Author’s response to reviews

Title: Conditional Disruption of Interactions Between G(α)i2 and Regulator of G Protein Signaling (RGS) Proteins Protects the Heart From Ischemic Injury

Authors:

Sergio Parra (sparra@vapogenix.com)
Xinyan Huang (XHUA@lundbeck.com)
Raelene A Charbeneau (raelenec@umich.edu)
Susan M Wade (suemwade@umich.edu)
Kuljeet Kaur (umkaurn@umich.edu)
Boyd R Rorabaugh (b-rorabaugh@onu.edu)
Richard R Neubig (rneubig@msu.edu)

Version: 3
Date: 1 May 2014

Author’s response to reviews: see over
May 1, 2014

Dr. Farb,

Thank you for the opportunity to revise our manuscript (manuscript # 1755746411217287; “Conditional Disruption of Interactions between Gai2 and Regulator of G Protein Signaling (RGS) Proteins Protects the Heart from Ischemic Injury” for publication in Biomed Central Pharmacology and Toxicology. The editor’s comments are addressed below:

1. Comment: Please remove the author suffixes from the title page.
   Response: The suffixes have been removed from the title page.

2. Comment: ARRIVE Guidelines: Please adhere to ARRIVE guidelines in your reporting and upload a completed checklist as an additional file with your revised submission.
   Response: We have adhered to the guidelines and uploaded the completed checklist.
   In addition, we have added the following sentence in lines 96 – 97 “Animals were housed in a specific pathogen free facility with a 12 hour: 12 hour light : dark cycle and free access to food and water.“

Thank you for the opportunity to report our findings in BMC Pharmacology and Toxicology.

Sincerely,

Boyd Rorabaugh, Ph.D.
Response to Reviewer Comments  
Manuscript # 1755746411217287

Reviewer: Martin C C Michel
Major compulsory revisions- none

Minor essential revisions
1. Ethics: While I have no doubt that the U Mich animal committee plays by the rules, it would be good to explicitly state whether the experimental protocols were in line with NIH guidelines.

The following sentence has been added to line 95 “Experimental procedures and animal husbandry were conducted in accordance with NIH guidelines.”

2. Ethics: Please explicitly state the doses of the anaesthetics in the bottom paragraph of p. 11.

The doses of pentobarbital and heparin have been added (line 231).

Discretionary revisions
3. That the conditional KO did not induce conversion in the brain was surprising to me. It apparently is not due to lack of CNS penetration by tamoxifen. While the authors discuss possible consequences of this, they do not comment on the basis for this lack of conversion. Can you compare it to other tamoxifen-driven conditional KO’s?

Other investigators using the Cre-ERT2 system have also observed low recombination rates in the brain. We have addressed this issue with the following sentence in the discussion (lines 380-384): “The reason for a lack of conversion to the G\(g_{i2}^{G184S}\) mutation in the brain is unclear. However, other investigators using the Cre-ER\(T2\) system have reported similar results. Seibler et al. confirmed that Cre-ER\(T2\) is expressed in the brain and suggested that the lack of recombination may reflect low local concentrations of 4-hydroxytamoxifen [27].”

4. I realize that indirect comparisons between studies can be problematic. Nonetheless, it would be nice to add some indications to the discussion how the present findings relate quantitatively to those with the constitutional knock-out. As both studies come from the same lab and have used the same techniques, that should be informative to some degree.

We have added a paragraph to the discussion (lines 357-366) comparing the cardioprotective phenotypes of the conditional and nonconditional knockin mouse models.

5. The Abstract emphasizes time course questions. In this regard it would be helpful to indicate at which time point, i.e. how many hours after tamoxifen dosing, the phenotypic experiments were done and/or clarify that ‘acute’ here
means ‘days rather than months’, as has been done on p. 16.

We have clarified the abstract to indicate that “acute” means days rather than months (line 43). We have also clarified the methods section to indicate that the Langendorff heart experiments were performed 14 days after the first tamoxifen injection (line 229).

6. Background, 1st paragraph: While not being a Gi protein, Go also belongs to the Gi family. If Go is not mentioned here, the sentence on PTX distinguishing Gi from other G-proteins becomes technically wrong as Go also is PTX-sensitive. Please reword.

