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REVIEW ARTICLE

Troponin Proteins and Their Role in Physiological and Aging Process?

Manoj G Tyagi*, Nikhil Shukla and Madhab Lamsal

Department of Pharmacology, Christian Medical College, Vellore 632002, Tamilnadu

ABSTRACT

The troponins are regulatory proteins and have been extensively studied in human medicine. They are an integral component of the contractile apparatus of skeletal and cardiac muscle tissue. Finding an elevated cardiac troponin level implies diagnostic and prognostic information for humans with cardiovascular disease. Troponin assays are used primarily to diagnose acute myocardial infarction in patients with ischemic symptoms such as chest pain. However, elevated blood levels of Troponin may be found without any cause of myocardial injury. Three types of troponins have been identified i.e troponin I, T, and C. Human assays may be used in most animals due to significant homology in the troponin proteins between species. This review deals with the role of troponins in the physiological and possibly in aging process.

Keywords: troponin, proteins, aging physiological

Corresponding author

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INTRODUCTION

Physiological role of Troponins in the cardiovascular system

Troponins are regulatory proteins that are part of the contractile apparatus of skeletal and cardiac muscle tissue. With the proteins actin and tropomyosin, they are part of the thin filaments within the myofibrils and are essential for the calcium-mediated regulation of muscle contraction. The troponin complex consists of 3 interacting and functionally distinct proteins (troponin I, T, and C). It has been shown that extreme physical stress such as marathon and ultra-marathon races may lead to transitory elevation of troponin levels. It is believed that the troponin part of cytosol could fit in with liberation of troponin from the filaments in normal breaking-down processes. Well trained individuals will have a larger contractile apparatus than those with no training, and they will therefore have larger amounts of troponin in their cytosol. Tissue-specific isoforms exist for each type of troponin. Within the thin filament, tropomyosin dimers form a continuous chain along the groove of the actin helix. The troponin complex lies at regular intervals along the filament. Tropomyosin acts to block the myosin binding sites on actin. Each troponin protein has specific functions that regulate muscle contraction. This article focuses on troponin I and T and C due to their specificity for various physiological processes. Troponin C (TnC) is present in 2 isoforms. One isoform is present in fast-twitch muscle fibers and the other is present in both cardiac and slow-twitch muscle fibers. Homology between the cardiac isoform and 1 of the skeletal muscle isoforms reduces the cardiac specificity of TnC and therefore limits its diagnostic usefulness in heart disease [1, 2].

Troponin C binds calcium to initiate muscle contraction. Several isoforms of troponin T (TnT) exist in skeletal muscle. Cardiac troponin T (cTnT) has a molecular weight of approximately 37,000 Da. In human cardiac tissue 4 isoforms exist, but only 1 is characteristic of the adult heart [3]. The other 3 cardiac isoforms are expressed in fetal tissue [4]. The fetal isoforms may be re-expressed during heart failure or in damaged skeletal muscle. Troponin T attaches the troponin complex to tropomyosin and actin. Three isoforms exist for troponin I (TnI). Two isoforms are present in skeletal muscle and the other is present only in cardiac muscle. The cardiac isoform (cTnI), with a molecular weight of 24,000 Da, is larger than the other isoforms as it contains an additional 32 amino acid N-terminal peptide. The rest of the protein has greater than 40% dissimilarity in its amino-acid sequence compared with skeletal muscle TnI unlike cTnT, cTnI is not expressed in fetal skeletal muscle during development, nor after damage and regeneration in adult skeletal muscle. Troponin I inhibits actomyosin ATPase and prevents the structural interaction of myosin with actin-binding sites. The binding of calcium to troponin C displaces troponin I and causes a conformational change in tropomyosin so that it no longer interferes with myosin/actin binding and muscle contraction can occur. Mutations in the genes encoding for cTnT and cTnI cause hypertrophic cardiomyopathy in humans [5]. Conversely, a knock out cTnI mouse model develops acute heart failure at 18 days of age. Cardiac troponins T (cTnT) and I (cTnI) are regulatory proteins that control the calcium-mediated interaction between actin and myosin [6]. The skeletal and cardiac isoforms of cTnT and cTnI are distinct, and skeletal isoforms are not easily detected by the monoclonal antibody-

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based assays currently in use. This specificity for cardiac isoforms is the basis for the clinical utility of cTnT and cTnI assays [7, 8].

