Hypothesis: troponin degradation is one of the factors responsible for deterioration of left ventricular function in heart failure

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Abstract

A hypothesis is presented that explains one of the mechanisms by which a heart starts to fail. The hypothesis is that myocardial function of an overloaded or otherwise stressed heart may become impaired by cellular troponin degradation in vital cardiomyocytes. The troponins (I, T and C) regulate actin–myosin interaction, thereby controlling contraction and relaxation. Troponins have been shown to be targets of activated calpain I. This enzyme, that is activated by elevated intracellular Ca\textsuperscript{2+} concentrations, such as occurs during ischemia, degrades troponins, leading to impaired interaction between actin and myosin and, thereby, less contractile force. Several reports about troponin degradation in viable myocardium support this hypothesis. Also, results are discussed that demonstrate the presence of immunoreactive troponin fragments in plasma under conditions in which myocardial necrosis can be excluded or is unlikely. The hypothesis implicates that release of troponin and/or troponin degradation products is not specific for necrotic myocardium but may occur from viable myocardium as well. To test this hypothesis, several lines of research are suggested. If the hypothesis is not rejected in the near future, the concept that a positive troponin test reflects ‘even microscopic zones of myocardial necrosis’ as used by the Joint ESC/ACC Committee for the Redefinition of Myocardial Infarction [The Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction. Myocardial infarction redefined—A consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the Redefinition of Myocardial Infarction. Eur Heart J 2000;21:1502–1513], should be withdrawn.

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1. Introduction

Several changes in myocardial composition, structure and function mark the transition of compensated left ventricular hypertrophy to heart failure. It is not always certain whether these changes contribute to the transition to heart failure, or are merely consequences of heart failure. It is well established that in patients with heart failure the renin–angiotensin system is activated and ACE-inhibitors are a common treatment of heart failure [1]. Angiotensin II induces hypertrophy of cardiomyocytes and hyperplasia of cardiac fibroblasts [2], and oxidation of accumulated catecholamines may lead to oxyradical formation and oxidative stress [3]. Heart failure is also associated with downregulation and desensitization of the \( \beta_1 \)-adrenergic receptor due to chronic overstimulation, leading to systolic dysfunction [4]. The therapeutic benefits of \( \beta \)-blockers in patients with heart failure have been demonstrated in the CIBIS-II and MERIT-HF trials [5,6].

Patients with severe chronic heart failure have increased plasma levels of tumor necrosis factor (TNF) that have been positively correlated with plasma renin activity [7] and with the functional class of heart failure [8]. In cardiomyocytes isolated from failing hearts, cellular calcium handling is compromised. This leads to a blunted contractile response to an increase in cell length (or an increase in end-diastolic volume), to an increase in heart

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rate, and to addition of β-adrenergic agonist (like isoproterenol). In addition, the rates of contraction and relaxation are also depressed. Trabeculae isolated from failing rat hearts have slower rates and lower peak values of force development, compared with those from control hearts [9]. The concomitant changes in the Ca\(^{2+}\) transient are considered to be adaptive, instead of detrimental to force development [9]. Slower tension development may be a consequence of lower myosin ATPase activity, due to a shift from α-myosin heavy chain (MHC) to βMHC expression (considered as a reinduction of a fetal gene program) [10]. This slowing in shortening velocity has a negative influence on cardiac output, and consequently stimulates other adaptational mechanisms, such as Starling’s law and catecholamine excretion. If these compensatory mechanisms fall short, heart failure develops. Apoptotic cardiomyocytes are abundant in hearts from patients with end-stage heart failure [11,12]. Continuously occurring apoptosis of cardiomyocytes has been held responsible for the transition of compensated hypertrophy to heart failure, and for the progressive worsening of heart failure [13,14]. Although this hypothesis seems attractive, several of the techniques used to detect apoptotic myocytes have been criticized [15].

