Complex Compartmental Behavior of Small Water-Soluble Uremic Retention Solutes: Evaluation by Direct Measurements in Plasma and Erythrocytes

Sunny Eloot, PhD, MScBME, MScCivE,[1] An Torremans, PhD, MSc,[2] Rita De Smet, MScBME,[3] Bart Marescau, PhD, MSc,[2] Peter Paul De Deyn, PhD, MD,[2] Pascal Verdonck, PhD, MScBME, MScCivE,[1] and Raymond Vanholder, PhD, MD[3]

Background: Although scanty data suggest that large solutes show kinetic behavior different from urea, there are virtually no data comparing the kinetics of urea with those of other small water-soluble uremic compounds, which are believed to behave similarly.

Study Design: Cross-sectional study of kinetics of urea and guanidino compounds in plasma and erythrocyte compartments during a single hemodialysis session.

Setting & Participants: Six stable hemodialysis patients on standard low-flux dialysis therapy.

Predictors: Reduction ratios (RRs) of urea calculated from plasma and erythrocyte concentrations.

Outcomes: RRs for guanidino compounds calculated from measurements of both plasma and erythrocyte concentrations.

Measurements: Blood samples were collected from the dialyzer inlet and outlet at 0, 5, 15, 30, and 120 minutes and at the end of the session. Plasma and erythrocyte concentrations of urea and guanidino compounds (creatinine [CTN], guanidinosuccinic acid [GSA], guanidinoacetic acid [GAA], guanidine [G], and methylguanidine [MG]) were determined.

Results: Postdialysis plasma RR was higher for GSA (82% $\pm$ 3%) compared with urea (77% $\pm$ 2%; $P < 0.01$), whereas CTN (69% $\pm$ 4%), GAA (49% $\pm$ 14%), G (55% $\pm$ 7%), and MG (55% $\pm$ 7%) showed smaller RRs ($P < 0.01$). In erythrocytes, GSA (45% $\pm$ 1%), G (10% $\pm$ 13%), and MG (27% $\pm$ 10%) showed markedly smaller RRs than urea (59% $\pm$ 6%; $P < 0.05$). Finally, significant differences were found between plasma and erythrocyte RRs for urea, GSA, G, and MG ($P < 0.01$).

Limitations: Discrepancies were found between the biochemical and mathematical approaches. Hence, the erythrocyte compartment does not necessarily conform to the kinetic nonperfused compartment.

Conclusions: Our data indicate by means of direct estimations that the compartmental behaviors of guanidino compounds and urea are substantially different. Hence, we should consider that not all changes in concentrations in uremia and dialysis are representatively reflected by urea kinetics, even when considering other small water-soluble substances, such as the guanidino compounds.

Am J Kidney Dis 50:279-288.

INDEX WORDS: Dialysis adequacy; urea kinetic modeling; uremic toxins; erythrocyte; intracellular.

Editorial, p. 181

The adequacy of dialysis is determined by the degree of decrease in concentrations of uremic retention solutes. Although not necessarily biologically active, urea currently is used as the standard marker for small-molecule removal by calculating the clearance index Kt/V_{urea}.[1] Although in a controlled[2] and uncontrolled study,[3] urea removal parameters were related to morbidity and mortality of dialysis patients, 2 more recently performed controlled studies showed no improvement in survival with increasing Kt/V_{urea}.[4,5]

As a consequence, it might be considered that molecules with kinetic behavior different from that of urea have an equal, if not more important, role in functional disturbances in patients with renal dysfunction. Differences in kinetic behavior are conceivable for retention solutes that are difficult to remove because of their high-molecular-weight and/or lipophilic properties. The question can be raised whether the kinetic behavior of

From the [1]Institute for Biomedical Technology, Ghent University, Ghent; [2]Laboratory of Neurochemistry and Behaviour, University of Antwerp, Antwerp; and [3]Nephrology Section, Ghent University Hospital, Ghent, Belgium.

Received December 22, 2006. Accepted in revised form May 18, 2007. Originally published online as doi: 10.1053/j.ajkd.2007.05.009 on June 28, 2007.

Address correspondence to Sunny Eloot, PhD, MScBME, MScCivE, Institute for Biomedical Technology, Ghent University, De Pintelaan 185, Ghent, 9000 Belgium. E-mail: sunny.eloot@ugent.be

0272-6386/07/5002-0013$32.00/0
doi:10.1053/j.ajkd.2007.05.009

small water-soluble molecules also differs from that of urea.

