Rationale of Mesenchymal Stem Cell Therapy in Kidney Injury

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Abstract

Hundreds of clinical trials are currently investigating the potential for mesenchymal stem cells (MSC) to treat human disease, including kidney disease. There is tremendous excitement over the therapeutic potential of this form of stem cell therapy and an improving understanding of how MSC act. This review will summarize our current knowledge concerning the mechanisms by which MSC accelerate kidney repair after acute injury. It will also survey the current MSC clinical trial landscape in nephrology. Finally, future challenges to a widespread application of MSC therapies for patients with kidney injury will be outlined.

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Introduction

Mesenchymal stem cells (MSC) are multipotent adult cells that have the ability to differentiate into cells of mesodermal origin, including chondroblasts, adipocytes and osteoblasts [1, 2]. MSC are best characterized in bone

marrow, but it is now clear that they exist in many adult organs. In addition to their progenitor properties, MSC regulate tissue repair by interacting with resident cells and secreting soluble factors. These functions are broadly immunomodulatory, pro-repair, anti-apoptotic and pro-angiogenic.

Mechanisms of MSC Protection against Acute Kidney Injury

Numerous rodent studies have consistently documented a protective effect of MSC on both acute and chronic injury models. In a systematic meta-analysis of 21 of these studies [3], the authors found a consistent reduction in serum creatinine (sCr) in MSC-treated animals versus controls. The studies differed in terms of time point and dose of MSC administered, time point of sCr measurement, route of MSC delivery, type of kidney injury and animal used (mice vs. rats). Subanalyses showed that the reduction in sCr in acute kidney injury (AKI)

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models was highest in ischemia-reperfusion injury (mean, 43 $\mu\text{mol/l}$ reduction) versus cisplatin- or glycerol-induced models (29 $\mu\text{mol/l}$), and all AKI models had a higher reduction than models of chronic kidney disease (5/6 nephrectomy, mean reduction 21 $\mu\text{mol/l}$). In terms of dosage, high-dose ($>10^6$ MSC) administration showed better results than low-dose ($<10^6$ MSC) administration, and the reduction in sCr was higher if MSC were given at a later time point (>1 day after injury) versus an earlier time point (<1 day after injury). If sCr was measured in the first 3 days after injury and treatment, the reduction in sCr was more pronounced than when measured at a later time point, and intra-arterial injection of MSC (vs. intravenous or intrarenal application) had the best protective effect on kidney function.

Early studies proposed that the mechanism by which MSC repair renal tubules was through direct engraftment and differentiation into epithelial cells. However, subsequent lineage analysis documented conclusively that MSC do not differentiate into epithelia. Indeed, one study found that either intraperitoneal MSC injection or injection of the conditioned media from MSC cultures could replicate the protective effect of MSC in AKI [4]. Thus, it is now accepted that MSC act by secreting paracrine factors and interacting with cells within the kidney interstitium (fig. 1).

Much recent progress has been made in defining the factors that mediate paracrine repair by MSC. Analysis of MSC-conditioned media showed a high expression of more than 40 cytokines [5, 6], among which were granulocyte colony-stimulating factor, vascular endothelial growth factor, hepatocyte growth factor, interleukin (IL)-10, epidermal growth factor and insulin-like growth factor (IGF)-1, which all have been shown to rescue mice from cisplatin-induced kidney injury. Exciting recent discoveries have implicated MSC-derived microvesicles (MV) in the amelioration of AKI [7]; in vitro, cisplatin-injured human tubular cells treated with MSC-MV expressed more anti-apoptotic genes such as Bcl-Xl (B-cell lymphoma-extra large), Bcl-2 (B-cell lymphoma 2) and Birc 8 (baculoviral IAP repeat containing 8), while pro-apoptotic genes such as caspase-1, caspase-8 and LTA (lymphotoxin α) were downregulated. MV and exosomes secreted from MSC not only contain cytokines, but also mRNA and microRNA, which are then taken up by host cells. Bruno et al. [8] did a gene array analysis on MSC-MV and found 239 different mRNA transcripts, of which 132 could be identified (see tables 1 and 2 in [8]).

Gatti et al. [9] treated rats with MV isolated from human adult MSC after uninephrectomy and unilateral

ischemia (45 min) and could show a protective effect of short- (48 h) and long-term (6 months) MV treatment; in the short-term treated group, MV injection resulted in more tubular epithelial proliferation and less apoptosis, as well as lower levels of sCr, BUN and proteinuria. This effect was abrogated when the MV were treated with RNase before injection.

It has long been known that IGF-1 secreted by MSC improved the viability of proximal tubular epithelial cells after cisplatin damage [10], and MSC silenced for IGF-1 lost their protective effect when injected into rodents after kidney damage; what was not known until recently is that MSC-MV also shuttle the mRNA for the IGF receptor (IGFR) [11]; MSC-secreted exosomes contain mRNA for IGFR as well as IGF-1. When treating IGFR-negative proximal tubular epithelial cells with MSC-conditioned media, these cells started to express IGFR and turned on IGF signaling.

