Administration of Mesenchymal Stromal Cell-derived Exosomes is an Effective Rescue Therapy for Progressive Acute Kidney Injury in Rats

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Abstract

Preclinical and clinical studies show Mesenchymal Stromal Cells (MSCs) to be effective for prevention of AKI [NCT00733876]. Yet studies where MSCs are given 48 hrs. post-insult, a time at which most patients with severe AKI are diagnosed and when no rescue therapy is available, show them to be ineffective or even damaging due to compromised renal blood flow in capillary beds [NCT01602328]. While MSCs' renoprotection is largely due to their release of beneficial cytokines and exosomes, their potential negative impact on renal blood flow is a concern. Administration of MSC-derived exosomes exerts beneficial effects similar to those of the parent cells. We hypothesized that since MSC-derived exosomes can prevent AKI, their small size and ability to move through the compromised microvasculature might allow them to also be an effective rescue therapy for late stage AKI where MSCs are ineffective.

Fat-derived MSCs (aka ASCs) from Sprague Dawley (SD) rats were used. Their purified exosomes were characterized for size by nanoparticle tracking analysis, protein concentration, gene expression of relevant markers, FACS (CD44 and CD29), and rtPCR. I/R AKI (50-52 min bilateral renal pedicle clamp) was induced in 3 groups of SD rats (6-8/group). SCr was assessed at baseline, Days (D) 1 and 2. If the SCr value on D2 was greater than that on D1, then on D3, rats were given via the suprarenal aorta either 1 ml of Vehicle, 4x10e10 exosomes, or 2x10e6 MSCs. Studied Endpoints: SCr at Days 0-9; survival and renal injury.

In contrast to what is found when MSCs are administered to rats immediately upon reflow, when administered to rats 48 hrs post-I/R AKI, 2x10e6 MSCs prove ineffective at ameliorating injury, while MSC-derived exosomes significantly and sustainably improve renal function by D5 post injury.

MSC-derived exosome therapy administered 2 days post-insult, when renal blood flow is compromised, but also when most clinical instances of AKI are diagnosed, is superior to MSC therapy for rescue of AKI, likely due to the mirrored paracrine content, but significantly smaller size of exosomes compared to MSCs. Our results support the hypothesis that MSC-derived exosomes could be used as a rescue therapy for non-spontaneously recovering and progressive AKI.

Introduction

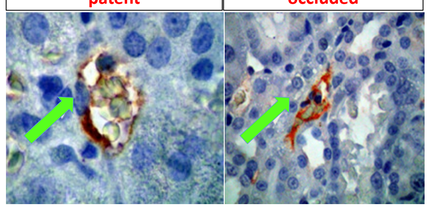
Preclinical and clinical studies have shown Bone-marrow and Adipose-derived Mesenchymal Stromal Cells (MSCs here is used interchangeably with ASCs) to be effective for prevention of Acute Kidney Injury (AKI) [NCT00733876] [1,2]. Yet studies in which MSCs are given 48 hrs. post-insult, a time at which most patients with severe AKI are diagnosed and when no rescue therapy is available, show them to be ineffective or potentially damaging due to compromised renal blood flow in capillary beds (**Fig 1**), where introduction of large cells (~50µm) has the potential to cause further deterioration of renal function [NCT01602328]. While MSCs' renoprotection is largely due to their release of anti-inflammatory, trophic, anti-apoptotic, mitogenic and vasculoprotective cytokines such as SDF-1, IGF-1, VEGF-α, etc., as well as their release of exosomes [2–6], their potential negative impact on renal blood flow is a concern.

As do most cells, MSCs release exosomes into their microenvironment. These are taken up by other cells where they effect paracrine signaling via lateral transfer of mRNAs, miRNAs, proteins, and lipids. Administration of MSC-derived exosomes is known to exert beneficial effects that are similar to those of the parent cells. Indeed, others have shown that like administration of MSCs, administration of MSC-derived exosomes upon reperfusion are protective and preventative against I/R AKI, with CD44 and CD29 surface expression being essential for their renoprotective effects[7–12].

We hypothesized that since MSC-derived exosomes can prevent AKI, their small size and ability to move through the microvasculature might allow them to also be an effective rescue therapy for late stage AKI where critically needed therapies currently do not exist, and where MSCs are ineffective (**Fig 1**).

