Calpain inhibition improves collateral-dependent perfusion in a hypercholesterolemic swine model of chronic myocardial ischemia

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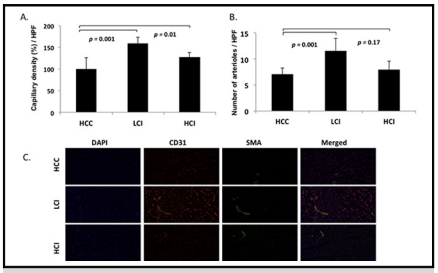
ABSTRACT

Purpose: Calpain overexpression is implicated in aberrant angiogenesis. We hypothesized that calpain inhibition (MDL28170) would improve collateral perfusion in a swine model with hypercholesterolemia and chronic myocardial ischemia.

Methods: Yorkshire swine fed a high cholesterol diet for 4 weeks underwent surgical placement of an ameroid constrictor to their left circumflex coronary artery. Three weeks later, animals received no drug, high cholesterol control group (n = 8); low-dose calpain inhibition (0.12 mg/kg; n = 9); or high-dose calpain inhibition (0.25 mg/kg; n = 8). The heart was harvested after 5 weeks.

Results: Myocardial perfusion in ischemic myocardium significantly improved with high-dose calpain inhibition at rest and with demand pacing ($P = .016$ and .011). Endothelium-dependent microvessel relaxation was significantly improved with low-dose calpain inhibition ($P = .001$). There was a significant increase in capillary density, with low-dose calpain inhibition and high-dose calpain inhibition ($P = .01$ and .01), and arteriolar density with low-dose calpain inhibition ($P = .001$). Calpain inhibition significantly increased several proangiogenic proteins, including vascular endothelial growth factor ($P = .02$), vascular endothelial growth factor receptor 1 ($P = .003$), vascular endothelial growth factor receptor 2 ($P = .003$), and talin, a microvascular structural protein ($P = .0002$). There was a slight increase in proteins implicated in endothelial-dependent (nitric oxide mediated) relaxation, including extracellular signal-regulated kinase, phosphorylated extracellular signal-regulated kinase, and inducible nitric oxide synthase with calpain inhibition.

Conclusions: In the setting of hypercholesterolemia, calpain inhibition improved perfusion, with a trend toward increased collateralization on angiography and increased capillary and arteriolar densities in ischemic myocardium. Calpain inhibition also improved endothelium-dependent microvessel relaxation and increased expression of proteins implicated in angiogenesis and vasodilatation. (J Thorac Cardiovasc Surg 2016;151:245-52)



Vessel density was significantly increased in the pigs with calpain inhibition versus controls.

Central Message

Calpain inhibition in the setting of chronic myocardial ischemia improves proangiogenic protein expression and myocardial perfusion.

Perspective

Moderate calpain inhibition in the setting of hypercholesterolemia and chronic myocardial ischemia improves proangiogenic protein expression, microvascular relaxation, and myocardial perfusion. These findings may have important clinical implications for the treatment of patients with severe coronary artery disease and microvascular dysfunction resulting from hypercholesterolemia.

See Editorial Commentary page 253.

Despite advances in percutaneous and surgical interventions in the treatment of coronary artery disease (CAD), up to one third of patients are not candidates for or receive suboptimal revascularization with these therapies.[1] The incidence of incomplete revascularization in patients with severe CAD who undergo surgical intervention is an

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Abbreviations and Acronyms

CAD = coronary artery disease
CI = calpain inhibition
ERK = extracellular signal-regulated kinase
HCC = high cholesterol control
HCI = high-dose calpain inhibition
iNOS = inducible nitric oxide synthase
LCI = low-dose calpain inhibition
LCx = left circumflex artery
pERK = phosphorylated extracellular signal-regulated kinase
RhoA = ras homolog gene family member A
VEGF = vascular endothelial growth factor
VEGFR1 = vascular endothelial growth factor receptor 1
VEGFR2 = vascular endothelial growth factor receptor 2

independent predictor for operative and perioperative morbidity and mortality.[2,3] With an increased prevalence of obesity and metabolic syndrome, the incidence of severe CAD not amenable to surgical treatment is likely to increase.[1,4,5] Inducing angiogenesis through medical therapies remains a promising therapeutic option for these patients. However, a deeper understanding of the proangiogenic and antiangiogenic pathways in the setting of hypercholesterolemia and chronic ischemic disease is necessary to treat this complicated and growing population of patients. Our laboratory has created a pig model for chronic myocardial ischemia in the setting of metabolic syndrome (weight gain, glucose intolerance, dyslipidemia, and hypertension).[4]

