

Renal Cell Therapy and Beyond

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ABSTRACT

Although current dialysis techniques have transformed acute and chronic renal failure from uniformly fatal clinical disorders into treatable diseases, these therapies replace only the water and solute clearance function of the kidney and have reached a point where little further therapeutic improvement can be anticipated. In addition to their metabolic and endocrine functions, renal tubule cells presumably play an important role in the systemic inflammatory balance by participating in the complex and dynamic network of leukocyte action and pro- and anti-inflammatory cytokines. Loss of this function may result in a propensity to develop systemic inflammatory response syndrome (SIRS), multiorgan dysfunction, and a high risk of death in acute kidney injury (AKI), and may relate to chronic inflammatory state in end-stage renal disease (ESRD). A renal tubule cell assist device (RAD) containing animal or human renal tubule cells has been recently developed with the purpose

of integrating the functions of tubule cells with the filtration function of current dialysis to offer a more complete renal replacement therapy. The viability and functionality of this device were confirmed in *in vitro* experiments and large animal studies, and recently the RAD's clinical therapeutic benefit was demonstrated with a series of FDA-approved human trials. Another novel synthetic membrane extracorporeal device that binds and inhibits circulating leukocytes has been developed with the purpose of reducing microvascular damage promoted primarily via activated circulating leukocytes in AKI and SIRS. This device, called a selective cytopheretic inhibitory device, mimics immunomodulation and duplicates RAD efficiency in preliminary studies. Both devices may become comprehensive treatments, replacing full renal function and correcting inflammatory imbalance in patients with acute and chronic renal disorders.

Acute kidney injury (AKI) affects up to 200,000 people in the United States annually, and approximately 5% of all long-term hospital patients. The development of AKI in a hospitalized patient results in a five- to eight-fold higher risk of death (1). The cause of death subsequent to AKI is generally the development of systemic inflammatory response syndrome (SIRS), frequently secondary to bacterial infection or sepsis, resulting in cardiovascular collapse and ischemic damage to vital organs, culminating in multiple organ failure (MOF; 2). An estimated 700,000 cases of sepsis occur each year, resulting in more than 210,000 deaths in the United States (3). The combination of AKI and sepsis is associated with a 70% mortality rate, as compared with a 4% mortality rate among patients with AKI alone (4).

Current therapy for AKI is predominantly supportive in nature. The therapeutic goals are the maintenance of

fluid and electrolyte balance, adequate nutrition, and treatment of infection and uremia when they are present. Uremia is treated with dialysis, either intermittent hemodialysis or continuous hemofiltration. Although this approach has substantially impacted this disease process over the last 40 years, patients with clinically severe AKI still have an exceedingly high mortality of > 50% (5). This high mortality, despite normal electrolyte balance and improvement in the uremic state, is because of the propensity of these patients to develop SIRS, most commonly secondary to bacterial sepsis, with resulting MOF from cardiovascular collapse and ischemia (6).

Limited Success of Current Dialysis Therapies

Current dialysis therapies are suboptimal for both AKI and end-stage renal disease (ESRD). These therapies do not meet the medical need for treatment of reducing mortality from AKI. Even in ESRD, the outcomes of patients receiving chronic dialysis therapy are still disappointing, with an annual mortality exceeding 25%, on average, and a drastically shortened life expectancy of only 5 years (7). Several groups have reported that the survival of critically ill patients with AKI could be improved by intensifying the dose of renal replacement therapy (8–10), but the initial excitement generated

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by these reports has waned following studies demonstrating that dialysis dose is not closely related to outcomes (11–13). Recent work by the VA/NIH Acute Renal Failure Trial Network intensified the skepticism (14). Regarding ESRD, current data from two large randomized controlled trials in ESRD, the Hemodialysis (HEMO) Study (15) and the ADEMEX (ADEquacy of PD in MEXico) Study in peritoneal dialysis (16), have also failed to show a survival benefit from increased dialysis dose. These results suggest that current therapies focusing only on fluid and small- and middle-molecule solute clearance are limited in their ability to further influence outcomes.

