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Received for publication: 26.5.08; Accepted in revised form: 31.12.08

Nephrol Dial Transplant (2009) 24: 2218–2224

doi: 10.1093/ndt/gfp023

Advance Access publication 11 February 2009

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

explain compartmentalization of creatinine or urea throughout the body during haemodialysis, although possible additional compartmentalization of urea in erythrocytes, and perhaps in the tissues, still needs to be accounted for. This new model should be applicable to modelling of other non-protein-bound candidate uraemic toxins, also.

Keywords: blood flow; creatinine kinetics; intercompartment clearance; membrane permeability; urea kinetics

Introduction

The regional blood flow (RBF) model is a two-compartment model that has been proposed to explain urea disequilibrium during dialysis [1–4]. This model assumes that disequilibrium of urea during dialysis is due to delay in bringing the urea from peripheral organs to the dialyzer when the urea is located in organs with relatively low blood flow, namely muscle, skin and bone. In contrast, rapid removal of urea occurs from organs with high relative rates of blood flow such as liver, heart, brain and kidneys [1]. Whereas both the RBF model and the standard serial two-compartment model (in which hindrance to urea transport is modelled as resistance to urea transport from intracellular to extracellular fluids) explain urea disequilibrium equally well, the RBF model has the advantage of explaining why urea disequilibrium is reduced under conditions of increased cardiac output or peripheral vasodilatation [5–8], and also why urea disequilibrium may be increased under conditions when blood flow to organs such as muscle is reduced [9]. Despite its advantages in describing urea transport, the original RBF model essentially fails when applied to solutes such as creatinine, in which the rate of transport from intracellular to extracellular space is much lower than that for urea [10]. For this reason, to date, creatinine kinetics have been better described by the serial two-compartment model. However, there is no reason why the realities of moving solute to the dialyzer from various organs by blood circulation, as modelled by the RBF model, should not apply to all solutes. What is needed, however, is to develop a model to account for the limited diffusibility between the intra- and extracellular spaces for creatinine and other candidate uraemic toxins. The goal of our analysis was to modify the RBF model to increase its generalizability to such creatinine-like solutes.

Material and methods

Description of the model

The new model builds on the RBF model that has been previously described [1,2,11]. Briefly, as shown in Figure 1, all organs in the body are divided into two groups based on specific perfusion, here defined as the rate of blood flow per tissue water volume. The low-blood flow group of organs includes muscle, skin and bone. This compartment has a total volume V_L . In the new model, V_L is divided into an extracellular space, V_{Le} , and an intracellular space, V_{Li} . Creatinine generation, since it takes place in muscle, is assigned to occur in V_{Li} . The high-flow group of organs, V_H , includes liver, brain, heart and all of the blood volume. In the new model this volume is also divided into extracellular, V_{He} , and intracellular, V_{Hi} , spaces. Urea generation is assigned to the V_{Hi} space as it takes place in the liver. Blood flow (Q_H and Q_L) through the compartments

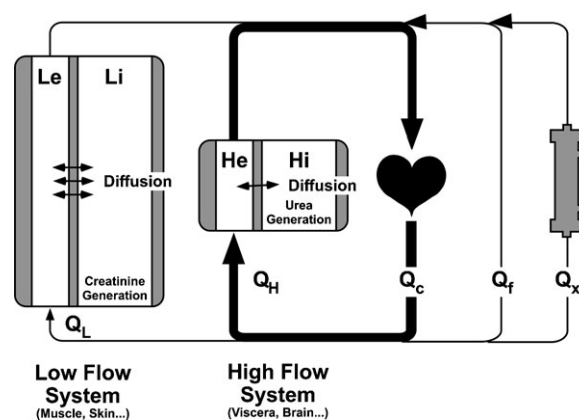


Fig. 1. Basics of the DA-RBF model. Q_H and Q_L are the blood flows to the high- and low-flow system. Q_C , Q_F and Q_X refer to cardiac output, fistula flow and extracorporeal blood flow, respectively. L_e and L_i are the extracellular and intracellular low-flow compartments, and H_e and H_i are the extracellular and intracellular high-flow compartments, respectively. See the text for additional explanations.

