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Resistance to intercompartmental mass transfer limits β_2-microglobulin removal by post-dilution hemodiafiltration

RA Ward[1], T Greene[2], B Hartmann[3,4] and W Samtleben[3,4]

[1]Department of Medicine, University of Louisville, Louisville, Kentucky, USA; [2]Department of Biostatistics and Epidemiology, Cleveland Clinic Foundation, Cleveland, Ohio, USA; [3]Nephrology, Department of Medicine I, Klinikum Grosshadern, University of Munich, Munich, Germany and [4]KfH Kidney Center, Neuried, Germany

Although clearance of β_2-microglobulin is greater with hemodiafiltration than with high-flux hemodialysis, β_2-microglobulin concentrations after long-term hemodiafiltration are only slightly less than those obtained with high-flux hemodialysis. Resistance to β_2-microglobulin transfer between body compartments could explain this observation. β_2-Microglobulin kinetics were determined in patients receiving on-line post-dilution hemodiafiltration for 4 h with 18 l of filtration. Plasma β_2-microglobulin concentrations were measured during and for 2 h following hemodiafiltration and immediately before the next treatment. The filter clearance of β_2-microglobulin was determined from arterial and venous concentrations. The β_2-microglobulin generation rate was calculated from the change in the plasma concentration between treatments. The intercompartmental clearance was obtained by fitting the observed concentrations to a two-compartment, variable volume model. The plasma clearance of β_2-microglobulin by the filter was 73 $\pm$ 2 ml/min. Plasma β_2-microglobulin concentrations decreased by 68 $\pm$ 2% from pre- to post-treatment (27.1 $\pm$ 2.2–8.5 $\pm$ 0.7 mg/l), but rebounded by 32 $\pm$ 3% over the next 90 min. The generation rate of β_2-microglobulin was 0.136 $\pm$ 0.008 mg/min. The model fit yielded an intercompartmental clearance of 82 $\pm$ 7 ml/min and a volume of distribution of 10.2 $\pm$ 0.6 l, corresponding to 14.3 $\pm$ 0.7% of body weight. Hemodiafiltration provides a β_2-microglobulin clearance of similar magnitude to the intercompartmental clearance within the body. As a result, intercompartmental mass transfer limits β_2-microglobulin removal by hemodiafiltration. This finding suggests that alternative strategies, such as increased treatment times or frequency of treatment, are needed to further reduce plasma β_2-microglobulin concentrations.

Kidney International (2006) **69,** 1431–1437. doi:10.1038/sj.ki.5000048; published online 4 January 2006

KEYWORDS: hemodiafiltration; β_2-microglobulin; kinetics

Correspondence: *RA Ward, Kidney Disease Program, University of Louisville, 615 S Preston Street, Louisville, Kentucky 40202-1718, USA. E-mail: richard.ward@kdp.louisville.edu*

Received 26 January 2005; revised 5 July 2005; accepted 11 August 2005; published online 4 January 2006

β_2-Microglobulin is an 11.8 kDa protein that comprises the light chain of the major histocompatibility complex present on the surface of all cells. After being shed from the cell surface, β_2-microglobulin is eliminated from the circulation by glomerular filtration, followed by reabsorption and catabolism in the proximal tubule. As renal function decreases, β_2-microglobulin is retained in the plasma and deposits as amyloid fibrils in many tissues. These amyloid deposits may cause carpal tunnel syndrome, arthropathy and bone cysts, and are a cause of morbidity in long-term hemodialysis patients.[1]

In general, the clearance of β_2-microglobulin by hemodialysis with low-flux membranes is negligible.[2,3] Clearance of β_2-microglobulin by high-flux membranes is reported to be in the range of 17–22 ml/min;[2,3] however, the rapid decrease in diffusive permeability with increasing molecular size limits β_2-microglobulin clearance by diffusion no matter which membrane is used. To address the limited β_2-microglobulin removal that can be obtained by diffusion, several investigators have turned to therapies based on convection, such as hemodiafiltration[4–8] or adsorption.[9,10] Hemodiafiltration provides dialyzer clearances of β_2-microglobulin that are approximately twice those obtained with high-flux hemodialysis.[7,11] In spite of this difference, predialysis β_2-microglobulin concentrations remain at about 20 mg/l following long-term on-line hemodiafiltration,[4–8] a level that is not markedly lower than that achievable with high-flux hemodialysis when comparable blood flow rates and membrane surface areas are used.[7]

