Measurement of Kinetic Parameters for Urea in End-Stage Renal Disease Patients Using a Two-Compartment Model

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ABSTRACT

Urea kinetic modeling depends critically on the parameters of the model used. When urea is removed during hemodialysis, the kinetic model is quite complex. This experiment describes for the first time the use of injected stable isotope-labeled urea to define kinetics in ESRD patients and compares the magnitude of the two urea compartments in these patients with those of control subjects. Such an experimental approach provides the kinetic data in the most direct manner. A gas chromatograph/mass spectrometer-based assay provided quantitation to as little as 0.2 mol% excess urea. The rapidly equilibrating fraction of the two urea compartments is quantified as 41.2% in ESRD patients and 33.4% in controls (P = 0.24). The rest is in a more slowly equilibrating pool. The urea clearances between these two compartments were near 1 L/min for both sets of subjects. The elimination of urea was due to the metabolic removal of the 15N label in both groups of subjects as well as renal elimination in the controls. The nearly threefold larger clearance (Cl) of labeled urea removal in controls (Cl = 74.6 mL/min; P = 0.015) shows the extent to which renal clearance is more important than metabolism. These direct analyses of the fractional volumes and intercompartmental clearances for urea agree closely with previous measurements during high-efficiency hemodialysis and indicate that ESRD patients do not differ significantly from control subjects in these parameters.

Key Words: Urea compartments, urea kinetics, ESRD, stable isotopes, mass spectrometry

There continues to be much discussion in the literature regarding the various mathematical models that can be used to describe hemodialysis. One debate focuses on whether one or two "compartments" are required for this modeling (1). To use the two-compartment model, a critical factor is the relative size of the two urea pools.

Estimates of the urea pool sizes in a two-compartment model arise from two different kinetic approaches: during hemodialysis when endogenous urea is removed or after the injection of isotopically labeled urea in nondialyzed subjects. Using high-efficiency hemodialysis, Heineken et al. determined that 28% of the urea was contained in the "extracellular space" and the remaining 72% was in an "intracellular" compartment (2). Using a 500-mL/min dialysis clearance and the equations of Heineken et al., Abramson et al. found that 30% of the urea was in the rapidly equilibrating central compartment and 70% was in the slowly equilibrating peripheral compartment (3). From one study using controls with stable isotope--labeled urea, the ratio of the two compartment fractions is nearly equal (4). From a more recent experiment involving a three-compartment model, also using controls, 37% of urea is in the two rapidly equilibrating compartments and 63% is in the slow compartment (5).

This study is the first to examine urea kinetics in ESRD patients after injections of stable-labeled material and to compare these data with those from normal subjects. The data have been subject to conventional pharmacokinetic analysis in order to ascertain fractional urea volumes and transfer constants in a direct manner.

MATERIALS AND METHODS

Experimental Protocol

Six patients with stable ESRD who were undergoing thrice-weekly hemodialysis and five control subjects were studied. Some of their characteristics are given in Table 1. The protocol was carried out when the patients were hospitalized for an incidental condition, such as asthma, infected access, pneumonia,
Two-Compartment Urea Kinetics

<table>
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<th>Subject</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>BUN (mg%)</th>
<th>k_{10} (min^{-1})</th>
<th>k_{12} (min^{-1})</th>
<th>k_{21} (min^{-1})</th>
<th>Cl (mL/min)</th>
<th>V_1 (L)</th>
<th>V_d (L)</th>
<th>V_d/Wt (L/kg)</th>
<th>%V_d</th>
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Significance (P) Patients versus Controls

* The designation P is for patients and C indicates controls. Figure 1 explains k_{10}, k_2, k_{21}, and V_d. Cl represents the total (i.e., renal and nonrenal) clearance of $^{13}$C,$^{15}$N$_2$-urea. V_d is the total volume of distribution for labeled urea. is also expressed normalized for weight, and is the denominator for the percentage of urea volume that is rapidly equilibrating. %V_d. To illustrate how several comparisons approach statistical significance, the results of the t-tests are given when the P value was less than 0.1. NS, not significant.

and cellulitis. The control subjects were, to a limited extent, matched for age and sex. No subject was edematous or had any known problem with cardiac output. Each was given 100 mg of $^{13}$C,$^{15}$N$_2$-labeled urea (MSD Isotopes, Montreal, Canada; >99% $^{13}$C,$^{15}$N$_2$ content; >99% chemically pure) in 5 mL of saline as a 2-min iv infusion. The urea solutions had been prepared and dispensed into syringes in the hospital pharmacy under sterile conditions. A heparin lock iv was placed in another site, through which 2-mL blood samples were taken before the labeled urea was administered and at 2, 5, 10, 15, 30, 60, 120, 240, 360, and 480 min after the infusion ended. The blood samples were collected in Vacutainer tubes (Becton-Dickinson, Rutherford, NJ) containing EDTA and kept on ice until the plasma was separated from the red blood cells and subsequently frozen at -70°C pending analysis. Most of the dialysis patients were studied in the afternoon after their routine dialysis procedure. This lessened interference from endogenous urea in the mass spectrometric assay and provided relatively “dry” weights. No special dietary restrictions were placed on either patients or controls. The protocol was approved by the George Washington University Committee on Human Research, and informed consent was obtained from all subjects.

