Improving Clearance for Renal Replacement Therapy

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Abstract

The adequacy of hemodialysis is now assessed by measuring the removal of the single solute urea. The urea clearance provided by current dialysis methods is a large fraction of the blood flow through the dialyzer, and therefore cannot be increased much further. Other solutes which are less effectively cleared than urea may however contribute more to the residual uremic illness suffered by hemodialysis patients. We here review a variety of methods which could be employed to increase the clearance of such non-urea solutes. New clinical studies will be required to test the extent to which increasing solute clearances improves patients' health.
We presume that an important part of the residual illness in patients maintained on hemodialysis is due to incomplete removal of uremic solutes.\textsuperscript{1-4} The variety of such solutes is enormous.\textsuperscript{5} Yet we now assess the adequacy of treatment by removal of the single solute urea. This review will describe potential means to improve clearance of non-urea solutes using mechanical devices. We will not discuss peritoneal dialysis and will deal only in passing with solute properties which prevent increases in their clearance from achieving proportional reductions in their plasma levels. We will separately consider the clearances of free low-molecular-weight solutes, middle molecules, and protein-bound solutes. In so doing, we employ the classification originally proposed by the European Uremic Toxin Work Group (EUTox), founded in 1999 to address question related to solute retention and removal in chronic kidney disease.\textsuperscript{2, 5, 6}

**Free low-molecular-weight uremic solutes**

Urea has served as a prototype for free, low-molecular-weight uremic solutes. Its use to assess treatment adequacy has directed the design of dialyzers and dialysis machines. With conventional hemodialysis a large portion of the urea is cleared from the blood on a single pass through the dialyzer. The urea clearance cannot be increased much further by increasing the dialyzer membrane capacity or dialysate flow.\textsuperscript{7, 8} The case is different, however, for non-urea solutes. Membrane capacity, as assessed by the mass transfer area coefficient $K_oA$, declines in approximate proportion to the square root of solute mass.\textsuperscript{9} A dialyzer's $K_oA$ for solutes with mass 240 Da is thus approximately half that of its $K_oA$ for urea with mass 60 Da. Viscosity further lowers the effective $K_oA$ of a dialyzer for removal of solutes from plasma as compared
to aqueous solutions. The predicted clearances of free solutes thus decline with increasing mass as depicted in Figure 1.

Because toxicities have not been proven for solutes in the mass range depicted in Figure 1, there has been limited effort to increase their clearances. Here we encounter a recurrent problem in dialysis research which is reminiscent of the conundrum which gave the novel "Catch 22" its title. We can't prove that solutes are toxic without lowering their levels, and we don't develop means to lower their levels without proof that they are toxic. The hope that dialysis can be miniaturized for ambulatory treatment has however stimulated the development of new membrane materials. Such materials could allow dialyzer $KoA$ values to be greatly increased without increasing dialyzer size.

**Middle Molecules**

Early dialysis membranes were impermeable to solutes with molecular weight much greater than 400 Da. Dialysis with these membranes reversed uremic coma and kept patients alive for years. Researchers hypothesized however that removal of larger solutes would improve health. It was initially suggested that toxic larger solutes had molecular weights in the range between 300 and 2000 Da, and they were thus designated "middle molecules." The meaning of "middle molecules" has changed over time to include solutes with molecular weights between 600 and 45,000 Da. Most of these are small proteins with molecular weights above 10,000 Da. Efforts to increase the clearance of such solutes were stimulated by the finding that accumulation of $\beta_2$ microglobulin caused amyloidosis in dialysis patients. $\beta_2$ microglobulin with molecular
weight 12,000 Da was indeed adopted as a prototypical middle molecule just as urea had been adopted as a prototypical free, low-molecular weight solute.

