Prominent Accumulation in Hemodialysis Patients of Solutes Normally Cleared by Tubular Secretion

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ABSTRACT

Dialytic clearance of urea is efficient, but other small solutes normally secreted by the kidney may be cleared less efficiently. This study tested whether the high concentrations of these solutes in hemodialysis patients reflect a failure of passive diffusion methods to duplicate the efficacy of clearance by tubular secretion. We compared the plasma concentrations and clearance rates of four solutes normally cleared by tubular secretion with the plasma concentrations and clearance rates of urea and creatinine in patients receiving maintenance hemodialysis and normal subjects. The predialysis concentrations (relative to normal subjects) of unbound phenylacetylglutamine (122-fold), hippurate (108-fold), indoxyl sulfate (116-fold), and p-cresol sulfate (41-fold) were much greater than the concentrations of urea (5-fold) and creatinine (13-fold). The dialytic clearance rates (relative to normal subjects) of unbound phenylacetylglutamine (0.37-fold), hippurate (0.16-fold), indoxyl sulfate (0.21-fold), and p-cresol sulfate (0.39-fold) were much lower than the rates of urea (4.2-fold) and creatinine (1.3-fold). Mathematical modeling showed that prominent accumulation of the normally secreted solutes in hemodialysis patients could be accounted for by lower dialytic clearance relative to physiologic clearance combined with the intermittency of treatment. Whether or not more efficient removal of normally secreted solutes improves outcomes in dialysis patients remains to be tested.

J Am Soc Nephrol 25: 615–622, 2014. doi: 10.1681/ASN.2013060597

Hemodialysis is now prescribed to provide a standard fractional reduction in the plasma urea concentration. The adoption of urea as our index solute has had the unintended consequence of maximizing the apparent efficacy of treatment. The dialytic clearance of urea is high, because it diffuses rapidly from both red cells and plasma. The native kidney clearance of urea, by contrast, is lower than the clearance of many solutes because of tubular reabsorption. As a result, conventional three times per week hemodialysis provides a time-averaged urea clearance, which is about one fourth of the clearance provided by the native kidneys. The efficacy of dialysis is reduced by intermittency, but predialysis plasma urea concentrations are still maintained within five to ten times the normal value.[1]

Recent reports have emphasized, however, that hemodialysis can be much less effective in controlling the levels of other small solutes.[2–5] Pretreatment levels of individual solutes have been reported to rise as high as 100 times the normal value. The current study examined why conventional treatment leaves the concentrations of some solutes so high. Mathematical modeling predicted that dialysis, which clears solutes by diffusion, would be relatively ineffective in controlling the levels of solutes that the native kidney clears efficiently by secretion. This prediction was tested by measuring the plasma concentrations and

Received June 10, 2013. Accepted September 2, 2013.

Published online ahead of print. Publication date available at www.jasn.org.

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clearance rates of four solutes normally cleared largely by secretion—phenylacetylglutamine, hippurate, indoxyl sulfate, and p-cresol sulfate—and comparing them with the concentrations and clearance rates of urea and creatinine. The dialytic clearances of the normally secreted solutes were low relative to their native kidney clearances. In accordance with the prediction of the model, conventional hemodialysis was relatively ineffective in controlling the levels of these solutes. Additional analysis showed that hemodialysis is particularly ineffective in controlling solute levels when a low dialytic-to-native kidney clearance ratio is combined with a high fractional reduction in the solute concentration during intermittent treatment.

RESULTS

Plasma solute concentrations in hemodialysis patients and normal subjects are summarized in Table 1. Hemodialysis patients had much higher concentrations of four solutes that are normally cleared largely by tubular secretion—phenylacetylglutamine, hippurate, indoxyl sulfate, and p-cresol sulfate. The total concentration of phenylacetylglutamine was most strikingly elevated followed by the total concentration of hippurate. Of note, the elevation of solute levels in hemodialysis patients was not correlated with the extent of protein binding. Hemodialysis patients exhibited more prominent increases in the total concentrations of phenylacetylglutamine and hippurate, which are only moderately bound, compared with indoxyl sulfate and p-cresol sulfate, which are more than 90% bound. Solute accumulation in hemodialysis patients, however, was attended by an increase in the free fraction of the bound solutes. The elevation of solute levels in hemodialysis patients was, thus, greater when free solute levels rather than total solute levels were compared.

