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Regular treadmill exercise inhibits mitochondrial accumulation of cholesterol and oxysterols during myocardial ischemia-reperfusion in wild-type and ob/ob mice

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ABSTRACT

Mitochondria play a central role in the irreversible damages induced to the heart by a prolonged period of ischemia followed by reperfusion. We previously demonstrated that (1) myocardial ischemia-reperfusion induces mitochondrial accumulation of cholesterol and oxysterols that are deleterious for the organelle; (2) inhibition of cholesterol and oxysterol accumulation prevents mitochondrial injury at reperfusion; (3) exercise is cardioprotective and remains efficient in the presence of co-morbidities such as obesity. The aim of this study was to investigate whether regular exercise limits mitochondrial cholesterol and oxysterol accumulation in wild-type and obese mice.

Wild-type C57BL/6J and obese (ob/ob) mice were assigned to sedentary conditions or regular treadmill exercise and submitted to 30 min of coronary artery occlusion followed by 15 min of reperfusion. Regular exercise improved oxidative phosphorylation, restored the antioxidant capacity of the heart by increasing the expression of SOD1 and catalase and reduced the mitochondrial generation of oxysterols in wild-type as well as in ob/ob mice. In wild-type animals, exercise limited the production of oxysterols. In ob/ob mice, despite hypercholesterolemia, chronic exercise abolished the mitochondrial accumulation of cholesterol and concomitantly reduced the generation of 7α-hydroxycholesterol, 7-ketocholesterol and cholesterol-5α,6α-epoxide.

In conclusion, regular exercise prevents the mitochondrial accumulation of cholesterol and oxysterols which occurs during early reperfusion of an ischemic myocardium in mice. This effect is observed in normo and hypercholesterolemic animals. It may be partly responsible for the antioxidant properties of regular exercise and contribute to its cardioprotective effect in obese conditions.

1. Introduction

The mitochondrial dysfunction resulting from an increase in the permeability of mitochondrial membranes is now considered as a major cause of cell death during myocardial reperfusion after a prolonged period of ischemia [1]. One of the main actors of this process is a non specific pore contained within the inner membrane of mitochondria, known as the mitochondrial permeability transition pore (mPTP) [2]. Ischemia causes adenine nucleotide depletion as well as calcium and phosphate accumulations which facilitate mPTP opening triggered by the burst of reactive oxygen species (ROS) and mitochondrial calcium overload at reperfusion [3]. Beside their harmful role during reperfusion, the production of small amounts of ROS has been reported to be necessary to trigger both preconditioning- and post-conditioning-related cardioprotection [4].

Previously, we demonstrated that the early reperfusion of the myocardium is associated with an accumulation of cholesterol into mitochondria leading to the production of oxysterols [5]. Cholesterol is highly sensitive to ROS attack and autoxidation resulting into the formation of oxysterols which have toxic effect on cells and mitochondria [6]. Oxysterols can promote deleterious mitochondrial effects such as lipid peroxidation of membranes and could contribute to the

Abbreviations: Ex, exercised; I/R, ischemia-reperfusion; mPTP, mitochondrial permeability transition pore; ob/ob mice, leptin deficient mice; ROS, reactive oxygen species; Sed, sedentary; WT, wild-type

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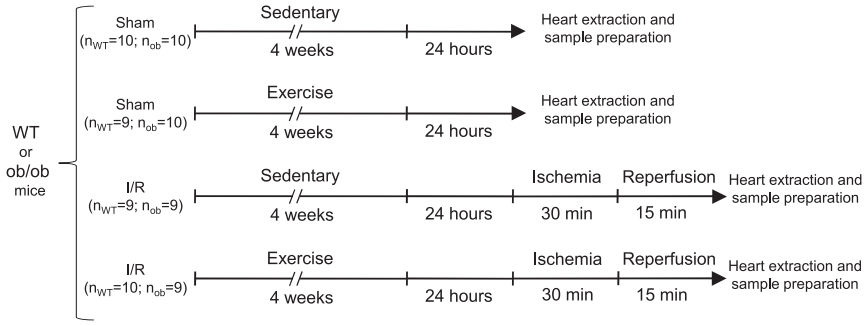

Fig. 1. Design of the experiments performed with wild-type (WT) and ob/ob mice.

increase in mitochondrial membrane permeability [5]. Conversely, the reduction of mitochondrial cholesterol accumulation and inhibition of oxidative stress by ligands of the mitochondrial translocator protein prevent mitochondrial injury at reperfusion [5,7].

The inhibition of mitochondrial cholesterol accumulation may therefore represent an interesting new strategy to protect mitochondria during myocardial ischemia-reperfusion (I/R) and this may be particularly attractive when myocardial I/R is associated with co-morbidities such as hypercholesterolemia, or obesity which render most of the cardioprotective strategies ineffective [8–10]. We recently demonstrated that regular exercise is a cardioprotective strategy which remains efficient in the presence of factors of co-morbidities. Regular treadmill exercise limited oxidative stress, restored cardioprotective signaling pathways despite obesity and increased resistance of mPTP opening independently of the improvement in associated co-morbidities [11,12]. The production of small amounts of ROS is critical to trigger the cardioprotective effect of exercise [12].

