄, which were present in tap water in unallowable concentrations, also decreased significantly after 2RO. Our data confirm the efficacy of RO, and we can consider the two passages through the RO safe for preparing dialysate and reinfusate.

The addition of mineral salts with the concentrates

### Table 2. Micropollutant anions (mg/l) in tap water (T), in water after two passages through reverse osmosis (2RO) and in dialysis/reinfusate fluid (D/R) determined by ion chromatography

<table>
<thead>
<tr>
<th></th>
<th>T</th>
<th>2RO</th>
<th>D/R</th>
<th>P &lt; (T vs 2RO)</th>
<th>P &lt; (D/R vs 2RO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F⁻</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>ns</td>
<td>&lt;1</td>
<td>ns</td>
</tr>
<tr>
<td>NO₃⁻</td>
<td>22.25 ± 7.64</td>
<td>1.41 ± 1.52</td>
<td>0.001</td>
<td>1.39 ± 1.36</td>
<td>(0.1–5.2)</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>ns</td>
<td>&lt;0.02</td>
<td>ns</td>
</tr>
<tr>
<td>PO₄³⁻</td>
<td>0.02</td>
<td>0.02</td>
<td>ns</td>
<td>&lt;0.02</td>
<td>ns</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>1.15 ± 0.02</td>
<td>0.01</td>
<td>0.001</td>
<td>2.44 ± 1.83</td>
<td>(0.1–4.7)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation, and minimum and maximum values (in parentheses). No. of determinations: 10 for tap water and 2RO; 18 for dialysate/reinfusate.

### Table 3. International standards for dialysis water

<table>
<thead>
<tr>
<th></th>
<th>AAMI</th>
<th>ASAIO</th>
<th>CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>F⁻ (mg/l)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>NO₃⁻ (mg/l)</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>SO₄²⁻ (mg/l)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Al (µg/l)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>As (µg/l)</td>
<td>5</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Cd (µg/l)</td>
<td>1</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Cr (µg/l)</td>
<td>14</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Cu (µg/l)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Hg (µg/l)</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Pb (µg/l)</td>
<td>5</td>
<td>5</td>
<td>50</td>
</tr>
<tr>
<td>Se (µg/l)</td>
<td>90</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Zn (µg/l)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

AAMI, Association for Advancement of Medical Instrumentation; ASAIO, American Society for Artificial Organs; CSA, Canadian Standards Association.
Table 4. Simultaneous determinations of trace elements at inflow (Din) and outflow (Dout) dialysis sites and in plasma (P) or whole blood (B) at the beginning of the on-line HDF run.

<table>
<thead>
<tr>
<th>Element</th>
<th>Din</th>
<th>Dout</th>
<th>P or B (*)</th>
<th>Dout/Din</th>
<th>P or B (*)/Din</th>
</tr>
</thead>
<tbody>
<tr>
<td>Al</td>
<td>1.1 ± 1.8</td>
<td>2.2 ± 2.1</td>
<td>17.1 ± 12.8</td>
<td>6.8 ± 6.1</td>
<td>15.8 ± 13.2</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>1.04 ± 0.41*</td>
<td>6.4 ± 0.41*</td>
<td></td>
</tr>
<tr>
<td>Cr</td>
<td>0.6 ± 0.5</td>
<td>0.7 ± 0.5</td>
<td>10.5 ± 11.1*</td>
<td>1.1 ± 0.3</td>
<td>9.7 ± 11.1*</td>
</tr>
<tr>
<td>Cu</td>
<td>6 ± 5.3</td>
<td>9.4 ± 0.4</td>
<td>980 ± 319</td>
<td>6 ± 10.2</td>
<td>793 ± 316</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>15.5 ± 6.5*</td>
<td>15 ± 65*</td>
<td></td>
</tr>
<tr>
<td>Se</td>
<td>&lt;2</td>
<td>&lt;2</td>
<td>73.1 ± 28.1*</td>
<td>71.1 ± 28.1*</td>
<td></td>
</tr>
<tr>
<td>Zn</td>
<td>11 ± 8</td>
<td>21.1 ± 6.4</td>
<td>6115 ± 1039*</td>
<td>3 ± 2.6</td>
<td>6104 ± 1039*</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation (μg/l). No. of determinations = 12.