The removal of the different solutes by hemodialysis was assessed to elucidate the cause of their variable accumulation in patients. Pre- and post-treatment plasma concentrations and the amount removed during treatment for each solute in a subset of subjects are summarized in Table 2. Table 2 also gives values for the reduction ratio, which is calculated as the preconcentration minus the postconcentration divided by the preconcentration. Hemodialysis reduced the total concentrations of phenylacetylglutamine and hippurate approximately as much as the concentrations of urea and creatinine. The reduction ratios for the more avidly bound solutes, indoxyl sulfate and p-cresol sulfate, were much lower. For the bound solutes, the decline in concentration during treatment tended to be accompanied by a reduction in the free fraction, and, therefore, the free concentrations tended to be reduced more than the total concentrations during treatment.

Dialytic clearance values are summarized in Table 3. Clearances were first expressed in terms of the total plasma solute concentration. As expected, the clearance was greatest for urea, which is removed from red cells as blood passes through the dialyzer.[6] Clearance values expressed in terms of the total solute concentration were lower for the normally secreted solutes and declined as the fraction of the solute bound to plasma proteins increased. A striking difference was observed when clearances were expressed in terms of the free solute concentrations. Clearances for the bound solutes were higher when expressed in terms of the free rather than the total solute concentrations, reflecting dialytic removal of solute molecules that dissociate from plasma proteins as blood passes through the dialyzer. The magnitude of the difference increased with the extent of protein binding. Thus, when expressed in terms of free solute concentration, the clearances of indoxyl sulfate and p-cresol sulfate were much higher than the clearance of creatinine.

Native kidney clearance values are also summarized in Table 3. For urea, the dialytic clearance greatly exceeds the native kidney clearance provided by glomerular filtration and partial tubular reabsorption. The dialytic clearance for creatinine, in contrast, is only slightly higher than the native kidney clearance. The relation of dialytic and native kidney clearances for the solutes that are normally cleared largely by secretion is variable. When expressed in terms of the total solute concentration, dialytic clearances of hippurate and phenylacetylglutamine are only a fraction of the native kidney clearances provided by secretion. For the more tightly bound solutes, indoxyl sulfate and p-cresol sulfate, the dialytic clearances, although lower, are a larger fraction of their native kidney clearances. Expression of clearances in terms of the free solute

Table 1. Solute concentrations in hemodialysis patients and normal subjects

Solute	Pretreatment Hemodialysis Patients			Normal Subjects			Hemodialysis/Normal	
	Total (mg/dl)	Free (mg/dl)	Free (%)	Total (mg/dl)	Free (mg/dl)	Free (%)	Total	Free
UreaN	65±25[a]	—	—	13±4	—	—	5	—
Creatinine	10.8±3.3[a]	—	—	0.84±0.18	—	—	13	—
Phenylacetylglutamine	5.2±2.5[a]	4.7±2.5[a]	88±12	0.05±0.02	0.04±0.02	79±14	112	122
Hippurate	5.3±3.0[a]	3.0±1.8[a]	54±10[a]	0.09±0.07	0.03±0.02	31±7	59	108
Indoxyl Sulfate	2.9±1.1[a]	0.27±0.19[a]	8.3±3.4[a]	0.10±0.04	0.002±0.002	2.2±0.7	30	116
p-Cresol Sulfate	3.7±1.6[a]	0.26±0.15[a]	6.9±2.5[a]	0.29±0.18	0.006±0.003	2.0±0.5	13	41

Values are mean±SD for 25 hemodialysis patients and 16 subjects with normal renal function. Demographic data and the patients' treatment prescriptions are summarized in Supplemental Table 1.

[a] $P<0.05$ for hemodialysis versus normal value.

