

May 02, 2014



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9512 rinse solution.doc).

Title: Polyethylene glycol rinse solution : an effective way to prevent ischemia reperfusion injury

Author: Mohamed Amine Zaouali, Mohamed Bejaoui, Maria Calvo, Emma Folch-Puy, Eirini Pantazi, Gianfranco Pasut, Antoni Rimola, Hassen Ben Abdennebi, René Adam and Joan Roselló-Catafau

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9512

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 and their comments are discussed below:

- (a) We agree with the reviewer's suggestion of explaining the reasons for justifying the PEG 35 concentrations used. The reasons by which we have chosen 1 g/L and 5 g/L are the following ones:
- 1 g/L: it is the concentration used in IGL-1 preservation solution. According to our experience, IGL-1 is a good alternative to UW solution in liver and pancreas graft preservation (Ben Mosbah I, Cell Death Dis. 2012; Zaouali MA, World J Gastroenterol. 2010; García-Gil FA et al., Transplantation 2014)
 - 5 g/L: This concentration was the best compromise; in order to avoid the viscosity problems of rinse solution and facilitate the efficient and suitable flushing of the liver graft when compared to other one more concentrated (10 g/L).

According to the reviewer comment, this was added in Discussion Section. Please see page 12.

- (b) According to Figures 6-8, we observed a better preservation of cytoskeleton, as reflected by an augmented ratio of F-actin/G-actin, and a decreased activity of metalloproteinases. In fact, this was confirmed by confocal microscopy findings that evidenced a more conserved shape of sinusoidal endothelial cells in the liver grafts washed with PEG35 rinse solutions. Consequently, these findings suggest that the cytoskeleton integrity has been better preserved by presence of PEG35 in rinse solutions. This agrees with Malhotra et al (Am J. Physiol. Heart Circ Physiol 300: H1733-H1742, 2011) that PEG is a potential agent to be used in clinical setting for decreasing IRL. In other experimental model (please see reference 43), it has been shown that PEG is able to interact directly with regulatory elements of the endothelial cell barrier, providing beneficial effects on the architecture of the endothelial cytoskeleton.
- In the present study, we aim to present not only the importance of liver graft washout, but also the concomitant protection against reperfusion injury. In this sense, the rinse solution composition is determinant for achieving a more favorable graft revascularization. Due to the interesting results that we have obtained here, in a future work we plan to enlarge the study in order to elucidate the

PEG 35 mechanisms for preventing IRI.

In accordance with the reviewer's comment, the role of PEG on cytoskeleton integrity is included in Discussion section, page 13.

(c): In response to the reviewer's suggestion, the errors have been corrected, as shown in page 3 (adenosine monophosphate-activated protein kinase (AMPK, heat shock protein 70 (HSP70) and heme oxygenase 1 (HO-1); page 12 (a growing body of evidence indicates that mitochondrial dysfunction is a critical pathological process in liver IRI); page 13 (We have now demonstrated that PEG-35 rinse solutions contribute to AMPK activation; furthermore, rinsing liver grafts with solutions containing PEG-35 resulted in activation of constitutive eNOS and subsequent NO generation).

3 References and typesetting were corrected

Thank you again for considering the manuscript for publication in *World Journal of Gastroenterology*.

Sincerely yours,

A handwritten signature in blue ink, appearing to read 'Joan Rosello-Catafau', written over a horizontal line.

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