Role of Calpains (Calcium-Dependent Proteases) on Coronary Artery Disease and Metabolic Syndrome

19

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Abstract

Coronary artery disease and metabolic syndrome together are two major causes of death in the United States and the numbers of people afflicted by these diseases are increasing around the world. Overactivation of calpain has been shown to contribute to cardiovascular disease and its associated comorbidities. Therefore, calpain inhibition may offer a novel potential medical therapy for treating not only coronary artery disease but also the individual aspects of the associated comorbidities.

Keywords

Coronary artery disease · Metabolic syndrome · Calpain · Pharmacologic therapy

1 Introduction

Metabolic syndrome is defined as having the presence of at least three of five risk factors for coronary artery disease (insulin resistance, obesity, hypertension, elevated triglycerides, and low high-density lipoprotein). Mortality from coronary vascular disease is increased in patients with metabolic syndrome [1]. The prevalence of metabolic syndrome in the adult US population is approximately 24% and is currently increasing [1]. Research has attempted to find a pharmacological therapy to reduce the incidence of coronary disease in patients with metabolic

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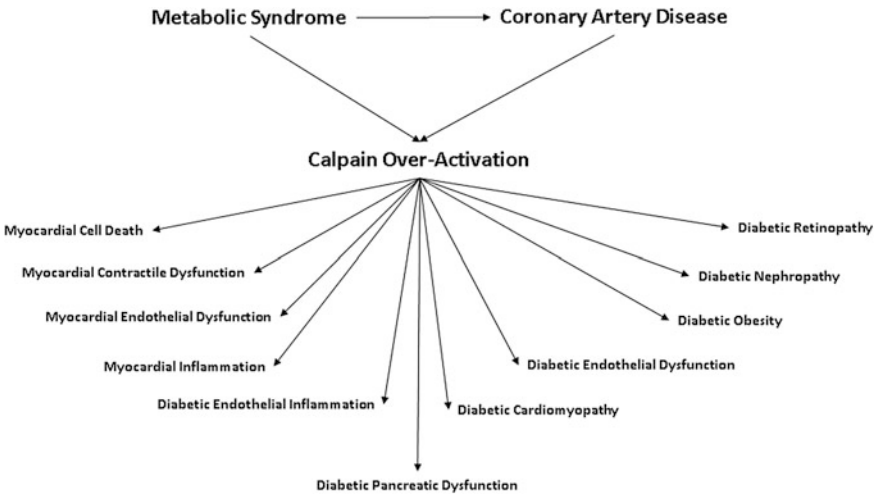

Fig. 1 Metabolic syndrome and coronary artery disease cause overactivation of calpain which has detrimental effects on various organs. Calpain is overactivated in various tissues during times of stress. This over activation of calpain has been shown to contribute to the organ dysfunction seen in patients with metabolic syndrome and coronary vascular disease

syndrome but so far the mechanism through which arterial disease is being accelerated in this group of patients has not been identified. Calpain is a protease whose overactivity has been found to promote coronary disease, insulin resistance, retinopathy, renal disease, and obesity. Overactivation of calpain has been shown to contribute to cardiovascular disease and its associated comorbidities [2–6] (Fig. 1). Moderate calpain inhibition in the setting of hypercholesterolemia and chronic myocardial ischemia has been found to improve proangiogenic protein expression, microvascular relaxation, and myocardial perfusion [5–7]. Therefore, calpain inhibition may be a mechanism through which to treat the coronary vascular disease associated with metabolic syndrome.

2 Calpains

Calpains are a class of calcium activated, intracellular, non-lysosomal cysteine proteases which play a key role in maintaining cellular homeostasis through their proteolytic activity [8]. In conjunction with all cysteine proteases, calpains contain the amino acid cysteine in their active site. The three major groups of cysteine proteases include caspases, cathepsins, and calcium-dependent calpains [4]. When not active, calpain is predominantly situated in the cytosol. Once calpain becomes activated it translocates to the membrane. Calpain works through cleavage of its substrates which results in proteolysis of several cytoskeletal proteins, membrane proteins, enzymes, cytokines, and transcription factors [9]. Excessive activation

of calpain has been found to be a part of the pathophysiology leading to several disorders including ischemia reperfusion injury, trauma, diabetes mellitus, coronary vascular disease, and inflammation [4, 8]. Therefore, an effective calpain inhibitor may serve as a beneficial potential medical therapy for patients suffering from a number of diseases including coronary artery disease and metabolic syndrome.

