Stunned peri-infarct canine myocardium is characterized by degradation of troponin T, not troponin I

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Abstract

Objective: Degradation of cardiac troponin I (cTnI) has been proposed to represent the underlying molecular mechanism responsible for post-ischemic contractile dysfunction of viable but ‘stunned’ myocardium. However, this concept is largely derived from models of brief, sublethal ischemia essentially devoid of necrosis, and there is speculation that defects in cTnI may be model-dependent. Accordingly, our primary aim was to evaluate the integrity of cardiac troponins—i.e., cTnI, as well as cTnT and cTnC—in viable but stunned peri-infarct tissue. In addition, we addressed the as-yet-unexplored issue of whether the profound reduction of infarct size evoked by brief preconditioning ischemia (PC) was accompanied by a favorable attenuation in ischemia/reperfusion-induced degradation of cTnI, cTnT or cTnC in the remaining viable subepicardium. Methods: Anesthetized open-chest dogs received 10 min of PC ischemia or a comparable control period, followed by 1 h of sustained coronary occlusion and 3 h of reperfusion. Subepicardial biopsies from the center of the soon-to-be ischemic territory were obtained at baseline and at 30 min and 3 h post-reflow, and myofilament protein integrity (intact cTnI, cTnT and cTnC, as well as degradation bands and covalent complexes) were assessed by Western immunoblotting. In addition, in all dogs, wall thickening was measured by echocardiography, collateral blood flow was assessed during sustained occlusion by injection of radiolabeled microspheres, and infarct size was delineated by tetrazolium staining. Results: Although PC was, as expected, cardioprotective (infarct size of 2% of the risk region vs. 17±6% in controls; p<0.05), both control and PC groups exhibited profound and comparable contractile dysfunction following reflow (mean wall thickening reduced to 20–22% of baseline values). There was, however, no significant degradation of cTnI in the viable but stunned, peri-infarct tissue. We did observe degradation of cTnT in the stunned subepicardium, an effect that was attenuated in dogs that received antecedent PC ischemia. However, there was no correlation between post-ischemic wall thickening and the immunoreactivity of the intact cTnT band, or wall thickening and the intensity of the cTnT degradation products. Conclusions: Our results suggest cTnI degradation is not a universal determinant of post-ischemic myocardial stunning. Moreover, the dissociation between cTnT degradation and wall thickening argue against a direct ‘cause-and-effect’ relationship between proteolysis of cTnT and acute, post-ischemic contractile dysfunction of stunned peri-infarct myocardium.

Keywords: Peri-infarct myocardium; Troponin T; Troponin I
If defects in cTnI are, indeed, model-dependent, it is noteworthy that all previous studies have utilized models of brief, sub-lethal ischemia essentially devoid of necrosis, and, among these, all but two studies [11,12] were conducted in isolated, buffer-perfused hearts subjected to global ischemia. There is a paucity of data obtained in the in vivo setting, and, in particular, the integrity of cTnI in viable but stunned, peri-infarct tissue has, to date, not been investigated. Accordingly, our primary aim was to establish, using a classic canine model of acute myocardial infarction (MI) characterized by sub-endocardial necrosis [17–21], whether degradation of cTnI was manifest in the viable but stunned peri-infarct region. Second, as degradation or proteolysis of multiple myofilament proteins, in addition to or in lieu of cTnI, may conceivably contribute to the pathogenesis of the stunned myocardium [5,16], we expanded our protocol to include the analysis of cardiac troponin T (cTnT) and troponin C (cTnC). Finally, as our third, ancillary aim, we addressed the as-yet unexplored issue of whether the profound cardioprotection evoked by brief, antecedent preconditioning (PC) ischemia extends beyond the well-characterized limitation of infarct size [17–22] and is accompanied by a favorable attenuation in the ischemia/reperfusion-induced degradation of cTnI, cTnT and/or cTnC.

