Extracellular vesicles derived from mesenchymal stromal cells may possess increased therapeutic potential for acute kidney injury compared with conditioned medium in rodent models: A meta-analysis

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Abstract. The potential involvement of the endocrine/paracrine mechanisms in the mesenchymal stromal cells (MSCs) therapy for acute kidney injury (AKI) has been increasingly studied. The aim of the present meta-analysis was to systematically review the therapeutic role of MSC-conditioned medium (CM) or MSCs released by extracellular vesicles (Evs) for the treatment of AKI in rodent models. Studies were identified using PubMed and Scopus databases using a custom search strategy and eligibility criteria. Data regarding serum creatinine (SCr) concentration, CM or Evs, measurement time point, AKI model (toxic or non-toxic) and other parameters, including delivery route, animal type and animal numbers, were extracted. Pooled analysis and subgroup analysis as well as multivariable meta-regression were performed. Heterogeneity and publication bias were also investigated. A total of 13 studies were included and analyzed. Pooled analysis showed reduced SCr (0.93 [0.67, 1.20], mg/dl) in rodent models of AKI after CM/Evs therapy. The results of the subgroup analysis suggested that Evs induced an increased therapeutic effect, in the form of SCr reduction, as compared with CM (P=0.05). There were also other significant influential factors for SCr reduction including measurement time point (P=0.0004) and therapeutic time point (P<0.0001) after surgery. By contrast, parameters such as delivery route, injury type and cell type were not significant influential factors. Multivariable meta-regression analysis showed that measurement time point (P=0.041), therapeutic time point (P=0.03), Evs or CM (P=0.0003) and cell type (P<0.0001) were influential factors in the reduction of SCr. The present meta-analysis indicates that CM or Evs derived from MSCs are able to improve the impaired renal function in rodents modelling AKI. Compared with CM, Evs may produce a more marked therapeutic effect in recovery from renal failure. In addition, CM or Evs administration in early stages of AKI may result in more evident effects.

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Introduction

Acute kidney injury (AKI) refers to a clinical syndrome characterized by a rapid (hours to days) reduction in renal excretory function, with the accumulation of creatinine and urea nitrogen and other waste products that are not commonly tested in clinical practice (1). AKI is commonly observed in clinical practice, particularly following major surgery and treatment in intensive care units (2). In addition, AKI mortality is high, ranging between 24 and 62% (3). Patients that survive AKI may have an increased long-term risk of developing chronic kidney disease with poor prognosis (4). There is therefore an urgent requirement for novel methods for the prevention and management of AKI.

In recent years, a promising and effective therapeutic strategy for AKI involves the use of mesenchymal stromal cells (MSCs) derived from various sources, such as bone marrow or adipose (5,6). However, the mechanisms are not understood well. It has been suggested that MSCs promote renal injury repair, predominantly via paracrine/endocrine mechanisms as opposed to direct transdifferentiation into kidney cells (7,8). In previous studies, MSC-conditioned medium and MSCs released by extracellular vesicles (Evs) were reported to exert renoprotective effects against AKI (9-11). The researchers attributed these effects to the favorable molecules, such as mRNA and miRNA (11), in the Evs or secreted soluble factors, such as hepatic growth factor. However, contradictory findings have

indicated that CM may not be able to protect against kidney injury (12,13).

EVs, such as exosomes and shedding vesicles (also known as microvesicles; Mvs) are membranous structures that deliver bioactive molecular content, including proteins, mRNA and micro (mi)RNA sequences (14). Evs is a term suggested in recent years which describes a novel pathway of cell-to-cell interaction, and it is also regarded as a crucial point of endocrine (14). Since Evs are released by cells and extracted from CM using differential centrifugation, in the present study Evs were regarded as a type of 'special CM' or 'improved CM'. On the basis of endocrine/paracrine mechanism of MSCs, CM/Evs could provide a novel strategy of cell-free therapy for tissue injuries (15).

Previous studies have produced inconsistent results regarding the effects of CM/Evs therapy on AKI in rodent models (8,12). This may be due to the variation of injury models, treatment models, delivery route and cell type. In present study, a meta-analysis was conducted to identify relevant literature regarding CM/Evs therapy applied to AKI in rodent models, using the serum creatinine (SCr) concentration, the classic index of kidney function, as an analyzed parameter. In addition, this study was intended to investigate the possible influential factors for the therapeutic effects by sub-group and regression analysis.

