In Vivo Fluorometric Assessment of Cyclosporine on Mitochondrial Function During Myocardial Ischemia and Reperfusion

Muneaki Matsubara, MD, Mahsa Ranji, PhD, Bradley G. Leshnower, MD, Mio Noma, MD, Sarah J. Ratcliffe, PhD, Britton Chance, PhD, Robert C. Gorman, MD, and Joseph H. Gorman III, MD

Gorman Cardiovascular Research Group, and the Departments of Surgery, Biostatistics and Epidemiology, and Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania; and the Biophotonics Laboratory, Department of Electrical Engineering and Computer Science University of Wisconsin-Milwaukee, Milwaukee, Wisconsin

Background. **Cyclosporine A (CsA) limits myocardial reperfusion injury and preserves mitochondrial integrity, but its influence on mitochondrial function has not been described in vivo. Auto-fluorescence of mitochondrial nicotinamide adenine dinucleotide and flavin adenine dinucleotide correlate with mitochondrial dysfunction. We hypothesized that CsA limits mitochondrial dysfunction and that fluorometry can quantify this influence.**

Methods. **Seventeen rabbits were studied: untreated (UnT, n = 7), CsA preinfarction (CsAp, n = 6), and CsA on reperfusion (CsAr, n = 4). Animals underwent 30 minutes of myocardial ischemia and 3 hours reperfusion. Infarct size was determined by staining. Nicotinamide adenine dinucleotide and flavin adenine dinucleotide fluorescence was continually measured in the risk area. The redox ratio was calculated [flavin adenine dinucleotide$_f$/(flavin adenine dinucleotide$_f$ + nicotinamide adenine dinucleotide$_f$)]. Electron microscopy evaluated mitochondria morphology.**

Results. **The infarct size by group was 39.1% ± 1.7% in CsAp, 39.1% ± 1.7% in CsAr, and 53.4% ± 1.9% in UnT ($p < 0.001$). During ischemia, the CsAp group demonstrated less hypoxic reduction, with the redox ratio decreasing to 75.6% ± 4.1% of baseline. The UnT and CsAr groups deceased to 67.1% ± 4.0% and 67.2% ± 3.6%, respectively ($p < 0.005$). During reperfusion the UnT group redox ratio increased to 1.59 ± 0.04 times baseline. This increase was blunted in the CsAp (1.17 ± 0.04, $p = 0.026$) and CsAr (1.35 ± 0.02, $p = 0.056$) groups. Electron microscopy revealed reduced mitochondrial disruption in CsAp (19.7% ± 7.6%) and CsAr (18.1% ± 7.1%) rabbits compared with UnT (53.3% ± 12.5%).**

Conclusions. **Fluorometric spectroscopy can be used in vivo to quantitatively assess the time course of CsA's influence on the mitochondrial dysfunction associated with myocardial ischemia and reperfusion.**

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The myocardial protective influence of cyclosporine A (CsA) has been described extensively in isolated hearts [1–3] and in in vivo animal experiments [4, 5] of ischemia-reperfusion injury. Interest in its clinical use has also recently been piqued by a report demonstrating CsA effectiveness in limiting infarct size in patients presenting with myocardial infarction and undergoing percutaneous coronary revascularization [6].

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Mitochondria are key regulators of necrotic and apoptotic cell death responsible for reperfusion injury [7, 8]. Evidence suggests that CsA protects mitochondrial function during the ischemic and reperfusion phases. Although the exact mechanism is unclear during ischemia, it has been proposed that CsA, given before ischemia, induces metabolic changes similar to hypoxia that result in a type of "pharmacologic" preconditioning [9]. During the reperfusion phase, CsA has been shown to protect against opening of the mitochondrial permeability transition (MPT) pore, which prevents collapse of membrane potential, uncoupling of the respiratory chain, mitochondrial disruption, and the release of cytochrome c, as well as other proapoptotic factors [4, 5].

To date, the affect of CsA on mitochondrial function has only been assessed in mitochondria isolated from postmortem myocardial specimens. To demonstrate the influence of CsA on an in vivo heart, we have developed an optical catheter device capable of continuously measuring the fluorescence signals of the intrinsic mitochondrial fluorophores nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD). The ratio of these fluorescence signals [FAD$_f$/(FAD$_f$ + NAD$_f$)], defined as the redox ratio (RR), correlates with different metabolic states and mitochondrial function [10–13]. In addition, the RR has been shown to undergo an oxidative

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Address correspondence to Dr Joseph H. Gorman III, Gorman Cardiovascular Research Group, Glenolden Research Laboratory, University of Pennsylvania School of Medicine, 500 S Ridgeway Ave, Glenolden, PA 19036; e-mail: gormanj@uphs.upenn.edu.

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Abbreviations and Acronyms

AR = area at risk
CsA = cyclosporine A
CsAp = cyclosporine A preinfarction
CsAr = cyclosporine A reperfusion
FAD = flavin adenine dinucleotide
FAD_f = FAD fluorescence
IS = infarct size
MPT = mitochondria permeability transition
NAD = nicotinamide adenine dinucleotide
NAD_f = NAD fluorescence
RR = redox ratio
UnT = untreated

shift in tumor cells with mitochondrial dysfunction associated with apoptosis [14].