1. Materials and methods

This study conforms to the principles endorsed in the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

1.1. Surgical preparation

Ten purpose-bred mongrel dogs (body weight: 18.9 ± 0.4 kg) were anesthetized with sodium pentobarbital (30 mg/kg IV), intubated and ventilated with room air. After cannulating the left carotid artery (for measurement of heart rate and arterial pressure) and jugular vein (for administration of fluids and supplemental anesthesia), the heart was exposed through a left lateral thoracotomy and suspended in a pericardial cradle. A segment of the mid-left anterior descending (LAD) coronary artery was isolated and served as the site of later coronary occlusion. A segment of the mid-left anterior descending (LAD) coronary artery was isolated and served as the site of later coronary occlusion. In addition, as collateral blood flow to the ischemic territory is variable in the dog, and is recognized to be the primary determinant of infarct size in this model [17–21], a cannula was positioned in the left atrial appendage for later injection of radiolabeled microspheres (141Ce or 103Ru) for measurement of regional myocardial blood flow (RMBF).

1.2. Protocol

After 10 min of stabilization, baseline measurements of heart rate and arterial pressure were obtained in all animals, and wall thickening (WT) in the center of the soon-to-be ischemic LAD territory was assessed by short-axis two-dimensional echocardiography [24]. In addition, a baseline transmural needle biopsy sample (approximately 100 mg tissue) was obtained from the center of the soon-to-be-ischemic LAD territory and cut into endo- and epicardial halves; the endocardial half was discarded, and the epicardial sample was immediately frozen in liquid nitrogen and stored at −80 °C for later analysis.

Each dog was randomly assigned to receive PC ischemia (10 min of LAD occlusion followed by 10 min of reflow; achieved by placement/removal of atrumatic vascular clamps) or a time-matched control period (n = 5 per group). All animals then underwent 1 h of sustained LAD occlusion followed by 3 h of reperfusion (Fig. 1). Radiolabeled microspheres were injected at 30 min into sustained ischemia for measurement of RMBF, and wall thickening was reassessed at end-occlusion and at 30 min and 3 h post-reflow. In addition, biopsies were obtained from the center of the previously ischemic (and obviously hypokinetic) LAD bed at 30 min and 3 h after relief of ischemia (immediately after echocardiography), and, as described above, the epicardial half was frozen and used for subsequent analysis.

To confirm cardioprotection with PC, infarct size was quantified using routine methods [18–21]. Briefly, at the end of the protocol, the LAD was reoccluded and the area of myocardium at risk of infarction (AR) was delineated by in vivo injection (via the left atrial catheter) of Unisperse Blue pigment (0.5 ml/kg). The dogs were euthanized under deep pentobarbital anesthesia by intracardiac injection of KCl, after which the hearts were rapidly excised, cut into 5–8 transverse slices, and photographed. The heart slices were then incubated in triphenyltetrazolium chloride (10 min at 37 °C) to distinguish necrotic from viable myocardium and re-photographed for later analysis.

1.3. Endpoints and analysis

1.3.1. Hemodynamics and RMBF

Hemodynamics (heart rate and arterial pressure) were measured at baseline, immediately before the onset of

**Protocol:**

<table>
<thead>
<tr>
<th>Control</th>
<th>1 hour</th>
<th>3 hours</th>
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<tr>
<td>Preconditioned</td>
<td>10'</td>
<td>10'</td>
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Fig. 1. Schematic illustration of the experimental protocol. Black boxes denote periods of coronary artery occlusion.
sustained LAD occlusion, at 30 min and 1 h into ischemia, and at 30 min and 3 h post-reflow.

RMBF was quantified, using routine methods, in sub-endo- and sub-epicardial tissue blocks cut from the center of the ischemic LAD and normally perfused circumflex beds [17–21].

1.3.2. Cardiac troponins
cTnI, cTnT and cTnC were assessed in a blinded manner, without knowledge of the treatment group, by standard Western blot analysis [7,8,10]. Briefly, biopsy samples were homogenized on ice in 6 M urea, 160 mmol/l Tris–HCl, pH 8.0, in the presence of protease (1 µg/ml leupeptin, 1 µg/ml pepstatin A, 0.1 mmol/l PMSF), kinase (1.0 mmol/l EDTA), and phosphatase (50 mmol/l NaF, 1.0 mmol/l Na3VO4) inhibitors. Protein content of the sample homogenates was determined using the Lowry assay [23], and equal loading of samples was confirmed by densitometry of coomassie-stained gels.

