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Immunomodulatory Device Therapy in a Pediatric Patient With Acute Kidney Injury and Multiorgan Dysfunction

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INTRODUCTION

Acute kidney injury (AKI) that requires continuous renal replacement therapy (CRRT) is a significant complication in critically ill patients with mortality rates approaching 50%. Although children are generally healthy, the development of AKI that requires CRRT in intensive care unit (ICU) pediatric patients results in a similarly high mortality rate.[1–3] AKI results in a systemic inflammatory response syndrome with the activation of circulating leukocytes, predominantly neutrophils and monocytes, which contribute to patient morbidity and mortality in multiorgan failure.[4,5] This dysregulated and excessive leukocyte (LE) activation promotes the activated white blood cells to bind to microvascular endothelium and to extravasate into tissue spaces to degrade injured tissue or kill invading pathogens. This process results in microvascular stasis and capillary leak, further propagating cardiovascular instability and hypotension, lung dysfunction, and renal function decline.[4]

In the past decade, therapies directed at treating systemic inflammatory response syndrome and severe AKI focused on inhibiting the soluble mediators of inflammation with little or modest efficacy when tested clinically.[6] Recent innovative strategies have focused on cell-based therapeutic approaches. One promising approach is a selective cytopheretic device (SCD) that promotes, when placed in an extracorporeal blood circuit, continuous cell processing of activated LEs to immunomodulate an excessive inflammatory response (Figure 1). This approach has demonstrated efficacy to improve solid organ dysfunction associated with acute and chronic inflammation in a variety of preclinical animal models.[7–9] SCD therapy has been evaluated in a number of clinical trials under Food and Drug Administration (FDA) and institutional review board approvals and has demonstrated encouraging clinical efficacy signals.[10–14] Several clinical studies have focused on the evaluation of the SCD in adult ICU patients who require CRRT to treat severe AKI and multiorgan dysfunction (MOD).[12–14] The encouraging results of a multicenter, randomized control trial that demonstrated that SCD treatment reduced 60-day mortality and dialysis dependency in adult ICU patients led to Investigational Device Exemption approval and funding (FDA Investigational Device Exemption G150179, FDA Grant 1R01 FD 005092) of a multicenter pilot study in pediatric ICU patients up to 22 years of age who weighed >20 kg and who had AKI that required CRRT. We present the first pediatric patient enrolled in this trial and treated with the SCD (ClinicalTrials.gov ID: NCT02820350). The primary endpoints of this trial were safety and 60-day mortality or dialysis-dependency.

This report does not intend to be proof of a testable hypothesis but is presented as an initial and important step in understanding a novel device as it begins the translation from bench to bedside in human disease. This initial case provokes more questions than answers but provides useful insights into the complex nature of severe AKI and MOD.

CASE PRESENTATION

Case

An 11-year-old girl underwent a 12-hour elective surgery procedure for posterior spinal fusion to repair her

