

A Systematic Review of Preclinical Studies on the Therapeutic Potential of Mesenchymal Stromal Cell-Derived Microvesicles

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Abstract

Background The therapeutic potential of mesenchymal stromal cells (MSCs) may be largely mediated by paracrine factors contained in microvesicles (MV) released from intracellular endosomes. A systematic review of controlled interventional animal studies was performed to identify models of organ injury where clinical translation of MSC-derived microvesicle therapy appears most promising as regenerative therapy.

Methods A total of 190 published articles were identified in our systematic search of electronic databases (MEDLINE, EMBASE, PUBMED). After screening for eligibility, a total of 17 controlled studies testing MSC-derived MVs as therapeutic interventions in animal models of disease underwent comprehensive review, quality assessment, and data extraction.

Results Thirteen studies addressed the regenerative potential following organ injury. Six studies were included on acute

kidney injury, 4 on myocardial infarction and reperfusion injury, 1 on hind limb ischemia, 1 on liver injury, and 1 on hypoxic lung injury. Four studies addressed immunological effects of MSC-derived MVs on inhibiting tumor growth. Twelve studies (71 %) provided explicit information regarding the number of animals allocated to treatment or control groups. Five studies (29 %) randomly assigned animals to treatment or control groups and only 1 study (6 %) reported on blinding. Therapeutic intervention involved isolation of exosomes (40–100 nm) in eight studies, while nine studies tested unfractionated microvesicles (<1,000 nm). In studies of tissue regeneration, all 13 reported that treatment with MSC-derived MVs improved at least one major/clinical parameter associated with organ dysfunction. Three of 4 studies evaluating the inhibition of tumor growth reported benefit.

Conclusions In preclinical studies, the use of MSC-derived MVs is strongly associated with improved organ function following injury and may be useful for inhibiting tumor growth. Improved preclinical study quality in terms of treatment allocation reporting, randomization and blinding will accelerate needed progress towards clinical trials that should assess feasibility and safety of this therapeutic approach in humans.

Keywords Mesenchymal stromal cells · Microvesicles · Exosomes · Preclinical · Animal models · Systematic review

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Introduction

The therapeutic potential and safety of mesenchymal stromal cells (MSCs) has been increasingly studied in the context of regenerative therapy and immune modulation [1]. Increasing numbers of publications have described the use of MSCs in clinical trials and there are many

additional registered ongoing studies [1]. The chief therapeutic attributes of MSCs are their ability to migrate to sites of tissue injury or inflammation, sense hypoxia and tissue damage [2], stimulate endogenous repair of injured tissues [3], and modulate immune responses [4,5]. Most studies, however, have reported low numbers of engrafted MSCs in recipients following administration in animal models of cardiac damage [6], kidney injury [7] or lung injury [8]. Moreover, several animal models of organ injury highlight the efficacy of conditioned media from MSC cultures [9,10]. Taken together, these observations support the emerging consensus that MSCs secrete bioactive factors that mediate beneficial therapeutic effects through a paracrine mechanism.

A central mechanism of cell to cell communication that has been recently recognized involves the packaging of bioactive factors in membrane bound vesicles, termed microvesicles (MVs) [11,12]. MVs include exosomes that arise from intracellular endosomes and shedding vesicles from the plasma membrane. MVs are secreted from many cell types and differ with respect to their origin within the cell, size, and contents. Exosomes are a subtype of MVs derived from budding of endosomal membranes and range in size from 40–100 nm, while shedding vesicles originate from plasma cell membranes and range from 100 nm–1 μ m in size. The diversity of proteins, lipids, and nucleic acids contained in MVs depends on their cell of origin and may be influenced by physiologic stress or other conditions such as cancer or infection [13–16]. MVs play an important role in intercellular communication and are capable of modifying the activity of target cells through surface receptor interactions and the transfer of proteins, mRNA and miRNA. Cellular processes that have been studied to date include the modulation of angiogenesis [15], cell proliferation [17], and immune regulation [14].

The therapeutic potential of MSC-derived MVs is particularly attractive as a strategy to harness the clinical benefits of MSC therapy using a cell-based product that reduces the risks associated with engraftment of MSCs, possible immune reactions against MSCs and the development of ectopic tissue. Moreover, the use of MSC-derived exosomes introduces the possibility of loading or changing the contents of bioactive factors to suit particular therapeutic needs. Indeed, MSC-derived exosomes have been studied in an increasing number of animal models of organ injury, models of inhibiting tumor growth and in the modulation of immune responses. We performed a systematic review of the literature and meta-analysis of preclinical data to evaluate the quality and strength of existing preclinical data. Our analysis is intended to provide an evidence-base to help accelerate the clinical development and translation of research addressing MSC-derived MVs.