The kidney is not merely a filtration organ. It also provides important transport, homeostatic, metabolic, and endocrinologic functions. The transport function of the kidney includes not only water and solute homeostasis but the important reclamation of metabolic substrates, including essential amino acids and glucose. The kidney serves as a critically important metabolic organ, synthesizing glutathione (GSH) and free-radical scavenging enzymes and providing gluconeogenic and ammoniogenic capabilities (17,18). Catabolism of low-molecular-weight proteins, including peptide hormones, cytokines, and growth factors, is also accomplished by the kidney (19), and this organ has an important hormonal function with the production and regulation of erythropoietin, vitamin D, and multiple cytokines critical to inflammation and immunologic regulation (20). The propensity of patients with acute renal failure (ARF) to develop SIRS and sepsis suggests the renal tubular cell has a critical immunomodulatory role under stress states (2,21).

For these reasons, “renal replacement therapy” is an inappropriate description for current dialysis treatments, which replace only the filtration function of the failed kidney without addressing its metabolic, endocrinologic, and immunologic roles. It is also not optimal to treat simply the volume, electrolyte, and uremic problems, and to focus only on kidney injury and recovery per se in situations in which AKI is combined with sepsis or SIRS and interacts with distant organs such as the lung and heart.

Less Recognized Role of the Kidney: Immunoregulation

The immunoregulatory role of the mammalian renal proximal tubule cells is under appreciated. The kidney is derived embryologically from dorsal mesoderm, a collection of cells also important in the development of bone marrow stem cells (22). Phylogenetically, in bony fish and amphibians without lymph systems, the kidney is the major antibody-producing organ (22) and, not surprisingly, mammalian renal proximal tubule cells are immunologically active. They are antigen-presenting cells (23) that have costimulatory molecules (24) that synthesize and process a variety of inflammatory cytokines (25,26).

GSH is resynthesized and returned to the systemic circulation by the proximal tubule cells (27). These cells are also the major source of synthesis of antioxidant GSH-

related enzymes (28). A variety of studies have clearly shown that excessive free-radical generation contributes to vascular and tissue damage in sepsis, which triggers a complex series of coagulation, complement, and cytokine cascades to defend against bacterial invasion (28,29). The loss of both GSH synthetic function and production of key free-radical scavenging enzymes in renal failure undoubtedly increases the risk of sepsis syndrome (28). The key role of the kidney in this GSH regulatory pathway is exemplified by the fact that renal failure patients have severely low plasma levels of GSH and its peroxidases (30), and therefore are at increased risk for oxidative stress, especially in bacterial infection.

1,25-dihydroxyvitamin D3 (vitamin D3) plays an important role in the regulation of the immune system (31,32). The immune system is recognized as a target tissue of this important hormone. High-affinity receptors are found in peripheral blood lymphocytes and thymocytes, and vitamin D deficiency impairs cell-mediated immunity (31). Neutrophils from patients with vitamin D deficiency have abnormal motility and phagocytic ability (32). Administration of vitamin D3 to patients on hemodialysis restores mitogen-stimulated T-cell responses to normal (33). A critical role of cytosolic calcium in the oxidative burst of granulocytes has been acknowledged (34).

The roles of the renal tubular cells in GSH metabolism, synthesis of GSH peroxidase and free-radical scavenging enzymes (17), regulation of vitamin D, and production and catabolism of multiple cytokines (20) are critical to immunoregulation to maintain tissue integrity and host defense under stress conditions (21).

AKI and ESRD as Systemic Inflammatory Diseases

AKI, or acute tubular necrosis (ATN), which results in the loss of the kidney's presumed immunoregulatory function, results in a propensity to develop SIRS, sepsis, MOF, and a high risk of death because of systemic immunologic or inflammatory imbalance caused by kidney cell injury or necrosis. Activation and release of inflammatory proteins from circulating activated leukocytes and imbalance between pro- and anti-inflammatory proteins are provoked and aggravated by kidney cell injury and possibly by dialysis. These conditions play a central role in the proinflammatory state in AKI with SIRS and/or MOF.

