

RESPONSE TO REVIEWERS

REVIEWER #1

The study by Chao and colleagues is a great comprehensive review of novel roles of the KKS in stem cell therapy to treat cardiovascular pathologies in humans. I have no major suggestions and problems with the manuscript. Only a couple of minor issues require authors' attention.

Comment 1. Spell out MI for the first time (Page 5)

Response: We have spelled out MI on page 5.

Comment 2. Introduction: It looks like references are missed for the last two sentences.

Response: We have modified the last two sentences in the Introduction to read: "Activated plasma kallikrein also initiates the intrinsic pathway of coagulation and the fibrinolytic system^[7,8]. In this review, we discuss the involvement of tissue kallikrein, plasma kallikrein and kinin peptides in promoting the mobility and functional capacity of stem cells, which may lead to enhanced protection against organ injury in human diseases."

Comment 3. As a follow up, a general recommendation: In many cases, references are given for the initial statement but not for the next 2-3 sentences. It might be confusing for a reader whether authors continue to discuss the same work or not. Please edit the manuscript to provide clearer referencing.

Response: We have provided clearer placement of references throughout the revised manuscript.

REVIEWER #2

In this manuscript, Chao et al. give a concise review about the potential role of the kallikrein-kinin system in improving the therapeutic effects of cell therapies. This is a very interesting and clinically relevant subject that was well presented and discussed by the authors. However, the following comments and suggestions should be considered:

Comment 1. Some sentences don't have any references. For instance: "Activated plasma kallikrein also initiates the intrinsic pathway of coagulation and the fibrinolytic system. Importantly, tissue kallikrein, plasma kallikrein and kinin peptides have been recently shown to be involved in enhancing the mobility and functional capacity of stem cells, thereby reducing multi-organ injury."

Response: We have modified the last two sentences in the Introduction to read: "Activated plasma kallikrein also initiates the intrinsic pathway of coagulation and the fibrinolytic system^[7,8]. In this review, we discuss the involvement of tissue kallikrein, plasma kallikrein and kinin peptides in promoting the mobility and functional capacity of stem cells, which may lead to enhanced protection against organ injury in human diseases." Moreover, we have provided clearer placement of references throughout the revised manuscript.

Comment 2. “Furthermore, tissue kallikrein gene delivery to the peri-infarct myocardium increased cardiac progenitor cell levels and promoted cardiac neovascularization and function in rats with post-MI heart failure[36]”. The regenerative capacity of cardiac progenitor cells in the adult heart has been intensively debated and seems to be very limited. The authors should be cautious when discussing these results.

Response: We appreciate the reviewer’s comments. Tissue kallikrein gene delivery increased cardiac progenitor cell (CPC) density in the peri-infarct myocardium, although CPC levels were low compared to other cardiac cells (Spillman et al. *Regen Med* 2006;1:235-254). We agree with the reviewer that the regenerative capacity of CPCs in the adult heart requires further investigation. We have included this information in the revised manuscript.

Comment 3. Please correct or further explain the following sentence: “The time window for treatment of stroke patients is limited, as drug administration has minimal value when given 24 hours after stroke onset.” The time window for neuroprotective strategies in stroke is really short. However, drug- or cell-based therapies that could improve the regenerative and restorative capacity of the brain could be effective as a late treatment option for stroke.

Response: We appreciate the reviewer’s constructive comment. The time window for treatment of stroke patients is limited, as the clinically accepted treatment regimen with tissue plasminogen activator requires initiation within 3 hours of symptom onset (Fisher M. *Therapie* 2002;57:564-568). Tissue kallikrein has a superior advantage over tissue plasminogen activator with a wide time window after stroke. In a double-blind clinical trial, human tissue kallikrein was shown to be effective in the treatment of patient with acute brain infarction when infused within 48 hours of established stroke (Ding et al. *Chin J Neurol* 2007;40:306-310). These findings indicate that tissue kallikrein therapy is a promising regimen in the treatment of ischemic stroke in humans. We have incorporated this information in the revised manuscript.

Comment 4. In the section “MESENCHYMAL STEM CELLS IN RENAL AND CARDIAC DISEASES” the authors have briefly conceptualized mesenchymal stem cells. The authors should expand the description of these cells (where they reside, potential sources for therapies, how they are phenotypically characterized).