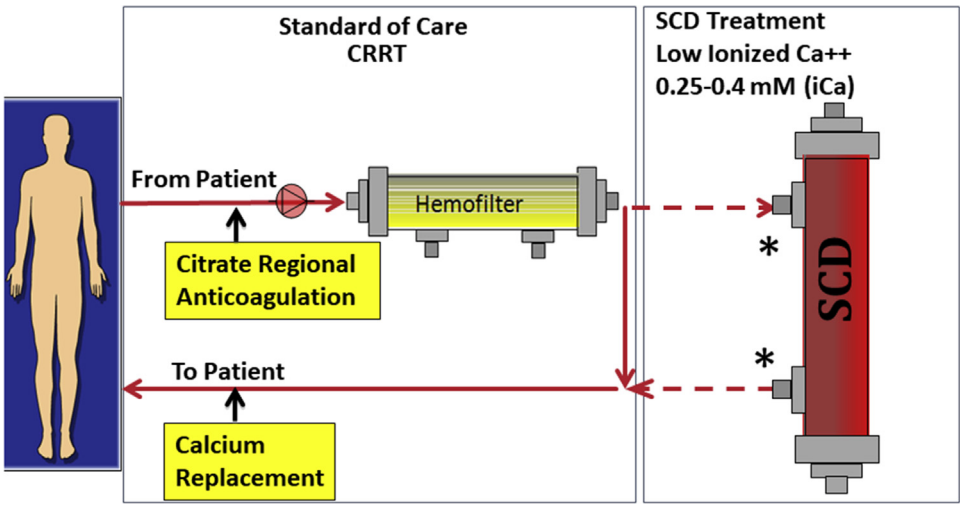

Figure 1. Extracorporeal blood circuit under standard continuous renal replacement therapy (CRRT) (solid lines) and after the integration of the selective cytopheretic device (SCD) into the circuit. The blood flow path in the SCD is in the extracapillary space along the outside surfaces of the hollow fibers. This flow path results in low shear force along the fibers, which allows for activated neutrophils and monocytes to selectively bind to the membrane. The low ionized calcium (iCa) environment promotes deactivation and release of the bound cells back to the systemic circulation. This process results in continuous leukocyte cell processing and immunomodulation of the excessive systemic inflammatory response state of acute kidney injury.[6–14] The SCD is changed every 24 hours for up to 7 days of treatment, a treatment time found to be effective in previous clinical studies.[6] *Side ports of SCD for elution sampling.

idiopathic scoliosis at an outside institution. She received anesthesia that included propofol (120–140 μg/kg per minute). Her estimated blood loss was 1.4 l and she received large quantities of blood products and crystalloid. At the end of the operation, she developed hypotension that required additional blood products (4 U packed red blood cells, fresh frozen plasma, cryoprecipitate, and platelets), together with 2 vasopressors (phenylephrine and norepinephrine) to maintain her blood pressure. She was extubated immediately postoperatively. On postoperative day 1, she developed disseminated intravascular coagulation (DIC), liver injury, and rhabdomyolysis with oliguria. Fluid resuscitation included 10 l of fluids, and the patient was reintubated to improve oxygenation in the face of fluid overload. She failed to respond to high-dose i.v. diuretics. Her dry weight was ∼44 kg before surgery and 54.6 kg at transfer. Because of the need for CRRT, she was transferred to a tertiary care hospital before initiating any renal replacement therapy (RRT) at the referring hospital (Table 1).

Upon transfer, she was found to have rhabdomyolysis, with a serum creatinine of 4.17 mg/dl, creatinine phosphokinase of 17,301 IU/l, phosphorus of 9.7 mg/dl, uric acid of 10.4, and urine myoglobin of 3845 mg/dl. She was acidemic, with an arterial pH of 7.32, partial pressure of carbon dioxide of 44 mm Hg, bicarbonate of 22 mEq/l, and lactate of 1.7 mmol/l. She had AKI with elevated levels of aspartate aminotransferase (3489 IU/l) and alanine aminotransferase (2593 IU/l), and acute pancreatitis with elevated levels of amylase (487 IU/l) and lipase (439 IU/l), as well as an elevated triglyceride level of 135 mg/dl (normal <90 mg/dl). She also had an elevated white blood count, together with DIC with an elevated protime and partial thromboplastin time, as well as an elevated lactate dehydrogenase of 5892 IU/l, D-dimer of >35 mg/dl, and low haptoglobin of <10 mg/dl. She was in respiratory failure that required mechanical ventilation, with a fraction of inspired oxygen of 50% with a partial arterial pressure of oxygen (PaO_2)/ fraction of inspired oxygen ratio of 290, but she was not on vasopressors (Table 1). The patient was placed on CRRT 5 hours after arrival at the tertiary care center, and she received minimal fluid after transfer before initiation of CRRT. Her estimated fluid overload at CRRT initiation was 24%. CRRT was performed with the Prismaflex CRRT machine (software v7.11, Baxter Healthcare, Deerfield, IL), using an HF-1000 filter with a blood pump flow of 150 ml/min, dialysis fluid rate of 3000 ml/h, and postfilter replacement fluid rate of 1100 ml/h (total prescribed clearance of 4729 ml/h per 1.73m^2). The postfilter solution contained calcium, and the replacement rate was protocolized to optimize the filtration fraction to lessen the need for calcium drips. This protocol was instituted during the calcium shortage before the trial. The total effluent rate was higher than typically prescribed at this institution (2000 ml/h per 1.73 m^2) and performed at the discretion of the attending nephrologist on service. Our institution commonly uses higher effluent flow rates with regional citrate anticoagulation and liver injury to prevent citrate lock. The working differential diagnosis was propofol infusion syndrome versus sequelae from intraoperative hypotension.