Equal amounts of protein (5 µg) from each sample were combined with sample buffer to a final concentration of 2% SDS w/v, 62.5 mmol/l Tris–HCl, pH 6.5, 10% glycerol w/v, 0.05% bromophenol blue w/v, 6 M urea, and 100 mmol/l DTT. Of note, this concentration of DTT is, in our experience, sufficient to disrupt thiol bonds that may form (and yield the development of covalent complexes) as an artifact during tissue processing. Proteins were resolved by 12% SDS-polyacrylamide gel electrophoresis, using the Biorad Mini-gel system, in electrode buffer containing 25 mmol/l Tris, 192 mmol/l glycine, and 0.1% SDS at 200 V for 2 h. Following gel electrophoresis, proteins were electroblotted to nitrocellulose (Micton Separation) using a wet transfer apparatus. Proteins were resolved by 12% SDS-polyacrylamide gel electrophoresis, using the Biorad Mini-gel system, in electrode buffer containing 25 mmol/l Tris, 192 mmol/l glycine, and 0.1% SDS at 200 V for 2 h. Following gel electrophoresis, proteins were electroblotted to nitrocellulose (Micton Separation) using a wet transfer apparatus (Biorad) with 20% methanol, 25 mmol/l Tris, and 192 mmol/l glycine buffer. Nitrocellulose membranes were blocked overnight at 4 °C in 10% blocking reagent (Boehringer Mannheim).

Western blot analysis was preformed using the following primary antibodies: anti-Tnl (Mab 8I-7, Spectral Diagnostics; 3E3, BiosPacific), anti-TnT (Pe P1 and Pe P3, BiosPacific), and anti-TnC (Biodiag). Two different antibodies against Tnl and TnT with different epitopes were used to maximize the probability of detecting degradation products, if present, in the sample. The antibodies bound the canine form of the various cardiac troponin subunits (data not shown). Primary antibodies were detected using anti-mouse IgG or anti-goat IgG conjugated to alkaline phosphatase (both from Jackson Immuno Research Labs). Signals were visualized using enhanced chemiluminescence substrate reagent (Boehringer Mannheim).

All variables assessed repeatedly throughout the protocols — i.e., myofilament protein integrity, hemodynamics and wall thickening — were compared between control and PC groups by two-factor analysis of variance (ANOVA: for group and time) with replication, and, if significant F-values were obtained between groups, over time, or for the group–time interaction, post-hoc pairwise comparisons were made using the Newman Keuls test. RMBF and AR/LV were compared by t-test, while infarct size was compared between control and PC-treated cohorts by both t-test and by analysis of covariance (ANCOVA), incorporating RMBF to the ischemic territory as the covariate [17–21]. Relationships between myofilament protein integrity and wall thickening post-reperfusion were assessed by linear regression analysis. All data are reported as mean ± S.E.M. (reflecting the average values and among-sample variability of five
hearts per group), and p-values < 0.05 were considered statistically significant.

2. Results

2.1. Hemodynamics and RMBF

There were no differences in heart rate or arterial pressure between groups at baseline, during sustained coronary artery occlusion, or following reperfusion (data not shown). Moreover, both groups were equally ischemic during LAD occlusion (Table 1), with collateral flow to the subepicardium of the LAD territory—the specific region sampled for analysis of myofilament protein integrity—averaging 0.23 ± 0.09 and 0.25 ± 0.09 ml/min/g tissue in the control and PC cohorts.

2.2. Risk region, infarct size and wall thickening

Area at risk of infarction was comparable, at 23% of the total LV weight, in both the control and PC groups (Fig. 2A).

Infarct size was, as expected [17–21], significantly reduced in dogs that received antecedent PC ischemia versus controls: AN/AR was 2 ± 1% versus 17 ± 6%, respectively; p<0.05 by t-test (Fig. 2A) and p<0.05 by ANCOVA (Fig. 2B). However, as further anticipated, preconditioning had no significant effect on the acute recovery of LV function in this model [18,19]: the LAD territory was rendered akinetic in all dogs during coronary artery occlusion, and, at 3 h post-reperfusion, WT remained profoundly depressed at 22 ± 11% and 20 ± 8% of baseline in the control and PC groups, respectively (p<0.01 vs. baseline; p=ns between groups; Fig. 3).