Table 2. Solute removal by hemodialysis

Solute	Concentration						Removal (mg per treatment)
	Total Pre (mg/dl)	Reduction Ratio (%)	Free Pre (mg/dl)	Reduction Ratio (%)	Free Pre (%)	Free Post (%)	
UreaN	47±22	75±5	—	—	—	—	$14.2\pm7.5\times10^3$
Creatinine	8.9±2.2	67±5	—	—	—	—	$1.9\pm0.7\times10^3$
Phenylacetylglutamine	4.7±3.1	80±5	4.3±2.8	80±4	89±8	89±9	$0.82\pm0.61\times10^3$
Hippurate	4.0±2.0	71±5[a]	2.1±1.2	79±5	51±7[b]	36±5	$0.55\pm0.28\times10^3$
Indoxyl Sulfate	2.2±0.9	36±13	0.15±0.12	47±17	6.4±2.8	5.0±1.8	109±64
p-Cresol Sulfate	4.5±2.3	31±13	0.26±0.21	43±19	5.1±2.2	4.0±1.3	171±119

Values are mean±SD for eight hemodialysis patients.
[a]$P<0.05$ for reduction ratio of total plasma concentration compared with reduction ratio of free plasma concentration.
[b]$P<0.05$ for percent free predialysis compared with percent free postdialysis concentration.

Table 3. Dialytic compared with native kidney clearances

Solute	Dialytic Clearance (ml/min)		Native Kidney Clearance (ml/min per 1.73 m^2)		Dialytic/Native	
	Total	Free	Total	Free	Total	Free
UreaN	292±52[a]	—	70±18	—	4.2	—
Creatinine	190±11[a,b]	—	148±35[b]	—	1.3	—
Phenylacetylglutamine	174±19[a,b]	196±14[a,b,c]	383±79[b,d]	532±167[b,c,d]	0.45	0.37
Hippurate	125±10[a,b,d,e]	287±39[a,c,d,e]	472±101[b,d]	1758±629[b,c,d,e]	0.27	0.16
Indoxyl Sulfate	32±6[a,b,d,e,f]	583±94[a,b,c,d,e,f]	58±18[d,e,f]	2776±1190[b,c,d,e]	0.55	0.21
p-Cresol Sulfate	23±5[b,d,e,f,g]	517±68[a,b,c,d,e,f]	23±8[b,d,e,f,g]	1319±597[b,c,d,f,g]	0.98	0.39

Values are mean±SD for eight hemodialysis patients and nine subjects with normal renal function. Values for native clearances are expressed per 1.73 m^2. Dialytic clearance values are unadjusted, because local practice is generally to not adjust blood flow, dialysate flow, and dialyzer size in proportion to a patient's body size. Free clearances for the bound solutes were compared with total clearance of urea and creatinine.
[a]$P<0.05$, dialytic versus native kidney clearance.
[b]$P<0.05$, clearances different from urea.
[c]$P<0.05$, clearances expressed in terms of free solute concentration compared with total solute concentration.
[d]$P<0.05$, clearances different from creatinine.
[e]$P<0.05$, clearances different from phenylacetylglutamine.
[f]$P<0.05$, clearances different from hippurate.
[g]$P<0.05$, clearances different from indoxyl sulfate.

concentration presents a different picture of the relation of dialytic to native kidney function. The clearance of even the tightly bound solutes by the native kidney is seen to be very high. The dialytic clearances for these solutes are lower relative to the native kidney clearance when expressed in terms of free solute concentration than when expressed in terms of the total solute concentration. The greater rise of their free concentrations in dialysis patients (reported in Table 1) is associated with their lesser clearance by dialysis compared with native kidney function.

Mathematical modeling predicted the failure of conventional hemodialysis to control levels of solutes that are efficiently cleared by secretion in the native kidney. Values for the average pretreatment solute level relative to normal were first estimated using the simplest model of treatment, which assumes that solutes are cleared by intermittent dialysis from a single compartment of fixed volume.[1] Results are depicted in Figure 1, which shows the predicted ratio of pretreatment solute levels relative to normal as a function of the ratio of the dialytic clearance to the native kidney clearance (K_D/K_{NL}) and the reduction ratio for the plasma solute concentration during each treatment. When the reduction ratio is small (equivalent to the assumption that the volume of distribution is large relative to the dialytic clearance), the solute level relative to normal approaches the native kidney clearance divided by the time-averaged dialytic clearance, which is the dialytic clearance multiplied by the fraction of time spent on dialysis during each week. As the reduction ratio increases, intermittent dialysis becomes less effective in controlling the pretreatment solute level. Of note, for any value of the reduction ratio, the predicted increase in the pretreatment solute level relative to the level that would be observed with a reduction ratio of zero is the same for all values of K_D/K_{NL} (Supplemental Figure 1). As the reduction ratio increases, time-averaged solute levels do not rise as much as pretreatment solute levels (Supplemental Figure 2).

