The plasma retinol levels as pro-oxidant/oxidant agents in haemodialysis patients

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Abstract

Background. Oxidative stress is a process involved in haemodialysis-related pathologies such as cerebrovascular diseases. Retinol is the major circulating form of vitamin A and it is elevated in haemodialysis (HD) patients. It is known that these patients present anaemia that is not totally responsive to erythropoietin. The aim of this study was to evaluate the influence of plasma retinol levels on oxidative stress biomarkers, especially on δ-aminolevulinate dehydratase.

Methods. Plasma retinol and malondialdehyde (MDA) levels were quantified by HPLC-UV/VIS; blood activities of catalase (CAT), superoxide dismutase (SOD) and δ-aminolevulinate dehydratase (ALA-D) were analysed by spectrophotometric methods, in HD patients (n = 29) and healthy subjects (n = 20).

Results. The MDA and retinol levels, SOD and CAT activities were significantly increased in HD patients. ALA-D activity was significantly decreased. Retinol levels were correlated with MDA levels (r = 0.68), CAT (r = 0.39), SOD (r = 0.40) and ALA-D (r = −0.55). A partial correlation between retinol levels with ALA-D (r = 0.43), SOD (r = 0.30) and CAT (r = 0.36) activity was found, utilizing MDA levels as co-variable.

Conclusion. Higher retinol levels may be associated with the increase of SOD and CAT activities, but this increase was not sufficient to prevent the lipid peroxidation and ALA-D thiolic group oxidation. In this manner, our results could suggest that high retinol levels contribute as an additional factor to the oxidative tissue damage.

Keywords: ALA-D activity; haemodialysis patients; MDA; oxidative stress; plasma retinol levels

Introduction

Haemodialysis treatment is the main resource for patients in the end-stage of renal disease, who are either waiting for or are not suitable to undergo renal transplantation [1]. In chronic renal failure (CRF) patients under haemodialysis (HD) treatment, the formation of reactive oxygen species (ROS) is amplified and the oxidative stress may be one of the most relevant complications occurring. This problem may not have immediate clinical effects although it may represent a long-term complication derived from the repetitive effects of the blood–membrane interaction [2–4]. Nevertheless, the multifactorial nature of this process [3] might include other factors peculiar to chronic HD treatment, such as the absence of a complete correction of the uraemic toxicity, malnutrition and the progressive worsening of the clinical condition due to ageing and comorbidity [2–4].

Retinol, the major circulating form of vitamin A, was shown to have some antioxidant properties [5] although...
recent studies demonstrated that at higher doses it is a pro-
oxidant and modulates antioxidant enzyme activity. Any-
way, the mechanism by which retinol can act as a pro-
oxidant is not well elucidated yet [6].

Vitamin A plays an essential role in maintaining mam-
malian health. It is required for many crucial biological
functions such as vision, reproduction, growth and immu-
nity [7,8]. These are generated intracellularly by two ox-
idative enzymatic reactions in which retinol is converted
first to retinaldehyde and then to retinoic acid [9]. Vi-
mamin A is normally transported in plasma as retinol linked
by a specific transport protein, which is known as retinol-
binding protein (RBP) [10,11]. When dietary vitamin A is
not available, RBP is able to mobilize retinol from vitamin A
stores in the liver to supply peripheral cells and tis-

sues with retinoids needed for various biological functions
[12].

The oxidative stress biomarkers include superoxide dis-
mutase (SOD), catalase (CAT) and glutathione peroxidase
(GPx); enzymes are the first endogenous antioxidants, and
they catalyze important defence reactions to clear up the
detrimental ROS in vivo. Any factors that undermine the
activities of antioxidant enzymes may lead to accumulation
of ROS and subsequently oxidative damage to biological
macromolecules [13].

On the other hand, δ-aminolevulinate dehydratase
(δ-ALA-D), a zinc metalloenzyme of the haem biosynthesis
pathway, requires reduced thiol groups for its activity [14].
For this reason, ALA-D has been suggested as a biomarker
for oxidative stress [15,16]. One of them, δ-aminolevulnic
acid (ALA), has been shown to induce pro-oxidant events
[17,18]. It is important to clarify that δ-ALA-D activity
is decreased in CRF, especially during HD treatment
[19–21]. Furthermore, malondialdehyde (MDA) is a more
specific and sensitive biomarker for the evaluation of the
lipid peroxidation status in many pathologies [22], includ-
ing in patients under chronic HD treatment [23].