The calpain family is similar in many different species (ranging from fungi to humans). In mammals, there are 15 isoforms of calpain. Some calpains are expressed ubiquitously (calpains 1, 2, 4, 5, 7, and 10) while others are thought be found in specific tissues. For example, calpain 1 and 2 are thought to be located in endothelial cells, calpain 3 in skeletal muscle, calpain 6 in placenta, calpain 8 in smooth muscle, calpain 9 in the stomach, calpain 11 in the testes, calpain 12 in the skin and calpain 13 in the testes and lung [9, 10]. Ten of the 15 calpain isoforms are thought have been found to be expressed in the heart. Calpain 1 (U-calpain) and calpain 2 (m-calpain) are the most well studied of the calpains [4].

Among the 15 isoforms of calpains found in mammals, 14 are large subunit members (80-kDa catalytic subunit), and there is 1 small subunit member (30-kDA subunit). One endogenous inhibitor (calpastatin) also exists [9]. Calpains can be divided into two groups based on the structure of domain IV (Typical and Atypical). Typical calpains (1, 2, 3, 8, 9, 11, 12, 14) contain a penta-EF motif in domain IV that can bind calcium, the calpain small subunit or calpastatin. Only calpains 1, 2, and 9 have the ability to bind to another calpain small subunit and "dimerize" Atypical calpains (5, 6, 7, 10, 12, and 15) do not have a penta-EF motif in domain IV. They are therefore unable to dimerize or be inhibited by calpastatin [9].

In general, calpains contain four structural domains. In some of the typical calpains (1, 2, and 9), domain I is cleaved after calcium activation. This cleavage is termed autolysis and it leads to autoactivation of the protease. In calpain 10, a calpain found to be a key player in diabetes, domain 1 contains a mitochondrial targeting sequence. The function of domain 1 for the atypical calpains is unknown [9]. Domain II contains the catalytic active site. This active site is described as the "catalytic triad" which consists of cysteine, asparagine, and histidine. The catalytic active site is the functional unit of the protease and it is present in both typical and atypical calpains (except for calpain 6 which lacks any proteolytic activity). Domain II also has the ability to assist in autoactivation by binding two atoms of calcium ion. Domain III contains a phospholipid-binding motif and two Ca^{2+} binding sites. These structures are also present in both typical and atypical calpains (except Calpain 10). Domain III also plays a role in substrate recognition and works to regulate calpain activity through specific electrostatic interactions [9] (Fig. 2). As mentioned, domain IV contains the penta-EF domain that can bind calcium, the calpain small subunit or calpastatin. As mentioned, atypical calpains have the same general structure for domain I, II, and III but they do not have a penta-EF motif on domain IV. Due to these differences in structure, it is thought that atypical domains have different activation and inhibition requirements than typical domains [9].

Calpain 4 has a unique structure consisting of domain V and VI. Calpain 4 is a small calpain subunit that dimerizes with domain IV of the typical calpains. Domain V binds the C-terminus region of domain IV on the large typical calpain

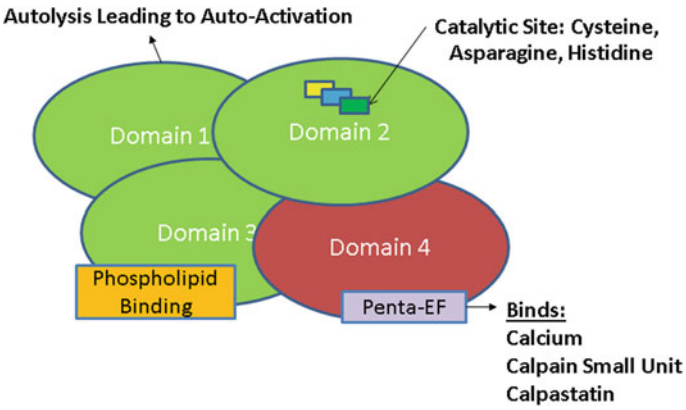

Fig. 2 Schematic of typical calpain structure. Most calpain isoforms contain four structural domains. Calpains are divided into two groups (typical and atypical) based on the structure of domain IV. Typical calpains contain a penta-EF motif in domain IV that can bind calcium, the calpain small subunit or calpastatin. Atypical calpains do not have a penta-EF motif in domain IV. Most calpain isoforms contain four structural domains. In some of the typical calpains, domain I is cleaved after calcium activation a term called autolysis which leads to autoactivation. Domain II contains the catalytic active site known as the "catalytic triad" of cysteine, asparagine and histidine. Domain III contains a phospholipid-binding motif and two Ca^{2+} binding sites

subunits. Domain VI contains a penta-EF domain that, similar to the penta-EF domain on domain IV of the typical calpains, can bind to dimerize with another calpain or bind calcium [9].