DISCUSSION

Relative to the native kidney function, dialysis provides a high clearance for urea. Urea diffuses out of erythrocytes as blood passes through the dialyzer; with conventional treatment, the urea clearance exceeds the plasma flow rate.[6] In the native

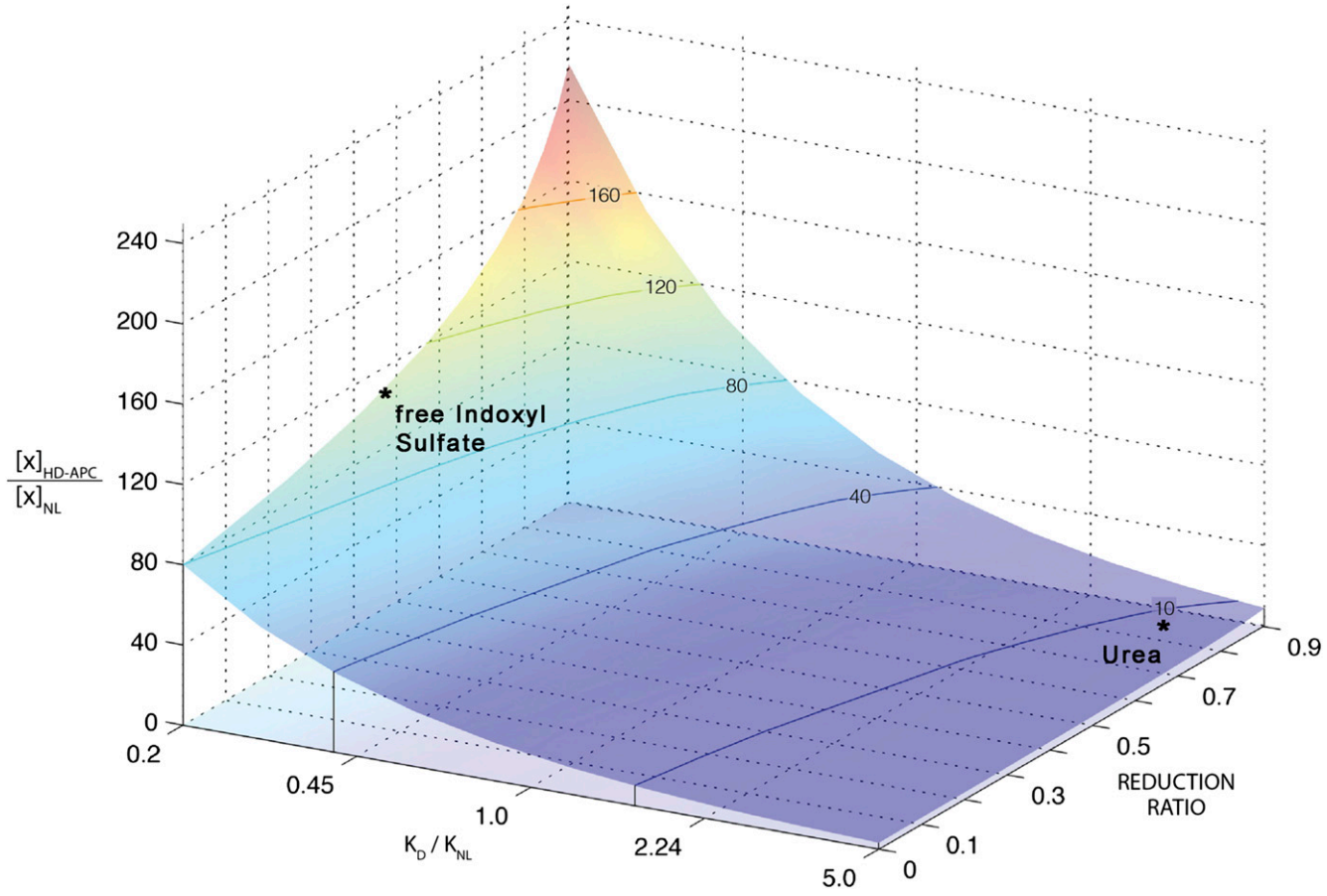

Figure 1. Determinants of plasma solute accumulation in hemodialysis patients. Predicted pretreatment solute levels in patients maintained on conventional hemodialysis relative to those levels in normal subjects. The ratio of the average pretreatment solute concentration in hemodialysis patients to the concentration in normal subjects ($[X]_{HD\text{-}APC}/[X]_{NL}$) is plotted on the vertical axis as a function of the ratio of the dialytic clearance to normal clearance (K_D/K_{NL}) and concentration reduction ratio defined as the pre- minus post-treatment concentration divided by the pretreatment concentration. Values were calculated assuming that treatment is performed three times per week for 3.5 hours, that solute production is constant and the same in patients and normal subjects, and that solute is removed from a single compartment. If a solute's concentration does not fall much during treatment, intermittency does not diminish the efficiency of treatment, and the solute's concentration in dialysis patients relative to normal subjects approaches the normal clearance K_{NL} divided by the time-averaged dialytic clearance, which is obtained by multiplying K_D by the fraction of the week for which treatment is applied (here 10.5 hours/168 hours). As the reduction ratio increases, intermittency limits the effect of treatment, and therefore, the solute concentration ratio is higher for any given value of K_D/K_{NL}. The asterisks illustrate the model's prediction in accordance with measured values that, although conventional three times per week hemodialysis restricts the average pretreatment concentration of urea to less than 10-fold normal, leaves the average pretreatment concentration of free, unbound indoxyl sulfate greater than 100-fold normal. If a solute is modeled as being removed from a single compartment, the reduction ratio is determined by the ratio of the dialytic clearance to the volume of distribution, K_D/V_D. A