Methods

Eligibility Criteria for Systematic Search

We included all controlled interventional studies describing *in vivo* experiments that tested the use of mesenchymal stromal cell-derived MVs, regardless of outcome, in animal models of organ injury, tumor growth or modulation of immune responses. Review articles, editorials, and studies describing only *in vitro* data were excluded. Articles written in languages other than English or French were excluded.

Search Strategy, Study Selection and Data Extraction

A systematic search of the literature was performed in accordance with recommendations by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [18]. A search strategy was developed to identify studies in MEDLINE, EMBASE, and PUBMED databases using the following search terms: (mesenchymal cell or stem or stromal or progenitor or multipotent or bone marrow or adipose or placenta) AND (exosomes or MVs or microparticles) AND (animals, animal experimentation, animal models of disease). Databases were searched from 1947 to May 2013. The electronic search strategy used is presented in Table 1. In addition, reference lists of relevant studies were searched manually to identify any studies that may have been missed in the database search. Titles and abstracts of studies identified in the systematic search were screened for relevance independently by two investigators. After initial screen, relevant articles were retrieved for complete assessment of eligibility criteria. Studies were classified based on the organ injury or disease studied: (1) myocardial ischemia and reperfusion (MI/R), (2) acute kidney injury (AKI), (3) tumor studies or, (4) other. Data was extracted independently by two individuals using standardized paper forms. Discrepancies were resolved through consensus.

Summary Measures and Synthesis of Results

We summarized the effects of MSC-derived MVs on outcomes by presenting pooled relative risks and 95 % confidence intervals using a DerSimonian and Laird random effects model. In the absence of sufficient data for pooling, results of individual studies were presented descriptively. Measures of study quality were extracted. We extracted key parameters of study design that would reduce the bias of investigators associated with the preclinical studies, including whether a clear description was provided in the methods section regarding the number of treated animals, whether randomization of the animals was performed, whether details regarding how many animals contributed to data were reported in the study, and whether blinding of investigators and/or lab personnel was

Table 1 Database: Embase Classic+Embase <1947 to 2013 May 21>, Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations and Ovid MEDLINE(R) <1946 to Present>Search Strategy