3 Calpastatin

Calpastatin is an endogenous protein calpain inhibitor. It is believed to be specific to typical calpains. Interestingly, calpastatin preferentially binds to calcium-activated calpains. This suggests that calpastatin inhibition does not interfere with basal calpain activity which is required for normal homeostasis [11]. Calpastatin has eight splice variants ranging in size from 18.7 to 85 kDA. The largest spice variant contains six domains (I, II, III, IV, L, and XL). Domains I–IV contain subdomains (A–C) that play an essential role calpain inhibition. These domains range in effectiveness as follows: I > IV > III > II. Domain XL contains three protein kinase A phosphorylation sites. The function of domain L is unknown. Calpastatin binds to domain II, IV or VI therefore it only inhibits typical calpains [9].

4 Calpain and Coronary Artery Disease

4.1 Calpain Overactivity and Myocardial Cell Death

In normal metabolic conditions, calpain 1 is active in myocytes. Calpain is responsible for preventing abnormal accumulation of proteins via the ubiquitin/proteasome protein degradation pathway. Overactivation of calpain is an important component of the mechanism that leads to detrimental mitochondrial permeability, aberrant apoptotic cell death, and ischemia/reperfusion injury in the heart [9].

Myocardial ischemia results in dysregulation of calcium homeostasis which leads to calpain overactivation and results in myocardial cell injury. Calpain activity is mainly regulated by calcium binding and that calcium binding is required for its proteolytic activity. It is also known that calcium overload causes auto proteolysis of the N-terminal peptide of calpain contributing to its overactivation [9]. However, studies also suggest that calpain activity is regulated by other means. For example, nicotinamide adenine dinucleotide phosphate (NADPH) is a co-factor used in anabolic reactions. NADPH oxidase has been found to induce calpain activation in stressed cardiomyocytes via upregulation of reactive oxygen species [11].

The mechanism through which calpain inhibition has a beneficial effect on the heart in the setting of stress is not fully known but there are many theories. One thought is that calpain cleaves proteins involved in apoptotic cell signaling including caspase 3, 7, 8, 9, 12, Bcl-2, Bcl-xl, Bid, Bax, and NF-kB [6, 11]. In the presence of a calpain inhibitor, the expression levels of these proteins are modulated and apoptosis is decreased [6, 11]. Additional calpain substrates include the transcription factors c-mos, YY1, c-fos, and c-jun and pro-apoptotic protein p53 [12, 13]. Specifically calpain cleaves the transcription factor YYI which decreases myogenic transcription [14].

Calpains also play a role in regulating mitochondrial function. In the setting of calcium overload (or mitochondrial stress), calpains located in the mitochondrion have been reported to cleave of Apoptosis Inducing Factor (AIF). This cleavage allows it to translocate to the nucleus where it functions to induce DNA degradation. In the presence of a calpain inhibitor, this process is reduced leading to reduced cardiac cell death [15].

4.2 Calpain Overactivity and Myocardial Contractile Dysfunction

Calpain overactivity has been found to cause cardiac dysfunction. Upregulation of calpain has been found to exacerbate the remodeling that takes place during myocardial infarction. This remodeling is associated with contractile dysfunction, chamber dilatation, and reduced overall function. Interestingly, one study found that calpain-mediated proteolysis is increased in the chronic phase (7 days or later) but

not in the acute phase (before 24 h) after a myocardial infarct and that calpain activity seemed to be located in the border zone of ischemia [8]. Calpain has also been implicated in the remodeling that leads to development of the atrial fibrillation seen in patients with diabetes and valvular heart disease [16]. There are a number of mechanisms through which this remodeling of cardiac tissue may occur.

In diseased myocardial tissue, ischemia induces changes to cytoskeletal, contractile, and myofibrillar proteins. Calpain has been found to cleave myofibrillar specific proteins including N-cadherin, connexin 43, troponin T, troponin I, troponin T, titin, α-fodrin, and desmin in cardiomyocytes [8, 11, 13, 17]. In the ischemic myocardial territory after a myocardial infarct, research has found that there is decreased expression of the important cytoskeletal protein N-cadherin and upregulation of molecular markers for cardiac hypertrophy and fibroses. Importantly, calpain is known to cleave N-cadherin and calpain overexpression is assocaited with decreased expression levels other cytoskeletal markers including B-catenin and connexin 43. These changes are attenuated in the presence of a calpain inhibitor [8].

Troponin I is a cardiac subunit of the troponin complex and is a component of cardiac muscle contraction. Calpain induces breakdown of troponin I in chronically ischemic cardiomyocytes [13]. This breakdown has been found to be associated with systolic dysfunction. Interestingly, overexpression of calpastatin and the presence of a calpain inhibitor have been found to improve this contractile dysfunction [13].

