

Review Article

Bioartificial kidney in the treatment of acute renal failure associated with sepsis

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SUMMARY: Acute renal failure (ARF) associated with sepsis has a high rate of mortality. It is not merely a surrogate marker for severity of disease but also an independent predictor of mortality and a separate pathogenic entity, even when nearly physiological doses of fluid and small-molecule clearance are maintained with currently available renal replacement therapies (RRT). The techniques to remove cytokines, including high-volume haemofiltration, haemodialysis using high-cut-off haemofilters, and absorptive techniques, lead to some improvement in outcome but are still insufficient to reverse the complicated biological dysregulation resulting from ARF associated with sepsis. The novel and exciting technique of cell therapy, which is based on the principle of using functional cells to replace a greater range of renal functions, may add significant benefit to current RRT in dealing with this disease process. Because renal tubule cells appear to play critical roles in immunoregulation, renal tubule cell therapy during ARF associated with sepsis should alter the detrimental multiple-organ consequences of sepsis. The development of a bioartificial kidney consisting of a conventional haemofiltration cartridge in series with a renal tubule assist device containing renal proximal tubule cells represents a new therapeutic approach to this clinical disorder. The results to date of large animal studies and recent Phase I/II and Phase II clinical trials show that such a device replaces multiple kidney functions and modifies the sepsis condition to improve survival in ARF.

KEY WORDS: acute renal failure, bioartificial kidney, haemodialysis, sepsis.

Acute renal failure (ARF) is common, affecting approximately 5% of all hospitalised patients and up to 20% of critically ill patients. The incidence goes up to 51% in patients with septic shock with positive blood cultures.¹ Furthermore, the combination of ARF and sepsis is associated with 75% mortality, compared with 45% mortality among patients with ARF alone.² This unacceptably poor prognosis persists despite the fact that the kidney was the first organ for which *ex vivo* substitutive therapy became available, when human dialysis was first performed in 1926.³ Substantial progress has been made toward understanding the pathogenesis of ARF associated with sepsis, and studies of new interventions with the potential to attenuate or even prevent this condition are ongoing. One of the most important interventions is renal replacement therapy (RRT), which ideally should replace all functions of the kidney. However, it is prohibitively difficult to replace the complicated biological functions of the kidney using synthetic

devices alone. Cell therapy techniques, including a bioartificial kidney currently in clinical trials, offer an alternative approach to conventional RRT.

CURRENT RENAL REPLACEMENT THERAPY IN ARF ASSOCIATED WITH SEPSIS

Current therapies for ARF with either intermittent or continuous RRT reduce death from hyperkalaemia, volume overload, and uraemic complications but are still suboptimal. Although survival rates are improved by increasing doses of treatment from alternate-day to daily intermittent haemodialysis⁴ and from a continuous venovenous haemofiltration (CVVH) rate of 20 mL/kg/h to 35 mL/kg/h,⁵ patients with ARF associated with sepsis still have high mortality. The cause of death in sepsis patients is usually the development of a systemic inflammatory response syndrome (SIRS) resulting in loss of kidney function and cardiovascular collapse, ischaemic damage to vital organs, and multiorgan failure (MOF).⁶ ARF usually follows from septic-related injury to the tubular epithelial cells, particularly in the S3 segment of the proximal tubule, resulting in acute tubular necrosis (ATN). Mechanisms of the pathogenesis of ARF associated with sepsis are multiple and have not been fully clarified. These include systemic and renal haemodynamic changes, an imbalance between proinflammatory and

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anti-inflammatory cytokines, oxidative stress and anti-oxidative stress factors, vasodilatation and vasoconstriction, thrombosis and bleeding, catabolic and anabolic activity and dysregulation of enzyme activity.^{7,8} The proinflammatory cytokines such as tumour necrosis factor (TNF), interleukin (IL)-1 and IL-6 overcome the anti-inflammatory cytokines and play an important role in the pathogenesis of ARF, including promotion of further cytokine release, induction of vasoconstriction, neutrophil aggregation, production of reactive oxygen species, induction of tissue factor and promotion of thrombosis.