The clearance of solutes in the size range of $\beta_2$ microglobulin was initially increased by making dialysis membranes permeable to larger solutes. Use of these "high flux" membranes provided $\beta_2$ microglobulin clearances in the range of 20 ml/min. The HEMO study failed to show clear benefit from increasing $\beta_2$ microglobulin clearance to this level as compared to the few ml/min achieved with "low flux" membranes. $^{12}$

Researchers however responded to this failure differently than to HEMO's failure to show benefit with increased clearance of urea. Efforts were made to further increase the clearance of low molecular proteins. Rapid passage of blood through the dialyzer does not allow sufficient time for large molecules to be cleared by diffusion even if the dialysis membrane is permeable to them. Their clearance however can be increased by hemodiafiltration which adds convective clearance to dialytic clearance. Convective clearance can also be added to dialytic clearance by manipulating blood and dialysate pressures within a dialysis cartridge. $^{13, 14}$

Increasing $\beta_2$ microglobulin clearances to approximately 80 ml/min by hemodiafiltration has so far failed to clearly improve outcomes in patients enrolled in clinical trials. $^{15}$ Proponents of the technique note that patients who have achieved the highest ultrafiltration volumes have appeared to benefit. $^{16, 17}$ A randomized trial is now being conducted to more rigorously test the benefit of high volume ultrafiltration. $^{18}$

Efforts to further increase the clearance of middle molecules are also ongoing. Some efforts have been made to increase the clearance of $\beta_2$ microglobulin and other low molecular weight proteins by passing blood over sorbent columns. $^{19}$ Currently, such
protein sorbent columns are being considered largely for treatment of sepsis and associated acute kidney insufficiency.\textsuperscript{20} Other efforts are directed toward increasing the clearance of low molecular weight proteins which are larger than $\beta_2$ microglobulin. New dialyzers can clear such solutes by using "medium cut-off" membranes which are permeable to solutes with molecular weight up to 50 kDa combined with designs which promote internal convection.\textsuperscript{21}

Our ability to increase clearances of $\beta_2$ microglobulin and even larger solutes has revealed a fundamental pathophysiological problem. Plasma levels may not fall in proportion to the increase in solute clearances, particularly when treatment is intermittent.\textsuperscript{22} In the HEMO study, increasing the average $\beta_2$ microglobulin clearance by more than fivefold reduced the average plasma level by only 20 percent.\textsuperscript{23} This apparent discrepancy may be attributable to two factors.\textsuperscript{24} First, a low but continually operating non-renal clearance accomplishes a large portion of $\beta_2$ microglobulin removal. Second, $\beta_2$ microglobulin movement from the interstitium to the plasma is restricted and plasma $\beta_2$ microglobulin levels rebound following rapid removal from the plasma during intermittent dialysis or hemodiafiltration. It seems likely that these factors also limit the extent to which high renal replacement clearances can lower levels of other middle molecules. It is notable that increasing the clearances of solutes with molecular weight greater than 20 kDa using "medium cut-off" membranes has generally failed to lower their plasma levels.\textsuperscript{25-27} These findings should stimulate further investigation of the largely unknown mechanisms by which low-molecular weight proteins are cleared outside the kidney at a low rate. We might be able to increase this non-renal clearance in patients whose kidneys have failed.
Protein-Bound Solutes

The protein-bound solutes are small molecules that bind to plasma proteins, with known examples binding largely to albumin.\textsuperscript{28-30} Conventional dialysis clears them poorly because only the free portion of the solute contributes to the concentration gradient driving their diffusion from the plasma to the dialysate.\textsuperscript{31} There has been much less clinical study of increasing the clearance of protein-bound solutes than of increasing the clearance of middle molecules. Looking back, it appears that this may have been because no single bound solute was shown to have a specific ill effect like the amyloidosis caused by accumulation of $\beta_2$ microglobulin.

The clearance of bound solutes can be increased by increasing the free fraction of the solute as blood passes through the dialyzer. One attractive means to accomplish this is addition of displacing agents to the blood entering the dialyzer.\textsuperscript{32} Madero et al.\textsuperscript{33} recently showed that infusion of ibuprofen could significantly increase the clearance of the bound solutes indoxyl sulfate and $p$-cresol sulfate during single dialysis treatments. Successful chronic treatment will require identification of displacing agents which can be repeatedly administered in sufficient concentrations without ill effect. Alternative agents have been considered but not yet shown to satisfy this requirement.\textsuperscript{34}

Imposing physical-chemical changes could also increase the free fractions of bound solutes as blood passes through the dialyzer. The free fraction of many bound solutes can be increased by lowering the blood pH.\textsuperscript{35} Clinical testing has been restricted to preventing a rise in blood pH during hemodialysis treatment rather than lowering the blood pH.\textsuperscript{36} This had only a limited effect on the clearance of protein-bound uremic solutes, and whether reduction of blood pH below physiologic levels
would have a greater effect remains to be tested. Another potential means to increase the free fraction of bound solutes is to increase the tonicity of the blood as it flows through the dialyzer. As with changes in pH, large changes in tonicity may be required to increase the free fractions of bound solutes, and the extent to which such changes can be safely imposed remains uncertain. It has also been suggested that the clearance of bound solutes can be increased by the imposition of electrical fields, possibly in conjunction with the use of new composite membrane materials.

