Author's response to reviews

Title: The Na+/Ca2+ Exchange Inhibitor SEA0400 Limits Intracellular Ca2+ Accumulation and Improves Recovery of Ventricular Function When Added to Cardioplegia

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Author's response to reviews: see over
Title: The Na⁺/Ca²⁺ Exchange Inhibitor SEA0400 Limits Intracellular Ca²⁺ Accumulation and Improves Recovery of Ventricular Function When Added to Cardioplegia

Authors: Jeanne Eggar BSc¹, Ahmad Ali MSc¹, Susan E Howlett PhD²,³, Camille Hancock Friesen MD, MSc⁴,⁵,⁶, Stacy O’Blenes MD, MSc¹,⁴,⁶.

Thank you for the constructive comments. We have attempted to address each comment in the revised manuscript as indicated below.

Referee 1

One minor aspect need to be clarified:
If there is statistical difference when the survival rates of cells arrested with cardioplegia with and without the SEA0400 addition were compared.
It is also important to emphasize that the control group is that of cardioplegia without SEA0400 in some data comparisons.

*Cells treated with cardioplegia containing SEA0400 had the highest survival rate which was significantly better than cells not protected with cardioplegia; however this did not reach significance when compared to cells arrested with cardioplegia not containing SEA0400. In order to clarify this, the last sentence of the first paragraph in the results section was modified to read “Cells arrested with cardioplegia not containing SEA0400 had an intermediate survival rate (10 of 24, 42%, p=NS vs. ischemia alone and vs. cardioplegia with SEA0400).”*

The results section has been modified to replace “control” with “hearts arrested with cardioplegia not containing SEA0400”

Referee 2

Method section:
1 – The isolated hearts were perfused with Krebs solution. What is the calcium concentration on this solution? The calcium content diverges widely in the literature. Please give a comment.

*The Ca²⁺ concentration in the Krebs solution is 2.5 mM. This has been clarified in the methods section by including the statement [Ca²⁺]=2.5mM.*

2 – the LV function was assessed by increments on intra-ventricular balloon. What were the rats weights? What was the volume increments? Was it easy to perform such volume increments? How accurate were those volume increments?
The rats weighed approximately 300g. This was clarified in the first paragraph of the methods section. The volume were increased incrementally by 25 µL to a total of 200 µL. The volumes were easily delivered using a calibrated chromatography syringe. This has been clarified in the methods section by adding the statement “...in 25 µL steps to a maximum of 200 µL...”

3 – The authors described an improvement on survival during ischemia. How was determined cell survival in the isolated cardiomyocyte model?

Upon death cells rounded up and did not exclude trypan blue. This has been clarified in the methods section by adding the sentence “Cell death was identified visually when cells lost the typical cardiomyocyte and rounded up into a ball, and was confirmed by trypan blue staining.”

Results section:
The Max LVDP, LV work, and Max and Min dP/dt figures comparing the groups with and without SEA0400 seem the baseline a little different. In other words, all hearts in the group without SEA0400 started at baseline with lower values of each variable. Are the groups comparable? How can you explain these differences at baseline? May these differences interfere in the final results? Were this heart (without SEA0400) in “bad shape” at the baseline of the experiment? Please explain.

All the animals were assigned to their experimental group before the experiment started so that there was no selection bias possible. Small, non significant differences in baseline parameters are most likely due to chance and related to relatively small sample size. Given the size of the differences observed after cardioplegia, we feel it is unlikely this affects the results.

This was clarified in the methods section by adding the sentence “Rats were assigned to their experimental group before the experiment was started.” and in the results section buy adding the sentence “There were no significant differences in baseline functional parameters between groups.”

Regarding the comments above, I would like to see explanation for the troponin findings and mitochondria evaluation

We have added the following sentences to the discussion section:
“We hypothesize that in the whole heart, the addition of SEA0400 to cardioplegia prevents death of a proportion of cardiomyocytes resulting in the lower troponin release observed. Furthermore, limitations of Ca\(^{2+}\) overload protects the mitochondria in surviving cells as evidenced by preserved mitochondrial morphology. These two features may both contribute to improved functional recovery in hearts arrested with cardioplegia containing SEA0400.”