The development of novel renal replacement modalities for sepsis generally focuses on correcting these imbalances in addition to replacing the filtration function of the kidney. One such therapeutic approach is high-volume haemofiltration, which is based on the principle of non-specific removal of soluble mediators, both pro- and anti-inflammatory, by increasing convective clearance. Clinical studies of this approach have shown some improvement in outcomes.⁵ Recently, RRT using high-cut-off (approximately 60 kDa) haemofilter membranes demonstrated incremental cytokine clearance in both convective (CVVH) and diffusive (CVVHD) modes, but the latter mode resulted in less protein loss.⁹ Plasmapheresis or plasma exchange is another approach aimed at restoring the imbalance of the pro- and anti-inflammatory cytokines. Survival benefits have been shown in several studies.^{8,10} However, the risks of exposure to pathogens and foreign antigens from the plasma should be considered. Finally, adsorptive treatments aim to remove harmful solutes in the blood by use of sorbent technologies, including both haemoperfusion, in which adsorbent is in direct contact with blood in the extracorporeal circuit, and coupled plasma filtration adsorption, in which filtered plasma is in contact with the adsorbent before returning back to the patient.¹¹ The adsorbent material may be non-specific or specific, such as polymyxin B bead adsorbent which binds specifically to lipopolysaccharide endotoxin.¹² Although these approaches aim to improve the outcome of ARF associated with sepsis by moving beyond renal filtration to correct the immunoregulation of SIRS, they are still non-specific in their effects. For example, beneficial mediators may be removed along with detrimental agents. These approaches rely entirely on artificial materials and systems that may perform specific tasks well but thereby fail to replicate the complex biological functions of the living body. These modalities do not substitute for the metabolic, endocrine and immunoregulation functions of the kidney thought to play a critical role in sepsis. Cell therapy, on the other hand, should better replicate these complex functions to offer more complete renal replacement treatment.

CELL THERAPY IN ACUTE RENAL FAILURE

Cell therapy is a new and exciting therapeutic approach to acute and chronic diseases.¹³⁻¹⁵ The potential success of the treatment is indicated by the growing appreciation that most disease processes are not due to the lack of a single protein but result from alterations in the complex interactions of a variety of cell products.

There is growing evidence that ARF is not merely a surrogate marker for severity of disease but also an independent predictor of mortality and a separate pathogenic entity, even when nearly physiological doses of small-molecule clearance are administered. This gives rise to the hypothesis that there are clinically important functions of the native kidney that are not replaced by dialysis or haemofiltration. These may include synthesis of cytokines,¹⁶⁻¹⁹ antigen presentation, reclamation of glutathione, synthesis of glutathione reductase, oxidative deamination and gluconeogenesis, 1,25-dihydroxyvitamin D₃ hydroxylation, trace mineral and element reclamation or other as yet undiscovered entities.²⁰ In ARF associated with sepsis, the detrimental consequences of sepsis arise not from a single mechanism but from complicated multiple mechanisms, including loss of kidney function, specifically proximal renal tubular cell function secondary to ATN.

Renal proximal tubule cells have been isolated from cadaveric kidneys and cultured for the purpose of integrating them with a filtration device to provide more complete renal replacement.^{21,22} These proximal tubule cells, obtained from adult tissue and having stem-cell-like characteristics, are grown in confluent monolayers along the inner surface of the hollow fibres in a conventional haemofiltration cartridge. The resulting construct containing these living cells is called the bioartificial renal tubule assist device (RAD).²³ Embryonic or adult stem cells exposed to the appropriate environmental cues *in vitro* have the potential to provide a better source of cells for clinical use (Fig. 1) and studies aimed at reproducibly guiding the differentiation of embryonic or adult stem cells into renal tubule cells are ongoing.

RENAL TUBULE ASSIST DEVICE

The RAD is clearly feasible when conceived as a combination of living cells supported on polymeric substrata acting as scaffolds for the cells. The renal tubule progenitor cells were cultured on the biomatrix-coated hollow fibre membrane of a standard high-flux haemofiltration cartridge. The membrane is both water- and solute-permeable, allowing for differentiated vectorial transport and metabolic and endocrine activity (Fig. 2). Immunoprotection of cultured progenitor cells is achieved concurrent with long-term functional performance as long as conditions support tubule cell viability.²⁶ Studies of RAD populated with porcine renal proximal tubule progenitor cells have demonstrated that the cells retain vectorial fluid transport properties as a result of Na⁺,K⁺-ATPase; other differentiated active transport properties, including active glucose and bicarbonate transport; differentiated metabolic activities, including intraluminal glutathione breakdown, constituent amino acid uptake and ammonia production; and the important endocrinological conversion of 25-OH-vitD₃ to 1,25-(OH)₂-vitD₃.²⁷