low reduction ratio is equivalent to the assumption that the volume of distribution is large relative to the dialytic clearance. The ratio of the pretreatment solute concentration in hemodialysis patients to the concentration in normal subjects ($[X]_{HD\text{-}APC}/[X]_{NL}$) can, therefore, alternatively be plotted as a function of K_D/K_{NL} and K_D/V_D (Supplemental Figure 3). Modeled values for solute concentration are somewhat higher if it is assumed that dialysis removes solute from a first accessible compartment and solute moves by diffusion between this compartment and a second compartment. The pattern of concentration dependence on the ratio of dialytic to native kidney clearance and the reduction ratio, however, remains the same (Supplemental Figure 2).

kidney, by contrast, urea is partially reabsorbed after glomerular filtration, and therefore, the urea clearance is less than the GFR and only a small fraction of the plasma flow. Use of urea to assess adequacy, thus, makes dialysis seem relatively effective in replacing renal function. Pretreatment plasma urea concentrations are maintained within five to ten times the normal value when dialysis is provided three times per week for 3–4 hours.

Recent reports have noted, however, that levels of other solutes may rise much higher in hemodialysis patients. A metabolomic study, which identified 44 solutes retained in uremia, found 10 solutes for which the concentration in uremic

patients was more than 10-fold the concentration in controls.[2] A study using targeted analysis showed that levels of 6 of 10 protein-bound solutes were more than 10-fold the normal value in hemodialysis patients.[3] A comprehensive review by Duranton *et al.*[4] identified 14 small solutes for which the average concentrations in uremic patients were reported to be more than 10-fold the normal value. In a recent study using high-resolution mass spectrometry, we detected more than 50 solutes for which the free, unbound concentrations were elevated above normal to this degree in hemodialysis patients.[5] Many of these solutes were characterized only by ion mass, but 11 solutes were chemically identified, including 6 solutes for which high concentrations had not previously been described.