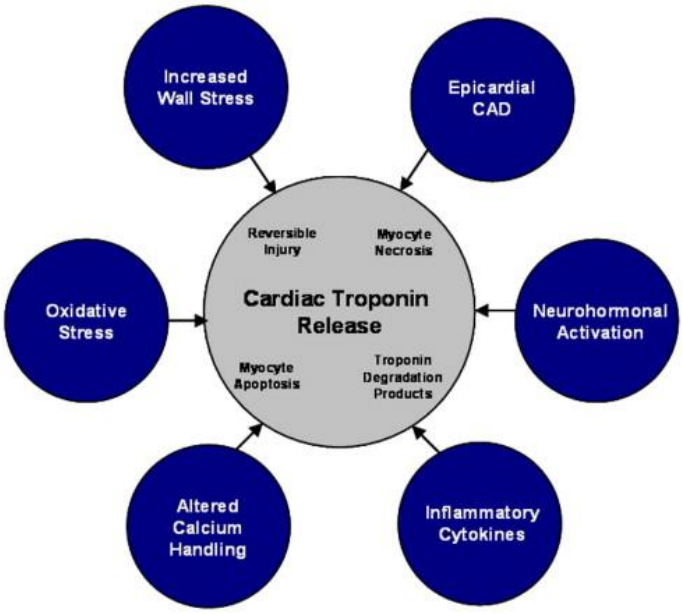

FIGURE 1

The troponin levels may be elevated in disease states. The most likely explanation is that elevated troponins reflect ongoing cardiomyocyte injury in HF [9]. Several processes could contribute to this injury. Reversible injury, from myocardial strain or subendocardial ischemia, could lead to transient changes in cell membrane permeability and leakage of cytosolic troponin. However, most of the troponin in cells is not free but bound to myofibrils, suggesting the presence of more severe injury. Myocyte necrosis may contribute to the release of troponins into the bloodstream of patients with HF [10,11], but it is controversial how much necrosis is sustainable over long periods of time in clinically stable patients [12]. Apoptosis also has been documented in failing hearts, although it is not known whether apoptosis leads to troponin release. Another interesting possibility is that troponin assays are detecting troponin degradation products that have been released into the bloodstream from proteolysis or turnover of myocardial contractile proteins [13,14]. Underlying triggers of either proteolysis or frank necrosis could possibly include oxidative stress, mechanical stretch, neurohormonal activation, or microvascular ischemia [15] (Figure 1).

Troponin T nuclear localization and its role in aging skeletal muscle

Troponin T (Tn-T) is known to mediate the interaction between Tn complex and tropomyosin (Tm), which is essential for calcium-activated striated muscle contraction [16]. This regulatory function takes place in the myoplasm, where Tn-T binds Tm. However, recent findings of troponin I and Tm nuclear translocation in Drosophila and mammalian cells imply other roles for the Tn-Tm complex. We hypothesized that Tn-T plays a nonclassical role through nuclear translocation. Immunoblotting with different antibodies targeting the NH2- or COOH-terminal region uncovered a pool of fast skeletal muscle Tn-T3 localized in the nuclear fraction

of mouse skeletal muscle as either an intact or fragmented protein. Construction of TnT3-DsRed fusion proteins led to the further observation that Tn-T3 fragments are closely related to nucleolus and RNA polymerase activity, suggesting a role for Tn-T3 in regulating transcription [17]. Functionally, overexpression of Tn-T3 fragments produced significant defects in nuclear shape and caused high levels of apoptosis. Interestingly, nuclear Tn-T3 and its fragments were highly regulated by aging, thus creating a possible link between the deleterious effects of Tn-T3 and sarcopenia. It is therefore proposed that changes in nuclear Tn-T3 and its fragments cause the number of myonuclei to decline with age, contributing to muscle damage and atrophy.

Molecular mechanism for troponin action

In vertebrate troponins, this regulation involves a Ca2+-dependent attachment of subunit TnC NH2 domain to a ~10-residue, amphipathic, "switch" helix of subunit TnI [18]. The switch helix is within a 9-kDa portion of TnI that appears to interact with actin and tropomyosin so as to shut off muscle contraction, specifically when Ca2+ alongwith the switch helix dissociate from the TnC regulatory domain. Ca2+-saturated cardiac and skeletal muscle troponin structures each have been solved by x-ray crystallography at high resolution [19]. Results agree with each other in many details, and the findings are important advances after many years when only much smaller portions of troponin had been solved at atomic resolution. On the other hand, the position of the TnC regulatory NH2 domain is different between these two structures. The present work represents a new approach to this subject: amide hydrogen exchange of troponin. Solvent-exposed amide hydrogens in peptides rapidly exchange with solution H (or D in D2O), with rates of ~10 s−1. In contrast, exchange is blocked by hydrogen bonding, such as that of most backbone amide hydrogens in folded proteins. Exchange rates in proteins do not correspond to unfolding or refolding rates. Rather, they are very much slower. This is because, under usual conditions, local refolding rates greatly exceed the H/D exchange rate of the unfolded region. Exchange at the slowest exchanging amides tends to be governed, i.e. protected from the solvent-exposed rate, by global protein folding stability. Other amide hydrogens exhibit faster exchange, intermediate between those of solvent-exposed hydrogens and core hydrogens, reflecting wide variation in local flexibility or folding stability across different regions of an overall folded protein [20]. The degree of H/D exchange protection is a measure of local folding stability within the context of a globally folded protein. By characterizing exchange rates at multiple sites, either by NMR or by mass spectrometry, the dynamic behavior of the specific protein can be mapped.