The aim of this study was to verify the possible influence
of plasma retinol levels on classical oxidative stress blood
biomarkers (enzymatic antioxidants and plasma MDA
levels—the most used lipid peroxidation biomarker) and
on erythrocyte ALA-D activity in HD patients, compared
to healthy subjects.

Materials and methods

Chemicals

δ-aminolevulinic acid (ALA), 2-thiobarbituric acid (TBA), dithiothreitol
(DTT) and retinol were purchased from Sigma (St. Louis, USA). All other
chemicals used in this study were of the highest purity available.

Subjects

Twenty-nine patients with diagnosis of CRF (19 men and 10 women)
undergoing regular HD treatment at Caridade and Casa de Saúde Hospitals,
located in Santa Maria, RS, Brazil were included in the study. The study
protocol was approved by the Human Ethics Committee of the Health
Science Center from the Federal University of Santa Maria (protocol no.:
091/2003), and all the patients gave their informed consent prior to their
inclusion in the study. Included in this study were patients on regular
hemodialysis. Exclusion criteria were excessive alcohol consumption, di-
abetes, smoking, viral hepatitis and HIV. Patients using any antioxidant
vitamin in the last 3 months were also excluded. The patients who par-
ticipated were using vitamin D, erythropoietin, statines and norpurpurin
(saccharate ferric hydroxide), and were performing dialysis three times
per week during the morning, with a duration of 4 h, each session, the last
one being two days before blood was drawn.

The control group consisted of 20 healthy subjects (10 men, 10 women),
who did not have clinical history of renal diseases or other pathologies.
All the volunteers were not taking antioxidant vitamins, were nonsmokers
and did not consume alcohol regularly.

Samples

Venous blood samples (10 mL) were drawn from HD patients, before the
haemodialysis session. In the control group the collection was held during
the morning. Then, these samples were divided into heparinized tubes,
EDTA-containing tubes and tubes without anticoagulant. Plasma-EDTA
and serum were obtained by centrifugation at 1500 g for 10 min at 4°C.

Hematological determinations

Haemoglobin (Hb) and haematocrit (Hct) were determined in the Cobas
Micsr os system, (Haematology Analyzer, Roche Diagnostics GmbH,
Mannheim, Germany).

Lipid peroxidation

Lipid peroxidation was estimated by measured malondialdehyde (MDA).
The measurement of plasmatic MDA was determined by high performance
liquid chromatography with visible detection (HPLC-VIS), according to
the method of Grotto et al. [24].

δ-ALA-D activity

δ-Aminolevulinate dehydratase activity was determined in the total blood,
with heparin as anticoagulant, according to the method of Sassa [25]
including some modifications. The enzyme activity was determined by
the rate of phorphobilinogen (PBG) formation in1ha t3 7
◦
C, in the presence and absence of the reductor agent dithiothreitol (DTT 2 mM
final concentration). The enzyme reaction was initiated after 10 min of
pre-incubation. The reaction was started by adding δ-aminolevulnic acid
(ALA) to a final concentration of 4 mM in a phosphate buffered solution
at pH 6.8; incubation was carried out for 1 h at 37°C and the reaction
product was measured at 555 nm. The reactivation index was estimated
using the formula A = B/A∗100, where A = absorbance ALA-D with DTT
and B = absorbance of ALA-D without DTT.

Retinol assay

Plasma retinol quantification was realized after liquid–liquid extraction
with a solution of n-butanol: ethanol (50:50) in BHT (2,6-di-ter-butyl-
4-methylphenol) mixed by vortex and followed by centrifugation. The
supernatant was analysed by HPLC with the UV/VIS detector, according
to Murata et al. with modifications [6]. The β-apo-8 caroteno were utilized
as internal standard.

Antioxidant enzyme activities

Enzyme assays were determined in the total blood with heparin. Super-
oxide dismutase activity was determined based on its ability to inhibit
the autoxidation of adrenaline into adrenochrome at an alkaline pH [26].
Catalase activity was determined using H2O2 as substrate [27]. They
were measured spectrophotometrically using a UV–VIS model Hitachi U
1800®.

