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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).

References

1. Lin HC, Liu SY, Lai HS, Lai IR. Isolated Mitochondria Infusion Mitigates Ischemia- Reperfusion Injury of the Liver in Rats. *Shock*. 2013 Jan 28.
2. Elmore SP, Nishimura Y, Qian T, Herman B, Lemasters JJ. Discrimination of depolarized from polarized mitochondria by confocal fluorescence resonance energy transfer. *Arch Biochem Biophys*. 2004 Feb 15; 422(2):145–152. [PubMed: 14759601]
3. Poot M, Zhang YZ, Kramer JA, Wells KS, Jones LJ, Hanzel DK, Lugade AG, Singer VL, Haugland RP. Analysis of mitochondrial morphology and function with novel fixable fluorescent stains. *J Histochem Cytochem*. 1996 Dec; 44(12):1363–1372. [PubMed: 8985128]
4. Lemasters JJ, Theruvath TP, Zhong Z, Nieminen AL. Mitochondrial calcium and the permeability transition in cell death. *Biochim Biophys Acta*. 2009 Nov; 1787(11):1395–1401. [PubMed: 19576166]

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5. Gunter TE, Pfeiffer DR. Mechanisms by which mitochondria transport calcium. *Am J Physiol.* 1990 May; 258(5 Pt 1):C755–C786. [PubMed: 2185657]