Response: We appreciate the reviewer’s suggestion. Besides bone marrow, mesenchymal stem cells (MSCs) have been documented to reside in adipose tissue, umbilical cord blood, placenta, amniotic fluid and amniotic membrane (Lee KD. *Chang Gung Med J* 2008;31:228-236). MSCs can be characterized by three main criteria: 1) adherent to plastic culture dishes; 2) expression of the cell surface markers CD73, CD90, CD105, and CD271; and 3) differentiation into lineages of osteoblasts, adipocytes, and chondroblasts *in vitro* (Lee KD. 2008). We have included this information in the revised manuscript.

Comment 5. The authors have not mentioned that mesenchymal stem cells are already being tested in clinical trials in patients with cardiovascular and cerebrovascular diseases.

Response: We thank the reviewer for the constructive comment. Clinical trials using human bone marrow-derived MSCs to treat various diseases are currently underway. These include treating patients with immune, renal, cardiovascular, neurodegenerative, gastrointestinal, bone/cartilage and blood disorders, and diabetes (<http://clinicaltrials.gov>). Efficacy can be maximized through pre-treatment of MSCs with drugs, cytokines, and growth factors, and genetically modified MSCs (Maestri et al. *World J Stem Cells* 2014;6:82-93). We have included this information in the revised manuscript.

Comment 6. Is there any evidence of the expression of kinin receptors by mesenchymal stem cells?

Response: Human MSCs possess kinin B2 receptors, as kinin stimulation increased intracellular calcium levels in MSCs, but its effect was blocked by icatibant, a specific kinin B2 receptor antagonist (Kim et al. *Cell Signal* 2008;20:1882-1889). We have included this information in the revised manuscript.

Comment 7. “Decreased numbers of circulating EPCs have been observed in patients with hypertension, chronic renal failure, CAD, and rheumatoid arthritis[72-75].” There are several studies in which the number of circulating EPCs could be associated with the outcome in stroke patients. Please include some of these references.

Response: We appreciate the reviewer’s constructive comment. The correlation of circulating EPC number and outcome of stroke patients is inconsistent. Decreased EPC numbers are associated with acute ischemic stroke (Tsai et al. *Clinica Chimica Acta* 2014;427:6-10), whereas higher EPC levels are reported in hemorrhagic stroke patients (Paczowska et al. *J Neurol Sci* 2013;325:90-99). We have included this information in the revised manuscript.

Comment 8. The following sentence should be corrected: “In addition, EPCs were recruited to the synovium at the acute phase of arthritis, and then differentiated into new blood vessels.” The EPCs differentiate into endothelial cells that form new blood vessels.

Response: We have corrected the sentence to read: “In arthritic Lewis rats, EPCs were recruited to the synovium at the acute phase of arthritis, and then differentiated into endothelial cells to form new blood vessels.”

Comment 9. “EPCs isolated from bone marrow of Lewis rats were observed to have higher expression levels of kinin B2 receptor than rats with experimental arthritis”. EPCs isolated from the bone marrow of control Lewis rats, compared to EPCs from Lewis rats with experimental arthritis?

Response: We have corrected the sentence to read: “EPCs isolated from bone marrow of Lewis rats were observed to have higher expression levels of kinin B2 receptor compared to control rat lung microvessel endothelial cells.”

Comment 10. In the conclusions, please include some considerations about the future directions of the field.

Response: As suggested, we have modified the Conclusion in the revised manuscript.

Comment 11. Please include references in Table 1.

Response: As suggested, we have included references in Table 1.

Comment 12. Please consider the possibility of including a figure.

Response: As suggested by the reviewers, we have included two new figures in the revised manuscript: one illustrating the kallikrein-kinin system for readers to better understand the system, and the other indicating the effects of kallikrein-kinin treatment on different stem cell populations.

REVIEWER #3

The manuscript “Kallikrein-kinin in stem cell therapy” is an exhaustive and well written review. The author deepen how the system kallikrein-kinin may enhance the efficacy of stem cell therapy for human diseases focusing their attention on renal, cardiac, vascular injury and ischemic stroke evaluating two type of adult stem cells namely mesenchymal stem cells and EPC. The manuscript is well presented and organized and has a good readability. The abstract reflect the major topic and content of the review. This review is interesting because the topic is much discussed in the literature but there are few articles that summarize the state of the art. Some points must be clarified or modify:

Comment 1. The manuscript is a review and not an original article. No experiments are performed by the authors and no result should be interpreted. Therefore please change the AUTHORS CONTRIBUTIONS that do not correspond to the content of the manuscript that reports the results of the articles focused on the topic specified in the title.

