Role of intramural platelet thrombus in the pathogenesis of wall rupture and intra-ventricular thrombosis following acute myocardial infarction

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Summary
Left ventricular thrombus (LVT) and rupture are important mechanical complications following myocardial infarction (MI) and are believed to be due to unrelated mechanisms. We studied whether, in fact, wall rupture and LVT are closely related in their pathogenesis with intramural platelet thrombus (IMT) playing a pivotal role. Male 129sv and C57Bl/6 mice underwent operation to induce MI, and autopsy was performed to confirm rupture deaths. Haemodynamic features of rupture events were monitored by telemetry in conscious mice. Detailed histological examination was conducted with special attention to the presence of IMT in relation to rupture location and LVT formation. IMT was detected in infarcted hearts of 129sv (82%) and C57Bl/6 (39%) mice with rupture in the form of a narrow streak spanning the wall or an occupying mass dissecting the infarcted myofibers apart. IMT often contained dense inflammatory cells and blood clot, indicating a dynamic process of thrombus formation and destruction. Notably, IMT was found extending into the cavity to form LVT. Haemodynamic monitoring by telemetry revealed that rupture occurred either as a single event or recurrent episodes. Importantly, the anti-platelet drug clopidogrel, but not aspirin, reduced the prevalence of rupture (10% vs. 45%) and IMT, and suppressed the degree of inflammation. Thus, IMT is a key pathological element in the infarcted heart closely associated with the complications of rupture and LVT. IMT could be either triggered by a wall tear or act as initiator of rupture. IMT may propagate towards the ventricular chamber to trigger LVT.

Keywords
Myocardial infarction, intramural thrombus, left ventricular thrombus, ventricular rupture, platelet, inflammation, anti-platelet drugs, haemodynamics

Introduction
Ventricular wall rupture and left ventricular thrombus (LVT) are important mechanical complications of acute myocardial infarction (MI) (1–4). LVT formation following acute MI was intensively investigated during the 1980s to 1990s, when LVT was detected in approximately 30% of patients with anterior/apical-localised MI (4, 5). Current routine therapy including primary coronary intervention in patients with acute MI has lowered this incidence to 1–3% and mortality of at least 70% (1, 2). In the clinical setting, whereas both LVT and wall rupture are generally regarded as independent complications of acute MI, there are reports that a fraction of patients diagnosed with LVT subsequently had a fatal wall rupture (5, 10). The mouse model has been widely used in current research on MI, and the mouse has been shown to be the only laboratory species that develops ventricular rupture, which occurs within a time-window of approx. 2–6 days after MI (11–14). Experimental studies, including ours, have convincingly shown that the central mechanism of rupture is regional inflammation and elevated activity of matrix metalloproteinases (MMP) that subsequently damage matrix collagen fibres and weaken tissue tensile strength (11, 13, 14). Histological features of the mouse MI model include massive inflammatory cell infiltration, intramural haemorrhage and significant LV remodelling (12).
tolological features in the mouse model are similar to that reported in human subjects who had post-infarct wall rupture (15–17).

Using the murine MI model, we have explored the possibility that both rupture and LVT occurring during the acute phase of MI are related in their pathogenesis. We also compared the histopathology between two strains of mice that differ in the risk of rupture following MI (12), and tested effects of anti-platelet drugs on cardiac inflammation and incidence of IMT and rupture.

Materials and methods

Animals and surgery

Male 129sv or C57Bl/6 mice at 14 weeks of age were used. They were housed 2–4 animals per cage at 21°C in a facility with 12/12 h light/dark cycle (06:00 to 18:00). We previously showed a higher incidence of wall rupture in 129sv than C57Bl/6 mice (12) and the use of both strains would allow us to ascertain whether histopathological differences might underlie the strain-related difference in the risk of rupture (12). The experiments were approved by a local animal ethics committee and conformed to the US NIH Guide for the Care and Use of Laboratory Animals (1996). As we described previously (12), after left thoracotomy and opening of the pericardium at the site of ligation, the left coronary artery was occluded with resultant MI at the anterior/lateral free wall and most of the apical region. Animals were closely monitored for seven days after surgery for the onset of rupture. Diagnosis of rupture death was indicated by autopsy findings of haemopericardium and large quantity of blood clot in the chest, as we described previously (11, 12). All remaining 129sv mice were killed at day 7, and hearts were collected for histological examination. Surviving C57Bl/6 mice were killed at week 1, 2 or 4, respectively, to detect the presence of LVT. Some mice were examined by echocardiography, as described previously (11, 12, 18). Infarct size was determined in all animals by the LV surface method as described previously (12).