Table 1. Initial conditions, times and model parameters

C_{ct0}	0.11	g/L	Creatinine concentration at $t = 0$ [14]
C_{ut0}	1.47	g/L	Urea concentration at $t = 0$ [14]
t_0	0	min	Treatment start
t_d	250	min	Treatment duration [14]
t_{di}	48×60	min	Duration of interval between treatments
V	28.6	L	Urea distribution volume after ultrafiltration
G_{24c}	0.00983	mol/24 h	Creatinine generation rate [13]
G_{24u}	0.31	mol/24 h	Urea generation rate [13]
Q_c	5.80	L/min	Cardiac output [5]
Q_f	1.00	L/min	Fistula flow [31]
Q_x	0.327	L/min	Extracorporeal blood flow [14]
V_x	0.25	L	Extracorporeal blood volume
V_b	5.90	L	Blood volume [32]
H	0.37		Haematocrit [14]
f_p	0.96		Fraction of packed cell volume
f_{pw}	0.93		Plasma water fraction [15]
f_{ew}	0.72		Erythrocyte water fraction [15,17]
f_{QH}	0.85		Fraction of high flow blood flow [2]
f_{VH}	0.20		Fraction of high flow volume [2]
f_{ecv}	0.33		Fraction of extracellular volume
V_{uf}	3.46	L	Ultrafiltration volume [14]
E_x	0.80		Dialyzer extraction [14]
k_{sc}	0.022	min^{-1}	Specific rate constant for creatinine [18]
k_{su}	158.000	min^{-1}	Specific rate constant for urea

moves solute between V_{Le} , V_{He} and the dialyzer. The effects caused by cardiopulmonary recirculation [12] are also accounted for. In each of the RBF compartments, solute moves between intra- and extracellular spaces by diffusion, the rate of which depends on a solute-specific rate constant, k_s . The equations for the model are detailed in the supplemental files.

Test data used

To test the model, data reported by Eloit *et al.* in two separate papers [13,14] were used to obtain dialysis parameters (Table 1) as well as creatinine and urea concentrations measured in erythrocytes and in plasma at six different times during haemodialysis. The solute concentrations at each time point represented the average of the values reported for six patients, with each value normalized to the initial concentration. It was

