Numerous protein-bound solutes are cleared by the kidney with high efficiency

Tammy L. Sirich[1], Pavel A. Aronov[2], Natalie S. Plummer[1], Thomas H. Hostetter[3] and Timothy W. Meyer[1]

[1]Departments of Medicine, VA Palo Alto HCS and Stanford University, Palo Alto, California, USA; [2]Vincent Coates Foundation Mass Spectrometry Laboratory, Stanford, California, USA and [3]Department of Medicine, Case Western Reserve University, Cleveland, Ohio, USA

The kidney clears numerous solutes from the plasma; however, retention of these solutes causes uremic illness when the kidneys fail. We know remarkably little about which retained solutes are toxic and this limits our ability to improve dialysis therapies. To explore this, we employed untargeted mass spectrometry to identify solutes that are efficiently cleared by the kidney. High-resolution mass spectrometry detected 1808 features in the urine and plasma ultrafiltrate of 5 individuals with normal renal function. The estimated clearance rates of 1082 peaks were greater than the creatinine clearance indicating tubular secretion. Further analysis identified 90 features representing solutes with estimated clearance rates greater than the renal plasma flow. Quantitative mass spectrometry with stable isotope dilution confirmed that efficient clearance of these solutes is made possible by the combination of binding to plasma proteins and tubular secretion. Tandem mass spectrometry established the chemical identity of 13 solutes including hippuric acid, indoxyl sulfate, and *p*-cresol sulfate. These 13 efficiently cleared solutes were found to accumulate in the plasma of hemodialysis patients, with free levels rising to more than 20-fold normal for all but two of them. Thus, further analysis of solutes efficiently cleared by secretion in the native kidney may provide a potential route to the identification of uremic toxins.

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Correspondence: *Tammy L. Sirich, Nephrology 111R, Palo Alto VAHCS, 3801 Miranda Avenue, Palo Alto, California 94303, USA.*
E-mail: tsirich@stanford.edu

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The kidney removes numerous waste solutes from the blood plasma. When kidney function is lost, these solutes accumulate in the body and cause uremic illness culminating in death unless renal function is partially replaced by dialysis. At present, we know remarkably little about which retained solutes are toxic, and this lack of knowledge limits our ability to improve treatment.[1,2] Progress has been slow in part because the number of solutes retained when the kidneys fail is very large.[1,3–6] This study employed untargeted mass spectrometry to find solutes that are efficiently removed from the plasma by the kidney. It revealed that there are many waste solutes for which renal clearance rates normally exceed the renal plasma flow. Such high clearances require a combination of binding to plasma proteins and active tubular secretion. Concentrations of such solutes can rise to high levels when renal function is replaced by hemodialysis that clears solutes only by diffusion. Presuming that evolution has provided for the kidney to remove toxic substances efficiently, identification of solutes with high renal clearance rates could provide a route to the identification of uremic toxins.

RESULTS

Measurements were made in four men and one woman with normal renal function as reflected by an average creatinine clearance of 142 ± 22 ml/min per $1.73\,m^2$. A total of 1808 features were detected in both urine and plasma ultrafiltrate by untargeted high-resolution mass spectrometry. Clearance rates for these features were estimated as the urinary excretion rate divided by the concentration in plasma ultrafiltrate. Clearance values are thus expressed in terms of the 'free' unbound solute concentration in plasma rather than the total solute concentration. The distribution of estimated clearance rates relative to the creatinine clearance is depicted in Figure 1. For 1082 features, estimated clearance rates were greater than the clearance of creatinine with a false discovery rate of $q < 0.05$. Because the creatinine clearance is slightly higher than the glomerular filtration rate (GFR), these features were considered likely to represent solutes secreted by the renal tubules. There were, in contrast, only 290 features with estimated clearance rates less than the creatinine clearance with a false discovery rate of $q < 0.05$.

For 163 features, estimated clearance rates were more than sevenfold the clearance of creatinine. This suggested that their clearances exceeded the renal plasma flow, which is approximately fourfold the clearance of creatinine. Among these 163 features, 90 were considered to represent unique chemical compounds after elimination of duplicates and features considered to represent dimers, adducts, isotopes, or artifacts on manual review of chromatograms (Supplementary Table S2 online). Compounds with matching mass values were sought in standard databases, and the chemical identities of 13 of these 90 features were established by comparison of their chromatographic retention times and tandem mass spectrometry (MS/MS) spectra with those of reagent standards (Table 1 and Supplementary Table S2 online). For 40 of the 90 features of interest, however, no candidate compounds were found among known human metabolites with mass within 3 parts per million (p.p.m.) (Supplementary Table S2 online).[7,8] Clearance values in excess of the renal plasma flow rate were possible because the 'free' concentrations of these solutes in plasma ultrafiltrate were lower than their total plasma concentrations, presumably reflecting binding to plasma proteins. Although all of the efficiently cleared solutes were protein bound, the extent of binding varied widely, with the free fraction ranging from 2 to 52% of the total plasma concentration. Calculation in terms of total plasma concentration yielded much lower clearance values for the bound solutes (Supplementary Table S2 online).

