Artificial Organs

Selective Cytopheretic Inhibitory Device With Regional Citrate Anticoagulation and Portable Sorbent Dialysis

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Abstract: Selective cytopheretic inhibitory device (SCD) therapy is an immunomodulatory treatment provided by a synthetic biomimetic membrane in an extracorporeal circuit, which has shown promise in preclinical large animal models of severe sepsis as well as in clinical trials treating patients with acute kidney injury and multiple organ failure. During SCD therapy, citrate is administered to lower ionized calcium levels in blood for anticoagulation and inhibition of leukocyte activation. Historically, citrate has been known to interfere with sorbent dialysis, therefore, posing a potential issue for the use of SCD therapy with a portable dialysis system. This sorbent dialysis SCD (sorbent SCD) would be well suited for battlefield and natural disaster applications where the water supply for standard dialysis is limited, and the types of injuries in those settings would benefit from SCD therapy. In order to explore the compatibility of sorbent and SCD technologies, a uremic porcine model was tested with the Allient sorbent dialysis system (Renal Solutions Incorporated, Fresenius Medical Care, Warrendale, PA, USA) and concurrent SCD therapy with regional citrate anticoagulation. The hypothesis to be assessed was whether the citrate load required by the SCD could be metabolized prior to recirculation from systemic blood back into the therapeutic circuit. Despite the fact that the sorbent SCD maintained urea clearance without any adverse hematologic events, citrate load for SCD therapy caused an interaction with the sorbent column resulting in elevated, potentially toxic aluminum levels in dialysate and in systemic blood. Alternative strategies to implement sorbent-SCD therapy will be required, including development of alternate urease-sorbent column binding chemistry or further changes to the sorbent-SCD therapeutic circuit along with determining the minimum citrate concentration required for efficacious SCD treatment. **Key Words:** Sorbent dialysis—Citrate—Cytopheresis—Immunomodulation.

Systemic inflammatory response syndrome from either sepsis or acute organ failure is a leading cause of death in critically ill patients in the United States (1,2). Despite prompt treatment with antibiotics, fluid resuscitation, and artificial organ function support, mortality rates still exceed 30% (3,4). Selective cytopheretic inhibitory device (SCD) therapy is an immunomodulatory treatment recently reported to improve survival time in a preclinical large animal model of severe sepsis (5) and in clinical trials treating patients with acute kidney injury and multiple organ dysfunction syndrome (MODS) (6–8). The SCD is composed of a synthetic biomimetic membrane that mimics the environment of capillary beds where leukocyte extravasation may occur in order to bind and sequester leukocytes from the systemic circulation in an extracorporeal blood circuit. The low-velocity, low-shear blood flow path around bundles of polysulfone fibers reproduces capillary shear conditions to bind activated leukocytes during a systemic inflammatory disease state. To further minimize the systemic effects of activated leukocytes, the blood is anticoagulated with regional citrate infusion to lower blood ionized calcium (iCa) levels to 0.2–0.5 mM, which inhibit the coagulation system of the blood. This lowering of blood iCa also has an inhibitory effect on neutrophil (NE) activation (9), thereby simultaneously combining the SCD effect to sequester activated circulating leukocytes and limit the potential activation of leukocytes entering the SCD and the low-iCa environment.

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Battlefield injuries, earthquakes, tsunami, explosions, and other causes of building collapse have the potential to produce many cases of attendant acute renal failure (ARF) in areas where access to purified water for dialysis may not be available (10,11). Portable sorbent dialysis systems have been developed, requiring as little as 6 L of tap water to regenerate dialysate, making dialysis treatment available at new sites including field hospitals (12–14). For a review of sorbent technologies, see Agar (12). A promising synergistic approach to treat sepsis, ARF, and MODS in water scarce settings is to combine SCD and portable sorbent dialysis therapies. In this sorbent dialysis-SCD (sorbent-SCD) treatment, the immunomodulatory properties of the SCD would be leveraged to counteract the progression of sepsis, ARF, or MODS, while the sorbent dialysis system would be used for small solute clearance, for removal of toxins, and to provide an extracorporeal platform for the SCD therapy.

