Calpain inhibition decreases myocardial apoptosis in a swine model of chronic myocardial ischemia

Brittany A. Potz, MD, MA, Ashraf A. Sabe, MD, Nassrene Y. Elmadhun, MD, Jun Feng, MD, PhD, Yuhong Liu, MD, Hunter Mitchell, BS, Peter Quesenberry, MD, M. Ruhul Abid, MD, PhD, *and* **Frank W. Sellke, MD,** *Providence, RI*

Introduction. *Calpain is a family of cysteine proteases that has an important role in the initiation, regulation, and execution of cell death. Our recent studies using a hypercholesterolemic swine model demonstrated that in the setting of the metabolic syndrome, calpain inhibition (CI) improved collateral-dependent perfusion and increased expression of proteins implicated in angiogenesis and vasodilation. In this study, we hypothesized that CI (by MLD28170) would decrease myocardial apoptosis in the same model.*

Methods. *Yorkshire swine, all fed a high-cholesterol diet for 4 weeks underwent placement of an ameroid constrictor on the left circumflex coronary artery. Three weeks later, animals received either no drug, termed the high-cholesterol control group (HCC;* n = *8); low-dose CI (0.12 mg/kg; LCI,* n = *9); or high-dose CI (0.25 mg/kg; HCI,* n = *8). The high-cholesterol diet and the CI were continued for 5 weeks, after which the pig was humanely killed and the left ventricular myocardium was harvested and analyzed via terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, oxyblot analysis, and Western blots. Data were analyzed using the Kruskal–Wallis test.*

Results. *The percentage of apoptotic cells to total cells in ischemic myocardial territory was decreased in the LCI and HCI groups compared with the HCC group as shown by TUNEL staining (*P = *.018). There was a decrease in proapoptotic proteins, including cleaved caspase 3, caspase 9, cleaved caspase 9, Bax, BAD, p-BAD, and Erk 1/2 (*P ≤ *.049 each), but no decrease in caspase 3 (*P = *.737). There was also an increase in antiapoptotic proteins, including BCL-2 and p-BCL2 (*P ≤ *.025 each). In the ischemic myocardium, several proangiogenic proteins were increased in the LCI and HCI groups compared with the HCC group, including p-AKT, p-eNOS, and eNOS (*P ≤ *.006 each) but there was no increase in AKT (*P = *.311). CI decreased tissue oxidative stress in both the LCI and HCI groups compared to the HCC group as shown by oxyblot analysis (*P = *.021).*

Conclusion. *In the setting of hypercholesterolemia, CI decreases apoptosis and the expression of proteins in proapoptotic signaling pathways. CI also increased expression of proteins implicated in anti apoptotic pathways and improves oxidative stress in ischemic myocardial tissue. (Surgery 2015;158:445-52.)*

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

The metabolic syndrome is associated with an increased incidence of and mortality related to coronary artery disease.[1] In patients who are not candidates for traditional revascularization strategies such as coronary bypass grafting or percutaneous coronary intervention, regenerative therapies are an attractive theoretic option; however, nearly all the attempts at regenerative therapy have met with little success.[2,3] Patients with end-stage coronary artery disease have advanced myocardial and endothelial dysfunction and abnormal myocardial and vascular signaling.[4,5] Similarly, patients with the metabolic syndrome have diminished angiogenic responses to chronic ischemia and alterations in a variety of mechanisms contributing to vascular bed dysfunction including the formation of collateral vessels.[6,7] We and other laboratories have reported that a major mechanism leading to vascular dysfunction in metabolic syndrome is increased oxidative stress leading to over production of reactive oxygen species and cellular apoptosis[7-9] (Fig 1).

Accepted for publication March 18, 2015.

Reprint requests: Frank W. Sellke, MD, 2 Dudley Street, MOC 360, Division of Cardiothoracic Surgery, Providence, RI 02905. E-mail: fsellke@lifespan.org.