It has long been recognized that disequilibrium develops between body compartments when dialytic removal exceeds the rate of transfer of solute from the extravascular compartment into the plasma.[12] This disequilibrium leads to a rebound in plasma concentrations, post-dialysis, as the solute re-equilibrates between compartments once dialytic removal ceases. Previous studies have shown a rebound in plasma β_2-microglobulin concentrations following modestly efficient high-flux hemodialysis.[13–15] Therefore, we hypothesized that the inability of long-term hemodiafiltration to markedly reduce plasma β_2-microglobulin concentrations is a consequence of a significant mass transfer resistance between

the vascular and extravascular compartments. To test this hypothesis, we determined the intercompartmental mass transfer coefficient for β_2-microglobulin using plasma concentrations of β_2-microglobulin obtained during and immediately following hemodiafiltration and a two-compartment kinetics model. The model was then used to assess the impact of changes in extracorporeal clearance and treatment frequency on β_2-microglobulin removal.

RESULTS

Ten patients (eight men and two women) were enrolled in the study. The patients ranged in age from 28 to 70 years (mean 56 years) and the etiology of their renal failure included glomerulonephritis (two patients), diabetes (two patients), hypertension (two patients), polycystic kidney disease (one patient) and was unknown for three patients. At the time of the study, nine of the patients were being treated with high-flux hemodialysis and one with hemodiafiltration. The duration of their dialysis therapy ranged from 24 to 74 months (mean 49 months).

Details of the study treatments are given in Table 1. The treatment dose, as determined by the equilibrated Kt/V for urea,[16] was 1.21 ± 0.07. The plasma clearance of β_2-microglobulin determined after 60 min of hemodiafiltration was 73 ± 2 ml/min (equivalent to a whole blood clearance of 109 ± 4 ml/min). β_2-Microglobulin concentrations decreased by $68 \pm 2\%$ during hemodiafiltration from 27.1 ± 2.2 to 8.5 ± 0.7 mg/l, then rebounded by $32 \pm 3\%$, corrected for generation, over the next 90 min by which time the rebound was complete (Figure 1). The post-hemodiafiltration concentration corrected for hemoconcentration by the method of Bergström and Wehle[17] was 7.4 ± 0.6 mg/l. The intercompartmental clearance and volume of distribution were estimated by fitting the model to the measured β_2-microglobulin concentrations for each patient. Overall, the estimated intercompartmental clearance was 82 ± 7 ml/min and the estimated volume of distribution was 10.2 ± 0.6 l, corresponding to $14.3 \pm 0.7\%$ of body weight. Estimated values of the two parameters for the individual patients are given in Table 2. Figure 2 presents the measured and modeled β_2-microglobulin concentrations for a typical patient. The overall generation rate of β_2-microglobulin, calculated using the estimated volume of distribution, was 0.136 ± 0.008 mg/min.

Table 1 | Details of study treatments

Treatment time (T_D, min)	240
Blood flow rate (Q_B, ml/min)	280
Hematocrit (t=60) (Hct)	0.35 ± 0.01
Plasma flow rate (t=60) (Q_P, ml/min)[a]	182 ± 3
Weight, pretreatment (kg)	73.4 ± 3.2
Weight, post-treatment (kg)	71.2 ± 3.3
Net ultrafiltration rate (Q_{UF}, ml/min)	9.1 ± 1.4
Total ultrafiltered volume (l)	18
Interdialytic interval (T_{ID}, min)	2327 ± 165
Weight, post-interdialytic interval (kg)	73.7 ± 3.1
Inter-dialytic fluid gain (α, ml/min)	1.14 ± 0.16

Data are presented as mean $\pm$ s.e.m. for n=10.
[a]Plasma flow rate (Q_P) was determined from the blood flow rate (Q_B) and the fractional hematocrit (Hct) at the inlet to the hemodiafilter as $Q_P = Q_B$ (1-Hct).

