Author's response to reviews

Title: The Effects of apoptosis vulnerability markers on the myocardium in depression after myocardial infarction

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

Many thanks for your help, the following responses to reviewers' comments have been addressed.

**Answer questions:**

Dr Guy ROUSSEAU comments:

1. Did some rats in the depression group present data in the behavioural tests that are similar to the MI group. In other words, did all rats in the depression perform similarly in the behavioural tests?

   Answer: Yes, all rats performed same behavioural test in all four groups. All rats performed similarly in the behavioural tests at baseline but not endpoint. *We have mentioned this in the Methods of Abstract, pg1, line 3-4, and in the Methods, pg 6, line 8-11, highlighted in red.*

2. In the sucrose consumption test, it is important to indicate the number of total ml of liquid taken for each group. Otherwise the difference in the sucrose consumption could be due to other parameters.

   Answer: *We have added this in the Results of Abstract, pg1, line 2, in the Methods, pg 6, line 10, and pg 9, line 7, highlighted in red.*

3. If I understand correctly, the measurements of caspase-3 or Bax-Bcl-2 were performed on tissues that were previously used in the determination
of infarct size (TTC staining). Do you know if TTC influences the results?

**Answer:** the left anterior descending coronary artery was ligated again at the same site, and the aorta was infused with 2ml of 0.5% Evan’s blue to determine the extent of the non-coloured ischemic risk area, then the myocardium was bisected into two parts from the apex to base along the left anterior descending coronary artery, which was frozen at -80°C for 5 minutes, and then sliced into 2mm transverse sections and stained using 2,3,5-triphenyl tetrazolium chloride (1.5%TTC) and myocardial infarction size (× 2) was thus confirmed. The rest of the myocardium was washed using DEPC (diethylypyrocarbonate) H2O. The anterior myocardial regional tissue sample (50 mg), which was non-coloured tissue using Evan’s blue was separated from the edge of the myocardial infarct risk area of the left ventricle.

We have revised and added the contents in the pg 9, the paragraph of myocardial infarct size, line 4-8, and the paragraph of Tissue preparation, pg 10, line 1-4, highlighted in red.

4. It is difficult to follow this section. The link between depression, MI and apoptosis is difficult to understand.

**Answer:** There is an increased incidence of major depressive disorder (MDD) (15-30%) in individuals after myocardial infarction (MI). Depression is linked to a 2.0-2.5 fold increased risk in new cardiovascular events and increased cardiac mortality. However the pathophysiological mechanisms underpinning the relationship between MI and depression remain poorly understood. Our previous study [24] demonstrated that in rats with chronic mild stress (CMS), there were significant behavioral deficits, an increase in Bax levels and a decrease in Bcl-xl levels in myocardium and hippocampus, suggesting an increase in pro-apoptotic pathways. This was reversed by venlafaxine. This study aimed to attempt to evaluate vulnerability markers of myocardial apoptosis, specifically the Bax:Bcl-2 ratio and caspase-3 levels in the myocardium post-MI depression, to clarify the molecular mechanisms, and as well as confirm whether the co-occurrence of myocardial infarction with MDD is associated with greater activation of apoptosis pathways.

We have revised the contents in the Background pg 4, line 4-7, 9-10, pg 5, line 15-18, pg 6, line 2-7, highlighted in red.
5. How did you divide the rats between MI and post-MI depression groups. What are the criteria.

Answer: The model of post-MI depression was determined using open field and sucrose preference tests. In this model of depression after MI, there were significantly decreased scores of both horizontal and vertical movement and sucrose consumption compared to sham group in the depression group.

We have amended this in pg 8, line 20-22, pg 9, line 7-10, highlighted in red.

Dr Ove Wiborg comments:

1. Authors have not found any difference in Caspase-3. But this is not the only caspase which can be involved in apoptosis. What about others? They comment this possibility in the discussion but they could do a bit more about it. F.ex. they could look in the literature whether other caspases expresses in the heart, or even to do their own test. If they could find other caspase they need to see whether it is affected by MI and depression.

   Answer:

   Other caspases may be involved. Lancel et al.[45] showed that endotoxin induced increases in ventricular cardiomyocyte caspase-3, -8, and -9-like activities. This was associated with sarcomeric structure damage and cleavage of components of the cardiac myofilament. Frantz et al.[46] noted that rats with deletion of the caspase-1 gene showed increased peri-infarct survival and lower rate of ventricular dilatation and a decreased rate of apoptosis after a model of myocardial infarction. However, we did not find a relationship between other caspases and the model of MI with depression. We have revised and added this section to pg 16, line 16-23, and pg 26, References 45, 46, highlighted in red.

2. What is the percentage of rats that survive after MI induction procedure?

   Answer: > 90%.
Michael Berk  cont’d…

We have added this in pg 7, last 2-3 lines, highlighted in red.

Other comments

1. The author names need to changed appropriately in the title page i.e first and last name.

Answer: We have revised the author names in the title page, highlighted in red.

2. Acknowledgements

We have revised the Acknowledgements in pg 19, line 2-3, pg 20, line 4-5, highlighted in red.

3. Authors' contributions

We have revised the Authors' contributions in pg 19, highlighted in red.

Yours sincerely

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