Urea Measurements

BUN was assayed in the Clinical Chemistry Laboratory by the urease method with Kodak Ektachem 700 (Eastman Kodak, Rochester, NY) and converted into urea concentrations. The isotopically labeled urea was measured by capillary gas chromatography/mass spectrometry with our modification of the method of Matthews and Downey (4). One hundred microliters of plasma is added to 1 mL of ice-cold ethanol in a microcentrifuge tube. After low-speed centrifugation, the ethanol/water layer is added to 1.5 mL of 1 M acetic acid. A Bio-Rad Poly-Prep AG 50W-X8 ion exchange column (Bio-Rad Laboratories, Richmond, CA) is first rinsed with 4 mL of 1 M acetic acid, and then, the ethanol/water/acetic acid mixture is loaded onto the column. The column is then rinsed with 4 mL of 1 M acetic acid and 1 mL of water. The urea elutes in the fourth milliliter of a 3 M ammonium hydroxide solution. The sample is dried under N$_2$ at 80°C, and after the addition of 50 µL of N-methyl-N-trimethylsilyl-trifluoroacetamide (MSTFA; Sigma Chemical Co., St. Louis, MO) and 50 µL of acetonitrile (Fisher HPLC Grade; Fisher Scientific, Pittsburgh, PA), the sample is derivatized at 100°C for 30 min.

The gas chromatography was carried out at 145°C on a 0.25-mm inside diameter fused silica capillary column coated with 0.25-µm DB-5 (J & W Scientific, Folsom, CA), giving a retention time of 2.5 min. Two microliters was injected with a 20:1 split. The instrumentation consisted of a Varian 3300 GC (Varian Analytical Instruments, San Fernando, CA) an Extrel C50/400 quadrupole mass spectrometer (Extrel Corp., Pittsburgh, PA), and a Teknivent Vector i data system. Masses 190, 191, and 192 were monitored in electron ionization mode and represent the loss of a methyl group from the molecular ions of the bistri-
methyisilyl (TMS)-urea isotopologs, i.e., [M-CH₃]⁺, containing primarily endogenous urea with one atom of ¹³C and/or ²⁹Si; endogenous urea with two heavy atoms; and tracer urea containing two atoms of ¹⁵N and one atom of ¹³C, respectively. The m/z (mass/charge) 189 signal representing the major isotopic component of bisTMS-urea was always offscale, so that its [M-CH₃+1]⁺ signal at m/z 190 was used to monitor the endogenous urea. The ion at m/z 190 also served to correct the observed m/z 192 signal for the contribution at [M-CH₃+3]⁺ from endogenous urea, the natural m/z 192/190 ratio being 0.0534. The ratio of m/z 191/190 was used as a monitor of analytical precision, because both represent endogenous urea. The concentration of the labeled urea was computed by reverse isotope dilution with the concentrations of endogenous urea that derived from the BUN values with the formula:

\[ [\text{tracer urea}] = [\text{endogenous urea}] \times \left( \frac{[M-\text{CH}_3+1]+}{[M-\text{CH}_3+1]} \right) - 0.0094 \]

where \( [M-189] \) and \( [M-190] \) refer to the integrated intensities of the m/z 192 and 190 mass chromatograms, respectively. Because \( [M-190] \) is not the base peak of unlabeled urea, the \( [M-190] \) value must be multiplied by the expected ratio of \( [M-189]/[M-190] \), which is 5.68, to calculate \( [M-189] \). The ratio of \( [M-192]/[M-189] \) for naturally abundant silylated urea is 0.0094. The chemical purity of the isotopically labeled urea was assessed by high-performance liquid chromatography with ultraviolet detection. On the basis of comparison of peak areas, no difference was found between it and a solution of ACS Reagent Grade urea (Sigma).

