

Cell-based therapies in kidney disease

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Cell-based therapy is an emerging field but one that has shown early promise for the treatment of human kidney diseases. The most widely used cell is the mesenchymal stem cell (MSC) given its ability to be harvested from bone marrow, expanded in culture, and used in allogeneic protocols. The beneficial effects of MSCs occur through differentiation-independent pathways that include increased cell survival and proliferation, decreased inflammation, and suppression of immune function. Acute kidney injury and kidney transplantation are the two conditions most frequently treated with MSC infusion. Although initial studies are promising, the long-term efficacy and safety of MSC infusion awaits further study.

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Diseases of the kidney result in cell damage and death leading to impaired kidney function. In many cases, kidney repair and regeneration can occur, but these processes are frequently incomplete and accompanied by fibrosis and long-term loss of function. Cell therapy has emerged as a strategy to enhance the renal regenerative response and to modulate the reaction to injury. Building on lessons learned in other organ systems, cell-based therapy is an emerging field in nephrology. The purpose of this review is to discuss the role of cell therapy in kidney diseases with a focus on how this therapeutic approach has been translated to human kidney disease.

CELL SOURCE FOR TREATMENT

Mesenchymal stem cells (MSCs), also known as mesenchymal stromal cells, are the most widely used cells for the treatment of kidney diseases.¹ MSCs originate from the mesodermal germ layer and can differentiate into both mesenchymal and non-mesenchymal cell lineages including bone, cartilage, muscle, fat, cardiomyocytes, and neurons. Bone marrow is the principal source of MSCs given its accessibility, but these cells can be isolated from other tissues such as liver, muscle, adipose tissue, and umbilical cord blood. MSCs can be easily expanded in culture and used for both autologous and allogeneic infusions given their low immunogenicity. Characteristics of the cells include their adherence to plastic, expression of CD90, CD73, CD105, and lack of expression for CD34, CD45, CD19, CD11a, and human leukocyte antigen-DR. MSCs express unique transcripts and proteins not expressed by hematopoietic stem cells.¹

MSCs can migrate to sites of injury and release factors that increase cell survival and proliferation, exert anti-inflammatory actions, and modulate the immune response. These actions combined with their accessibility and *in vitro* expansion ability make MSCs an attractive cell to use for therapeutic purposes. Evidence from preclinical and clinical studies has shown that treatment with MSCs is safe, and in many cases, effective. Clinical studies have been successfully performed in graft-versus-host disease, Crohn's disease, amyotrophic lateral sclerosis, multiple sclerosis, stroke, coronary artery disease, heart failure, cirrhosis, chronic obstructive pulmonary disease, and osteogenesis imperfecta.¹

ACUTE KIDNEY INJURY

Acute kidney injury (AKI) is a common complication of hospitalized patients contributing to significant morbidity

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and mortality. In addition, AKI has emerged as a significant factor leading to the progression of chronic kidney disease. Recovery of kidney function following an episode of AKI is dependent on renal regeneration. The source of regenerating cells is most likely surviving tubular epithelial cells that undergo dedifferentiation, proliferation, migration, and redifferentiation to reline the denuded tubule restoring the structural and functional integrity of the kidney.² Stem cells and extrarenal cells were thought to have a role in the regenerative response. However, available evidence has failed to demonstrate a role for these cells.

Cellular therapy has been used to enhance the regenerative response following AKI. The initial rationale was to replace renal tubular epithelial cells by transdifferentiation of infused cells. The most commonly infused cell has been the MSC although other cell types such as hematopoietic stem cells, endothelial cells, and unfractionated bone marrow containing multiple stem cell types have been used with varying degrees of efficacy.³⁻⁵ A protective effect of MSC infusion has been demonstrated in many preclinical models of AKI including ischemia-reperfusion, cisplatin nephrotoxicity, myoglobinuric AKI, and sepsis-induced AKI. The most important discovery in these studies was that the beneficial effect was not due to transdifferentiation of infused cells into tubular epithelial cells, but rather it occurred through differentiation-independent pathways. MSCs home to the injured kidney and exert anti-inflammatory, anti-apoptotic, mitogenic, and pro-angiogenic effects in the injured kidney accounting for less injury and faster recovery of kidney function.^{3,6} There is an increasingly recognized systemic inflammatory response to AKI leading to adverse effects in other organs such as the lung, brain, and heart. MSCs may also be capable of dampening this inflammatory response leading to beneficial effects that extend beyond the kidney.