SIRS is a catastrophic sequela of a variety of clinical insults and is usually present with AKI. In the past decade, a large amount of data has provided new insights into the inflammatory response that seems to underlie the MOF syndrome. There are now data linking patient outcome to initial plasma levels of tumor necrosis factor (TNF)- α , interleukin (IL)-6, and other proinflammatory cytokines (35–37). Septic shock is an acute syndrome that is characterized by hypotension, coagulopathy, and eventual MOF primarily because of ischemic tissue injury. This disorder is associated with dramatic elevations in inflammatory cytokines, including TNF- α , IL-1 β , IL-6, IL-8, and interferon- γ (17). This reactive and

uncontrolled inflammatory response results in the adverse hemodynamic and metabolic disturbances in septic shock. If this balance between pro- and anti-inflammatory mediators is lost, the patient may proceed to cardiovascular collapse if proinflammatory processes are excessive, or develop profound immunosuppression with increased risk of recurrent or continuing infection if the anti-inflammatory cascade overreacts (36,37).

ESRD is recognized as a disease associated with chronic inflammation that predisposes patients to cardiovascular diseases and acute infectious complications, the two most common causes of death, despite adequate hemodialysis. Clinically, the chronic inflammatory state in ESRD patients, which is independent of dialyzer membrane activation and clearance, is represented by elevated levels of C-reactive protein and proinflammatory cytokines, including IL-1, IL-6, and TNF, equivalent to a chronic proinflammatory state (38,39). All these parameters are associated with enhanced mortality in ESRD patients and might have many causes, including the clearance of GSH, negative nitrogen balance and energy loss in the clearance of peptides and amino acids, loss of tubular cell function in oxidative deamination, gluconeogenesis, and loss of cytokine and hormone metabolic activity in the kidney.

Because renal tubule cells may play an important role in the systemic inflammatory balance by participating in the complex and dynamic network of leukocyte action and pro- and anti-inflammatory cytokines, our research group turned its attention to the effort to replace this immunomodulatory function in addition to the kidney's filtration, metabolic, and endocrine functions. Our focus is not confined to the kidney but extends to the whole body.

Renal Bio-Replacement Therapy in AKI and SIRS

The replacement of the functions of renal tubule cells during the AKI episode in conjunction with hemofiltration provides more complete renal replacement therapy as compared with the "partial" replacement therapy of current dialysis treatments (Fig. 1). The addition of met-

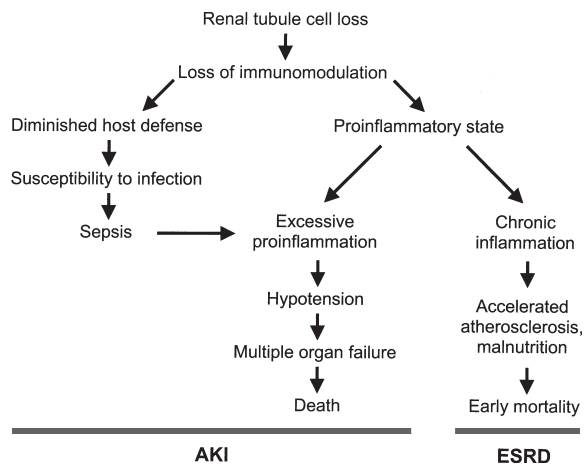


FIG. 1. Schematic representation of the sequelae of inflammatory dysregulation in acute kidney injury (AKI) and end-stage renal disease (ESRD). The intervention of renal proximal tubule cell therapy, renal bio-replacement, may interrupt the pathophysiologic spiral that leads to death in AKI, and may possibly interrupt the chronic proinflammatory state of patients with ESRD.

abolic activity, such as ammoniogenesis and GSH reclamation, activation of vitamin D3, low levels of which seem to correlate with high mortality rates in hospitalized patients (40), immunoregulatory support, and cytokine homeostasis may replace important physiologic activities to change the current natural history of this disease process (Fig. 2; 21). We have developed an extracorporeal device utilizing a standard hemofiltration cartridge seeded with approximately 10⁸ renal tubule cells which grow in confluent monolayers along the inner surface of the fibers (41–45).

