Author's response to reviews

Title: Bradykinin and adenosine receptors mediate desflurane induced postconditioning in human myocardium: role of reactive oxygen species

Authors:

Sandrine Lemoine (sand.lemoine2@wanadoo.fr)
Clément Buléon (buleon-c@chu-caen.fr)
René Rouet (rene.rouet@unicaen.fr)
Calin Ivascau (ivascau-c@chu-caen.fr)
Gérard Babatasi (babatasi-g@chu-caen.fr)
Massimo Massetti (massetti-m@chu-caen.fr)
Jean-Louis Gérard (gerard-g@chu-caen.fr)
Jean-Luc Hanouz (hanouz-ji@chu-caen.fr)

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Author's response to reviews: see over
Dear Dr Sabina Alam,

We have now revised the manuscript MS: 2576476136707922. You and the reviewers pointed out several key issues which we have addressed below. We have also enclosed a point by point response to each of the Reviewers’ comments. We have truly appreciated the time and expertise that you and the Reviewers put into our manuscript.

We hope that the revised manuscript will be improved.

Responses to Reviewer #1, P Pagliaro, MD, PhD

First we thank you very much for the time and expertise you spent in reading the present manuscript.

Major Compulsory Revisions
1) Although the literature data strongly support a role for A2b adenosine receptors (AR) in cardioprotective signaling in ischemic postconditioning, A2aAR may also play a role. At this time specific receptors antagonists must be used to show which of these receptors are responsible of the effects.

The present study was not designed to assess whether specific adenosine receptors are involved in desflurane induced postconditioning. One of the major aims was to explore the relationship between adenosine and bradykinin receptor activation and ROS generation in desflurane induced postconditioning, moreover at our knowledge; these precise relationships have never been investigated in anesthetic myocardial postconditioning.

Additionally, controversy exists on the receptor subtypes involved, because a really selective agonist or antagonist for each subtype is not available at the moment (Cohen MV, Downey JM. Basic Res Cardiol 2008; 103: 203-15; Peart JN, Headrick JP. Pharmacol Ther 2007; 114: 208-21). Therefore, we decided to block all of the receptors with a nonselective blocker. Consequently, we cannot exclude that the results would have been different had a synthetic antagonist been used. However, we think that the evaluation of specific activation of adenosine receptor subtype in desflurane induced postconditioning could be a very interesting feature study.

Then, studies on human atrial myocardium are difficult, and require a long time to be performed because atrial appendage cannot be obtained from all patients scheduled for cardiac surgery with cardiopulmonary bypass. Thus experimental studies from our laboratory exclude patients with diabetes mellitus (either with or without insulin administration) and chronic atrial arrhythmia because these diseases profoundly modify structure and function of the myocardium (see for review Dobrev D et al. Basic Res Cardiol 2003; 98:137-48 and Amour J et al. Anesthesiology 2008; 108:524-30). Moreover, we also exclude patients scheduled for mitral and tricuspid valve replacement, and emergency cardiac surgery. Thus we hope to select patients with the less “remodelled” heart and then right atrial tissue. Furthermore, it is one of the major interest of this work the use of human atrial species excludes the difference species between the clinical and experimental results.

2) The authors should discuss controversy on anti-stunning effects of postconditioning. Moreover, authors should consider that adenosine protection may not include ROS-signaling.

The "discussion" section has been rewritten taken this comment into account, now we have discussed the contradictory results concerning the anti-stunning effects of myocardial postconditioning (page 13, paragraph 3), and we have added the reference "Couvreur N et al.

Moreover, as the reviewer suggested, we have added and discussed the Cohen and Downey study (Cohen MV et al. Circ Res 2001; 89: 273-8; Reference N°36) where the adenosine induced preconditioning did not require the implication of ROS generation as a trigger (“discussion” section - page 13, paragraph 2)

3) How authors choose the doses of antagonists and scavenger? Please, quote some articles.

This point and the references appear already in the original manuscript in "Experimental protocol" of the "method" section (page 7, paragraph 5).

4) Correction performed.

As suggested by the reviewer, the sentence “Importantly, our investigation included a control group that was equally affected by any of these potentially modifying factors” have been deleted in “discussion” section (page 14, paragraph 2) of the revised version of the manuscript.

5) We must apologize for the language and grammar errors in the manuscript. We have tried to correct all language and grammar errors through several rereading of the manuscript. We hope that the manuscript will be improved.

Responses to Reviewer #2, Franz Kehl MD, PhD

We thank you very much for the time and expertise you spent in reading the present manuscript.

1) As you suggested we have cited the reference "Yao et al., Mol Biol Rep. 2009 in press” (Reference N° 8) you suggested in the “background” section (page 4, paragraph 2) of the revised version of the manuscript.

2) Regarding legend to figure 3: Are the data for control and desflurane group the same as shown in figure 2? If yes, this should be stated in the legends.

Yes, the data for control and desflurane group are the same in figure 2 and 3.
Correction performed. We have specified this point in figure 3 legend (page 25).

3) We must apologize for the language and grammar errors in the manuscript. We have tried to correct all language and grammar errors. We hope that the manuscript will be improved.

Sincerely yours,
Sandrine Lemoine Ph.D.
Jean-Luc Hanouz M.D.,Ph.D.