Intracoronary infusion of high calcium levels leads to overload-induced cardiac dysfunction through proteolysis of α-fodrin. A-fodrin is a member of the sarcolemma membrane. Calpain inhibition downregulates this proteolysis and protects the heart from ischemia/reperfusion injury possibly through a conformational change to the L-type calcium channel receptor on the cell membrane which results in protection of left ventricular function [2, 18].

4.3 Calpain Overactivity and Myocardial Endothelial Cell Dysfunction

Endothelial cells are integral to multiple homeostatic functions including blood vessel formation, coagulation, vascular tone, angiogenesis, inflammation, and cell–cell barrier. Calpain plays an integral role in modulating endothelial cell function [19]. Interestingly, calpain is known to cleave numerous cytoskeletal proteins of the endothelial cell including filamin, talin, paxillin, spectrin, vinculin, α tubulin, and vimentin [9, 11, 12, 20]. Angiotensin II is an effector peptide in the renin–angiotensin system which is often dysregulated in the setting of arterial disease and metabolic syndrome. Angiotensin II release induces matrix metalloproteinase-2 (MMP-2) and calpain-1 expression and activity in the smooth muscle of the arterial wall. It is thought that the calpain-1 overactivity induces MMP-2 activation which modulates extracellular matrix remodeling and causes increased collagen I and III production leading to vascular calcification [21]. Calpain is also thought to lead to the increase of transforming growth factor beta 1 (TGF-β1) expression which is a

major profibrotic factor causing the activation of downstream signaling pathways including collagen I synthesis [22].

Additionally, oxidized low-density lipoprotein (oxLDL), which is present in many patients with vascular disease and metabolic syndrome, induces apoptosis in endothelial cells leading to the development of atherosclerosis. OxLDL activates calpain which cleaves proteins on the cytoskeletal membrane and many apoptotic proteins. Inhibition of calpain reverses these negative effects in endothelial cells [23].

In hypoxic retinal endothelial cells, hypoxia-induced calpain activity has been found to lead to a disorganized actin cytoskeleton which leads to neovessel formations that are defective both functionally and architecturally. Moderate calpain inhibition has been found to reduce these architectural abnormalities, reduce vascular leakage, and ultimately lead to a more functional vessel that reduces overall hypoxia. It is thought that this calpain inhibition works by stabilizing and organizing the actin cytoskeletal in the retinal endothelial cells that are undergoing capillary morphogenesis. The result is improved actin cables within the new blood vessels [19, 24]. Interestingly, while moderate calpain inhibition has been shown to be beneficial, complete suppression results in nonfunctional endothelial cells. The optimal amount of calpain inhibition appears to be about 30–35% inhibition [19, 24].

Vascular Endothelial Grown Factor-A (VEGF-A) therapy has been found to induce angiogenesis, however this new vascular growth has been found to be highly abnormal in pathological settings. Interestingly, this vascular growth has been associated with increased calpain activity in endothelial cells. Calpain inhibition has been found to lead to a functional angiogenesis which reduces myocardial infarct size and improves contractile function and hemodynamics in large animal model of cardiac ischemia/reperfusion [25].

A large animal model of chronic myocardial ischemia in the setting of metabolic syndrome shows that calpain activity is increased in the ischemic myocardium. Calpain inhibition improves collateral dependent perfusion, improves endothelial-dependent microvessel relaxation and increases expression of proteins involved in vasodilatation. Furthermore, calpain inhibition promotes expression of the survival proteins, decreases oxidative stress and inhibits apoptotic pathways in animals with metabolic syndrome and chronic myocardial ischemia [5, 6].

4.4 Calpain Overactivity and Myocardial Inflammation

Calpain activity also promotes inflammatory pathways [20]. The proinflammatory cytokine tumor necrosis factor-alpha (TNF-α) is released after myocardiali schemia/reperfusion injury possibly leading to cardiomyocyte apoptosis [11]. In the setting of sepsis, calpain activation is associated with overexpression of TNF-α and nitric oxide (NO) which leads to depressed myocardial contractile function [4, 11].

Calpain cleaves and leads to the degradation of IkBα. This degradation allows transcription factor NF-κB to translocate from the cytosol to the nucleus [20, 26]. NF-kB then binds to the promoter region of genes which serve as inflammatory mediators including interleukin-1 (Il-1), interleukin-6 (IL-6), vascular molecule-1

(VCAM-1), inducible nitric oxide synthase (iNOS), TNF-α and cyclooxygenase-2 (Cox-2) [4, 9].

