Higher levels of collagen and facilitated healing protect against ventricular rupture following myocardial infarction

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ABSTRACT

The mechanism of cardiac rupture after MI (myocardial infarction) is not fully understood. Rupture has not been reported in most laboratory species, including the rat, but does occur in mice. We have reported previously that β2-TG mice (transgenic mice with cardiac-restricted overexpression of β2-adrenergic receptors) had a lower incidence of rupture compared with NTG (non-transgenic) littermates. We hypothesized that the difference in the incidence of rupture between rodents and specific mouse strains is due to the difference in collagen content following MI. In the present study, we compared the difference in matrix remodelling post-MI between β2-TG and NTG mice and between mice and rats. MI was induced by ligation of the left main coronary artery. Following MI, tensile strength, insoluble and soluble collagen content and gelatinase expression were determined in the infarcted and non-infarcted myocardium. Better preserved tensile strength measured as TTR [tension-to-rupture; 88 ± 14 and 58 ± 3% of the respective sham group values for β2-TG compared with NTG mice (P < 0.05); 108 ± 7 and 32 ± 4% of the respective sham group values for rats compared with 129sv mice (P < 0.01)] and less severe acute infarct expansion after MI were found in rats compared with mice or in β2-TG compared with NTG mice. These differences were associated with a higher content of pre-existing fibril collagen in the normal myocardium of β2-TG compared with NTG mice (1.6-fold) or rats compared with 129sv mice (2-fold) and an accelerated fibrotic healing in the infarcted myocardium. Additionally, a less pronounced increase in MMP-9 (matrix metalloproteinase-9) activity was observed in the infarcted myocardium of rats compared with 129sv mice. We conclude that a higher collagen level is associated with facilitated fibrotic healing of an infarct and preserves the tensile strength of infarcted myocardium, thereby preventing cardiac rupture and acute ventricular remodelling.

INTRODUCTION

Acute MI (myocardial infarction) is one of the most common causes of cardiac morbidity and mortality. In the past 30 years, there has been a significant decrease in the early mortality following acute MI due to the development of effective monitoring and therapeutic interventions. However, cardiac rupture remains a fatal complication during the acute period after MI [1]. In fact, thrombolytic therapy increases early rupture deaths [2–4]. Furthermore, certain types of cardiac rupture can be viewed as an extreme form of infarct expansion (infarct

Key words: collagen, matrix metalloproteinase, myocardial infarction, remodelling, rupture, tensile strength.

Abbreviations: LV, left ventricular; MI, myocardial infarction; MMP, matrix metalloproteinase; NTG, non-transgenic; SD, Sprague–Dawley; β2-TG mice, transgenic mice with cardiac-restricted overexpression of β2-adrenergic receptors; TTR, tension-to-rupture.

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wall thinning and regional dilation) [5,6], which also contributes to persist remodelling and congestive heart failure [7]. Thus a better understanding of the mechanism of cardiac rupture in the short term is not only helpful to prevent rupture itself, but also to attenuate the progression of chamber dilation and heart failure in the long term.

Interestingly, post-MI rupture has not been reported in commonly used laboratory species, such as rats, rabbits, dogs, pigs and sheep [8–10], but has been reported recently in mice [11,12]. In a mouse infarct model, we reported previously that β2-TG mice (transgenic mice with cardiac-restricted overexpression of β2-adrenergic receptors), which have higher levels of myocardial collagen, had a much lower incidence of cardiac rupture after MI than NTG (non-transgenic) littermates (2 compared with 20%), despite having markedly enhanced LV (left ventricular) contractility and heart rate [13]. The mechanisms for such differences in the risk of rupture between β2-TG and NTG mice and between rats and mice are not known, but would provide important insights into the pathogenesis of cardiac rupture.

Accumulating studies have suggested that MMP (matrix metalloproteinase) activation plays an important role in the pathogenesis of cardiac rupture [12,14–17], and we have shown recently that damage of the pre-existing fibrillar collagen is the underlying pathogenesis of rupture [18]. On the basis of these findings, we hypothesize that the differences in rupture incidence between β2-TG and NTG mice and between rats and mice, are also due to the difference in the collagen remodelling they undergo following MI.

**MATERIALS AND METHODS**

**Animals and surgery**

We used 4-month-old male β2-TG and NTG littermate mice (genetic background C57B6 × SJL) [13], male 129sv mice and male SD (Sprague–Dawley) rats. Animals were anaesthetized by intraperitoneal injection of a mixture of ketamine, xylazine and atropine (100, 20 and 1.2 mg/kg of body weight respectively) and ventilated via an endotracheal tube. MI was induced by ligation of the left main coronary artery, as we have described previously [13,19]. Sham operation in controls involved a thoracotomy without coronary artery ligation.

