Assessment of cardiac function and infarct size following myocardial infarction in mitochondrial cyclophilin-D knockout mice

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Introduction:

Coronary heart disease (CHD) remains one of the leading causes of death in both men and women throughout the world[1]. A principal symptom of CHD is myocardial infarction (MI), which leads to a complex process of ventricular remodelling and ultimately heart failure. Novel treatment strategies which are capable of limiting myocardial infarct size, preventing LV remodelling and preserving cardiac function are needed to improve clinical outcomes. The mitochondrial permeability transition pore (mPTP), a critical mediator of cell death, has emerged as an important therapeutic target in ischemic-reperfusion injury. The inhibition of mitochondrial cyclophilin-D (*CypD*), a key regulator component of the mPTP, has been reported to reduce infarct size in pre-clinical studies [2-4]. Here we present the first MRI assessment of cardiac function and infarct size in *CypD*$^{-/-}$ mice at 48 hours following myocardial infarction.

Methods:

Animal preparation: All experiments complied with the UK Animals (Scientific Procedures) Act, 1986 and local ethical guidelines. Male wild type (B6Sv129) and mice lacking cyclophilin-D (*CypD*$^{-/-}$) were anaesthetised (i.p.) with ketamine (75mg/kg) and medetomidine hydrochloride (1mg/kg). Ligation of the left anterior descending coronary artery was performed during mechanical ventilation using a rodent Minivent (type 845, Harvard Apparatus) and supplementary oxygen. Mice were then recovered for 48 hours. Imaging was performed on a 9.4T Varian (VNMRS) system equipped with 60mm 1000mT/m G_{max} gradients and 33mm transmit/receive cardiac-optimized volume coil (Rapid Biomedical GmbH). For delayed contrast enhancement 0.6 mmol/kg Gd-DTPA (Magnevist, Schering AG, Germany) was injected (i.p.) following initial functional cine scans.
Cardiac MRI: Global cardiac function was assessed using a double gated spoiled gradient echo sequence (TE/TR=1.2/4-5ms, 20 cine frames, 200µm in-plane resolution, slice thickness=1mm, 10 short-axis slices, NA=1). Infarct volume was assessed with late gadolinium enhancement (LGE) using an inversion recovery gated GRE sequence, with inversion time optimized on an individual basis using an additional Look-Locker (LL) GRE sequence, as described in [5] (200µm in-plane resolution, slice=1mm TE=1.2ms, TR=~1s, FA=90°/10(LL), NSA=1, TA~3 minutes).
Analysis: Global cardiac function was assessed from the full stack of short-axis cine images using freely available software Segment (http://segment.heiberg.se/). Infarct volume was calculated from the late gadolinium images using Segment and expressed as percentage of left ventricular volume. Infarct and LV volumes were analyzed by two observers blinded to treatment groups. Data are presented as mean ± SEM, and where appropriate mean values analysed using an unpaired t-test, with a $P<0.05$ considered to be statistically significant.
Histology: The effect of *CypD* ablation on myocardial apoptosis was evaluated by determining caspase-3 protein cleavage within the infarcted myocardium by Western blotting.

Results and discussion:

LGE images revealed that all mice subjected to MI had discrete transmural infarcts in the free LV wall and around the apex (Figure 1). Significant differences in mean infarct size were observed between the WT and *CypD*$^{-/-}$ groups, albeit with a larger variation in infarct size in the knockout mice (Table 1 & Figure 2). In addition, there were significant differences in both EDV and ESV between MI groups, but at this stage no significant differences are observed in either SV or EF. WT mice also showed a significant increase in caspase-3 protein cleavage following MI compared to sham and over *CypD*$^{-/-}$ MI mice (Figure 3) indicating increased cardiomyocyte apoptosis.

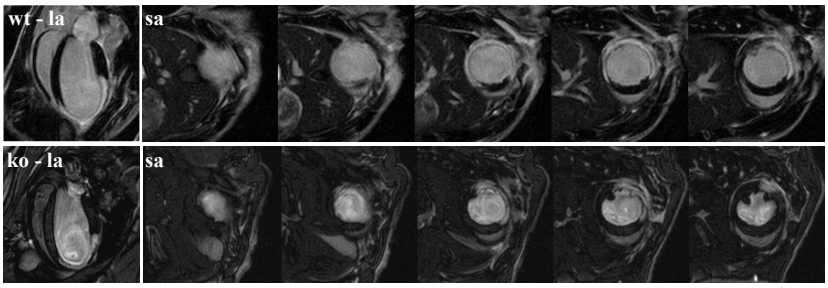

Figure 1. Example late gadolinium enhancement images from a wild type (top) and *CypD*$^{-/-}$ mouse (bottom) 48 hours following MI. 5 contiguous short axis (sa) slices from apex are shown alongside the 4-chamber (la) view revealing the extent of infarction.

	MI		Sham[#]	
	WT (n=5)	KO (n=6)	WT (n=4)	KO (n=3)
EDV (ul)	107.1 ± 4.8	86.9 ± 6.3*	64.5 ± 8.0	48.3 ± 6.1
ESV(ul)	76.9 ± 6.6	57.8 ± 6.3*	22.7 ± 7.2	19.8 ± 7.7
SV (ul)	30.0 ± 2.1	29.2 ± 2.0	38.7 ± 2.9	28.8 ± 3.7
EF (%)	28.7 ± 3.3	34.4 ± 3.6	60.7 ± 3.2	60.0 ± 5.2
Infarct (%)	40.4 ± 1.5	27.6 ± 4.7*	-	-

Table 1. Global cardiac function parameters, mean ± SEM, *P<0.05 vs WT MI, ([#]no significant differences shown by Mann-Whitney U test between shams).

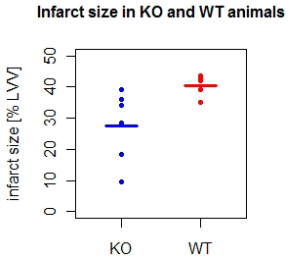

Figure 2. Infarct size by LGE.

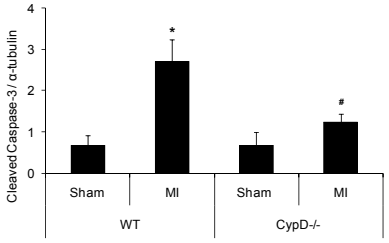

Figure 3. Cleaved caspase-3 concentrations in the infarct (*P<0.05 vs WT sham, [#]P<0.05 vs WT MI).

Conclusion:

Following non-reperfused myocardial infarction, *CypD*$^{-/-}$ mice have smaller infarct sizes and show signs of better preserved cardiac function with less LV remodelling and reduced cardiomyocyte apoptosis compared to WT mice at 48 hours. Therefore, mitochondrial *CypD* may prove to be a novel therapeutic target for the treatment of heart failure following myocardial infarction.

References: [1] WHO, World Health Report 2004. [2] Baines CP et al, Nature 2005;434:658-662. [3] Nakagawa T et al, Nature. 2005;434:652-658 [4] Hausenloy DJ et al, Cardiovasc Res. 2002;55:534-543. [5] Price AN et al, Proc. ISMRM 2009 #3737.