Functional Characteristics of the Renal Assist Device (RAD)

In vitro studies of this renal tubule assist device have demonstrated that the cells retain differentiated active transport properties, differentiated metabolic activities, and important endocrine processes (Table 1). Additional studies have shown that the RAD, when incorporated in series with a hemofiltration cartridge in an

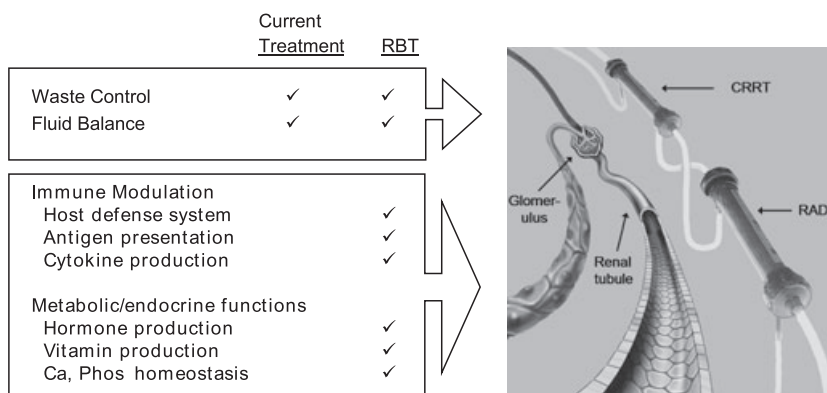


FIG. 2. Advantages of renal bio-replacement therapy compared with the current treatment. Renal bio-replacement therapy replicates the structure and complete function of the nephron and provides more complete renal replacement therapy.

TABLE 1. In vitro and ex vivo properties of renal tubule cell assist device

	Transport	Metabolic	Endocrinologic
In vitro	Sodium—ouabain inhibitable Bicarbonate—acetazolamide inhibitable Glucose—phlorizin inhibitable PAH—probenecid inhibitable	Ammoniogenesis—pH-sensitive Glutathione synthesis—acivicin inhibitable	1-hydroxylation of vitamin D ₃ -PTH, Pi-sensitive
Ex vivo	Sodium Potassium Bicarbonate Glucose	Ammonia excretion Glutathione reclamation	1-hydroxylation of 25-OH vitamin D ₃

extracorporeal blood perfusion circuit, replaces filtration, transport, metabolic, and endocrine functions of the kidney in acutely uremic dogs (42). RADs consisting of either human or porcine cells have been successfully fabricated and tested (43).

Renal Cell Therapy in Animal Model of AKI and Sepsis

Our recent studies have demonstrated that renal bio-replacement therapy with the RAD ameliorates endotoxin or bacterial septic shock in acutely uremic animals. Nephrectomized Mongrel dogs treated with continuous venovenous hemofiltration (CVVH) and either a RAD or sham cartridge were given endotoxin to simulate gram-negative septic shock (44). Mean peak levels of an anti-inflammatory cytokine, IL-10, and mean arterial pressures (MAP) were found to be significantly higher in cell-treated animals. To further assess the effect in ARF with bacterial sepsis, dogs were nephrectomized and 48 hours later administered intraperitoneally with *Escherichia coli* (45). Immediately after bacteria administration, the animals were placed in a CVVH circuit with either a RAD or a sham cartridge. RAD treatment maintained better cardiovascular performance, as determined by MAP and cardiac output, for longer periods than sham RAD therapy. All sham animals expired within 2–10 hours after bacteria administration, whereas all cell RAD-treated animals survived greater than 10 hours. Levels of IL-10, an anti-inflammatory cytokine, were significantly elevated in the RAD group, and a significant correlation was observed between the rise in plasma IL-10 levels and the decline in MAP. The RAD maintained renal metabolic activity throughout the septic period. In another study, pigs with normal kidney function were administered *E. coli* intraperitoneally (46). One hour later, the animals were placed in a CVVH circuit containing either a RAD or a sham cartridge. All animals developed ARF with anuria within 2–4 hours after bacteria administration. RAD treatment maintained better cardiovascular performance, as determined by cardiac output and renal blood flow, for longer periods than sham therapy. Consistently, the RAD group survived longer than the controls (10 ± 2 hours versus 5 ± 1 hour, respectively). RAD treatment was associated with significantly lower plasma circulating levels of IL-6, a proinflammatory cytokine, and interferon- γ . These data demonstrate that septic shock results in early ARF and that renal bio-replacement therapy improves the cardiovascular performance associated with changes