Biochemistry assay

Serum creatinine and urea were determined in the Cobas Integra system
(Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Statistical computations were performed with the Statistica® 6.0 software
system (Statsoft Inc., 2001). The results are expressed as mean ± standard
error medium (SEM). Comparisons between groups were achieved by the
Student t-test or Mann–Whitney test, depending on the variable distribu-
tion. Pearson’s correlation or Spearman’s rank order correlation was used
to evaluate the relationship between pairs of variables, following the vari-
able distribution. Partial correlation was used to evaluate the relationship
Table 1. General characteristics of the studied groups (healthy subjects and haemodialysis patients)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy subjects (n = 20)</th>
<th>HD patients (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>43.15 ± 1.30</td>
<td>51 ± 2.17</td>
</tr>
<tr>
<td>Time of HD treatment (months)</td>
<td>–</td>
<td>45.68 ± 7.27</td>
</tr>
<tr>
<td>Sex (men/women)</td>
<td>10/10</td>
<td>19/10</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>27.35 ± 1.58</td>
<td>164.54 ± 7.45*</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.67 ± 0.07</td>
<td>10.17 ± 0.62*</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± standard error medium (SEM). *Significantly different from controls (P < 0.001).

Results

The general characteristics of HD patients and healthy subjects such as age, sex distribution, haemodialysis time and biochemical parameters are presented in Table 1.

Retinol assay

Plasma retinol levels were significantly increased in HD patients compared to healthy subjects, almost three fold, being 6.86 ± 0.60 versus 2.41 ± 0.12 μmol/L, respectively (P < 0.0001). On the other hand, plasma MDA levels, the biomarker of lipid peroxidation, were also significantly increased in HD patients (Figure 1). Moreover, plasma retinol levels were positively correlated with MDA levels (Figure 2) and presented a negative correlation with the ALA-D activity (Figure 3).

To verify whether plasma retinol levels and plasma MDA levels are indeed independent variables influencing ALA-D activity, the partial correlation between each of these variables and ALA-D activity was estimated, while controlling for the other variable. Controlling for plasma retinol levels, partial correlation analysis revealed no significant relation between MDA levels and ALA-D activity (r = −0.11, P > 0.05). However, the partial correlation analysis revealed a negative relation between plasma retinol levels and ALA-D activity (r = −0.43, P < 0.05), controlled for MDA levels. Additionally, when controlling for retinol levels no linear correlation between the activities of the antioxidant enzymes and MDA levels was observed.

The retinol levels also correlated with the catalase and SOD activity, being r = 0.39 and r = 0.40, P < 0.05, respectively. Moreover, while carrying out the partial correlation analysis a positive correlation was observed between plasma retinol levels and SOD activity (r = 0.30, P < 0.05) such as catalase activity (r = 0.36, P < 0.05), controlled by MDA levels.

Lipid peroxidation

The results of the lipid peroxidation assessed by MDA measurement were significantly higher in HD patients compared to the healthy subjects, being 6.92 ± 0.35 versus 4.53 ± 0.16 μmol/L, respectively (P < 0.0001). Plasma MDA levels presented a negative correlation with the
The plasma retinol levels in HD patients

Table 2. Activities of the ALA-D enzyme obtained with and without reductant agent (DTT) and of the antioxidant enzymes

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>Healthy subjects (n = 20)</th>
<th>HD patients (n = 29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA-D (U)</td>
<td>20.00 ± 1.49</td>
<td>11.57 ± 0.55*</td>
</tr>
<tr>
<td>ALA-D index of reactivation (%)</td>
<td>21.05 ± 2.88</td>
<td>11.74 ± 7.35**</td>
</tr>
<tr>
<td>SOD (U SOD/mg Hb)</td>
<td>0.7 ± 0.05</td>
<td>0.90 ± 0.03*</td>
</tr>
<tr>
<td>CAT (K/mg Hb)</td>
<td>43.17 ± 9.20</td>
<td>56.98 ± 4.25*</td>
</tr>
</tbody>
</table>

Results expressed in mean ± standard error medium.
*Significantly different from controls (P < 0.05).
**Significantly different from controls (P < 0.001).