This has been corrected (line 57).

7. P. 5, l. 6: I recommend a paragraph break after mentioning reference #14, as you switch to a new thought.

A paragraph break has been added (line 81).

8. P. 12: Please add version of Prism being used.

The software version has been added (line 240)

9. I recommend using the same y-axis scaling for panels A-C of figure 4 for ease of comparison.

The scales have been adjusted so that they are all equal.

Reviewer: Ulrike Mende

Minor Essential Revisions:
1. A “deleter mouse line” with generalized tamoxifen-dependent Cre expression was used to assess the effectiveness of endogenous to mutant Ga2 conversion in various tissues. What was the rationale for using a generalized knock-in model for assessing the cardioprotective effect? Utilization of mice with cardiac-restricted Cre-ERT2 expression could alleviate questions about if and to what extent Ga2 G184S knock-in in non-cardiac tissues may contribute to the observed phenotype. Please discuss.

We agree that this would have been advantageous. In fact, we attempted to make two additional Gi2 G184S knockin mouse models in addition to the conditional model described in this manuscript. One model was a cardiac specific Gi2 G184S knockin and the other model was fibroblast specific Gi2 G184S knockin. The goal was to determine whether both cardiac myocytes and cardiac fibroblasts contribute to the cardioprotective phenotype that results from Gi2 G184S expression. Unfortunately, we were unable to use the cardiac-specific model because it produced very low recombination rates in the
heart. We were unable to use the fibroblast specific Gi2 G184S knockin model because we found that it was not sufficiently selective for fibroblasts.

2. In addition to the developed pressure, other important functional measurements obtained from the Langendorff preparations (including +dP/dT, -dP/dT, EDP and heart rate) should be provided and discussed for a more complete picture of the cardioprotective effect.

We have added Table 1 which includes preischemic and postischemic values for developed pressure, +dP/dT, -dP/dT, end diastolic pressure, and coronary flow rate. These data are discussed in lines 327-339. Heart rate was not included in the table because all of the hearts were paced at a constant rate of 500 bpm.

3. The 1st sentence in the abstract ("Regulator of G protein signaling (RGS) proteins suppress G protein coupled receptor signaling by catalyzing the hydrolysis of Gai-bound guanine nucleotide triphosphate.") could be wrongly interpreted as if all RGS proteins suppress Gi signaling and/or as if RGS proteins only suppress Gi signaling. Please rephrase to clarify. This has been corrected so that the statement is not specific for Gi (line 26).

4. Please clarify the last sentence on page 12: “Blots were hybridized to 5’ and 3’ probes, EcoNI (13.0 kb fragment) (Fig. 1D) and AflIII -digested genomic DNA (Fig. 1E, this probe hybridizes to a 7.9 kb fragment from the wild type Gai2 allele and an 11.5 kb fragment from a correctly targeted Gai2 allele), respectively.”

This has been clarified (line 256-260).

5. Please relate the results from the cAMP assay in adult fibroblasts from the conditional knock-in model to published data from the non-conditional model.

The following sentence has been added (lines 315-318): “These data are consistent with our previous finding that LPA-induced inhibition of adenylate cyclase activity is enhanced in embryonic fibroblasts in the nonconditional Gai2$^{G184S}$ model [13], and they provide evidence that agonist-induced Gai2 signaling is enhanced in cardiac fibroblasts that conditionally express the Gai2$^{G184S}$ allele.”

6. The genotypes of mice used for Western blot analysis shown in Figure 4 are inconsistent and must be reconciled between the result section (Page 17) and Figure 4A-C (are the bar graph annotations truncated?)

Fig. 4 has been corrected so that the full names of each genotype are consistently used.

7. The labeling above the molecular weight markers in Figure 4D should be corrected to “Mr (kDa)".
This has been corrected.

8. Please fix the following additional minor errors: (i) The comma on lane 5 of page 17 should be a full stop; (ii) “Western” in the legend for Figure 4 should be capitalized; and (iii) “derivation” in the legend for Fig. 5 should be “determination”.

These errors have been corrected.

Discretionary Revisions:
1. The marked change in infarct size could be visually supported by representative images of infarced hearts from each group.

Representative images have been added to Fig. 6B.