In this study we hypothesized that our continuous fluorescent spectroscopy technique could be used to quantify the time course of CsA on limiting myocardial mitochondrial dysfunction during ischemia-reperfusion in a rabbit model. A method to assess mitochondrial dysfunction at the time of reperfusion therapy would provide a potentially valuable clinical tool given its important role in reperfusion injury. Such a device would allow for an immediate quantitative measure of therapeutic success and could potentially be used to guide acute and long-term management decisions.

Material and Methods

The animals in this study were treated under experimental protocols approved by the University of Pennsylvania's Institutional Animal Care and Use Committee and in compliance with National Institutes of Health Publication No. 85-23, revised 1996.

Surgical Protocol

Seventeen male New Zealand white rabbits (weight, 3.2 to 4.0 kg) were sedated with intramuscular ketamine (50 mg/kg), glycopyrrolate (0.01 mg/kg), and buprenorphine (0.05 mg/kg). They were intubated and then ventilated with a mechanical respirator (Hallowell EMC Model AWS; Pittsfield, Massachusetts) using room air enriched with oxygen at 0.6 L/min. Anesthesia was maintained with an intravenous infusion of ketamine (20 mg/kg/h) and supplemental pentothal (2.5 to 5 mg/kg) as needed.

A left thoracotomy was performed. A coronary snare was placed around a large branch of the circumflex coronary artery at approximately 50% of the distance from base to apex of the heart and threaded through a small piece of polyethylene tubing.

A hyper/hypothermia unit was used to maintain core temperature between 39.0° and 40.0°C. Arterial blood gases were measured and pH was maintained between 7.35 and 7.45 throughout the protocol.

Experimental Protocol

The animals were divided into three groups: the untreated group (UnT, n = 7), the preischemia CsA group (CsAp, n = 6), and the CsA-on-reperfusion group (CsAr, n = 4). The UnT and the CsAp animals received a 1-hour, continuous 20-mL infusion of phosphate buffered saline vehicle (UnT) or 25 mg/kg of CsA. The CsAr animals received a 25-mg/kg intravenous bolus of CsA immediately at the onset of reperfusion.

The coronary snare was tightened to produce an ischemic region of the left ventricle (LV). Ischemia was confirmed by a visible color change in the ischemic myocardial region, ST elevations on the electrocardiogram, and regional wall motion abnormalities on the echocardiogram. At the end of the 30-minute ischemic period, the coronary snares were loosened and the previously ischemic myocardium was reperfused for 3 hours.

Myocardial Fluorescence Spectroscopy

Fluorescence spectroscopy of rabbit myocardium was conducted with a fluorometer (Fig 1). This fluorometer is a mobile optical-electrical apparatus that collects fluorescence signals of any type of tissue through a 3-mm-tip light guide catheter. The incident light is a broadband mercury arc lamp that can be filtered at 2 pairs of excitation/emission wavelengths by an air turbine filter wheel rotating at 50 Hz. Consequently, up to 4 signals can be multiplexed to a photodetector to make 4-wavelength channel optical measurements of tissue metabolism. In this experiment, 2 channels were used for excitation and 2 for emission signals. The light intensity that is incident on tissue at the fiber tip is 3 $\mu W/mm^2$.

In cardiac fluorometry experiments, the excitation wavelengths of FAD and NAD are obtained by filtering

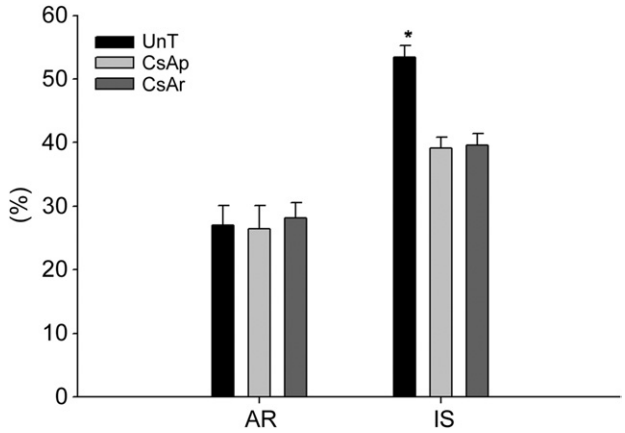

Fig 1. Comparison of area at risk (AR) and infarct sizes (IS) among the untreated (UnT), cyclosporine A (CsA) preinfarction (CsAp), and CsA reperfusion (CsAr) groups. Mean values are shown with the standard error of the mean (error bars). The area at risk is expressed as a percentage of the left ventricle, and the infarct size is expressed as a percentage of the area at risk. The area at risk was not significantly different between groups. Infarct size was significantly greater in the untreated group than in either of the CsA groups ($p < 0.01$). Infarct size was the same in both CsA groups.*