Materials and methods

Search strategy. PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and Scopus (http://www.scopus.com) were employed as searched databases. The last search was updated on October 1st, 2014 using the following key words and search terms: ([extracellular vesicles or Evs or micro vesicles or micro-vesicles or microvesicles or Mvs or exosome or shedding vesicles] or [conditioned medium or conditioned culture media]) and (mesenchymal stromal cells or mesenchymal stem cells or MSCs) and (acute kidney injury or acute kidney disease or acute renal failure).

Eligibility criteria and data extraction. AKI models in rats or mice were screened. The animal experiments that investigated the effect of MSC-derived CM/Evs therapy on impaired renal function as determined by the level of SCr were analyzed. In addition, a sham- or placebo-operated control group was a requirement for study inclusion. The exclusion criteria for the studies were the following: i) Large animal/non-rodent experiments; ii) renal function was not determined by SCr; iii) SCr estimation was not included; iv) cell behavior was altered by genetic modification. Comments, reviews, and editorials were excluded. Only published English-language studies were considered for inclusion.

The following data were extracted from the complete manuscripts of the qualified studies: Basal characteristics of the study, SCr concentration, CM or Evs therapy, time of the therapy after injury and measurement time. If necessary, SCr data were estimated using graphics, as previously described (16,17). Accordingly, standard deviations were determined or recalculated based on the standard error. SCr concentrations are expressed herein as mg/dl (original data presented as μmol/l were changed accordingly). All literature searching, screening and data extraction were performed by two independent individuals, and determined after discussion.

Data analysis. The outcome was presented in teh form of the different in mean SCr between the control (AKI) and experimental (CM/Evs therapy) animals. A random-effects model was applied according to the results of heterogeneity tests. Continuous variables are presented as weighted mean differences with 95% confidence intervals (CIs) between the MSC-treated and control groups. In the case of multiple experimental groups compared with one control group within a single study, the number of animals in the control group was divided equally by the number of experimental groups. When there were multiple measurements, one study was regarded as separate assessment (16,17). $P<0.05$ was considered to indicate a statistically significant difference in two-sided tests.

Sub-group analysis and multivariate meta-regression were performed. The analyzed influential factors included: CM or Evs; injury type, toxic or ischemia-reperfusion (IRI); cell type, bone marrow MSC (BMSC) or non-BMSC; delivery route, intravenous or others; time point of therapy after injury, <1, 1-24 and >24 h; and time point of measurement, ≤2, 3-4 and >4 days. Furthermore, as publication bias is of concern for present meta-analysis, publication bias was investigated by a funnel plot.

All analysis was performed using Review Manager (version 5.2.9; The Nordic Cochrane Centre, Cochrane Collaboration, 2012) and SPSS software, version 18.0 (SPSS, Inc., Chicago, IL, USA). Meta-regression analysis was conducted using the 'MetaReg' macro written by David B. Wilson (http://mason.gmu.edu/~dwilsonb/ma.html).

Results

Literature characteristics. A total of 45 studies were retrieved from the PubMed database and 254 from the Scopus database. After excluding duplicate studies, a total of 274 remained. By excluding 96 review articles, 17 books, 14 book chapters and 4 short surveys, a total of 143 research articles remained. After screening for inclusion eligibility based on reading titles and abstracts, there were 13 papers eligible for our review. Among the included animals, only 461 animals met the inclusion criteria and were analyzed. Characteristics of the enrolled studies are described in Table I.

Meta-analysis. SCr data were continuous, as shown by the mean and standard deviation. Pooled analysis showed a SCr reduction of 0.93 mg/dl (95% CI, 0.67-1.20 mg/dl) in the CM/Evs therapy groups, as compared with the control groups with significant heterogeneity ($P<0.00001$; $I^2=96\%$; Fig. 1). Overall, no significant difference in SCr at baseline between the control and therapy groups was detected ($P=0.83$). In addition, several subgroup analyses were performed in order to determine whether CM or Evs have comparable therapeutic effects, and the optimum choice in CM/Evs therapy time after injury, time point measurement, delivery route, cell type and animal species.

The multivariable meta-regression analysis showed that measurement time point ($P=0.041$), therapeutic time point ($P=0.03$), Evs or CM ($P=0.0003$) and cell type ($P<0.0001$) were independent influential factors of SCr reduction.

Table I. Characteristic of the included studies.