Homing of MSCs to the site of injury is mediated by upregulated expression of homing receptors CD44 for hyaluronic acid, and CXCR4 for stromal-derived cell factor-1. Both hyaluronic acid and stromal-derived cell factor-1 are produced in the injured kidney.³ The mediators for the beneficial effects of MSCs have been defined and include molecules such as vascular endothelial growth factor, hepatocyte growth factor, insulin-like growth factor-1, interleukin-10, basic fibroblast growth factor, and transforming growth factor- α ('MSC secretome').^{3,7} MSC can also downregulate expression of pro-inflammatory molecules such as interleukin-1 β , tumor necrosis factor- α , inducible nitric oxide synthase, and interferon- γ . Despite the ability of MSCs to home to the site of injury and to function in a paracrine and endocrine manner, the route of administration can affect renal outcomes. For unclear reasons, in models of AKI and CKD, intra-arterial administration of MSCs has been associated with better preservation of kidney function compared with intravenous or intrarenal administration.^{3,6}

Various preconditioning strategies have been performed to increase the efficacy of MSC infusions in AKI. The rationale behind many of these studies was to increase homing of

infused cells to the site of injury thereby reducing the number of cells that have to be infused.^{8,9} Other studies have genetically altered MSCs to make them more resistant to oxidant stress. To avoid genetic alteration of the cells, another strategy has been to preincubate the cells with different trophic factors. For example, preincubation of MSCs with insulin-like growth factor-1 before infusion resulted in enhanced migration capacity and restoration of renal function after cisplatin-induced AKI.¹⁰ A similar added protective effect was seen by preincubation of MSCs with darbepoetin.¹¹

In view of the positive preclinical studies, a phase 1 clinical study has been performed in 16 patients undergoing on-pump cardiac surgery who were identified as being at high risk for postoperative AKI (NCT00733876).³ Risk factors included underlying CKD, diabetes, chronic obstructive pulmonary disease, and predicted cardiopulmonary bypass time >2 h. In a dose-escalation protocol, groups of five patients received a pre-determined dose of allogeneic MSC (average dose 2×10^6 /kg body weight) infused into the suprarenal aorta through a femoral catheter after they came off cardiopulmonary bypass. The cell infusions were safe at all tested dosages. Preliminary data demonstrated MSCs protected kidney function and reduced length of hospitalization and need for readmission compared with historical case controls. These early impressions will need confirmation in adequately powered randomized controlled studies.³ Another human trial is testing the effects of MSCs in cisplatin-induced AKI (NCT01275612).

KIDNEY TRANSPLANTATION

MSC infusion has been used in a number of human kidney transplant studies based on the efficacy of these cells in preclinical studies and the effects of MSCs in modulating the immune response. These cells have been demonstrated to prolong the time to rejection of skin grafts, to inhibit T-cell proliferation, inhibit monocyte differentiation to dendritic cells, modulate B-cell functions, suppress natural killer cell cytotoxic effects, and to induce tolerance in some animal models.¹

The first study examining the safety and clinical feasibility of MSC in kidney transplantation was first published in 2011 and involved two patients who safely received MSC infusion on post-transplant day 7.¹² Both patients developed an increase in serum creatinine 7-14 days following cell infusion but otherwise did well. An expansion of regulatory T cells and a restriction of memory T-cell expansion occurred in both patients.

A randomized controlled trial examining the effects of induction therapy with MSCs was performed in 159 living-related kidney transplant patients.¹³ The source of cells was autologous having been harvested from bone marrow 1 month before transplantation. Three groups of subjects were studied, two receiving MSCs as induction therapy along with maintenance treatment with steroids, mycophenolate mofetil, and either regular dose or low-dose calcineurin inhibitor. The non-MS group received anti-interleukin 2

receptor antibody as induction therapy along with the same maintenance therapy that included regular dose calcineurin inhibitor. The primary outcome was incidence of biopsy proven acute rejection and estimated glomerular filtration rate within the first year. Secondary outcomes were 1-year patient and graft survival and incidence of adverse events.

The incidence of acute rejection was improved at 6 months in the MSC-treated groups but not at the predetermined end point of 1 year. When acute rejection did occur, it was more responsive to therapy. Renal function was better preserved in the MSC-treated groups compared with control at 1 year. Patient and graft survival was similar between the groups. There appeared to be faster recovery of kidney function in the MSC-treated groups in the first post-transplant month, although the incidence of delayed graft function was not different. Interestingly, the risk of opportunistic infection was decreased in MSC-treated groups.