The data were examined for their ability to fit the two-compartment pharmacokinetic model shown in Figure 1 with PCNONLIN (Clintrials Inc., Lexington, KY), a personal computer-based nonlinear regression program for kinetic analyses. Because the labeled urea concentrations were measured with a constant coefficient of variation throughout the range of calibration, they were weighted by the inverse square of their values in the kinetic fitting procedure (6). In general, kinetic analysis does not provide anatomical information (7). Therefore, the concepts of rapidly and slowly equilibrating compartments based on their relative perfusion rates (8), as indicated in Figure 1, are preferable to calling the two compartments “extracellular” and “intracellular.” Certainly, there are some intracellular pools in well-perfused tissues, e.g., heart or kidney, that equilibrate before some less well-perfused extracellular pools, e.g., muscle, thus making inappropriate any particular distinction about where the urea in each compartment is found. Therefore, we have two compartments designated only by their kinetic characteristics. The program library included an appropriate model that allowed the inclusion of the 2-min infusion.

The percentage of the urea space that was contained in the central, rapidly equilibrating compartment, \%V₁, was computed as 100 times the ratio of \( V₁ \) as generated by the kinetics program to the total kinetic volume of distribution \( V_d \), which was computed as (7):

\[ V_d = V₁ \cdot \frac{(1 + k_{12}/[k_{21} - \beta])}{[M-\text{CH}_3]+} \]

where \( k_{12} \) and \( k_{21} \) are explained in Figure 1 and \( \beta \) is the slope of the slower phase of the urea decay curve. All data are presented as means ± standard deviations. The t test was used to compare urea volumes and clearances.

RESULTS

Analytical

This method was successful in providing a means to assay tracer amounts of urea in the presence of high endogenous concentrations of urea, as found in patients without renal function. On the basis of BUN values, weight, and the observed urea space, the 100-mg dose of labeled urea was 0.25 to 1.2% of the total body urea, a value that clearly represents a tracer dose and would not have perturbed the normal physiologic disposition of urea. We could analyze the ¹³C,¹⁵N₂-labeled urea tracer out to 480 min in every subject. The lowest enrichment observed was a mol% excess of 0.8. The 12 samples needed for each study could be processed and analyzed in 1 day. The average error of the \( [M-191]/[M-190] \) ratio was less than 2%, indicating a good ability to measure these isotope ratios precisely by this method. The consistency of this ratio means that the isotopic contributions of the urea that is degraded and is therefore labeled with less than three heavy atoms were below our limits of precision.

We made two modifications of the precedent procedure for urea (4), each of which made the method yet more efficient. First, we substituted MSTFA for bis-trimethylsilyl-trifluoroacetamide as a derivatiz-
ing reagent. MSTFA is the most volatile silylating reagent available. This reduced the width of the solvent peak to permit observing the urea clearly at an earlier retention time, thus making the total analysis time shorter. Second, we used urea labeled in a way to allow us to monitor an ion three masses above the endogenous urea, rather than one or two. The natural abundance of the isotopes for the ion from endogenous bisTMS-urea that was measured [(M-CH₃)+ at m/z 192; +3 masses] is only 11.5% of that at m/z 191 (+2 masses) and 5.3% of that at m/z 190 (+1 mass). With less background interference, we were able to quantify our labeled urea tracer with as little as 0.2 mol% excess, or below 1 μg/mL. Despite the approximately doubled price per milligram of [¹³C,¹⁵N₂]urea compared with [¹⁵N₂]urea, these experiments are substantially less expensive because the size of the dose administered to subjects was substantially reduced. As proof, we note that Odeh et al. (5) used 2-g infusions of [¹⁵N₂]-labeled urea and reported a 5-μg/mL minimum concentration, whereas we used 100-mg doses and have at least a fivefold better detection limit.

Kinetics

Both control and ESRD patients yielded plasma concentrations that were well fit by the two-compartment kinetic model. The results of all fits and the averages of the six patients and five controls are shown in Table 1. The appearances of the data and the fit to one patient and one control are shown in Figure 2.

As typified in Figure 2, the kinetic model fit the observed points very well. Although not presented here, the quality of each subject's parameter fits as provided by PCNONLIN was good; typical standard errors for parameters were 20 to 30%, and correlation coefficients of the fits to the points were all > 0.97.

DISCUSSION

Although there was considerable variability in the rate constants of both the patients and the controls, this is not unexpected for a heterogenous population. With an invasive protocol having an 8-h duration, our search for volunteers was limited to colleagues in the medical center. Given a relatively elderly group of ESRD patients, we were unable to fully duplicate their age and weight characteristics in the control subjects. We do not notice any trends in the %V₁ values with regard to age or weight, so we think that our study populations suitably support our conclusions. There was a nonsignificant (P = 0.24 by t test) difference in %V₁ between patients (41.2) and controls (33.4).