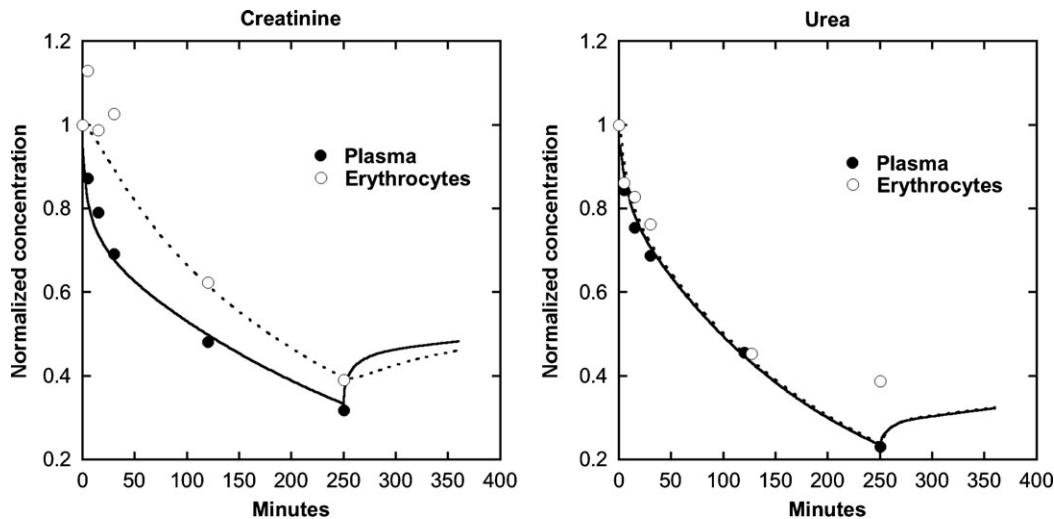


Fig. 2. DA-RBF model. Predicted versus reported creatinine and urea data. Time course of creatinine (left panel) and urea concentrations (right panel) predicted by the diffusion-adjusted RBF model, using dialysis parameters and solute concentrations reported by Eloot *et al.* [14]. The predicted concentrations are shown as solid (plasma) and dotted (erythrocytes) lines, whereas the measured values are shown as solid (plasma) and open (erythrocytes) circles. All concentrations are normalized to initial plasma concentrations measured at $t = 0$.

assumed that plasma concentrations reported in [14] corresponded to arterial concentrations and that erythrocyte concentrations corresponded to intracellular concentrations in the high-flow system.

We used well-known standard values for the fractional water content in plasma (f_{pw}) at 0.93 [15] as well as the fractional water content in erythrocytes (f_{ew}) at 0.72 [15–17]. Within each flow compartment, we maintained the well-accepted ratio of 1/3 extracellular space to 2/3 intracellular space, and so f_{ecv} was set at 0.33.

The value for the intercompartment diffusive transfer coefficient for creatinine, k_{sc} , was set at 0.022 min^{-1} based on measured creatinine equilibration between erythrocytes and plasma [18]. The value for the intercompartment diffusive transfer coefficient for urea, k_{su} , was also derived from data measured in erythrocytes and computed from the product of urea permeability and specific erythrocyte surface area as described elsewhere [19]. Data for urea permeability range from $3.3 \times 10^{-5} \text{ cm/s}$ in murine [20] to $20.6 \times 10^{-5} \text{ cm/s}$ in human [21] erythrocytes. With a specific surface area of human erythrocytes between $1.87 \times 10^4 \text{ cm}^{-1}$ [19] and $2.26 \times 10^4 \text{ cm}^{-1}$ [21], the specific rate constant for urea was in the range between 0.62 and 4.66 s^{-1} ($37\text{--}279 \text{ min}^{-1}$). For the model, the intercompartment diffusive transfer coefficient for urea, k_{su} , was set at 158 min^{-1} that was the average of the values measured in erythrocytes. Thus, with the exception of specific rate constants, initial concentrations, and solute generation rates the same numerical values for the different model parameters were used in the same model to describe the kinetics of either creatinine or urea.

The total distribution volume (V) was assumed as identical for both solutes and modelled as 28.6 L from the classic two-compartment model and plasma urea concentrations reported in [14] using the Berkeley-Madonna X software (version 8.3.19, Kagi, Berkeley, CA, USA). The fractional size of the central compartment f_{V1} was derived from data given in the paper by Eloot *et al.* [13] and set at 0.14 for creatinine and 0.15 for urea.

Once the initial parameters were accepted and the compartment volume was identified, the diffusion-adjusted RBF (DA-RBF) model was solved by numerical integration using the Berkeley-Madonna X software to predict relative erythrocyte and plasma concentrations throughout the modelled dialysis session, as well as 2 h after dialysis both for urea and for creatinine. These values were then compared to normalized plasma and erythrocyte concentrations as derived from actually measured concentrations reported by Eloot *et al.* [14].

Finally, to examine the relative importance of parameters on model output we calculated time-averaged sensitivities for the DA-RBF model with regard to the magnitude of the solute disequilibrium during dialysis (see supplemental files). This was done for creatinine where $k_{sc} = 0.022 \text{ min}^{-1}$ and repeated for urea where $k_{su} = 158 \text{ min}^{-1}$. Solute disequilibrium was defined as the ratio of plasma water concentration over equilibrated concentration.

Results

RBF model predictions versus test data

The time course of creatinine concentrations predicted by the DA-RBF model was quite similar to that actually reported by Eloot *et al.* [14], as shown in the left panel of Figure 2. During dialysis a large concentration gradient developed between the erythrocytes and the plasma. The intradialytic profile of plasma creatinine deviated substantially from first-order kinetics and there was a large creatinine rebound after the end of haemodialysis that lasted for $\sim 1 \text{ h}$.