Fig. 1

Normal Renal Capillary: patent	Renal Capillary in AKI: occluded



Objectives of Study:

1. **Characterize MSC-derived exosomes.**
2. **Compare the therapeutic efficacy of MSC-derived exosomes to that of MSCs given late to rats with severe, non-spontaneously recovering IRI AKI.**

Methods and Materials

Cells and Exosomes
All experiments were conducted using inguinal fat derived-MSCs from adult wt Sprague Dawley (SD) rats, passage 2-4, cultured in DMEM-F12 + 10% FBS under standard conditions.

MSC-derived exosomes were isolated from P4 MSCs cultured 24 hrs. in serum free medium, and purified using the ExoQuick-TC kit (SBI).
Purified exosomes were characterized for size by nanoparticle tracking analysis (Nanosight), protein concentration (Pierce BCA protein assay), gene expression of relevant markers, FACS (CD44 and CD29 Exo-Flow capture kits; SBI; Becton Dickinson), and rtPCR by standard techniques (ABS).

Induction of AKI, Treatment, and Assessment
I/R AKI (50-52 min bilateral renal pedicle clamp) was induced in 3 groups of SD female rats (209-264 g; 6-8 animals per group). SCr was assessed at baseline, Days (D) 1 and 2. If the SCr value on D2 was greater than that on D1, then on D3, rats were administered via left carotid artery either 1 ml of

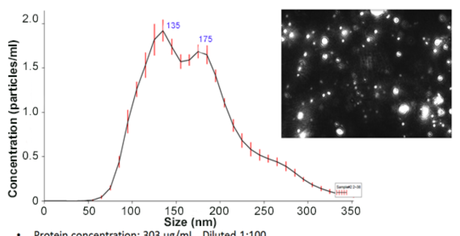
1) **Vehicle** (1xPBS; n=8),
2) **Exosomes** (200 µg protein-equivalent; ~4x10e10 exosomes; n=6), or
3) **MSCs** (2x10e6 SD MSCs; n=6).

Studied Endpoints: SCr at Baseline, D1, D2, D3, D5, D7, D9; and Mortality rates, Renal injury.

Results

Characterization of MSC-derived Exosomes
As shown in **Figs 2** and **3**, MSC-derived exosomes have a mode size of 136.7 nm (**Fig. 2**), and > 95% express both CD44 and CD29 (**Fig. 3**), necessary for preventing AKI[7]. Furthermore, as shown in **Fig. 3**, MSC-derived exosomes carry the mRNA for genes known to be involved in rescue of AKI.
Fig. 2: **Nanosight histogram (left) and representative image (right) of exosomes secreted from 2x10e6 MSCs**. Exosomes are a mode of 135 nm in diameter. In this sample, 1x10e6 MSCs secrete ~4.86x10e10 exosomes overnight.

Fig. 2



- Protein concentration: 303 ug/mL. Diluted 1:100
- Mean: 175.2 +/- 2.1 nm
- Mode: 136.7 +/- 5.3 nm
- SD: 64.6 +/- 1.2 nm
- Particle count (2.43e+009 +/- 2.59e+007 particles/ml) x 100 = 2.43e+011 +/- 2.59e+009 particles/ml

Results

Fig. 3: Expression profiles of MSC-derived exosomes for factors known to be involved in prevention of AKI (A): FACS histograms of CD44 and CD29 surface expression on SD rat MSC-derived exosomes. Green, exosomes; grey, negative control beads incubated with respective antibodies. Exosomes are 97% and 99% positive for CD44 and CD29, respectively. (B) mRNA profiles, raw cycle numbers (ct mean) from 60 ug RNA indicate that with the exception of tnf-α, MSC-derived exosomes, like their parent cells (MSCs) contain mRNA for genes involved in AKI prevention.

Fig. 3A: CD44 **CD29**

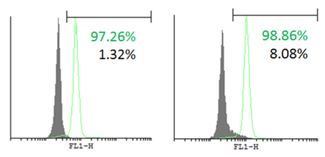


Fig. 3B: ct mean

Gene	MSCs	Exo.
actb	14.8	27.6
gapdh	19.3	25.5
cxcl12	18.1	32.0
cxcr4	24.8	ND
vegf-α	20.1	33.3
igf-1	21.3	37.1
fgf-2	21.3	34.4
ho-1	19.5	32.0
tgfβ-1	20.2	33.4
tnf-α	26.4	ND
il-1β	32.3	35.9
il-6	24.2	33.4

Exosomes vs. MSCs treatment 48 hrs post-AKI induction
As shown in **Fig. 4**, while both treatments reduce mortality compared with vehicle treatment (**Fig 4A**), renal function is significantly protected and improved by MSC derived exosomes administered 48 hrs. post-injury, while MSCs are ineffective compared to vehicle treatment (**Fig.4B and C**).

