Response letter.

Reviewer's code: 03475779

SPECIFIC COMMENTS TO AUTHORS

The article deals with a current topic but I have to express some concerns:

1)Being a review, it would have been useful to describe the methodology with which the reference articles were chosen. In fact, lacking this aspect, the choice of the articles taken into consideration is arbitrary and therefore of little significance. 2)The article is limited to considering only cold storage and does not take into account the methods of preservation of the livers to be transplanted with the system of perfusiuone machines in normo and sub-thermothermics, which represent the new line of research aimed at reducing damage from reperfusion.In fact, it would have been interesting to evaluate the effectiveness of the UPS in the various perfusion temperature conditions if, even after these methods, reperfusion damage was present 3)Even in the absence of an analysis of the efficacy of the UPS in normo or sub-normomothermics, the authors do not consider the different outcomes that different reperfusion solutions can determine and therefore evaluate if and how the UPS could be used in association with different reperfusion solutions while holding the criterion of cold storage.

4)The article concludes proposing to evaluate the proteasome inhibitors but does not propose a study protocol on the subject and is therefore an important analysis of their action but without making their use concrete.

ANSWERS:

We appreciate your comments.

1) Our manuscript is a Minireview that follows the guidelines of a narrative review, searching for the answer to a particular question in the existing scientific literature. In no case we pretended to perform a meta-analysis to compare and combine the findings of previously published studies, since we did not intend to asses on the effectiveness of an intervention or mode of treatment. Obviously, a systematic literature search was performed: PubMed, Scopus (Elsevier) and Web of Science (Clarivate Analytics) database searches were conducted with MeSH keywords, combined with various terms for organ

transplantation, oxidative stress and proteasome. The end date for the literature search was March 2018.

Due to the concerns of the reviewer, we have added the following sentence at the end of the introduction:

"In this review we outline the current data of the literature, previous search in databases -Pubmed, Web of Science and Scopus- that support the hypothesis on the potential involvement of the Ubiquitin proteasome system and oxidative stress in ischemia/reperfusion injury"

2 and 3) The role of UPS in normothermic and subnormothermics perfusion is poorly studied. As far as we know, only our previous work [1] has proven the role of UPS in subnormothermal perfusion, whereas in UPS normothermal perfusion the efficacy of UPS has not already been tested.

Related to normo and sub-normothermic machine preservation, we have added a new paragraph in page 5.

"*Protective strategies to reduce hepatic IRI include the use of the machine perfusion, which represents a new line of research opposed to static cold storage.*

Perfusion machine involves a pulsatile perfusion of the liver with a cold (subnormothermic, hypothermic) perfusate ([1–6]); with normothermic perfusate [7,8]; or with a gradual increase in of the perfusate temperature [9]. These references confirm that machine perfusion protects against IRI damage in animal models. These references confirm that machine perfusion protects against IRI damage in animal models. Livers preserved by subnormothermic machine perfusion at 20 ° C showed significantly less liver damage at the end of reperfusion compared to cold storage. The release of GDH was reduced while the production of bile, ATP levels, glycogen and glutathione content increased in preserved livers by subnormothermic machine perfusion than livers submitted to cold storage[2].

Normothermic machine perfusion has also been assessed in discarded human liver grafts[10] . The reported data demonstrate the viability of normothermic perfusion, which results in the continuous production of bile, the fall of lactate levels and the preservation of hepatic morphology. However, the safety and efficacy of machine perfusion is yet to be assessed by randomized controlled clinical trials"

4. We agree with the suggestion of the reviewer, about proposing a study protocol on the subject and we have added it in the conclusion.

Reviewer's code: 00034989

SPECIFIC COMMENTS TO AUTHORS

Well structured paper with clear clinically applicable message. Minor corrections are required and all are pointed out at the attached .pdf. The extent of the references and the length of the test may be an issue but this is up to the editor to decide.

ANSWER

We greatly appreciate your comments and corrections complemented in the pdf. All your comments have been corrected and are underlined in the text.

Reviewer's code: 02566971

SPECIFIC COMMENTS TO AUTHORS

This manuscript presents current information about using antioxidants and proteasome inhibitors to improve organ preservation solutions to reduce the severity of IRI. Overall this is an interesting manuscript. However, there are several defects in this manuscript, which need to be addressed. 1. In keywords, "Autophagy" is not the focus of this manuscript. 2. Page 6, line 6, Before "Autophagy ..." there should be added a period. 3. Ubiquitination is a well-known progress that the authors describe too much detail. However, it is not clear that how UPS acts as a defense system against cellular oxidative stress. 4. Page 11, line 4, "MG32" should be corrected by "MG132".

ANSWER

The authors want to thank you for your review. All your comments have been reviewed.

1, 2, 4) We have solved the mistakes reported.

3) Regarding the description of the UPS, we have added its value as an antioxidant defense.