In cultured rat cardiomyocytes, lipopolysaccharide induces calpain overactivity and is associated with increased TNF-α expression. The same study showed that calpastatin attenuates this TNF-α expression and improves cardiac function in a small animal model of endotoxemia [11].

5 Calpain and Metabolic Syndrome

Interestingly, calpain overactivation has been implicated in the pathogenesis of many of the aspects of metabolic syndrome including diabetes, obesity, diabetic nephropathy and diabetic retinopathy [10].

5.1 Calpain Overactivity and Diabetic-Induced Inflammatory Pathways

Overactivation of the inflammatory response causes damage to blood vessels and surrounding tissues. The endothelial cells of the vascular tract direct leukocytes to the site of injury. This is referred to as leukocyte trafficking. Pathologic leukocyte trafficking leads to vascular disease [20]. The inappropriate activation of calpain has been found to change platelet function, partially degrade proteins resulting in hyperaggregability and increase leukocyte trafficking in the microcirculation of NIDDM (noninsulin-dependent DM). Interestingly, inhibition of calpain activity attenuates leukocyte–endothelium interactions [10]. Therefore, inhibition of calpain activity may improve myocardial function by the means of the attenuation of tissue leukocyte infiltration and endothelial cell activation in the inflamed coronary vasculature [4].

5.2 Calpain Overactivity and Diabetic Pancreatic Dysfunction

Glucose metabolism triggers the entry of calcium into the pancreatic islets of Langerhans β-cells leading to exocytosis of insulin granules. Hyperglycemia in type 2 diabetes is caused by both impaired insulin secretion from these β-cells and insulin resistance. Patients with diabetes demonstrate not only a deficit in β-cell function, but they also experience an increase in β-cell apoptosis.

Calpain-10 is the first diabetic gene identified. Calpain 10 plays a role in the actin reorganization required for glucose-stimulated insulin release from the pancreatic β-cells. Calpain 10 regulates both insulin-stimulated glucose metabolism and insulin secretion in pancreatic islet cells [27]. Calpain inhibition has been found to lead to decreased insulin secretion [28, 29]. Calpain also plays a key role in the pathophysiology of beta-cell death in patients with type 2 diabetes [30]. It is

thought that elevated plasma-free fatty acids lead to β-cell apoptosis via an interaction between calpain-2 and endoplasmic reticulum stress-induced apoptotic factor (CHOP) [31].

5.3 Calpain Overactivity and Diabetic Cardiomyopathy

Diabetic cardiomyopathy is distinct from the coronary vascular disease that develops in patients with metabolic syndrome. Diabetic cardiomyopathy is defined as the ventricular dysfunction that occurs in patients with diabetes [32]. Calpain activity is increased in the acute inflammatory processes associated with the cardiac hypertrophy of diabetic cardiomyopathy [20]. Elevated glucose levels lead to increased levels of free fatty acids and growth factors. This results in dysregulation of substrate supply, calcium levels and lipid metabolism. Elevated glucose levels also increase production of reactive oxygen species causing oxidative stress leading to cardiomyocyte apoptosis. Reactive oxygen species also lead to stimulation of connective tissue growth factor which causes myocardial fibrosis and the formation of glycation end products. Together this leads to the increased cardiac stiffness associated with diabetic cardiomyopathy [32]. Calpain inhibition is associated with decreased (1) left ventricular hypertrophy, (2) perivascular inflammation and (3) fibrosis in myocardial tissue in mouse models of diabetes [33, 34].

5.4 Calpain Overactivity and Diabetic Endothelial Dysfunction

Calpain is overexpressed in diabetic vasculature. Inhibition of this overactivity attenuates the vascular dysfunction associated with chronic diabetic mellitus [20, 35].

High glucose levels lead to calcium overload and overactivation of calpain in endothelial cells. This is associated with the formation of reactive oxygen species, mitochondrial superoxide generation, cellular apoptosis, and endothelium-dependent vascular dysfunction in the setting of diabetes. Inhibition of calpain prevents glucose-induced reactive oxygen species expression and production of mitochondrial superoxide generation. This leads to decreased endothelial apoptosis and improved endothelium-dependent relaxation [35, 36].

A proposed mechanism of this beneficial effect on endothelial cells is that calpain inhibition leads to the increased expression of endothelial nitric oxide (NO) and attenuates expression of Intercellular Adhesion Molecule 1 (ICAM-1) and VCAM-1 in the diabetic vasculature [20, 37]. In diabetic rats there is decreased expression levels of endothelial heat shock protein 90 (hsp90) and endothelial nitric oxide synthase (enols). It is thought that calpain regulates hsp90 which serves as a dock site for eNOS activation by AKT. Inhibition of calpain activity in these vessels restores eNOS/hsp90 interaction, increases NO release and attenuates leukocyte trafficking [20, 37].