The hypothesis that failing myocardium is energy depleted has been investigated intensively [16,17]. In failing human myocardium the creatine pool and total creatine kinase (CK) activity are decreased, suggesting impaired ability to deliver ATP to energy-consuming systems [18]. Left ventricular myocardium of pressure-overloaded human hearts, studied by \(^{31}\)P-nuclear resonance spectroscopy, showed depressed myocardial CrP/ATP ratio [19,20] that improved upon relief of pressure-overload [20]. Myocardial deformation itself is a sufficient stimulus for intracellular signalling [21] and growth [22,23] in cardiomyocytes, and for collagen production in cardiac fibroblasts [24]. Cell stretch activates integrins, thereby transmitting mechanical signals to and from the cytoskeleton [25], but whether such stretch-induced activation of integrins plays a role in the transition to heart failure is unknown. Myofibrillar dysfunction in heart failure may also be caused by alterations in the properties and concentrations of troponins (Tn). Three subtypes of troponin exist: TnI (inhibitory), TnT (tropomyosin binding) and TnC (Ca\(^{2+}\) binding), all playing a particular role in the regulation of muscle contraction. Fine-tuning of the functions of troponins in vivo occurs by phosphorylation of specific amino acids in specific domains of the molecules. In failing myocardium, the phosphorylation state (i.e. the quantity of the phosphorylated protein relative to the total quantity of that protein) of TnI is lower (56%) than that in healthy myocardium (84%), leading to increased myofibrillar calcium sensitivity and lower maximal Mg\(^{2+}\)-ATPase activity [26]. cTnI released from injured myocardium is released in both oxidized and reduced forms, the oxidation being the result of intramolecular disulfide formation of two cysteines. Human cTnT contains no cysteine groups, and is, therefore, unable to form disulfide bonds [27]. Thus, changes in the state of phosphorylation and/or oxidation by extracellular and intracellular factors may modulate the function of troponins, thereby disturbing normal actin–myosin interaction and subsequent (force of) contraction. Moreover, any loss of troponins from the myofibrillar system will impair control of actin–myosin interaction, leading to impairment of contractile function.

2. Proposed mechanism of impaired ventricular function (Fig. 1)

In hypertrophied and failing myocardium, diastolic Ca\(^{2+}\) levels are increased due to multiple alterations in Ca\(^{2+}\) homeostasis. At increased intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)), neutral Ca\(^{2+}\)-activated protease I (calpain I) becomes activated. A prolonged activation of calpain I has deleterious consequences for its target proteins, such as troponin-I and troponin-T. The troponins are responsible for the interaction between actin and myosin, thereby controlling the formation of cross-bridges. If the troponins undergo proteolysis by activated calpain I, their regulatory function is lost, leading to impairment of contraction and relaxation. These effects will become evident only if synthesis of new troponins cannot keep pace with their destruction by calpain. The consequences will be (i) deterioration of myocardial contractile function, leading to ventricular dilatation and eventually heart failure, and (ii) appearance of troponin degradation products in the circulation that, if containing the immunogenic epitope, react positively with the anti-troponin antibodies used in currently available troponine tests.

![Fig. 1. Hypothetical scheme of events leading to heart failure.](image-url)
The following sections show evidence that supports the hypothesis.

2.1. Increase of calpain I activity

Calpain I is an enzyme that becomes activated by Ca\(^{2+}\) ions in \(\mu\)mol concentrations. However, the chance that \([Ca^{2+}]_i\), becomes as high as 1 \(\mu\)M is small. To date it is unknown whether a small activation of calpain I by sub-micromolar \(Ca^{2+}\) concentration is sufficient to attack troponins, or whether intracellularly \(Ca^{2+}\) concentration is higher than average in or near specific compartments. It has been demonstrated that calpain I activity rises in the early phases of ‘chemical anoxia’ of cardiomyocytes incubated with iodoacetamide (to block glycolysis) and sodium cyanide (to block mitochondrial oxidation). This rise of calpain I activity started before onset of cell death [28]. Recently, calpain activity has been shown to be increased in right atrial appendages of patients with atrial fibrillation, and increased calpain activity has been correlated with several parameters of structural and electrical remodeling of atrial tissue [29]. In the pathogenesis of atrial fibrillation, calcium overload plays a key role [30]. One of the mechanisms that cause increased \([Ca^{2+}]_i\), is stretch of the myocardium [31], and subsequent calpain I activation may lead to proteolytic degradation of cytoskeletal [32], contractile [33,34], and L-type \(Ca^{2+}\) channel proteins [35].

