Discussion of 2021-1764

NUCLEAR FACTOR ERYTHROID 2–RELATED FACTOR 2 ACTIVATION AND BURN–INDUCED CARDIAC DYSFUNCTION

**DR JON D SIMMONS** (Mobile, AL): Similar to the research presented today, my lab has concentrated on mitochondrial oxidative DNA damage and cell‑fate responses for the past decade. I would like to emphasize to the audience that the amount of expertise and resources needed to conduct this study were outstanding and enormous. Additionally, getting this study completed during the COVID pandemic is truly remarkable to me.

Overall, I thought the study design was appropriate, and I have no suggestions regarding the technical aspects and the methodology. So, I will keep my comments and questions brief.

I did not see a power analysis in the manuscript. Please comment on how you derived the number of mice in each group. Also, the methods stated there were 6 to 9 in each group. Why did this vary and were any animals excluded from the analysis?

The mice in this study received a 30% TBSA burn to induce mitochondrial and cardiac dysfunction. What TBSA burn in humans is needed to induce similar cardiac dysfunction, and how often do patients with 30% TBSA burns have these same features?

Is the mechanism of cardiomyopathy seen in this model a result from the general SIRS response that we often see in trauma and sepsis, or do you believe this is specific to burn injury?

The drug used in this study has previously been used in a phase 2 clinical trial. What do you believe are the next steps in obtaining equipoise for its use in burn patients as you have suggested today?

Finally, I think it is important to mention that increased oxidative stress within the mitochondria is not always bad. One important role of oxidative stress is to cause conformational change within DNA promotor regions which regulates gene expression by turning it on or off. Conversely, most of the cell death pathways are initiated by oxidative damage within mitochondrial DNA, presumably because of the likelihood of germline nuclear DNA damage.

My biggest fear for using any specific drug that inhibits or abrogates cell death from oxidative damage is that it may cause downstream effects such as early cancer. Stated differently, it is not always a good thing to prevent the cell from killing itself. Therefore, giving a similar medication to any patient that does not have a high certainty of death may have inadvertent consequences, such as early death due to cancer. Can you please comment on this?

**DR JAY COLLINS** (Norfolk, VA): I am presenting this on behalf of Dr Cindy Downard, who could not be here.

Does your study examine the role of Nrf2 in the heart after burn injury? You have used the Nrf2 depletion model with an exogenous Nrf2 activator to examine this. Have you considered a Nrf2 transgenic model to examine the effect of Nrf2 overexpression on the heart after burn injury?

A Nrf2 activator like oltipraz seems to offer a promising therapy for improving cardiac function after burns. However, a small molecule like oltipraz may have a mechanism action beyond Nrf2 activation that may improve cardiac function. How do you plan to examine that?

Lastly, have you been able to verify your findings in human burn patients?

**DR HERB CHEN** (Birmingham, AL): I might have missed it, but you mentioned in your disclosure that you were doing a clinical trial. I would be interested in more details about your clinical trial and how this will be applied to patients.

**DR HAROLD BO LOVVORN, III** (Nashville, TN): Did you happen to do any survival studies? How durable is the effect of olti over time? Also, olti was given at time of injury. Did you vary the time of administration? Finally, is this specific to the heart or is this an explanation for multisystem organ failure?

**DR RAVI S RADHAKRISHNAN** (Galveston, TX): Dr Simmons, to answer your question about a power analysis, our power analysis was based on prior work that we have done. Briefly, we looked at maximal mitochondrial respiratory capacity in cardiomyocytes in some of our preliminary work. We showed a 72% reduction in respiratory capacity. Based on this decrease, our power analysis determined we needed 6 animals per group to find statistical significance with an alpha of .05 and power of 95%.

To address the confusion in the number of animals in each group, we had at least 6 animals in each group. In our prep, we typically plan for 7 to 8 in the burn group because, for many numerous reasons, we tend to lose one of these animals during the preparation. In our study, our burn groups have 6 to 7 animals in each group while the sham group each has 7 animals.

I apologize for the confusion in the methods. Each group is at least 6 animals, usually 6 to 7. When we conducted the histologic and electron microscopy analysis, we selected 9 images from each animal to ensure we had a broad enough area to assess inflammation and fibrogenesis. I will make sure I update this in our methods before submitting the paper.