One might initially be surprised that the ESRD patient data in Figure 2 and Table 1 do not differ more from the controls than they do. In Figure 2, the steep slope in the 0- to 30-min region is primarily due to redistribution of the injected urea tracer from the rapidly equilibrating central compartment to the slowly equilibrating peripheral compartment. This does not differ substantially in the two populations. The shallower slope is due to the loss of labeled urea. The decay of labeled urea concentrations in the ESRD patients is explained by the realization that the clearance of the [¹³C,¹⁵N₂]-labeled urea is not just due to renal mechanisms, but to a long-known (9–11) but less well-recognized nitrogen-carbon cleavage reaction of urea that is initiated by urease in intestinal microflora.

It is believed (10) that not all of this urea degradation generates CO₂ and N₂. Some partly metabolized urea remains as carbamyl phosphate that is subsequently recycled to urea. Thus, a fraction of the [¹³C,¹⁵N₂]-labeled urea would follow this reaction sequence:

\[¹⁵NH₂-¹³CO-¹⁵NH₂ \rightarrow ¹⁴NH₂-¹³CO-¹⁵NH₂ \rightarrow ¹⁴NH₂-¹³CO-¹⁴NH₂\]

In observing the mass spectrum, this represents m/z 192 → 191 → 190. Mass 190 could also be [¹⁴NH₂-¹²CO-¹⁵NH₂ as a result of recycled [¹⁵N₂]. As mentioned above, the amounts of m/z 191 and 190 that derive from the degradation of the [¹³C,¹⁵N₂]-labeled tracer were too small to be detected in the large background of endogenous urea isotopologs.

Some of the ESRD patients were anuric, whereas others had varying degrees of oliguria; all required hemodialysis. Thus, in comparing [¹³C,¹⁵N₂]-labeled urea clearance in the patients and controls subjects (25.4 versus 74.5 mL/min; P = 0.015), the majority, but not all, of this 25.4 mL/min can be assigned to the urease-mediated mechanism in the gastrointes-
tinal tract. Literature values for the percent metabolized urea average about 20 (4), whereas we find that the clearance values in ESRD are 34% of control subjects. Urea recycling is reported to be increased in uremic subjects, 3.7 mmol/h, compared with controls, 2.3 mmol/h (12).

The clearances between Compartments 1 and 2 for urea can be computed by multiplying the volumes by their appropriate rate constants, i.e., \( C_{12} = k_{12} \cdot V_1 \) and \( C_{21} = k_{21} \cdot V_2 \). In this way, we find that \( C_{12} = 0.89 \) L/min and \( C_{21} = 0.91 \) L/min for patients and \( C_{12} = 1.31 \) L/min and \( C_{21} = 1.35 \) L/min for controls. There is no significant difference between patients and controls. These data are gratifying for two reasons. First, they show the expected equal bidirectional clearance for urea between compartments. Second, they nicely match the bidirectional urea clearance that was measured in our dialysis kinetics studies, 1.28 L/min (3).

Much of the long-standing debate about the number of urea compartments is the result of limitations in the experimental designs of most previous studies. As Figure 2 shows, after urea injection, the decay phase that is attributed to the rapidly equilibrating compartment falls quickly; its \( t_{1/2} \) is 6.4 ± 4.1 min (mean ± SD, including both patients and controls). Therefore, after about 30 min, only the terminal kinetic phase is visible. Few studies of urea kinetics have acquired much data in the first 30 min and therefore have only observed one compartment, despite there actually being two. As early as 1953 (13), studies of stable isotope-labeled urea kinetics showed a rapid decay phase, but that part of the data was unimportant to the intended results of estimating urea space after the tracer had distributed throughout the body.

The principal purpose of these experiments was to obtain data about urea kinetics that could be compared with previous data obtained during high-efficiency hemodialysis (3). When urea is modeled during dialysis, the kinetic parameters for urea may be obscured by the use of a much more complex model that includes volume shifts between compartments, both convective and diffusive losses of urea, and blood flow through the dialyzer. We find here that, for ESRD patients, somewhat more urea is in the rapidly equilibrating compartment, 41.2%, than was observed in the dialysis study, 30.0% (3). This latter value may be limited in accuracy by the use of a predetermined total urea volume, 0.52 x body weight (14), whereas the 41.2% value reflects an experimentally determined total urea space. This difference is not trivial, but the limited range of the size of \( V_1 \), along with the consistency of the urea intercompartmental clearances between these two types of experiments, leads us to believe that the evaluation of the two-compartment behavior of urea in ESRD patients is fairly well determined. In this way, we confirm the appropriateness of a two-compartment model when describing urea kinetics for any purpose and validate our estimates of the errors in high-efficiency hemodialysis that are associated with using the one-compartment model (3). Furthermore, ESRD patients do not appear to have substantially different two-compartment characteristics than control subjects. This means that much of the literature on urea kinetics, which was obtained with control subjects, may be considered applicable to ESRD patients.

REFERENCES