0039-6060/$ - see front matter

http://dx.doi.org/10.1016/j.surg.2015.03.034

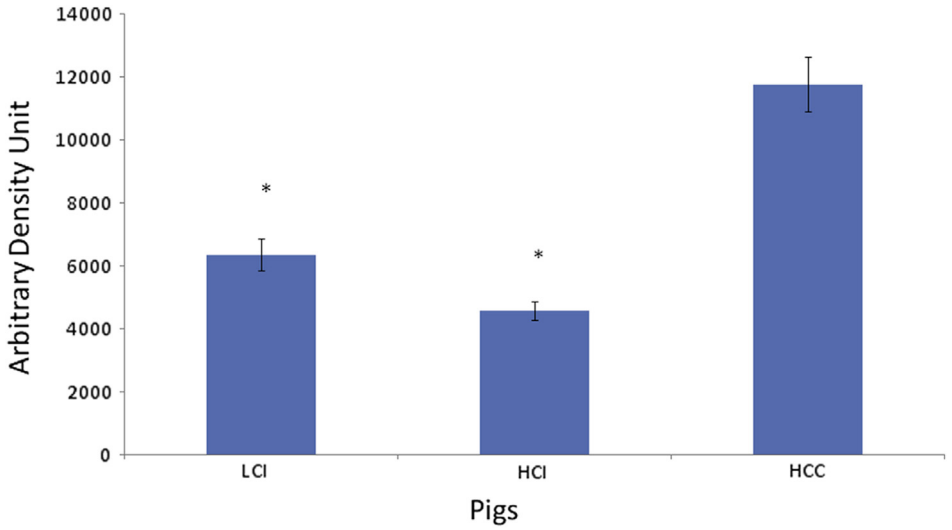

Fig 1. Results of calpain inhibition (CI) on oxidative stress in ischemic myocardial hypermetabolic pig tissue. CI decreased tissue oxidative stress in both the low CI (LCI) and high CI (HCI) groups compared with the control (HCC) group as shown by oxiblot analysis ($P = .021$). *$P < .05$ versus HCC by analysis of variance (Kruskal–Wallace test).

Recent studies have suggested that the family of calpains control fundamental cellular functions, such as cytoskeleton remodeling, cell-cycle regulation, gene expression, and cell death in all tissue.[10] Myocardial calpain plays an essential role in the ubiquitin/proteasome protein degradation pathway that removes proteins whose abnormal accumulation causes cardiomyocyte apoptosis and heart failure.[11,12] In ischemic heart disease, calpain activation has been found to promote left ventricular remodeling after myocardial infarction by disassembling cell–cell adhesion via degradation of N-cadherin.[10] Uncontrolled activation of calpain, however, is involved in the pathogenesis of myocardial ischemia and dysfunction.[13] Similarly, in the metabolic syndrome, hyperactivation of calpain has been linked with myocardial and vascular inflammation and impaired collateral formation.[14] Conversely, complete knockout of calpain abolished neovessel integration and lumen formation. Taken together, these findings suggest that a regulated level of calpain is necessary for basic physiological function and that overactivation of calpain leads to tissue dysfunction.[15] In support of this concept, moderate inhibition of calpain can improve neovasculature intervention and lumen formation during pathologic angiogenesis and can improve global hemodynamics and left ventricular contractility in myocardial ischemia.[13,16] The mechanism by which uncontrolled activation of calpain leads to cardiac dysfunction is under investigation. Therefore, calpain represents an important target for rectifying key vascular defects associated with pathologic angiogenesis.[16]

Recently, we found that calpain activity was increased significantly in the ischemic myocardium of pigs with the metabolic syndrome and that CI improved collateral-dependent perfusion and increased expression of proteins implicated in angiogenesis and vasodilatation.[17] Because collateral vessel formation is in part regulated by apoptosis, the purpose of this study was to examine the role of calpain on myocardial apoptosis in the setting of chronic ischemia and MS conditions. In the current study, we hypothesized that CI could affect the heart not only via angiogenic pathways, but also by helping to inhibit ischemia-induced cell death. The findings reported herein implicating a role of calpain on myocardial apoptosis may in part identify the molecular mechanisms by which CI may exert a beneficial effect on chronic myocardial ischemia.

In this study, we hypothesized that dose-dependent CI would have a protective effect on the heart against chronic, ischemia-induced myocardial apoptosis in a clinically relevant model in pigs with the metabolic syndrome.

METHODS

Animal model. Twenty-five juvenile (7-weeks-old) Yorkshire swine (E.M. Parsons and Sons, Hadley, MA) were fed a high-fat/high-cholesterol diet for 4 weeks. The swine then underwent placement of an ameroid constrictor on the left circumflex coronary artery to induce chronic myocardial ischemia. Three weeks later, animals received either no drug (the high cholesterol control group [HCC]; $n = 8$), a low dose of the of Calpain