Sorbents provide an alternate means to remove uremic solutes which bind to plasma proteins. Early workers attempted to clear uremic solutes by direct passage of blood over activated carbon. Contact of blood with carbon however caused platelet consumption and other complications. These complications were largely avoided by coating carbon granules with cellulose acetate or other materials. Hemoperfusion using coated carbon cartridges has since been used largely to remove poisons. Cartridges remain available but evidence for efficacy is lacking and their use has declined where hemodialysis is available.

Hemoperfusion over coated sorbent granules provided limited clearance because solutes which diffuse through the coating cannot readily permeate the interior of the granules. Several strategies have been envisioned to improve plasma solutes' access to sorbents. The first is to create sorbents which allow direct hemoperfusion by taking up solutes of interest without adversely affecting other blood constituents. Modern materials science provides a variety of means to create such sorbents. Clinical testing, however, has been limited and ability to enhance the removal of protein-bound uremic solutes has not been demonstrated.
A second strategy for sorbent removal of bound solutes is to separate the plasma from the cellular components of the blood using a membrane with a molecular cut-off of 250 to 300 kDa. The plasma stream created by this "plasma fractionation" can then be passed over sorbents to remove bound solutes. This strategy was developed largely for the treatment of liver failure and its effect was measured by removal of bilirubin and bile acids. Limited trials showed that it could increase the clearance of protein-bound solutes from patients with end stage renal failure (ESRD). Testing in ESRD was however complicated by coagulation abnormalities and therefore abandoned.

A third strategy for sorbent removal of bound solutes in hemodialysis is to add a sorbent to the dialysate compartment. This has the effect of reducing the solute concentration in the dialysate compartment toward zero and thereby increasing the concentration gradient across the dialysis membrane. Because the free solute concentration of a highly bound solute in the plasma remains low a high capacity membrane is required to achieve high clearances of bound solutes with sorbent addition to the dialysate compartment. Indeed adding a sorbent to the dialysate compartment has the same effect on bound solute clearances as greatly increasing the dialysate flow. Pilot clinical studies have shown that the bound solute clearances achieved with conventional hemodialysis can be increased significantly by increasing dialyzer membrane capacity together with dialysate flow.

An obvious candidate sorbent for addition to the dialysate compartment is albumin. Solutes bound to albumin in a patient's plasma would pass through the dialysis membrane and be absorbed onto albumin in the dialysate compartment. Two
designs for "albumin dialysis" have been considered. In "single pass albumin dialysis" the patient is dialyzed against an albumin solution which is discarded after passage over the dialysis membrane. In "sorbent recirculating dialysis" the patient is dialyzed against an albumin solution which is itself then dialyzed against standard dialysate in a second dialyzer to remove unbound solutes and electrolytes and then passed through sorbent cartridges to remove bound solutes before being recirculated to dialyze the patient. Like plasma fractionation, albumin dialysis has been developed as a short term treatment for liver failure. Questionable efficacy and great expense have discouraged consideration of its use as renal replacement therapy.

