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Tissue kallikrein is required for the cardioprotective effect of Cyclosporin A in myocardial ischemia in the mouse

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ABSTRACT

Clinical and experimental studies suggest that pharmacological postconditioning with Cyclosporin A (CsA) reduces infarct size in cardiac ischemia and reperfusion. CsA interacts with Cyclophilin D (CypD) preventing opening of the mitochondrial permeability transition pore (mPTP). Tissue kallikrein (TK) and its products kinins are involved in cardioprotection in ischemia. CypD knockout mice are resistant to the cardioprotective effects of both CsA and kinins suggesting common mechanisms of action. Using TK gene knockout mice, we investigated whether the kallikrein-kinin system is involved in the cardioprotective effect of CsA.

Homozygote and heterozygote TK deficient mice ($TK^{-/-}$, $TK^{+/-}$) and wild type littermates ($TK^{+/+}$) were subjected to cardiac ischemia–reperfusion with and without CsA postconditioning. CsA reduced infarct size in $TK^{+/+}$ mice but had no effect in $TK^{+/-}$ and $TK^{-/-}$ mice. Cardiac mitochondria isolated from $TK^{-/-}$ mice had indistinguishable basal oxidative phosphorylation and calcium retention capacity compared to $TK^{+/+}$ mice but were resistant to CsA inhibition of mPTP opening. TK activity was documented in mouse heart and rat cardiomyoblasts mitochondria. By proximity ligation assay TK was found in close proximity to the mitochondrial membrane proteins VDAC and Tom_{22}, and CypD.

Thus, partial or total deficiency in TK induces resistance to the infarct size reducing effect of CsA in cardiac ischemia in mice, suggesting that TK level is a critical factor for cardioprotection by CsA. TK is required for the mitochondrial action of CsA and may interact with CypD. Genetic variability in TK activity has been documented in man and may influence the cardioprotective effect of CsA.

1. Introduction

Cardiac ischemia secondary to coronary occlusion leads to myocardial necrosis, arrhythmias and heart failure and is a leading cause of death worldwide. Restoring blood flow to the ischemic myocardium can prevent ischemia-related death. Experimentally however, both myocardial ischemia and reperfusion result in myocardial damage [1,2]. In the past decades progress has been made in the understanding and prevention of ischemia–reperfusion injury by description of the "ischemic" and "pharmacological postconditioning" phenomena (IPC, PPC respectively). PPC with several classes of agents has been demonstrated experimentally and in some cases clinically [3–10] to reduce infarct size and consequently to improve cardiac functional recovery after ischemic insult.

There is large evidence that opening of the mitochondrial permeability transition pore (mPTP) plays a key role in lethal reperfusion injury and inhibition of this phenomenon during IPC

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and PPC is cardioprotective [11,12]. Indeed, administration of Cyclosporin A (CsA), that binds to the mitochondrial chaperone cyclophilin D (CypD) and inhibits Ca^{2+}-dependent mPTP opening at the time of reperfusion, reduces infarct size to a similar extent as IPC in experimental studies [4,11].

The Kallikrein Kinin System (KKS) is involved in cardioprotection in the setting of cardiac ischemia–reperfusion. Several studies have shown that bradykinin (BK) and the serine protease responsible for its production, tissue kallikrein (TK) exert vascular and myocardial effects limiting necrosis [3,5,7,13–19]. The cardioprotective effect of TK was documented by studying genetically modified mice with TK deficiency [5,7,15,17] or transgenic rats with TK gene overexpression [16,18,19]. Together, these studies document the critical role of TK level in myocardial tolerance to ischemia–reperfusion. Lim et al. reported that while BK and CsA reduced infarct size when administered at reperfusion in wild-type mice, they both failed to do so in CypD knockout mice [12], suggesting a possible link between CsA and kinin's actions. Therefore, using TK deficient mice, we addressed the question of whether the KKS is involved in the cardioprotective effect of CsA in cardiac ischemia–reperfusion injury. Our data suggest a critical role of TK in CsA's effect.

2. Materials and methods

2.1. Genetically modified, TK deficient mouse model

TK-deficient mice have been engineered in our laboratory and bred in the C57BL/6 strain for more than 15 generations as previously described [15,20]. The 22-month-old male TK deficient ($TK^{-/-}$, $TK^{+/-}$) and wild-type ($TK^{+/+}$) littermate mice used in the experiments were obtained by heterozygous crossing. The mice had unrestricted access to standard chow (A03, UAR, Epinay-sur-Orge, France) and drinking water, and were housed in a temperature- and light-controlled environment. All animal experiments were performed in accordance with the European Community Council directive (86-609/87-848EEC) and were approved by the University animal care and use committee.

2.2. Cardiovascular phenotype of $TK^{+/+}$ and $TK^{-/-}$ mice

2.2.1. Blood pressure and heart rate

Systolic blood pressure (SBP) and heart rate (HR) were measured in conscious animals using a tail cuff blood pressure analyzer (BP-2000, Visitech system, Bioseb, Vitrolles, France). After a week of adaptation SBP was measured on 3 consecutive days and averaged [15].