2.2. Troponin degradation

The definition of ‘stunned’ myocardium is postischemic prolongation of contractile dysfunction in the absence of necrosis [36]. Several explanations of this phenomenon have been given [37]. One of these explanations, presented by Gao et al. [38], is that ischemia and subsequent reperfusion cause \(Ca^{2+}\)-desensitisation of the actin–myosin system, which implies lower sensitivity of calcium-dependent contraction and relaxation for \(Ca^{2+}\) ions. If actin–myosin interaction would have less sensitivity for \(Ca^{2+}\) ions, higher (than normal) calcium concentration would normalize contractile function. Indeed, Ito et al. have demonstrated elegantly that after plasma calcium concentration had been raised, stunned myocardium responded by an increase in its contractile performance [39]. If a certain amount of troponins has been proteolysed by calpain I activated during ischemia (when \([Ca^{2+}]_i\), is elevated), resumption of contraction, promptly after restoring blood flow, is prohibited, and restoration of normal actin–myosin interaction will depend on synthesis of new troponins. The results obtained by Ito et al. [39] can be explained by an activation of unaffected troponins upon increased \(Ca^{2+}\) availability. If troponins are degraded in stunned myocardium, degradation products from troponin proteolysis should be present. These have been demonstrated in rat ventricular myocardium that had been ischemic for 20 min and had been reperfused for 20 min [34]. Gao et al. also showed that the same troponin degradation products were produced when permeabilized trabeculae, isolated from normal heart, were treated with exogenous calpain I [34]. Solaro advocated the use of antibodies with appropriate epitopes to examine whether cTnI breakdown is associated with the generation of cardiac stunning [40]. In human cardiac tissue that was incubated at 37 °C for varying times, at least seven TnI fragments were detected using Western blot and a panel of anti-TnI-antibodies directed against specific epitopes of the TnI molecule [41]. These authors concluded that proteolytic degradation of cTnI occurs during incubation of myocardial tissue, and that the N-terminal and C-terminal ends of cTnI are highly susceptible to proteolytic degradation. In left ventricular biopsies taken from patients undergoing coronary artery bypass surgery before and 10 min after removal of aortic cross-clamp, at least seven degradation products of TnI and three covalent complexes of TnI with other troponin subunits were detected [42].

Results obtained in skeletal muscle ‘fatigue’ models resemble results obtained in stunned myocardium. Compared to control muscle strips, the muscle strips that underwent a hypoxia/fatigue protocol had lower (by 45%) maximal \(Ca^{2+}\) activated force \((F_{max})\) and lower tissue concentrations of TnI (by 32%) and TnC (by 18%), whereas tissue concentrations of actin and TnT remained unaffected [43]. As the hypoxia/fatigue protocol is followed by slow recovery of muscle function, the authors concluded that this slow recovery is due to resynthesis or repair of troponins [43].

2.3. Release of troponin degradation products from the ischemic/infarcted heart

Degradation products of troponins have been demonstrated in the effluent of isolated rat hearts that had been ischemic for 20 min and were reperfused for 20 min [34], and in effluent of isolated rat hearts that had been ischemic for 15 or 60 min and were reperfused for 45 min [44,45]. In the latter experiments the presence of necrotic myocardium cannot be ruled out. Likewise, in blood from patients with acute myocardial infarction (AMI), degradation products of troponin have been observed [46], but here the presence of necrotic myocardium is certain. The anti-cTnI antibodies used bound to different degradation products of cTnI, which may be the reason that the many cTnI test kits currently available have different upper limits of reference levels. Less certain is the presence of necrotic myocardium in patients during and after coronary artery bypass surgery. In blood obtained before aortic cross clamping and 10 min, 30 min, 3 h, 24 h, and 72 h after cross clamp removal, troponin degradation products were found [42]. Although troponins undergo proteolysis in the circulation, the left ventricular biopsies showed troponin degradation products
already [42] which may be released from viable cells into the circulation. Sobel and LeWinter [47] were the first to recognize that interpretation of positive serum troponin tests may depend on whether breakdown products of troponins are released from viable cardiomyocytes subjected to ‘repeated, subclinical bouts of ischemia and myocardial stunning with activation of calpain’.