ALA-D activity. The MDA levels positively correlated with CAT and SOD activities (r = 0.46, P < 0.05 and r = 0.49, P < 0.05) respectively.

ALA-D activity

The blood δ-ALA-D activity was significantly decreased in HD patients compared to healthy subjects (Table 2). The involvement of SH groups in ALA-D inhibition was examined by testing the effect of DTT on the enzyme. The addition of DTT (2 mM) into the assay mixture caused an increase of 74.74% and 21.05% in the ALA-D activity in patients and healthy subjects, respectively, corresponding the ALA-D reactivation index (%). Moreover, the ALA-D activity correlated with the CAT activity (P = 0.003; r = −0.46), while the SOD activity was not correlated with ALA-D activity (P > 0.05).

Enzyme activity assay

Table 2 shows that the SOD activity was significantly higher in HD patients compared to healthy subjects (P < 0.05). The catalase activity also was significantly higher in HD patients compared to healthy subjects (P < 0.05).

Biochemistry assay

Table 1 shows the results of serum creatinine and urea levels. Both uremic markers were significantly increased (seven times higher) in the HD patients compared with the control group. Moreover, positive correlations were found between creatinine with MDA and retinol levels (r = 0.63 and r = 0.65; P < 0.05), respectively, and urea with MDA and retinol levels (r = 0.61 and r = 0.68; P < 0.05), respectively.

Discussion

Several reports have documented that the plasma vitamin A concentration is often elevated in patients with CRF, either untreated or treated with haemodialysis or peritoneal dialysis [28]. In accordance with Zima et al., in a group with 14 HD patients, all possessed increased retinol levels which were three fold higher in comparison to controls [29]. In another study with 40 HD patients, almost all possessed toxicant levels of plasma retinol and it was also verified that there were not any significant differences among the analysis predialysis and postdialysis, while alpha-tocopherol was significantly decreased in the postdialytic state [30]. In our study, an increase of retinol levels was observed, three times higher in the HD patients compared to healthy subjects (P < 0.001) (Figure 1).

It is known that vitamin A is transported from its hepatic stores to peripheral target sites in the form of retinol bound to its specific carrier protein, retinol-binding protein (RBP) and transthyretin (TTR). The retinol-binding protein 4 (RBP4) is a 21 kDa plasma protein that is mainly synthesized in the liver and adipose tissue and is known to transport retinol (ROH) in the blood. The binding of ROH to RBP4 guarantees the homeostatic regulation of plasma ROH levels, which is an essential aspect for a variety of physiological processes. In healthy individuals RBP4 is mainly synthesized in the liver and secreted into the circulation in a 1:1:1 complex with ROH and transthyretin (TTR). The binding with TTR increases the molecular weight of RBP4 and thus prevents its glomerular filtration and catabolism in the kidney. After releasing ROH into the target cells the remaining apo-RBP4 (unbound ROH) is rapidly filtered through the glomeruli and subsequently reabsorbed in the proximal tubular cells via the megalin–cubulin receptor complex and then catabolized [31]. Thomas et al. 1991 observed an increase in circulatory retinol in rats with experimental acute renal failure and have established that this increase is almost entirely due to retinol in the retinol–RBP–TTR complex. This retinol is derived from the hepatic pool of retinol newly acquired from the diet, suggesting that the kidney modulates its release. In this manner, normal kidney function influences the release of hepatic retinol into circulation and contributes to circulatory vitamin A homeostasis [32]. In this line, clearance and catabolism of retinol-binding protein depend on normal renal function [33]. In fact, a correlation between plasma vitamin A and serum creatinine concentration has been observed [28], suggesting an association with increased severity of renal failure [33]. In our study, HD patients had high levels of urea and creatinine (Table 1) that correlated with the levels of retinol (r = 0.68 and r = 0.65, respectively) and MDA (r = 0.61 and r = 0.63, respectively). In this way, Frey and collaborators demonstrated that the kidney, the main site of RBP4 catabolism, contributes to an elevation of RBP4 levels during chronic kidney disease (CKD) and regarding the kidney function, there was a strong correlation between serum creatinine and RBP4 levels [31]. Another factor, but less relevant, that may contribute to an elevation in vitamin A concentration is a decrease in the enzymatic transformation of retinol into retinoic acid [24,25].