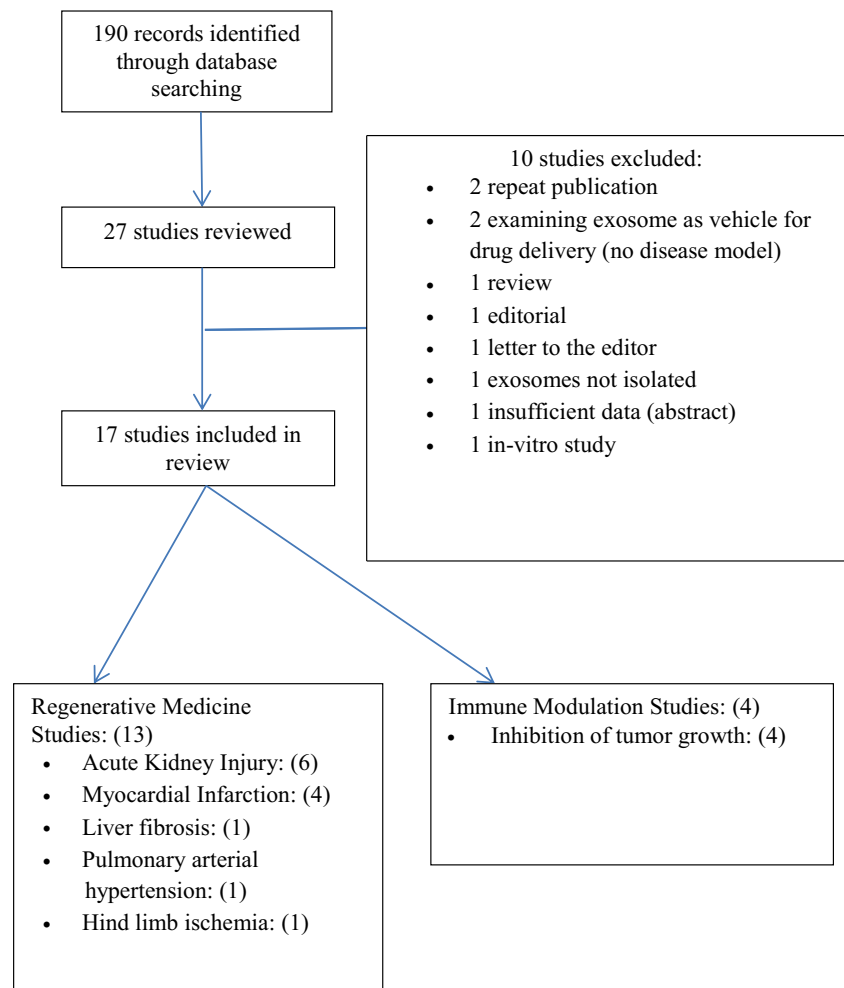
1	Mesenchymal Stromal Cells/ (14089)	30	(mesenchymal adj5 (cell\$ or stem or stromal or progenitor or multipotent or bone marrow or adipose or placenta\$)).tw. (73956)
2	Mesenchymal Stem Cell Transplantation/ (8703)	31	(MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs).tw. (28218)
3	Multipotent Stem Cells/ (5773)	32	(marrow stroma\$ adj2 cell\$).tw. (12043)
4	(MSC or MSCs or ADMSC or ADMSCs or BM-MSC or BM-MSCs or BMD-MSC or BMD-MSCs or BMDMSC or BMDMSCs).tw. (28218)	33	(colony forming unit fibroblast\$ or cfu f or cfu fibroblast\$).tw. (1347)
5	(mesenchymal adj5 (cell\$ or stem or stromal or progenitor or multipotent or bone marrow or adipose or placenta\$)).tw. (73956)	34	or/26–33 (98519)
6	((multipotent or multi-potent) adj (stroma\$ cell\$ or stem cell\$)).tw. (2396)	35	exosome/ (2095)
7	colony-forming unit fibroblast\$.tw. (376)	36	exosome\$.tw. (4412)
8	marrow stroma\$ cell\$.tw. (11509)	37	(cell adj2 vesicle\$).tw. (1851)
9	Mesoderm/cy (5096)	38	microvesicle\$.tw. (3342)
10	or/1–9 (99431)	39	microvesicle\$.tw. (3342)
11	Exosomes/ (2916)	40	microparticle\$.tw. (18371)
12	exosome\$.tw. (4412)	41	or/35–40 (27478)
13	(cell adj2 vesicle\$).tw. (1851)	42	34 and 41 (335)
14	microvesicle\$.tw. (3342)	43	animal experiment/ (1696590)
15	microparticle\$.tw. (18371)	44	exp animal/ (35988046)
16	Cell-Derived Microparticles/ (1765)	45	animal model/ (723735)
17	or/11–16 (27779)	46	(animal\$ or mouse or mice or murine or rat or rats or rodent\$ or pigs or pig or swine\$).tw. (5927841)
18	10 and 17 (329)	47	or/43–46 (36511573)
19	Animals/ (6986160)	48	42 and 47 (299)
20	Animal Experimentation/ (1696590)	49	48 use emczd (179) EMBASE
21	models, animal/ or disease models, animal/ (991495)	50	25 or 49 (271)
22	(animal\$ or mouse or mice or murine or rat or rats or rodent\$ or pigs or pig or swine\$).tw. (5927841)	51	remove duplicates from 50 (190)
23	or/19–22 (10568190)		
24	18 and 23 (179)		
25	24 use prmz (92) MEDLINE		
26	mesenchymal stem cell/ (33030)		
27	mesenchymal stem cell transplantation/ (8703)		
28	multipotent stem cell/ (5773)		
29	mesenchymal stroma cell/ (1096)		

described. These key parameters were recently identified as threats to validity in a systematic review of guidelines for preclinical studies [19].

Results

A total of 190 citations were identified by our systematic literature search. After screening for eligibility, 27 studies underwent comprehensive review. Ten studies

were subsequently excluded for the following reasons: repeat publications (2 reports), use of MSC-derived MVs as a vehicle for delivery only without a specific disease or injury model (2 reports), review article with no primary data (1 report), editorial (1 report), letter to the editor with no primary data (1 report), study did not isolate exosomes or MVs from MSCs (1 report), insufficient data in the report to draw conclusions (1 report), and lack of in vivo data (1 report). A summary of the study selection process is provided in Fig. 1. A total of 17 studies met eligibility.

Fig. 1 Results of systematic search of the literature

The animal models examined in these 17 studies included 13 studies of tissue regeneration (Table 2) addressing AKI (6 studies) [20–25], MI/R injury (4 studies) [26–29], hind limb ischemia (1 study) [30], liver fibrosis (1 study) [31], and pulmonary arterial hypertension (1 study) [32]. Four studies were identified that addressed aspects of immune modulation, all focusing on the inhibition of tumor growth [16,33,34].