BIOARTIFICIAL KIDNEY

The bioartificial kidney consists of a filtration device (a conventional high-flux haemofilter) followed in series by the

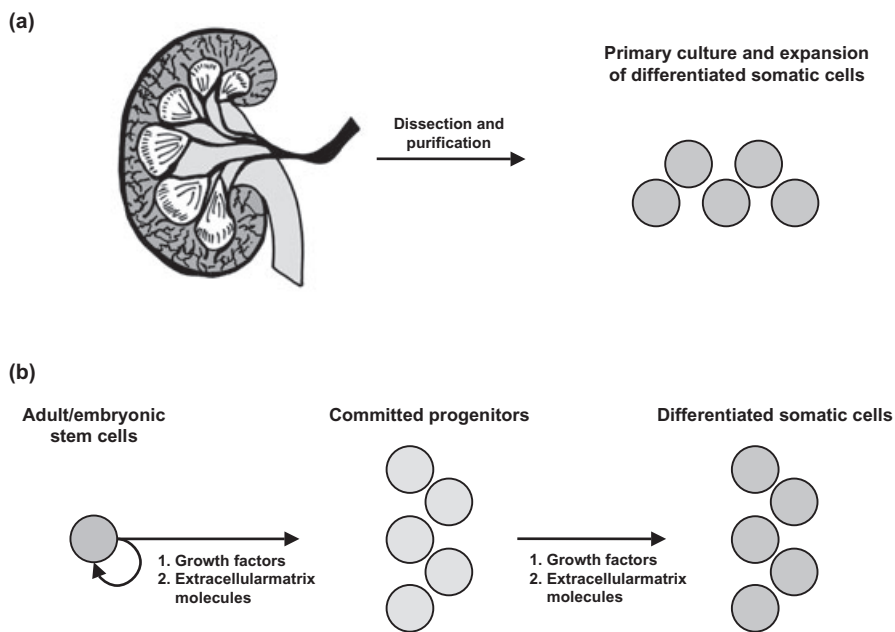


Fig. 1 (a) A methodology for the purification and expansion of primary cells from adult kidney exists. Primary cells are retrieved from post-mortem kidneys through a regimen that includes enzymatic digestion, sieving and centrifugation. Cells are expanded *in vitro* prior to seeding into a bioartificial renal tubule assist device (RAD). Progenitor and stem cells have been purified from other tissue sources, including muscle and skin. (b) Alternative methodologies for generation of fully functional differentiated somatic cells are currently being investigated by researchers in the stem cell field. The working hypothesis is that adult or embryonic stem cells can differentiate into somatic cells if placed into the appropriate chemical and physical environment. A major challenge of this effort is to reproduce the complex temporal, spatial and signalling events that occur during development. (Reproduced from Humes and Szczyzka,²⁴ with permission from Elsevier.)

RAD. Blood pumped out of the patient enters the fibres of the haemofilter, where ultrafiltrate (UF) is formed and delivered into the fibres of the tubule lumens within the RAD, downstream of the haemofilter. Processed UF exiting the RAD is collected and discarded as 'urine.' The filtered blood exiting the haemofilter enters the RAD through the extracapillary space port and disperses among the fibres of the device. Upon exiting the RAD, the processed blood is returned to the patient's body via a third pump (Fig. 3). The RAD is orientated horizontally and kept in a 37°C temperature-controlled environment to ensure optimal functionality of the cells.