The effects of autologous bone marrow-derived MSCs for the treatment of allograft rejection after kidney transplantation was studied in six human leukocyte antigen-mismatched living donor kidney transplant recipients.¹⁴ Patients were selected on the basis of sub-clinical rejection and/or an increase in interstitial fibrosis and tubular atrophy present in a protocol biopsy performed at either 4 weeks or 6 months. Autologous cells were cultured for 20–31 days and 1×10^6 infused twice, 7 days apart. As with the other studies described above, the cells were tolerated without adverse effects. Two patients with rejection had resolution of the rejection. Three patients developed an opportunistic infection. Five of the six patients demonstrated a decrease in peripheral blood mononuclear cell proliferation assay in response to donor cells. The conclusions of this study were that MSC treatment is tolerated and feasible and has immunosuppressing effects. In another small study of three patients, donor-specific MSC infusion achieved successful desensitization in recipients with a positive pretransplant cross match.¹⁵

These human trials suggest that MSC infusion can be effective induction therapy and is a candidate treatment for acute rejection and desensitization, although further studies are needed. Interestingly, MSCs were associated with both an increase and a decrease in opportunistic infections. A review of clinicaltrials.gov turns up ongoing studies examining the effects of MSC to induce renal transplant tolerance, for treatment of chronic allograft nephropathy, and as induction therapy.

OTHER DISEASES

A beneficial effect of MSC infusion has been demonstrated in such animal models of kidney disease as glomerulonephritis, Alports, focal segmental glomerulosclerosis, lupus nephritis, diabetic nephropathy, and the subtotal nephrectomy model of CKD.¹⁶ In the glomerulonephritis model, maldifferentiation of intraglomerular MSCs into adipocytes occurred and was accompanied by glomerular sclerosis.¹⁷ Currently, there

are 225 registered clinical trials using MSCs to treat various human conditions. From a kidney perspective, there are a number of ongoing studies using MSCs in AKI and kidney transplantation as mentioned above, but also in atherosclerotic renal artery stenosis, lupus nephritis, and diabetic nephropathy (clinicaltrials.gov).

RISKS OF CELL THERAPY

Theoretical and observed risks of cell therapy in kidney diseases dictates that wide spread adoption of this therapy proceeds with caution and with careful monitoring for adverse effects. Potential adverse events include over immunosuppression leading to opportunistic infections, chromosomal aberrations in the injected cells leading to neoplastic transformation. Concern has also been raised whether MSC can induce the growth of pre-existing but occult tumors. Adverse events associated with the injection of cells can occur including pulmonary emboli. There may be lack of standardization of cell preparations leading to inconsistent clinical results. Until beneficial effects of cell therapy are definitively proven, cell injections should only be used only as part of a carefully monitored clinical trial.

OTHER APPROACHES

An alternative strategy to infusing MSCs has been to use conditioned media from MSC cultures as the treatment. This approach has yielded beneficial effects in acute and chronic models of kidney injury.^{18,19} Cells can also be used in more complex ways as part of the design of integrated renal replacement devices. For example, renal tubular epithelial cells were manufactured to be used in a renal assist device designed to treat AKI.²⁰ Also implantable bioartificial kidney devices have been envisioned in which renal tubular cells are part of the device and are linked to a glomerular membrane.^{21,22} The goal of these approaches is to restore the metabolic, immune, and transport functions of the kidney. Kidney cells are also critical components of a bioengineered kidney designed by injecting endothelial and neonatal kidney cells into a decellularized kidney scaffold.²³ Such a device has demonstrated *in vitro* and *in vivo* function.

CONCLUSION

Cell-based therapy, especially with MSCs, is an emerging field but one that has shown early promise for the treatment of human kidney diseases. The most studied areas have been the use of MSC infusion in AKI and kidney transplantation. In the latter cells have been used as induction therapy, for desensitization, and in the treatment of acute rejection. The mechanism for beneficial effects is related to paracrine and endocrine effects of the cells on pathways of cell proliferation, survival, inflammation, angiogenesis, and immunity. Beneficial effects are not due to transdifferentiation of injected cells into kidney cells. Ongoing and future studies need to carefully assess efficacy and monitor for adverse effects before cell therapy is widely adopted.

DISCLOSURE

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