A linkage beween Troponin and Sirt1?

Sirtuin 1 (Sirt1), known as NAD-dependent deacetylase, belongs to class III histone/protein deacetylases and is a member of the silent information regulator (Sir2) family [21]. Sirt1 plays a pivotal role in a wide variety of cellular processes such as apoptosis/cell survival, endocrine signalling, chromatin remodelling, and gene transcription. Recent studies have suggested that Sirt1 is an important endogenous apoptosis inhibitor in cardiomyocytes. However, it is unclear whether Sirt1 also protects cardiomyocytes against apoptosis induced by palmitate and whether miR-195 targets Sirt1 in cardiomyocytes [22]. It would be interesting to

speculate an interaction between the Sirt 1 and troponins in the aging and cellular senescence. In particular, recent findings obtained from transgenic mice with cardiac-specific over expression of Sirt1, which demonstrated delayed aging and protection against oxidative stress in the heart [23]. It is proposed that activation of known longevity mechanisms in the heart may represent a novel cardioprotection strategy against aging and certain types of cardiac stress, such as oxidative stress.The most striking protein found differentially expressed was troponin T. The immuno-cytochemistry to confirm this data, first in kidneys from untreated rats. Troponin T was observed in the smooth muscle cells along the afferent arterioles in the kidneys.

Troponin phosphorylation and telomerase activity in cellular senescence

Cellular senescence is defined as an irreversible arrest of cell proliferation with maintenance of cell function, as against the apoptosis, which is defined as controlled autodigestion of the cell leading to the formation of apoptotic bodies within the intact cell plasma membrane [24]. Senescence and apoptosis are two distinct pathways. Senescent cells are resistant to programmed cell death which leads to accumulation of these cells in injured tissue, and may have substantial effects on healthy neighboring tissue, with long-term consequences for the aging process and disease states. Cellular senescence is an important phenomenon in decreased cellular function. Recently, it was shown that cellular senescence is induced in proliferating cells within a short period of time by oxidative stresses. It is interesting to note a co-relation in the interaction of troponins and telomerase activity. Is it possible that with aging there is decreased phosphorylation of troponins and it causes a subsequent decrease in telomerase activity and alters the telomere function and shape. A decreased cardiac troponin I phosphorylation and telomerase activity were also observed in aged cardiomyocytes [25]. Decreased troponin I phosphorylation and changes in the expression of various genes have been reported in cardiomyocytes from aged rat heart [26].Telomerase replaces telomeric repeat DNA lost during the cell cycle, restoring telomere length. This enzyme is thought to be expressed only during periods of cell replication, and its activity reflects the extent of proliferation. However, some recent studies have shown that telomerase activity is detectable in cardiomyocytes and may play an important role in protecting against cell death. It was reported that aging decreases telomerase activity by 31% in male rat heart cells. Oh & Schneider reported that telomerase activity and TERT expression are both markedly down-regulated in the adult rat heart [27].Therefore, the changes in telomerase activity as a marker of senescence is getting prominent attention in scientific circles.

Troponins and renal function

It appears that troponins do play a role in the kidney during pathophsysiological crisis. Many investigators hypothesize that uremic-induced skeletal myopathy may be responsible for increased troponins in renal failure. The hypothesis centers on the notion that uremia may promote re-expression of cardiac TnT from injured or regenerating skeletal muscle fibers. Indeed, the skeletal muscle from patients on maintenance hemodialysis has significant morphological changes by both electron and light microscopy. Early reports describe elevated serum TnT levels in patients with skeletal muscle injury or inflammatory myopathies in the

absence of any obvious history of myocardial ischemia. It is unlikely that elevated serum troponin is the result of decreased clearance by the failing kidney. Free TnT and bound TnT are relatively large molecules (37 and 77 kDa, respectively), similar in molecular weight to albumin (60 kDa), making it improbable that the kidney would be responsible for their clearance. Creatine kinase and its isoforms are of similar size and are mainly cleared by the reticulo-endothelial system, whereas myoglobin is smaller (18 kDa) and cleared by the kidney. It is possible that smaller immunoreactive troponin fragments are cleared by the kidney, but this remains to be clarified. Improvement in renal function after renal transplant does not appear to alter the occurrence of elevated serum troponin.

CONCLUSION

In conclusion this study indicates a novel role for troponin proteins in the physiological regulation and in aging process and cellular senescence. It appears to be a key protein in aging and cellular senescence. A comprehensive investigation into the cellular and molecular mechanisms of troponin in aging process is required.

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