Studies have shown that the bioartificial kidney using a RAD consisting of either porcine or human cells replaces filtration, transport, metabolic, and endocrine functions of the kidney in acutely uraemic dogs following bilateral nephrectomies.^{28,29} The dogs were treated with haemofiltration and either a RAD cartridge containing tubule cells or a sham control cartridge containing no cells. Fluid and small solutes, including urea nitrogen (BUN), creatinine (Cr) and electrolytes, were adequately controlled in both groups. However, potassium and BUN levels were more easily controlled during RAD treatment compared with sham treatment. Furthermore, active reabsorption of K^+ , HCO_3^- , and glucose and excretion of ammonia were accomplished only in RAD treatments. Glutathione reclamation from UF was

greater than 50% in the RAD. Finally, uraemic animals receiving cell therapy attained normal $1,25-(OH)_2\text{-vitD}_3$ levels, whereas sham treatment resulted in a further decline from the already low plasma levels.²⁸

BIOARTIFICIAL KIDNEY IN ARF ASSOCIATED WITH SEPSIS

Patients presenting with sepsis and ARF have an exceedingly high mortality rate⁷ due to their propensity to develop SIRS followed by MOF,⁶ despite maintenance of normal electrolyte balance and improvement of the uraemic state. The sequential failure of organ systems apparently unrelated to the site of the initial insult has been correlated with altered plasma cytokine levels.³⁰⁻³²

Because ARF secondary to sepsis, as well as other ischaemic and/or nephrotoxic insults, arises from ATN, replacement of the functions of renal proximal tubule cells during the episode of ATN and in conjunction with haemofiltration would provide almost full renal replacement therapy. The addition of metabolic activity such as ammoniogenesis and glutathione reclamation, endocrine activity such as activation of vitamin D_3 (low levels of which appear to correlate with high mortality rates in hospitalized patients),³³ cytokine homeostasis, and immunological

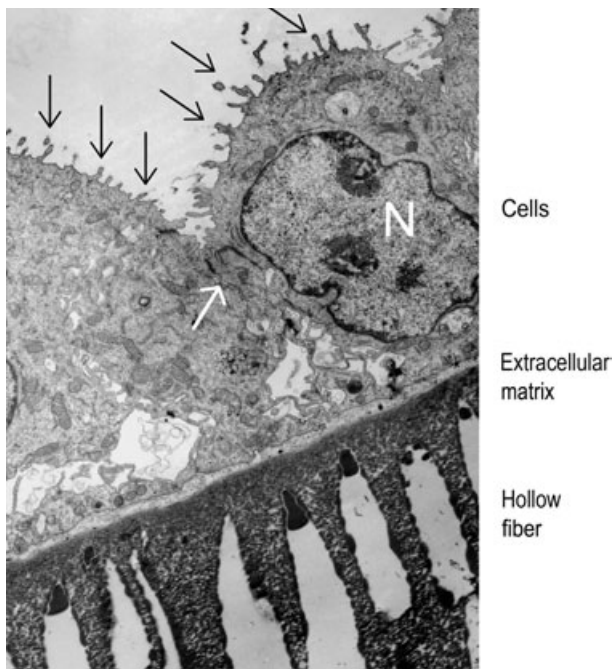


Fig. 2 Electron micrograph of a tissue-engineered bioartificial renal tubule. The nucleus (N) is shown; black arrows delineate apical microvilli (a differentiated morphological characteristic of proximal tubule cells), and the white arrow identifies the tight junctional complex of a transporting epithelium. (Adapted from Humes,²⁵ with permission from Elsevier.)

activity may change the current natural history of this disease process.²⁴

Preclinical animal studies

In a series of animal experiments to investigate whether the bioartificial kidney can protect against the high mortality of sepsis complicated by ARF, surgically nephrectomised dogs were administered endotoxin *i.v.* and treated with CVVH and either a RAD or an identically prepared sham cartridge containing no cells. Mean arterial pressures were found to be significantly higher in RAD-treated animals. Mean peak levels of an anti-inflammatory cytokine, IL-10, were also significantly higher in these animals. Levels of a proinflammatory cytokine, TNF- α , were on average lower among RAD-treated versus sham-treated animals but not statistically significant.³⁴

To further assess the effect of the bioartificial kidney in ARF with bacterial sepsis, dogs were nephrectomised and 48 h later administered with 3×10^{11} *Escherichia coli* cells/kg bodyweight *i.p.*³⁵ Immediately after bacteria administration, animals were placed in a CVVH circuit with either a RAD with cells or a sham cartridge without cells. RAD treatment maintained better cardiovascular performance, as determined by mean arterial blood pressure and cardiac output, for longer periods than sham therapy. All sham animals

expired within 2–10 h after bacteria administration, whereas all RAD-treated animals survived greater than 10 h. Plasma cytokine levels of IL-10 were significantly higher in the RAD group compared with the control group.³⁵