Fig 4: *In Vivo*, renal Function as assessed by (B) SCr levels and (B) renal histology is protected and improved by MSC derived exosomes. SCr levels at baseline and 1, 2, 3, 5, 7 and 9 days post I/R AKI. Vehicle (red), Exosomes (blue), or MSCs (green) were administered at 48 hrs. post injury as described in **Methods**. Compared to Vehicle treatment, Exosomes significantly improve renal function, while MSCs are ineffective.

Fig. 4A

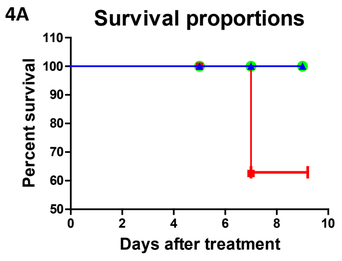


Fig. 4B

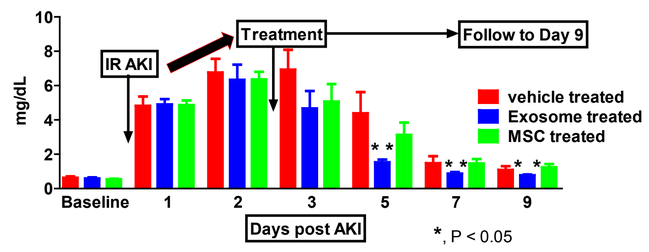
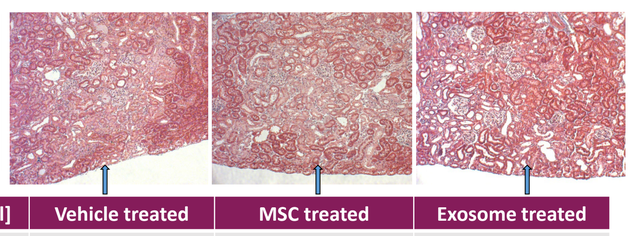


Fig. 4C: Significant functional and histological improvement post MSC-derived exosome therapy of progressive AKI.
Corresponding functional data (SCr) over time are shown in the table below the sections.

Trichrome stained on Day 9 post AKI (10x)



SCr [mg/dl]	Vehicle treated	MSC treated	Exosome treated
Baseline	0.51	0.65	0.43
Day 1	3.4	4.26	4.3
Day 2	5.1	5.1	5.1
Day 9	0.85	1.9	0.69

Discussion

1) With overnight culture in serum free medium, MSCs secrete ~ 4.9x10e10 exosomes and other microvesicles per million cells in a range of 50 to 300 nm in diameter, with a mode of 136.7 nm.
2) >95% of MSC exosomes express CD44 and CD29, and carry mRNAs for many genes previously shown to be important for AKI protection.
3) In contrast to what is found when A/MSCs are administered to rats immediately upon reflow, when administered to rats 48 hrs. post-I/R AKI, 2x10e6 MSCs prove ineffective at ameliorating injury, while MSC-derived exosomes secreted by 1x10e6 cells in overnight culture, significantly and sustainably improve renal function by day 5 post-injury.

Conclusions

1) MSC-derived exosome therapy administered 2 days post-insult, when renal blood flow is compromised, but also when most clinical instances of AKI are diagnosed, is superior to MSC therapy for rescue of AKI, likely due to the mirrored paracrine content, but significantly smaller size of exosomes compared to MSCs.
2) While survival rates at the end of the study were identical in both groups, animals treated with MSC-derived exosomes showed fully recovered SCr levels and more normal renohistopathology as compared to MSC treated animals, and thus would be less likely to develolp CKD as a consequence of AKi.
3) Our results support the hypothesis that MSC-derived exosomes could be used as a rescue therapy for non-spontaneously recovering AKI.
4) Further optimization of the exosome therapy is expected to identify optimal treatment doses for advanced AKI.

References

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