Measurements using liquid chromatography/tandem mass spectrometry (LC/MS/MS) with isotopically labeled standards confirmed the finding of very high clearance rates for the bound solutes hippurate, indoxyl sulfate, and *p*-cresol sulfate. Clearance values for these solutes along with urea and creatinine are summarized in Table 2. For comparison, clearance values were also measured for phenylacetylglutamine that previous studies had shown to be secreted by the renal tubules but largely unbound.[9] As expected, the clearance of urea was less than the creatinine clearance, reflecting tubular reabsorption after glomerular filtration. The clearance of phenylacetylglutamine in contrast averaged 455 ± 62 ml/min per 1.73 m^2, or approximately

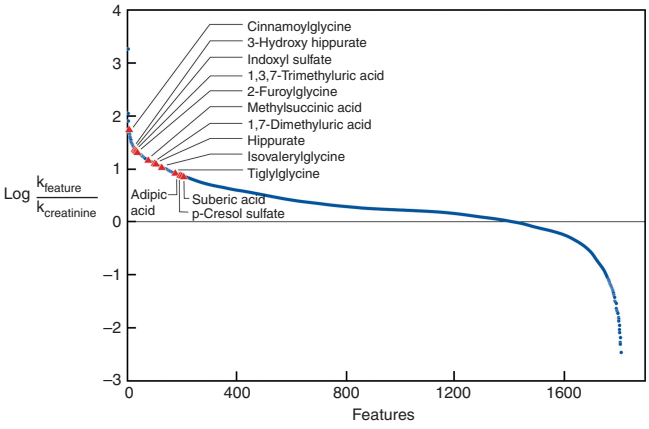

Figure 1 | The blue line represents the distribution of estimated clearance rates relative to the creatinine clearance for the 1808 features found in the urine and plasma ultrafiltrate of normal subjects. The red triangles represent the 13 features for which identity was confirmed by analysis of reagent standards.

Table 1 | Solutes efficiently cleared by the native kidney and their accumulation in hemodialysis patients

	Values in normal subjects			Hemodialysis/normal	
Solute	Clearance (ml/min per 1.73 m^2)	Clearance/$clearance_{cre}$	Free fraction (%)	Total	Free
Cinnamoylglycine	7343 ± 567	55 ± 5	4 ± 1	28[a]	123[a]
3-Hydroxy hippurate	3617 ± 3618	23 ± 19	35 ± 6	34[a]	34[a]
Indoxyl sulfate	3023 ± 533	22 ± 4	2 ± 1	26[a]	77[a]
1,3,7-Trimethyluric acid	2896 ± 1117	21 ± 7	9 ± 3	3	5[a]
2-Furoylglycine	2733 ± 1282	21 ± 11	49[b]	64[a]	82[a]
Methylsuccinic acid	1988 ± 447	14 ± 4	11 ± 4	11[a]	91[a]
1,7-Dimethyluric acid	1977 ± 2238	13 ± 12	39 ± 30	7[a]	8[a]
Hippurate	1868 ± 1312	12 ± 6	27 ± 5	31[a]	53[a]
Isovalerylglycine	1498 ± 324	11 ± 2	30 ± 6	9[a]	47[a]
Tiglylglycine	1148 ± 235	8 ± 2	52 ± 19	27[a]	50[a]
Adipic acid	1100 ± 304	8 ± 2	4 ± 1	13[a]	23[a]
p-Cresol sulfate	1055 ± 148	8 ± 1	2 ± 1	11[a]	34[a]
Suberic acid	1041 ± 147	8 ± 1	7 ± 2	6[a]	30[a]

Values are mean ± s.d. Clearance/$clearance_{cre}$ is the average ratio of solute clearance to creatinine clearance. Free fraction is the level in plasma ultrafiltrate as a percent of the total plasma level. Hemodialysis/normal is the ratio of the average pretreatment concentration in hemodialysis patients to the average concentration in normal subjects.

[a]Indicates $q < 0.05$ for elevation of the solute concentration in hemodialysis patients above the level in normal subjects.

[b]Indicates that free fraction for furoylglycine was calculated in only one subject because peaks in plasma samples from other subjects were too small to quantify.

Table 2 | Clearance values obtained using LC/MS/MS assay

Solute	Clearance (ml/min per 1.73 m^2)	Clearance/$clearance_{cre}$	Plasma free concentration (μmol/l)	Free fraction (%)	$Clearance_{total}$ (ml/min per 1.73 m^2)
Urea	57 ± 11	0.4 ± 0.1	2.4 ± 0.6 × 10^3	—	—
Creatinine	142 ± 22	1.0	74 ± 13	—	—
Phenylacetylglutamine	455 ± 62	3.1 ± 0.1	1.8 ± 0.8	78 ± 10	357 ± 73
Hippurate	1257 ± 193	9 ± 1	1.5 ± 0.9	41 ± 2	518 ± 102
p-Cresol sulfate	990 ± 151	7 ± 2	0.46 ± 0.26	2.4 ± 0.4	22 ± 3
Indoxyl sulfate	1944 ± 389	14 ± 2	0.13 ± 0.05	2.7 ± 0.4	53 ± 7

Abbreviation: LC/MS/MS, liquid chromatography/tandem mass spectrometry.

Values are mean ± s.d. Clearance/$clearance_{cre}$ is the average ratio of solute clearance to creatinine clearance. Free fraction is the level in plasma ultrafiltrate as a percent of the total plasma level. $Clearance_{total}$ is the value that would be obtained if clearance was calculated using the plasma total concentration rather than the plasma free concentration.