DISCUSSION

Developing treatment strategies to remove β_2-microglobulin, and thereby prevent or slow amyloid formation in patients with end-stage renal disease, requires an understanding of

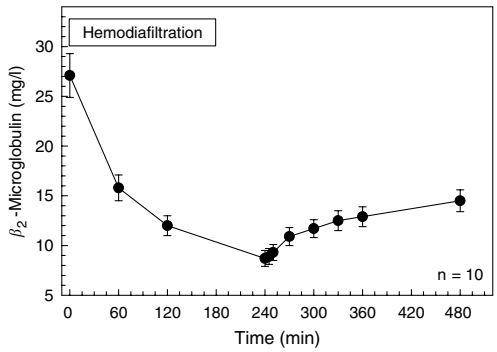

Figure 1 | Plasma β_2-microglobulin concentrations during a 4-h hemodiafiltration treatment and for 2-h following hemodiafiltration. Data are presented as mean $\pm$ s.e.m. for 10 patients.

Table 2 | Estimated kinetic model parameters for β_2-microglobulin

Patient	Intercompartmental Clearance, K_{IC} (ml/min)	Distribution volume, V_D=V_P+V_{NP} (l)	Generation rate, G (mg/min)
1	100	13.27	0.131
2	86	7.52	0.131
3	63	8.10	0.144
4	75	12.31	0.091
5	53	8.57	0.140
6	57	9.25	0.125
7	108	11.99	0.131
8	102	9.91	0.165
9	74	9.31	0.182
10	107	11.37	0.115

Data are presented as mean $\pm$ s.e.m. for n=10.

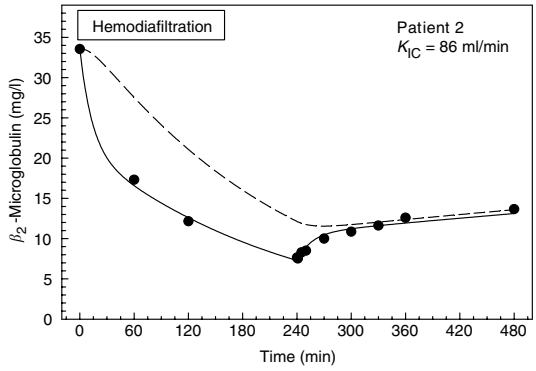

Figure 2 | Concentration profiles of β_2-microglobulin in the perfused (solid line) and non-perfused (broken line) compartments obtained by fitting the kinetic model to measured plasma concentrations of β_2-microglobulin (●) for a typical patient.

β_2-microglobulin kinetics. Various kinetic models have been developed to describe β_2-microglobulin kinetics during hemodialysis[2,18–21] and some of these models have been used to simulate β_2-microglobulin removal with different treatment strategies.[2,22] Two-compartment models have been the most widely used.[18–20,22] Single-compartment models are too simplistic based on the observation of a significant post-dialysis rebound in plasma β_2-microglobulin concentration immediately following high-flux hemodialysis (Figure 1 and Yasuhiro *et al.*[14]), while models with three or more compartments suffer from problems of parameter estimation. The utility of two-compartment models depends on the estimation of several parameters that cannot be measured directly, including the compartment volumes, the intercompartmental clearance and the generation rate of β_2-microglobulin. Previous efforts to estimate these parameters have relied on infusing ^{125}I-β_2-microglobulin and fitting the plasma disappearance curve to the model.[2,23,24] This method has several drawbacks, including the need to prepare and infuse radiolabeled protein, the need to separate free ^{125}I from ^{125}I-β_2-microglobulin in plasma samples and the assumption that kinetics following intravenous injection are the same as those occurring during and after an extracorporeal blood purification treatment. An alternative strategy involves fitting the model equations to plasma solute concentrations measured during and immediately following hemodialysis. The usefulness of this method has been limited by the relatively small reduction in plasma β_2-microglobulin concentration that is effected by dialysis even with high-flux membranes.[2,14] Hemodiafiltration causes a much more pronounced decrease in plasma β_2-microglobulin concentrations than does high-flux hemodialysis,[7] making the latter approach more amenable to estimating the model parameters.

The model for β_2-microglobulin kinetics used in this study differs from the two-pool models used by other investigators in several ways. In the present work, generation of β_2-microglobulin was assumed to occur in both the perfused and the non-perfused compartments. Previous investigators have assumed that β_2-microglobulin is generated in only one compartment.[2,18–22] However, β_2-microglobulin is expressed on the surface of most nucleated cells making it more likely that free β_2-microglobulin is generated in both compartments. Most prior investigations[2,18,22] have assumed that mass transfer of β_2-microglobulin between the two compartments occurs by diffusion, only. We, along with Gotch and Keen,[25] have assumed that mass transfer also occurs by convection in conjunction with fluid movement between the two compartments. Finally, many prior studies of β_2-microglobulin kinetics have assumed that the distribution volume for β_2-microglobulin corresponds to extracellular fluid and they have also assumed that volume to be equal to one-third of the total body water.[18,20–22] We allowed the volume of distribution to vary and obtained an estimate based on the best fit of the model equations to the experimental data.