Haemodynamic monitoring in conscious and infarcted mice by telemetry

To define the haemodynamic features in mice present at the onset of rupture, blood pressure (BP) and heart rate (HR) were monitored in a sub-group of 129sv mice by radiotelemetry. The selection of 129sv strain in this experiment was because of its high incidence of rupture post-MI, which helped in collecting more haemodynamic data at the time of rupture onset. A PA-C10 pressure telemetry probe (Data Sciences International) was implanted in mice with the catheter positioned in the right carotid artery (19). After recovery for one week, animals were single caged and placed on receiver pads for continuous recordings of BP, HR and physical activity using a data acquisition (Universal DAQ) and analysis program written in Labview (19). Following a 24-h baseline recording, open-chest surgery was performed to induce MI and continuous telemetry recordings commenced from day 2 after surgery.

Histological analyses

Heart tissues were fixed (10% buffered formaldehyde), paraffin embedded, and then serially cut with sections (5 μm) collected every 0.3 mm. Each heart had 4–7 sections that included the infarcted region. Carstair’s stain was used to identify platelet thrombus (purple/light blue in colour) as well as other structures (20). Haematoxylin and eosin staining was also used where indicated. Images were acquired and analysed using Olympus Image Pro Plus6 (Media Cybernetics). The accuracy of identifying platelet thrombus by Carstair’s stain has been well documented previously (20). The severity of myocardial inflammation and intramural haemorrhage was evaluated using a score system, as described previously (12). The observer was blinded to animal information and after inspecting the entire infarct region, a score was assigned as: 1=minimal; 2=mild, 3=moderate; 4=severe, 5=extreme for inflammation or presence of haematoma.

In a separate batch of mice, hearts were collected 72 h after MI. After fixation in 4% paraformaldehyde and embedding in OCT compound (Tissue-Tek, Sakura), frozen sections (5 μm) were cut. Inflammatory cells were identified by immunohistochemical stain with rat anti-mouse CD45 primary antibody (BD Biosciences, 1:50) with 2nd Alexa Fluor 555 goat anti-rat antibody (Invitrogen). Nuclei were stained with DAPI (Invitrogen). Multiple images (8–11 fields/heart) covering the entire infarcted regions were acquired digitally using Olympus BX61 fluorescence microscopy and AnalySIS FICE software (Olympus). CD45+ cell number was counted in a blind fashion.

Anti-platelet therapy and determination of bleeding time

Tablets containing either clopidogrel (75 mg, Sanofi Aventis) or prasugrel (5 mg, Eli Lilly) were ground into fine powder and freshly made in an emulsion in 0.5% methylcellulose solution. Starting 3 h after surgery, animals were gavaged once daily for three days with clopidogrel (50 mg/kg loading dose followed by 15 mg/kg/day) or prasugrel (5 mg/kg/day). For aspirin (Sigma) treatment, animals were gavaged 3 h after surgery at 90 mg/kg and subsequent drug delivery was done by dissolving aspirin in drinking water (850 mg/L, fresh every second day till day 7) based on a daily drinking of 3 ml/animal. These dosages were selected according to previous reports showing a 90% inhibition of platelet activity (21–23).

For measurement of bleeding time, mice were anesthetised (ketamine/xylazine/atropine at 100/10/1.2 mg/kg, respectively, i.p.) and a 10-mm segment of the tail tip was cut off with a scalpel with the tip of the tail immersed in saline (37°C). Bleeding was moni-
tored till its cessation or the experiment was terminated after 20 minutes, as previously reported (20).

Two separate experiments were done in C57Bl/6 mice. The first experiment involved histological examination of infarcted hearts. Twenty mice with MI were randomly assigned to clopidogrel, prasugrel or aspirin following the regimen as described above or no treatment (4 groups). Animals were killed at 72 h after MI and hearts harvested for examination as described above. In the second, the effect of clopidogrel or aspirin on the incidence of ventricular rupture was examined. After induction of MI, mice were then randomly assigned to either untreated control or treatment and were monitored closely for seven days. Autopsy was performed to confirm reason of death, as described previously (11, 12).

