The Next Step from High-Flux Dialysis: Application of Sorbent Technology

James F. Winchestera  Claudio Roncob  James A. Bradya  Larry D. Cowgillc
Jamie Salsberga  Eric Youshaa  Mike Choquettea  Robert Albrighta
Jonathan Clemmera  Vadim Davankovd  Maria Tsyurupad
Ludmila Pavlovad  Mikhail Pavlov  Gerald Cohene  Walter Hörl  
Frank Gotchf  Nathan W. Levin
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aRenalTech International, New York, N.Y., USA; bOspedale San Bortolo, Vicenza, Italy; cVeterinary Medicine Teaching Hospital, University of California Davis, Sacramento, Calif., USA; dRussian Academy of Sciences (INEOS), Moscow, Russia; eUniversity of Vienna, Austria; fUniversity of California San Francisco, Calif., USA and gRenal Research Institute, New York, N.Y., USA

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Abstract
The current foci of renal replacement therapy with dialysis are middle molecular weight toxins, consisting of small proteins, polypeptides and products of glycosylation and lipoygenation. Conventional high-flux dialysis is not efficient at removing these molecules, explaining the increased interest in using sorbents to supplement dialysis techniques. Prototype biocompatible sorbents have been developed and investigated for middle molecule removal; these have been shown, in man, to remove β2-microglobulin, angiogenin, leptin, cytokines and other molecules, without reducing platelets and leukocytes. Extensive clinical studies are underway to demonstrate the clinical utility and safety of adding routinely a sorbent hemoperfusion device to hemodialysis.

Introduction
In the last three decades, sorbent technology has been applied to increase the efficiency of dialysis, or replace it, in renal replacement therapy. Regeneration of dialysate with sorbents is an accepted modification of dialysis, and sorbent hemoperfusion is gaining ground as a valuable adjunct to dialysis, especially as new sorbents are developed. The National Cooperative Dialysis Study (NCDS) established the first rational basis for prescription of dialysis based on the quantity of urea removed – Kt/V urea [1]. Despite improved dialysis prescription and delivery, benefit survival for long-term dialysis patients remains of concern [2]. The HEMO study, currently underway in the USA, is evaluating outcomes between two levels of Kt/V urea higher than in the NCDS, high- and low-flux dialyzers [3], dialyzer reuse [4], vascular access [5] and nutrition [6]. The discovery of uremic toxins [7, 8], other than urea and creatinine, has stimulated several investigations on alternatives to standard or high-flux hemodialysis, to remove these molecules. These methods include hemodiafiltration with [9] or without [10, 11] dialysate regen-
eration using sorbents, as well as hemoperfusion using adsorbents such as charcoal [12] and resins [13].

Interest in middle molecular weight substances currently dominates research in hemodialysis. Inflammatory mediators (CRP, cytokines [14, 15], advanced glycosylation end-products (AGEs), advanced lipoxygenation compounds (ALEs), carbonyl and oxidative stress [16]) may produce malnutrition [17], wasting and increased levels of β2-microglobulin [18]. High-efficiency hemodiafiltration may reduce some of these proteins, with the high cost of ultrapure replacement fluid; on-line generation of replacement fluid may become practical in the future. Sorbent hemoperfusion for removal of these toxins is attractive in offering low cost and high-efficiency removal for certain substances. β2-Microglobulin (11.8 kDa) is a candidate toxin for sorption. It is implicated in dialysis-related amyloidosis (DRA) [19]; its glycosylation [20], and deposition in amyloid fibrils, with delayed release from a deep body pool [21], produces bone cysts, severe carpal tunnel syndrome and crippling arthritis [22].

While it appears there is a survival advantage to reduced β2-microglobulin concentrations in dialysis patients [23], it is known that cellulose membranes do not allow removal of β2-microglobulin, while reuse of PMMA and other membranes impairs its mass transfer [24, 25] and CAPD does not contribute to its removal [26]. Transplantation with normal renal function causes reduction in β2-microglobulin concentrations.

**Sorbents: Theory and Practice**

Sorbents remove solutes from solution through adsorption. The efficiency of this process is dependent both on the nature of the solute and the sorbent. The attractive forces between solute and sorbent can be generally classified as specific or nonspecific adsorption. Specific adsorption relies on the presence of tailored ligands or antibodies that have high selectivity for their targets. Specific adsorbents have seen clinical use in the treatment of autoimmune disorders such as idiopathic thrombocytopenic purpura [27] and for the removal of lipids in familial hypercholesterolemia [28]. Specific adsorbents, though highly selective, are often expensive and have a low capacity for toxin removal.