2.2.2. Cardiac morphology and function

Echocardiographic analysis was performed in isoflurane-anaesthetized animals using a Sequoia ultrasound device (Acuson, Moutin view, CA, USA) equipped with a specifically designed 13–15 MHz short-focus linear array probe. Each parameter was evaluated by calculating average of three cardiac cycles: end-diastolic and end-systolic left ventricular (LV) diameter (LVIDd, LVIDs), septum thickness (IVSd, IVSs), posterior wall thickness (LVPWd, LVPWs) and fractional shortening [FS = (LVIDd − LVIDs)/LVIDd × 100] were measured as described previously [20].

2.2.3. Histological analysis of the myocardium

Dissected hearts of dedicated groups of mice sacrificed by cervical dislocation were fixed in paraformaldehyde, embedded in paraffin and cut into 4–5-μm sections. The sections were mounted on slides and stained with Sirius Red F3BA (0.1% solution in saturated aqueous picric acid) for evaluation of fibrotic area (collagen fibers stained in red) using NIS-element software (Nikon, Champigny, France). Percentage of fibrosis was calculated as ratio between total red surface over total section surface.

2.3. Ischemia–reperfusion injury model

The mice were anesthetized with sodium pentobarbital (Sanofi, Chilly-Mazarin, France, 60 mg/kg, ip). The procedure was performed essentially as previously described [5]. Briefly, the left anterior descending coronary artery was reversibly occluded. Successful coronary occlusion was attested by the development of a pale color in the distal myocardium and by S-T segment elevation and QRS widening on the ECG (ECG Biotach, Gould Instruments, Cleveland, OH, USA). After 30 min of sustained ischemia (I), removing the ligature restored blood flow. Reperfusion (R) was confirmed by reappearance of a bright red color of the epicardium and normalization of ECG. Reperfusion was maintained for 2 h. Measurements of area at risk (AAR) and infarct size (IS) were performed by Evans blue injection and 1% TTC (2,3,5-triphenyltetrazolium chloride, Sigma–Aldrich, Saint Quentin Fallavier, France) staining respectively, as previously described [5]. IS/AAR ratios were calculated and IS was expressed as a percentage of AAR. In a separate set of experiments aimed at performing molecular studies, the reperfusion phase was carried out for 7 min.

2.4. Pharmacological postconditioning with CsA

2.4.1. Measurement of CsA concentration in blood

Cyclosporin A (#30024, Fluka Analytical, Sigma–Aldrich, Saint Quentin Fallavier, France) was dissolved in polyethoxylated castor oil (Cremophor EL, 650 mg/ml, Fluka Analytical), and ethanol 95%. The solution was further diluted with saline for in vivo use at dosage of 10 mg/kg. The same solution lacking CsA was used for vehicle treatment. CsA or vehicle were administered through a catheter implanted in the jugular vein.

In preliminary experiments using anesthetized C57BL/6 male mice we monitored blood level of CsA at different time points up to 2 h after an intravenous bolus administration of 10 mg/kg (n = 4–10 mice per time point). Blood was collected in heparinized tubes. CsA concentration (μg/ml of blood) was measured immunologically using Flex reagent cartridge (Siemens healthcare diagnostics, Newark, DE, USA) according to the manufacturer's protocol.

2.4.2. Ischemia–reperfusion and CsA postconditioning

Animals of the three genotypes ($TK^{+/+}$, $TK^{+/-}$, $TK^{-/-}$) were subjected to myocardial IR injury (30-min coronary occlusion followed by 2 h reperfusion). They received either vehicle or CsA, 10 mg/kg (n = 5–7 per group) given as an intravenous bolus 5 min before reperfusion.

2.5. Study of TK in heart and cardiomyoblasts

2.5.1. Measurement of TK activity

Tissue kallikrein activity was measured in hearts of $TK^{+/+}$ and $TK^{-/-}$ mice. After cervical dislocation, hearts were rapidly dissected, rinsed and minced into buffer A containing 100 mM KCl, 50 mM 3-(N-morpholino) propanesulfonic acid (MOPS), 1 mM EGTA, 5 mM $MgSO_4$, and 1 mM ATP, pH 7.4 at 4 °C. Ventricular tissue was isolated, placed in buffer A supplemented with 0.2% bovine serum albumin (BSA) and homogenized with a polytron tissue processor (Kinematica GmbH, Luzern, Switzerland). The homogenate was centrifuged at 500 × g and cardiac cytoplasmic and mitochondrial fractions were isolated using differential centrifugation, as previously described [21]. Protein content was measured according to Biorad procedure using bovine serum albumin as a standard. Mitochondria were kept on ice and used within 4 h. Tissue kallikrein activity was measured in cytoplasm