The right panel of Figure 2 shows modelled versus measured erythrocyte and plasma concentrations for urea. Here the same parameters were used as for creatinine, except for the assumption of a very high specific rate constant for urea diffusion, $k_{su} = 158 \text{ min}^{-1}$, across all cell membranes. It is clear that for plasma, the predicted and measured values closely agree. For erythrocytes, Eloot *et al.* [14] found a progressively increasing gradient in urea concentration as dialysis progressed in both inlet and, especially, outlet samples. Such a large gradient cannot be explained by the model, given the high rate constant for urea diffusion in erythrocytes. One possible explanation for this discrepancy is presented in the Discussion section (also see the note added in proof). Despite the very high diffusion coefficient used in this model, a substantial post-dialysis urea rebound was still observed, and it was basically all due to a compartment effect related to the heterogeneity in regional perfusion, in accordance with what has been previously described [3].

Standard diffusion model versus test data

The standard two-compartment diffusion model can be made to fit both plasma and erythrocyte solute concentrations for urea as well as for creatinine, but the degree of the fit depends on how the intercompartment rate constant

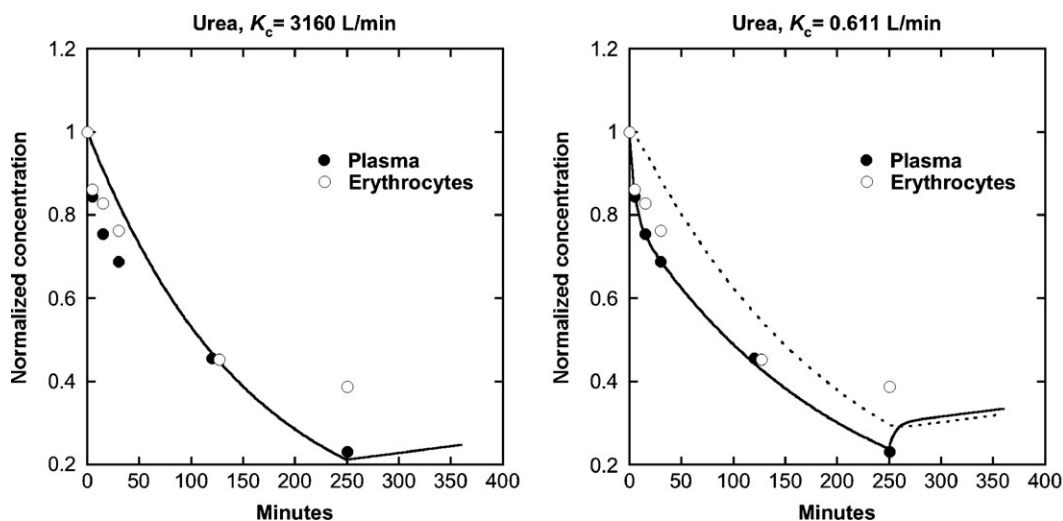


Fig. 3. Standard model. Predicted versus reported urea data using two different values of K_c . Time course of plasma (solid lines) and erythrocyte (dotted lines) concentrations predicted by the standard model compared to actual data (solid circles for plasma and hollow circles for erythrocytes) as per Eloit *et al.* [14]. Actual concentrations were averaged and normalized to initial plasma concentrations as described in the Material and methods section. In the left panel a K_c value of 3160 L/min (K_c derived from erythrocyte-plasma k_s) was used. At such a high value of K_c , the kinetics are very similar to that for a single-pool model. In the right panel, a K_c value of 0.611 L/min was used to fit the modelled concentration to the experimental data.