Other sorbents can also be added to the dialysate compartment to increase the diffusive clearance of protein-bound solutes. This has so far been tested only in vitro with activated carbon being the sorbent most frequently used. Various configurations for addition of activated carbon to the dialysate stream have been envisioned, as illustrated in Figure 2. Perhaps the simplest design is for addition of a sorbent to the dialysate stream. This is equivalent to "albumin dialysis" with the use of a sorbent other than albumin. Alternate designs would fix the sorbent in different positions in the dialysate stream. As is the case with plasma separation, sorbent addition to the dialysate stream has been considered more extensively for the treatment of liver failure than kidney failure. In one design, part of the dialysate stream would be passed over a sorbent and then added to the fresh dialysate being pumped past the dialysis membrane (Fig 2B). The effect would be to greatly increase the effective dialysate flow for solutes taken up by the sorbent and increase their clearances by keeping their concentrations low in the dialysate compartment. This design might have particular
application in home hemodialysis in which low dialysate flows are commonly prescribed to limit the cost and complexity of in-home dialysate production. Another design would be to insert a sorbent cartridge in the dialysate path of two dialyzers used in series (Fig 2C). This configuration has the advantage that it could be tested using standard dialyzers and dialysis machines. Perhaps the optimal configuration for sorbent addition to the dialysate compartment would be to fix sorbent in the dialysate compartment along the length of a dialyzer (Fig 2D). Of note, sorbent fixation to the dialysis membrane was tested early during the development of hemodialysis however its effect on bound solute clearances was not evaluated. The recent development of a mixed matrix hemodialysis membrane in which activated carbon is incorporated into the membrane material represents a technical advance along these lines. The performance of mixed matrix membranes could potentially be enhanced by an "outside-in" design, with dialysate flowing through hollow fibers while blood flows outside the fibers.

While activated carbon has been the sorbent most commonly considered for addition to the dialysate stream, other materials could provide special benefits or greater safety. Addition of lipids to the dialysate was initially evaluated as a means to improve removal of drugs which bind to both lipids and plasma proteins. Addition of lipid to the dialysate could potentially increase the clearance of as yet unknown uremic toxins that bind to circulating lipids more than to proteins. It has also recently been suggested that liposomes could be added to the dialysate to absorb uremic solutes and thereby increase their dialytic clearance.

**What Next**
We have means, as described above, to increase the clearances of various types of solutes. We have not however identified those solutes which are most toxic and therefore most important to remove. This lack of information is a major impediment to progress. If we knew which solutes were toxic, we could refine our proposed methods for solute clearance. Sorbents which remove specific solutes from the blood or dialysate, displacing agents which release specific solutes from binding proteins, and active membrane materials which selectively pass or chemically degrade specific solutes could be devised. Solutes whose behaviors are not adequately characterized under our current classification scheme may require additional consideration, including solutes that bind to plasma lipids and solutes that move into or out of erythrocytes during conventional dialysis. 68, 69

"Metabolomic" studies employing untargeted mass spectrometry have provided a new means to identify toxic uremic solutes. This analytic method has increased the number of known uremic solutes to more than 250 and additional solutes continue to be identified. 3, 70-73 Large scale studies will be required, however, to associate levels of individual solutes with clinical and physiological endpoints. An alternate means to identify toxic solutes is to try to increase the clearance and lower the levels of whole groups of solutes. Positive clinical effects could both improve current treatment and provide direction to our search for specific toxins. Efforts to improve the removal of large middle molecules are ongoing as described above. Means to improve the removal of protein-bound solutes have been much less extensively tested in patients. A clinical trial of adding activated carbon to the dialysate stream using the configurations depicted in Figure 2B or 2C might speed progress in this area. Such a trial could be
performed with only modest modifications to existing hemodialysis equipment. A positive result would spur development of more effective means to clear bound solutes. A question attracting current clinical interest is the relative value of residual native kidney function to dialysis.\textsuperscript{74, 75} The ratio of residual to dialytic clearance for individual solutes is highly variable.\textsuperscript{24, 76} Better knowledge of the extent to which residual function allows dialysis to be curtailed could help identify the solutes which are most toxic.

Finally, we face the question of the how solute levels respond to changes in their extracorporeal clearances. The failure of $\beta_2$ microglobulin levels to fall in proportion to increases in the extracorporeal $\beta_2$ microglobulin clearance has been noted above. Other studies suggest that plasma levels of the commonly studied bound solute p-cresol sulfate are unaffected by large changes in its time-averaged dialytic clearance.\textsuperscript{77-80} This phenomenon remains unexplained but could reflect changes in solute production combined with non-renal clearance and/or a complex compartmental distribution. A question of particular current interest is the value of continuous clearance supplied by wearable or implantable dialysis machine.\textsuperscript{10, 81} The value of continuous as opposed to intermittent clearance can depend on a solute's dialytic clearance relative to its volume of distribution within the body as depicted in Figure 3. Overall, we need more knowledge not only of solute toxicity but also of solute generation and disposition within the body to improve our methods for solute removal.
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Figure Legends