Optimal SCD therapy is dependent on the administration of citrate, which is currently thought to modulate leukocyte adhesion to the polysulfone hollow fibers of the SCD, as well as modulate NE release from noncirculating pools (5). Heparin is the preferred anticoagulation therapy during sorbent dialysis because previous studies have shown that citrate can interact with sorbent columns, resulting in the release of aluminum complexes (15). High aluminum blood levels may thereby occur (16–18). An approach to integrate the two potentially incompatible therapies into a combined sorbent dialysis-SCD therapy is to use systemic heparin coagulation for sorbent compatibility and regional citrate administration within the SCD extracorporeal circuit in order to attempt to isolate the citrate from the sorbent column. The working hypothesis being that the citrate administered postsorbent column would not be exposed to the sorbent column during the first pass of circulation, avoiding citrate-sorbent column interactions, and furthermore that citrate would be metabolized in the body prior to blood recirculation into the therapeutic circuit.

MATERIALS AND METHODS

Porcine model of uremia

A uremic state was achieved via bilateral renal artery ligation with the organs left in situ, along with administration of a urea load in a porcine model. All procedures were approved by the University Committee for Use and Care of Animals at the University of Michigan.

Pigs (60 kg) were placed under general anesthesia using xylazine hydrochloride (2.2 mg/kg i.m.) and Telazol (Phoenix Pharmaceutical Inc., Burlingame, CA, USA) (6 mg/kg i.m.), then intubated and maintained on isoflurane (2–4%) with the balance oxygen for the duration of the study period. A catheter was placed into the right carotid artery and the right external jugular vein provided access for drug administration and blood pressure monitoring. An 11-Fr 20-cm Brevia short-term hemodialysis catheter was inserted into the left external jugular vein using a modified Seldinger technique and connected to the hemodialysis circuit. The renal pedicle was then approached via ventral midline laparotomy for bilateral renal artery ligation with the organs left in situ.

Urea was administered intravenously to achieve elevated blood urea-nitrogen (BUN) levels required by the Allient sorbent system (Renal Solutions Incorporated, Fresenius Medical Care, Warrendale, PA, USA), which was designed to remove urea and other low molecular weight toxins from uremic patients (14). A 10% urea solution in 5% dextrose in water was administered intravenously to achieve systemic BUN >75 mg/dL at the beginning of the study, simulating a uremic condition. The premixture and infusate were calculated according to total body water, starting BUN, and anticipated urea clearance by the Allient system in order to maintain higher than normal BUN throughout a 6-h study.

Following systemic heparinization (100 U/kg i.v.), 6 h of hemodialysis was administered using the Allient sorbent system connected in series with the SCD. Six liters of tap water was used to create the dialysate, which was regenerated throughout the study. Citrate regional anticoagulation was achieved with anticoagulation citrate dextrose-A (ACD-A, Baxter, Deerfield, IL, USA), which was infused into the blood line pre-SCD while 2% $CaCl_2$ was infused into the venous return post-SCD to compensate for iCa bound by citrate. Arterial electrolytes, BUN, creatinine, and citrate levels were monitored in the systemic circulation, in dialysate, and at various locations in blood circuit. Dialysate was tested hourly to detect ammonia breakthrough. Blood samples were collected systemically and in the extracorporeal circuit, which was sampled presorbent, postsorbent, pre-SCD, and post-SCD to determine efficacy and compatibility of the sorbent-SCD treatment.

SCD

The SCD is a column with an inlet and outlet for flow, containing porous polysulfone hollow fibers with an inner diameter of 200 μm, a wall thickness of 40 μm, and a molecular weight cutoff of 40–50 kDa

(5). Blood flow is directed to the extracapillary space (ECS) of the SCD. SCD used for these studies had a calculated outer membrane surface area of 0.98 m^2 and was supplied by CytoPherx, Inc. (Ann Arbor, MI, USA).

Allient sorbent dialysis system

An Allient sorbent dialysis system was utilized with either SORB+ or HISORB+ sorbent columns (Renal Solutions Incorporated). SORB+ columns have an approximate total urea-nitrogen capacity of 9.5–23.5 g, which were used in studies 1–5, while HISORB+ columns have a total urea-nitrogen capacity of 23.5–35.0 g, which were used for studies 6 and 7.

Extracorporeal circuit design

Two extracorporeal circuit designs were evaluated in a pilot study, with the sorbent dialysis system and SCD either in parallel or in series. In the sorbent-SCD parallel circuit configuration, blood was perfused at a rate of 300 mL/min, divided in parallel circuits such that 200 mL/min was diverted to the sorbent system and 100 mL/min to the SCD system (Fig. 1). This circuit was maintained over a 6-h time course.