2.3. Cardiac troponins

In all dogs, myocardial necrosis produced by 1 h of LAD occlusion was, as expected from previous studies [18–21], confined to the subendocardial half of the LV wall: i.e., the maximum transmural extent of necrosis was 43 ± 3% of the LV wall thickness in controls versus 20 ± 5% in the preconditioned group (p<0.01). Thus, tissue sampled for analysis of cardiac troponins was obtained exclusively from the viable, peri-infarct region.

Post-translational modifications of cTnI, cTnT and/or cTnC in the viable but stunned subepicardium would presumably be manifest by degradation and/or significant reductions in the immunoreactivity of the intact, 26, 42 and/or 20 kDa bands, respectively. In this regard, no degradation of cTnC was observed: density of the intact, 20 kDa bands remained unchanged at 100% of baseline values in all samples, irrespective of the treatment group or the sampling time (Figs. 4 and 5).

Although cTnT from canine myocardium can be degraded (data not shown), we observed no significant degradation or differences in the density of the 26 kDa bands in samples obtained at 30 min and 3 h post-reperfusion versus baseline (F=1.37 and p=0.29 over time), and, although there was a trend toward lower immunoreactivity in the PC group following reflow, there was no significant group difference at any time during the protocol (F=2.72 and p=0.14 between groups; Figs. 4 and 5). In addition,

Table 1

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<th>Ischemic LAD bed</th>
<th>Remote circumflex bed</th>
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<tr>
<td></td>
<td>Endo</td>
<td>Epi</td>
</tr>
<tr>
<td>Control</td>
<td>0.07 ± 0.03</td>
<td>0.23 ± 0.09</td>
</tr>
<tr>
<td>Preconditioned</td>
<td>0.09 ± 0.03</td>
<td>0.25 ± 0.09</td>
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Endo = subendocardium; Epi = subepicardium; LAD = left anterior descending coronary artery.

Fig. 2. (A) Area at risk (AR) expressed as a % of the total left ventricular (LV) weight and area of necrosis (AN) expressed as a % of the AR for control and preconditioned (PC) groups. (B) Relationship between AN/AR and regional myocardial blood flow to the ischemic subendocardium (Endo RMBF) for control and PC dogs. ANCOVA = analysis of covariance.
regression analysis revealed no correlation between WT and density of the intact cTnI band for paired data obtained for all dogs at 3 h post-reperfusion (Table 2), or paired data obtained at 30 min following reflow (data not shown).

Interestingly, 3/10 dogs exhibited qualitative evidence of high molecular weight (42 kDa) covalent complexes in post-reperfusion samples, purportedly formed by the bonding of cTnT to TnI degradation products (example shown in Fig. 4). All 3 animals displaying covalent complex formation were in the control cohort; however, this apparent trend toward a group difference was not significant ($p=0.16$ by Fisher’s exact test). Moreover, when data from all dogs (both control and PC) was considered, there was no difference in recovery of wall thickening in the three dogs that displayed covalent complex formation versus the seven animals that did not (i.e., WT at 3 h post-reperfusion: $13 \pm 7\%$ vs. $24 \pm 6\%$, respectively; $p=0.45$ by $t$-test).