All procedures used were approved by the local Animal Ethics Committee, which adheres to the Australian Code of Practice For the Care And Use of Laboratory Animals for Scientific Purposes.

**Autopsy and infarct size determination**

In the mouse infarct model, cardiac rupture occurs during the first week of infarction, peaking at days 3–4 [11]. Heart tissues were therefore collected at early day 4 for tensile strength, collagen and MMP measurement. Autopsy was performed on each animal found dead, as described previously [13]. Cardiac rupture was confirmed by the presence of a large amount of clotted blood in the chest cavity and perforation of the infarcted wall [13]. Heart failure was considered by the presence of severe congested lung and chest fluid accumulation [11]. Infarcted and non-infarcted LV areas were measured from digital images, and infarct size was calculated as a percentage of the infarct area over the entire LV area, as described previously [11].

**Determination of infarct expansion and TTR (tension-to-rupture)**

Animals with or without infarction were killed at day 4 after surgery, and their hearts were used to determine infarct expansion [13] and TTR [11], as reported previously. To estimate the extent of infarct expansion, two parameters were measured from a single H&E (haematoxylin and eosin)-stained LV section around the equator of the left ventricle: the largest endocardial circumference and the ratio of the thickness of the thinnest infarcted wall to average thickness of the ventricular septum [13]. For TTR measurement, the left ventricle was sectioned transversely into four rings (1 or 3 mm in thickness for mice and rats respectively). The rings were then stretched over a device connected to a force transducer, with TTR being the force required to rupture a ring [11].

**Assay for soluble and insoluble collagen**

Under a surgical microscope, the infarct zone of the left ventricle could be clearly demarcated from the non-infarcted zone. The left ventricles from sham-operated animals and the infarct zone from infarcted animals were subjected to step-wise enzymatic and acidic digestion, and the content of insoluble (cross-linked) and soluble collagen was determined using a hydroxyproline assay, as described previously [20]. Briefly, heart tissue was cut into small pieces, freeze-dried, weighed and then digested with pepsin (200 µg/ml; Sigma) at 37°C for 24 h, followed by centrifugation to separate the supernatant fraction (containing pepsin-soluble collagen) from the pellet fraction (containing insoluble collagen). Both fractions were then acid-hydrolysed with 6M HCl for 18 h at 110°C, before samples were filtered using a Dowex/charcoal mixture. The hydroxyproline content was determined colorimetrically, calculated from a standard curve and expressed as µg of hydroxyproline/mg of dry weight.

**Gelatin zymography**

MMP-2 and MMP-9 activities in the left ventricle (the left ventricle from sham-operated animals, and the non-infarct zone and infarct zone from infarcted animals) were determined by gelatin zymography, as we have described previously [21]. Briefly, MMPs were extracted from LV tissues, and samples were fractioned by electrophoresis using 7.5% (w/v) acrylamide gels containing 0.5 mg/ml
gelatin. Coomassie-Blue-stained gels were scanned and densitometry levels were determined by using Optimas 6.2 image analysis software (Media Cybernetics).

Statistical analysis
Results are expressed as means ± S.E.M. One- or two-way ANOVA was used to detect differences among groups, followed by a Newman–Keuls or Bonferroni post-hoc test or unpaired Student’s t test. Differences at $P < 0.05$ were considered significant.

RESULTS
Previous studies from our laboratory have documented the incidence of rupture in β2-TG and NTG mice (2 compared with 20 %) [13] and 129sv male mice (up to 70 %) [11,18]. Post-MI rupture has not been reported in rats. The present study focused on comparing matrix remodelling and TTR between these models.

Animal allocations
For comparison between β2-TG and NTG mice, a total of 51 male mice (25 β2-TG and 26 NTG mice) were included in the study. Of these, 19 β2-TG and 20 NTG mice were subjected to coronary artery ligation, whereas six β2-TG and six NTG mice underwent sham operation. Of the animals subjected to MI, three mice (one NTG and two β2-TG mice) died of surgery-related reasons within 24 h, and eight mice (six NTG and two TG mice) died of heart failure. The surviving mice were killed at early day 4 post-surgery for heart collection. The lack of rupture death in NTG mice in the present study might be due to the fact that cardiac rupture peaks at late day 4 after MI in this strain [11].