Statistics

Results are presented as frequency of events (%) or mean ± SD unless otherwise specified. Frequency of events between groups was compared using Chi-square or Fisher’s exact test. Scores were compared by Rank-Sum test and cell densities were compared by ANOVA. Telescope parameters were also analysed by one-way ANOVA for repeated measures. P < 0.05 was regarded as statistically significant.

Results

Incidence of rupture

A total of 168 mice (n=70 129sv mice including those used for telemetry study; n=98 C57Bl/6 mice) underwent surgery to induce MI. After excluding mice that died of surgery-related reasons within the first 24 h (about 5%) or with a small infarct size (<25% of LV, about 7%), the remaining mice were included in analysis. All mice developed transmural infarcts with infarct size between 25–60%. The incidence of rupture was higher in 129sv than that in C57Bl/6 mice (p<0.01, Table 1). Rupture developed mostly during 2–4 days for 129sv and during 4–6 days for C57Bl/6 mice after MI.

Findings from telemetry experiments

Of 40 129sv mice with telemetry implants, five were excluded due to poor telemetry signals. Rupture occurred in 28 mice within five days after MI, confirmed by autopsy. Of the mice that died of rupture with recordings by telemetry, 22/28 (79%) had rupture occurring at night starting from 17:00 when mice were active. Further, telemetry recording revealed a significant increase in HR, but not MBP or activity, immediately prior to rupture onset (Fig. 1C).

In 16 (57%) of these rupture occurred as a single and fatal event indicated by a sudden collapse of BP and HR (Fig. 1A). In contrast, the remaining 12 mice (43%) displayed 1–3 periods of hypotension and bradycardia prior to a fatal rupture (Fig. 1B). Of seven mice surviving to day 7, two showed a single episode of hypotension and bradycardia, and autopsy revealed a small blood clot attached to the infarct wall in both mice, confirming non-fatal rupture as the reason for the haemodynamic changes.

Inflammation and haemorrhage

In mice that died of rupture at day-4, the severity of inflammatory cell infiltration or intramural haemorrhage was estimated using a score system (12). There was a trend for a higher inflammatory score in 129sv than C57Bl/6 mouse hearts (p=0.057) whilst the extent of intramural haemorrhage was significantly more severe in the former (p<0.05, Table 1).

Incidence of IMT in infarcted mouse hearts

All hearts from mice that died of rupture or were killed at various time post-MI were analysed histologically focusing on the detection of IMT. We identified the presence of IMT ranging in size from 50 to 500 μm within the infarcted wall from their distinct structure and purple/blue colour by Carstair’s stain. Such platelet-rich IMT was detected in over 80% of 129sv mice that died of rupture but only in 39% of C57Bl/6 mice with rupture (p<0.01, Table 1). To test whether timing of rupture influenced the presence of IMT, ruptured hearts of 129sv mice were re-grouped as early (2–3 days, n=25) or late rupture (4–6 days, n=20). The frequency of IMT was not significantly different between both subgroups (92% vs. 70%, p=0.11). Furthermore, the detection rate of IMT was similar in

Table 1: Summary of the between-strain differences in the incidence of rupture and histopathological findings in infarcted mouse hearts.

<table>
<thead>
<tr>
<th></th>
<th>129sv mice</th>
<th>C57Bl/6 mice</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>63</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Rupture %</td>
<td>71 (45/63)</td>
<td>33 (28/85)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rupture onset time (days)</td>
<td>3.4 ± 0.9</td>
<td>4.8 ± 1.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Intramural thrombus (IMT) %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMT/rupture</td>
<td>82 (37/45)</td>
<td>39 (11/28)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>IMT/no rupture (day-7)</td>
<td>11* (2/18)</td>
<td>0* (0/19)</td>
<td>0.23</td>
</tr>
<tr>
<td>Left ventricular thrombus (LVT) %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LVT/IMT</td>
<td>79 (31/39)</td>
<td>73 (8/11)</td>
<td>0.69</td>
</tr>
<tr>
<td>LVT/no IMT</td>
<td>25* (6/24)</td>
<td>31* (11/36)</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Inflammation score (day-4): 4.4 ± 0.9 (n=9) vs. 3.6 ± 0.8 (n=15) P=0.057
Haemorrhage score (day-4): 4.5 ± 0.6 (n=9) vs. 3.7 ± 1.1 (n=15) P=0.041

*p<0.01 vs. data in the raw above from the same strain. P values denote between-strain comparison. *Only 19 hearts of C57Bl/6 mice were killed at day-7 and histologically examined.