cTnT appeared to be sensitive to trauma associated with surgical preparation and the sampling procedure per se: i.e., all dogs showed modest evidence of cTnT degradation products in baseline samples, as demonstrated by the presence of low molecular weight (~26 kDa) immunoreactive bands that averaged $3 \pm 1\%$ and $13 \pm 6\%$ of total TnT reactivity in control and PC groups, respectively (Figs. 4 and 6). Two lines of evidence suggest that degradation of cTnT was exacerbated following reflow in control—but not PC—animals. First, immunoreactivity of the intact, 46 kDa cTnT band tended to decline over time ($F=3.50$ and $p=0.06$), with a trend toward a group difference between control and PC cohorts ($F=2.54$ and $p=0.12$; Figs. 4 and 5). Furthermore, at 3 h post-reperfusion, intensity of the low molecular weight degradation bands increased to $39 \pm 10\%$ of total cTnT immunoreactivity in control animals ($p<0.05$ vs. baseline), but remained unchanged at $12 \pm 4\%$ of total cTnT immunoreactivity in the PC group ($p=ns$ vs. baseline;
Figs. 4 and 6). There was, however, no correlation between WT and the intensity of the intact cTnT band, or WT and intensity of the cTnT degradation products at either 3 h following reflow (Table 2) or 30 min post-reperfusion (data not shown).

3. Discussion

In this study, we report that the stunned, peri-infarct canine myocardium is not characterized by significant deg-

radation or proteolysis of cTnI. Second, we make the novel observation of cTnT degradation in the viable but stunned subepicardium, an effect that was attenuated in dogs that received brief, antecedent PC ischemia. However, as the deficit in wall thickening was comparable in control and PC groups, and regression analysis revealed no correlation between wall thickening and cTnT degradation, our results argue against a direct, ’cause-and-effect’ relationship between proteolysis of cTnT and acute, post-ischemic contractile dysfunction of stunned myocardium during the initial hours following reperfusion in this model.

3.1. Validity of the model

Our specific aim was to assess myofilament protein integrity in viable but stunned, peri-infarct myocardium. It is therefore important to document, first, that we sampled viable (rather than necrotic) tissue. In this regard, we observed that, as expected in this canine model [18,19], all infarcts (even in the most severely ischemic controls) were confined to the subendocardium, with the maximum transmural extent of necrosis occupying < 50% of the LV wall thickness in all animals. A second issue is whether this viable peri-infarct region was, in fact, stunned. Although wall thickening measured by echocardiography represents an integrated assessment of akinesis/dyskinesis of the in-

Table 2

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<th>Wall thickening (% of baseline)</th>
<th>p-value</th>
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<tr>
<td>vs. intact cTnI (% of baseline)</td>
<td>0.031</td>
<td>0.62 (ns)</td>
</tr>
<tr>
<td>Wall thickening (% of baseline) vs. intact cTnT (% of baseline)</td>
<td>0.078</td>
<td>0.50 (ns)</td>
</tr>
<tr>
<td>Wall thickening (% of baseline) vs. degraded cTnT (% of total)</td>
<td>0.044</td>
<td>0.59 (ns)</td>
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$r^2 =$ correlation coefficient; ns = not significant.
farcted subendocardium and hypokinesis of the remaining viable tissue, previous studies in which sonomicrometry was utilized to quantify subepicardial segment shortening have conclusively demonstrated that, in this model, the viable subepicardium is stunned [18]. Indeed, the persistent deficit in wall thickening following relief of ischemia seen in the current study by echocardiography is consistent with the impaired segment shortening reported previously by sonomicrometry [18].

3.2. cTnI and stunned myocardium

Troponin consists of three subunits, TnI, TnT, and TnC, which interact with each other and with other thin filament proteins (i.e., tropomyosin), and serve a regulatory role in the mediation of calcium-dependent contraction in cardiac and skeletal muscle [25]. It is, therefore, perhaps not surprising that post-translational modifications of one or more of these myofilament proteins have been proposed to contribute to the profound but transient contractile dysfunction of the stunned myocardium.

Initial evidence in support of this hypothesis—and, in particular, the focal role of cTnI—was obtained in isolated buffer-perfused rat hearts subjected to a transient, 15–20 min period of global ischemia. Specifically, myocardial stunning in this model was associated with proteolytic cleavage of cTnI, with the primary degradation product, cTnI−193, produced from a C-terminal clip of intact cTnI [6–8], as well as the presence of high molecular weight covalent complexes formed between cTnI−193 and cTnT [26]. The concept that proteolysis of cTnI contributes to contractile dysfunction was bolstered by a seminal report in which the classic phenotype of the stunned myocardium was displayed, in the absence of ischemia/reperfusion, in transgenic mice overexpressing the major degradation product, cTnI−193 [9]. Moreover, the potential importance of these findings was corroborated by observations of cTnI proteolysis in myocardial biopsy samples obtained from patients after cross-clamp removal during coronary artery bypass surgery [10], and by the presence of degraded forms of cTnI in serum from patients with acute myocardial infarction [27].