K_c is related to the diffusion rate constant (k_s) in erythrocytes. Based on a relationship reanalysed in a recent paper [18] and outlined in the supplemental files, one of us derived the intercompartment clearance K_c between intra- and extracellular spaces from the following relationship:

$$K_c = k_s(1 - f_{ecv}). \quad (1)$$

If one assumes that V is ~ 30 L, and the fraction of extracellular volume f_{ecv} is $1/3$, this simplifies to

$$K_c \approx 20k_s. \quad (2)$$

If one uses the erythrocyte diffusion constant value for urea ($k_{su} \sim 158 \text{ min}^{-1}$), then an enormously high value of K_c is computed, ~ 3160 L/min. When applying this to the standard model (Figure 3, left panel) one does get identical values for plasma and erythrocyte urea levels during dialysis, but essentially no deviation of intradialytic urea concentrations from first-order kinetics and no rebound. If on the other hand one uses a K_c value of ~ 0.611 L/min, to fit the model to the experimental plasma concentrations, this will correspond to a k_s value for urea of $\sim 0.031 \text{ min}^{-1}$, or ~ 5000 -fold less than measured in erythrocytes. When the standard model is solved using such values for K_c and k_s (Figure 3, right panel), the familiar intradialytic plasma urea profile is shown (dropping below that predicted from first-order kinetics), but then substantial erythrocyte to plasma urea gradients are predicted [compare the dotted (erythrocyte) versus solid (plasma) lines in the right panel of Figure 3] that are not found experimentally [compare the hollow circles (erythrocytes) and solid circles (plasma) in Figure 3] during the early phase of dialysis. At the end of dialysis, the predicted erythrocyte to plasma gradient is in agreement with, but markedly underestimates, the measured erythrocyte-plasma gradient found by Eloit *et al.* [14].

For creatinine a similar set of results is found, as shown in Figure 4, though here the discrepancy might be classed

as 'minor', and conceivably within the error of the method used to derive K_c from k_s . Using a K_c value of 0.618 L/min reported in [13], corresponding to a k_s value of $\sim 0.031 \text{ min}^{-1}$, one can match the predicted erythrocyte concentrations during dialysis, but the plasma concentrations are not well explained (Figure 4, left panel). Lowering the K_c value to 0.390 L/min, corresponding by Eq. 2 to a k_s value of $\sim 0.020 \text{ min}^{-1}$, matches plasma as well as the erythrocyte profiles, but then the distribution volume V also has to be lowered to 25.2 L so that this volume is different from that assumed for urea (Figure 4, right panel).

Effective dialyzer clearance for urea and creatinine

Even though the same fractional plasma extraction of $E_x = 0.80$ was assumed for both solutes, dialyzer clearance was quite different for urea and creatinine. In the case of urea where k_s was high, the effective blood water fraction passing the dialyzer was high so that the dialyzer clearance was calculated as $K_d = 0.227$ L/min. However, in the case of creatinine where k_s was low, the dialyzer clearance was only 0.162 L/min, essentially because erythrocyte water barely equilibrates with plasma during the very brief period of transit through the dialyzer [22,23].

Sensitivity analysis: factors affecting solute disequilibrium

To better understand the relative importance of the different model parameters listed in Table 1, a sensitivity analysis was done for the DA-RBF model for both creatinine and urea. Briefly, sensitivity analysis examines how the variation in the magnitude of individual parameters affects the calculated output of the model. Such an analysis is helpful to identify parameters that are important for the model output.

Here, the sensitivity was computed for the magnitude of the solute disequilibrium measured as the ratio of plasma