The mice in the study regarding the 30% TBSA burn, a few studies published in the literature have looked at cardiac function after burn injury in both the acute and chronic settings. All of these studies had an inclusion criteria of patients with at least a 30% TBSA burn. In these studies, the TBSA average was about 50% to 60%. These are the patients where I quoted the 70% cardiac dysfunction numbers. While the number of patients with the burn injury close to 30% were small in these studies, we did see significant decreases in cardiac function in them.

Regarding mechanism of cardiac myopathy and is this a general SIRS response similar to trauma or sepsis, a multicenter study in 2011 showed that there is a nearly 98% similarity in upregulated genes after severe trauma and burn injury. Interestingly, they saw that while this response was elevated in trauma, it appeared to start decreasing at 1 month post‑injury. They did not, as far as I could tell, look at it beyond this. Our longest-term studies in burn from our centers showed that this response is elevated as far as 1 to 2 years post burn injury. We believe this is a similar inflammatory storm that you see after severe trauma or sepsis, but it is much more long lasting in burn injury.

Regarding the phase 2 clinical trial and the equipoise for its use in severe burn patients, there are animal studies that have shown the benefits of oltipraz in wound healing after burn injury. There is a phase 2 study looking at its effect in insulin resistance and hyperglycemia in the setting of nonalcoholic fatty liver disease. This is now transitioning to phase 3 trials.

Interestingly, burn patients have a similar problem like this with hepatic steatosis and hepatomegaly. To my knowledge, there are no trials of oltipraz in burn patients, so its effects are still unknown. This raises the possibility of us conducting a clinical trial to evaluate its efficacy in burn patients, if we continue to see benefits in our preclinical work.

I will address Dr Chen's comment. I was just quoting a different study. That is not my clinical trial. We have not started any trials yet in humans using this medication.

Finally, Dr Simmons, your last comment about oxidative stress and modulating any of these pathways and some untoward effects of doing that, your comment is very well taken. An even more immediate effect of inhibition of oxidative stress within the mitochondria may be susceptibility to infection, which is the leading cause of death in burn patients. There are numerous studies looking at the role of reactive oxygen species generated by mitochondria and how they play a response to LPS and intracellular bacteria. It is possible that by preventing one severe consequence of burn injury we make the patient more susceptible to another.

It is important to remember all of these pathways exist for a reason, and we just do not fully understand them yet, that blockade or activation of a pathway is not simply good or bad or should be the goal of our study. These exist in a balance, and we must carefully weigh the consequences of our manipulation of these pathways to ensure we are not trading one bad problem for another.

Dr Collins' comments regarding Nrf2, we have used a Nrf2 depletion model, and have we considered a Nrf2 transgenic model? We have started working on the Nrf2 overexpression transgenic model. We are doing this in a cardiomyocyte cell line. Hopefully, in the next few months, we will start doing this in an in vivo model.

The Nrf2 activator like oltipraz, does it have other effects beyond Nrf2 activation? Oltipraz activates Nrf2 by stimulating translocation of Nrf2 and DNA binding. In addition, it has some effect on Keap1, which is the endogenous mechanism to keep Nrf2 from continuing to work. In addition to using a Nrf2 overexpression model to try to see if this is truly acting by Nrf2 both in vitro and in vivo, we are planning to use a different Nrf2 activator, like sulforaphane, which has a slightly different mechanism of action.

Finally, for the last question from Dr Collins, have you been able to verify your findings in burn injury? We have been able to verify cardiac dysfunction in burn injury. This is from historic studies that we have done. We have some preliminary ECHO data that replicates what was shown many years ago both in the acute and chronic setting.

We have not yet, though, verified the circulating redox state in burn patients after injury. We plan to assay their patient plasma for levels of Nrf2 and its associated antioxidants as well as markers of cardiac hypertrophy, fibrogenesis, and cardiomyopathy to correlate it with our in vivo work.

Finally, Dr Lovvorn's comments. In this study, this was a relatively acute burn injury model. We have animals that we have studied out to one month. We don't have anything longer than that looking at this, but this would be something that we would plan as we continue to work with this.

Varying the time of oltipraz administration, in this study, we used it just right around the burn injury. We have not yet used it as what would be a more clinically relevant one, maybe a day or two after burn injury. So that is a good comment, and we will plan to do that in our further studies.