The observed rebound in plasma β_2-microglobulin concentration following hemodiafiltration was similar to that observed by Yasuhiro *et al.*[14] following high-flux hemodialysis, 31 versus 24%, respectively. The volume of distribution for β_2-microglobulin derived from fitting the model to the experimental data was significantly less than that estimated from the anthropometric formulae of Watson *et al.*[26] (10.2 ± 0.6 l or 14.3 ± 0.7% of body weight compared to 12.8 ± 0.6 l or 18.0 ± 0.4% of body weight, $P = 0.0016$). A similar discrepancy between the volume of distribution derived from experimental data and that calculated from anthropometric relationships has also been observed for urea.[27,28] The reasons for these differences are not known, but may include failure of the anthropometric method to account for the protein content of the plasma.[27,28] The calculated generation of β_2-microglobulin (0.136 ± 0.008 mg/day) is similar to that determined in normal subjects (0.162 ± 0.016 mg/min[29]) and in dialysis patients using radiolabeled β_2-microglobulin (0.158 ± 0.015 mg/min,[2] 0.180 ± 0.024 mg/min,[23] 0.148 ± 0.009 mg/min[24]), and in hemodialysis patients using methods similar to ours (0.132 ± 0.006 mg/min,[13] 0.088 ± 0.003 mg/min[30]). The estimated intercompartmental clearance for β_2-microglobulin was 82 ± 7 ml/min, similar to the 66 ± 5 ml/min obtained by Floege *et al.*[24] following infusion of radiolabeled β_2-microglobulin.

Our model incorporates assumptions that may limit its applicability. Two assumptions concern fluid and solute transport between the perfused and non-perfused compartments. Changes in total fluid volume are assumed to be distributed between the perfused and non-perfused compartments in proportion to their volumes with no delay in fluid movement between the compartments. Delayed vascular refilling in response to ultrafiltration during a treatment would violate this assumption and reduce the accuracy of the model. A delay in vascular refilling would be marked by an increase in plasma protein concentration during the treatment followed by a prompt decrease in the immediate post-treatment period. We observed a small increase in serum albumin concentration during hemodiafiltration (3.7 ± 0.2 to 3.9 ± 0.2 g/dl) and no decrease in the immediate post-treatment period, indicating no delay in vascular refilling. Leypoldt *et al.* obtained a similar result in one study of high-flux hemodialysis,[15] but not in another,[31] suggesting that delayed vascular refilling may occur under some circumstances. The nature of these circumstances is unclear, making it difficult to incorporate a lag term in the equations used to describe compartment volumes as a function of time. Unlike some previous models of β_2-microglobulin kinetics,[2,18,22] our model assumes that solute transfer between the perfused and non-perfused compartments occurs by both diffusion and convection. The perfused and non-perfused compartments are generally assumed to represent plasma and the interstitium, in which case the transfer barrier is the capillary wall. We are unaware of any sieving coefficient data for low molecular weight proteins, such as β_2-microglobulin, and the

capillary wall. Harper *et al.*[32] have shown that a uremic environment significantly increases the protein permeability of the microvasculature. Given this observation, and in the absence of data, we have assumed that the sieving coefficient of the transfer barrier between the two compartments for β_2-microglobulin is one. The impact of these two assumptions on the accuracy of the model is likely to depend on the magnitude of the ultrafiltration rate, with the impact being limited at the low net ultrafiltration rates used in our study.

Another assumption in our model is that the extracorporeal clearance of β_2-microglobulin is constant throughout the treatment. In hemodiafiltration, clearance of β_2-microglobulin occurs through a combination of convection, diffusion and adsorption to the membrane. We combined these three mechanisms into a single total clearance, which was measured after 1 h of treatment. We have found that the clearance of the Polyflux S membrane used in this study remains essentially constant over the course of a single treatment given constant operating conditions (unpublished observations). However, this observation may not hold true for other membranes. Moreover, the practice of dialyzer reuse has been associated with both increases and decreases in β_2-microglobulin clearance from treatment to treatment,[33] which would impact the accuracy of the model predictions shown in Figure 3 if hemodiafiltration was performed with reused hemodiafilters.

