

## Experimental Physiology

# Critical appraisal of the mouse model of myocardial infarction

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**In order to critically evaluate the utility of a mouse model of myocardial infarction (MI) for therapeutic studies, we investigated survival, haemodynamic measurements and histopathology in mice with an occluding suture placed at one of three distinct sites along the left anterior descending coronary artery. The suture was placed at the atrioventricular juncture (High), or at two sites more distally towards the base (Middle and Low). In the High group, only 33% of animals survived 7 days after MI ( $P < 0.05$  compared to all other groups). Only the Middle group had significantly reduced haemodynamics compared to sham-operated animals (maximum left ventricular pressure:  $55.9 \pm 3.5$  versus  $80.8 \pm 5.1$  mmHg, maximum change in pressure over time:  $2003 \pm 172$  versus  $4402 \pm 491$ ,  $P < 0.01$ ). Histological examination showed morphological changes in all MI groups. The Middle group had larger lesions than the Low group ( $P < 0.05$ ). Lesions in the anterior and lateral walls correlated, albeit weakly, with cardiac function. Power calculations indicated that, despite a certain amount of intragroup variation, the Middle Suture model may be useful for therapeutic studies to assess the effects of treatment on cardiac function and overall lesion size.**

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Myocardial ischaemia is one of the leading causes of morbidity and mortality in humans in the Western world. Existing treatments, such as coronary artery bypass graft (CABG) or percutaneous transluminal coronary angioplasty (PTCA), are often insufficient, particularly in patients with multiple small vessel disease. Thus there is a clear need for the development of novel treatment approaches, such as therapeutic angiogenesis using protein or gene therapy (Losordo *et al.* 1998; Laham *et al.* 1999; Rosengart *et al.* 1999a,b; Symes *et al.* 1999; Vale *et al.* 2001). With the increasing range of potential therapeutic molecules and delivery vectors, animal models of ischaemic heart disease are therefore necessary to support further advancement in this field.

Preclinical models of myocardial ischaemia have been reported in several large animal species, including dogs (Schaper *et al.* 1970; Shou *et al.* 1997) and goats (Mannion *et al.* 1996). The model that most closely resembles the

response seen in humans is the pig ameroid model (Ware & Simons, 1999), which has been used in a variety of therapeutic studies (Giordano *et al.* 1996; Hariawala *et al.* 1996; Lopez *et al.* 1998; Mack *et al.* 1998; Tio *et al.* 1999; Patel *et al.* 1999; Laham *et al.* 2000; Sato *et al.* 2000, 2001). However, the expense and practical demands of porcine surgical facilities severely limit the extent of such studies, precluding the use of this model for larger scale screening studies of novel therapeutic approaches.

A rodent model of acute myocardial infarction (MI) was first developed in the rat (Pfeffer *et al.* 1979). More recently a murine equivalent has been described (Michael *et al.* 1995), providing the means to exploit the increasing availability of many useful transgenic and knockout mouse strains. Complete occlusion of the left anterior descending (LAD) coronary artery induces an acute MI. The resulting ischaemia in the left ventricular wall has been visualized with Evans blue/TTC perfusion assays

(Du *et al.* 1998; Lutgens *et al.* 1999; Michael *et al.* 1999; Scherrer-Crosbie *et al.* 1999), which allows quantification of the infarct area. Further work by Guo *et al.* (1998) has demonstrated ischaemic preconditioning after short-term LAD occlusion in mouse, thus validating the physiologic relevance of this infarct model. Previous studies show that the size of the infarct varies between 20–65% (Michael *et al.* 1995), 8–54% (Du *et al.* 2000), 14–65% (Patten *et al.* 1998) and 15–55% (Gao *et al.* 2000) in individual studies. Because of this, some studies divide animals into subgroups based on infarct size (e.g. Large Infarct *versus* Moderate Infarct) for further functional analysis (Patten *et al.* 1998; Du *et al.* 2000). Clearly this would not be appropriate, however, for studies aimed at assessing the effect of novel therapies.