In another study, pigs with normal kidney function were administered with 3.0×10^{11} *E. coli* cells/kg bodyweight *i.p.* One hour later, animals were placed in a CVVH circuit containing either a RAD with cells or a sham cartridge without cells. All animals developed ARF with anuria within 2–4 h after bacteria administration. RAD treatment maintained better cardiovascular performance, as determined by cardiac output and renal blood flow, for longer periods than sham therapy. The RAD-treated group consistently had significantly longer survival times than control animals. RAD treatment was associated with significantly lower plasma circulating proinflammatory cytokine levels of IL-6 and interferon- γ . Together, these data demonstrate that septic shock results in early ARF and that RAD treatment in a bioartificial kidney circuit improves cardiovascular performance associated with changes in cytokine profiles and confers a significant survival advantage.³⁶

Phase I/II clinical trial

These encouraging preclinical animal data led to a Food and Drug Administration (FDA)-approved Phase I/II clinical trial to evaluate the safety and efficacy of this new system on 10 critically ill patients with ARF and MOF receiving CVVH.³⁷ The predicted hospital mortality rates for these patients averaged greater than 85%. The devices used in this study were seeded with human renal proximal tubule cells isolated from kidneys donated for cadaveric transplantation but found to be unsuitable due to anatomic or fibrotic defects. The RAD perfusion pump system was connected in series to a CVVH extracorporeal pump system, following the principle tested in the preclinical animal studies (Fig. 3), but with a minor adaptation of the circuit to maintain the original CVVH prescription in terms of blood flow rate from the patient and UF rate from the haemofilter (Fig. 4). The postfiltered blood from the CVVH circuit was pumped with a peristaltic pump system at a rate of 150 mL/min to the extracapillary space of the RAD and dispersed among the fibres of the device. Upon exiting the RAD, the processed blood travelled through an additional pump and was delivered back to the patient. The UF formed from the synthetic haemofilter was delivered into the fibers of the tubule lumen within the RAD downstream to the haemofilter at rate of 10 mL/min. The hydraulic pressures within the RAD were adjusted to reabsorb and return UF to the patient at a rate of 5 mL/min. Processed UF exiting the luminal space of the RAD was collected and discarded as 'urine' (Fig. 4).

The results of this clinical trial demonstrated that the experimental treatment could be delivered safely under study protocol guidelines for up to 24 h when used in conjunction with CVVH.³⁷ The clinical data also indicate that the RAD maintains and exhibits viability, durability and

