

ANSWERING REVIEWERS

November 12, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: Author responses to referee comments MS-12976.doc).



Title: THE ROLE OF NITRIC OXIDE IN THE MAINTENANCE OF PLURIPOTENCY AND REGULATION OF THE HYPOXIA RESPONSE IN STEM CELLS

Author: Beltran-Povea A, Caballano-Infantes E, Salguero-Aranda C, Martín F, Soria B, Bedoya FJ., Tejedó JR., Cahuana GM.

Name of Journal: *World Journal of Stem Cells*
ESPS Manuscript NO: 12976

The manuscript has been improved according to the suggestions of reviewers:

- 1 Format has been updated
- 2 Revision has been made according to the suggestions of the reviewer (1) and (2)
- 3 References and typesetting were corrected
- 4 The manuscript was edited by a native English speaker

Thank you again for publishing our manuscript in the *World Journal of Stem Cells*.

Sincerely yours,

A handwritten signature in blue ink, appearing to read 'G. Cahuana', written over a horizontal line.

Gladys M. Cahuana Macedo, PhD

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ESPS Manuscript No: 12976

Title: THE ROLE OF NITRIC OXIDE IN THE MAINTENANCE OF PLURIPOTENCY AND REGULATION OF THE HYPOXIA RESPONSE IN STEM CELLS

Reviewer number: 1

This review is well written, providing a large volume of information as well as in-depth considerations regarding the biological effects of nitric oxide (NO). I believe that this review will be a great guide to “the science of NO” for readers of broad fields.

Nevertheless, this manuscript contains numerous grammatical errors and syntactic problems. Before publication in WJSC, the manuscript should be thoroughly checked by native speakers.

Response from authors:

The manuscript was edited by Dr. John Pearson, a native English speaker.

Major concerns

Authors describe detailed explanations regarding the distinct effects of high and low concentrations of NO, which is indeed a matter of great interest. It seems that the phrase “low concentration” indicates “physiological” or “normal” concentration, while “high concentration” indicates “pharmacological” concentration obtained by NO donors.

It would be of great help for readers to understand the physiological/pathological significance of “high concentration” if authors would add some comments regarding its *in vivo* relevance”.

Response from authors:

Since there is little evidence regarding the *in vivo* relevance of the effects of high NO concentrations on differentiation and development *in vitro*, we have added the following sentence (Page 4, Lines 11-19):

Moreover, higher NO concentrations, induced by the inflammatory response, can cause oxidative and nitrosative stress, and apoptosis. These actions are

partly responsible for cell death in chronic and degenerative diseases. Pharmacological treatment with high NO concentrations promote embryonic stem cell (ESC) differentiation [9, 20-22]. However, the functional significance of high NO concentrations on differentiation in vivo has not yet been demonstrated.”

Minor concerns

Comments 1 to 7: We agree with the comments of the referee and have changed the text according to their recommendations

- 1) In page 3, lines 6-7, the sentence "which are now considered not inert products" should be rewritten as "which are now considered as non-inert (or chemically active) products"
- 2) In page 3, lines 8-13, the sentence "This gas acts as second messenger and has multiple biological effects implicated in numerous physiological functions in mammals such as smooth muscle relaxation, dilation of blood vessels, neurotransmission and inhibition of platelet aggregation, vascular tone, blood pressure, immune response, and oxidation-sensitive mechanisms" seems wordy. Also, the construction of a sentence is distorted.

It should be rewritten, for example, as "In addition to serving as a germicide in the immune system and also a neurotransmitter in the central nervous system, NO acts as a second messenger and has multiple biological effects implicated in numerous physiological functions in mammals such as regulation of blood pressure via smooth muscle relaxation and an inhibition of platelet aggregation."
- 3) In page 3, line 21, the sentence "Critical points in the effect of NO production on cellular processes" should be rewritten as "The critical factors that influence the effects of NO on cellular processes".
- 4) In page 4, lines 24-25, the phrase "cyclic guanosine monophosphate dependent (cGMP) or cGMP independent" should be written as "cyclic guanosine monophosphate (cGMP)dependent or cGMP independent"
- 5) In page 5, line 5, the phrase "cGMP dependent effects" should be rewritten as "cGMP-dependent effects".
- 6) In page 5, line 20, the phrase "bone marrow stem cell pluripotency" should be rewritten as "bone marrow stem cell multipotency".