For comparison between 129sv mice and SD rats, 27 129sv male mice were operated on (22 for MI and five for sham). One mouse died within 24 h due to surgery-related reasons and another 11 mice died of cardiac rupture. There was no death from heart failure. Of a total of 32 male SD rats operated on (26 for MI and six for sham), six rats died within 24 h due to surgery-related or arrhythmic reasons. By the time of tissue collection at early day 4 after surgery, eight rats had died of heart failure and one had died of unknown reasons (probably arrhythmias). There was no cardiac rupture death according to autopsy findings in SD rats.

All of the surviving animals were killed early on day 4 post-MI and the heart tissue was collected for histochemical analysis, TTR measurement, hydroxyproline determination and gelatin zymography.

Acute infarct expansion and TTR
At day 4 after MI, compared with NTG mice, β2-TG mice had smaller endocardial circumference (8.4 ± 0.4 mm; $P < 0.05$) and larger wall thickness ratio (0.47 ± 0.04 compared with 0.35 ± 0.03; $P < 0.05$). In comparison between SD rats and 129sv mice, SD rats also had a greater wall thickness ratio (0.82 ± 0.17 compared with 0.32 ± 0.01; $P < 0.01$). These results indicated attenuated infarct expansion in infarcted β2-TG compared with NTG mice and in SD rats compared with 129sv mice (Figure 1A). Infarct size was comparable between β2-TG and NTG mice (36.8 ± 3.2 compared with 38.7 ± 4.1 %; $P = $ not significant) and between SD rats and 129sv mice (35.7 ± 2 compared with 32.8 ± 4.4 %; $P = $ not significant).

The tensile strength of LV rings with and without MI was determined as the TTR of the ring. At day 4 post-MI, TTR, measured from infarcted LV rings of both β2-TG and NTG mice, showed a significant decrease from the respective sham-control values, but remained higher in the β2-TG group (Figure 1B). Interestingly, unlike the profound decrease in TTR observed post-MI in 129sv mice, TTR in SD rats remained unchanged from the sham controls (Figure 1B). These results indicate better preserved tensile strength after MI in β2-TG compared with NTG mice and particularly in SD rats compared with 129sv mice, which is in keeping with an

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Figure 2  Content of insoluble (A), soluble (B) and total (C) collagen, and the insoluble/soluble collagen ratio (D) in the left ventricle of sham-operated and infarcted myocardium of β2-TG and NTG mice at day 4 after surgery. Values are means ± S.E.M., n = 5–6/group. *P < 0.05 compared with NTG mice. MI, infarcted myocardium; SH, sham-operated.

Figure 3  Content of insoluble (A), soluble (B) and total (C) collagen, and the insoluble/soluble collagen ratio (D) in the left ventricle of sham-operated and infarcted myocardium of 129sv mice and SD rats at day 4 after surgery. Values are means ± S.E.M., n = 5–6/group. *P < 0.05 compared with the respective sham value. #P < 0.05 compared with 129sv mice. MI, infarcted myocardium; SH, sham-operated.

attenuated acute LV remodelling and lower risk of cardiac rupture.

Strain- and species-dependent changes in collagen

We next compared collagen content and cross-linking in the myocardium between β2-TG and NTG mice and between 129sv mice and rats. The left ventricle of sham-operated β2-TG mice contained higher levels of collagen, particularly insoluble collagen (approx. 60% higher) than that of NTG mice (Figure 2). In the infarct zone collected at day 4 after MI, collagen content, particularly the insoluble collagen content and the insoluble/soluble collagen ratio, was significantly higher (over 2-fold) in β2-TG compared with NTG mice (Figure 2).

A similar, yet much greater, difference in collagen content was observed between sham-operated 129sv mice and SD rats. The levels of insoluble, soluble and total collagen were markedly higher (approx. 2-fold) in the left ventricle of sham-operated SD rats compared with that of 129sv mice (Figures 3A–3C), whereas the ratio of insoluble/soluble collagen was comparable between 129sv mice and SD rats (Figure 3D). In the infarct zone collected at day 4 after MI, insoluble collagen content and the insoluble/soluble collagen ratio were significantly higher in SD rats compared with 129sv mice (Figure 3). Importantly, in contrast with a marked decrease (up to 70%) in insoluble collagen and the insoluble/soluble collagen ratio in the infarct zone of 129sv mice relative to the sham values, > 2-fold increases in these two parameters were detected in the infarct zone of SD rats at day 4, indicating a facilitated fibrotic healing in the rat heart following MI.