Calpains, calcium-dependent thiol proteases expressed ubiquitously in mammals, are an important potential mediator of these angiogenic pathways. When activated, calpains regulate a broad spectrum of functionally important protein targets that involve cytoskeletal organization, cell adhesion, and cell migration. Hypoxia is known to induce calpain activity, resulting in disruption of cardiac endothelial cell cytoskeletal structure and function.[6-10] Modest suppression of calpain activity has been shown to improve functional neovasculature.[7,8] Although the mechanism for this improvement remains largely unknown, there is evidence in small animal (rodent) models that calpain inhibition (CI) allows for upregulation of proangiogenic proteins and scaffolding proteins that are essential for new vessel growth and maturation.[7,8] Although these studies are promising, they have been performed only in small, otherwise healthy animal models. Given the considerable potential for the proangiogenic effects of CI, we sought to investigate their effects in a clinically relevant porcine model of metabolic syndrome. We hypothesized that in the setting of chronic myocardial ischemia, CI would result in improved collateral-dependent myocardial perfusion and vascular function.

MATERIALS AND METHODS

Animal Model and Surgical Interventions

Juvenile male Yorkshire swine (Parsons Research, Amherst, Mass) were divided into 3 groups, fed a high cholesterol diet for 4 weeks, and then underwent surgical placement of a titanium ameroid constrictor (Research Instruments SW, Escondito, Calif) on the proximal left circumflex artery (LCx). Male pigs were used in an effort to limit variables (male vs female) between pigs. Three weeks later, animals received no drug, high cholesterol control (HCC) group (n = 8); an oral form of a low-dose CI (LCI) (0.12 mg/kg; n = 9); or an oral form of a high-dose CI (HCI) (0.25 mg/kg; n = 8) (CI MDL28170; EMD Millipore, Danvers, Mass). The diets and oral form of the CI were continued for 5 weeks until completion of the study, and then the animals were anesthetized and underwent x-ray coronary angiography. The heart was then exposed through a midline sternotomy, and microspheres were injected at rest and with ventricular pacing (160 beats/min). The animals were euthanized, and their hearts were harvested. Tissue samples from chronically ischemic myocardium (LCx territory) and nonischemic myocardium were rapidly frozen in liquid nitrogen. Tissue samples for microvessel reactivity studies were placed in Krebs solution. Detailed methods on surgical procedures, anesthesia, and tissue harvesting have been described.[11] 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*.

Microvessel Studies, Coronary Angiography

Our group has previously described detailed methods for microvessel studies and coronary angiography.[11] Coronary arterioles taken from ischemic myocardium were isolated and microvascular relaxation responses were measured after being exposed to endothelium-dependent and endothelium-independent agents. Relaxation responses were defined as percent relaxation of the preconstricted (thromboxane analog U46619) diameter. Coronary angiography was performed during the final procedure to demonstrate occlusion of the LCx. A cardiologist interpreted recorded images in a blinded fashion to assess collateral formation. Collaterals were graded according to the well-validated Rentrop system of 0 to 3.[5,12] Rentrop score is a scoring system to grade collateral filling vessels: grade 0 (no visible filling of any collateral channels), grade 1 (collateral filling branches of vessel without any dye reaching the epicardial segment of that vessel), grade 2 (partial collateral filling of the epicardial segment of the vessel being dilated), and grade 3 (complete collateral filling of the vessel being dilated).

Myocardial Perfusion

Methods for myocardial perfusion have been described at rest and during rapid cardiac pacing.[11] Briefly, gold isotope-labeled microspheres (Biophysics Assay Laboratory, Worcester, Mass) were injected into the left atrium during transient LCx occlusion at the time of initial ameroid placement to determine the territory at risk. Lutetium and Europium isotope-labeled microspheres were injected at rest and with demand pacing (160 beats/min), respectively (all microspheres obtained from