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Basic Science

Metformin protects against infection-induced myocardial dysfunction

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ABSTRACT

Background and Purpose. Metformin administration is associated with myocardial protection during ischemia and/or reperfusion, possibly via inhibition of inflammatory responses in the heart. Exposure to pathogens, in addition to the activation of the immune system and the associated metabolic dysfunction, often results in compromised myocardial function. We examined whether metformin administration could maintain the normal myocardial function in experimental moderate Gram negative infection, induced by lipopolysaccharide (LPS) administration.

Experimental Approach. 129xC57BL/6 mice were divided into control groups that received either vehicle or a single intraperitoneal (i.p.) injection of low dose LPS (5 mg/kg body wt), and metformin treated groups that received either daily metformin (4 mg/kg/animal) i.p. injections for five days prior to LPS administration [Experiment 1], or a single metformin injection following same dose of LPS [Experiment 2].

Key Results. LPS alone caused cardiac dysfunction, as confirmed by echocardiography, whereas metformin administration, either before or after LPS, rescued myocardial function. LPS caused marked reduction of the cardiac metabolism-related genes tested, including Prkaa2, Cpt1b, Ppargc1a and Ppargc1b; reduction of fatty acid oxidation, as reflected by the regulation of Ppara, Acaca and Acacb; increased glucose transport, as

Abbreviations: ACC, Acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; Cpt, Carnitine palmitoyltransferase; EF, Ejection fraction; eNOS, Endothelial nitric oxide; FAO, Fatty acid oxidation; FS, Fractional shortening; GLUT, Glucose transporter; IL, Interleukin; LPS, Lipopolysaccharide; LV, Left ventricle; PDK, Pyruvate dehydrogenase kinase; PEPCK, Phosphoenolpyruvate carboxykinase; PGC, Peroxisome proliferator-activated receptor γ coactivator; PPAR, Peroxisome proliferator-activated receptor; TNF, Tumor necrosis factor.

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shown by Slc2a4 levels; reduction of ATP synthesis; significant increase of inflammatory markers, in particular IL6; and reduction of autophagy. Pretreatment with metformin normalized the levels of all these factors.

Conclusions and Implications. We show for the first time that metformin protects the myocardium from LPS-associated myocardial dysfunction mainly by supporting its metabolic activity and allowing efficient energy utilization. Metformin can be a potential cardioprotective agent in individuals susceptible to exposure to pathogens.

1. Introduction

Bacterial infections initiate a systemic inflammatory response that leads to cardiac dysfunction, characterized by impaired contractility, diastolic dysfunction and reduced ejection fraction [1]. As demonstrated in a number of studies on Gram negative bacterial infection, the LPS-induced cardiac dysfunction is associated with reduced free fatty acid (FFA) and glucose oxidation, which compromise ATP availability and may eventually lead to ATP depletion [2,3].

Under physiological conditions, cardiovascular function is strongly dependent on energy availability provided by FFA oxidation (FAO) [4]. Fatty acids (FAs) account for 70%, while glucose, lactate and ketones cover for the remaining 30% of the myocardial ATP production [5]. Cardiac FAO is regulated by peroxisome proliferator-activated receptor (PPAR)α which, similar to PPARγ and PPARβ/δ [6], is activated by FAs and controls the expression of genes that regulate cardiac FAO [7]. In states of increased energy demand, such as in heart failure, glucose becomes the predominant source of energy for the heart. However, opposite to what happens in heart failure, when reduced FAO is, at least partially, compensated by increased glucose oxidation, the LPS-mediated suppression of FAO is not counterbalanced by an increase in glucose catabolism [4,8]. The enzyme 5′ AMP-activated protein kinase (AMPK), a serine–threonine kinase, is thought to act as a molecular sensor, modulating the changes in cellular energy and coordinating the enzymes involved in carbohydrate and fat metabolism to enable ATP conservation and synthesis [9].

0.15pt?>Metformin (1,1-dimethylbiguanide hydrochloride), an insulin-sensitizing biguanide that is well tolerated and has minimal adverse effects reported, is currently believed to prevent cardiovascular complications associated with type 2 diabetes [10,11]. It has been suggested that the effects of metformin are either mediated via activation of AMPK [12], which then stimulates FAO, promotes glucose transport, accelerates glycolysis, and inhibits synthesis of both triglyceride and protein [13]; or via AMPK-independent mechanisms [14]. Metformin also improves hyperglycemia and increases insulin sensitivity and FAO [13]. Emerging evidence from mouse studies highlights the protective role of metformin against myocardial damage when administered either before ischemia or at the reperfusion phase [15–17]. In addition, beneficial effects of metformin have been shown in the survival of mice following LPS-induced sepsis [18].

Based on the above, we set out to determine whether metformin could be useful as a protective agent in acute myocardial dysfunction due to typical Gram-negative infection, as simulated by a sub-lethal LPS dose, and identify the mechanisms involved.

2. Methods

2.1. Mice

Wild-type male mice of 129xC57BL/6 genetic background weighing 30 to 35 g and 10 to 14 weeks old were used. Animal housing and care were conducted according to National Institutes of Health guidelines; all experimental procedures were approved by the Animal Care and Use Committee of the Biomedical Research Foundation of the Academy of Athens in Athens, Greece. The animals were housed in plastic cages under controlled conditions (temperature 22 °C; 12-h light/dark cycle; lights on at 7 am) and were given free access to standard laboratory pellet formula and tap water. Daily inspection of cages for food spillage and monitoring of body weight and food intake were performed for the duration of the study. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals [19]

Euthanasia was performed by isoflurane (IsoFlo®, Abbott) inhalation followed by cervical dislocation. The left ventricle was excised and either stored at −80 °C for RNA extraction, or fixed overnight at 4 °C in 10% formalin and embedded in paraffin.

An average of ten 2.5–3 month-old male mice per experimental group was used in all experiments and experiments were repeated to confirm the reproducibility of the findings and their statistical significance.

2.1.1. LPS-Induced Infection

Infection was induced by intraperitoneal (i.p.) injection of LPS in 0.9% NaCl at a dose of 5 mg/kg of BW. Control mice were injected with the vehicle. Echocardiograms were performed at 0 h and 24 h after LPS administration.

2.1.2. Metformin Administration

Metformin was administered i.p. either before or after LPS administration. In the first case, metformin (4 mg/kg of BW) in 0.9% NaCl was injected daily for 5 days prior to LPS administration. Last dose of metformin was administered 40 min prior to LPS administration, while 16 h past-LPS administration, mice received one more dose of metformin [Experiment 1]. In the latter case, metformin was administered 6 h after LPS administration, as a single dose of 4 mg/kg of BW [Experiment 2]. In both cases, mice were sacrificed 24 h after LPS administration. To measure the effects of LPS on myocardial function, as well as those of metformin on LPS-induced myocardial dysfunction two