Author, year	Injury type	n	CM or Evs	Cell type	Delivery route	Time point of therapy after injury	Time point of SCr measurement	Animal model	Ref.
Milwid *et al*, 2012	Cisplatin	14	CM	BMSC	Intravenous	3, 9, 24, 48 and 72 h	3 and 5 days	Rat	9
Bruno *et al*, 2009	Glycerol	32	Evs	BMSC	Intravenous	3 days	5 and 8 days	Mouse	11
Gheisari *et al*, 2011	Cisplatin	113	CM	BMSC	Intravenous	1 d	4 days	Mouse	12
Xing *et al* 2014	IRI	104	CM	BMSC	Intravenous	1 day	2 and 3 days	Mouse	13
Bruno *et al*, 2012	Cisplatin	24	Evs	BMSC	Intravenous	8 h and 8 h, 2 days	4 days	Mouse	18
Gatti *et al*, 2011	IRI	12	Evs	BMSC	Intravenous	<1 h	2 days	Rat	19
Kilpinen *et al*, 2013	IRI	26	Evs	hu-UCBMSC	Intra-arterial	<1 h	1 and 2 days	Rat	20
Kim *et al*, 2012	Cisplatin	15	CM	ADMSC	Intraperitoneal	1-2 days	3 days	Rat	21
Reis *et al*, 2012	Gentamicin	20	CM	BMSC	Intravenous	1 day	5 days	Rat	22
Zarjou *et al*, 2011	Cisplatin	23	CM	BMSC	Intraperitoneal	6 h	3 days	Mouse	23
Zhang *et al*, 2014	IRI	12	Evs	hu-WJMSC	Intravenous	<1 h	14 days	Rat	24
Zhou *et al*, 2014	cisplatin	54	CM and Evs	hu-UCMSC	Renal capsule injection	1 day	3, 4 and 5 days	Rat	25
Zou *et al*, 2014	IRI	12	Evs	hu-WJMSC	Intravenous	<1 h	14 days	Rat	26

CM, conditioned medium; Evs, extracellular vesicles; SCr, serum creatinine; BMSC, bone marrow mesenchymal stromal cells; IRI, ischemia-reperfusion injury; hu-UCBMSC, human umbilical cord blood mesenchymal stromal cells; ADMSC, adipose derived mesenchymal stromal cells; hu-WJMSC, human Wharton Jelly mesenchymal stromal cells; hu-UCMSC, human umbilical cord mesenchymal stromal cells.