Despite these seemingly compelling data, disparate results have been obtained, most notably in vivo, large animal models of regional ischemia, thereby suggesting that alternative protein changes may be involved. No evidence of cTnI degradation was found in porcine myocardium stunned by a brief, 10 min episode of coronary artery occlusion [11], in porcine and canine models of myocardial stunning produced by prolonged (1–5 h) periods of subtotal coronary artery stenosis [11,12], or in stunned porcine myocytes isolated following 90 min of coronary stenosis [14]. Our results obtained in stunned, peri-infarct canine myocardium—i.e., no significant decrease in the immunoreactivity of the intact cTnI band—are consistent with these observations.

Although these aforementioned results suggest that cTnI was not degraded in our model, we did observe, in three animals, the presence of high molecular weight covalent complexes. This represents a paradox: i.e., as covalent complexes are purportedly composed, at least in part, of cTnI fragments bonded to cTnT [26], the presence of covalent complexes implies that some proteolysis to yield cTnI−193 presumably occurred. One potential explanation for this apparent discrepancy is that the covalent complexes may have been formed via other as-yet unidentified secondary modifications to cTnI and/or cTnT. Alternatively, the sensitivity of standard Western immunoblotting may be insufficient to resolve subtle changes in intact cTnI. In this regard, it is perhaps noteworthy that we did observe, in some blots, faint immunoreactivity that may represent near-threshold detection of low concentrations of cTnI degradation products (example shown in Fig. 4).

In any case, despite this paradox, our results revealed that the presence versus absence of covalent complexes was not a significant determinant of postischemic dysfunction in our model. That is: although subtle cTnI proteolysis may have been manifest in some animals, degradation of cTnI is not essential for, or the primary mechanism of, stunning in this canine model.

How can the apparent incongruity in the contribution of cTnI degradation between the rodent versus porcine and canine models be reconciled? One possibility is that the molecular mechanisms responsible for post-ischemic stunning are model- and/or species-dependent: i.e., degradation of cTnI may play a role in isolated buffer-perfused hearts subjected to global ischemia, but does not contribute importantly to myocardial stunning in the setting of regional ischemia in the dog and swine. This is not, however, the sole explanation. Data obtained in isovolumic rat hearts suggests that degradation of cTnI may be due to increased preload rather than brief ischemia/reperfusion per se [13], while, in isolated buffer-perfused rabbit hearts, there was no relationship between post-ischemic contractile function and the integrity of cTnI [15]. Thus, although evidence from the transgenic mouse model suggests (albeit does not prove) that the presence of cTnI degradation products may be sufficient to evoke myocardial dysfunction and the stunned phenotype [9], these discrepancies, together with our current results, support the concept that cTnI degradation is not a universal determinant of post-ischemic myocardial stunning. Indeed, there may not be a single, common molecular determinant, but, rather a series or collection of protein modifications that can induce or contribute to the stunned myocardium.

3.3. Involvement of cTnT, cTnC?

These emerging conflicts with regard to cTnI have prompted speculation that the complex phenotype of the stunned myocardium is, in all likelihood, not mediated by a single protein abnormality [5,16]. However, only one preliminary report has, to date, specifically addressed this issue:
a loss in immunoreactivity of intact cTnC, as well as increased immunoreactivity of cTnT (attributed to some form of post-translational modification)—in the absence of cTnI degradation—was described in pig myocardium stunned by 90 min of coronary artery stenosis [28].