Difference in the activation of MMP-2 and MMP-9

As the gelatinases MMP-2 (gelatinase-A) and MMP-9 (gelatinase-B) have been implicated in the pathogenesis of rupture [12,15–17], MMP-2 and MMP-9 activities were compared in these rodent models of MI. In sham-operated mice, MMP-2 was significantly higher in β2-TG than NTG mice (Figure 4), whereas MMP-9 levels were comparable between the groups. In mice following MI, MMP-9 expression and activity were markedly increased in the infarct zone, but to a similar degree in both β2-TG and NTG mice (Figure 4), whereas MMP-2 expression and activity was modestly increased in the infarct zone of NTG mice and to a greater extent in the infarct zone of β2-TG mice (Figure 4).

In contrast, there was a significant difference in MMP-9 and MMP-2 expression and activity in the infarcted LV tissues at day 4 post-MI between 129sv mice and SD rats (Figure 5). Unlike the remarkable increase in MMP-9 activity that was observed in infarcted mouse hearts (Figure 5A), the increase in MMP-9 activity was rather modest in infarcted SD rat hearts (Figure 5B). However, the increment in MMP-2 activity was more pronounced in the infarct zone of SD rats (Figure 5B).
DISCUSSION

Currently, the mechanism for cardiac rupture is not fully understood. Rodent models of MI with varying risk of cardiac rupture have thus become very useful tools to explore mechanisms that contribute to cardiac rupture. The present study demonstrates several important findings. First, a lower risk of rupture in \( \beta_2 \)-TG mice [13] or lack of rupture in SD rats following MI was associated with the partial or complete preservation of tensile strength and less severe infarct expansion. Secondly, \( \beta_2 \)-TG mice or SD rats had a higher content of pre-existing collagen in the normal myocardium and facilitated fibrotic healing of the infarct compared with NTG mice or 129sv mice respectively. Furthermore, in contrast with a marked decrease in insoluble collagen in the infarct zone of 129sv mice relative to the sham values at day 4 after MI, a >2-fold increase in insoluble collagen was detected in the infarct zone of SD rats. Thirdly, the infarcted myocardium of SD rats had a much less remarkable activation of MMP-9 compared with that of mice. These results suggest that a higher level of pre-existing collagen levels, together with facilitated infarct healing, preserves tensile strength and prevents infarct expansion and cardiac rupture.

We have investigated previously the mechanism responsible for a sex difference in the rupture incidence in 129sv mice with MI and demonstrated that damage of the pre-existing fibrillar collagen, which occurs during the acute phase prior to infarct healing, leads to decreased LV wall tensile strength and infarct expansion, and contributes to the pathogenesis of rupture [18]. This notion is supported further by the findings of the present study.
A higher level of pre-existing collagen was observed in the myocardium of β_2-TG mice or SD rats after sham operation compared with that of NTG mice or 129sv mice respectively. Furthermore, accelerated infarct healing was also observed in the infarcted myocardium of β_2-TG compared with NTG mice or SD rats compared with 129sv mice. Collagen levels, particularly insoluble collagen, in the infarcted myocardium were much higher in β_2-TG compared with NTG mice at day 4 following MI. In fact, insoluble collagen was increased rather than decreased in the infarcted myocardium in β_2-TG mice at day 4 after MI, indicating accelerated fibrotic healing in these mice. In 129sv mice, substantial collagen deposition occurred at day 7 after infarction, corresponding to the restoration of tensile strength and cessation of cardiac rupture [18]. In contrast, excessive collagen deposition in the infarcted myocardium occurred as early as day 4 following MI in SD rats, indicating that fibrotic healing proceeds faster in the rat heart. Using the 129sv mouse strain for a comparison with the SD rat was based on the fact of a higher rate of post-MI rupture in the 129sv than other strains [11,18]. 129sv is a commonly used strain for making genetically modified mice and for cardiovascular research. Although there are reports on the between-strain difference in some areas, such as cholesterol absorption [22], atherosclerotic lesion [23], platelet aggregation [24] or neuronal activity [25], whether these genetic differences influence the cardiac healing process or composition of collagen matrix requires further investigation. In keeping with findings between species differences, a recent study also observed differences between mice and dogs in collagen deposition in the infarcted area [26]. Dogs, which have not been reported to develop rupture after MI, had more extensive collagen accumulation in the infarcted area compared with mice [26]. Thus facilitated infarct wound healing is probably one of the contributors to a lower incidence of rupture in β_2-TG mice or the lack of rupture in the SD rat. A low infarct collagen content is known to be associated with more pronounced remodelling and greater ventricular dilation [27,28].

