Published in final edited form as:
Shock. 2013 June ; 39(6): 543. doi:10.1097/SHK.0b013e318292300d.

MitoTracker Probes and Mitochondrial Membrane Potential

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In the recent article by Lin *et al.* (1), the authors demonstrate protective effects of splenic infusion of isolated mitochondria against hepatic ischemia/reperfusion injury in a rat model. One of their conclusions is that the isolated mitochondria from donor animals maintained an intact membrane potential in the livers of recipient animals even at 4 hours after infusion, as assessed by Mito-Tracker Orange CMTMRos staining.

Cationic fluorophores like rhodamine 123 and tetramethylrhodamine methylester (TMRM) are readily sequestered in the matrix space of polarized mitochondria, and these probes are released once mitochondria experience a loss in membrane potential. MitoTracker dyes are also cationic fluorophores that accumulate electrophoretically into mitochondria in response to the highly negative mitochondrial membrane potential. However, unlike TMRM and rhodamine 123, MitoTracker dyes possess a reactive chloromethyl group that forms a covalent bond with thiols on proteins and peptides, which traps MitoTracker dyes within mitochondria. Thus, mitochondria retain MitoTracker dyes like MitoTracker Orange CMTMRos after loss of their membrane potential (2;3). Hence, retention of MitoTracker staining does not signify that infused mitochondria remain polarized, as was concluded in (1). Indeed, high serum free Ca^{2+} concentration, which is 10,000 times greater than cytosolic free Ca^{2+}, will lead quickly to mitochondrial Ca^{2+} overload, respiratory inhibition and mitochondrial dysfunction from onset of the mitochondrial permeability transition with loss of the mitochondrial membrane potential (4;5).

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