Animal Allocation and Randomization

Twelve studies (71 %) provided explicit information in the methods section on the number of animals allocated to each treatment group and they accounted for all the animals in their results (6 of 6 studies addressing AKI; 3 of 4 studies addressing inhibition of tumor growth; 1 of 4 studies addressing MI/R; and 1 of 1 study of hind limb ischemia and 1 of 1 study of liver fibrosis) while the remaining studies provided specifics regarding treated animals only in the pertinent areas of the results section where they contributed data. Five studies randomly assigned animals to treatment groups or control

groups (3 studies of tumor growth, 1 of hind limb ischemia and 1 study of AKI). None of these studies provided information on the method of randomization or concealment of allocation. One study of MI/R [26] reported that the surgeon was blinded with respect to treatment allocation prior to reperfusion of the heart. No baseline characteristics were provided on any of the animals in any of the studies to ensure treatment groups and controls were balanced whereas, all studies reported outcomes and/or animal characteristics at particular time intervals following injury that were consistent for both treatment and control groups. Details of the animals (ie. strain and source) were provided in all studies and similar animals were used for treatment and control arms in all studies. None of the studies specified a predicted treatment effect in their methods or provided a power calculation and sample size determination.

Acute Kidney Injury Studies

Studies of treatment with MSC-derived MVs in animals with AKI were conducted in a murine model of AKI (3 studies) or

Table 2 Summary of all studies identified

Ref	Disease model	Outcomes reported (n)	Improved, MV vs control	Improved, MV vs MSCs	Improved, MV-MSC vs MV-fibroblasts	Animal allocation and Flow described	Randomized	Blinded
[20]	AKI	5	Yes (5/5)	↔	Yes	Yes		
[21]	AKI	5	Yes (5/5)			Yes		
[22]	AKI	6	Yes (6/6)		Yes	Yes		
[23]	AKI	4	Yes (4/4)		Yes	Yes	Yes	
[24]	AKI	2	Yes (2/2)			Yes		
[25]	AKI	2	Yes (2/2)		Yes	Yes		
[26]	MI	1	Yes (1/1)			Yes		Yes
[29]	MI	1	Yes (1/1)					
[27]	MI	1	Yes (1/1)					
[28]	MI	1	Yes (1/1)		Yes			
[31]	Liver injury	5	Yes (4/5)			Yes		
[32]	Lung injury	5	Yes (5/5)					
[30]	Hindlimb ischemia	2	Yes (2/2)	↔		Yes	Yes	
[33]	Tumor growth	1	Yes (1/1)		Yes	Yes	Yes	
[16]	Tumor growth	1	Yes (1/1)					
[35]	Tumor growth	1	Yes (1/1)	Yes		Yes	Yes	
[34]	Tumor growth	2	No (0/2)		Yes	Yes	Yes	

MV, microvesicles

rats (3 studies). Several methods were used to induce AKI across the studies. Monolateral nephrectomy and/or arterial occlusion were used in 1 study while 5/6 subtotal nephrectomy was reported by 1 study and injection with nephrotoxins including glycerol (1 study), cisplatin (2 studies) or gentamicin (1 study) was reported in the remaining studies. MSCs were expanded from bone marrow in 5 of 6 studies of AKI: 3 from human and 2 from homologous animals. One study reported the use of MSCs derived from human umbilical cord blood. Five studies isolated MSC-derived vesicles by ultracentrifugation of conditioned culture medium while one study used sucrose density gradient centrifugation. Two studies treated their animals with isolated MVs ranging in size from 80–1,000 nm whereas three studies treated animals with smaller microvesicles or purified exosomes (40–135 nm). In the 3 studies using human bone marrow-derived MSCs, MVs were characterized using similar membrane surface markers such as CD44, CD29, and CD73.

All studies reported on at least two measures of renal function and/or tissue injury following treatment with exosomes or MVs in comparison to controls. All of the reported outcomes concerning renal function and/or the extent of kidney injury were significantly improved by exosomes/MVs in comparison to non-treated controls. Measures of kidney function that improved in these studies (Table 3) included blood urea nitrogen levels (BUN) (reported in 5 studies), serum creatinine levels (5 studies), levels of cell proliferation (5 studies), extent of tubular necrosis (4 studies), the

development of cast formation (3 studies), and proteinuria (2 studies).

Myocardial Infarction and Reperfusion Studies

Four studies examined the therapeutic effect of MSC-derived exosomes in mouse models of myocardial infarction and reperfusion injury (MI/R) and were published by research teams collaborating with the same group in Singapore. Although initial studies from this group described the use of conditioned media from MSCs and were performed in pigs, subsequent studies of purified exosomes and/or MVs were all performed in mice and were included in this analysis. Myocardial infarction was induced by left coronary artery occlusion in all studies. In three reports, an ex-vivo Langendorff model of MI/R was also studied in parallel, however, these ex vivo results were not included in our summary of the data. MSCs were derived from human embryonic stem cells in 3 studies and 1 of these studies used MSCs that were immortalized following transformation of the cells by transducing the c-Myc gene. One study described the use of fetal tissues (limb, kidney and liver) to derive MSCs. All 4 studies purified MSC-derived exosomes by high performance liquid chromatography (HPLC) using the fraction with a hydrodynamic diameter of 50–65 nm in the therapeutic intervention in comparison to saline administration.

