Glycogen synthase kinase 3β inhibition reduces mitochondrial oxidative stress in chronic myocardial ischemia

Brittany A. Potz, MD, Laura A. Scrimgeour, MD, Sharif A. Sabe, MD, Richard T. Clements, PhD, Neel R. Sodha, MD, and Frank W. Sellke, MD

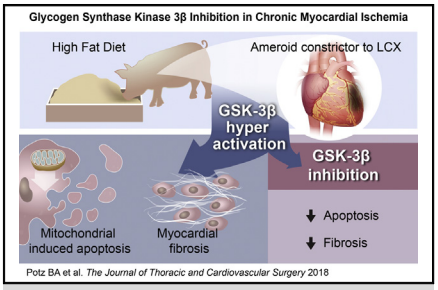
ABSTRACT

Objectives: Glycogen synthase kinase 3β (*GSK-3β*) inhibition has been reported to increase microvascular density and improve myocardial blood flow in a porcine model of chronic myocardial ischemia and metabolic syndrome. Inhibition of GSK-3β can also be cardioprotective by modulating fibrosis signaling and mitochondrial-induced apoptosis. We hypothesized GSK-3β inhibition would have a beneficial effect on myocardial fibrosis and oxidative stress in a porcine model of chronic myocardial ischemia and metabolic syndrome.

Methods: Pigs were fed a high fat diet for 4 weeks followed by placement of an ameroid constrictor to the left circumflex coronary artery. Three weeks later animals received either no drug or a GSK-3β inhibitor. The diets and placebo/GSK-3β inhibition were continued for an additional 5 weeks, the pigs were then euthanized, and the myocardial tissue was harvested. Collagen expression was analyzed via Picrosirius staining. Oxidative stress was analyzed via Oxyblot analysis. Protein expression was analyzed via Western blot.

Results: GSK-3β inhibition was associated with decreased collagen expression and oxidative stress in the ischemic and nonischemic myocardial tissue compared with control. There was a decrease in profibrotic proteins transforming growth factor-β, p-SMAD2/3, and matrix metalloproteinase-9, and in proapoptotic and oxidative stress proteins, apoptosis inducing factor, the cleaved caspase 3/caspase 3 protein ratio and phosphorylated myeloid cell leukemia sequence-1 in the GSK-3β inhibited group compared with the control.

Conclusions: In the setting of metabolic syndrome and chronic myocardial ischemia, inhibition of GSK-3β decreases collagen formation and oxidative stress in myocardial tissue. GSK-3β inhibition might be having this beneficial effect by downregulating transforming growth factor-β/SMAD2/3 signaling and decreasing mitochondrial induced cellular stress. (J Thorac Cardiovasc Surg 2018;■:1-12)



GSK-3β inhibition decreases fibrosis in ischemic myocardium in pigs with metabolic syndrome.

Central Message

GSK-3β inhibition might serve as a potential therapy to decrease myocardial fibrosis and oxidative stress in patients suffering from coronary artery disease and metabolic syndrome.

Perspective

In the setting of metabolic syndrome and chronic myocardial ischemia, inhibition of GSK-3β decreases collagen formation and oxidative stress in myocardial tissue. GSK-3β inhibition is associated with decreased profibrotic and mitochondrial-induced stress protein signaling. GSK-3β inhibition might serve as a therapy to inhibit myocardial fibrosis for patients suffering from coronary artery disease.

See Editorial Commentary page XXX.

BS

Metabolic syndrome is a diagnosis encompassing a host of pathologic conditions observed in cardiovascular disease states including hypertension, obesity, hyperlipidemia, and glucose intolerance/diabetes mellitus type 2. The incidence of metabolic syndrome is approximately 24% in the United States.[1] Patients with metabolic syndrome are at increased

Scanning this QR code will take you to a supplemental video for the article.

From the Division of Cardiothoracic Surgery, Department of Surgery, Cardiovascular Research Center, Rhode Island Hospital, Alpert Medical School of Brown University, Providence, RI.

Funding for this research was provided by the National Heart, Lung, and Blood Institute (RO1HL128831, RO1 HL46716) (to F.W. Sellke); NIH T32 GM065085 training grant to (to L.A. Scrimgeour); and National Institutes of Health/National Institute of General Medical Sciences training grant 2T32 GM065085 to (to B.A. Potz); American Heart Association Grant-in-Aid GRNT20460376, and National Institute of General Medical Sciences U54GM115677 (to R.T. Clements).

Read at the 43rd Annual Meeting of The Western Thoracic Surgical Association, Colorado Springs, Colorado, June 21-24, 2017.

Received for publication May 11, 2017; revisions received Nov 21, 2017; accepted for publication Dec 29, 2017.

Address for reprints: Frank W. Sellke, MD, Division of Cardiothoracic Surgery, 2 Dudley St, MOC 360, Providence, RI 02905 (E-mail: fsellke@lifespan.org).