Figure 1. The effect of increasing solute molecular weight on clearance of solutes from the plasma with conventional hemodialysis. The solid line depicts clearance values obtained with a standard dialyzer with $KoA_{\text{urea}}$ in aqueous solution of 1400 ml/min as assessed by the manufacturer and the dashed line depicts clearance values obtained with a dialyzer with a $KoA_{\text{urea}}$ twice that high. $KoA$ values in plasma were reduced by a factor of 0.52 as compared to $KoA$ in aqueous solution and $KoA$ was assumed to decrease in proportion $m^{-0.46}$ where $m$ is the solute molecular weight as described by Schneditz.\textsuperscript{9} Clearance values were modeled for a plasma flow of 250 ml/min and a dialysate flow of 600 ml/min using a published model. Doubling $KoA$ has little effect on the clearance of very small solutes but a larger effect on the clearance solutes with molecular weight in the range 400 to 2000. The clearance of such solutes, which includes the lower end of the range now classified as "middle molecules" could be increased simply by increasing the size of dialyzers made with current membrane materials. The addition of convection, which is now employed to increase the clearance of low molecular weight proteins like $\beta_2$ microglobulin with molecular weight approximately 12,000 Da, would not be required.

Figure 2. Potential configurations for sorbent addition to the dialysate compartment to improve the clearance of protein bound solutes. A). In standard hemodialysis without any sorbent, blood (pink shaded) flows at a rate $Q_B$ past a semipermeable membrane (dashed line) with size $KoA_x$ for solute x with dialysate (blue shaded) flowing at a rate $Q_D$ in the opposite direction on the other side of the membrane. Uremic solutes (not shown) diffuse from the blood into the dialysate which goes down the drain. Different configurations for addition of a sorbent to lower the concentrations of bound solutes in the dialytic compartment have been considered. B). Part of the dialysate stream now is diverted and flows at a rate $Q_{DR}$ over a sorbent (gray shaded area) before being reintroduced into the stream of fresh dialysate entering the system at a flow rate of $Q_D$. The effective dialysate flow for a given solute is determined by the extent to which the sorbent takes up that solute. C). Blood passes thought two dialyzers in series and a sorbent cartridge is inserted in the dialysate
stream between the two dialyzers. D). Sorbent material is fixed along the dialysate path within a dialyzer.

Figure 3. Predicted plasma solute levels with continuous dialysis using a wearable dialyzer (blue lines) compared to 8 hours of nocturnal dialysis providing 10 fold higher solute clearances (red lines, with time averaged concentrations as dashed lines). Levels are depicted over the course of 24 hours for urea and two solutes which are normally cleared by tubular secretion. Solute A is not protein-bound and normally cleared at 540 ml/min by the kidneys with a volume of distribution of 14 liters. Solute B is normally 98 percent bound and has a kidney clearance of 23 ml/min and a volume of distribution of 13 liters in terms of its total plasma levels. The figure is scaled so that plasma free levels would be 1.0 for each solute in humans with normal kidney function. The continuously operating wearable dialyzer provides a urea clearance of 17 ml/min equal to that of the device described by Gura et al.\textsuperscript{82} Dialytic clearances of the secreted solutes are adjusted downwards relative to urea to 10 ml/min for the unbound solute and 1.3 ml/min for the bound solute based on dialytic clearances of phenylacetylglutamine and p-cresol sulfate observed by Sirich et al.\textsuperscript{83} The figure shows first that plasma levels of solutes normally cleared by secretion are poorly controlled by dialysis whether provided continuously or intermittently. Levels of urea are maintained within 4-fold normal by both treatments and must be plotted on an expanded scale for their diurnal variation be apparent, while levels of the secreted solutes remain more than 20-fold normal. Compared to continuous dialysis, a higher clearance during nocturnal treatment can control average solute levels but will allow wide diurnal variation in the levels of those solutes for which the dialytic clearance is high relative to their volume of distribution. The control of a solute’s plasma level with continuous dialysis compared to intermittent dialysis is highly dependent on the solute’s volume(s) of distribution and compartmental behavior.
Figure 1
Figure 3

Urea vs. Secreted Solutes over Hours