Metformin alters the insulin signaling pathway in ischemic cardiac tissue in a swine model of metabolic syndrome

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Objective: The purpose of this study is to evaluate the effect of metformin on insulin signaling in ischemic cardiac tissue in a swine model of metabolic syndrome.

Methods: Ossabaw miniswine were fed either a regular diet (Ossabaw control [OC]) or a hypercaloric diet (Ossabaw high cholesterol [OHC], Ossabaw high cholesterol with metformin [OHCM]). After 9 weeks, all animals underwent placement of an ameroid constrictor to the left circumflex artery to induce chronic ischemia. OHC animals were continued on a hypercaloric diet alone; the OHCM group was supplemented with metformin in addition to the hypercaloric diet. Seven weeks after ameroid placement, myocardial perfusion was measured and ischemic cardiac tissue was harvested for protein expression and histologic analysis.

Results: The OHC and OHCM groups had significantly higher body mass indices and serum insulin levels compared with the OC group. There were no differences in myocardial perfusion in the chronically ischemic territories. In the OHC group, there was upregulation of both an activator of insulin signaling insulin receptor substrate 1, and an inhibitor of insulin signaling phosphorylated insulin receptor substrate 2. In the OHCM group, there was upregulation of activators of insulin signaling including phosphorylated adenosine monophosphate-activated protein kinase α, protein kinase B, phosphorylated protein kinase B, mammalian target of rapamycin, phosphorylated mammalian target of rapamycin, and phosphoinostitide 3-kinase, and upregulation of inhibitors including phosphorylated insulin receptor substrate 1, phosphorylated insulin receptor substrate 2, and retinol binding protein 4. Histologic analysis demonstrated increased expression of glucose transporter 1 at the plasma membrane in the OHCM group, but there was no difference in cardiomyocyte glycogen stores among groups.

Conclusions: Metformin treatment in the context of metabolic syndrome and myocardial ischemia dramatically upregulates the insulin signaling pathway in chronically ischemic myocardium, which is at the crossroads of known metabolic and survival benefits of metformin. (J Thorac Cardiovasc Surg 2013;145:258-66)

Despite advances in treatment, diabetes mellitus still affects 25.8 million Americans and is a major cause of morbidity and mortality in the United States.[1] Patients with diabetics often experience a group of metabolic derangements including dyslipidemia, hypertension, and obesity (also known as metabolic syndrome), which more than doubles their risk of developing cardiovascular disease.[2-5] A central component in the development of type 2 diabetes mellitus and metabolic syndrome is insulin resistance.[6] One of the mainstay treatments of type 2 diabetes mellitus is metformin, an orally administered biguanide. Metformin reduces blood glucose levels by reducing hepatic glucose production and increasing peripheral glucose uptake.[7] Metformin also has direct cardioprotective properties that are independent of its antihyperglycemic effects. Epidemiologic studies have shown that patients with type 2 diabetes mellitus treated with metformin had reduced all-cause mortality.[8,9] In animal studies, metformin has been shown to reduce infarct size and attenuate myocardial remodeling, preserve myocardial function, limit cardiac hypertrophy, and reduce the development of heart failure after myocardial infarction.[10]

Studies have shown that the cardioprotective properties of metformin are mediated by protein kinase B (AKT) and adenosine monophosphate-activated protein kinase (AMPK) by promoting cell survival during periods of ischemia.[10] Interestingly, both AKT and AMPK are also key protein kinases in the insulin signaling cascade. Therefore, it is prudent to investigate further the effects of metformin at the junction of its cardioprotective and glucose lowering effects: the insulin signaling cascade. We developed a clinically relevant swine model of metabolic syndrome and chronic myocardial ischemia to investigate the effects of metformin on the insulin signaling pathway in ischemic myocardium.