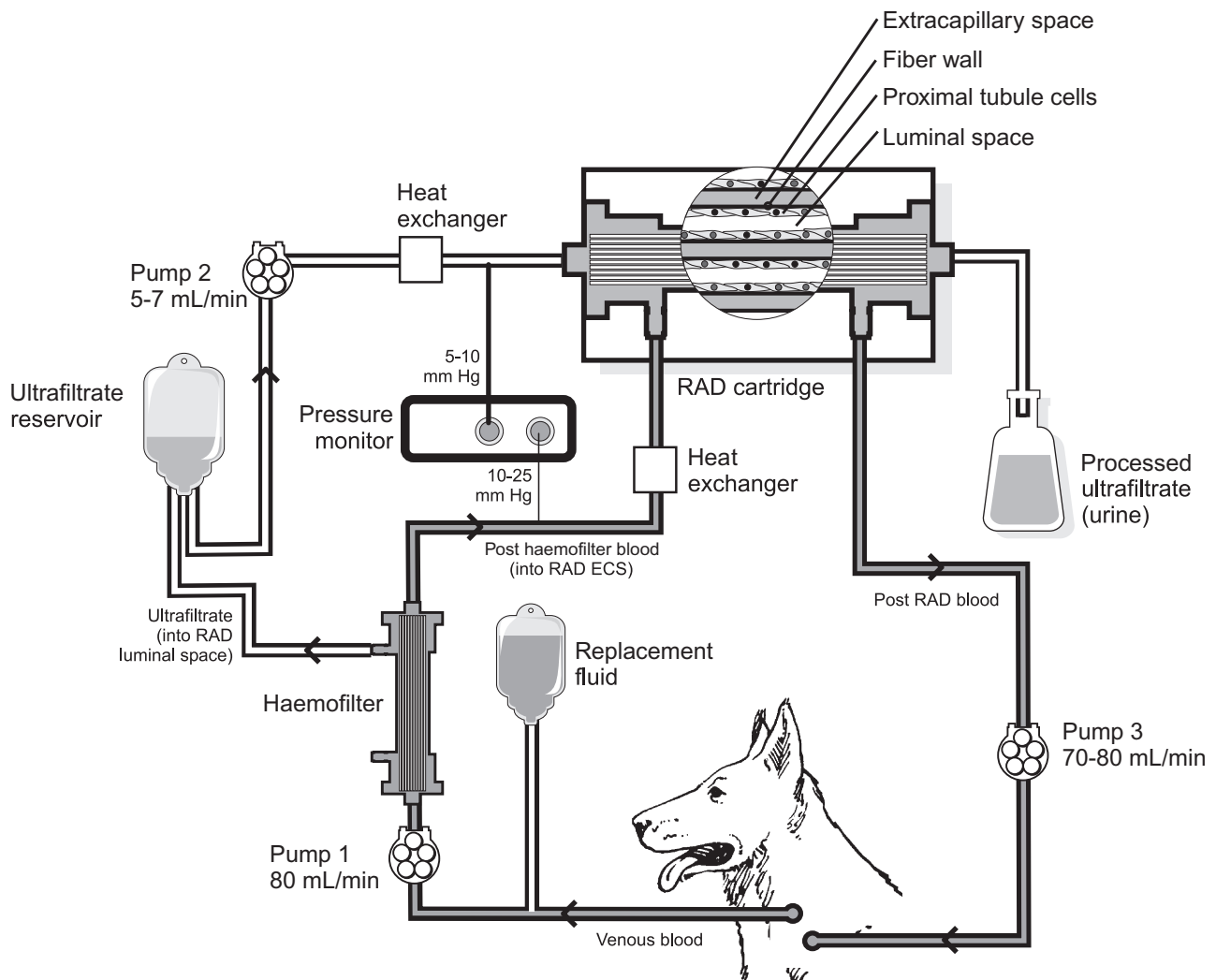


Fig. 3 Schematic of the extracorporeal circuit for the bioartificial kidney that was used in preclinical studies consists of a synthetic haemofilter and the renal tubule assist device (RAD) cartridge. ECS, extracapillary space. (Reproduced from Fissell *et al.*²³ with permission from Blackwell Publishing.)

functionality in this clinical setting. Cardiovascular stability of the patients was maintained, and increased native kidney function, as determined by elevated urine outputs, temporally correlated with RAD treatment. The device also demonstrated differentiated metabolic and endocrinological activity, with glutathione reclamation and endocrinological conversion of 25-OH-vitD₃ to 1,25-(OH)₂-vitD₃. All but one treated patient with more than a 3-day follow up showed improvement, as assessed by acute physiological scores. Six of the 10 treated patients survived past 28 days with kidney function recovery. One patient expired within 12 h after RAD treatment due to his family's request to withdraw ventilatory life support. Three other patients died due to non-recoverable complications unrelated to RAD therapy and ARF, including toxic megacolon in one patient, fungal pericarditis and vancomycin-resistant enterococcus septicaemia in another patient, and ischaemic colitis with

bowel perforations in the third patient. Plasma cytokine levels suggest that RAD therapy produces dynamic and individualised responses in patients depending on their unique pathophysiological 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.

These favourable Phase I/II trial results led to an FDA-approved, randomised, controlled, open-label Phase II investigation at 10 clinical sites to determine whether this cell therapy approach alters patient mortality. This Phase II study involved 58 patients, of whom 40 were randomised to RAD therapy and 18 made up a control group with comparable demographics and severity of illness. The early results have been as compelling as the Phase I/II results. Renal cell