In page 7 we have added:

"*The activity of the UPS is necessary so that the cells can cope with oxidative stress, but in turn, the activity of the UPS are also modulated by the redox state*"

And in page 11

"*The proteasome is responsible for the selective degradation of oxidatively damaged proteins. In this sense it has been shown that certain oxidized proteins degrade faster than their native counterparts* [11] *and, furthermore, it has been shown that inhibition of the proteasome stabilizes the oxidized proteins*[12]. "

Reviewer's code: 03755443

SPECIFIC COMMENTS TO AUTHORS

GENERAL COMMENTS: - Please review available literature on MACHINE COLD PRESERVATION and relate it to potential effects on PROTEASOME UBIQUITIN SYSTEM. - Please review available literature on NORMOTHERMIC LIVER PRESERVATION and relate it to potential effects on PROTEASOME UBIQUITIN SYSTEM. SPECIFIC COMMENTS: - Please spell the abbreviation UPS on page 2. - Insert a comma after DURING ISCHEMIA on row sixth of page 3. - Replace BUT by HOWEVER on fourth row of COLD STORAGE on page 4. - Replace HAVE by HAS on first row of page 5. - Replace MANY by SEVERAL on row 14th of the section OXIDATIVE STRESS IN IRI (page 5). - Insert a point before the word AUTOPHAGY on fifth row of page 6. - Replace MANY by SEVERAL on third row of section UBIQUITIN (page 6). - Replace ORGANS by ORGAN (last row of page 8). - Insert RAT between the words NON-STEATOTIC and LIVER. - Insert the words FROM RATS between the words FATTY LIVER and THROUGH.

ANSWER

1. We have solved the errors reported by the reviewer.

2.- Related to cold and normothermic machine perfusion, we have added a new paragraph in the Introduction section, page 3 (underlined in the manuscript) and the following text in the page 5.

"*Protective strategies to reduce hepatic IRI include the use of the machine perfusion, which represents a new line of research opposed to static cold storage.*

Perfusion machine involves a pulsatile perfusion of the liver with a cold (subnormothermic, hypothermic) perfusate ([1–6]); *with normothermic perfusate* [7,8]; *or*

with a gradual increase in of the perfusate temperature [9]. These references confirm that machine perfusion protects against IRI damage in animal models. Livers preserved by subnormothermic machine perfusion at 20 ° C showed significantly less liver damage at the end of reperfusion compared to cold storage. The release of GDH was reduced while the production of bile, ATP levels, glycogen and glutathione content increased in preserved livers by subnormothermic machine perfusion than livers submitted to cold storage[2].

Normothermic machine perfusion has also been assessed in discarded human liver grafts[10] . The reported data demonstrate the viability of normothermic perfusion, which results in the continuous production of bile, the fall of lactate levels and the preservation of hepatic morphology. However, the safety and efficacy of machine perfusion is yet to be assessed by randomized controlled clinical trials"

3.- About the role of UPS on machine perfusion:

In the search performed we have not found any experimental work on the subject. We can only hypothesize that the finding that ATP negatively regulates proteasome activity can explain the protection found in livers submitted to machine perfusion. This could be associated with an energy metabolism recovery. Machine perfusion keeps the ATP/ADP ratio and glycogen content. Moreover, glycogen may help in maintaining tissue stores of high-energy adenylates over the course of preservation.

If the reviewer can provide us with any reference, we will be very grateful.

Reviewer's code: 03475120

SPECIFIC COMMENTS TO AUTHORS

1. Cold ischemia/warm reperfusion injury is confirmed at least several hours, and early posttransplant period is important for liver regeneration. This point should be clearly mentioned. 2. Also, shear stress affects on posttransplant oxidative stress. Please mention this point. 3. Graft size is a key factor for oxidative stress after liver transplantation. Behaviors of oxidative stress markers are different according to graft size. This point should be mentioned with related articles. Pretreatment of Small-for-Size Grafts In Vivo by γ - Aminobutyric Acid Receptor Regulation against Oxidative Stress-Induced Injury in Rat Split Orthotopic Liver Transplantation. Int J Hepatol. 2013;2013:149123. PMID: 24223309 Pretreatment of liver grafts in vivo by γ-aminobutyric acid receptor regulation reduces cold ischemia/warm reperfusion

injury in rat. Ann Transplant. 2013;18:299-313. PMID: 23792534

ANSWER

The authors want to thank you for your review. All your comments have been reviewed and added in the Introduction section.

"*Variables related to the donor (age, steatosis) and surgery (prolonged ischemia times* [13]*are the most commonly reported risk factors for graft dysfunction. Other factors such as shear stress and graft size*[14] *have been recognized as important factors associated with oxidative stress, lack of primary function, early dysfunction allograft and biliary complications after liver transplantation* [15] ·

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