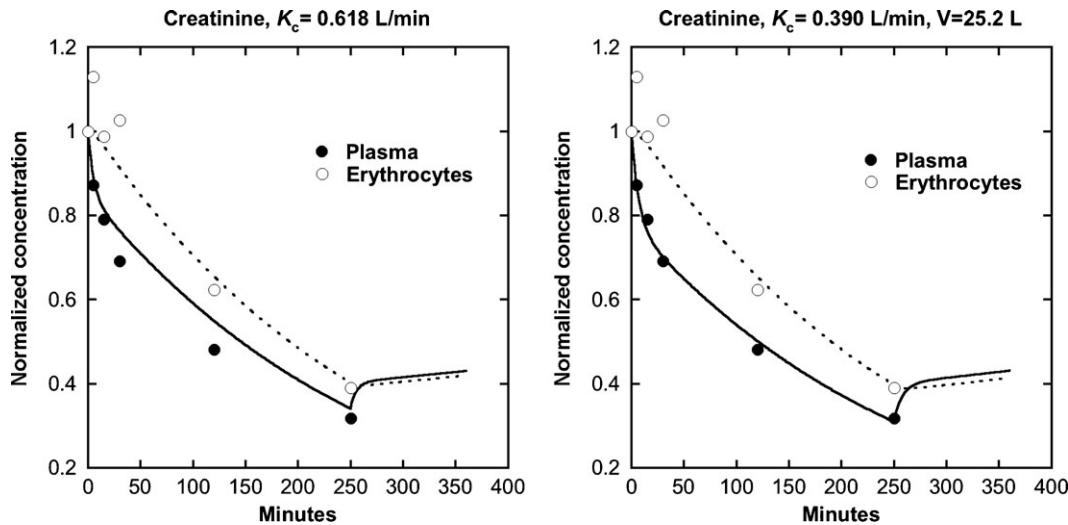


Fig. 4. Standard model. Predicted versus reported creatinine data using two different values of K_c . Time course of creatinine in plasma (solid lines) and erythrocytes (broken lines) predicted by the standard two-compartment model using the intercompartment clearance $K_c = 0.618$ L/min reported elsewhere [13] (left panel) or $K_c = 0.390$ L/min together with a distribution volume of 25.2 L identified from fitting the model to experimental plasma and erythrocyte concentrations (right panel). Measured values shown as solid (plasma) and open (erythrocytes) circles are identical to those shown in the left panel of Figure 2.

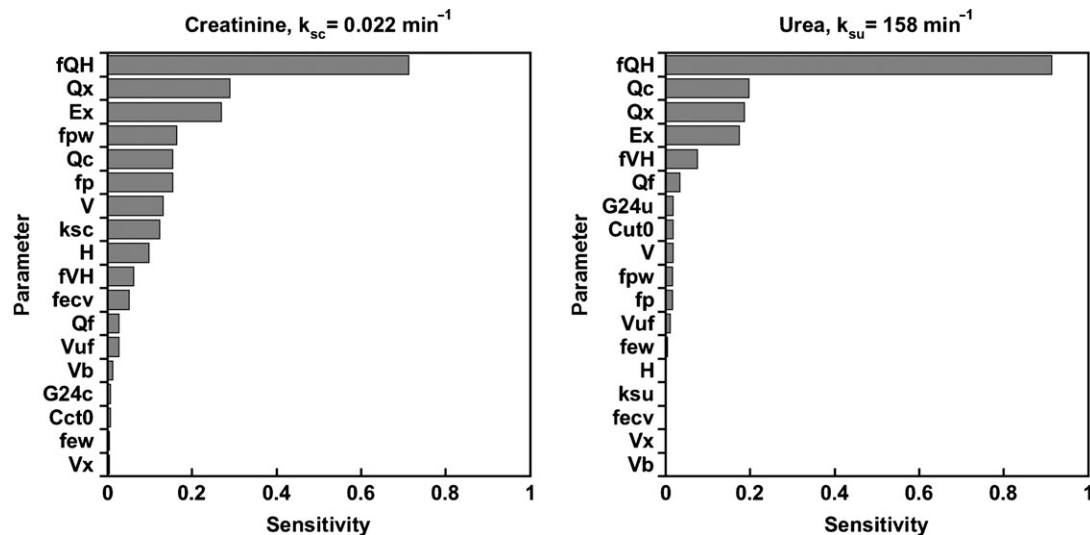


Fig. 5. Global sensitivity. Ranking of time-averaged sensitivities computed for the degree of solute disequilibrium for creatinine (left panel) and urea (right panel) during dialysis with regard to model parameters and initial conditions explained and listed in Table 1. A high sensitivity for a given parameter implies that a variation in that parameter has a large effect on solute disequilibrium.

concentration to equilibrated concentration. A ranking of time-averaged sensitivities for the two solutes calculated for the duration of dialysis is shown in Figure 5. The sensitivity analysis suggests that for both creatinine and urea, fractional perfusion of the high-flow compartment, f_{QH} , as well as the cardiac output, Q_c , are of great importance in determining the amount of solute 'disequilibrium'. The same is true for extracorporeal clearance, calculated as the product of solute extraction E_x and extracorporeal blood flow, Q_x . For other parameters, however, there is a difference between the solutes. The creatinine model is sensitive to the specific diffusion rate constant k_s and to the haematocrit H while the urea model is insensitive to these and most other model parameters.