Recognizing that convection provides higher clearances of large solutes, such as β_2-microglobulin, many investigators have turned to hemofiltration and hemodiafiltration in the expectation that these therapies would decrease plasma β_2-microglobulin concentrations and ameliorate the adverse effects of β_2-microglobulin amyloid in long-term end-stage renal disease patients. The benefits obtained with these therapies, however, have been relatively modest, at least in terms of reducing pre-treatment plasma β_2-microglobulin concentrations below those obtained with high-flux hemodialysis.[4,5,7] Even with 60 l of convection in the post-dilution mode, Wizemann *et al.*[6] could not reduce the pretreatment β_2-microglobulin concentration below about 18 mg/l after 24 months. (It should be noted that the predialysis concentrations of β_2-microglobulin reported in Figures 1 and 2 indicate nothing about the long-term effectiveness of hemodiafiltration because, with one exception, the patients participating in this study were normally treated with high-flux hemodialysis.)

Our results show that the extracorporeal plasma clearance of β_2-microglobulin obtained with hemodiafiltration (73 ± 2 ml/min) approached the intercompartmental clearance (82 ± 7 ml/min). Our kinetic analysis also shows that a substantial disequilibrium develops between the perfused and non-perfused compartments when the extracorporeal clearance and the intercompartmental clearance are similar in magnitude (Figure 2). The extent of this disequilibrium will increase as the extracorporeal clearance is increased relative to the intercompartmental clearance and the return on

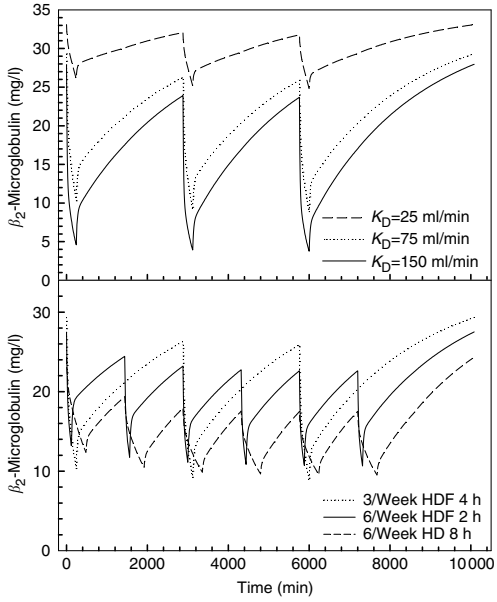

Figure 3 | (a) Predicted plasma β_2-microglobulin concentration profiles over a one week period for thrice weekly high-flux hemodialysis ($K_D = 25$ ml/min, dashed line), hemodiafiltration ($K_D = 75$ ml/min, dotted line) and hemoadsorption ($K_D = 150$ ml/min, solid line). (**b**) Predicted plasma β_2-microglobulin concentration profiles over a 1-week period for three 4-h hemodiafiltration treatments ($K_D = 75$ ml/min, dotted line), six 2-h hemodiafiltration treatments ($K_D = 75$ ml/min, solid line) or six 8-h high-flux hemodialysis treatments ($K_D = 25$ ml/min, dashed line). All simulations assume a distribution volume of 10 l, a generation rate of 0.14 mg/min, an intercompartmental clearance of 82 ml/min, a non-renal clearance of 3 ml/min and a weekly fluid removal volume of 9 l.

increasing the extracorporeal clearance will be limited as the greatest resistance to mass transfer limits solute removal. This situation is shown in Figure 3a, which depicts the plasma β_2-microglobulin concentration profile predicted using our model over a 1-week period for thrice-weekly high-flux hemodialysis (extracorporeal clearance 25 ml/min), hemodiafiltration (extracorporeal clearance 75 ml/min) and hemoadsorption (extracorporeal clearance 150 ml/min). These calculated profiles show a diminishing return in terms of reducing the plasma β_2-microglobulin concentration as clearance increases and suggest that the benefits to be derived from hemoadsorption may be limited. On the other hand, application of our model suggests that increasing the frequency of treatment may have a marked effect on the plasma concentration of β_2-microglobulin (Figure 3b). The model predicts mid-week serum β_2-microglobulin concentrations of 23 and 17 mg/l for 2 h of hemodiafiltration and 8 h of high-flux hemodialysis, 6 days per week, respectively, compared to 25 mg/l for 4 h of hemodiafiltration, 3 days per week. These model predictions are in good agreement with clinical observations reported in the literature. We have previously reported a mid-week serum β_2-microglobulin concentration of 23 mg/l in 24 patients treated with thrice-weekly on-line hemodiafiltration for 12 months.[7] Maduell