in cytokine profiles and confers a significant survival advantage.

Clinical Experiences of Renal Cell Therapy in Critically Ill Patients with AKI

The Food and Drug Administration (FDA) approved an Investigational New Drug application to study the RAD containing human cells in patients with ATN receiving CVVH. Human kidney cells were isolated from kidneys donated for cadaveric transplantation but found unsuitable for this purpose because of anatomic or fibrotic defects. The initial results in the first 10 treated patients demonstrated that the RAD can be delivered safely for up to 24 hours (47). Cardiovascular stability was maintained, and increased native renal function, as determined by elevated urine outputs, temporally correlated with RAD treatment. The cells demonstrated differentiated metabolic and endocrinologic activity in this ex vivo treatment. GSH degradation and endocrinologic conversion of 25-OH-D₃ to 1,25-(OH)₂-D₃ by the RAD tubule cells were demonstrated. All 10 patients were critically ill with AKI and MOF, with predicted hospital mortality rates between 80% and 95%. One patient expired within 12 hours after RAD treatment following his family's request to withdraw ventilatory life support. Another expired after a surgical catastrophe, toxic megacolon, required discontinuation of RAD treatment after only 12 hours. Of the remaining eight patients, six survived past 28 days with renal function recovery.

Plasma cytokine levels suggest that RAD therapy produces dynamic and individualized responses in patients depending on their unique pathophysiologic conditions. For the subset of patients who had excessive proinflammatory levels, RAD treatment resulted in significant declines in granulocyte-colony stimulating factor, IL-6, IL-10, and especially IL-6/IL-10 ratios, suggesting a greater decline in IL-6 relative to IL-10 levels and a less proinflammatory state. Subsequently, a Phase II, randomized, controlled, open-label trial involving 58 patients who had AKI and required continuous renal replacement therapy (CRRT) was carried out at 12 clinical sites (48). Despite the critical nature and life-threatening illnesses of the patients enrolled in this study, the addition of the RAD to CVVH resulted in a substantive clinical impact on survival compared with a conventional CRRT group. RAD treatment for up to 72 hours promoted a statistically significant survival advantage over 180 days of follow-up in intensive care unit (ICU) patients with AKI and demonstrated an acceptable

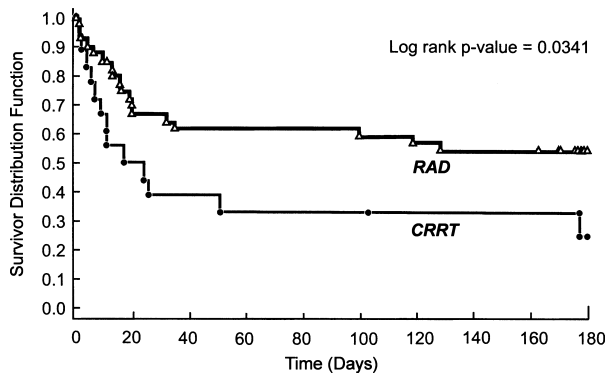


FIG. 3. Kaplan–Meier estimates of survival of patients in the renal tubule cell assist device (RAD) and conventional continuous renal replacement therapy (CRRT) groups. The mortality rate at day 28 was 61% in the CRRT group compared with 33% in the RAD group, and at day 180 mortality was 76.5% in the CRRT group compared with 50% in the RAD group. The hazard ratio for death in the RAD group compared with the CRRT group adjusted for disease cause was 0.481 (95% confidence interval, 0.23–0.99). Adapted from Tumlin et al. (48).

safety profile. Cox proportional hazards model suggested that the risk of death was approximately 50% of that observed in the CRRT-alone group (Fig. 3). A follow-up Phase IIb study to evaluate a commercial manufacturing process was not completed owing to difficulties with the manufacturing process and clinical study design. This approach will be further evaluated when an improved scale-up manufacturing process is established.