The current study examined the hypothesis that prominent solute accumulation in hemodialysis patients reflects the failure of dialysis to replicate high clearances normally achieved by tubular secretion. We first assessed the plasma concentrations and dialytic clearances of four normally secreted solutes: phenylacetylglutamine, hippurate, indoxyl sulfate, and p-cresol sulfate. They were selected to exhibit a wide range of protein binding because of the reported effect of binding on dialytic clearance.[7–9] Predialysis plasma concentrations for the secreted solutes were much higher relative to normal values than the concentrations of urea. For all but the slightly bound phenylacetylglutamine, their free concentrations were higher relative to normal values than their total plasma concentrations. This finding is consistent with previous observations that the extent of protein binding declines as solutes accumulate.[4,10–12] The resultant greater rise in the free than the total concentrations of bound solutes has potential clinical importance, because it is presumably the free concentration of a solute that is available for interaction with body tissues. This presumption is strongly supported by studies of pharmaceutical agents.[13,14] Perhaps, the best known example in nephrology is the dependence of the effect of phenytoin on its free rather than its total plasma concentration.[15]

A notable finding of this study was that hemodialysis patients had relatively higher levels of phenylacetylglutamine and hippurate than the more tightly bound indoxyl sulfate and p-cresol sulfate. This finding is consistent with the recent report by Itoh *et al.*,[3] but it is contrary to our initial expectation. Lesaffer *et al.*[7] initially showed that protein binding greatly reduces the clearance of small solutes. Subsequent studies confirmed their results and showed that dialytic clearance falls as the avidity of binding increases.[8,16,17] We initially expected that, because avidly bound solutes have low dialytic clearances, they would accumulate to particularly high levels in hemodialysis patients. We should have realized, however, that it is the ratio of the dialytic clearance to the native kidney clearance rather than the absolute value of the dialytic clearance that determines the extent to which a solute's concentration is elevated in hemodialysis patients. The importance of this clearance ratio is made clear by the current results. In terms of total solute concentration, the dialytic clearances of indoxyl sulfate and p-cresol sulfate are much lower than the clearances of hippurate and phenylacetylglutamine. They are, however, higher in proportion to the native kidney clearances than those concentrations of hippurate and phenylacetylglutamine, and the total plasma levels of indoxyl sulfate and p-cresol sulfate do not rise as high as those levels of hippurate and phenylacetylglutamine in hemodialysis patients. The dialytic clearances for indoxyl sulfate and p-cresol sulfate are higher when expressed in terms of the free rather than the total solute concentration. The native kidney, however, achieves an even higher ratio of free-to-total solute clearance for these solutes than conventional dialysis, and the relative increase in their free concentrations in hemodialysis patients is greater than the increase in their total concentrations.

The predicted dependence of solute concentrations on the ratio of the dialytic to native kidney clearance is depicted in Figure 1. As shown in Figure 1, when dialysis is intermittent, solute accumulation also depends on the extent to which the solute concentration falls during treatment, because as the concentration falls, less solute is removed per unit time, even if the dialytic clearance remains constant. The concentration reduction ratio during treatment, thus, provides an index of the extent to which intermittency limits the effectiveness of treatment. If we assume that solute is being removed from a single compartment, the solute reduction ratio depends on the ratio of the dialytic clearance to the volume of that compartment. If the clearance is large relative to the volume of distribution, the solute's concentration declines rapidly during treatment, and the latter part of the treatment removes relatively little solute. The dialytic behavior of some solutes is more accurately described by the assumption that they are cleared from a readily accessible compartment linked to a larger reservoir.[18] For such solutes, the dependence of solute concentrations on the ratio of the dialytic to native kidney clearance and concentration reduction ratio is quantitatively different but directionally the same (Supplemental Figure 2).

Although knowledge of the molecular mechanisms that provide tubular secretion has grown rapidly, the contribution of secretion to the removal of waste solutes has received relatively little attention. The majority of studies in this area have analyzed the secretory clearance of pharmaceutical agents.[19–22] Recent metabolomic studies, however, suggest that secretion is also responsible for the clearance of a large number of naturally occurring solutes.[5,23,24] We recently found that the concentration of many such solutes in hemodialysis patients is very high relative to normal.[5] The current study shows that this result is a predictable consequence of replacing the native kidney's secretory mechanisms by dialysis treatment, which clears solutes by diffusion.

