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Cyclosporine A Cardioprotection: Mechanisms and Potential for Clinical Application

Rolf Bünger, M.D., Ph.D.¹ and Robert T. Mallet, Ph.D.²

¹Emeritus Member of the American and German Physiological Societies McLean, Virginia, U.S.A

²Department of Integrative Physiology and Cardiovascular Research Institute Univ. North Texas Health Science Center, Fort Worth, TX

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The report by Gill *et al.* (1) provides insights on the pathogenesis of a clinically important problem, that of cardiac dysfunction following perinatal asphyxia, and on the cardioprotective mechanisms of acute cyclosporine A (CsA) treatment in this setting. CsA is a cyclic, 1.2 kD oligopeptide, naturally synthesized in certain fungi. Marketed under the trade names Sandimmune and Neoral, CsA is soluble in ethanol and chloroform, and has been used extensively as an immunosuppressant to minimize host-graft rejections (2, 3). The LD₅₀ for intravenous CsA is between 10 and 100 mg/kg in mice, rats and rabbits (4).

Basic biomedical research has repeatedly demonstrated CsA's cytoprotective properties in various heart preparations (5) including isolated cardiomyocytes (6), crystalloid-perfused rodent hearts (7), allogeneically transplanted swine hearts (8), and *in situ* swine hearts subjected to ischemia and reperfusion (9, 10). The principal mechanism of CsA's cytoprotection appears to be attenuation or prevention of the opening of the mitochondrial permeability transition pore (MPTP). Opening of these pores permits passage of molecules up to 1.5 kD across the inner mitochondrial membrane; consequently, the electrochemical H⁺ gradient, the driving force for ATP synthesis according to Mitchell's hypothesis, collapses, and water enters the mitochondrial matrix, causing matrix swelling, unfolding of cristae and rupture of the outer mitochondrial membrane (11). MPTP opening is thought to be initiated by mitochondrial reactive oxygen species (ROS) which are generated by hypoxia-reoxygenation and ischemia-reperfusion, especially upon reintroduction of oxygenated blood to the myocardium. These changes are likely associated with a decrease in the cytosolic ATP phosphorylation potential ($[ATP]/\{[ADP][P_i]\}$) and, thus, Gibbs free energy of ATP hydrolysis (ΔG_{ATP}), the only energy source for sarcoplasmic reticular Ca²⁺ sequestration. Thus it appears clear that pathologic opening of the MPTP in ischemia-reperfusion scenarios can compromise the ATP-dependent sarcoplasmic reticular Ca²⁺ uptake culminating in impaired diastolic relaxation and weakened systolic contraction of the myocardium.

Address for Correspondence: Rolf Bünger, M.D., Ph.D., Emeritus Member of the American and German Physiological Societies, 1922 Kenbar Ct., McLean, Va 22101, telephone: 703-241-1254, rolfbunger@gmail.com.

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As for known mitochondrial effects of CsA, it has been reported that CsA stabilizes the inner mitochondrial membrane by preventing cyclophilin D binding to adenine nucleotide translocase, a pivotal event in MPTP formation (12). In addition, CsA could conceivably protect the mitochondria via its inhibition of the calcium-activated phosphatase calcineurin (13, 14). Like ROS, increased cytosolic Ca^{2+} concentration can trigger MPTP opening (15). Phospholamban, a physiological modulator of sarcoplasmic reticular Ca^{2+} ATPase (SERCA), is a calcineurin target (16); phospholamban dephosphorylation by calcineurin would dampen sarcoplasmic reticular Ca^{2+} sequestration, thereby increasing cytosolic Ca^{2+} concentration which may trigger MPTP opening. The resultant failure of mitochondrial ATP synthesis and myocardial energy depletion could generate hypoxanthine, an ATP degradation product that can generate ROS via the enzyme xanthine oxidase. Moreover, mitochondrial Ca^{2+} accumulation activates mitochondrial dehydrogenases to generate NADH; transfer of NADH's electrons to the respiratory chain provides free electrons for univalent reduction of O_2 to the oxyradical $\cdot\text{O}_2^-$ (17). CsA suppression of calcineurin may serve to minimize intracellular Ca^{2+} accumulation and, thus, dampen Ca^{2+} -induced ROS formation.

A 2008 pilot study (18) in 58 patients undergoing percutaneous coronary intervention for acute myocardial infarction was the first clinical trial of CsA directed toward cardioprotection. Here, intravenous injection of 2.5 mg CsA/kg less than 10 min before coronary intervention decreased myocardial injury as judged from a 40% decrease in serum creatine kinase activity over 72 h post-intervention, and from a 20% decrease in the mass of hyperenhanced tissue detected by MRI. However, CsA did not decrease cardiac troponin I release, and other independent measures of myocardial infarct, *e.g.* PET imaging of metabolism, were not reported. In the current study, asphyxiated piglets received a fourfold higher dose of *iv* CsA, which prevented myocardial cytochrome *c* accumulation and aconitase inhibition, and attenuated plasma troponin I increases during reoxygenation; left ventricular hydroperoxide levels were also normal in the CsA group. These authors (19) also reported that CsA treatment in the asphyxiated piglet model afforded near-complete recovery of cardiac hemodynamics, whilst in controls cardiac output fell to 60% of the pre-asphyxiation value. These results confirm a systemic cardiovascular benefit resulting from CsA treatment in asphyxiated piglets. Collectively, the current results strongly suggest, but do not unequivocally prove, that in the current asphyxia model, CsA was cardioprotective by a mechanism consistent with inhibition of MPTP opening. To substantiate the mitochondrial protection more direct evidence of attenuation of MPTP pore opening is required. Parameters of normal mitochondrial competence include a high cytosolic $[\text{ATP}]/[\text{ADP}][\text{P}_i]$ and ΔG_{ATP} , low rates of ATP catabolism (monitored, *e.g.*, by coronary venous adenosine release) and the absence of intracellular acidosis (20, 21). It is the paucity of this kind of specific metabolic evidence that makes acceptance of the proposed blockade by CsA of the myocardial mitochondrial pore opening in the current piglet model difficult.

The authors raise the possibility of clinical implementation of CsA as a cardioprotective intervention based on piglet data. While *iv* CsA administration in the newborn asphyxiated piglet clearly has a systemic hemodynamic benefit, it remains unclear if this effect is permanent and whether other asphyxia-induced morbidities such as kidney or brain ischemia-reperfusion injury, impaired cognitive function or lack of growth and development can be attenuated by CsA. CsA is a complex drug with potentially adverse side effects, especially at high dosages and/or prolonged treatment (13, 22, 23). Great caution seems warranted in initiating clinical implementation of CsA as a cardioprotectant, despite the apparently favorable results of preclinical studies. In addition, it is also not known whether the interesting findings in the piglet can be directly extrapolated to an asphyxiated human newborn infant who was resuscitated with 100% oxygen immediately after birth.

Nevertheless the focus of neonatal asphyxia research on mitochondrial function and preservation is important for all organs with high metabolic demands, particularly brain, heart and kidney, the organs most adversely affected by perinatal asphyxiation (24, 25). As translational research in neonatal asphyxia-reoxygenation progresses, CsA should prove to be a valuable investigative tool to probe subcellular mitochondrial injury. On the other hand, its application as an established clinical regimen, approved for use by the practicing neonatologist, is not yet certain.

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