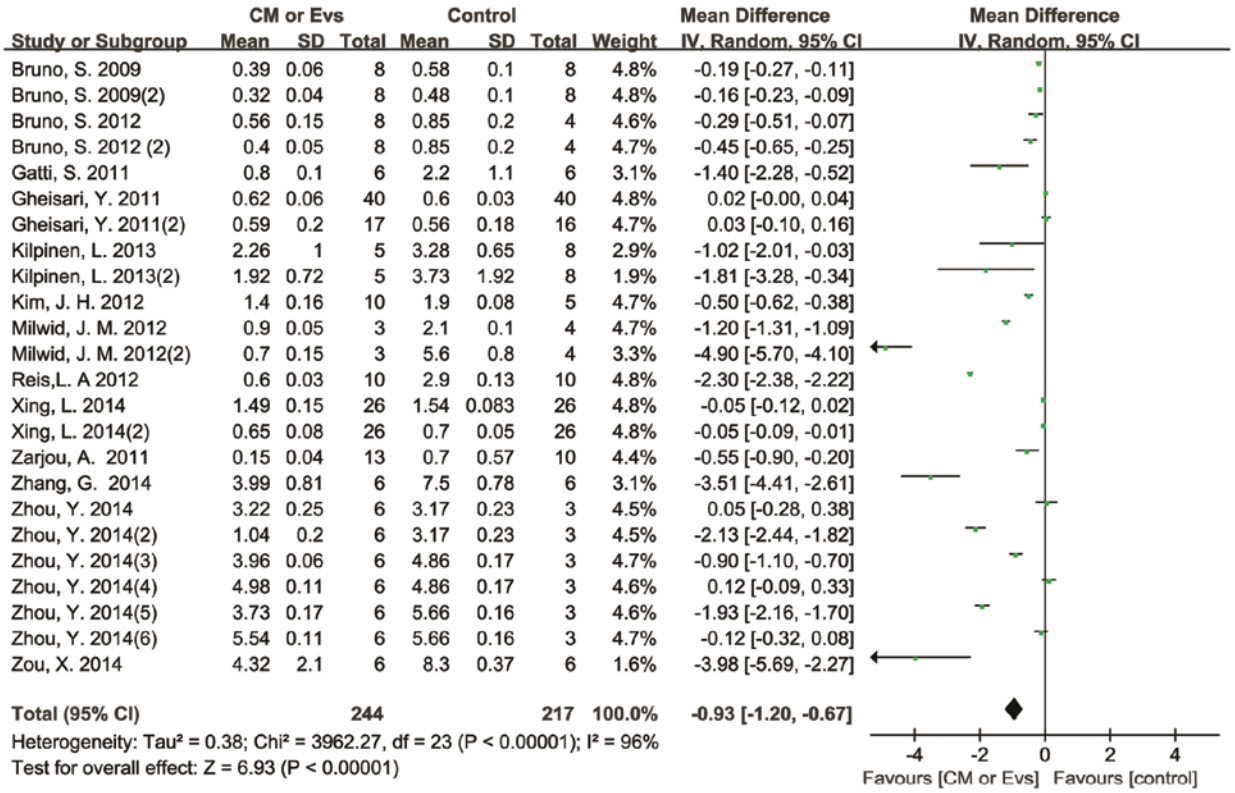

Figure 1. Forest plot shows the impact of CM/Evs derived from mesenchymal stromal cell injection on serum creatinine reduction compared with controls, 95% CI: 95% confidence interval. CM, conditioned medium; Evs, extracellular vesicles; SD, standard deviation; IV, inverse variance; CI, confidence interval.

