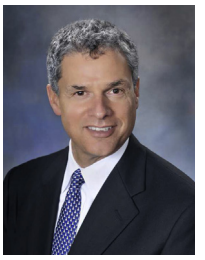
3. Finkel T, Menazza S, Holmström KM, Parks RJ, Liu J, Sun J, et al. The ins and outs of mitochondrial calcium. *Circ Res*. 2015;116:1810-9.
4. Kornfeld OS, Hwang S, Disatnik MH, Chen CH, Qvit N, Mochly-Rosen D. Mitochondrial reactive oxygen species at the heart of the matter: new therapeutic approaches for cardiovascular diseases. *Circ Res*. 2015;116:1783-99.
5. Zamzami N, Larochette N, Kroemer G. Mitochondrial permeability transition in apoptosis and necrosis. *Cell Death Differ*. 2005;12(suppl 2):1478-80.
6. McCully JD, Cowan DB, Pacak CA, Toumpoulis IK, Dayalan H, Levitsky S. Injection of isolated mitochondria during early reperfusion for cardioprotection. *Am J Physiol Heart Circ Physiol*. 2009;296:H94-105.
7. Masuzawa A, Black KM, Pacak CA, Ericsson M, Barnett RJ, Drumm C, et al. Transplantation of autologously derived mitochondria protects the heart from ischemia-reperfusion injury. *Am J Physiol Heart Circ Physiol*. 2013;304:H966-82.
8. Preble JM, Pacak CA, Kondo H, MacKay AA, Cowan DB, McCully JD. Rapid isolation and purification of mitochondria for transplantation by tissue dissociation and differential filtration. *J Vis Exp*. 2014;91:e51682.
9. Pacak CA, Preble JM, Kondo H, Seibel P, Levitsky S, Del Nido PJ, et al. Actin-dependent mitochondrial internalization in cardiomyocytes: evidence for rescue of mitochondrial function. *Biol Open*. 2015;4:622-6.
10. Cowan DB, Yao R, Akurathi V, Snay ER, Thedsanamoorthy JK, Zurakowski D, et al. Intracoronary delivery of mitochondria to the ischemic heart for cardioprotection. *PLoS One*. 2016;11:e0160889.
11. McCully JD, Levitsky S, Del Nido PJ, Cowan DB. Mitochondrial transplantation for therapeutic use. *Clin Transl Med*. 2016;5:16.
12. Cowan DB, Yao R, Thedsanamoorthy JK, Zurakowski D, Del Nido PJ, McCully JD. Transit and integration of extracellular mitochondria in human heart cells. *Sci Rep*. 2017;7:17450.
13. Emani SM, Piekarski BL, Harrild D, Del Nido PJ, McCully JD. Autologous mitochondrial transplantation for dysfunction after ischemia-reperfusion injury. *J Thorac Cardiovasc Surg*. 2017;154:286-9.
14. Kaza AK, Wamala I, Friehs I, Kuebler JD, Rathod RH, Berra I, et al. Myocardial rescue with autologous mitochondrial transplantation in a porcine model of ischemia/reperfusion. *J Thorac Cardiovasc Surg*. 2017;153:934-43.
15. McCully JD, Cowan DB, Emani SM, Del Nido PJ. Mitochondrial transplantation: from animal models to clinical use in humans. *Mitochondrion*. 2017;34:127-34.
16. Shin B, Cowan DB, Emani SM, Del Nido PJ, McCully JD. Mitochondrial transplantation in myocardial ischemia and reperfusion injury. *Adv Exp Med Biol*. 2017;982:595-619.
17. Emani SM, McCully JD. Mitochondrial transplantation: applications for pediatric patients with congenital heart disease. *Transl Pediatr*. 2018;7:169-75.
18. Moskowitzova K, Shin B, Liu K, Ramirez-Barbieri G, Guariento A, Blitzer D, et al. Mitochondrial transplantation prolongs cold ischemia time in murine heart transplantation. *J Heart Lung Transplant*. 2019;38:92-9.
19. Ramirez-Barbieri G, Moskowitzova K, Shin B, Blitzer D, Orfany A, Guariento A, et al. Alloreactivity and allorecognition of syngeneic and allogeneic mitochondria. *Mitochondrion*. 2019;46:103-15.
20. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging*. 2015;16:233-70.
21. Risum N, Ali S, Olsen NT, Jons C, Khouri MG, Lauridsen TK, et al. Variability of global left ventricular deformation analysis using vendor dependent and independent two-dimensional speckle-tracking software in adults. *J Am Soc Echocardiogr*. 2012;25:1195-203.
22. Suzuki Y, Lyons JK, Yeung AC, Ikeno F. In vivo porcine model of reperfused myocardial infarction: in situ double staining to measure precise infarct area/area at risk. *Catheter Cardiovasc Interv*. 2008;71:100-7.
23. Cung TT, Morel O, Cayla G, Rioufol G, Garcia-Dorado D, Angoulvant D, et al. Cyclosporine before PCI in patients with acute myocardial infarction. *N Engl J Med*. 2015;373:1021-31.
24. Shin B, Saeed MY, Esch J, Moskowitzova K, Ramirez-Barbieri G, Cowan DB, et al. A novel mode of mitochondrial transplantation: intracoronary delivery of autologous mitochondria provides prolonged increase in coronary blood flow. American Heart Association's 2017 scientific sessions and resuscitation science symposium. *Circulation*. 2017;136(suppl 1):abstr19402.