At the end of the pilot study, the circuit was altered to run in series with the SCD following the sorbent dialysis (Fig. 2). Citrate was infused into the blood line pre-SCD with 2% calcium chloride being infused into the venous return post-SCD to compensate for the ionized calcium bound by the citrate.

Pressure measurements in both circuits were assessed with a Mesa Labs 90XL (Mesa Labs, Lakewood, CO, USA) at various locations including presorbent and postsorbent system, as well as pre- and post-SCD.

Assessment of sorbent-SCD efficacy and compatibility

In six additional studies in the porcine model of uremia, the series sorbent-SCD setup was utilized. In order to assess sorbent cartridge efficacy in removing uremic waste, samples were collected presorbent and postsorbent cartridge and assayed for urea nitrogen to determine urea clearance. Postcartridge ultrafiltrate was also analyzed for ammonia breakthrough, an indication of cartridge exhaustion.

SCD efficacy was assessed by complete blood count (CBC), manual differentials, NE apoptotic state, NE activation state, and quantification of leukocyte binding to SCD cartridge to determine if the SCD remained effective during sorbent dialysis compared with historical SCD-citrate values.

Compatibility of the sorbent dialysis and SCD systems was assessed by sampling of the blood outflow, prior to entering the sorbent cartridge (presorbent), to determine if there are detectable levels of citrate in the blood returning to the sorbent system, which might interact with the sorbent column. In addition, presorbent and postsorbent dialysate was assessed for aluminum levels, as well as systemic blood levels of aluminum, to assess possible aluminum complex formation and to monitor the porcine model for possible aluminum toxicity.

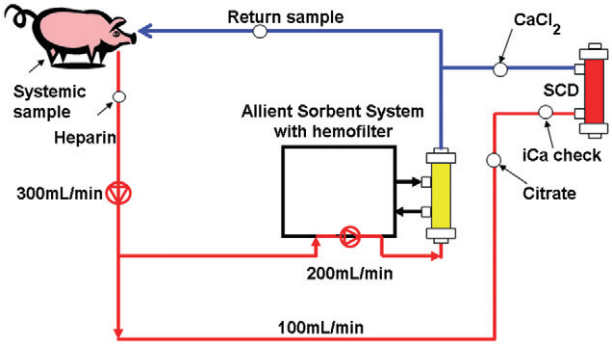

FIG. 1. Sorbent-SCD parallel circuit configuration.

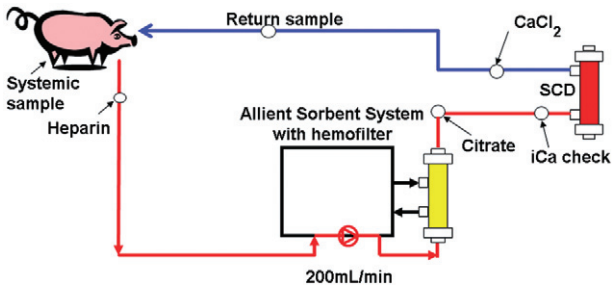

FIG. 2. Sorbent-SCD series circuit configuration.

Regional citrate anticoagulation

ACD-A was infused pre-SCD to maintain the iCa concentration in the circuit between 0.2 and 0.5 mM. Calcium chloride (2% concentration) was infused to maintain systemic iCa values between 1.1 and 1.3 mM. These iCa levels were monitored utilizing an i-STAT System (Abbott Point of Care, Inc., Princeton, NJ, USA).

Analyte quantification

Dialysate ammonia was detected through the use of dip sticks supplied by Renal Solutions Incorporated to estimate concentration by color change via 2- and 5-mg indicators. BUN was measured by i-STAT analyzer with 6+ cartridges.

Citrate analysis

A citrate analysis kit (#K655-100, BioVision, Mountain View, CA, USA) was used according to the manufacturer's instructions to determine concentration of citrate by colorimetry. The colored product was quantified using a SpectraMax M5 multimode microplate reader (MTX Lab Systems, Vienna, VA, USA) at a wavelength of 570 nm.