Table 1. Laboratory parameters during selective cytopheretic device treatment

	Day 0[a]	Day 1	Day 2	Day3	Day 4	Day 5	Day 6	Day 7
Absolute complete blood counts								
WBC	13.6	8.8	7.8	10.4	10.4	11.8	14.9	16.6
Neutrophil	12.7	6.6	5.2	7.4	7.1	8.7	10.9	11.7
Monocyte	0.7	0.8	0.8	1.4	1.5	1.9	2.4	2.4
Lymphocyte	0.9	1.2	1.2	1.0	1.2	1.1	1.3	1.9
Disseminated intravascular coagulation parameters								
PT (9.3–12.0 s)	17.8	11.9	11.6	10.5	10.3	10.6	10.9	11.2
PTT (22.0–30.0 s)	33.3	27.7	27.2	28.2	31.7	33	30.1	27.7
Platelet count/μl	44,000	84,000	68,000	67,000	71,000	100,000	153,000	162,000
Fibrinogen (150–450 mg/dl)	198	227		335		308	313	348
Liver function tests + pancreatic enzymes								
AST (5–60 IU/l)	3489	1329	484	255	157	105	65	52
ALT (<35 IU/l)	2593	1815	1250	973	659	504	379	277
Bilirubin T (≤1.0 mg/dl)	3.0	2.4	1.1	1.0	0.9	1.1	1.3	1.3
Albumin (3.2–5.2 g/dl)	3.0	3.1	2.8	2.9	3.6	3.9	4.1	4
Amylase (30–100 IU/l)		487	245	148		117		
Lipase (5–50 IU/l)		439	186	120	109	124		
Respiratory-related parameters								
Mechanical ventilation FiO_2 (%)	35%	35%	35%	35%		Extubated		
Nasal cannula (O_2 flow l/min)					18	16	7	2
PO_2 (mm Hg)	99.1	90.9	112	91	56	113	88	162
PCO_2 (mm Hg)	41	44	36	41	39	35	36	40
pH	7.42	7.47	7.44	7.46	7.52	7.53	7.47	7.51
Acute kidney injury–related parameters								
Urine output (ml/24 h)	372	27	58	51	97	53	48	82
Body weight (dry weight: 44.2 kg)	54.6	53.4	52.3	50.9	48.7	46.9	46.2	44.5
Creatinine phosphokinase (26–180 IU/l)	17,301	8808	3030	1314	827	799	529	384
Urine myoglobin (ng/ml)	3845				284			

ALT, alanine transaminase; AST, aspartate transaminase; FiO_2, fraction of inspired oxygen; PCO_2, partial pressure of carbon dioxide; PO_2, partial pressure of oxygen PT, protime; PTT, partial prothrombin time; WBC, white blood cell.
[a]Continuous renal replacement therapy and the selective cytopheretic device were initiated between day 0 and day 1 for all parameters.
Normal values are in parentheses.

She was evaluated by the clinical investigator of the institutional review board–approved Investigational Device Exemption trial and met enrollment criteria, which included a pediatric patient (younger than 22 years old) in the ICU with severe AKI and multiorgan failure that required CRRT. Full inclusion and exclusion criteria are available on clinicaltrials.gov (NCT02820350). Her parents provided informed consent, and she was enrolled into the study. After 7 hours of CRRT, to obtain circuit ionized calcium consistently at goal (<0.4 mM), the SCD with a 1.0 m^2 surface area was incorporated into the extracorporeal blood circuit on the day of the transfer (Figure 1). The SCD was changed every 24 hours for up to 7 days of treatment per clinical protocol. By the next morning, she had stable blood pressure on CRRT and SCD therapy. Circuit ionized calcium (measured immediately postfilter by blood gas analyzer) was maintained consistently at <0.4 mM during therapy, as required by protocol. Her clinical picture showed elements of stabilization or improvement. Oxygen requirement stabilized, white blood count normalized, coagulopathy improved, liver injury diminished, and net fluid volume removal was achieved (Table 1).