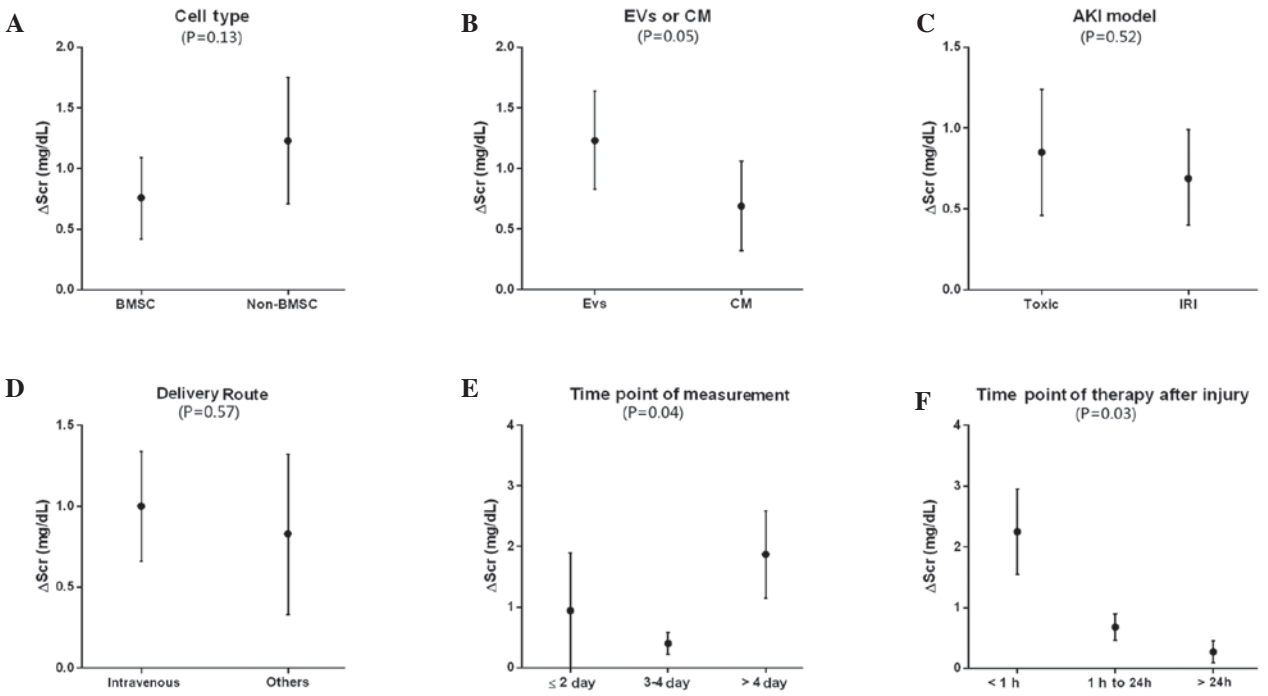

Figure 2. Sub-group analysis of influential factors for SCr reduction (ΔSCr in the figure). (A) SCr reduction from BMSC-treated and non-BMSC-treated sub-groups. (B) SCr reduction from EVs-treated and CM-treated subgroups. (C) SCr reduction from Toxic-AKI model and IRI-AKI sub-groups. (D) SCr reduction from intravenous treated and other delivery sub-groups. (E) SCr reduction from different time points of SCr mearsurement. (F) SCr reduction from different time points of therapy after injury. P-value was calculated using χ^2 test for sub-group difference. BMSC, bone marrow mesenchymal stromal cells; Evs, extracellular vesicles; CM, conditioned medium; AKI, acute kidney injury; IRI, ischema-reperfusion injury.

In the sub-group analysis, no difference in SCr reduction was detected between BMSC and non-BMSC therapy groups (0.76 mg/dl [1.09,0.42] vs. 1.23 mg/dl [1.75,0.71]; P=0.13). (Fig. 2A). These results were inconsistent with the

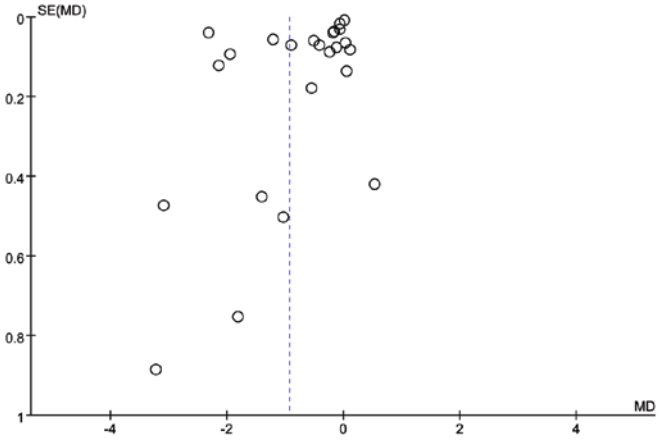

Figure 3. Funnel plot for serum creatinine reduction. Dotted line indicates the overall estimated mean difference. No obvious evidence for publication bias was detected. MD, mean difference; SE, standard error.

result of meta-regression, which indicated that cell type was an influential factor for SCr reduction. The sub-group analysis also detected significant differences between Evs and CM (P=0.05), which showed the SCr reduction (0.7 mg/dl, [0.32,1.07] vs. 1.23 mg/dl, [0.84,1.63], for Evs and CM respectively; Fig. 2B). Furthermore, the SCr reduction induced by Evs (1.23 mg/dl, [0.84,1.63]) was increased compared with CM (0.7 mg/dl,[0.32,1.07]). In the present study, AKI model or CM/Evs delivery route were not identified to be associated with SCr reduction (Fig. 2C and D). As shown in Fig. 2E, SCr measurement at >4 days after therapy was associated with more favorable effects (1.87 mg/dl, [1.14,2.59]), while measurement at ≤2 days showed no beneficial effects (-0.94 mg/dl, [-1.89,-0.00]). Significant differences were also detected in the sub-group analysis (P=0.041). In addition, the therapeutic time point was an influential factor for SCr reduction (Fig. 2F). It was observed that CM/Evs injected within 1 h after injury were associated with a favorable outcome (2.25 mg/dl, [2.95,1.55]), while at 1 day after injury the therapeutic effects were reduced (0.27 mg/dl, [0.09,0.45]). The subgroup analysis demonstrated the significant differences associated with treatment time point (P=0.03). In present study, two species of animal were investigated, namely rats and mice. The sub-group analysis showed no significant difference between these animals (P=0.72), which was consistent with the meta-regression analysis.

As shown in Fig. 3, the funnel plot for SCr indicated no publication bias.

Sensitivity analysis. Sub-group and multivariable meta-analyses were performed to investigate the source of significant heterogeneity among the involved studies.

Analyzed factors included CM or Evs, injury type, animal type, cell type, delivery route, therapeutic time point (after injury) and measurement time point. Meta-regression showed that measurement time point (P=0.041), therapeutic time point (P=0.03), Evs or CM (P=0.0003) and cell type (P<0.0001) were independent influential factors of SCr reduction. No trend in SCr reduction was observed regarding animal model (P=0.72).