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Abbreviations and Acronyms

ACC = acetyl coenzyme A carboxylase
AKT = protein kinase B
AMP = adenosine monophosphate
AMPK = adenosine monophosphate-activated protein kinase
ATP = adenosine triphosphate
CoA = coenzyme A
FAS = fatty acid synthase
FOX01 = foxhead box 01
GLUT1 = glucose transporter 1
GLUT4 = glucose transporter 4
IRS = insulin receptor substrate
MTOR = mammalian target of rapamycin
OC = Ossabaw control
OHC = Ossabaw high cholesterol
OHCM = Ossabaw high cholesterol with metformin
PI3K = phosphoinostitide 3-kinase
p-IRS = phosphorylated insulin receptor substrate
p-MTOR = phosphorylated mammalian target of rapamycin
RBP4 = retinol binding protein 4

METHODS

Animal Model

Twenty-four intact male Ossabaw miniswine (Purdue Ossabaw Facility, Indiana University, Indianapolis, Ind) were divided into 3 groups according to diet at 6 weeks of age. The control group was fed 500 g/day of regular chow (Ossabaw control [OC], n = 8). The high-cholesterol animals (Ossabaw high cholesterol [OHC], n = 8) were fed 500 g/day 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). High-cholesterol metformin animals (Ossabaw high cholesterol with metformin [OHCM], n = 8) were also fed high-cholesterol chow. After 9 weeks 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 were continued on their respective diet. Seven weeks after ameroid constrictor placement, all animals were weighed and underwent functional cardiac and hemodynamic measurements, 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.

Surgical Interventions

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

Ameroid constrictor placement. Animals were given a single dose of intravenous enrofloxacin 5 mg/kg for antibiotic prophylaxis, 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 and pericardiotomy. The left atrial appendage was retracted and the left circumflex artery was dissected at the takeoff of the left main coronary artery. The circumflex artery was occluded for 2 minutes, during which time 5 mL isotope-labeled gold microspheres (BioPhysics Assay Laboratory, Worcester, Mass) was injected into the left atrium to establish shadow labeling of the ischemic myocardium. The ameroid constrictor was placed around the left circumflex artery (Research Instruments SW, Escondito, Calif). The pericardium was reapproximated loosely 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 a 72-hour Fentanyl patch (4 μg/kg). All animals received 325 mg aspirin daily starting 1 day preoperatively and continuing for a total of 5 days for prophylaxis against thromboembolic events. All animals continued perioperative antibiotics of enrofloxacin 68 mg orally daily for 5 days.

Cardiac harvest. Under general anesthesia, coronary angiography of the left and right coronary circulation was performed as described previously.[11] Complete occlusion of the left circumflex artery was confirmed angiographically in all cases. A blinded interventional cardiologist then assessed the TIMI, Rentrop, and Blush scores. The heart was exposed via a median sternotomy. Global and regional myocardial function and contractility were measured by placing sonomicrometer crystals (Sonometrics Corporation, Ontario, Canada) in the normally perfused left ventricle as described previously.[11] Pressure catheters were also placed in the descending aorta through a right femoral sheath and into the left ventricle through an apical puncture.[11] After microsphere injection (see Myocardial Perfusion), animals were euthanized by exsanguination, and chronically ischemic myocardial samples in the left circumflex territory were collected for further analysis. 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.*[12]

Serologic Studies

Blood samples were drawn from the jugular vein prior to euthanasia and tissue harvest for insulin measurement. The chemistry laboratory at the Rhode Island Hospital, Providence, RI, analyzed the serum samples.

Myocardial Perfusion

Myocardial perfusion was measured by injecting gold isotope-labeled microspheres (Biophysics Assay Laboratory) into the left atrium at the time of ameroid placement during a brief left circumflex artery occlusion. At the final operation, prior to cardiac harvest, lutetium microspheres were injected while simultaneously withdrawing blood from a femoral artery catheter. Samples of the left ventricle and blood were dried at 60°C for > 48 hours, and microsphere density was quantified with a gamma counter after exposure to neutron bean radiation (Biophysics Assay Laboratory). Myocardial blood flow to each sample was calculated using the following equation:

$$\text{Blood flow} = \frac{\text{Withdrawal rate}}{\text{Tissue weight}} \times \frac{\text{Tissue microsphere count}}{\text{Blood microsphere count}}$$

Protein Expression

Forty micrograms of the radio-immunoprecipitation assay (Boston BioProducts, Ashland, Mass) soluble fraction of myocardial lysates from the chronically ischemic territory were fractionated by SDS-PAGE using 3% to 8% Tris-acetate gel (NuPage Novex Mini Gel; Invitrogen, Carlsbad, Calif) for molecular weight targets > 100 kDa and 4% to 12% Bis-Tris