As SCD treatment progressed, liver and pancreatic enzymes continued to improve. Within the first day of therapy, capillary leak improved so that net volume removal with CRRT was able to be consistently achieved at a rate of 50 ml/h on the second day, and 100 ml/hour during the third day of treatment. With this net fluid removal, her respiratory function improved, with extubation occurring on day 4 of therapy. On day 4 of treatment, she spiked a temperature to 38.4°C; her white blood count became elevated and continued to rise on day 5 with a procalcitonin level of 2.64 ng/ml (normal, 0–2.25 ng/ml). Accordingly, broad spectrum antibiotics were started, although her blood cultures ultimately showed no growth of bacteria. She was able to transition to room air after day 7 upon completion of the full SCD 7-day treatment course according to clinical protocol. Her renal function improved throughout the therapy. She completed a full treatment of 7 days with the SCD; no significant device-related adverse events were observed.

At admission to this tertiary care hospital, the patient was oliguric. She became nonoliguric with 618 ml/24 h urine formation 3 days (10th hospital day) after

completing SCD therapy. She continued on CRRT until her urine output was >1000 ml/24 h at 7 days post-treatment (14th hospital day), so that CRRT was discontinued, and she required no further RRT. She continued to show improvement and was transferred to a general medical floor on day 15 of her hospitalization. On the 20th day of hospitalization, she was discharged home with full recovery from liver, lung, kidney, and hematologic dysfunctions. Her serum creatinine was 0.9 mg/dl at discharge and 0.51 mg/dl at clinic follow-up 48 days after the initiation of therapy.

DISCUSSION

This patient presented with 4 multiorgan failures, including rhabdomyolytic AKI, respiratory failure, severe liver injury, and coagulopathy. The primary differential diagnosis for this patient was propofol infusion syndrome (PRIS) versus sequelae from prolonged recumbency and intraoperative hypotension during the operation, which resulted in disseminated intravascular coagulation and multiorgan failure. PRIS was first described in pediatric patients, but it was also recognized later in adults.[15] This syndrome is uncommon, but upon development, a number of serious adverse events occur that are characterized by rhabdomyolytic AKI, liver abnormalities, and hyperlipidemia. With prolonged administration of propofol (days vs. hours), cardiac failure also develops. PRIS and this degree of AKI have a high likelihood of death.[1,15] Treatment options are limited, consisting of supportive cardiopulmonary and RRT support.

Although the inciting event in this patient, either PRIS or DIC from intraoperative hypotension, was not clear, the patient developed AKI with multiorgan failure. The subsequent excessive systemic inflammatory response associated with AKI resulted in microvascular dysfunction with DIC and capillary leak. Subsequent tissue ischemia and LE infiltration with release of toxic byproducts promoted severe liver injury and pulmonary dysfunction. Aggressive fluid resuscitation in the face of oliguric AKI further compromised respiratory function.

SCD treatment is targeted toward reducing the excessive LE activation that occurs with AKI.[4,5] This modulation reduces the progression of microvascular dysfunction and tissue damage that arises from LE infiltration, thereby limiting the degree of tissue ischemia and toxic damage.[6] In this regard, SCD therapy in this patient was consistent with these effects, as reflected by the rapidity of improvement of DIC parameters and lessening of the capillary leak, which allowed for net volume removal with CRRT. Cause and effect could not be established in this individual case and would require further evaluation in control clinical trials.

The SCD is a membrane-based, cell-processing device. This device, when incorporated into an extracorporeal blood circuit, preferentially binds activated LEs, and in the presence of regional citrate anticoagulation, it deactivates the bound LEs and releases them back into the systemic circulation. The low ionized calcium in the blood circuit produced with regional citrate anticoagulation establishes an environment to deactivate the LEs bound to the membranes. This deactivation step appears to result in release of less inflammatory phenotypes of white blood cells to the systemic circulation.[7,8,10,13] This device is similar to a polysulfone membrane dialyzer, but it directs the blood flow to the outside of the hollow fiber membrane rather than the inside of the membrane. The blood flow path results in low shear forces similar to capillary shear; therefore, the membrane has selectivity to bind activated LEs. This continuous cell processing activity results in measurable diminution of excessive inflammatory responses in a variety of disease states.[7,8,10,13,14]