The aim of the present study was to investigate whether exercise-induced cardioprotection involves a limitation of mitochondrial cholesterol and oxysterol accumulation in wild-type and obese animals.

2. Methods

All animal procedures used in this study were in strict accordance with the Directives of the European Parliament (2010/63/EU-848 EEC) and were approved by the Animal Ethics Committee Afssa/ENVA/Universite Paris Est-Créteil (approval number: 11/12/12-7).

2.1. Regular treadmill exercise

Male 5–10 week-old wild-type C57BL/6J (WT) and obese (ob/ob) mice (R. Janvier, Le Genest St Isle, France) were housed in an air-conditioned room with a 12 h light–dark cycle and received standard rodent chow and drinking water ad libitum.

Both WT and ob/ob mice were randomly subjected to regular treadmill exercise (Ex-WT and Ex-ob/ob, respectively) or to sedentary conditions (Sed-WT and Sed-ob/ob, respectively). As previously described [12], exercised animals run 5/7 days for 4 weeks without any exhaustion. The first week was an adaptation period with gradual increase in the speeds of the treadmill (4° slope) for 30 min (10, 14, 18, 22, 26, and 30 cm/s, 5 min each step for WT and 8, 8, 10, 14, 18, and 20 cm/s, 5 min each step for ob/ob). For the three other weeks, mice ran for 1 h per day (30 and 20 cm/s for WT and ob/ob, respectively, 4° slope). The speed of the treadmill was slower for ob/ob than for WT mice. This allows taking into account the differences in body weight in order to obtain similar vertical work of the animals. The distances run by exercise mice submitted or not to I/R were similar (sham and I/R WT mice, 18,818 ± 3.48 and 18,766 ± 28.5 m, respectively; sham and I/R ob/ob mice, 12,477 ± 0.33 and 11,717 ± 1.60 m, respectively).

2.2. Coronary artery occlusion-reperfusion

After 4 weeks of exercise or sedentary conditions, mice were subjected to 30 min of ischemia followed by reperfusion. Mice were anesthetized with sodium pentobarbital (80 mg/kg i.p.), intubated and mechanically ventilated. The depth of anesthesia was monitored using the tail pinching response and the pedal reflex. A left thoracotomy was performed at the fourth intercostal space. A surgical needle with a 7–0 Prolene thread was passed around the left coronary artery and the ends of the suture were passed through a polypropylene tube to form a snare. Tightening the snare induced coronary artery occlusion and its release initiated reperfusion for 15 min, except in the sham group in which the surgical procedure was identical to others but the coronary artery was not occluded. This reperfusion duration was chosen because mPTP opening was shown to occur early during reperfusion [13]. After 15 min reperfusion, the heart was removed and mitochondria were prepared from the left ventricular area at risk (≈40 mg of tissue) which was conservatively estimated by the position of the suture. The design of the experiments was illustrated in Fig. 1.

2.3. Isolation of mitochondria and cytosols

For mitochondrial oxygen consumption experiments, left ventricle tissues were removed, immediately immersed in ice cold 0.9% NaCl, scissor minced and homogenized using a Polytron homogenizer in cold buffer (4 °C, pH 7.4) containing: mannitol (220 mM), sucrose (70 mM), Hepes (10 mM) and EGTA (2 mM). The samples were further homogenized for 10 consecutive times using a Potter homogenizer at 1500 rev/min. The homogenates were then centrifuged at 1000*g* for 5 min at 4 °C to remove tissue debris and nuclei. The supernatants were centrifuged for 10 min at 10,000*g*. The final mitochondrial pellets were resuspended in homogenization buffer with only 0.01 mM of EGTA. Isolated mitochondria were kept on ice until assaying for mitochondrial respiration. Cytochrome c oxidase (mitochondrial complex IV) activity was evaluated in each mitochondrial preparation to ensure that the quantity of mitochondria included in the different assays was identical. Cytochrome c oxidase activity was identical between WT and ob/ob mice (1.36 ± 0.10 vs 1.27 ± 0.05 µmol/min/mg proteins, n=9–10, respectively) and unchanged after I/R (1.48 ± 0.08 and 1.47 ± 0.09 µmol/min/mg proteins in WT and ob/ob mice, n=9–10, respectively).

For Western blot analysis, fresh left ventricular tissues were placed in medium containing 220 mM mannitol, 70 mM sucrose, 10 mM HEPES, 2 mM EGTA, 5 µl/ml of protease inhibitor cocktail, 1 mM sodium orthovanadate, 5 mM sodium fluoride, 1 mM sodium Na_2β-glycerol phosphate (Sigma-Aldrich, St. Louis, MO, USA), pH 7.4 at 4 °C and the tissues were scissor minced. For mitochondrial extraction, the samples were homogenized and centrifuged as described in the previous paragraph. For cytosol preparation, the samples were homogenized on ice using a Teflon Potter homogenizer and the cytosols were obtained after centrifugation of the homogenates at 17,600*g* for 30 min at 4 °C. Protein concentrations were determined by the Advanced