2.4. Release of troponins from hearts inflicted by doxorubicin toxicity

Treatment of patients with cancer using high-dose chemotherapy has cardiotoxic side-effects. In particular the anthracyclins, like doxorubicin, become highly cardiotoxic if the cumulative dose exceeds 550 mg/m². In several studies it has been demonstrated that chemotherapy, either as new therapy or applied to patients who have had chemotherapy before, causes elevation of cardiac troponins in serum. Cardinale et al. have shown that if chemotherapy-treated patients had serum levels of cTnI and CK-MBmass below the upper limits of their reference ranges, left ventricular ejection fraction was only slightly and transiently depressed [48]. If cTnI was above reference range and CK-MBmass was not, patients had prolonged and more marked LV dysfunction, which may be reversible on a longer term [49,50], indicating that—instead of necrosis—vacuolar degeneration and/or loss of myofibrils have occurred [51]. In only three of 139 patients serum cTnI and CK-MBmass levels were above their respective reference ranges, and in these three patients left ventricular ejection fractions were below 30% [48]. Missov et al. used a very sensitive cTnI test that enabled determination of serum cTnI levels in healthy subjects [52]. They found serum cTnI values in the range of 13.5–25.5 ng/l and concluded that these serum cTnI levels are unlikely the result of myocardial injury. Rather ‘they reflect a hitherto unsuspected physiologic turnover of the protein that is detected in the systemic circulation’ [52]. Patients who had received anthracyclin therapy for the first time had maximal serum cTnI levels above reference range, but their maximal serum CK-MBmass levels were within reference range. Subsequent doses of anthracyclin increased serum cTnI even further, but maximal serum CK-MBmass levels remained within reference range [52]. This 2–4-fold increase in maximal cTnI levels observed in patients on anthracyclin therapy appears to be the result of myocardial injury, rather than myocardial necrosis, given the within reference limits of CK-MBmass. Herman et al. investigated this issue in spontaneously hypertensive rats that received doxorubicin intravenously weekly for up to 12 weeks [53]. After 4 weeks serum cTnT levels were not significantly increased, but after 7 weeks and 10–12 weeks serum cTnT levels were elevated. Immunohistochemical staining for cTnT and myocardial lesion scoring in cardiac tissue sections showed moderate but focal decreases in cTnT after 7 weeks, and a marked but still focal decrease in cTnT after 10–12 weeks. The authors concluded that their ‘immunohistochemical study demonstrated that the cardiac myocytes exhibiting the typical lesions of doxorubicin toxicity also have decreased staining for cTnT. The findings indicate that a loss of cTnT from damaged but nonnecrotic cells is the cause of the elevation in the serum levels of this protein’ [53].

2.5. Release of troponins from hearts with myocarditis

In mice with autoimmune myocarditis, serum cTnI levels were elevated. The histological severity of myocarditis paralleled serum cTnI elevations, but this relation was not statistically significant [54]. In 53 patients with myocarditis, proven by endomyocardial biopsy analysis, serum cTnI levels were higher and more often elevated than in 35 patients with chronic heart failure but no evidence of myocarditis [54]. When these data are used, the sensitivity of an elevated cTnI for the diagnosis of myocarditis is 0.34, the specificity is 0.89, and the predictive value of a positive test is 0.82. In the myocarditis group only three patients had increased CK-MBmass, whereas none of the patients in the nonmyocarditis group had increased CK-MBmass. Like in their study with mice having myocarditis, the myocardial biopsies from patients with myocarditis showed no significant correlation between histological severity of myocarditis and serum cTnI levels [54]. Given the low proportion of patients with myocarditis who have increased serum CK-MBmass levels, the release of cTnI from myocardial tissue with myocarditis may originate from affected cardiomyocytes that undergo myocytolysis without necrosis.

2.6. Release of troponins from hearts of patients with unstable angina pectoris

Troponins (both I and T) may also be released by patients with unstable angina pectoris. Although this diagnosis is difficult to make in the acute setting, and is to be confirmed in the next 24 h, the combination of a CK-MBmass concentration in plasma in the reference range and a troponin concentration in plasma higher than the upper limit of the reference range may imply that troponin is released from cardiomyocytes that were deeply ischemic, but not necrotic. Interestingly, the highest troponin concentration found in these patients in the first 24 h after referral to the emergency department has strong predictive power as to complications (death and AMI) occurring during follow up [55–58]. The so-called ‘microinfarctions’ that, according to several authors, have caused the release of troponins and CK-MB in small quantities (too low for the CK-MB level to reach the upper limit of the reference range), are unlikely responsible for this high power in predicting death and AMI during follow up. It is more
likely that the plasma levels of troponin found in patients with unstable angina pectoris provide information about the severity of myocardial ischemia that caused cellular troponin degradation and release of troponin degradation products in the circulation. In a ‘viewpoint paper’, Sobel and LeWinter stated that the ‘immunoassays used to characterize concentrations in blood of these macro-molecular markers detect not only TnI and TnT, but also proteolytic degradation products of each, as well as covalently bound complexes of TnI–TnC and TnT–TnC’ [47].

