20th January 2014

Dear Ya-Juan Ma,

Please find enclosed the edited manuscript in Word format (file name: WJBC_6552_edited.docx).

Title: Activated protein C, a regulator of human skin epidermal keratinocyte functions

Authors: Kelly McKelvey, Christopher John Jackson and Meilang Xue

Name of Journal: World Journal of Biological Chemistry

ESPS Manuscript NO: 6552

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

Reviewer 00289641:
While the article was well written and important, the following issue need to be addressed: Subtitle 5 "PC/APC function and regulation" should be combined with Subtitle 3 "PC system on keratinocytes". It is about the expression, activation and regulation of PC/APC in keratinocytes. Subtitle 6 should be "the functions of PC/APC in keratinocytes". Please make the subtitles more specific and accurate, and avoid repetition.

Response:
Subtitle 5 "PC/APC function and regulation" (bold and italicised; page 10) is within title 3 "PC system on keratinocytes" (bold; page 8). No revision made.

Subtitle 6 was modified to "The functions of PC/APC in keratinocytes" (page 12).

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Reviewer 02770184:

The review was well written. However, many reviews regarding the same topic were published in last several years. The author should acknowledge these reviews and indicate how the current review is different from the previous reviews. Here is a list of a few reviews published recently: 1. Montes R, Puy C, Molina E, Hermida J. Thromb Haemost. 2012 May;107(5):815-26. 2. van der Poll T, Levi M. Curr Vasc Pharmacol. 2012 Sep;10(5):632-8. 3. Della Valle P, Pavani G, D'Angelo A. Thromb Res. 2012 Mar;129(3):296-300. 4. Gleeson EM, O'Donnell JS, Preston RJ. Cell Mol Life Sci. 2012 Mar;69(5):717-26. 5. Navarro S, Bonet E, Estellés A, Montes R, Hermida J, Martos L, Espana F, Medina P. Thromb Res. 2011 Nov;128(5):410-6. 6. Shahzad K, Isermann B. Hamostaseologie. 2011 Aug;31(3):179-84. 7. Suzuki K. FEBS J. 2010 May;277(10):2223-9. 8. Sarangi PP, Lee HW, Kim M. Br J Haematol. 2010 Mar;148(6):817-33. 9. Wang J, Li J. Am J Transl Res. 2009 Jul 15;1(4):381-92. 10. Gupta A, Williams MD, Macias WL, Molitoris BA, Grinnell BW. Curr Drug Targets. 2009 Dec;10(12):1212-26. 11. Castellino FJ, Ploplis VA. J Thromb Haemost. 2009 Jul;7 Suppl 1:140-5. 12. Mann HJ, Short MA, Schlichting DE. Am J Health Syst Pharm. 2009 Jun 15;66(12):1089-96. 13. Neyrinck AP, Liu KD, Howard JP, Matthay MA. Br J Pharmacol. 2009 Oct;158(4):1034-47. 14. Loubele ST, Spronk HM, Ten Cate H. Mini Rev Med Chem. 2009 May;9(5):620-6.

Response:

There are many reviews in relation to the topic of APC as listed by this reviewer. However, our focus is on APC's effects on skin keratinocytes, which has not been reviewed previously. We have cited relevant articles to cover the actions and therapeutic potential of APC in other settings. No revision made.

Reviewer 02610229:

This is a review article that summarizes the role of activated protein C in keratinocyte function. It is well written and reasonably describes the current literature. Minor comments: There ought to be more discussion by the authors of their own thoughts and ideas (pros and cons) as it relates to pharmaceutical potentials of APC. On example is as follows. Despite the importance of APC in anticoagulation, recombinant APC (Xigris) failed in clinical trials in the setting of sepsis. Bleeding was one of the problems. Could APC applied in the setting of skin modulate bleeding in the open wound settings?

Response:

We have added the below text to the section of "Prospective therapeutic potential of PC/APC." Please see pages 15-16 for details. References 101-117 were added to the reference list (pages 30-31).

"In late 2011, rhAPC (Xigris; drotrecogin alfa [activated]; Eli Lily) was withdrawn from the market after failure to significantly improve patient outcome in a clinical trial of septic shock[101], in an attempt to replicate earlier favourable results[102]. One concern was the observation of serious bleeding in patients, although there was no significant difference between patients treated with rhAPC and placebo[101, 102]. Most *in vivo* studies, including our own, show that systemic rhAPC does not induce any

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bleeding side-effects[71, 82, 100, 103-5]. Bleeding has occurred in a subset of near-death sepsis patients with recent surgery and although APC efficacy and safety is controversial in treatment of sepsis patients, it is beneficial and safe in clinical trials for chronic wound healing[82, 100], acute lung injury[106, 107], and solid organ transplantation[108]. Recently APC mutants (3K3A-APC and APC-2Cys) with minimal anticoagulant activity, but normal cytoprotective activity have been generated[109, 110] and shown pre-clinically to be safe[111-17]. Although both variants are yet to be assessed in the field of skin inflammatory diseases. The notion that rhAPC may increase bleeding during wound healing could be circumvented by use of APC variants lacking anticoagulant activity.

Nevertheless, the future for utilising exogenous APC as a topical treatment for skin inflammatory conditions remains a novel and exciting avenue of investigation."

Additional changes by author:

Manuscript title: modified to "Activated protein C; a regulator of human skin epidermal keratinocyte function"

Thank you again for publishing our manuscript in the *World Journal of Biological Chemistry*.

Yours sincerely,

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