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mice that had recurrent or a single mode of rupture (63% vs. 83%, P=0.33). Detection rate of IMT was not significantly different in mice with moderate versus large infarct size (129sv: 73% vs. 48%, p=0.08; C57Bl/6: 50% vs. 28%, p=0.198). In mice that survived through the rupture time-window (2–6 days) and were killed at day 7, the detection rate of IMT was much lower than those that had ventricular rupture in both strains (Table 1).

Histopathology of MI changes rapidly with time (12). To control for the timing of MI, comparison for the extent of inflammation and haemorrhage was made in hearts from 129sv and C57Bl/6 mice that died of rupture at day 4. Our results showed a strong trend for higher scores of cardiac inflammation and haemorrhage in 129sv than C57Bl/6 mice (Table 1).

**Morphological features of intramural thrombus**

Whereas diffuse platelet aggregation in the infarcted myocardium was found in about 30% of hearts from mice with rupture in both strains (image not shown), thrombus formation (IMT) was by far the most common form of platelet accumulation within the infarcted myocardium (Fig. 2). In 60% hearts, IMT was transmural in distribution (Fig. 2A, C, G, H). Interestingly, two types of IMT were evident: narrow and transmural thrombus (Fig. 2A, B) and conglomeration thrombi (Fig. 2D-I). In 68% hearts, broken ends of dead myofibers that formed the border of platelet thrombi were curly in shape (Fig. 2A, C, G, H), indicating that IMT acted as a blunt dissector contributing to structural damage.

**Figure 1:** Haemodynamic monitoring by telemetry in conscious mice revealed two distinct modes of rupture events in mice with myocardial infarction (MI). Representative traces of telemetry recording of mean arterial pressure (MAP), heart rate (HR) and physical activity of mice with MI. Two modes of rupture events were apparent based on haemodynamic changes: A) Single and fatal episode; B) Repeated episodes of rupture with hypotensive and bradycardiac intervals that lasted for hours. Arrows indicate time of rupture episode. C) HR, MAP and activity of 129sv mice from 20 minutes prior to the onset of rupture events. Results are mean ± SEM. *p<0.001 by one-way ANOVA for repeated measures.
and wall rupture. Another feature of IMT, as shown in Figure 2G-I, was co-existence at the same location of homotypic platelet thrombus (a), heterotypic aggregates of platelet and neutrophils (b), and red blood clot (c). IMT of this type requires considerable time to form implying a dynamic process of wall rupture. Infiltrated inflammatory cells either formed a dense rim around a blood clot or were evenly embedded in a platelet-rich thrombus (Fig. 2G-I).

**Presence of LVT in relation to intramural thrombus**

In hearts of both strains of mice harvested within the first week, LVT at its early phase of development was detected more frequently in hearts with IMT than those without (p<0.05, Table 1). We paid special attention to the link between IMT and LV chamber-localised blood clot at the rupture site. Although a blood clot in the LV cavity of mice that died of rupture event could have occurred post-mortem, in all hearts examined, we found the presence of platelet thrombi in a blood clot at the interface facing the infarcted wall. The characteristic mosaic or laminated appearance of platelet thrombi in the LVT (Fig. 3A) were in keeping with Lines of Zahn (24) excluding the possibility of clot formation post-mortem. In over 80% of heart sections collected at the rupture site, IMT was found to extrude into the ventricular chamber and connected tightly with a LVT (Fig. 3B). LVT and IMT were usually linked by a narrow neck (Fig. 3B), or alternatively with a wide base tightly and deeply adherent to the infarcted wall (Fig. 3C). While LVT in 55% of hearts was not directly adherent to the infarcted myocardium, their shape and proximity to adjacent border of the infarcted wall implies that they were originally connected with an IMT and separated from the infarct wall during tissue processing (Fig. 3A). The separation of LV cavity-localised thrombi from IMT might also have occurred if a section had missed the rupture site.