Retinoids have redox-related properties and they influence the oxidative status of the cell. Many authors suggested that retinol and related molecules, such as beta-carotene, act in biological systems as antioxidants. Thus, they could be potential clinical agents in antioxidant therapies for treatment and prevention of malignant and neurodegenerative diseases. However, during clinical trials it was observed that retinoids can also be deleterious and are associated with the activation of proto-oncogenes, leading to an incidence increase of neoplasias. There are also several reports in the literature which clearly show that the retinol works as
pro-oxidant, increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and also maximizes the oxidative damage in lipids, proteins, DNA and modulation of iron turnover [34]. Due to this conflict of information, more studies regarding the action of retinol are still needed since there are several therapies based on the use of retinol for several diseases. These diseases are related to cell cycle disruption/cell death and increased reactive oxygen species, including skin cancer, lung cancer, Parkinson’s disease and Alzheimer’s [34–36].

Retinol has been related to the increase of superoxide anion and oxygen peroxide and consequently lipid peroxidation [6]. Furthermore, in agreement with Oliveira et al. [37], the sharp and chronic supplementation with vitamin A caused an augment of 1.8 and 2.7 in the striatum lipid peroxidation. In other studies, an increase in chromatin sensitivity to DNase I [34] and significative changes in nuclear protein phosphorylation [35] in Sertoli cells treated with retinol were demonstrated. Many of these effects were inhibited by the addition of 1.0-phenanthroline (iron chelator), suggesting the participation of a Fenton reaction in these retinol-induced effects [36].

In HD patients, the levels of retinol and MDA were increased when compared to controls observed in Figure 1. These findings are in agreement with the previous studies that revealed increased oxidative stress and retinol levels in HD patients [38]. Moreover, the present work demonstrated a positive correlation between the levels of retinol and the lipid peroxidation (Figure 3). In this line, the increase of retinol levels may act, in association with other factors, as pro-oxidant. It was shown in other studies that high levels of retinol involve a drastic increase in the production of \( \text{O}_2^- \), ROS, which might produce the radical hydroperoxyl, as occurs at the proximity of biomembranes [36].

In Table 2, it is possible to observe that the ALA-D activity in controls (healthy subjects) was similar to previous studies [40] and it was significantly increased compared to HD patients (\( P < 0.001 \)). Additionally, it has been known that the ALA-D enzyme is involved in the haem synthesis, and more recently, its inhibition has been related to oxidative stress in humans [39]. Also, HD patients are in continuous oxidative stress and normally are anaemic. In our work we found a negative significant correlation between vitamin A and haematocrit (\( r = -0.61 \)). These results are in agreement with Ono and collaborators [24] who demonstrated that the plasma vitamin A levels were inversely correlated with haematocrit (\( r = -0.5 \)). According to Ono and collaborators, the increase in vitamin A could be a factor contributing to anaemia in patients on regular dialysis.

In agreement with Valentini et al. [39], in this work a significant inverse linear relation between ALA-D activity and MDA levels was also found. The influence of retinol levels was evaluated that also revealed a significant and inverse linear relation with ALA-D activity (\( P < 0.001 \)). In this manner, the results from this work demonstrated that plasma retinol levels might influence MDA formation and ALA-D activity inhibition. This way, the partial correlation test was carried out to verify whether plasma retinol and MDA levels were indeed independent variables influencing the ALA-D activity, estimating between each one of these variables and the ALA-D activity, while controlling for the other variable. When controlling for retinol levels, a partial correlation analysis revealed no significant relation between MDA levels and ALA-D activity. However, the partial correlation analysis revealed a negative relation between retinol levels and ALA-D activity (\( r = -0.43, P < 0.05 \)), controlled by MDA levels. These results suggest that the plasma retinol levels are an independent variable affecting ALA-D activity, since MDA levels had no relation with ALA-D activity when controlling for retinol. In accordance with preview works published from our other factors also inhibited ALA-D activity, such as high aluminum levels that presented a negative correlation with ALA-D (\( r = -0.31, P < 0.05 \)) [39] and time of haemodialysis treatment that showed a positive correlation with the reactivation (\( r = +0.30; P < 0.05 \)), which is also a marker of the ALA-D inhibition [23]. The present work demonstrated that plasma retinol levels might be a new factor, because the correlation was strong when compared to other factors. The test with DTT, a reducing agent that has been used in vitro in order to prevent and/or reverse the oxidation of thiolic groups [41], verified that the ALA-D reactivation index was increased in HD patients compared to controls. The hypothesis is that the overproduction of oxidant species due to CRF and/or haemodialysis is responsible for the increase of oxidative stress in HD patients [42] and may be contributing to ALA-D–SH groups oxidation. The reduced ALA-D activity in HD patients was found to be related to the oxidation of the –SH group which is essential for enzyme activity as described in another work [39]. Besides, other mechanisms seem to be involved in enzyme inhibition, since DTT could not completely restore the ALA-D activity.

