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Metformin mitigates apoptosis in ischemic myocardium

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ABSTRACT

Background: Epidemiologic data has shown that metformin confers a survival advantage in patients with cardiovascular disease. Although the underlying cardioprotective mechanism is unclear, it appears to be independent of metformin's insulin-sensitizing effect. The purpose of this study was to evaluate the effect of metformin on the apoptosis pathway in the ischemic and nonischemic cardiac tissue in a swine model of metabolic syndrome.

Materials and methods: Ossabaw miniswine were fed either a regular diet (Ossabaw control, $n = 8$), a high-cholesterol diet (Ossabaw high cholesterol, $n = 8$), or a high-cholesterol diet supplemented with metformin (Ossabaw high-cholesterol metformin, $n = 8$). After 9 wk, all animals underwent placement of an ameroid constrictor to the left circumflex coronary artery to induce chronic ischemia. Seven weeks after ameroid placement, animals underwent cardiac harvest.

Results: In the chronically ischemic myocardium, metformin significantly upregulates prosurvival proteins: extracellular signal-regulated kinases, nuclear factor κB, phosphorylated endothelial nitric oxide synthase, and P38. Metformin also significantly inhibits or downregulates proapoptosis proteins: FOXO3 and caspase 3. Metformin decreased the percent apoptotic cells in the ischemic and nonischemic myocardium. There was no difference in arteriolar density, capillary density, intramyocardial fibrosis, or collagen deposition in the ischemic or nonischemic myocardium.

Conclusions: Metformin selectively alters the apoptosis pathway by inhibiting FOXO3 and decreasing the active form of caspase 3, cleaved caspase 3. Metformin also upregulates mitogen-activated kinase proteins p38 and extracellular signal-regulated protein kinases 1 and 2, which are considered cardioprotective during ischemic preconditioning. Perhaps, the altered activation of the apoptosis pathway in ischemic myocardium is one mechanism by which metformin is cardioprotective.

1. Introduction

Metformin is a widely prescribed antihyperglycemic drug for the treatment of type 2 diabetes. Epidemiologic studies have shown that metformin reduces all cause and cardiovascular mortality in treated diabetics [1,2]. Despite similar glycemic control, obese patients with type 2 diabetes treated with metformin monotherapy had greater reduction in mortality

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compared with insulin or sulfonylureas [1]. The same observational studies have also shown that diabetics with a history of prior myocardial infarction who were treated with metformin had a lower mortality than similar patients treated with sulfonylureas. These findings are significant since patients with type 2 diabetes are at an increased risk for developing coronary artery disease and suffer worse outcomes after a myocardial infarction, angioplasty, or coronary artery bypass grafting [3–5].

Although the mechanism is not entirely well understood, metformin has direct cardioprotective properties independent of its glucose lowering effect. Animal studies have investigated the effects of metformin on myocardial ischemia–reperfusion injury and have found that metformin administration reduces infarct size, limits cardiac hypertrophy, preserves myocardial function and attenuates myocardial remodeling [6]. To further elucidate metformin's cardioprotective mechanism, we developed a clinically relevant animal model of metabolic syndrome and chronic myocardial ischemia to evaluate the effect of metformin on the apoptosis and cell survival pathways.

2. Materials and methods

2.1. Animal model

Twenty-four intact male Ossabaw miniswine (Purdue Ossabaw Facility, Indiana University, Indianapolis, IN) were split into three groups according to diet at 6 wk of age. Male swine were selected to minimize the sex hormone–induced variability on ischemic heart disease and metabolic syndrome. The control group was fed 500 g/d of regular chow (Ossabaw control [OC], $n = 8$). The high-cholesterol animals were fed 500 g/d of high-cholesterol chow consisting of 4% cholesterol, 17.2% coconut oil, 2.3% corn oil, 1.5% sodium cholate, and 75% regular chow (Sinclair Research, Columbia, MO; Ossabaw high cholesterol [OHC], $n = 8$). High-cholesterol metformin animals were also fed high-cholesterol chow (Ossabaw high-cholesterol metformin [OHCM], $n = 8$). After 9 wk of diet initiation, all animals underwent surgical placement of an ameroid constrictor to induce chronic myocardial ischemia (see surgical interventions). Postoperatively, the OHCM group was supplemented with 500 mg metformin orally twice daily, and all animals continued on their respective diets. Seven weeks after ameroid constrictor placement, all animals underwent euthanasia and cardiac tissue harvest. All animals were observed to ensure complete consumption of food and supplement, had unlimited access to water, and were housed in a warm nonstressful environment for the duration of the experiment.

2.2. Surgical interventions

2.2.1. Anesthesia

Anesthesia was induced with an intramuscular injection of telazol (4.4 mg/kg). Animals were endotracheally intubated, mechanically ventilated at 12–20 breaths/min, and general anesthesia was maintained with a gas mixture of oxygen at 1.5–2 L/min and isoflurane at 0.75–3.0% concentration.

2.2.2. Ameroid constrictor placement

Animals were given a single dose of antibiotic prophylaxis, intravenous enrofloxacin 5 mg/kg, and general anesthesia was induced and maintained. Animals were prepped and draped in the usual sterile fashion. The heart was exposed through a left minithoracotomy. The left atrial appendage was retracted, and the proximal left circumflex artery was dissected at the take off of the left main coronary artery. The ameroid constrictor was placed around the left circumflex artery (Research Instruments NW, Escondido, CA). The pericardium was loosely reapproximated followed by a layered closure of the surgical incision. Postoperative pain was controlled with a single dose of intramuscular buprenorphine (0.03 mg/kg) and 72 h fentanyl patch (4 μg/kg). All animals received 325 mg of aspirin daily for thromboembolic prophylaxis starting 1 d preoperatively and continuing for a total of 5 d. All animals continued perioperative antibiotics: enrofloxacin 68 mg orally daily for 5 d.

2.2.3. Cardiac harvest

Under general anesthesia, the heart was exposed via a median sternotomy, and animals were euthanized by exsanguination. Of note, before euthanasia and harvest, myocardial perfusion was measured by injecting isotope-labeled microspheres at rest and with demand pacing at 150 beats/min. We previously reported that there was no difference in myocardial perfusion in the normally perfused or chronically ischemic myocardium [7].

Full-thickness sections from the chronically ischemic left ventricle immediately adjacent to the left circumflex artery, distal to the ameroid constrictor were collected. Also, full-thickness sections from the normally perfused left ventricle immediately adjacent to the left anterior descending artery were collected for analysis. The previously reported myocardial perfusion analysis confirmed that the ischemic territory had the lowest microsphere counts, and the normally perfused normal ventricle had the highest microsphere counts [7].

The Institutional Animal Care and Use Committee of the Rhode Island Hospital approved all experiments. Animals were cared for in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" (National Institutes of Health publication no. 5377-3 1996).

2.3. Immunohistochemical staining for angiogenesis

Frozen myocardium was sectioned (10-μm thickness) and fixed in 10% formalin for 10 min. Sections were blocked with 1% bovine serum albumin in phosphate-buffered saline for 1 h at room temperature and incubated with antibodies against porcine endothelial marker CD-31 (R&D Systems, Minneapolis, MN) and smooth muscle actin (Sigma–Aldrich, St. Louis, MO), followed by the appropriate alexa fluor–conjugated antibody (Jackson ImmunoResearch, West Grove, PA) for 45 min. Slides were then mounted with VECTASHIELD with 4′,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA). Images were captured at ×20 magnification with a Nikon E800 Eclipse microscope (Nikon, Tokyo, Japan) at the