Aluminum analysis

Presorbent and postsorbent system dialysate samples were stored in an ultralow freezer at –80°C until batched samples were ready for analysis. Systemic blood samples were collected in ethylenediaminetetraacetic acid tubes and sent fresh for analysis. Initial baseline and end of study samples were shipped to Spectra Laboratories (Milpitas, CA, USA) for heavy metal analysis consisting of aluminum, antimony, arsenic, barium, beryllium, cadmium, calcium, chloride, chromium, copper, fluoride, lead, magnesium, mercury, nitrate, potassium, selenium, silver, sodium, sulfate, thallium, and zinc. Based on this preliminary screening where most heavy metal concentrations remained low and unchanged, all subsequent samples were analyzed for aluminum content alone, due to the known possible interaction of the aluminum within the sorbent column with citrate.

Leukocyte analysis

Automated CBCs were measured with a Hemavet 950 (Drew Scientific, Waterbury, CT, USA). Total manual white cell counts were determined using the Unopette system (BD Biosciences, San Jose, CA, USA) and differentials were determined from blood smears after ethanol fixation and Wright stain (Richard-Allen Scientific, Kalamazoo, MI, USA). NE activation was assessed by CD11b expression. Briefly, fluorescein isothiocyanate conjugated antiporcine CD11b antibody (AbD SeroTec, Raleigh, NC, USA) was added to prechilled peripheral blood. Red cells were then lysed and the leukocytes fixed by addition of fluorescence-activated cell sorting lyse solution (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Cells were collected by centrifugation and resuspended for flow-cytometry analysis. CD11b expression was quantitatively assessed as a mean fluorescent intensity with a flow cytometer (Accuri, Ann Arbor, MI, USA).

SCD elution

SCD elution was performed by a previously established method (5). Briefly, at the end of each experiment, the circuit was disconnected and normal saline was flushed continuously through the ECS of the SCD until fluid was free of visible blood. Fluid was drained from the device and a stabilization buffer containing a calcium chelating agent was added. Adherent cells were then mechanically removed from the cartridge eluent for analysis.

RESULTS

Extracorporeal circuit selection: sorbent-SCD series configuration

The sorbent-SCD series extracorporeal circuit configuration allowed for a lower total blood flow rate (only 200 mL/min) and the resultant pre- and post-SCD pressures were lower than in the parallel circuit configuration. Due to the lower pressures and lower blood flow rates of the series circuit, it was selected for use in subsequent studies, as low flow rates and low pressures are desirable in the clinical setting, as they would have less negative impact on severely ill patients.

Assessment of sorbent SCD in a porcine model of uremia

Efficacious sorbent dialysis during sorbent-SCD therapy

The sorbent-SCD series circuit (Fig. 2) was used in the subsequent uremic porcine studies ($n = 6$), with careful measurements to determine compatibility of sorbent system with SCD treatment with regional citrate administration. Dialysate ammonia (Table 1) and systemic BUN (Table 2) were measured as markers of sorbent dialysis efficacy. Ammonia release from the sorbent cartridge was used as one measure of sorbent system failure, in addition to samples being

TABLE 1. *Dialysate ammonia (mg/dL) summary for sorbent-SCD porcine studies: study 1 (parallel circuit) and studies 2–7 (series circuit)*

	Time course		
	4 h	5 h	6 h
Study 1	0	0	0
Study 2	0	0	1
Study 3	0	0	0
Study 4	1	4	4
Study 5	0	1	4
Study 6	0	0	0
Study 7	0	0	0

Studies 1–5 utilized SORB+ sorbent columns and studies 6 and 7 utilized HISORB+ columns. Note that for all studies, $t = 0$–3 h, all ammonia measurements were 0 mg/dL. For study 4, ammonia breakthrough occurred at $t = 4$ h, and for study 5, ammonia breakthrough occurred at $t = 5$ h.

TABLE 2. *Systemic BUN (mg/dL) summary for sorbent-SCD porcine studies: study 1 (parallel circuit) and studies 2–7 (series circuit)*

	Time course								
	0 h	0.25 h	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
Study 1	101	55	—	39	49	37	35	26	24
Study 2	100	100	—	57	46	41	33	27	23
Study 3	98	82	72	69	58	49	41	36	31
Study 4	120	94	64	76	59	50	40	33	27
Study 5	98	82	72	69	58	49	41	36	31
Study 6	79	79	—	49	45	36	30	21	21
Study 7	106	—	66	54	42	35	28	23	19

Studies 1–5 utilized SORB+ sorbent columns and studies 6 and 7 utilized HISORB+ columns.

taken for assessment of any heavy metal bleed-off from the sorbent cartridge. Study 2 had a trace amount of ammonia in the last hour of study. Studies 4 and 5 had trace amounts of ammonia at hour 4, which increased to 4 mg/dL at last hour (Table 1). This may have been caused by the high serum BUN (over 100 mg/dL) (Table 2) and lower ammonia binding capacity of the SORB+ cartridges used. In the last two studies, 6 and 7, the sorbent cartridge was switched to the HISORB+, which had a higher ammonia binding capacity. For both of these studies, no ammonia was detected throughout the time course.