The guanidino compounds are a large group of such solutes, resulting from protein and amino acid metabolism. They can interfere with neuronal,[6] cardiovascular,[7] leukocyte,[8] platelet,[9] and erythrocyte function.[10] Of note, guanidino compounds were shown to modify albumin structure, liberating homocysteine,[11,12] which was linked to cardiovascular damage in the general population.[13] This finding, together with the activation of baseline leukocyte activity by guanidino compounds,[8] relates this solute group to accelerated vascular disease in patients with uremia.[14]

A recent study based on mathematical kinetic analyses suggested that the kinetic behavior of guanidino compounds during hemodialysis was markedly different from that of urea.[15] The present study checks this hypothesis by means of direct estimations of concentration. Because erythrocytes are virtually the only easily accessible intracellular compartment, an in vivo study was undertaken measuring plasma and whole-blood solute concentrations during dialysis.

METHODS

Patients and Dialysis Strategies

The study included 6 stable hemodialysis patients (3 women, 3 men) without residual renal function, corresponding to patients in the previous kinetic study.[15] Because in the latter study, patient 7 represented a pilot experiment to fine-tune the sampling time points and for whom we did not estimate the content inside erythrocytes, this additional patient was not included in the present study. The limited patient sample size is related to the large number of labor-intensive analytical evaluations needed for this study. The study was approved by the local ethical committee, and written informed consent was obtained.

Conventional 2-needle hemodialysis was performed using low-flux polysulfone dialyzers: F8 (n = 2) and F10HPS (n = 4; Fresenius Medical Care, Bad Homburg, Germany). All patients had an arteriovenous fistula as vascular access. Dialysate composition was 38.5 mEq/L (mmol/L) of bicarbonate, 138 mEq/L (mmol/L) of sodium, 104 mEq/L (mmol/L) of chloride, 4 mEq/L (mmol/L) of acetate, 5 mg/dL (1.25 mmol/L) of calcium, 1.22 mg/dL (0.5 mmol/L) of magnesium, and 100 mg/dL (5.55 mmol/L) of glucose. Dialysate potassium concentration was adapted to the needs of the patients and ranged from 1 to 3 mEq/L (mmol/L). A constant dialysate flow rate of 500 mL/min was applied using a Fresenius FO1 or FO3 dialysis machine. Mean Kt/V_{urea} in this population immediately before the study was 1.77 ± 0.07 by means of routine monthly assessment according to the single-pool Daugirdas formula.[16]

Blood and Dialysate Sampling

For each patient and during a single dialysis session, blood samples were obtained from the inlet blood tubing immediately before the onset of dialysis and from the inlet and outlet after 5, 15, 30, and 120 minutes and at the end of the dialysis session (either 240 or 270 minutes). A fraction of blood samples was immediately centrifuged during 10 minutes at 1,900*g* (CR 412; Jouan, Saint-Herblain, France), after which plasma was stored at −75°C until analysis. The remaining part of the samples, used for determination of whole-blood concentrations, was immediately frozen.

Analyses

Hematocrit was measured by using the capillary centrifugation technique. Plasma urea concentrations were determined using the method of Ceriotti.[17] Plasma concentrations of creatinine (CTN), guanidinosuccinic acid (GSA), guanidinoacetic acid (GAA), guanidine (G), and methylguanidine (MG) were determined by using a Biotronic LC 6001 amino acid analyzer (Biotronic, Maintal, Germany) adapted for guanidino compound determination.[18]

For determination of guanidino compounds in whole blood, a 600-μL whole-blood sample was transferred to an Eppendorf tube and immediately frozen at −75°C. The sample was later thawed, gently shaken, and frozen again. This procedure was repeated 3 times. Finally, before analysis, the sample was thawed, 250 μL of the whole-blood lyzate was transferred to an Eppendorf tube, and 250 μL of 30% trichloroacetic acid was added for deproteinization. The sample was centrifuged further at 4°C and 20,800*g*. The supernatant was used for determination of guanidino compound concentrations.[19]

Blood cell concentration was determined after subtraction of plasma contents from whole-blood contents, taking into account the volumes occupied by packed cells and serum based on instantaneous hematocrit determinations. Although measured blood cell concentrations comprise all blood cells, erythrocytes constitute 95% of total blood cell volume; therefore, the following discussion is written in terms of erythrocyte concentrations.

This determination method was preferred over the determination on washed blood cells to avoid diffusion of urea or guanidino compounds out of blood cells during the washing procedure,[20] whereas dilution of intracellular contents by remnants of the washing solution could have skewed the results further.

Table 1. Patient and Dialysis Characteristics

	Characteristics
Patient age (y)	69 ± 12
Time on dialysis (mo)	73 ± 32
Predialysis body weight (kg)	76 ± 10
Predialysis hematocrit (%)	37 ± 3
Time of dialysis session (min)	250 ± 15
Blood flow (mL/min)	327 ± 27
Ultrafiltration flow (L/h)	0.83 ± 0.18