Among 57 C57Bl/6 mice with MI that were killed at different time points from week 1, 2 and 4, mature LVT (Fig. 4) was found in four mice (7%). Histological components of the thrombus varied with its aging and included platelets and inflammatory cells during early phase and fibrotic infiltration and organisation at late phases. Chronic LVT was associated with enlarged LV chamber size and poor contractile function, as detected by echocardiography (data not shown).

**Effects of anti-platelet drugs on rupture incidence, inflammation and histopathology**

Mice without treatment or treated with clopidogrel or aspirin (n=10 each) were killed at 72 h after MI and hearts processed for histological examination. This time-point was chosen as wall rupture in this strain starts from late day-3 (12), thereby eliminating the possibility that IMT was formed following wall tear or post-mortem. The bleeding time was similarly prolonged by clopidogrel and aspirin at the doses tested (Table 2), but treatment with clopidogrel and aspirin had no significant effect on the degree of intramural haemorrhage. Clopidogrel prevented the formation of IMT and alleviated the extent of inflammation (Table 2), actions not shared by aspirin. Immunohistochemical finding of a lower inflammatory cell density in the infarcted myocardium of mice treated with two different thienopyridines, clopidogrel (Table 2) and prasugrel (1,234 ± 56 cell/mm², p<0.01 vs. control) further
confirmed the anti-inflammatory action of thienopyridines. Again, aspirin had no such effect. Prasugrel treatment also prolonged bleeding time (all >1200 seconds, n=5).

We then tested whether clopidogrel or aspirin treatment reduced the risk of rupture. Our results from a total of 68 mice with MI showed that oral clopidogrel for three days reduced incidence of rupture whilst treatment with aspirin for seven days failed to lower the incidence of rupture (Table 2).

**Discussion**

We have made several important findings in this study. (i) The presence of previously unrecognised extra-vascular platelet thrombi, IMT, is a key pathological change in the infarcted heart. (ii) Whilst rupture is generally viewed as a single and fatal event, haemodynamic monitoring of mice revealed a recurrent mode of rupture characterised by periods of hypotension and bradycardia. This could be explained by the histopathological finding of IMT plugging the rupture tunnel. (iii) Wall rupture could be initiated by intramural haemorrhage and formation of growing and dissecting platelet thrombi. (iv) IMT extends into the ventricular cavity at the rupture site to form LVT, a finding indicating LVT and wall rupture as mechanical complications with their pathogenesis closely related to IMT. (v) Anti-platelet drug clopidogrel, but not aspirin, reduced the incidence of rupture, an efficacy associated with prevention of IMT formation and attenuation of inflammation.

Table 2: Effect of clopidogrel and aspirin on histopathology of infarcted mouse hearts and incidence of rupture.

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Clopidogrel</th>
<th>Aspirin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histopathology (72 h)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>38.6 ± 3.8</td>
<td>34.4 ± 4.3</td>
<td>37.4 ± 3.5</td>
</tr>
<tr>
<td>Bleeding time (sec)</td>
<td>172 ± 9</td>
<td>all &gt;1,200*</td>
<td>1164 ± 26*</td>
</tr>
<tr>
<td>Intramural thrombus</td>
<td>5/10</td>
<td>0/10*</td>
<td>4/10</td>
</tr>
<tr>
<td>LV thrombus</td>
<td>1/10</td>
<td>0/10</td>
<td>3/10</td>
</tr>
<tr>
<td>Inflammation score</td>
<td>3.7 ± 0.6</td>
<td>2.4 ± 0.6*</td>
<td>3.7 ± 0.4</td>
</tr>
<tr>
<td>Haemorrhage score</td>
<td>2.7 ± 1.2</td>
<td>3.0 ± 1.4</td>
<td>2.8 ± 1.5</td>
</tr>
<tr>
<td>CD45+ cells (number/mm²)</td>
<td>1763 ± 111 (8)</td>
<td>1237 ± 153* (5)</td>
<td>1456 ± 67 (5)</td>
</tr>
<tr>
<td><strong>Wall rupture (7 days)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number</td>
<td>20</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>Infarct size (%)</td>
<td>37.8 ± 5.1</td>
<td>39.3 ± 5.7</td>
<td>41.5 ± 6.2</td>
</tr>
<tr>
<td>Rupture %</td>
<td>45% (9/20)</td>
<td>10% (2/20)*</td>
<td>54% (15/28)*</td>
</tr>
<tr>
<td>*P&lt;0.01 vs. untreated group. Numbers in brackets indicate group size.</td>
<td></td>
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</table>