Nonspecific adsorbents, typically charcoal and resins, attract solutes through a variety of forces including hydrophobic interactions, ionic (or electrostatic) attraction, hydrogen bonding, and van der Waals interactions. Manipulating the porous structure can increase the selectivity of nonspecific adsorbents for particular solutes. In this case, solute molecules are separated according to their size by their ability to penetrate the porous network of the beaded sorbent. For targeting middle molecular weight uremic toxins (MW 4–30 kDa), a population of pores between 4 and 10 nm will permit the entrance of these toxins to the adsorbent interior of the bead, while excluding larger proteins, such as albumin (MW 69 kDa, molecular diameter 22.6 nm), from the adsorptive surface [29]. The introduction of ion exchange groups can also significantly increase the affinity of resin sorbents for bilirubin [30] and endotoxin [31].

Adsorption capacity for resins and charcoal is often quite high, in excess of 500 m²/g of adsorbent. These materials are also relatively cheap to produce and are used in several commercial applications. Until recently, poor hemocompatibility, as evidenced by thrombocytopenia and neutropenia [32], has been the major clinical limitation of these materials. Newer resin adsorbents appear to have solved this issue with the addition of a hemocompatible coating [33].

**In vitro Results and Animal Testing**

To optimize resin performance (maximum middle molecule removal with minimum impact on albumin), porosity has been adjusted to maximize the capacity for middle molecular weight uremic toxin uptake. In the high-grade polymer formulation designed for clinical studies, the surface area (BET analysis) is 650 m²/g. In perfusion experiments, human β2-microglobulin (Cortex Biochem, Calif., USA) was added to human plasma to achieve an initial β2-microglobulin concentration of 38 mg/l. The resin has reduced the concentration of β2-microglobulin by 99.2% after 4 h of perfusion (fig. 1). In vitro, cytochrome C – a surrogate marker for β2-microglobulin – uptake was 82.2 mg cytochrome C/g of dry polymer after 3 h of incubation (fig. 2).

Safety has been demonstrated by testing for biocompatibility in vivo in healthy canines. In fifteen 300-min hemoperfusion treatments using 5 normal dogs and a device containing 100 ml of polymer, leukocytes and platelets decreased initially (13% and 27% at 15 min, respectively) with complete recovery of white blood cells by the end of the treatment period. No adverse effects were associated with any of the treatments, and the prototype resin was deemed highly compatible with canine blood [34].
Clinical Results

Using nonspecific agents such as activated charcoal [35], hemoperfusion has been shown to enhance ‘middle molecule’, amino acid and creatinine removal in dialysis patients. All sorbent systems are limited in sorption of water, urea and acidic species, and must be combined with dialysis.

We now have experience with a prototype hemoperfusion device, containing 300 g of a hydrated cross-linked polystyrene divinylbenzene resin, of pore structure designed to remove molecules between 4 and 30 kDa. The resin beads were prepared with a blood-compatible coating, confirmed to be biocompatible in vivo in animals [34], and to possess superior biocompatibility ex vivo in human volunteers [36]. In two long-term dialysis patients with high plasma concentrations of β2-microglobulin, we combined the device in series with high-flux hemodialysis for 3–6 weeks in 2 patients. Combined hemodialysis and hemoperfusion reduced β2-microglobulin by approximately 70% in each separate session; predialysis β2-microglobulin was reduced by 29.6% in 1 patient over a period of 3 weeks (fig. 3). It should be pointed out that the treatment period of 3–6 weeks is very short and further reductions could be possible with more prolonged treatment. We also calculated that an average of 140 mg β2-microglobulin was removed (average of two sessions) by analysis of the plasma time concentration profiles (table 1). The device was well tolerated clinically, producing no changes in platelet or leukocyte counts during the procedure. No changes in total protein and albumin concentrations were observed.