Another interesting finding is that MSC-MV change their content depending on the environment in which they are cultured; Kilpinen et al. [12] compared MSC-conditioned media in the presence or absence of interferon- γ mimicking an inflammatory microenvironment. They compared the ability of MSC to protect rats from reperfusion injury in vivo; while the MSC cultured under normal conditions had a protective effect on kidney function, this effect was abrogated when the cells were cultured in the presence of interferon- γ . Proteome analysis of the different conditioned media showed that the protein content differed by 50–66%.

Harnessing the Beneficial Effects of MSC Clinically – Ongoing and Past Trials

To date, there are 10 active clinical trials (phase 1/2) registered that involve intravascular application of MSC in acute or chronic kidney disease, autoimmune disease affecting the kidney or kidney transplantation (clinicaltrials.gov, summarized in table 1). The first trials to report results are those in kidney transplantation. In a pilot study in 2 patients, autologous bone marrow-derived MSC were infused intravenously for 7 days after living-related donor kidney transplantation [13]; 3 patients with the same immunosuppressive therapy and living donor kidney transplantation served as a control group. The authors found a temporary decrease in graft function following MSC in both patients, leading them to conclude that the protocol should be changed to MSC infusion before transplant. All patients were followed up for 1 year;

Fig. 1. Model for paracrine actions of MSC on the injured tubule and surrounding tissue. Injections of MSC, MSC-MV or MSC-conditioned media accelerate repair by supporting proliferation and inhibiting apoptosis of tubular cells, induce vascular growth and inhibit inflammation/modulate immune responses, e.g. in

AKI patients after kidney transplantation. The secreted MV contain both cytokines and mRNA/microRNA (miRNA). HGF = Hepatocyte growth factor; TGFβ = transforming growth factor β; EGF = epidermal growth factor; IRI = ischemia-reperfusion injury; Casp-1 = caspase 1; VEGF = vascular endothelial growth factor.

at this time point, all patients had a comparable kidney function, yet the 2 MSC-treated patients had a decreased population of memory CD8+ T cells within the CD8+ T-cell population in the peripheral blood, which was also associated with reduced CD8+ T-cell activity. The authors concluded that MSC infusion in kidney transplant recipients restricts memory T-cell expansion, although

with a study group consisting of 2 patients, this is rather anecdotal evidence.

In a second pilot study with again 2 patients in the experimental group [14], MSC infusion before transplant did not lead to acute graft dysfunction in 1 patient; a basiliximab-free induction therapy did not lead to expansion of the CD4+FoxP3+ regulatory T-cell pool; for the next

Table 1. Ongoing/completed clinical trials registered at clinicaltrials.gov (as of February 2014) involving intravascular MSC application and kidney disease or function among the endpoints

Topic/ NCT No.	Title	Conditions	Kidney-related outcome measures	Recruit- ment	Enroll- ment (planned)	Comple- tion date (planned)
<i>Acute kidney injury</i>						
NCT01275612	MSC in cisplatin-induced acute renal failure in patients with solid organ cancers	Cisplatin-induced AKI	sCr, NGAL, NAG	Recruiting	9	March 2014
NCT01602328	Evaluate the safety and efficacy of AC607 for the treatment of kidney injury in cardiac surgery subjects	AKI after coronary artery bypass grafting	Time to kidney recovery (sCr), all-cause mortality or dialysis (composite endpoint)	Recruiting	200 (until December 2014)	January 2016
<i>Autoimmune diseases</i>						
NCT01539902	Phase 2 study of human umbilical cord-derived MSC for the treatment of lupus nephritis	Lupus nephritis	Efficacy and safety	Recruiting	25	May 2013
NCT01741857	Umbilical cord-derived MSC transplantation for active and refractory systemic lupus erythematosus	Systemic lupus erythematosus	Renal function (GFR, BUN, urinalysis), BILAG score, lupus serology	Recruiting	40	December 2013
<i>Chronic renal failure</i>						
NCT01840540	MSC for occlusive disease of the kidney	Atherosclerotic renal artery stenosis	Renal blood flow and function in the treated kidneys	Recruiting	15	April 2017
NCT01876017	Safety and efficacy of MSC in patients with chronic renal failure	Chronic renal failure	Incidence of uremia and dialysis requirement; serum urea and sCr	Not yet recruiting	15	December 2014
<i>Kidney transplantation</i>						
NCT01429038	MSC after renal or liver transplantation	Kidney failure after transplantation	Biopsy-proven (Banff) rejection rates, graft function, feasibility and safety of weaning immunosuppression, immune function, anti-MSC donor HLA antibodies	Recruiting	40	February 2017
NCT00752479	MSC under basiliximab/low-dose RATG to induce renal transplant tolerance	Kidney transplant	Graft function, graft rejection; T-cell response/anergy, peripheral blood; regulatory T cells	Terminated	4	December 2013
NCT00658073	Induction therapy with autologous MSC for kidney allografts	Kidney transplant	Incidence of acute rejection (biopsy), early renal function recovery, patient and graft survival, prevalence of adverse events	Completed	165	October 2010
NCT00734396	MSC and subclinical rejection	Kidney transplant	Late acute rejection (6-month vs. 4-week biopsy), renal cortical matrix accumulation, immunologic response to MSC infusion	Completed	15	December 2012

study, the protocol will again be altered to include basiliximab to prevent patients from the risk of an early rejection. Overall, kidney function was again as good as in the MSC-free control patients after 1 year, and no other adverse effects of MSC injection were reported.