Response: We have changed the author contributions appropriately.

Comment 2. In the INTRODUCTION the authors state: “Activated plasma kallikrein also initiates the intrinsic pathway of coagulation and the fibrinolytic system. Importantly, tissue kallikrein, plasma kallikrein and kinin peptides have been recently shown to be involved in enhancing the mobility and functional capacity of stem cells, thereby reducing multi-organ injury.” Please add references.

Response: We have modified the last two sentences in the Introduction to read: “Activated plasma kallikrein also initiates the intrinsic pathway of coagulation and the fibrinolytic system^[7,8]. In this review, we discuss the involvement of tissue kallikrein, plasma kallikrein and kinin peptides in promoting the mobility and functional capacity of stem cells, which may lead to enhanced protection against organ injury in human diseases.”

Comment 3. A figure (a drawing) in the Introduction that explains the kallikrein-kinin system could be very helpful for a better understanding of the system.

Response: We appreciate the reviewer's constructive suggestion. We have modified the Introduction to better explain the kallikrein-kinin system, and have incorporated a figure illustrating the kallikrein-kinin system in the revised manuscript.

Comment 4. In the paragraph "Tissue Kallikrein-Kinin in vascular injury" the authors state: "Tissue kallikrein levels in the circulation are significantly higher in patients with coronary artery disease (CAD) and correlate with disease severity[37], suggesting that circulating tissue kallikrein levels are an independent predictor for CAD." Please clarify this point.

Response: We appreciate the reviewer's constructive comment. Tissue kallikrein levels in the circulation are significantly higher in patients with coronary artery disease (CAD) compared to non-CAD patients, and increase with disease severity, from moderate CAD to multi-vessel CAD with acute obstruction of one major coronary artery (Yao et al. *Clinica Chimica Acta* 2013;423:90-98). This study suggests that circulating tissue kallikrein levels may serve as a predictive tool to assess the presence and extent of CAD. We have included this information in the revised manuscript.

Comment 5. In the paragraph "Tissue Kallikrein-modified MSCs provide enhanced protection in kidney injury" from line 6 to line 14 (end of paragraph) there are no references. Please add references.

Response: The text from lines 6 to 14 refers to the study of Hagiwara et al. (*Hum Gene Ther* 2008;19:807-19). We have included this reference in the revised manuscript.

Comment 6. In the paragraph "Tissue Kallikrein-modified MSCs in Lupus Nephritis protection" from line 5 to line 13 (end of paragraph) there are no references. Please add references.

Response: The text from lines 5 to 13 refers to the study of Li et al. (*PLoS One* 2013;8:e67790). We have included this reference in the revised manuscript.

Comment 7. EPC are progenitor cells but these cells are considered adult stem cells since contributing to the regeneration of vascular endothelium. Please add a reference that define EPC as adult stem cells. This is required so that the title can match fully with the content of the manuscript.

Response: We thank the reviewer for the constructive comment. EPCs participate in postnatal angiogenesis, and are thus considered to be adult stem cells (Kopp et al. *Curr Opin Hematol* 2006;13:175-181). We have included this reference in the revised manuscript.

REVIEWER #4

There some small flaws need to modify.

Comment 1. A figure in the Introduction that explains the kallikrein-kinin system could be very helpful for a better understanding of the system.

Response: We appreciate the reviewer's constructive suggestion. We have modified the Introduction to better explain the kallikrein-kinin system, and have incorporated a figure illustrating the kallikrein-kinin system in the revised manuscript.

Comment 2. The reference was 90 in total. However, there were 51 articles which focused on the kallikrein-kinin system contributed to renal, cardiac, vascular injury and ischemic stroke. More presentation should be focused on the stem cell therapy.

Response: As suggested by the reviewer, we have incorporated additional references regarding stem cells, such as a more detailed description of MSCs and the correlation between circulating EPCs and the outcome of stroke patients in the revised manuscript. The background information on the kallikrein-kinin system was provided for readers to better understand its significance on renal, cardiac, vascular injury and ischemic stroke and how it relates to stem cell therapy for these pathological conditions.

Comment 3. The stem cells included many classifications of cells. However, in the paper, only three kinds of stem cells were presented, especially MSCs and EPCs. Therefore, whether the title is proper.

Response: We agree with the reviewer that only three kinds of stem cells are discussed. However, as this is an invited review, we believe this is the best title that reflects the current research of the effect of kallikrein-kinin on these different types of stem cells.