et al.[34] reported a mid-week serum β_2-microglobulin concentration of 23 mg/l in eight patients treated for 6 months with 2–2.5 h of on-line hemodiafiltration 6 days per week, whereas Canaud *et al.*[35] reported a concentration of around 25 mg/l in seven patients treated daily with 3 h of hemofiltration. It should be noted that the β_2-microglobulin clearance was lower in the latter study than is assumed in Figure 3. Finally, Raj *et al.*[36] reported a mid-week serum β_2-microglobulin concentration of 14 mg/l in 13 patients treated for 9 months with 8 h of high-flux hemodialysis six nights per week.

In summary, the results of this kinetic study suggest that the development of on-line convective therapies, such as hemodiafiltration, has led to the situation where β_2-microglobulin removal is controlled as much by intercompartmental transfer within the body as by the extracorporeal clearance of β_2-microglobulin. This finding, as illustrated by the simulations in Figure 3, suggests that returning serum β_2-microglobulin concentrations to a more normal level will be best achieved by concentrating on the time and frequency of therapy rather than through strategies designed to increase the extracorporeal clearance.

MATERIALS AND METHODS

Experimental design

Plasma β_2-microglobulin concentrations were determined during a single mid-week hemodiafiltration treatment and the following intertreatment period. Hemodiafiltration was performed in the post-dilution mode using on-line preparation of substitution solution (AK 100 ULTRA, Gambro, Hechingen, Germany). All treatments were performed with dialyzers containing high-flux membranes based on polyarylethersulfone (Polyflux 17S, Gambro). The treatment time was 240 min, with blood and total dialysate flow rates fixed at 280 and 500 ml/min, respectively. The total filtration volume was set at 18 l. Net fluid removal was set according to the patients' clinical needs. Other aspects of the treatment were according to the patient's routine prescription. Patients were required to have a hematocrit of at least 30% and a blood access capable of delivering a flow rate of 280 ml/min to be eligible for participation in the study. Exclusion criteria included a residual urine output of more than 200 ml/day or an infection within 30 days of the study.

Blood samples were collected following insertion of the first access needle and from the arterial blood line after 60, 120 and 240 min of hemodiafiltration. A venous blood sample was also collected at 60 min to allow calculation of the plasma water clearance of β_2-microglobulin by the dialyzer. Immediately after collection of the 240 min blood sample, the blood flow rate was reduced to 80 ml/min and a blood sample was collected 20 s later. Subsequent blood samples were obtained at 5, 10, 30, 60, 90, 120, and 240 min after the end of the treatment, and immediately before the next treatment. All samples were placed in tubes containing ethylenediaminetetraacetic acid and the plasma was separated by centrifugation. Samples were stored at -70°C until analysis for β_2-microglobulin by nephelometry.

The Ethics Committee of the University of Munich reviewed the study protocol and each patient gave informed consent before participating in the study.

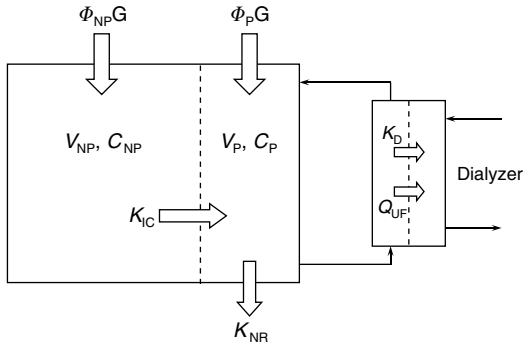

Figure 4 | Schematic representation of the two-pool model for β_2-microglobulin kinetics. See text for an explanation of the symbols.

Kinetic modeling

β_2-Microglobulin kinetics were determined using a modification of a two-pool model previously described by Kanamori and Sakai[18] (Figure 4). The model assumes that β_2-microglobulin is distributed in two compartments: a perfused compartment, to which the dialyzer has direct access, and a non-perfused compartment. Transfer of β_2-microglobulin between the two compartments is assumed to occur by diffusion, with an intercompartmental clearance, K_{IC}, and convection, with an intercompartmental sieving coefficient of one. Generation (G) is assumed to occur in both compartments in proportion to their volumes. Non-renal clearance (K_{NR}) of β_2-microglobulin is assumed to occur in the perfused compartment. The change in mass of β_2-microglobulin in the two compartments can be described as a function of time (t) by the following two equations.