In this study, activities of erythrocyte enzymes that scavenge superoxide radicals (SOD) and hydrogen peroxide (CAT) were measured in HD patients and controls. The results demonstrated a significant increase of the blood SOD and CAT activity. Some authors also demonstrated that the activity of erythrocyte SOD and CAT and plasma SOD and CAT increased significantly [43–48]. However, another work showed a reduction of antioxidant enzymes in plasma and RBC of CRF patients [49–55].

These antioxidant enzymes are mainly involved in intracellular antioxidant defence. Several publications describing enzyme participation in free radical metabolism have yielded wrong and mixed results. The authors explained this by adaptive mechanisms to oxidative stress. This mechanism can also be explained by another finding, in accordance with Murate and collaborators, who demonstrated the increase of \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) by retinol increase [6]. Our results tend to confirm these observations. The activities of these enzymes (CAT and SOD) were significantly correlated with the retinol levels (\( r = 0.39 \) and \( r = 0.40, P < 0.01, \) respectively). In agreement with Dal-Pizzol [36], the treatment with retinol only induces the increase of the CAT activity in high doses; this may suggest that retinol levels could be a factor inducing oxidative stress in HD patients. In this line, we also verified that CAT activity was negatively associated with ALA-D activity, suggesting that the production of \( \text{H}_2\text{O}_2 \) due to elevated retinol levels could contribute to inhibit the thiol groups from ALA-D enzyme.
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In summary, high lipid peroxidation in the plasma of HD patients could be occurring due to the process of haemodialysis, which has already been mentioned in other articles. Furthermore, the results of this work demonstrated the correlation between high plasma retinol levels and lipid peroxidation and also the induction of antioxidant enzyme activity and inhibition of thiol group-dependent enzyme, ALA-D. In this line, the increase of plasma retinol levels in HD patients tends to act as additional effect, as a pro-oxidant agent. However, more works will be necessary to evaluate the influence of retinol levels with other factors on oxidative stress biomarkers and possible damages, as lipid peroxidation, protein oxidation and DNA injury in HD patients.

Conflict of interest statement. None declared.

References

A diffusion-adjusted regional blood flow model to predict solute kinetics during haemodialysis

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Abstract

Background. Sequestration of creatinine, in both erythrocytes and other cells, has complicated the widespread application of creatinine kinetics in haemodialysis. The goal of this study was to determine whether creatinine kinetics could be described using a regional blood flow (RBF) model that also incorporated diffusion between intra- and extracellular fluids.

Methods. Transport between intra- and extracellular spaces was modelled by diffusion using a specific rate constant \(k_s\) for creatinine equilibration in whole blood (0.022 min\(^{-1}\)) determined in a separate study. This \(k_s\) was applied to all body spaces and to creatinine removal from blood coursing through the dialyzer. Erythrocyte and plasma creatinine and urea concentrations during haemodialysis measured and reported by others were used to test the model.

Results. The model accurately predicted the reported time course of creatinine in plasma and erythrocytes as well as the time course of urea in plasma when using the much higher \(k_s\) for urea (158 min\(^{-1}\)). However, it did not explain an increased erythrocyte to plasma urea gradient found at the end of haemodialysis.

Conclusion. The results suggest that a diffusion-adjusted regional blood flow (DA-RBF) model can be used to

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