We found, in our canine model, marginal degradation of cTnT even in baseline samples. Similar observations have been made by others for cTnI [10,11]; this is presumably a consequence of the invasive sampling procedure or, alternatively, may reflect the normal turnover of cardiac troponins in the heart [15]. More notably, our results further demonstrated progressive degradation of cTnT in the viable but stunned peri-infarct region of control animals following relief of ischemia. However, the mechanistic relevance of this observation is unclear, as regression analysis revealed no correlation between the immunodensity of cTnT degradation products and the deficit in wall thickening following relief of ischemia. This dissociation between cTnT proteolysis and post-ischemic function in the canine preparation, together with the apparent maintenance of cTnT integrity in isolated buffer-perfused rat heart models [6], argues against the concept that degradation of cTnT may play an integral role in the pathogenesis of the stunned myocardium.

3.4. Preconditioning, stunning and cardiac troponins

Overwhelming evidence has demonstrated that brief episodes of antecedent ischemia paradoxically protect or ‘pre-condition’ the heart and render the myocardium resistant to a later, more sustained ischemic insult. The undisputed hallmark of this phenomenon, observed in all models and species [2,17–22,29]—and, as expected, recapitulated in the present study—is reduction of infarct size. In contrast, an issue that has generated some controversy is the effect of preconditioning on recovery of contractile function. In some models—most typically, in isolated buffer-perfused rat hearts subjected to global ischemia—acute recovery of LV function during the initial hours following relief of ischemia is reportedly enhanced in preconditioned groups versus controls [29,30]. However, in in vivo models of regional ischemia, it is well-established that preconditioning has no acute, beneficial effect on the immediate functional recovery of post-ischemic, stunned myocardium [18,19,22,29–31]. Our current results, showing no difference in wall thickening between control and PC groups, are consistent with this concept.

Although wall thickening following relief of sustained coronary artery occlusion was comparable in both control and PC cohorts, we made the intriguing observation that cTnT in the viable but stunned sub-epicardium was better-preserved in dogs that received antecedent PC ischemia. The functional consequences of this finding are, at present, unknown. However, it is interesting to note that, in the in vivo, chronically instrumented rabbit model, preconditioning, despite having no acute benefit, was associated with an enhanced recovery of systolic contractile function at 24–72 h following reflow [22]. It is well-established that single point mutations in amino acid residues or either N- or C-terminal truncations in cTnT can detrimentally affect contractile function [32–34], thereby suggesting that better maintenance of cTnT in PC hearts versus controls may serve to accelerate the temporal recovery of the stunned, peri-infarct region in the initial days—but not hours—post-reperfusion. Further prospective studies would, however, be required to substantiate this concept.

3.5. Summary, limitations and future directions

We observed no significant degradation or proteolysis of cTnI, as assessed by standard immunoblotting, in stunned but viable, peri-infarct canine myocardium. We cannot, however, exclude the possibilities that: (1) subtle degradation of cTnI—below the resolution detectable by our methods—may have been manifest; and (2) as illustrated in the transgenic mouse model [9], even a modest increase in the presence of cTnI degradation products may be sufficient to evoke a decrease in contractile function. Second, although our results argue against a direct, ‘cause-and-effect’ relationship between the observed proteolysis of cTnT in our model and acute, post-ischemic contractile dysfunction of the stunned subepicardium, we cannot rule out the concept that cTnT degradation plays some deleterious role that was blunted by other as-yet unidentifiable, favorable and compensatory mechanisms. Third, we focused exclusively on proteolysis of cardiac troponins; other non-structural and/or post-translational modifications in cTnI, cTnT or cTnC—i.e., phosphorylation—were not explored, and may have profound consequences on myocardial contractile performance. In this regard, it is interesting to note that, despite the lack of cTnI degradation in stunned porcine myocardium, purified troponin complexes isolated from stunned pig heart evoked a significant decrease in calcium responsiveness when reconstituted into rabbit skeletal muscle fibers, thereby implying that ischemia/reperfusion in the intact pig did elicit as-yet unresolved modifications in one or more troponin subunits [35]. Finally, although statistical significance was not achieved, several as-yet unexplained and potentially intriguing ancillary observations emerged from our study, including the apparent trends toward a greater incidence of covalent complex formation in controls and better maintenance of cTnT in preconditioned hearts. All of these issues and unanswered questions merit further prospective investigation, ideally using a comprehensive proteomic approach.

References