An obvious question is whether prominent accumulation of normally secreted solutes contributes to residual illness in hemodialysis patients. The four solutes examined in this study were chosen, because they were known to be cleared by tubular

secretion in the native kidney and exhibit widely variable protein binding. There is substantial, albeit incomplete, evidence that indoxyl sulfate and p-cresol sulfate are toxic in hemodialysis patients, whereas studies in normal subjects suggest that high levels of hippurate and phenylacetylglutamine have little adverse effect (at least over the short term).[25–27] We know very little, however, about most of the large number of secreted solutes, and additional studies will be required to determine which of them are toxic.

One potential approach to the problem of toxicity is to compare hemodialysis with peritoneal dialysis. Clinical outcomes obtained with the two modalities are not widely different, suggesting that solutes that one modality clears less well than the other do not contribute importantly to illness. Peritoneal dialysis is nearly as effective as hemodialysis in removing unbound solutes but much less effective in removing tightly bound solutes. We might, therefore, expect levels of tightly bound solutes to rise much higher in peritoneal dialysis than hemodialysis and exclude such solutes from consideration as major uremic toxins. We previously found that levels of indoxyl sulfate and p-cresol sulfate are not much higher in peritoneal dialysis than hemodialysis.[28] This result remains unexplained, but it could reflect nondialytic solute clearance or differences in solute production.[29] Levels of other, normally secreted solutes in patients on peritoneal dialysis and hemodialysis remain to be compared.

A related question is the extent to which normally secreted solutes accumulate in patients with CKD. The secreted solute that has been most extensively studied in CKD is para-aminohippurate, which has long been used as a measure of renal plasma flow. On average, its clearance declines only slightly less than the GFR.[30] The clearances of indoxyl sulfate and p-cresol sulfate have also recently been shown to decline in parallel with the estimated GFR in CKD.[31] We found that urinary indoxyl sulfate and p-cresol sulfate clearances also had approximately the same relation to estimated GFR in hemodialysis patients with residual kidney function and normal subjects.[32] We might, thus, expect plasma levels of secreted solutes to rise in proportion with GFR markers before the initiation of dialysis and then rise further as residual function is lost. As is the case with peritoneal dialysis, however, measurements of indoxyl sulfate and p-cresol sulfate do not clearly follow this pattern, and data on other normally secreted solutes are not available.

If we presume that some solutes that the native kidney clears efficiently by secretion are toxic, we face the question of how their levels can be reduced in patients on hemodialysis. When the solute reduction ratio is not high, the plasma solute concentration can be reduced by increasing the clearance. For tightly bound solutes, it can be accomplished by several means, including increasing the dialyzer mass transfer area coefficient and dialysate flow above conventional levels.[9,33] Reducing the concentration of solutes for which conventional dialysis provides high reduction ratios poses a more difficult problem. In the current study, such solutes are represented by phenylacetylglutamine and hippurate. The methods that have been shown to raise the clearances of more avidly bound solutes would not greatly increase their clearances. Additionally, even if their clearances could be increased, plasma solute levels at the end of each treatment would not be much reduced. An increase in treatment frequency would be required to significantly reduce the plasma levels of these solutes.

An alternate means to reduce solute levels is to reduce solute production. In Table 4, the elevations in solute concentration that we observed are compared with values modeled assuming that solute production is the same in hemodialysis patients and normal subjects. We presume that lower protein intake and lesser muscle mass accounted for the failure of the observed urea and creatinine levels to rise as high as the modeled values. Indoxyl sulfate, p-cresol sulfate, and phenylacetylglutamine are produced largely and hippurate is produced partially by intestinal microbes.[34–37] Their production is, thus, potentially influenced by not only diet but also, changes in the colon microbiome and bowel habits.[38,39] We presume that some combination of such changes accounted for the finding in dialysis patients of hippurate levels that were lower and phenylacetylglutamine levels that were higher than the modeled values.

In summary, concentrations of solutes secreted by the native kidney remain high in patients maintained on conventional hemodialysis prescribed using urea as the sole marker of adequacy. Whether reducing their concentrations would improve outcomes remains to be tested.