In this regard, the evaluation of the number and phenotype of LEs bound to the SCD membrane at the end of treatment days 1, 3, 5, and 7 demonstrated that the cells bound were almost exclusively neutrophils or monocytes (Figure 2). Lymphocytes were not bound to the membranes. The absolute number of bound cells on the SCD membranes varied between 1×10^8 and 2×10^9 increasing numbers with treatment duration were most likely due to higher LE activation during the clinical course of this patient (see the following). These cell numbers and selectivity were similar to preclinical data observed in both acute and chronic inflammation in large animal disease models.[7,8,10] Although the number of bound cells were <5% of the circulating pool of LEs, constant cell processing of the entire circulating pool of LEs occurred, with membrane exposure occurring more than once per hour at the blood flow rates used for CRRT.

The cytometric analysis during treatment was noteworthy. Neutrophils[16,17] and monocytes[18,19] mobilized intracellular stores of CD11b to the cell surface as they became activated (primed), which allowed a real-time measurement of systemic acute neutrophil (priming) and monocyte activation. The CD11b MFI for both circulating neutrophils and monocytes bound to the SCD decreased from day 1 to day 3, but trended upward at day 5 and day 7, coincident with development of a clinically observed systemic infection (Figure 3). This observation, if reproduced in other patients, might be a cell biomarker for systemic inflammation in critically ill patients. The results from day 7 of treatment were