Several assays of cardiac function have also been developed in this model. Haemodynamic measurements, such as heart rate, blood pressure and contractility (maximum change in pressure over time,  $dP/dT_{\max}$ ), have been carried out following catheterization of the left ventricle (Gao *et al.* 2000). Further functional assessments have been made using echocardiography (Scherrer-Crosbie *et al.* 1999). Although variability was fairly high, these end-points have been sufficient to distinguish the MI mouse from sham-operated or wild-type control animals (Patten *et al.* 1998; Lutgens *et al.* 1999; Du *et al.* 2000; Gao *et al.* 2000). However, in order to discern possible treatment effects, the intrinsic variability of the model must be kept to a minimum.

Before embarking on interventional studies, it is important first to evaluate the model's baseline characteristics. Here we attempted to critically appraise the usefulness of the model for future therapeutic studies. Since the placement of the occluding suture is likely to be a key factor in the extent of the resulting cardiac insult, we used three distinct occlusion sites along the LAD coronary artery. We then selected end-points suitable for relatively high-throughput preclinical screening studies. We assessed the effects of suture placement on survival and disease phenotype. We further evaluated end-points of haemodynamic function, lesion size in different regions of the left ventricle, and the correlation between infarct size and cardiac function.

## Methods

### Induction of myocardial infarction

All procedures were approved by the British Home Office, in accordance with the Animals (Scientific Procedures) Act 1986, and by the Imperial College Ethical Review Panel.

Pain and distress were limited by regular administration of analgesia in accordance with advice from Imperial College Named Veterinary Surgeons. C57B6/J mice (male, 20–25 g, 6–10 weeks old) were used. Animals were anaesthetized with 250 mg kg<sup>-1</sup> Avertin (Papaioannou & Fox, 1993; 0.1 ml 2.5% Avertin per 10 g body weight injected intraperitoneally). The analgesic buprenorphine was given preoperatively (10–20 µg (kg body weight)<sup>-1</sup>). Occlusion of the left anterior descending coronary artery (LAD) was performed as previously reported (Michael *et al.* 1999), under sterile conditions, with minor alterations. Briefly, the anaesthetized animal was intubated endotracheally in a supine position, and ventilated with a Harvard Mouse Mini-Vent (Harvard Apparatus, March-Hugstetten, Germany), which supplied 0.2–0.25 ml room air 120 times per minute. The animal was moved onto its right side, and a left thoracotomy provided access to the beating heart. After removing the pericardium, the LAD was visualized with a stereomicroscope (Leica MZ6, Heerbrugg, Switzerland), and occluded with 8/0 (0.4) Prolene suture (Ethicon, Johnson & Johnson, Brussels, Belgium). Occlusion was confirmed by observation of left ventricular pallor immediately post ligation. The chest was closed, the lungs re-inflated and the animal moved to a prone position until spontaneous breathing occurred. Open-chest surgery itself has been shown to cause haemodynamic changes observable 7 days after surgery (Lutgens *et al.* 1999). Thus, control (sham) animals underwent a thoracotomy with removal of the pericardium, but no LAD occlusion. No suture was placed in the sham animals' hearts, in order to avoid unintended vessel damage or occlusion. Animals were monitored closely for signs of infection at the surgical site; none were observed in any animal.

### Suture placement

After experience of approximately 100 procedures had been gained, and the operator was able to locate the LAD in every animal, it became possible to choose finely distinguished suture target sites. A total of 46 animals were divided into three different groups: High, Middle and Low Suture ( $n = 10–15$  per group). In the High Suture group, the occluding suture was placed at the atrioventricular junction. This was located at the edge of the atrial appendage. In the Middle Suture group, the suture was placed 0.3 mm distal to the atrioventricular junction. Accurate measurement of this distance was made possible by use of the stereomicroscope, using the suture needle (width 0.3 mm) as a guide. In the Low Suture group, the suture was placed 0.6 mm distal to the atrioventricular

junction. In all groups, this resulted in the suture being placed 'upstream' of the first major branching of the artery. Figure 1 shows a schematic representation of suture placement sites. Previous studies have reported suture placement 1–3 mm from the atrial appendage (Michael *et al.* 1999; Du *et al.* 2000; Gao *et al.* 2000), whereas here the most distal suture was placed 0.6 mm from the atrial appendage. Apart from suture placement, all other aspects of the procedure were identical for each group. Mice were randomised to each group in order to avoid the effects of any minor variation in age or size.