Endpoints in the studies of MI/R included infarct size as the ratio of ischemic tissue (IS) to the total tissue area at risk

Table 3 Summary of preclinical studies of MSC-derived microparticles in AKI

Ref	Animals (n)	MSCs	Particles (nm)	Purification method	Injury	Outcome
[20]	Mouse (230)	human BM	80–1,000,	UC	glycerol	improved BUN and Cr, increased cell proliferation, reduced tubular necrosis, reduced cast formation
[21]	Mouse (40)	human BM	<135	UC	cisplatin	improved BUN and Cr, increased cell proliferation, reduced tubular necrosis reduced cast formation
[22]	Rat (28)	human	80–1,000	UC	nephrectomy±arterial occlusion	improved BUN and Cr, increased cell proliferation, reduced tubular necrosis, reduced cast formation, reduced proteinuria
[23]	Mouse (22)	mouse BM	<100	UC	5/6 nephrectomy	improved BUN and Cr, decreased uric acid, reduced proteinuria
[24]	Rat (40)	rat BM	<100	UC	gentamicin	reduced cell proliferation and reduced cast formation
[25]	Rat (36)	human UCB	40–100	DGC	cisplatin	improved BUN and Cr

BM bone marrow, UCB umbilical cord blood, UC ultracentrifugation, DGC density gradient centrifugation, BUN blood urea nitrogen level, Cr creatinine

(AAR) in both treatment and control groups. In all four studies IS/AAR was significantly reduced in the group receiving treatment with MSC-derived exosomes compared to saline injection (see Table 4). Following a pooled effects analysis, the mean absolute reduction in the infarct size in relation to the IS/AAR was 18 % (12.5–23 %, 95 % confidence interval) in exosome treated mice compared with saline-treated animals, reducing the reported range of means of the infarct area in relation to the AAR (34–49 % to 17–23 % in favor of MSC-derived MV treatment (see Table 5). The visual inspection of the funnel plot reveals some asymmetry, implying the presence of publication bias (Fig. 2). However, the classic fail safe N, or number of missing studies needed to make the p value > 0.05, is 347 (Z value -16.4 and $p=0.000$ for 5 studies; Z value 1.96 for alpha of 0.05), which makes the likelihood of publication bias less probable. Taken together, some publication bias may be present in the identified studies, but seems unlikely to be significant such that it would overturn our conclusions.

Other Studies of Tissue Injury

One report addressed MSC-derived exosome treatment in a mouse model of tetrachlorohydrate-induced liver injury [31].

Exosomes were isolated from human cord blood-derived MSCs by ultracentrifugation and characterized by CD9 and CD81 surface expression and transmission electron microscopy. Exosomes were 40–100 nm in size and were injected directly into the mouse liver 6 weeks following injury (250 µg dose in 330 µl phosphate buffered saline (PBS)), leading to significant improvement in hepatic fibrosis measured 6 weeks after injection compared with mice injected with PBS alone.

One report described treatment of mice with hypoxia-induced lung injury and pulmonary hypertension using exosomes from murine marrow-derived MSCs isolated using size exclusion chromatography [32]. Intravenous injection of MSC-derived exosomes (10 µg per mouse, 30–100 nm particles) prevented hypoxia-induced inflammatory alveolar infiltrates and prevented pulmonary hypertension compared with fibroblast-derived exosomes and PBS controls.

Exosomes isolated by ultracentrifugation from human umbilical cord blood-derived MSCs accelerated repair of hindlimb ischemia in rats following intramuscular injection of exosomes 24 h after femoral artery occlusion in one study [30]. Exosomes were characterized by surface expression of CD29, CD44 and CD73 and were 80–150 nm as measured by