In a safety and feasibility study (phase 1 [15]), patients with acute allograft rejection at control biopsies (4 weeks or 6 months after kidney transplantation) received intravenous injections of autologous bone marrow-derived MSC and were followed up for 24 weeks (n = 6). Infusions were tolerated without adverse effects; follow-up biopsies in 2 patients showed resolution of tubulitis, and 5 of 6 pa-

tients had less donor-specific peripheral mononuclear cells, pointing towards a possible immune-modulatory effect of the treatment.

The largest published study to date enrolled 159 patients undergoing living donor-related kidney transplantation (NCT00658073 [16]). The two groups receiving autologous bone marrow-derived MSC at the time point of reperfusion and 2 weeks after kidney transplantation differed by having a standard or low dose (80% of standard) of calcineurin inhibitor treatment; the control group did not receive MSC, but an anti-IL-2 receptor antibody and a standard dose of calcineurin inhibitor. After

1 year, the incidence of biopsy-confirmed acute rejection was 7.5 and 7.7%, respectively, in the two MSC-treated groups, whereas it was 21.6% in the control group receiving IL-2 receptor antibody and a standard dose of calcineurin inhibitor. Also, kidney function at 1 year was better in the MSC-treated groups, and the patients in these groups had less opportunistic infections. The rate of adverse effects was lower in the MSC-treated group than in the control group within the 360-day follow-up period.

In the field of AKI, a phase 1 study has been finished in which 16 cardiac surgery patients at high risk for post-operative AKI were safely treated with allogeneic MSC (NCT00733876 [17]) injected into the suprarenal aorta. During a 6-month follow-up period, no adverse effects of treatment were reported, and MSC-treated patients seemed to have less kidney injury and a shorter hospital stay. Currently, a larger trial is underway including 200 patients undergoing coronary artery bypass grafting (NCT01602328, www.allocure.com/clinical/index.php); enrollment is likely to close in December 2014, and results are to be expected in January 2016.

Overall, intravenous injection of autologous MSC seems to be safe within the 1st year after treatment, yet long-term results and a longer follow-up than 1 year are needed to judge whether this holds true on the long run. Unfortunately, there are no results published yet from studies involving MSC treatment in autoimmune kidney disease or chronic renal failure.

Future Challenges to Human Application of MSC in Kidney Injury

Despite the number of ongoing MSC-based human clinical trials, long-term safety concerns remain based upon results from animal studies. Breitbach et al. [18] injected green fluorescent protein (GFP)-labeled mouse bone marrow-derived MSC into the heart in myocardial infarction mouse models and traced them over 132 days; they did not find any GFP-positive cardiomyocytes or GFP-positive vascular smooth muscle cells, which argues against transdifferentiation of MSC into these cell types. Instead, they found GFP-positive cells and amorphous material in encapsulated structures in the scars, and these structures contained calcifications, which were verified by von Kossa staining, suggesting maldifferentiation of injected MSC into bone tissue.

In a different study using a rat model of glomerulonephritis, the authors reported a short-term improvement in kidney function after MSC injection on day 10 [19] –

yet, at 2 months, they found intraglomerular adipocytes that had transdifferentiated from MSC, leading to glomerulosclerosis. Also, while early investigations reported homing of MSC to injured tissue, more recent studies predominantly show that the majority of injected MSC (67.2%) embolize in the lung [5] and only a small fraction (5.4%) is found in the kidney 1 h and 1 day after injection: after 7 days, MSC were undetectable in the lung or kidneys – they did not engraft.

Given the strong data emphasizing a paracrine mechanism of action of MSC, clearly more work is needed to identify the soluble factors secreted by MSC that mediate repair, since these may be therapeutic alone. Yet MSC show great promise, and most concerns regarding their long-term safety are theoretical at this point. Indeed, one reason for the number of MSC clinical trials existent is their excellent safety profile to date.

Conclusion

The area of MSC therapy for kidney injury is very promising with – so far – good safety profiles in clinical studies, even with the relatively crude method of injecting whole cells intravenously. MSC have the advantage of relatively easy isolation and culture conditions, making them a potentially low-cost and personalized therapy option for patients with kidney injury. Still, adverse effects of treatment with MSC are possible and have been described in animal studies, and most data suggest that engraftment of the cells into the diseased organs is transient.

Despite the absence of long-term safety data, MSC therapy for kidney injury is one of the most exciting areas involving new therapeutics and the results of current clinical trials for kidney indications are sure to better define the potential of these cells to treat human disease.

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