In the sorbent-SCD parallel circuit (pilot, study 1), serum BUN levels declined throughout the study and no ammonia was detected from the sorbent cartridge ultrafiltrate during the 6-h treatment. In the subsequent sorbent-SCD series studies, the decrease in BUN values over time (Table 2) indicates that urease in the sorbent cartridge was not negatively affected by the circuit citrate levels required for effective SCD therapy and that the sorbent dialysis system was efficacious.

SCD treatment during sorbent-SCD therapy

The SCD was demonstrated to function in the sorbent-SCD series circuit with regional citrate administration with no adverse cardiovascular or immunological events, such as leukopenia, in studies to date. Briefly, in this uremic porcine model with renal artery ligation, because no pathogens were administered, no major impact was shown in NE quantification throughout the time course of the study (Fig. 3A). Furthermore, activation as assessed by CD11b expression in isolated NE demonstrated no statistical difference in activation status over the duration of the sorbent-SCD treatment ($P > 0.05$) (Fig. 3B). SCD elution after 6 h of sorbent-SCD treatment resulted in a total recovery of $3.5 \pm 0.9 \times 10^9$ cells.

Assessment of the compatibility of sorbent-SCD therapy

Citrate concentrations were measured for all studies in various circuit locations to assess regional citrate administration for the SCD. Of critical impor-

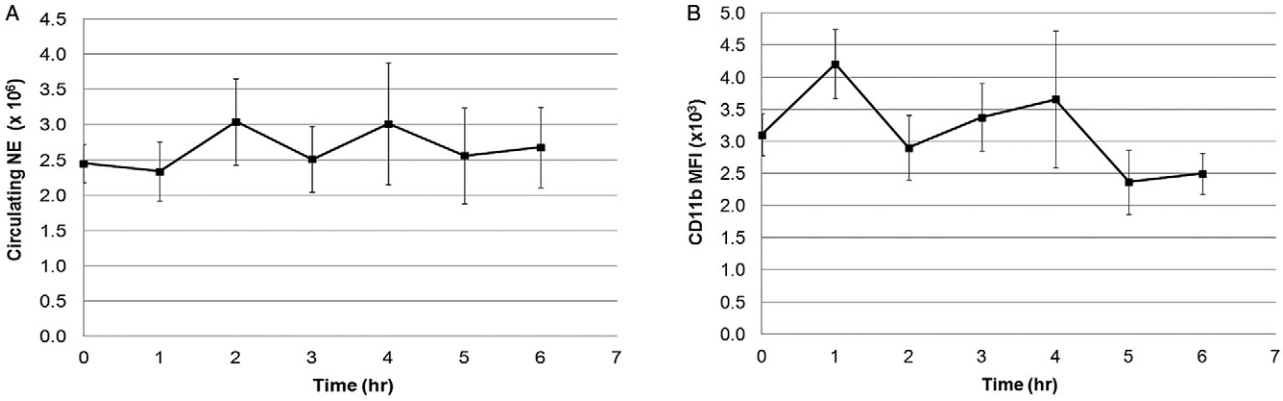

FIG. 3. Total neutrophils in systemic circulation during the sorbent-SCD porcine study were reported as average ± standard error (S.E.), $n = 6$ (A), and neutrophil activation as assessed by CD11b expression was also reported as average ± S.E., $n = 6$ (B). MFI, mean fluorescence intensity.