0022-5223/$36.00

https://doi.org/10.1016/j.jtcvs.2017.12.127

Abbreviations and Acronyms

AIF = apoptosis inducing factor
CON = control group
GAPDH = glyceraldehyde 3-phosphate dehydrogenase
GSK-3β = glycogen synthase kinase- 3β
GSK-3βI = glycogen synthase kinase-3β inhibitor
HSF-1 = heat shock factor-1
MCL-1 = myeloid cell leukemia sequence-1
MMP-9 = matrix metalloproteinase-9
mPTP = mitochondrial permeability transition pore
p-MCL-1 = phosphorylated myeloid cell leukemia sequence-1
TGF-β = transforming growth factor-β

risk for cardiovascular disease.[2,3] Furthermore, coronary artery disease, vascular disease, and total mortality are increased in adults with metabolic syndrome.[2,3] The mechanism by which metabolic syndrome leads to the previously mentioned cardiovascular conditions is multifactorial.[4]

Glycogen synthase kinase 3β (GSK-3β) is a serine/threonine kinase that regulates a diverse range of cellular pathways. GSK-3β hyperactivation is associated with several pathologic conditions including type 2 diabetes, obesity, retinal ischemia, coronary artery disease, myocardial fibrosis, and elevated levels of inflammation and oxidative stress.[5-8] Although hyperactivation of GSK-3β is detrimental, some level of physiologic GSK-3β activity is necessary for life because global GSK-3β knockout is lethal.[7,9,10] Inhibition of GSK-3β overactivity has been reported to beneficially reverse some of these pathologic conditions by modulating insulin signaling, improving glucose disposal in diabetes by improving liver glycogen synthesis, decreasing transforming growth factor-β (TGF-β)/SMAD3–induced fibrosis after renal ischemia/reperfusion, and improving neovascular formation in the setting of retinal ischemia.[7,10-13]

Hyperglycemia and ischemic coronary disease contribute to myocardial remodeling via apoptosis and the accumulation of myocardial collagen leading to aberrant myocardial architecture ultimately resulting in cardiac dysfunction and heart failure.[14,15] Inhibition of stress-induced myocardial fibrosis is therefore cardioprotective.[15-18] GSK-3β activity has been implicated in the fibrosis that develops in ischemia/reperfusion injury.[13] Similarly, GSK-3β knockout mice have reduced left ventricular remodeling and preserved left ventricular dysfunction in the setting of permanent myocardial infarction.[19] GSK-3β is thought to regulate fibrosis by β catenin-dependent and TGF-β–SMAD 3-dependent mechanisms.[20]

Mitochondrial oxidative stress leads to opening of the mitochondrial permeability transition pore (mPTP), which plays a critical role in myocardial apoptosis.[21,22] Suppression of mPTP opening is cardioprotective against reperfusion injury in rats.[21] GSK-3β phosphorylates and inhibits myeloid cell leukemia sequence-1 (MCL-1), which regulates formation of mPTP.[6,8,23] GSK-3β inhibition is also thought to be cardioprotective by modulating fibrosis signaling and cardiomyocyte hypertrophy, limiting infarct size in animals models of acute myocardial infarction, reducing oxidative stress, and preventing mPTP opening in myocardial reperfusion injury.[13,24-27]

In this study we used a porcine model of chronic coronary artery disease and myocardial ischemia in the setting of metabolic syndrome. This model represents the circulatory and metabolic dysfunction observed in adult patients with metabolic syndrome and chronic coronary artery disease.[28] Using this model, we have found that inhibition of GSK-3β increases blood flow and vessel density in ischemic and nonischemic myocardial tissue.[29]

The objective of this study was to examine the effects of inhibition of GSK-3β on myocardial fibrosis and oxidative stress in the setting of chronic myocardial ischemia and metabolic syndrome. We hypothesized that GSK-3β inhibition would have a protective effect on myocardial collagen formation and oxidative stress.

METHODS

Animal Model

Sixteen Yorkshire swine (E.M. Parsons and Sons, Hadley, Mass) were fed a 500 g/d high fat/high cholesterol diet for 4 weeks (Sinclair Research, Columbia, Mo).[28] This diet consists of 4% cholesterol, 2.3% corn oil, 17.2% coconut oil, 1.5% sodium cholate, and 75% regular chow.

The swine received aspirin (10 mg/kg) 1 day preoperatively and 5 days postoperatively and cephalexin (30 mg/kg) 1 day preoperatively and 5 days postoperatively. Anesthesia was then induced with telazol (4.4 mg/kg) and xylazine (2.2 mg/kg) and maintained with isoflurane (0.75%-3%). A 72-hour fentanyl patch (4 μg/kg) was placed before surgery. A left mini thoracotomy was then performed, the left circumflex artery was identified and manually occluded for 2 minutes while gold microspheres (5 cc, BioPal, Worcester, Mass) were injected into the left atrium to allow for microsphere labeling of the nonischemic territory. Heparin (80 IU/kg) was injected before occlusion. To induce chronic myocardial ischemia, a titanium ameroid constrictor ring (Research Instruments SW, Escondido, Calif) was placed on the left circumflex coronary artery. The hydrophilic core of the ameroid constrictor enlarges gradually over the course of 10 to 20 days to reproduce a chronic model of ischemia. Amiodarone (10 mg/kg) was used as needed for arrhythmias that developed as a result of isolation and manipulation of the left circumflex artery at the time of ameroid placements.

Two weeks later, the animals were split into 2 groups and received either a placebo (high cholesterol control group, 1.5 mg/kg/d of DMSO, termed as "CON" group; n = 8); or a GSK-3β inhibitor (*GSK-3βI*; 1.5 mg/kg/d dissolved in DMSO, termed as "GSK-3βI" group; n = 8).