5.5 Calpain overactivity and Obesity

Calpain 10 plays an important role in regulating diabetic obesity. This obesity has been partially contributed to inadequate diet and sedentary lifestyle. However, one study has shown that in obese patients there is a significant association between having the genotype 1/1 (SNP19 of Calpain10) and the phenotype of excess weight. This relationship exists even in patients who have an active lifestyle [38]. Similarly, calpain expression is greater in obese rats with diabetes than in lean rats with diabetes. Exercise and weight reduction have been found to be associated with decreased calpain expression in the skeletal muscle of diabetic rats [10]. In fact, exercise reduces calpain mRNA expression in a rat model of diabetes. Finally, calpain activity is thought to reduce glucose transport turnover in skeletal muscle. Therefore, calpain may modulate muscle glucose equilibrium and mass [10].

5.6 Calpain overactivity and Diabetic Nephropathy

Diabetic nephropathy is the leading cause of end stage renal disease [27]. The kidney is responsible for maintaining ion, water, and metabolic substrate homoeostasis. Diabetic nephropathy is characterized by the loss of the charge barrier and the expansion of the molecular matrix of the glomerular basement membrane [27, 39]. Calpains degrade cytoskeletal proteins leading to increased plasma membrane permeability, water influx, and eventual necrosis in renal proximal tubular cells [9, 15, 40–43].

Calpain overactivation is also associated with acute renal cell death [15]. In the late phase of renal proximal tubule cell injury there is an influx of calcium into the cell. This influx leads to the overactivation of calpain. Inhibition of calpain blocks this renal proximal tubule cell death [40]. Interestingly, calpain 10 protein expression is decreased in the kidney of aging rats. This decreased expression is attenuated by caloric restriction. In humans, calpain 10 protein and mRNA levels decrease linearly in kidney samples with age as renal function decreases [44]. This contributes to the idea that basal calpain expression is necessary for appropriate cell function but overactivity of calpain in the setting of stressful cellular conditions is detrimental.

5.7 Calpain Overactivity and Ischemic Retinopathy

Retinopathy caused by endothelial dysfunction is a serious comorbidity of both coronary artery disease and metabolic syndrome. Diabetic retinopathy is a leading cause of blindness worldwide [45]. Patients with diabetic retinopathy develop diabetic macular edema, proliferative retinopathy, and retinal blood vessel dysfunction [46]. Calpain hyperactivation has been implicated in this retinal pathology. Calpain expression has been found to be increased in patients with diabetic retinopathy and cataract formation [47]. In a mouse model of ischemic retinopathy

vascular endothelial growth factor induced neovessel formation was not found to relieve hypoxia. However, in the same mouse model, the addition of moderate calpain inhibition normalized pathological retinal neovessel formation and relieved the underlying hypoxia. Calpain inhibition functions by (1) improving the architecture and function of the new vessels and (2) improving vascular regrowth. A mechanism through which calpain inhibition is thought to work is by improving the organization of the endothelial tau protein and actin cytoskeleton in the newly formed vessels [19, 24]. In cultured monkey cells, hypoxia/reoxygenation was found to lead to apoptotic rod, cone, and Muller cell death by activation of calpain. This retinal cell death was inhibited by adding a calpain inhibitor [48].

6 Conclusion

Coronary artery disease and metabolic syndrome together are two major causes of death in the United States and the number of people afflicted by these diseases is increasing around the world [10]. Calpain overactivity is implicated in the pathophysiology of coronary artery disease and its associated comorbidities (obesity, hypertension, hyperlipidemia, insulin intolerance). Currently, there are no medications available that will treat both the components of metabolic syndrome and its associated coronary artery disease. In fact, there is limited evidence that treatment with metformin has a beneficial effect on the risk factors for the development of atherosclerosis [49]. Therefore, calpain inhibition may offer a novel potential medical therapy for treating not only coronary artery disease but also the individual aspects of the associated comorbidities.