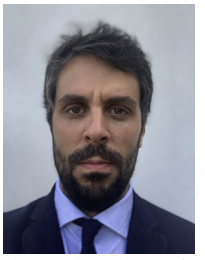
Key Words: ischemia/reperfusion injury heart, myocardial ischemia, myocardial protection

Discussion



Dr Todd K. Rosengart *(Houston, Tex)*. Thank you for that great work, and I appreciate you sharing the paper in advance. This is at least the second time I have had the privilege of reviewing this work by Dr del Nido's lab, and I find it fascinating. In prior publications and discussions, you posited that the mechanism of action in this mitochondrial transplantation is essentially a rescue technique; it is essentially taking healthy, viable mitochondria to replace those that are damaged by the ischemia or the ischemic event. This is different, though. You are pretreating with the mitochondria, so presumably they would be exposed to the same ischemia-reperfusion effects as the naive or the native mitochondria.

So why is this working?



Dr Alvise Guariento *(Boston, Mass)*. Thank you Dr Rosengart. This is really the key question. In terms of the mechanism, we still have no definitive answers, but we think that somehow the mitochondria we are injecting can change the balance in cell homeostasis. What we do know is that mitochondria can enhance the proteomic expression of some important cytokines. These data were obtained from what you just mentioned, postinjection studies. It is therefore possible that they act in the same way after a preischemia injection, somehow activating preconditioning pathways or other pathways that can induce an enhancement in cell function during the ischemic phase. We also noticed that during the ischemic phase, we could obtain better results when we injected them before. This was actually not the first study that we did with this strategy. In fact, we did a similar study where we injected mitochondria before a prolonged period of cold ischemia, and we had similar results. So I totally agree with you that understanding the mechanism should be the next step of our research.

Dr Rosengart. So one alternative possibility—and I apologize, I have not looked through all your articles to the extent to know whether or not you have looked at this—is perhaps the skeletal muscle mitochondria are different in some way than the cardiac. So, have you looked beyond the skeletal? Have you looked at, A, is there a difference and, B, are there other tissue that might be equally relevant?

Dr Guariento. In the early stages of developing this technology, we did a bunch of studies where we added mitochondria obtained from either skeletal muscle or liver, and also cardiac mitochondria. We didn't notice a great

difference in terms of ATP production or oxygen production. So, there may be some reasons for this, but we don't know yet.

Dr Rosengart. I will ask one last quick question before Dr Sellke. So, the other thing that was dramatic about this paper was the very significant decrease in myocardial infarction. You rarely see that much improvement in any intervention. Is there any specific reason why you think that was so?

Dr Guariento. This strategy seems very effective. This is the only answer I can give for now. We usually considered mitochondrial transplantation as a replacement of the native damaged mitochondria. In this study, everything was different in terms of what we speculated in the past.

Dr Frank W. Sellke *(Providence, RI).* Remind me, have you looked at the effects of the mitochondrial injection postischemia, because it is difficult to predict when somebody is going to have a myocardial infarction? Have you injected the mitochondria after the onset of the ischemic event?

Dr Guariento. This is the first study in which we injected them before. All our previous studies were focused on postischemia strategies, both at the immediate start of reperfusion or in a delayed fashion two hours after reperfusion.

Dr Sellke. The other question I had is that with all the stem cell studies, the benefit is not because of developing new myocytes, but more due to a trophic effect. I was wondering if that could have an effect as well, rather than increased energy utilization? Maybe there is some trophic effect from these transplanted mitochondria.

Dr Guariento. I totally agree with you. We know that in recent studies, others showed that stem cell infusion can also be closely related to some sort of mitochondrial mechanism. They didn't call this process mitochondrial transplantation but instead called it mitochondrial transfer, but the concept is quite the same.

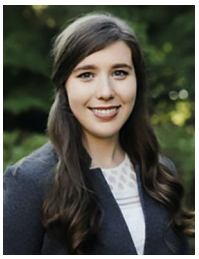
Dr Sellke. A very nice study.

Dr Guariento. Thank you very much, Dr Sellke.

Dr Marek A. Deja *(Katowice, Poland).* Maybe I missed it in the presentation, but what actually happened to these mitochondria? Do they survive in between the cells, do they get into the cells, how long do they survive? It is quite interesting, and I can't really understand, what are they doing there?

Dr Guariento. I didn't have the chance to show this. We investigated this in a previous paper, and the current study was mainly related to this new approach, the preischemia injections. We know that mitochondria can enter the cells within 5 minutes after the injection through an actin-dependent mechanism. We also know that they are rapidly integrated in the cells and they can fuse with the resident mitochondria. Subsequently, they can be found in the cells over a period of 28 days after injection. This is what we know so far.

We have also demonstrated this with F-18 rhodamine labeling of the mitochondria, and you can actually see them for quite a long period. Surely, a limitation of this study is that this is only a 2-hour reperfusion experiment, and we know that we will need to extend it to have more definitive results.



Katherine Driscoll *(Ithaca, NY).* I was thinking about this talk and then also the earlier talk on the increased mitochondrial DNA in pericardial fluid, and I was wondering if you could speculate on whether maybe the body has a mechanism kind of similarly mitochondrially related, and maybe that could be the related to why this therapy is working and also why you will see increased mitochondrial DNA in that area.

Dr Guariento. We know that we have tried to inject just components of the mitochondria, such as mitochondrial DNA, and have not achieved the same results.