2.7. Release of troponins from hearts of patients with congestive heart failure

In patients with congestive heart failure (CHF), plasma troponin (I or T) concentrations may exceed the upper limit of the reference range [59–61]. Is this caused by ongoing myocardial necrosis? Using a very sensitive cTnI assay, Missov et al. found in patients with CHF 10-fold higher serum cTnI values than in healthy blood donors, although serum CK-MB<sub>mass</sub> levels were comparable [59]. In the study of Setsuta et al., cTnT was detectable in 51.7% of the patients with CHF, and in 4.1% of the healthy control subjects [60]. Serum CK-MB values did not differ significantly between two groups of patients with CHF, those with cTnT≥20 ng/l and those with cTnT<20 ng/l. However, the severity of CHF in terms of cardiothoracic ratio, serum atrial natriuretic peptide level, and 12-months cardiac event rate differed between the two groups [60]. Recently, Feng et al. demonstrated that, besides ischemia, increased preload (left ventricular end-diastolic pressure) of isolated, Langendorf-perfused rat hearts caused myocardial TnI degradation, which was prevented by the calpain inhibitor calpeptin [62]. If this information is combined with the recent findings of Brundel, who showed that atrial tissue obtained from patients with atrial fibrillation demonstrated (stretch-induced?) structural and electrical remodeling associated with increased calpain activity [29], the association of myocardial stretch with calpain activation and troponin degradation in viable myocardial tissue, followed by release of troponin fragments from stretched tissue, is plausible.

According to the proposed mechanism of CHF, troponin concentration in plasma of patients with CHF is caused by ongoing degradation of myocardial troponin, leading to progressive impairment of contractile function. Ricchiuti et al. have shown that surviving left ventricular myocardium of infarcted porcine hearts, when undergoing overload-induced remodeling, contained substantially less troponins I and T compared to normally functioning left ventricular myocardium from sham-operated pigs [63]. Thus, overloaded viable myocardium may release troponin (or troponin degradation products) in the circulation and become partially depleted of troponin if the rate of release of troponins exceeds the rate of synthesis of troponins.

2.8. Release of troponins from hearts of athletes engaged in ultra-endurance exercise

Endurance athletes have increased plasma levels of cTnI or cTnT after ultra-endurance exercise, which have been attributed to asymptomatic focal myocardial necrosis accompanied by depressed left ventricular ejection fraction and abnormal wall motion [64–67]. Others have found that ultra-endurance exercise-induced cardiac dysfunction is transient [68,69] and named this condition ‘cardiac fatigue’. So, there is evidence that cardiac consequences of ultra-endurance exercise are due to reversible cardiac injury, rather than cardiac cell death. Chen et al. measured plasma cTnT and myocardial cTnT in male rats that were forced to swim with a weight attached to their tail for 3.5–5 h [70]. The highest plasma cTnT levels were found immediately after termination of exercise, and these levels were dependent on duration of exercise and training status. If measured immediately after termination of exercise, myocardial cTnT concentrations were lower by 10–14% compared to controls, but not different from controls at 24 h and 48 h after termination of exercise [70]. In combination with the observation of transient cardiac dysfunction after ultra-endurance exercise [68,69] these results suggest that troponin is released by viable myocytes: the depression of cellular troponin concentration may be responsible for depressed cardiac function, and, after exercise, cellular troponin is replenished by troponin synthesis leading to restoration of normal myocardial troponin levels and normal cardiac function.

3. Issues for further research

1. Which degradation fragments are produced by intracellular proteolysis of troponin (for which calpain I is held responsible)?
2. Can stunning be prevented by exogenous inhibitors of calpain I, administered before ischemia?
3. Do isolated cardiomyocytes, if subjected to ischemia, anoxia or ‘chemical anoxia’, release troponins (or troponin degradation products) and is this release a measure of present or future cardiomyocyte death?
4. What are the myocardial concentrations of troponins in human and/or animal hearts with compensated left ventricular hypertrophy, and in hearts that fail?
5. What relation exists between actual concentration of TnI or TnT and left ventricular function in transgenic mice in which synthesis of myocardial troponins is switched off for some time?
6. Is left ventricular function impaired in transgenic mice in which cardiac synthesis of calpain I is switched on for some time?
References


