Author's response to reviews

Title: Preconditioning offers cardioprotection in hyperlipidemic rat hearts: possible role of Dopamine D2 signaling

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Author's response to reviews: see over
To,
The Editor
BMC Cardiovascular Disorders

Subject: Submission of Revised manuscript for publication..

Sir / Mam,
In view of the comments raised by two reviewers, the corrections have been incorporated into the manuscript. Therefore, I am hereby submitting the revised manuscript as original research article entitled “Preconditioning offers cardioprotection in hyperlipidemic rat hearts: possible role of Dopamine D2 signaling” for publication. The Authors of research paper are Varun Gupta, Rohit Goyal and Pyare Lal Sharma.

The point to point responses to each comment are also being supplied.

I shall feel grateful, if you kindly acknowledge receipt of revised manuscript.

Thanking you.
Yours Sincerely

Rohit Goyal
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Responses to the Comments:

Reviewer 1:

Comment 1: Number of results should be reported in the abstract.

Answer: The total number of results reported from the study is five which are: body weight, lipid profile, LDH, CK-MB, coronary flow rate and infarct size. Hence the corrections have been incorporated into the abstract.

Comment 2: Given the small sample size, parametric distribution should be checked for

Answer: The sample size was initially taken to be 8, but since the success of induction of hyperlipidemia in rats through High Fat Diet is approximately 80%, only 6 out of 8 animals could develop hyperlipidemia and the sample size was found to be 6. The correction for actual sample size n=8 is incorporated into the manuscript.

Statistical analysis using one-way ANOVA and Two-Way ANOVA were used. One way ANOVA test was applied for infarct size assessment because this was estimated one time after the completion of treatment.

For LDH, CKMB and CFR, the readings were taken at 2 time points i.e. i) basal ii) after 5 min of reperfusion (for LDH & CKMB) and after end of reperfusion (for CFR). So Two Way ANOVA was applied.

Comment 3: It not clear the rationale for high vs normal diet and interactions with IPC

Answer: The ischemic preconditioning (IPC) is reported to provide cardioprotection against ischemia-reperfusion in normal rat hearts. But the protective effect of IPC gets blunted if the subjects are hyperlipidemic. The effect of dopamine preconditioning was tested on normal and high fat treated rat hearts. For this the effect of IPC on normal and high fat treated rat hearts were employed to estimate as control measures in comparison to dopamine preconditioning.

Comment 4: Analysis for all animals with IPC vs. no IPC should be added

Answer: The relation of I/R with rat hearts treated with normal diet and high fat diet is already established. The effect of IPC with I/R on rat hearts treated with normal diet and high fat diet is already established. The aim of the present study was to investigate the role of dopamine (in the form of dopamine preconditioning) in cardioprotective effect of IPC against hyperlipidemia in rat hearts.

Hence the comparison of:

- effect of IPC in normal rat heart with HFD treated rat heart,
- effect of IPC in HFD treated rat heart with dopamine treated rat hearts, and
- effect of combination IPC and dopamine in HFD treated rat hearts against I/R
were already done.

**Comment 5:** Use of troponin may have also been important and should be added as limit

**Answer:** Lactate dehydrogenase, troponin and CK-MB are the characteristic markers for the myocardial necrosis during ischemic diseases and hence significantly prevalent in condition like ischemic heart disease, as also suggested by reviewer. The markers for cardiac ischemic injury LDH and CKMB have been carried out in the study, as also suggested by most of the literature available. The study was not funded by any external agency, perhaps it was a part of M Pharm dissertation work. Hence, estimation of troponin using ELISA or radio assay would not be possible.


**Comment 6:** Extent of disease should be assessed (comment on D’Ascenzo et al, Eurointervention 14)

**Answer:** D’Ascenzo et al very effectively explained the use of remote IPC in cases of periprocedural myocardial infarction. Such cases are usually high due to the increasing use of percutaneous coronary interventions. Brief episodes of ischemia–reperfusion is reported to provide protection to myocardium on prolonged ischemia followed by reperfusion. This concept of myocardial protection derived different types of cardiac interventions: preconditioning, post-conditioning, pharmacological preconditioning and remote precondition.

These intervention are useful for providing protection in conditions like myocardial infarction, coronary arteries blockade, organ transplantation etc. The extent of disease was assessed in present study as evidenced from the damage cause in rat hearts treated with normal diet and HFD respectively. The proximity of cardioprotection in rats with HFD is very less as characterized by excessive coronary blockade. Cardiac preconditioning is therefore a possible methodology to avoid such risk.

The extent of damage is depicted through the measure of infarct size. The animals subjected to ischemia reperfusion injury had an infarct area of 45.64 ± 5.93 whereas those treated with IPC showed an infarct size of 21.22 ± 4.36. Similarly for all the groups, the extent of damage caused by ischemia reperfusion injury has been mentioned in the results.

**Comment 7:** Clinical translation of these results in everyday clinical practice

**Answer:** Preconditioning to heart in the form of ischemic-, post and remote preconditioning in clinical practice has been shown to be relevant during cardiac abnormalities like myocardial
infraction and coronary artery diseases. Dopamine is a drug being used for congestive heart failure and further detailed investigations on its role in preconditioning can be established as protective effect of varying doses of dopamine also found. Moreover this study also focuses on establishing the role of D2 receptors which helps in understanding the basic physiological mechanism of IPC.

Clinically, IPC induced cardioprotection has been reported to be blunted in hyperlipidemic or obese subjects. The findings from present study shows a possible way for the development of therapeutic interventions by dopamine cascades.

**Comment 8:** Figure number 2 is not clear

**Answer:** Figure number 2 explains about the schematic experimental protocol for the various sessions of ischemia, reperfusion and drug treatment at isolated heart preparation using Lagendorff’s apparatus. The image submitted was in monochrome. Therefore, I am enclosing a colored version of Fig no 2 for your reference. All the authors have been consented to incorporate mono colored version of Figure No 2. The explanation of figure no 2 is as under:

- Blue color signifies 10 min stabilization period for all the groups. Green color refers to reperfusion (5, 40 or 120 min). Pink color refers to Ischemia (5 or 30 min). Grey color signifies Dopamine (5 min). Downward arrow refers to Clozapine. Inverted triangle refers to perfusate collection.
- The detailed explanation of the proceeding of every group has been mentioned in the Experimental Procedure.
- However, in the black and white version of the image, all the different zones have been marked with respective treatments related to them and denominations have been mentioned in the legends.
Reviewer 2:

Report 1: I would like to thank the authors for this nice article. It really handles an important and interesting issue.
Answer: The appreciation for the article submitted is duly received and duly acknowledged by all the contributing authors.