CD45+ cells (number/mm²) 1763 ± 111 (8) 1237 ± 153* (5) 1456 ± 67 (5)
Platelets and post-infarct complications

is apparently unable to prevent lethal rupture. Thus, the recurrent mouse hearts studied, the presence of thrombi at the infarcted wall components that require time to form. However, in the majority of the co-existence at the rupture site of thrombi with different a dynamic process. This is in keeping with the histological feature haemodynamic monitoring, in some animals rupture presented as sympathetic withdrawal and parasympathetic activation (34). Whereas rupture can be a single and fatal event, as revealed by our investigation. Considering the increasing recognition of platelets as an important class of inflammatory cells (31–33), the between-strain differences in the incidence of IMT and the trend for a higher inflammatory score are in keeping with our recent report of strain differences in the incidence of IMT and the trend for a higher inflammatory score are in keeping with our recent report of (18).

We observed, in conscious mice with MI, single or multiple episodes of hypotension and bradycardia. A similar haemodynamic pattern lasting up to two days was also reported in 20–30% of patients who later on developed rupture (5, 10, 25, 26). The mechanisms may involve loss of blood and, more importantly, activation of the Bezold-Jarisch reflex by a wall tear with sustained sympathetic withdrawal and parasympathetic activation (34). Whereas rupture can be a single and fatal event, as revealed by our haemodynamic monitoring, in some animals rupture presented as a dynamic process. This is in keeping with the histological feature of the co-existence at the rupture site of thrombi with different components that require time to form. However, in the majority of mouse hearts studied, the presence of thrombi at the infarcted wall is apparently unable to prevent lethal rupture. Thus, the recurrent mode of rupture is most likely the outcome of interaction between formation of IMT and on-going tissue damage and rupture penetration.

As far as we are aware, this is the first experimental study that has addressed the pathogenesis of post-infarct LVT. In the majority of mice of both strains that had wall rupture, we revealed the presence of IMT at the rupture site that was either structurally connected or adjacent with a LVT. These findings strongly suggest IMT as one of the pivotal pathological changes, which mechanistically links wall rupture to the formation of LVT. Our findings in the murine MI model are likely relevant to the clinical situation for several reasons.

(i) Earlier studies reported onset of fatal rupture in patients initially diagnosed with LVT by echocardiography (5, 10).

(ii) A recent clinicopathological study reported that rupture usually started with an endocardial tear and that fresh or mature thrombi were found in the majority of rupture entries (35). Further, a recent case-report on a patient with LV rupture found the presence of a large intramural thrombus-like structure adjacent to the rupture site (36).

(iii) There is a report stating the presence of LVT in a patient who died of rupture at day 5 after MI (37).

Regional inflammation and consequent damage of myocardial matrix collagen network constitute the central mechanism for reduced tensile strength and wall rupture (11, 12, 14). According to the histopathological findings from the infarcted mouse hearts, we propose two distinct roles of platelet thrombosis in the onset of post-infarct mechanical complications (Fig. 5).

(i) Ventricular rupture might start from an endocardial tear that then triggers thrombosis representing platelet’s haemostatic action (Fig. 5A) (30).

(ii) Rupture might also be initiated by a centrifugal tear within the wall forming an occupying mass of IMT and haemorrhage, eventually contributing to endo- and epi-cardial tears (Fig. 5B).
We observed a high incidence of intramural haemorrhage together with IMT formation, changes that could lead to rupture by promoting regional inflammation and creating an occupying thrombotic mass within the infarcted wall. In both cases (Fig. 5A, B), while leading to wall rupture and formation of IMT, an endocardial tear would simultaneously allow IMT growing towards the LV chamber and triggering a blood clot, ultimately forming a LVT (Fig. 5C). We propose that LVT seen in the majority of patients during the acute phase of MI is due to this mechanism.