Table 4 Summary of preclinical studies of MSC-derived microvesicles in MI/R

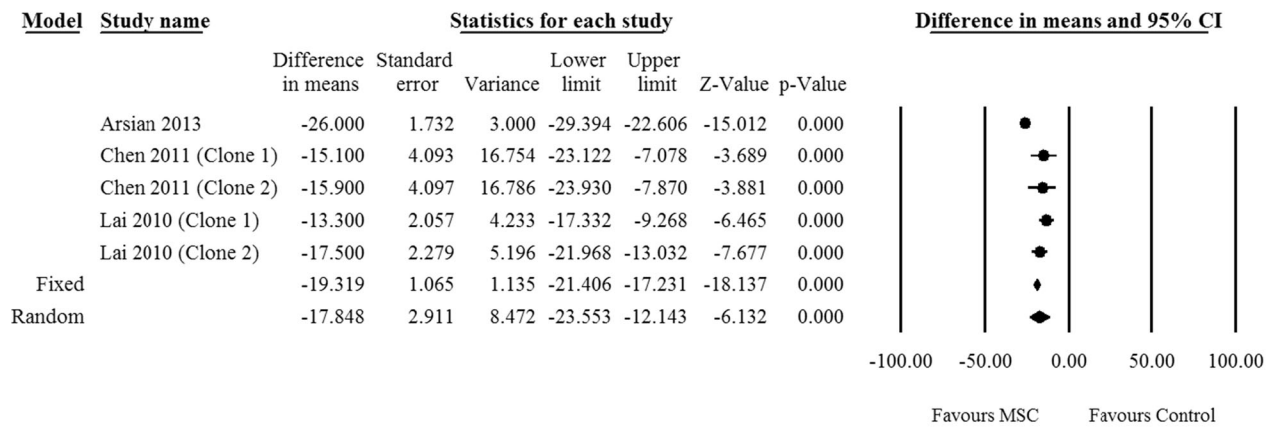
Ref	Animals (n)	MSCs	Particles (nm)	Purification method	Injury	Outcome IS/AAR
[26]	Mouse (63)	hESC-derived	50–65	HPLC	LCA occ	23±1.5% vs 49±5.3% (saline), $p<0.002$
[29]	Mouse (19)	transformed hESC-derived	50–65	HPLC	LCA occ	23.4±8.2% (clone 1) and 22.6±4.5% (clone 2) vs 38.5±5.6% (saline treated), $p<0.002$
[27]	Mouse (32)	Hu fetal tissue derived	50–65	HPLC	LCA occ	21.3±3.3% vs 34.5±3.3% (no treatment), $p<0.05$
[28]	Mouse (21)	hESC-derived	55–65	HPLC	LCA occ	17.1%±3.6% vs. 34.5±3.3% (saline), $p<0.004$

hESC human embryonic stem cells, HPLC high performance liquid chromatography, LCA occ left coronary artery occlusion

Table 5 Forest plot of preclinical studies of MSC-derived microvesicles in myocardial ischemia / reperfusion. Mean values of the % ischemic area per AAR is provided for MSC-derived microvesicles and controls. Where

separate MSC-derived microvesicles were reported, the results from individual clones are provided

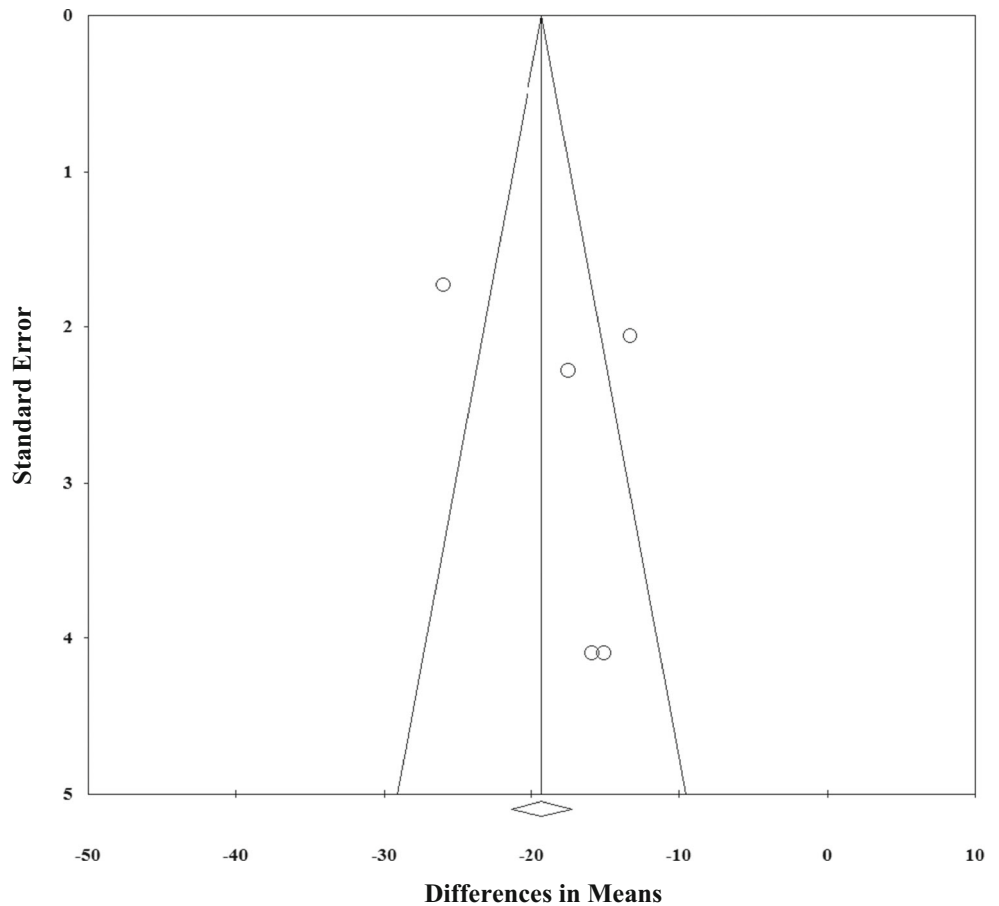
Forest Plot of preclinical studies of MSC-derived microvesicles in myocardial ischemia/reperfusion