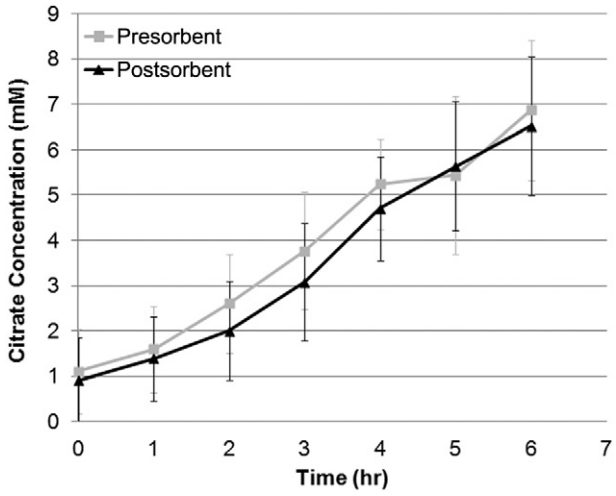

FIG. 4. Sorbent dialysate citrate levels presorbent (gray) and postsorbent (black) (average ± standard error).

tance, presorbent and postsorbent dialysate samples were measured to verify low citrate concentration. The citrate concentrations in both the presorbent and postsorbent dialysate were higher than expected and continued to increase throughout the duration of the sorbent-SCD therapy, from 0 mM when the circuit was initially started to an average of approximately 7 mM after 6 h of therapy, which was statistically significant ($P < 0.05$, $n = 3$, Fig. 4). Presorbent and postsorbent dialysate citrate concentrations were not significantly different ($P > 0.05$, $n = 3$).

In order to assess if the increasing citrate concentrations within the sorbent system led to an interaction with the sorbent cartridge, a heavy metal panel analysis was carried out. Of the heavy metals analyzed, the only metal with measurable values that changed significantly from $t = 0$ h to $t = 6$ h in a selected study was aluminum. The analysis of studies 5–7 for aluminum found that presorbent and postsorbent dialysate samples showed statistically significant increases in aluminum levels at all times after 2 h of therapy ($P < 0.05$, $n = 3$, Fig. 5A), regardless of the occurrence or absence of ammonia breakthrough.

To assess if increased aluminum levels in dialysate also led to higher aluminum levels in systemic blood, further heavy metal analysis was conducted. Serum aluminum levels increased significantly over the duration of the study, from 31.4 ± 7.6 to final values of 406.5 ± 78.4 μg/L ($P < 0.05$, $n = 3$, Fig. 5B).

DISCUSSION

Sorbent-SCD therapy is a promising approach to treat sepsis, ARF, and MODS by combining existing SCD and portable sorbent dialysis technologies. The immunomodulatory properties of the SCD with regional citrate anticoagulation, which has previously been shown to reduce circulating levels of activated NE, could be leveraged to counteract the progression of sepsis, ARF, or MODS, while the concurrent use of sorbent dialysis system would provide small toxin clearance using only 6 L of tap water, which would be especially helpful in care settings where purified water is not available.

In uremic porcine studies with conventional citrate anticoagulation protocol for SCD treatment (5), citrate was administered pre-SCD at a rate sufficient to maintain iCa between 0.2 and 0.5 mM. In the studies described in this report under similar regional citrate anticoagulation conditions, both presorbent dialysate and postsorbent dialysate citrate and aluminum levels increased over time, suggesting incomplete first pass citrate metabolism, citrate accumulation, and that citrate-sorbent column interaction occurred, releasing aluminum. Furthermore, similar concentrations of citrate presorbent and postsorbent suggest a well-mixed circuit that equilibrates over time; therefore, attempting to regionally isolate sections of the circuit by regional administration of citrate was not effective. Assessment of ammonia release from the sorbent cartridge was not necessarily an indicator of the start of sorbent column breakdown or correlative with released aluminum. For example, ammonia breakthrough occurred in study 5 at hour 4, but the initial rise in aluminum levels occurred even earlier, at $t = 2$ h. In the last two

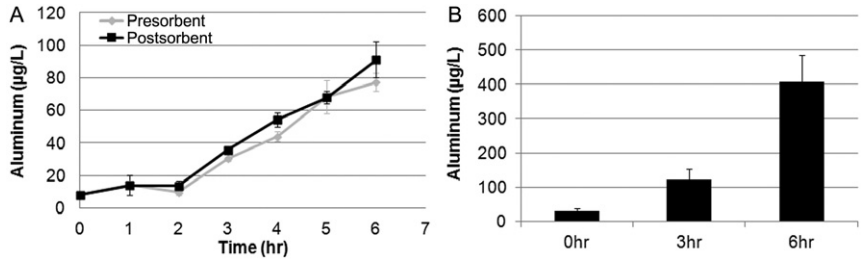

FIG. 5. Aluminum in presorbent dialysate (gray) and in postsorbent dialysate (black) (average ± standard error [S.E.], $n = 3$) (A), and systemic serum aluminum (average ± S.E., $n = 3$) (B) in the sorbent-SCD series circuit.

sorbent uremic porcine studies (studies 6 and 7), the sorbent cartridge was switched to HISORB+, with a high ammonia binding capacity, and no ammonia release was detected; however, high aluminum levels persisted, suggesting release of aluminum from sorbent column filler materials.