In addition to removal of β2-microglobulin, we also observed reductions in leptin, measured by immunoassay (Human Leptin Quantikine Immunoassay Kit, R&D Systems, Minneapolis, Minn., USA) of approximately 32%, and sequential reduction of predialysis leptin (16 kDa) of 22% after 3 weeks in 1 patient (fig. 4) and 37% after 3 weeks in the other patient. A 30.5% average reduction in angiogenin (homologous to degranulation inhibitory protein I (DIP I), 14.4 kDa) was observed; an immunoassay (Human Angiogenin Quantikine Immunoassay Kit, R&D Systems) formed the basis of the measurement.

Another resin, cellulosic beads to which ligands trapping β2-microglobulin are attached (Kaneka Corp., Osaka, Japan), hemoperfusion device has also been shown to reduce β2-microglobulin concentrations during extracorporeal treatment, with removal of β2-microglobulin and partial regression of DRA over 6–13 months; no data were given on platelet and leukocyte function [37]. In a recent report, modification of the device (reduced priming volume from 177 to 85 ml) has demonstrated 123–190 mg removal of β2-microglobulin with the larger device and 80–98.6 mg with the smaller device [38] (table 1). The device is approved for established DRA in Japan for 1 year of treatment. Its high cost has precluded more widespread use after the first year of treatment.
Fig. 3. Response of plasma concentrations of β₂-microglobulin to three times weekly combined hemoperfusion and hemodialysis using a prototype resin hemoperfusion device (BetaSorb™) over a short period of 3–6 weeks.

Fig. 4. Response of plasma concentrations of leptin in 1 patient to three times weekly combined hemoperfusion and hemodialysis using a prototype resin hemoperfusion device (BetaSorb™).

Table 1. Comparative changes in blood components of prototype BetaSorb™ hemoperfusion device and Lixelle hemoperfusion device (S-15)

<table>
<thead>
<tr>
<th>Parameter reduction per session</th>
<th>BetaSorb™ prototype</th>
<th>Lixelle S-15</th>
</tr>
</thead>
<tbody>
<tr>
<td>β₂-Microglobulin, mg</td>
<td>140</td>
<td>80.5–98.6</td>
</tr>
<tr>
<td>Angiogenin, %</td>
<td>30.5</td>
<td>Not reported</td>
</tr>
<tr>
<td>Leptin, %</td>
<td>32</td>
<td>Not reported</td>
</tr>
<tr>
<td>Platelets</td>
<td>None*</td>
<td>Not reported</td>
</tr>
<tr>
<td>WBC</td>
<td>None*</td>
<td>Not reported</td>
</tr>
</tbody>
</table>

* Not corrected for hemoconcentration.

Discussion

Hemodialysis using the synthetic membranes Arylante (Hospal Renal Care, Lyon, France) or Fresenius Polysulfone (Fresenius Medical Care, Bad Homburg, Germany), in addition to removing β₂-microglobulin (170 and 110 mg per session, respectively), are associated with moderate removal of other small molecular weight proteins (10 and 7 g, respectively) [39]. Studies have shown that a 53% reduction of β₂-microglobulin can be achieved using synthetic membranes (T-sulfone, Toray, Japan) [40]. Lornoy et al. [41] have recently shown that after 10 years of either hemodiafiltration (which removes about 340 mg β₂-microglobulin per session) or biocompatible membrane hemodialysis, that β₂-microglobulin-associated bone disease is present in 25% and carpal tunnel syndrome in 12.5%. To our knowledge, the biocompatibility of the adsorbent resin used in the device used in our clinical study, as measured by platelet and leukocyte counts, is superior to devices used currently or abandoned. In view of this we intend to seek application of hemoperfusion for chronic use in dialysis to achieve sustained reduction in β₂-microglobulin, to measure β₂-microglobulin kinetics, and to examine whether a decrease in β₂-microglobulin prevents and reverses DRA. The inability to demonstrate adsorption of albumin in our study contrasts with protein removal as described in the hemodiafiltration study of Lornoy et al. [41]. Our device will therefore likely not contribute to any existing or developing malnutrition. The adsorptive resin used here, in addition to removing leptin...
and angiogenin, is also capable of removing other middle molecular weight uremic toxins, such as the pro-inflammatory cytokines TNF-α and IL-1β [42].

Much more clinical experience is needed with sorbent devices in uremia. Clinical trials are planned for regulatory approval, and trials are being devised to answer important clinical outcome questions, such as improvement in infection, atherosclerosis, DRA and other dialysis-related complications.

References