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References

1. Malik S (2004) Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. Circulation 110(10):1245–1250
2. Yoshikawa TMY, Hagihara H, Ohga Y et al (2004) S Calpain inhibitor-1 protects the rat heart from ischemia-reperfusion injury: analysis by mechanical work and energetics. AJP Heart Circ Physiol 288(4):H1690–H1698
3. Hayashi M, KasaiY, Kawashima S (1987) Preferential localization of calcium-activated neutral protease in epithelial tissues. Biochem Biophys Res Commun 148(2):567–574
4. Tissier S, Lancel S, Marechal X et al (2004) Calpain inhibitors improve myocardial dysfunction and inflammation induced by endotoxin in rats. Shock 21(4):352–357
5. Sabe AA, Potz BA, Elmadhun NY et al (2016) calpain inhibition improves collateral dependent perfusion in a hypercholesterolemic swine model of chronic myocardial ischemia. J Thorac Cardiovasc Surg 151(1):245–252

6. Potz BA, Sabe AA, Elmadhun NY et al (2015) Calpain inhibition decreases myocardial apoptosis in a swine model of chronic myocardial ischemia. Surgery 158(2):445–452
7. Potz BA, Sabe AA, Abid MR et al (2016) Calpains and coronary vascular disease. Circ J80(1):4–10
8. Kudo-Sakamoto Y, Akazawa H, Ito K et al (2014) Calpain-dependent cleavage of N-cadherin is involved in the progression of post-myocardial infarction remodeling. J Biol Chem 289(28):19408:19419
9. Smith MA, Schnellmann RG (2012) Calpains, mitochondria, and apoptosis. Cardiovasc Res 96(1):32–37
10. Pandurangan M, Hwang I, Orhirbat C, Jieun Y et al (2014) The calpain system and diabetes. Pathophysiology 21(2):161–167
11. Li X, Li Y, Shan L et al (2009) Over-expression of calpastatin inhibits calpain activation and attenuates myocardial dysfunction during endotoxaemia. Cardiovasc Res 83(1):72–79
12. Kubbutat MH, Vousden KH (1997) Proteolytic cleavage of human p53 by calpain: a potential regulator of protein stability. Mol Cell Biol 17(460):468
13. Kositprapa C, Zhang B, Berger S et al (2000) Calpain-mediated proteolytic cleavage of troponin I induced by hypoxia or metabolic inhibition in cultured neonatal cardiomyocytes. Mol Cell Biochem 214(1–2):47–55
14. Walowitz JL, Bradley ME, Chen S et al (1998) Proteolytic regulation of the zinc finger transcription factor YY1, a repressor of muscle-restricted gene expression. J Biol Chem 273(12):6656–6661
15. Liu X, Rainey JJ, Harriman JF et al (2001) Calpains mediate acute renal cell death : role of autolysis and translocation. Am J Physiol Ren Phsiology 281(4):728–738
16. Zhao Y, Cui G, Zhou N et al (2016) Calpain-calcineurin-nuclear factor signaling and the development of atrial fibrillation in patients with valvular heart. Dis Diab J Diab Res 1:7
17. Streng AS, De Boer D, van der Velden J et al (2013) Posttranslational modifications of cardiac troponin T: an overview. J Mol Cell Cardiol (63):47–56
18. Takeshita D, Tanaka M, Mitsuyama S et al (2012) A new calpain inhibitor protects left ventricular dysfunction induced by mild ischemia-reperfusion in in situ rat hearts. J Physiol Sci 113:123
19. Hoang MV, Nagy JA, Fox JE et al (2010) Moderation of calpain activity promotes neovascular integration and lumen formation during VEGF-induced pathological angiogenesis. PLoS One 5(10)
20. Stalker TJ, Skvarka CB, Scalia R (2003) A novel role for calpains in the endothelial dysfunction of hyperglycemia. FASEB J 17(11):1511–1513
21. Jiang L, Zhang J, Monticone RE et al (2012) Calpain-1 regulation of matrix metalloproteinase 2 activity in vascular smooth muscle cells facilitates age-associated aortic wall calcification and fibrosis. Hypertension 60(5):1192–1199
22. Li FZ, Cai PC, Song LJ et al (2015) Crosstalk between calpain activation and TGF-β1 augments collagen-I synthesis in pulmonary fibrosis. Biochim Biophys Acta 1852(9):1796–1804
23. Porn-Ares MI, Saido TC, Andersson T et al (2013) Oxidized low-density lipoprotein induces calpain-dependent cell death and ubiquitination of caspase 3 in HMEC-1 endothelial cells. Biochem J 411(374):403–411
24. Hoang MV, Smith LE, Senger DR (2011) Calpain inhibitors reduce retinal hypoxia in ischemic retinopathy by improving neovascular architecture and functional perfusion. Biochim Biophys Acta 1812(64):997, 1549–1557
25. Khalil PN, Neuhof C, Huss R et al (2005) Calpain inhibition reduces infarct size and improves global hemodynamics and left ventricular contractility in a porcine myocardial ischemia/reperfusion model. Eur J Pharmacol 528(1–3):124–131
26. Letavernier E, Zafrani L, Perez J et al (2012) The role of calpains in myocardial remodelling and heart failure. Cardiovasc Res 96(1):38–45
27. Wan TT, Li XF, Sun YM et al (2015) Role of the calpain on the development of diabetes mellitus and its chronic complications. Biomed Pharmacother 74(187):190
28. Pablo P, Salazar AM, Burns AL et al (2014) Role of calpain-10 in the development of diabetes mellitus and its complications. Arch Med Res 45(2):103–115