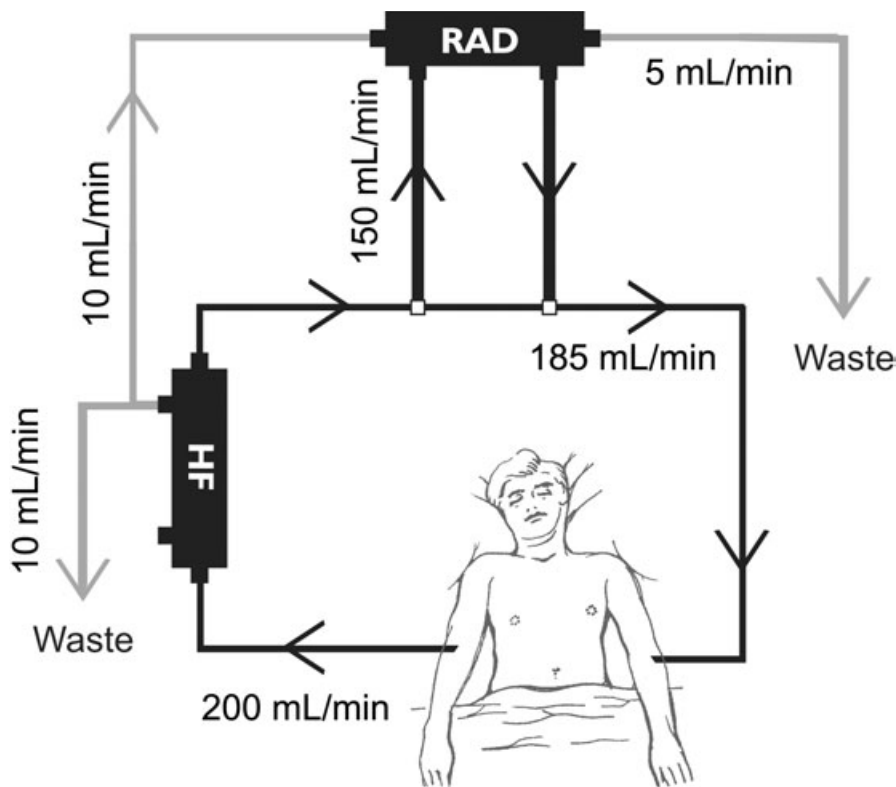


Fig. 4 Schematic of the extracorporeal circuit for the perfusion of the bioartificial kidney that was used in the Phase I/II clinical trial. HF, haemofilter; RAD, renal tubule assist device. (Reproduced from Humes *et al.*³⁷ with permission from Nature Publishing Group.)

therapy improved the 28-day mortality rate from 61% in the conventional haemofiltration-treated control group to 34% in the RAD-treated group.³⁸ The results of these clinical trials have shown a safety profile comparable to CVVH alone. Hypoglycaemia and hypotension, both treatable in the intensive care unit, have been observed due to insulin release from cell culture media during maintenance of the RAD in manufacturing and the increase in extracorporeal blood volume required by the second cell-containing cartridge, respectively. The addition of a second cell-containing cartridge necessitates an additional extracorporeal pump system to maintain adequate filtrate and blood flow to the cells. This pump system requires additional expertise to maintain safe functionality and interface with the standard CVVH circuit. In an extension of this approach to chronic renal failure, an evaluation of renal tubule cell therapy in an exploratory Phase I/II trial in ESRD patients on chronic haemodialysis is planned to begin in early 2006. This trial will focus on safety issues as well as assess the influence of short-term cell therapy on the key biomarkers, C-reactive protein and IL-6, as surrogates of the proinflammatory state of ESRD. This protocol will test the hypothesis that the excessive proinflammatory state of ESRD, the root cause of accelerated atherosclerosis, may be owing to renal tubule cell loss rather than a decline in renal clearance function.

CONCLUSION

Acute renal failure is a common complication of sepsis. The loss of kidney function – including fluid and electrolyte

homeostasis and metabolic, endocrine and immunological activities – leads to further deterioration and increased mortality in sepsis patients. Despite advances in current RRT, which improve only the fluid and electrolyte homeostasis function of the kidney, mortality rates have remained fairly stable over the last two decades. These therapies fail to address the complicated pathophysiology of sepsis coupled with ARF. Cell therapy has the potential to overcome many of the limitations of existing treatments. The bioartificial kidney, a cell therapy approach to renal replacement therapy, has been tested in large animal studies and Phase I/II and Phase II clinical trials. The results to date have demonstrated the RAD's ability to replace multiple kidney functions and confer a survival advantage in ARF associated with sepsis, seemingly due to modulation of inflammatory mediators. Further studies are required to confirm these results and elucidate detailed mechanisms underlying the RAD's effects.

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