Discussion

At present, adult stem cells have been extensively investigated with regard to their potential implications in regenerative medicine (27). MSCs from various tissues have been applied to the therapy for kidney injury, ischemia myocardial infarction and other diseases in clinical trials, a number of which produced favorable results (28). However, there remain a number of limitations associated with MSC transplantation, including immune-mediated rejection, senescence-induced genetic instability or loss of function and limited cell survival (29). Besides these issues, the primary problem related to the use of MSCs in clinical applications is the possibility of malignant transformation (30). On the basis of the endocrine/paracrine mechanism that may be involved in MSC therapy, CM/Evs may offer a strategy which avoids a number of the risks and limitations mentioned above (15).

The present meta-analysis comprised 13 published studies concerning on CM/Evs for AKI, and the pooled analysis showed a more marked SCr reduction (0.93 mg/dl [0.67,1.20]) in CM/Evs therapy groups compared with control groups, suggesting that CM/Evs were able to protect rodent model animals against AKI. The sub-group analysis showed that CM and Evs administration could lead to SCr reduction (0.7 mg/dl [0.32,1.07] and 1.23 mg/dl [0.84,1.63], respectively). Furthermore, SCr reduction in Evs sub-group was significantly elevated compared with the CM sub-group (P=0.05). Thus, Evs may offer more substantial therapeutic effects compared with CM. After the long-time concerning on growth factors and cytokines which is an important part of the cellular secretome, it now appears that the cells secreted Evs instead of soluble factors, which has previously been regarded as the main cellular secretome with a more important function. Evs, including exosomes and shedding vesicles, have been shown to deliver genetic information and functional proteins as well as bioactive membrane. Previous studies have attributed the therapeutic effects of Evs to their role in cell-to-cell communication (14) or the capability to reprogram injured cells (31). Evs can be extracted from CM *in vitro* using differential centrifugation, although the protocol may vary between studies. Thus, we hypothesize that the more marked protective role of Evs may be attributed to higher concentration of effective ingredient, such as functional protein, mRNA, miRNA and DNA, in Evs compared with CM.

The sub-group analysis showed that the rapid delivery of CM/Evs (1 h after injury) may lead to greater SCr reduction (Fig. 2F), and the therapeutic effects may emerge after 4 days (Fig. 2E), while there was no significant SCr reduction after 2 days. Furthermore, the review data suggested that the delivery route and kidney injury type might not affect SCr reduction. Notably, in a previous meta-analysis concerning MSCs therapy for impaired renal function in small animal models (16), increased SCr reduction was observed using an arterial delivery route compared with an intravenous route. For MSCs transplantation, intravenously delivered cells were retained in the lung capillaries (32), while intra-arterial delivery may lead to more efficient infusion. This may explain why arterial injection therapy is able to produce improved treatment effects. By contrast, no retained cells were detected in the lung capillaries after intravenous injection in CM/Evs therapy (11). In addition,

Evs were able to migrate toward injured tissue, thus functioning in a similar manner to MSCs (15). Therefore, the results mentioned above indicate that delivery route may not affect the therapeutic efficacy of an Evs-based treatment for AKI.

Thus far, cell-free therapy using CM/Evs for AKI experiments have been performed only in small animals. Therefore, further animal experiments involving different species are necessary in order to assess the safety and efficiency of CM/Evs therapy, prior to human clinical trials. Meta-analysis of animal studies was not common, yet they were recommended in several settings (33-35), and could often guide research (36), even clinical endeavors. Based on the present meta-analysis, our recommendations for MSCs cell-free MSCs therapy (CM/Evs) for AKI are as follows: i) Compared with CM, Evs have the priority as they possess greater therapeutic potential; ii) the time point of treatment should be as early as possible after injury; iii) the therapeutic effects may emerge at a later time; and iv) the delivery route could not affect the therapeutic effects.

However, there were still limitations of present study. The limitation of meta-analysis is well known (37), our analysis was based on study outcomes, and we did not have access to individual data. Another limitation is that some data were estimated using graphics during data extraction. Besides, there was significant heterogeneity, which might be due to other unknown influential factors varied in the included studies. Nevertheless, by using the random-effect analysis, the risk of finding erroneous estimates was minimized.

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