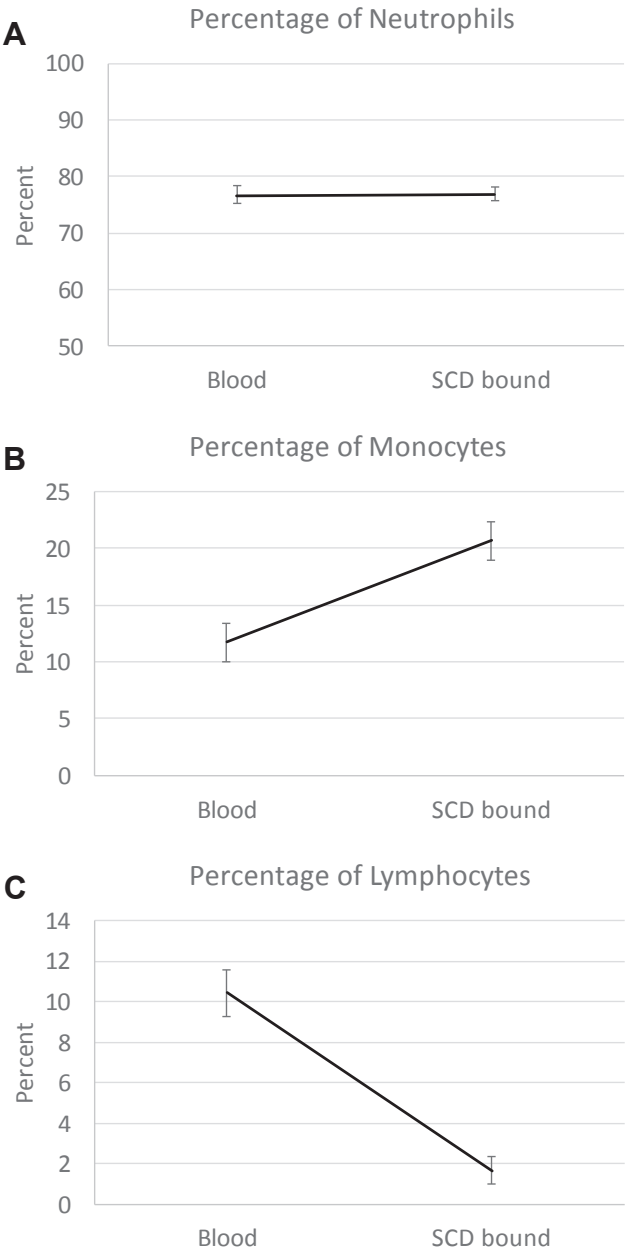

Figure 2. Leukocyte characteristics associated with a selective cytopheretic device (SCD) compared with blood. (a–c) Leukocytes that were eluted from the SCD after treatment days 1, 3, 5, and 7. Elution of the cells from the SCD was accomplished as previously described after placing elution buffer into the SCD via the perfusion side ports.[9] The percentage of neutrophils, monocytes, and lymphocytes of total cells in peripheral blood and the SCD elutions are presented as mean ± SE for n = 4 as measured at 1, 3, 5, and 7 days of SCD treatment, when the SCD bound cells were evaluated after elution. The total number of SCD eluted cells and the selectivity of binding of neutrophils and monocytes to the SCD were similar to preclinical animal models.[7,8,10]

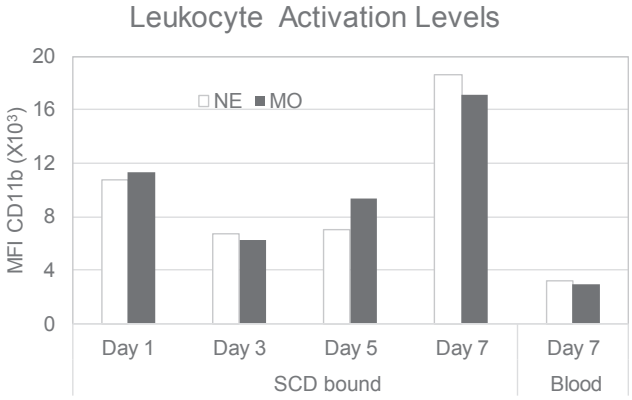

Figure 3. The acute activation state of neutrophils and monocytes were assessed with cytometric analysis as previously described.[7,8,10,13] The higher mean fluorescent intensity (MFI) of CD11b on the cell surface was associated with higher activation of these leukocytes.[16–19] CD11b is a membrane protein involved in the adherence of leukocytes to activated endothelium as the first step to extravasation to a site of inflammation.[7,8,10] The increase in MFI CD11b at day 5 and day 7 correlated with the development of sepsis clinically. As demonstrated, the activation levels of both neutrophils and monocytes bound to the selective cytopheretic device (SCD) membranes were elevated throughout the time course of treatment. On the final day of treatment (day 7), the selectivity of binding of the highest activated leukocytes was demonstrated compared with the CD11b MFI of the circulating cells in the peripheral blood. This selectivity was repeatedly observed in preclinical animal models.[7,8,10]

especially illuminating. The level of activation, as measured by the CD11b mean fluorescent intensity (MFI) of SCD, bound the neutrophils and monocytes compared with circulating LEs. This demonstrated that the SCD membranes selectively bound the subpopulation of the most highly activated pool of circulating neutrophils and monocytes (Figure 2), because the MFI of bound LEs were >4 times the levels seen in circulating blood. This observation was similar to what was seen in preclinical animal models.[7,8,10]

This initial case of SCD treatment in a critically ill pediatric patient provided insights into its potential clinical usefulness. No device-related adverse events were observed similar to the safety characteristics observed in adult patients.[11,12,14] The cytometric analysis of circulating and SCD membrane-associated leukocytes demonstrated the quantification and selectivity of cell binding within the device. This analysis confirmed similar SCD-related effects on leukocytes as observed in large animal models, thereby translating observations seen in animal models to human disease.

This singular case does not intend to demonstrate that SCD therapy provides added benefit to conventional RRT. This case is presented to demonstrate how initial evaluation of key parameters of a therapeutic device observed in preclinical animal models may be related to human disease parameters to better interpret preclinical animal findings to the human experience. This case also presents the initial steps to correlate innate immunologic cell activity as measured by cytometric analysis of circulating blood as a diagnostic and potentially prognostic index in a critically ill patient.

This report is presented to emphasize the importance of evaluating novel devices in pediatric patients after safety and efficacy clinical trials have occurred in adult patients. The relevance of these findings will be further developed as the experience of this therapy is expanded in further clinical studies.

DISCLOSURE

HDH is a shareholder of CytoPherx and Innovative BioTherapies, Inc. University of Michigan has financial interest in CytoPherx.

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