Discussion

This study shows that the kinetics of creatinine can be described by a DA-RBF model. The model accurately predicts the concentration of creatinine in plasma as well as in erythrocytes when using a value for creatinine membrane permeability that has been reported in the literature in erythrocytes [19,22]. For urea the model predicted the intradialytic plasma profile but did not account for the large erythrocyte to plasma urea gradient measured at the end of dialysis [14].

When the DA-RBF model is applied to creatinine, an attractive feature is that if one begins from an experimentally determined erythrocyte diffusion constant (k_s) for

creatinine of 0.022 min^{-1} , both the measured intradialytic plasma and erythrocyte profiles can be explained. Also, the model is consistent with the removal of creatinine from the plasma only during passage of blood through the dialyzer.

The specific rate constant for the equilibration of urea in whole blood has been measured to range between 37 and 279 min^{-1} [20,21]. This is 10^3 – 10^4 times larger than that of creatinine [22,24,25]. The rapid equilibration of urea across the cell membrane is essentially due to facilitated transport by selective urea transporters [26–28]. The average $k_s = 158 \text{ min}^{-1}$ assumed in this study corresponds to an inter-compartment clearance $K_c = 3160 \text{ L/min}$ (Eq. 1). At this value, K_c is much larger than blood flow so that overall clearance is flow-controlled. In this situation, it is not important to know the precise value of K_c and the specific rate constant for urea, k_{su} , as long as K_c is much larger than blood flow. This is also revealed by the low sensitivity of k_{su} on model output (Figure 5, right panel).

Because of the very high permeability of urea across the erythrocyte membrane, there should be no measurable urea gradient between erythrocytes and the plasma during haemodialysis (Figure 2, right panel). The existence of such a gradient in inlet blood at the end of dialysis, as well as a progressive increase in the erythrocyte to plasma urea gradient in outlet blood with dialysis that was found by Elood *et al.* [14], cannot be explained by this model. In earlier studies it was observed that this gradient did not dissipate even after extended periods of time [29], and it was therefore assumed that some fraction of urea in excess of that freely diffusible in erythrocyte water was sequestered or tightly bound to haemoglobin. Others have associated this discrepancy with the interference of haemoglobin on enzymatic and colorimetric urea measurements [30]. On the other hand, tracer experiments using radio-labelled urea that are insensitive to haemoglobin interference showed that urea rapidly equilibrated across a fractional erythrocyte volume of 0.72 in normal and in uraemic blood samples without binding to haemoglobin [16]. This value corresponds to the fractional water content of erythrocytes used in this study and in other studies [17], and it is this value that counts for mass balance considerations.

The compartment effect in the whole body observed for urea is essentially caused by the heterogeneous perfusion of low- and high-flow systems as explained by the RBF model [1]. While the overall compartment effect for urea can also be explained by the standard two-compartment model, the standard model requires a whole body diffusion rate constant ($K_c = 0.611 \text{ L/min}$) that is ~ 5000 -fold lower than that expected if one were to derive K_c from the diffusion rate constant for urea in erythrocytes (3160 L/min). Thus, the standard two-compartment model requires these two markedly different diffusion constants for tissues and erythrocytes, respectively, to adequately describe the experimental changes in urea concentrations during dialysis.