Renal Bio-Replacement Therapy for ESRD

A major disease process that increases the accelerated morbidity and mortality of ESRD patients is generalized atherosclerosis. Recent literature has shown an important role for free-radical-induced oxidation of lipoproteins in atheroma formation (49). Renal tubule cells are critical in maintaining the circulating antioxidant compounds of the body (28), including the plasma levels of GSH and its peroxidases, some of the most important free-radical-scavenging substances in the blood. The replacement of these key moieties by use of the RAD may significantly alter the accelerated atherogenesis in ESRD patients. The use of renal tubule cells in a chronic cell therapy device to produce $1,25(\text{OH})_2\text{-D}_3$, which is regulated by normal physiologic parameters such as parathyroid hormone and inorganic phosphate levels, would provide a long-term treatment program to minimize the progression of renal osteodystrophy. Finally, the ability of renal proximal tubules cells to take up and catabolize β_2 -microglobulin provides an opportunity to treat the subset of patients who develop dialysis-related amyloidosis secondary to high circulating levels of β_2 -microglobulin (50). These are just a few examples of the commonly associated morbidity of patients on chronic renal substitution therapy and the potential for RAD treatment to improve these pathophysiologic disease processes.

Novel Immunomodulatory Device: Selective Cytopheretic Inhibitory Device (SCD)

Leukocytes, especially neutrophils, are major contributors to the pathogenesis and progression of many clinical inflammatory disorders, including SIRS, sepsis, ischemia/reperfusion injury, and adult respiratory distress syndrome (ARDS) (51,52). A large number of therapeutic approaches are under investigation to limit the activation and tissue accumulation of leukocytes at sites of inflammation to minimize tissue destruction and disease progression. For example, cardiopulmonary bypass (CPB) is a strong inducer of SIRS, with activation of complement and coagulation systems and stimulation of cytokine production, and lung and kidney damage can be ameliorated during CPB surgery with the use of leukocyte depletion filters (53). Disruption of the activation process of circulating leukocytes may limit microvascular damage and multiorgan dysfunction. We have developed a synthetic membrane device with the ability to bind and inhibit activated neutrophils along a continuous renal replacement extracorporeal circuit. This device, called an SCD, improved septic shock survival times in preclinical animal models (54) and improved the survival outcome of ICU patients with MOF in an exploratory, randomized, blinded, multicenter trial (55).

Conclusion

For many decades, dialysis has benefited both chronic and acute renal failure patients. However, further development of this approach, which is based solely on water and solute removal, cannot be expected to yield further therapeutic advantage in terms of reducing morbidity and improving survival in either acute or chronic diseases. We have recently developed a cell therapy device containing living animal or human renal tubule cells. We have confirmed its viability and functionality in *in vitro* and large animal studies, and recently demonstrated its therapeutic benefits in a series of FDA-approved multicenter human trials.

Our research group is now focused on the role of kidney as an immunomodulatory organ and the possibility of renal bio-replacement therapy for the systemic treatment of inflammatory dysregulation. The RAD appears to influence systemic leukocyte activation and the balance of inflammatory cytokines, and may alter the proinflammatory state of AKI and ESRD and, ultimately, improve the morbidity and mortality of these disorders. In addition, a novel synthetic membrane device, the SCD, mimics immunomodulation and duplicates RAD efficacy. We believe both devices will become comprehensive treatments to more fully replace renal functions and correct inflammatory imbalance in patients with acute or chronic renal disorders, especially those patients with critical illnesses such as sepsis, SIRS, or MOF. The future focus of renal care must go beyond replacement of the small-solute clearance function of the kidney.

Disclosure

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