29. Derbel S, Doumaguet C, Hubert D et al (2006) Calpain 10 and development of diabetes mellitus in cystic fibrosis. J Cyst Fibros 5(1):47–51
30. Huang CJ, Gurlo T, Haataja L et al (2010) Calcium-activated calpain-2 is a mediator of beta cell dysfunction and apoptosis in type 2 diabetes. J Biol Chem 285(1):339–348
31. Cui W, Ma J, Wang X et al (2013) Free fatty acid induces endoplasmic reticulum stress and apoptosis of beta-cells by Ca^{2+}/Calpain-2 pathways. PLoS One 8(3)
32. Falcão-Pires I, Leite-Moreira AF (2012) Diabetic cardiomyopathy: understanding the molecular and cellular basis to progress in diagnosis and treatment. Heart Fail Rev 17(3):325–344
33. Letavernier E, Perez J, Bellocq A et al (2008) Targeting the calpain/calpastatin system as a new strategy to prevent cardiovascular remodeling in angiotensin II-induced hypertension. Circ Res 102(6):720–728
34. Li Y, Ma J, Zhu H et al (2011) Targeted inhibition of calpain reduces myocardial hypertrophy and fibrosis in mouse models of type 1 diabetes. Diabetes 60(11):2985–2994
35. Chen B, Zhao Q, Ni R et al (2014) Inhibition of calpain reduces oxidative stress and attenuates endothelial dysfunction in diabetes. Cardiovasc Diabetol 13(88):1–12
36. Kumar SS, Kain V, Sitasawad SL (2012) High glucose-induced Ca^{2+} overload and oxidative stress contribute to apoptosis of cardiac cells through mitochondrial dependent and independent pathways. Biochim Biophys Acta 1820(7):907–920
37. Stalker TJ, Gong Y, Scalia R (2005) The calcium-dependent protease calpain causes endothelial dysfunction in type 2 diabetes. Diabetes 54 (4):1132–1140
38. Orozco AC, Muñoz AM, Velásquez CM et al (2014) Variant in CAPN10 gene and environmental factors show evidence of association with excess weight among young people in a Colombian population. Biomedica 34(4):546–555
39. Hovind P, Rossing P, Tarnow L et al (2001) Progression of diabetic nephropathy. Kidney Int 59(2):702–709
40. Waters SL, Sarang SS, Wang KKW et al (1997) Calpains mediate calcium and chloride influx during the late phase of cell injury 2. J Pharmacol Exp Ther 283(3):1177–1184
41. Harriman JF, Waters-williams, Chu DL et al (2000) Efficacy of novel calpain inhibitors in preventing renal cell death. J Pharmacol Exp Ther 294(3):1083–1087
42. Harriman JF, Liu XL, Aleo MD et al (2002) Endoplasmic reticulum Ca^{2+} signaling and calpains mediate renal cell death. Cell Death Differ 9(734):741
43. Liu X, Schnellmann RG (2003) Calpain mediates progressive plasma membrane permeability and proteolysis of cytoskeleton-associated paxillin, talin, and vinculin during renal cell death. J Pharmacol Exp Ther 304(1):63–70
44. Covington MD, Arrington DD, Schnellman RG (2009) Calpain 10 is required for cell viability and is decreased in the aging kidney. Am Physiol Ren Physiol 296(3):478–448
45. Ahsan H (2015) Diabetic retinopathy—biomolecules and multiple pathophysiology. Diabetes Metab Syndr 9(1):51–54
46. Lois N, McCarter RV, O'Neill C et al (2014) Endothelial progenitor cells in diabetic retinopathy. Front Endocrinol (Lausanne) 2014(5):44
47. Ahn YJ, Kim MD, Chung DK (2016) Calpain and caspase-12 expression in lens epithelial cells of diabetic cataracts. Am J Ophthalmol 167(31):37
48. Nakajima E, Hammond KB, Rosales JL et al (2011) Calpain, not caspase, is the causative protease for hypoxic damage in cultured monkey retinal cells. Investig Ophthalmol Vis Sci 52(10):7059–7067
49. El S, Rongen GA, De Boer RA et al (2011) The cardioprotective effects of metformin. Curr Opin Lipidol 22(445):453