electron microscopy. Fifty micrograms of exosomes were injected per animal and significantly improved blood flow

compared with sham injected rats. A higher dose of 200 μ g yielded similar results to 1×10^6 MSCs [30].

Fig. 2 Funnel plot of studies of myocardial ischemia/reperfusion. Difference in the means between treatment group and control group are plotted against the standard error of the mean



Tumor Growth Studies

Four studies examined the effect of MSC-derived MVs in models of tumor growth. All studies utilized a mouse xenograft model of tumor growth, however the tumor cell lines used in each study were different. In one study, mice received subcutaneous injections of three different human tumor cell lines (hepatoma cells HepG2, ovarian cancer cells Skov-3, and Kaposi cells) [33], while in a second study T24 cells derived from a bladder tumor cell line were administered [35]. Mice received cells from a gastric carcinoma cell line SG7901 or colon cancer cells SW48 in the third study [34], and human multiple myeloma cell lines (MM.1S, RPMI.8226 or U266) in a fourth study [16]. MSCs were derived from human bone marrow in 3 studies and from human cord blood Wharton's jelly in another study. Three studies isolated MSC-derived MVs by ultracentrifugation and 1 study isolated exosomes using a precipitation solution (ExoQuick, System Biosciences, USA). Two studies reported isolating MVs characterized by the presence of CD44, and CD73, while two studies characterized isolated exosomes on the basis of size and using the surface markers CD63 and CD81 and/or CD9. The ability of MSC-derived MVs to inhibit tumor growth was compared with a control group of untreated animals in all studies. In 3 studies, tumor growth was significantly inhibited compared with controls, whereas tumor growth was not altered by MV treatment in the study involving injection of gastric carcinoma cells or colon cancer cells (see Table 6). In one study, exosomes from MSCs in patients with multiple myeloma contributed to tumor growth whereas MSC-derived exosomes from normal bone marrow samples inhibited tumor growth compared with controls [16].

Comparing Therapeutic Effects of MSCs and Their Secreted Vesicles

The benefit of MSC-derived MVs was compared with the administration of MSCs in 3 studies of AKI [20], hind limb

ischemia [30] and inhibition of bladder tumor cell growth [35]. One study reported that treatment with MSC-derived exosomes/MVs produced greater benefit (less tumor growth) compared to the corresponding MSCs from which they were derived. Indeed, this study [35] reported greater benefit with 200 µg of MV-derived protein in the animal model compared with 1×10^7 MSCs while 2 other studies reported no difference between MSCs and MSC-derived exosomes using greater relative doses of MSC-derived exosomes compared to MSCs (15 µg of exosome protein vs 7.5×10^4 MSCs [20]; and 200 µg of exosomes vs 1×10^6 MSCs [30]).

Specificity of MSC-Derived Vesicles

Seven studies compared the therapeutic potential of MSC-derived MVs with MVs derived from fibroblasts to determine the specificity of MSC-derived MV signaling. In all of these studies, treatment with MV-derived from fibroblasts produced no significant benefit with outcomes similar to untreated controls [20,22,23,25,28,33,34].