Systemic aluminum levels found in this porcine model were indicative of a potentially toxic concentration. Briefly, the Association for the Advancement of Medical Instrumentation standards suggest a maximum aluminum concentration of 10 μg/L in water for hemodialysis and the minimum serum concentration associated with dialysis aluminum toxicity to be 0.06 mg/L (19) or 60 μg/L. The suggested maximum aluminum level in dialysate has been specified to prevent accumulation of this toxic metal in the patient (20). Aluminum toxicity is a serious and potentially fatal disease state. Uptake of aluminum from the dialysate is associated with bone disease (18), anemia (17), and the dialysis encephalopathy syndrome, which is usually fatal (16). However, aluminum toxicity in patients on chronic dialysis for end-stage renal disease may be completely different from toxicity associated with ARF patients in an intensive care unit setting treated a few times with sorbent-SCD therapy.

All sorbent column technologies currently employ aluminum chemistry to immobilize urease. Results in this report suggest that SCD therapy with the current level of regional citrate anticoagulation and sorbent dialysis are currently incompatible technologies due to the interaction of citrate and the sorbent column. Although the cause of aluminum displacement from the sorbent column was not directly evaluated in this study, previous studies have investigated citrate's interaction with these types of sorbent columns. Citrate interaction with aluminum-containing sorbent cartridges is three pronged (15): (i) citrate uncouples urease from alumina, which is the material holding the urease enzyme in place within the cartridge; (ii) citrate complexes with the aluminum, forming a soluble complex which can be released from the cartridge; and (iii) citrate competes with the released urease for binding to the hydrated zirconium oxide, therefore the urease circulates in the dialysate, which breaks down urea, releasing ammonia. Changes to sorbent column urease immobilization chemistry could correct these issues.

Citrate is rapidly metabolized to bicarbonate by the liver. Therefore, a simple working hypothesis that was tested for sorbent-SCD combined therapy was that citrate entering the systemic circulation from the extracorporeal circuit would be quickly metabolized and would not result in systemic accumulation and citrate exposure to the sorbent system upon recirculation. However, the literature suggests that an infusion of 0.5 mmol/kg/h citrate solution is not able to be fully eliminated from the body (21). The infusion rate in our porcine model was approximately 0.97 mmol/kg/h to meet the required amount for SCD therapy, exceeding the metabolic capability of the average animal tested. These results suggest that a strategy that needs to be developed to mitigate citrate-aluminum interactions is critical to successful implementation of sorbent systems and SCD therapy.

CONCLUSIONS

Regional citrate anticoagulation required for optimal SCD therapy is currently incompatible with sorbent dialysis technology due to an inability to exclude citrate from interacting with sorbent columns containing aluminum. The strategy employed attempted to isolate and eliminate citrate by systemic metabolism prior to blood recirculation into the therapeutic circuit. However, citrate was not fully eliminated by liver metabolism, leading to increasing systemic concentrations and therefore presorbent concentration, allowing for aluminum-citrate complex formation. Aluminum liberated from sorbent columns led to increased aluminum levels in dialysate and then in systemic blood, causing potential aluminum toxicity. This study confirms that sorbent-SCD therapy maintains urea clearance while avoiding hematologic adverse reactions. Thus, sorbent-SCD combined therapy remains promising as an approach to sepsis, ARF, and MODS treatment where purified water for dialysis is not available. Alternate strategies to implement sorbent-SCD therapy in order to eliminate citrate interaction with sorbent columns will be required, including development of alternate urease-sorbent column binding chemistry or further changes to the sorbent-SCD therapeutic circuit and determining minimum citrate concentration required for efficacious SCD treatment.

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Conflict of Interest: H.D.H. is a shareholder of Innovative BioTherapies, Inc. and CytoPherx, Inc. C.J.P., A.F., L.L., P.L.S., K.J., and D.A.B. are employees of Innovative BioTherapies, Inc.

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