An important difference between the DA-RBF model and the standard two-compartment model refers to the interpretation of intercompartment clearance. In the standard two-compartment model, transport within the patient, which is determined by blood flow (convection) as well as by diffusion, is lumped into one whole-body clearance. In contrast, in the DF-RBF model, solute transport by blood flow and between intra- and extracellular spaces is more appropri-

ately modelled as two separate processes. The overall transport rate is not determined by the sum of both processes but rather is limited by the process with the lower transport rate. Therefore, in the case of urea, the rate constant for overall transport is limited by blood flow. The importance of RBF heterogeneity in urea transport is supported by studies of urea kinetics in situations where the blood flow heterogeneity was altered from the norm, e.g. when muscle blood flow was increased during dialysis while patients were exercising [6,7] or alternatively, when muscle blood flow was reduced in seriously ill patients being dialyzed in an intensive care setting who were markedly vasoconstricted [9].

For solutes where transport across the cell membrane is slow compared to blood flow, even with the DA-RBF model, overall transport is limited by the rate of diffusion. The case of creatinine lies between those extremes (perfusion predominance versus diffusion predominance), as its intercompartment clearance K_c (Eq. 2) of 0.440 L/min is close to the magnitude of RBFs of 0.870 and 4.93 L/min , respectively. This also explains the importance of RBF parameters such as f_{QH} and Q_c in the sensitivity analysis of creatinine kinetics (Figure 5, left panel).

The DA-RBF model output is highly non-linear especially with respect to the specific rate constant k_s describing the transport of solute across the cell membrane. This is seen in the change of sensitivities computed for individual parameters when k_s changes from 158 (the value used for urea) to 0.022 min^{-1} (the value used for creatinine) (Figure 5). At low k_s which is used to describe the kinetics of creatinine, global sensitivity is high for plasma water fraction, haematocrit and membrane permeability k_s . A high sensitivity for a given parameter implies that small variations in that parameter have large effects on model output. On the other hand, parameters such as the fractional volumes, f_{VH} and f_{ecv} , have only the minimal effect on the degree of solute disequilibrium.

Despite the apparent success of scaling erythrocyte-derived diffusion constants to whole body values for both urea and creatinine using the DA-RBF approach, the results should not be taken to imply that true resistance to diffusion is equivalent in tissues and red blood cells. In fact, anatomical considerations make this possibility unlikely. It is quite possible that some of the components of compartment disequilibrium that the RBF model is attributing to flow/volume differences may be due to a diffusive component. The generalizability of the DA-RBF approach to other solutes will require more data and most likely modification in the model structure for the important class of protein-bound solutes.

In conclusion, the DA-RBF model can be used to explain the compartment effects of creatinine and urea during haemodialysis and predict both erythrocyte and plasma levels of creatinine with a minimum of additional assumptions. Possible erythrocyte sequestration of urea during dialysis may also need to be taken into account, but this might be limited to urea and not be a property of small diffusible solutes in general. The DA-RBF model might be useful to predict the kinetics of other, non-protein-bound solutes of interest once their diffusion constants in erythrocytes are known. Ultimately, this model could be of help in measurement and prescription of a more comprehensive dose of dialysis.

Note added in proof

During the submission of this manuscript the technique to measure urea in erythrocytes was revisited and it was concluded that urea to plasma gradients developing during dialysis and reported in (14) were most likely caused by haemoglobin interference of the urea assay (S Eloot, B Marescau, and R Vanholder, personal communication). This explains the discrepancy between urea concentrations measured in (14) and those modelled in this report and also rejects the long held belief of urea sequestration in erythrocytes.

Acknowledgements. The source code of a stand-alone Javascript calculator (open source license) to determine model outputs is provided in the supplemental files. The help of Paul Silisteanu in developing this Javascript application is gratefully acknowledged.

Conflict of interest statement. There are no relationships with pharmaceutical firms or other entities that could be perceived to represent a financial conflict of interest with regard to the topic discussed in this manuscript. There was no financial support for this study except for support provided by the authors' affiliation. The results presented in this paper have not been published previously in whole or part, except in abstract format.

Supplementary data

Supplementary data is available online at <http://ndt.oxfordjournals.org>.

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Received for publication: 9.10.08; Accepted in revised form: 12.1.09

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