contributes to the increase in the concentration of some trace elements. In our samples, the increase was significant only for SO$_3^-$-ions. However, the end product (dialysate/reinfusate) analysed after the distribution circuit can be considered free of contaminants, when compared with international standards reported in Table 3. It has been underlined that the purity of the salts cannot be improved globally without enormous efforts (in terms of costs) [7]. Research must now identify elements, other than Al, responsible for intoxication in order to justify a further reduction in their concentrations in dialysate. Padovese et al. [3] demonstrated that weekly exposure for several trace elements appeared to be 50- to 12 000-fold greater than the corresponding values for the amount absorbed via the diet (for dialysate in haemodialysis: Al, Ba, Be, Ce, Ga, La, Sc, Ta, Th, V, Zr; for dialysate in continuous ambulatory peritoneal dialysis: Au, Be, Ce, Ta, Th, V, Zr). None of these trace elements was studied by us, and the exposure is not equal to contamination since other factors, such as the chemical form of the element or its ability to bind carriers, may be important.

In haemodialysis, the balance of trace elements is influenced mainly by the gradient between dialysate and the free part present in the plasma. Electrical charges may influence the passage across the low-flux membrane [21]. In HDF, there are additional factors regulating the balance: some trace elements can pass through the high-flux membrane together with protein carriers and, further, the reinfusate may dilute or augment the free form of the ions. These considerations can explain some differences between haemodialysis and HDF.

Salvadeo et al. found a reduction of metal concentrations in the fluid leaving the dialyser and thus attributed the elevated plasma values to the positive dialytic balance [8]. On the other hand, Bonomini et al. [22] found that with low-flux cuprophan membranes the Dout of Se was not significantly lower than Din, and they considered the low plasma levels of Se independent of replacement treatment to be a result of the high molecular weight of Se-binding proteins.

The chemical form of trace elements in the fluid and the ability of the metal to be bound by carriers can also be involved [3]. For example, Cr rapidly binds to proteins and does not back diffuse [23]. When Dout/ Din, as a mean, is close to 1, it is very difficult to determine the direction of the diffusion, as plasma values may also be influenced in part by haemoconcentration. This is particularly true in on-line HDF which is characterized by an elevated haemoconcentration. However, when Dout/Din is very elevated, as in our case for Al, Cu and Zn, a loss of trace elements is very probable. Although the gradient from plasma or blood to dialysate is dependent on the free form, the ratio, when positive, may confirm the possibility of losing trace elements. The loss of some trace elements into the dialysate of on-line HDF patients can balance the accumulation due to the absence of renal function or to the transfer from plasma to the dialysate. Only long-term studies can clarify this problem.

A comparison between our concentration of trace elements in dialysate/reinfusate and the data of commercial substitution fluid for haemofiltration [3] or HDF [12] is difficult to do for different analytical techniques. The concentrations of trace elements and pollutants in our substitution fluid were acceptable according to international standards. The safety can be evaluated by comparing the plasma values of on-line HDF patients with normal or uraemic subjects and performing longitudinal studies in on-line HDF patients [15].

In conclusion, NO$_3^-$ and SO$_3^2^-$ were very elevated in our tap water; trace elements and micropollutants decreased (NO$_3^-$, SO$_3^2^-$, Al, Cr, Zn significantly) after two passages through RO. Due to the impurity of concentrate salts, trace elements and anions increased (significantly only Al, Cr and SO$_3^2^-$) in reinfusate and dialysate. Trace elements and anions were acceptable according to international standards so that our dialysate and reinfusate can be considered safe.

Acknowledgements. We thank all nurses and technicians from the dialysis centre of Seregno as well as Fresenius Medical Care Italia for technical support. We are also grateful to Dr G. Orlandini and Dr A. Gauy for reviewing the manuscript.

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