Author's response to reviews

Title: Possible involvement of coveolin in attenuation of cardioprotective effect of ischemic preconditioning in diabetic rat heart

Authors:

Preeti Ajmani (hnyadav@gmail.com)
Harlokesh Narayan Yadav Asst. Professor (hnyadav@gmail.com)
Manjeet Singh Professor (hnyadav@gmail.com)
P L Sharam Emeritus professor (hnyadav@gmail.com)

Version: 2 Date: 11 May 2011

Author's response to reviews: see over
To,
The Editor-in-Chief
BMC Cardiovascular disorders
Respected Sir,

Subject – Old manuscript no 3225652425173078, **Point to point changes made in the manuscript based on the comments of the referees.**

Dear Sir,

This is with reference to your E-mail regarding the manuscript (3225652425173078) the point to point changes made in the manuscript are as follows.

**Reviewer; Edward Lesnefsky**

**Major compulsory revisions;**

The significant values of the one-way ANOVA is \( P < 0.05 \) followed by significance value of secondary comparisons test (Tuky’s test) is \( P < 0.05 \) has been incorporated, as per the suggestions made by the reviewer.

The specificity and limitations of the agents has been discussed. The doses have been selected on the basis of earlier published studies. The focus of this study was a mechanistic approach, therefore the dose response relationship was not studied.

The proposed caveolin-eNOS protein-protein interaction should be assed by co-immunoprecipitation studies. Alternatively caveolin can be isolated.

The above mentioned comment has been included under limitation of our study at the end of the discussion.


**Minor Essential Revisions;**

Caveolin-1and caveolin-2 is co-expressed in many cell types including adipocytes, endothelial cells, epithelial cells and fibroblasts (Scherer et al., 1994, Scherer et al., 1997) whereas is in cardiac myocytes Caveolin-3 is in abundant which may decrease the availability of NO by
making caveolin-eNOS protein protein complex (Scherer et al., 1994; Song et al., 1996; Minetti et al., 1998; Galbiati et al., 1999, Galbiati et al., 2001).

The caveolin and eNOS knockout rats were not used because these have to be imported and need authorization from CPCSEA India.

The main focus in our study was reduction of infarct size, release of LDH and CK-MB, hence functional data was not obtained.

Role of caveolin in cardiac protection have been included in discussion.
Reviewer: Birendra Kumar Roy

Minor Essential revisions;

The spelling mistakes and needful corrections have been carried out.
Reviewer; Derek J Hausenloy

Reviewers report;

The spelling mistakes have been corrected.

In discussion the observed data on results has been incorporated.

In an earlier study from our laboratory, 7 day treatment with daidzein had no significant effect on ischemia-reperfusion induced myocardial infarct size, release of LDH and CK-MB (Gupta I (2010) Involvement of heme oxygenase-1 in attenuation of the cardioprotective effect of ischemic preconditioning in diabetic rat heart, Thesis accepted for award of M.Pharm degree (2010), Punjab Technical University Jalandhar, India).

Caveolae are the specialised membrane domains, serve as centres for cellular signal transduction which facilitates the interaction and organization of signalling molecules so as to provide coordinated and efficient signal transduction (Lisanti et al., 1994; Okamoto et al., 1998). Preconditioning with bradykinin induces the formation of caveolar signaling platform (signalosomes) that contains the enzymes of the signaling pathway which interact with mitochondria to induce the opening of mito K\textsubscript{ATP} channel (Garlid et al., 2008; Quinlan et al., 2008). Involvement of caveolin in the myocardium has been reported to mimic preconditioning by activating PI-3K (Patel et al., 2006; Tsutsumi et al., 2008).

Bucci et al (2004) noted an increased expression of caveolin-1 in diabetic mouse. This has been added in the discussion section.
Reviewer; Amiteshwar Jaggi

Reviewer’s report;

In earlier work in our laboratory, STZ-induced diabetes mellitus in the rat did not produce a significant increases in I/R induced myocardial infarct size or in release of LDH and CK-MB, (Unpublished data).

In our study, the main end-point used to access the protective effect of IPC was measurement of myocardial infarct size. In addition we have used two secondary marker of myocardial injury (LDH and CK-MB) in the coronary effluent. This being a mechanistic study, we did not measure myocardial functioning parameters.

The animal mortality data due to diabetes, both short term and long term has been added in materials and methods section.

It is noted in our laboratory that peak release of LDH occurs immediately after reperfusion and peak CK release is reported to occur 5 min after reperfusion (Parikh and Singh, 1997, 1998; Sharma and Singh, 2001). Similarly in present study the release of LDH and CK-MB mesured at 0 and 5 min. was significantly higher than sham control group, there was no any ischemia was given (Yadav et al., 2010).

In the present study, the release of LDH and CK-MB obtained at different time intervals was compared with sham control. The timed results were compared between themselves. Therefore use of one-way ANOVA is quite appropriate.
Reviewer; Vishal Diwan

Reviewer’s report;

That the Nitrate/nitrite concentration can be measured in perfusate from the Laggendorff is stated in two references already cited in this paper i.e. Parikh V, Singh M 48 and Szabo et al 47.


In case, any further clarification is required please do let me know.

Thanking you in advance

Yours faithfully

(Harlokesh Narayan yadav)

I.S.F. College of Pharmacy,
Moga - 142001,
Punjab, INDIA.

Tel- +919888036775