Our experiment testing the anti-platelet therapy revealed novel efficacy, i.e. clopidogrel treatment prevented IMT, attenuated inflammatory infiltration in the acutely infarcted myocardium and reduced rupture incidence. These actions indicate a direct cardiac protection via clopidogrel's anti-inflammatory efficacy (31–33). Inhibition of IMT by clopidogrel was observed as early as day 3, immediately prior to the time window of rupture in C57Bl/6 mice, further suggesting a causal role of IMT in rupture pathogenesis. Whereas the use of clopidogrel is common, there are certain situations that platelet inhibition is unsatisfactory (e.g. anti-platelet resistance). A high percentage of patients at the time of acute MI are not receiving anti-platelet treatment. Thus, under these conditions, the existence of IMT in patients with acute MI is likely. In contrast, these effects of clopidogrel were not shared by aspirin implying significant differences in the anti-inflammatory potency of anti-platelet drugs in the setting of acute MI. This warrants further investigation. Bleeding is a major side-effect of anti-platelet therapy. Indeed, intramyocardial bleeding has been reported in patients with acute MI by magnetic resonance imaging (MRI) (38). Interestingly, our study showed that the extent of intramural haemorrhage was not worsened by treatment with clopidogrel or aspirin although tail bleeding time was markedly prolonged.

The incidence of post-infarct ventricular rupture has declined from 5–10% during 1960s to 1980s to current 1–3% (1, 2). The consensus is that this is attributable to the current routine therapy, particularly primary coronary intervention. Studies on the mouse rupture model support the effectiveness of current therapies in lowering the risk of rupture. For example, reperfusion following a period of coronary artery occlusion (1, 2 and 4 h, respectively) prevents rupture (our unpublished data). Our present study demonstrated reduction in rupture incidence by clopidogrel treatment.

Great caution is required when extrapolating the findings from the murine model to the clinical situation. Our findings, however, would provide important clues to future clinical studies. It is worthwhile to re-examine hearts of patients who died of post-infarct rupture to detect presence of IMT. It also remains to be studied whether anti-platelet therapy is associated with a lowered

**What is known about this topic?**
- Histopathology of acutely infarcted hearts and mechanical complications (e.g. wall rupture, ventricular thrombus) have been well described by clinic-pathological studies.
- Pro-inflammatory action of platelets has been increasingly appreciated.
- Anti-platelet therapy has become a routine to patients with acute myocardial ischaemia and infarction with the aim of preventing coronary thrombosis.

**What does this paper add?**
- This study has revealed the presence of platelet-rich thrombus in the infarcted myocardium as an important pathological component that has not been identified previously.
- The two major mechanical complications of acute myocardial infarction, wall rupture and left ventricular thrombus, are not independent events, but similar in their pathogenesis closely related to the intramural thrombus.
- The significance of intramural thrombus, as shown in this study, would justify the use of image detection of platelet aggregation within the infarcted myocardium for the purposes of diagnosis and risk assessment.
- This is the first study to demonstrate that the anti-platelet drug clopidogrel suppressed incidence of post-infarct wall rupture, development of intramural thrombus and regional inflammation, actions independent of coronary thrombosis.
risk of ventricular rupture in patients with MI. A recent study revealed that the use of clopidogrel is significantly less in patients who had heart rupture than those without rupture (30% vs. 42%, p<0.001) (39). Although the COMMIT trial reported that addition of clopidogrel to routine medication in patients with acute MI had no effect on the incidence of rupture deaths (0.8% vs. 0.9%) (40), this trial was limited by the lack of a loading dose of clopidogrel or confirmative measures of fatal or non-fatal rupture events. In addition, studies are warranted to investigate the potential risk of exacerbated intramyocardial bleeding by anti-platelet therapy, albeit this was not observed in the mouse MI model, and the differential anti-inflammatory action of different anti-platelet drugs. Finally, since the formation of IVT during the acute phase of MI may represent the extension of an IMT towards the chamber, detection of IMT by MRI might provide important information on monitoring and therapy of patients.

References


Thrombosis and Haemostasis 105.2/2011 © Schattauer 2011

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