Higher levels of collagen in β_2-TG mice or SD rats were associated with the partial or complete preservation of tensile strength and a less severe infarct expansion in the infarcted myocardium, which, in turn, contributed to a much lower risk of rupture in β_2-TG mice [13] or the lack of rupture in SD rats following MI. Interestingly, previous experimental studies also observed unchanged tensile strength of the infarcted myocardium in other species, such as the rabbit and dog [5,8,9], in accordance with the lack of rupture in these species. These findings may explain the clinical observation that the risk of post-MI rupture is lower in patients with a previous MI or LV hypertrophy [29,30], conditions associated with increased interstitial collagen in the myocardium. Indeed, studies using various mouse models have also shown that collagen content or procollagen gene transcription is inversely related to the incidence of rupture [13,31–33].

These results also highlight the importance of insoluble collagen in preserving tensile strength and preventing cardiac rupture. Although soluble collagen indicates newly synthesized collagen unable to provide mechanical strength, insoluble collagen represents cross-linked collagen fibrils that endow the myocardium with tensile strength. Despite unchanged or even increased total collagen, as observed in previous studies [13,34], a significant decrease in insoluble collagen in the infarcted myocardium in 129sv mice was observed in the present study, in keeping with a high incidence of rupture. In contrast, in infarcted SD rats that do not develop rupture, an increased level of insoluble collagen in the infarcted myocardium was observed. Similarly, β_2-TG mice in which cardiac rupture is almost abolished [13] also had a higher level of insoluble collagen in the infarcted myocardium compared with NTG littermates. In the above rodent models of MI, the levels of insoluble collagen correlated very well with tensile strength and were inversely related to the degree of infarct expansion. Interestingly, by combining the results from the present study and our previous study [18], it was found that, in five rodent models with different risk of rupture (129sv male, 129sv female, β_2-TG mice, NTG mice and SD rats), insoluble collagen levels in the infarcted myocardium were inversely related to rupture incidence (Figure 6).

In addition to a higher pre-existing collagen content and facilitated fibrotic infarct healing, SD rats had a less marked increase, relative to 129sv mice, in MMP-9 content and activity in the infarcted myocardium at day 4 after MI. The role of MMP-9 in rupture has been suggested by previous studies [15,17]. During the time window of rupture in 129sv mice, MMP-2 was only modestly increased, whereas MMP-9 was markedly up-regulated.
in the infarcted myocardium [18]. MMP-9 was also significantly higher in male than female mice, consistent with a higher incidence of rupture in male mice [18]. Therefore less degradation of collagen due to a less remarkable increase in MMP-9 is another probable reason for the lack of post-MI rupture in rats. There was no significant difference in MMP-9 between \( \beta_2 \)-TG and NTG mice; however, levels of myocardial MMP-2 were significantly higher in \( \beta_2 \)-TG than NTG mice in both the sham-operated and infarcted groups. Thus the lower incidence of rupture in \( \beta_2 \)-TG than NTG mice is not likely to be due to the difference in MMP activation and subsequent collagen breakdown, but rather due to the difference in levels of pre-existing collagen and faster infarct healing post-MI. Whereas, in the rat, higher pre-existing collagen, less severe MMP activation and faster fibrotic healing all contribute to the absence of rupture and preservation of tensile strength of infarcted tissue in this species.

Taken together, several factors need to be considered regarding the risk of rupture: collagen content, extent of inflammation/MMP activation and resultant collagen degradation, and the process of fibrotic healing. Results from our previous study [18] and the present study suggest that protecting the pre-existing collagen network in the infarcted area during the acute phase of MI until extensive fibrotic healing occurs is important in preventing the occurrence of rupture. Thus, in clinical practice, caution should be given to the administration of any drugs or therapies that would promote collagen degradation or interfere with infarct healing during this vulnerable period. Indeed, thrombolytic agents are associated with increased cardiac rupture [2–4], which is probably due to their ability to activate plasmin and MMPs, thereby stimulating collagen degradation [35].

In conclusion, a higher pre-existing collagen content in the normal myocardium and accelerated fibrotic infarct healing in \( \beta_2 \)-TG mice and SD rats are associated with better-preserved tensile strength and a lower risk of rupture after MI. In addition, a much less pronounced increase in MMP-9 activity is observed in the infarcted myocardium of rats compared with that of mice. These results suggest that the protection of pre-existing collagen content and adequate healing are important for preserving tensile strength and preventing acute LV remodelling and cardiac rupture.

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**REFERENCES**


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31 Hwang, M. W., Matsumori, A., Furukawa, Y. et al. (2001) Neutralization of interleukin-1β in the acute phase of myocardial infarction promotes the progression of left ventricular remodeling. J. Am. Coll. Cardiol. 38, 1546–1553


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