Discussion

Our systematic review of preclinical studies of MSC-derived exosomes and MVs provides a timely summary of evidence from several animal models of organ injury and immune modulation. Studies suggest MSC-derived MVs can be safely administered in animals and contribute to improved organ function following myocardial or AKI and may be useful in the context of inhibiting tumor growth. MSC-derived MVs appear at least as effective as MSCs in these preclinical animal models of organ injury and may account for the beneficial paracrine effects ascribed to MSCs. The studies report the derivation of MVs of various sizes from MSCs expanded from several tissue sources and from both humans and animals, underscoring the broad applicability of this potential

Table 6 Summary of studies of MSC-derived microvesicles in animal models of tumor growth

Ref	Animals (n)	MSCs	Particles(nm)	Purification method	Tumor model	Outcome
[33]	Mouse (66)	Human, marrow	<145	UC	Sc injection of tumor cell lines (Hep G2, Skov-3, Kaposi cells)	Inhibited tumor progression compared to controls
[16]	Mouse (15)	Human, marrow, with myeloma	100	Exoquick™	Xenograft, multiple myeloma (MM.1S, RPMI.8226, U266)	Inhibited progression of myeloma compared to controls.
[35]	Mouse (24)	Human, placenta-derived	30–50	UC	Xenograft bladder tumor cell line (T24)	Inhibited tumor progression compared to controls
[34]	Mouse (36)	Human, marrow	30–100	UC	Injection of gastric or colon carcinoma cell lines (SG7901, SW48)	Promoted vascularity and tumor growth compared with controls

UC ultracentrifugation, Sc subcutaneous

treatment approach. In the case of AKI, studies were performed using a range of methods to induce organ injury and by numerous independent research groups, highlighting the reproducibility of the findings summarized in our study. Some potential issues regarding the quality of preclinical studies in this area were identified, including a lack of information regarding description of the allocation and randomization of animals. In particular, the results of our systematic review provide an important foundation to support future translational clinical research using MSC-derived MVs to repair organ injury and modulate immune responses or suppress tumor growth.

The paracrine effects of MSC therapy have been previously reported in studies describing the use of conditioned media from MSC cultures in a wide range of disease and injury models [3,9]. Characterizing the size and content of the MVs appears feasible and more straightforward in comparison to characterizing conditioned media and may be appealing in the context of meeting stringent health regulatory requirements [36]. Importantly, clinical trials using MSC-derived MVs could reduce potential risks associated with cellular therapies, including issues related to ectopic tissue formation, infusional toxicities due to cells lodging in the pulmonary microvasculature and cellular rejection or unwanted engraftment [37]. Although some risks associated with MSC therapy may be acceptable [38], infusing third party cell-based products that can be manufactured in large batches would obviate the need for costly patient-specific personalized cellular products. Moreover, MV therapy could involve concentration of the product in a minimal volume which is attractive for storage and transportation and could limit infusional issues and simplify dosing studies. Manipulating the content of MVs may also be envisioned and may be more easily controlled than the content of conditioned media or cells. Comprehensive insight regarding the full scope of molecules packaged in MSC-derived MVs and their role in tissue regeneration remains to be studied in greater detail.

The specificity of MSC-derived MVs likely relates to the content of the vesicles. Small RNA molecules or proteins that can specifically modulate repair mechanisms and immune responses likely underscore the observed beneficial effects of MSC-derived MVs [29,39,40]. Although few studies have systematically profiled the content of MSC-derived exosomes or MVs, paracrine factors that may be critical in vascular repair or immune modulation have been characterized [16].

We acknowledge that systematic reviews of preclinical studies may present a bias towards an overestimation of favourable outcomes since results of negative preclinical studies are less likely to be published. The quality of preclinical studies is typically reduced in comparison to human clinical

trials and several threats to validity have been recently reported [19] and have been considered. Threats to the validity of preclinical studies can complicate or delay the translation of preclinical studies into the clinical realm. For example, preclinical studies are less likely to involve robust randomization methods or blinding and this was observed in studies included in our systematic review. Moreover, the relative homogeneity of subjects in a preclinical study is in stark contrast to the heterogeneity that characterizes the realities of clinical studies. This may explain the observation that studies in our review did not provide baseline characteristics of animals with regard to organ function and this appears to be the reality of preclinical trials at this juncture. It is also important to acknowledge that all studies described in this review did not systematically screen for potential serious adverse events in other tissues and the safety of MSC-derived MV therapy remains unclear. Standardized reporting of safety data from preclinical animal studies is strongly encouraged but cannot ensure the safety of initial studies in humans. More work is needed with input from experts in the field to develop specific guidelines for reporting of safety data in preclinical animal models.

In conclusion, our study provides new insight regarding the collective preclinical experience using MSC-derived MVs to treat AKI and myocardial infarction with possible benefit in animal models of attenuating tumour growth. The current foundation of preclinical data is encouraging for planning and executing feasibility studies in humans. Barriers to clinical studies include robust and standardized characterization of MSC-derived MVs and the reporting of standardized safety data from preclinical animal models. Early phase proof-of-principle clinical studies may be possible in the near future.

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