Perfused compartment

$$\frac{d(V_P C_P)}{dt} = \Phi_P G + K_{IC}(C_{NP} - C_P) - \Theta K_D C_P - K_{NR} C_P + \Theta \Phi_{NP} Q_{UF} C_{NP} - (1-\Theta)\Phi_{NP}\alpha C_P \quad (1)$$

Non-perfused compartment

$$\frac{d(V_{NP} C_{NP})}{dt} = \Phi_{NP} G + K_{IC}(C_P - C_{NP}) - \Theta \Phi_{NP} Q_{UF} C_{NP} + (1-\Theta)\Phi_{NP}\alpha C_P \quad (2)$$

where V_P and V_{NP} are the volumes of the perfused and non-perfused compartments, respectively, C_P and C_{NP} are the concentrations in the perfused and non-perfused compartments, respectively, K_D is the dialyzer clearance, Q_{UF} is the ultrafiltration rate, α is the rate of fluid intake in the interdialytic period, Φ_P and Φ_{NP} are the fractions of the total distribution volume in the perfused and non-perfused compartments, respectively, and, Θ is an indicator variable such that $\Theta = 1$ during dialysis and $\Theta = 0$ in the interdialytic period.

A similar pair of equations describes the change in volume of the two compartments during dialysis as a function of time. Intradialytic fluid removal and interdialytic fluid intake are assumed to occur in both the perfused and non-perfused compartments in proportion to their relative volumes.

Perfused compartment

$$\frac{dV_P}{dt} = -\Theta \Phi_P Q_{UF} + (1-\Theta)\Phi_P \alpha \quad (3)$$

Nonperfused compartment

$$\frac{dV_{NP}}{dt} = -\Theta \Phi_{NP} Q_{UF} + (1-\Theta)\Phi_{NP} \alpha \quad (4)$$

The generation rate of β_2-microglobulin (G) was calculated from the distribution volume ($V_D = V_P + V_{NP}$) and the change in plasma concentration between the end of the post-treatment rebound and the beginning of the next treatment. For this purpose, the post-treatment rebound was assumed to be complete 240 min after the end of the treatment.

$$G = \frac{(K_{NR} + \alpha)\left(C_{NEXT} - C_{480}(V_{D480}/V_{DNEXT})^{(K_{NR}+\alpha)/\alpha}\right)}{1 - (V_{D480}/V_{DNEXT})^{(K_{NR}+\alpha)/\alpha}}$$

where the subscripts 480 and NEXT refer to 240 min after the end of the treatment and the beginning of the next treatment, respectively.

The plasma clearance of β_2-microglobulin by the dialyzer, K_D, was calculated using the following standard equation for clearance:

$$K_D = Q_B(1 - \mathrm{Hct})\frac{(C_A - C_V)}{C_A} + Q_{UF}\frac{C_V}{C_A}$$

where Q_B is the blood flow rate, Hct is the fractional hematocrit, and C_A and C_V are the β_2-microglobulin concentrations at the inlet and the outlet of the dialyzer, respectively.

The non-renal clearance of β_2-microglobulin, K_{NR}, was assumed to be 3 ml/min.[2,37]

Equations (1)–(4) were solved numerically using a fourth order Runge–Kutta method.[38] The ratio of the perfused volume to the non-perfused volume was assumed to be 1:3, such that $\Phi_P = 0.25$ and $\Phi_{NP} = 0.75$. The intercompartmental clearance, K_{IC}, and the volume of distribution, $V_D = V_P + V_{NP}$, were estimated by determining the best fit of the equations to the plasma concentration versus time data using a least-squares method.

The rebound in plasma β_2-microglobulin concentration following the end of hemodiafiltration was calculated as

$$\text{Percent Rebound} = 100 \times \frac{(C_T - C_{240})}{C_{240}}$$

where C_{240} and C_T are the solute concentrations at the end of dialysis and T minutes after the end of dialysis, respectively.

Data are presented as mean $\pm$ s.e.m. for n observations.

ACKNOWLEDGMENTS

We thank Christina Dengler for her help in sample collection and analysis and the nursing staff of the KfH Kidney Center Neuried.

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