

# Cardioprotective Effects of mPTP Inhibition on Myocardial Ischemia/Reperfusion Injury in Perfused Rat Heart

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**Target audience** Researchers who are interested in myocardial ischemia/reperfusion injury.

**Background/Purpose** Ischemia/reperfusion (IR) injury is a well-known contributor to myocardial cell death. There is a substantial body of evidence suggesting that the opening of a nonspecific conductance channel in the inner mitochondrial membrane, the mitochondrial permeability transition pore (mPTP), is the primary factor leading to cell death. mPTP inhibition during reperfusion has been shown to have the beneficial effects of reduced infarct size. In this study, we measured the cardioprotective effects of mPTP inhibition at early reperfusion on functional and energetic parameters measured through <sup>31</sup>P MRS and magnetization transfer.

**Methods** Male Sprague-Dawley rats were anesthetized. The heart was excised, cannulated, and perfused with the Krebs-Henseleit buffer equilibrated with 95% O<sub>2</sub>/5% CO<sub>2</sub> at 37°C. Left ventricular pressure was continuously recorded via water filled balloon catheter line connected to a pressure transducer. The perfusion column was placed into a vertical bore 9.4T Bruker scanner. The perfusion protocol comprised of 30 min no-flow global ischemia, followed by 45 min reperfusion. Hearts were randomized to receive 0.2 μM cyclosporin A (CsA), an mPTP inhibitor, for the first 15 minutes of reperfusion (CsA-treated group, n=8) or no treatment (control group, n=6). <sup>31</sup>P MR spectra were acquired with a 20 mm volume coil.

Acquisition parameters for MRS were: TR, 6 s; spectral width, 5700 Hz; NAV, 16; flip angle, 90°. Magnetization transfer (MT) measurements were acquired at baseline and 7, 20, and 40 min after reperfusion. The MT acquisition comprised of 6 measurements: 5 measurements with progressive γATP saturation by continuous wave, with saturation time ranging from 0.4 to 6 s; 1 control measurement with the saturation pulse positioned at equal distant from P<sub>i</sub> resonance opposite of γATP. All spectroscopy data was processed using in-house developed Matlab-based code. Spectra were processed with 10 Hz line broadening, Fourier transformed, and phase corrected. The area of each resonance peak was calculated by fitting the peak by Lorentzian curve. The Bloch equations that described the evolution of magnetization of phosphocreatine (PCr) and inorganic phosphate (P<sub>i</sub>) were modified to account for MT effects. Reaction rates of ATP synthase and creatine kinase were determined by least-square fitting of the model to experimentally measured MT data.

**Results** Hearts that failed to recover after reperfusion (1 from each group) were excluded from analysis. CsA treated hearts showed a trend of increased functional recovery after reperfusion (Fig. 1). Average rate-pressure-product (RPP), an index of ventricular function, was 0.48±0.38% of baseline in CsA-treated hearts and 0.27±0.22% in the controls 2 min after reperfusion. 40 minutes after reperfusion, RPP was 0.76±0.18% and 0.56±0.28% in CsA-treated and control hearts, respectively. Post-ischemia PCr recovery (Fig. 2) and decrease of intracellular inorganic phosphate (P<sub>i</sub>) (Fig. 3) was faster in CsA treated hearts. Without CsA treatment, ATP synthase rate (k<sub>f</sub>) showed progressive decrease (Fig. 4), while k<sub>f</sub> of CsA-treated hearts remained constant during the course of reperfusion. At 40 min after reperfusion, CsA-treated hearts showed significantly higher k<sub>f</sub> than untreated hearts (0.11±0.04 vs. 0.05±0.03 s<sup>-1</sup>, p<0.05). The recovery of intracellular pH, calculated from the chemical shift of P<sub>i</sub>, was also faster in CsA-treated hearts (p<0.05).

**Discussion/Conclusion** Compared to the untreated group, CsA treatment during reperfusion showed improved functional recovery, increased phosphocreatine and decreased inorganic phosphate, higher ATP synthase rate, and faster intracellular pH restoration. While statistical power is small due to the small sample size, these results suggest that CsA treatment during reperfusion leads to improved metabolic and functional recovery.

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## References

1. Griffiths EJ, Halestrap AP. Protection by Cyclosporin A of ischemia/reperfusion-induced damage in isolated rat hearts. *J Mol Cell Cardiol* 1993; 25: 1461–69.
2. Halestrap AP. A pore way to die: the role of mitochondria in reperfusion injury and cardioprotection. *Biochem Soc Trans* 2010;38:841–60. PM:20658967.
3. Murphy E, Steenbergen C. Mechanisms underlying acute protection from cardiac ischemia-reperfusion injury. *Physiol Rev* 2008;88:581–609. PM:18391174.
4. Kingsley-Hickman P, Sako E, Foker J, From AHL, and Ugurbil K. <sup>31</sup>P NMR measurements of mitochondrial uncoupling in isolated rat hearts. *J Biol Chem* 1990; 265: 1545–1550.

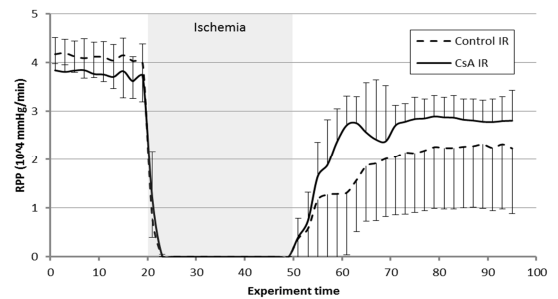


Fig. 1. Time course of RPP, an index of LV function

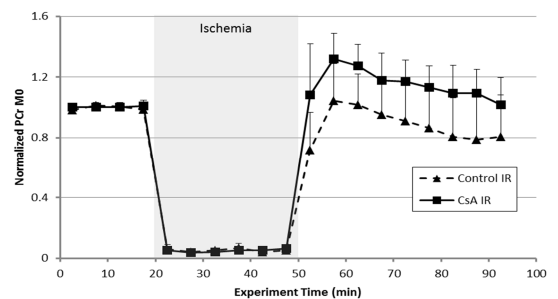


Fig. 2. Time course of PCr signal through IR protocol

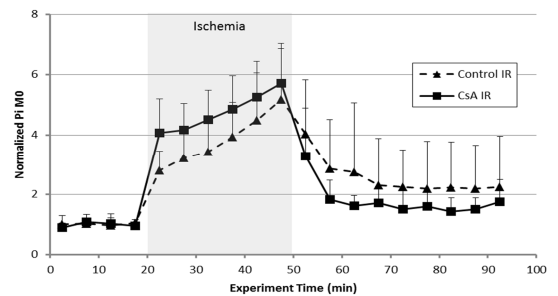


Fig. 3. Time course of Pi signal through IR protocol

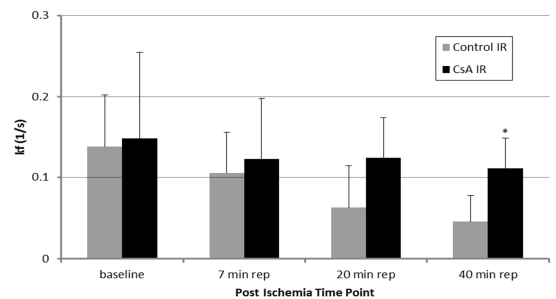


Fig. 4. ATP synthase rate constant before and after ischemia. \*p<0.05