CONCISE METHODS

Plasma was collected pretreatment from 25 patients maintained on hemodialysis and 16 control subjects with no history of renal disease. Studies were performed in accordance with the Declaration of Helsinki. Demographic data and prescriptions for the patients are summarized in Supplemental Table 1. Patients were included in the current analysis if they were stable with minimal residual kidney function, had no active gastrointestinal disease or recent antibiotic use, and were treated with a dialysate flow of at least 700 ml/min. Many of the patient samples were baseline samples from an ongoing study of the effect of diet on solute production in hemodialysis patients. Subjects with normal renal function were hospital colleagues. Additional samples were collected in a subset of eight hemodialysis patients and nine control subjects to determine clearance values. Demographic and prescription data for these subsets are also summarized in Supplemental Table 1. Plasma samples were collected pre- and post-treatment from the dialysis patients, and spent dialysate was collected in drums. Clearance values were calculated assuming a logarithmic reduction in plasma levels during treatment as previously described.[33] Clearance values in subjects with normal renal function were obtained by collecting blood samples during timed 3- to 4-hour urine collections after an overnight fast.

Table 4. Observed and predicted ratios of predialysis solute concentrations in hemodialysis patients relative to normal subjects

	Hemodialysis/Normal Observed		Hemodialysis/Normal Predicted	
	Total	Free	Total	Free
UreaN	5	—	8	—
Creatinine	13	—	22	—
Phenylacetylglutamine	112	122	79	94
Hippurate	59	108	113	214
Indoxyl Sulfate	30	116	39	113
p-Cresol Sulfate	13	41	21	58

Observed ratios were obtained from measured solutes levels in 25 hemodialysis patients and 16 subjects with normal renal function, and they are repeated from Table 1. Predicted values for a patient treated three times weekly for 3.2 hours were obtained using the average values for clearance and reduction ratio summarized in Tables 2 and 3. Values were predicted assuming that solute production is the same in hemodialysis patients and normal subjects, that dialysis patients have no residual function and there is no extrarenal solute clearance, and that dialysis clears solute from a single compartment.

UreaN was measured in plasma and urine by the clinical laboratory and dialysate using a commercial kit (1770–500; Thermo Electron Corp.). Creatinine was measured in plasma and urine by the clinical laboratory and dialysate by HPLC. Levels of hippurate, phenylacetylglutamine, indoxyl sulfate, and p-cresol sulfate in plasma, plasma ultrafiltrate, urine, and dialysate were measured by stable isotope dilution liquid chromatography–tandem mass spectrometry using a previously described method.[5] Plasma was deproteinized by the addition of three parts methanol to one part plasma, dried by lyophilization, and resuspended in water for analysis. Plasma ultrafiltrate was obtained using Nanosep 30K Omega separators (Pall, Ann Arbor, MI). Ultrafiltrate from control subjects was concentrated 10-fold by lyophilization and resuspension in water; other samples were analyzed at the original concentration or diluted as necessary to ensure that values were within assay range. Values for the limits of detection and coefficients of variation of the liquid chromatography–tandem mass spectrometry assays are proved in Supplemental Table 2.

Solute concentration profiles in hemodialysis patients were modeled as described by Depner[1] using MATLAB (R2012a). Concentrations were calculated assuming that solute production is constant and the same in dialysis patients and normal subjects, that there is no nonrenal or nondialytic clearance, and that the dialysis patients have no residual renal function. When a single compartment is assumed, the standard model yields a unique value for the concentration ratio for each value of K_D/K_{NL} and the reduction ratio. If it is assumed that solute is dialyzed from an accessible compartment and diffuses between this compartment and a second compartment, different concentration values will be obtained depending on the assumed values for the ratios among the volumes of the two compartments and the intercompartmental mass transfer area coefficient governing diffusion between them.

Unpaired comparisons between hemodialysis patients and normal subjects were performed using the Mann–Whitney *U* test. Paired comparisons within the dialysis and normal subject groups were made using the Wilcoxon rank sum test. Statistical analyses were performed using SPSS 21.0. Clearances of different solutes within the dialysis and normal subject groups were log transformed and compared by ANOVA, with significance of pairwise comparisons made using the Student Newman Keuls method.

ACKNOWLEDGMENTS

T.L.S. was supported by the Mitsubishi Tanabe Pharma Corporation National Kidney Foundation Fellowship for the Study of Uremia. Other support was provided by National Institutes of Health Grants R01DK80123 (to T.H.H.), R21DK84439 (to T.W.M.), and R01DK80123 (to T.W.M.).

DISCLOSURES

None.

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