- 7) In page 9, line 21, the words “0,5 mM” should be rewritten as “0.5 mM”.
- 8) In page 10, line 20, the phrase “....function, however other signaling ...” should be rewritten as “....Function; however, other signaling ...”.

Response from the authors:

Following a thorough revision of the manuscript by a native English-speaking scientist this sentence has been changed as follows

(now Page 11, Line 4):

“These studies indicate that NO is important for later endothelial development and function, although other signalling pathways appear to play a greater role in early development^[52].”

Comments since 9 to 23: We agree with the comments of the referee and we have changed the text according to its recommendations

- 9) In page 11, line 18, the words “pluripotency of MAPCs” should be corrected as “multipotency of MAPCs”.
- 10) In page 11, line 22, the words “through an independent cGMP pathway mechanism” should be rewritten as “through a cGMP-independent mechanism”.
- 11) In page 13, line 10, the word “cito-protective genes” should be corrected as “cytoprotective genes”.
- 12) In page 16, line 15, the phrase “... pathways, that regulate...” should be corrected as “... pathways, which regulate...”.
- 13) From page 16, lines 22 to page 17 line 1, the sentence “Pyruvate conversion to acetyl-CoA by PDH and its entry into mitochondria is blocked in hypoxic cancer cells by hypoxia inducible factor-1 α (HIF1 α) because induces pyruvate dehydrogenase kinase 1 (PDK1) expression and thus the inactivation of PDH phosphorylation” should be rewritten as “PDH-mediated conversion of pyruvate to acetyl-CoA and its entry into mitochondria is blocked in hypoxic cancer cells by hypoxia inducible factor-1 α (HIF1 α), which induces pyruvate dehydrogenase kinase 1 (PDK1) expression and inactivates PDH phosphorylation”.
- 14) In page 17, line 8, the phrase “it is still unknow” should be corrected as “it is still unknown”.
- 15) In page 18, line 13, the phrase “in some physiological conditions” should be corrected as “under some physiological conditions”.
- 16) In page 19, line 5, the phrase “AMP-activated kinase (AMPK) which ...” should be corrected

- as “AMP-activated kinase (AMPK), which ...”.
- 17) In page 19, line 8, the phrase “oxygen alternative energy sources” should be rewritten, for example, as “energy sources other than oxygen”.
 - 18) In page 19, line 20, the phrase “apoptosis cell protection” should be rewritten as “protection against apoptosis”.
 - 19) In page 20, lines 11, the word “throughout” should be corrected as “through”.
 - 20) In page 21, lines 23, the words “others authors” should be corrected as “other authors”.
 - 21) In page 22, lines 7, the phrase “in a dependent concentration manner” should be corrected as “in a concentration-dependent manner”.
 - 22) In page 22, lines 7-10, the sentence “The endogenous NO (400 nM), in hypoxia destabilizes HIF-1 α by inhibiting mitochondrial respiration and increase cytosolic oxygen concentration, where PHD2 is mainly located” should be rewritten as “In hypoxia, endogenous NO (400 nM) destabilizes HIF-1 α by inhibiting mitochondrial respiration and thus increases the oxygen concentration in the cytosol, where PHD2 is mainly located”.
 - 23) In page 26, line 1, the phrase “...up-regulation of glycolytic genes, that helps...” should be corrected as “...up-regulation of glycolytic genes, which helps ...”.
 - 24) Throughout this manuscript, there are numerous grammatical errors and unlogical phrases in addition to above-mentioned points. Authors should consult native speakers to get English proofreading.

Response from authors:

The manuscript has been edited by Dr. John Pearson, a native English speaker.