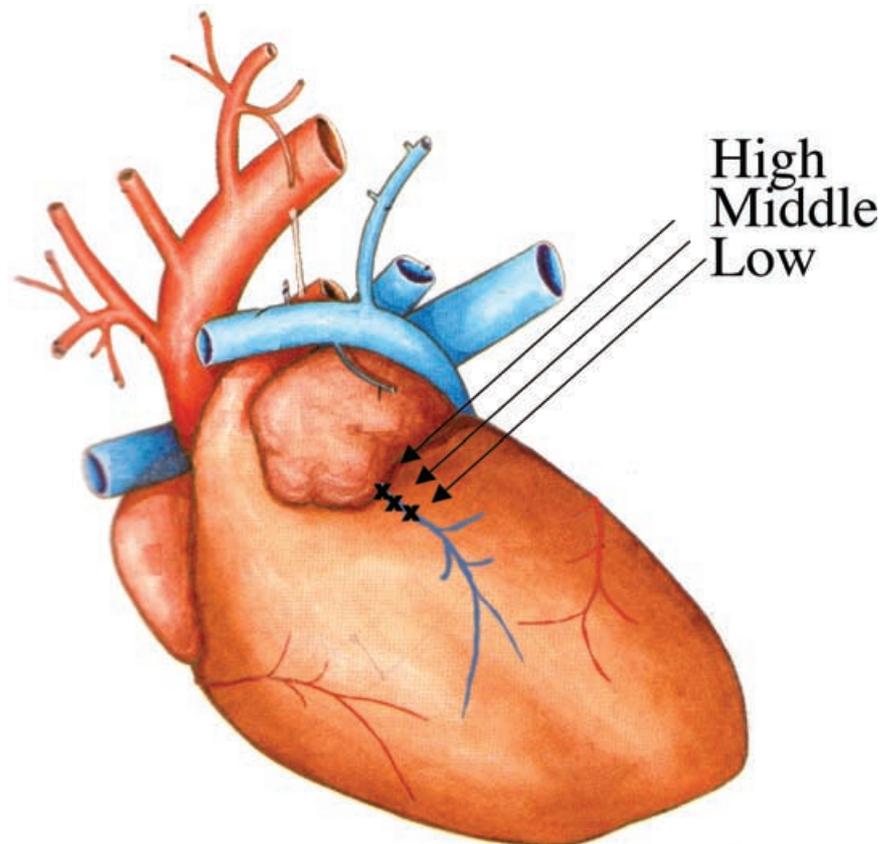
### Phenotypic and haemodynamic assessment

Seven days after surgery, the phenotype of each animal was scored semiquantitatively as follows: 3 = phenotypically indistinguishable from a wild-type animal; 2 = at least one sign of illness (hunched posture or ruffled coat), but otherwise as active as a wild-type animal; 1 = hunched posture, ruffled coat and less active than a wild-type animal; 0 = dead. All surviving animals were anaesthetized with Avertin, intubated and ventilated as above for haemodynamic measurements. Access to the apex of the heart was gained via the abdominal cavity after removal of the diaphragm. A 23 gauge needle filled with heparinized

saline (200 IU ml<sup>-1</sup> heparin in 0.9% saline) was inserted through the apex into the left ventricle. The needle was connected to a Digi-Medical Heart Performance Analyser (Micro-Med Inc., Louisville, KY, USA), which recorded maximum left ventricular pressure (LVP) and maximum change in pressure over time ( $dP/dT_{max}$ ). All measurements and analysis were performed in a blinded manner.

### Histologic analysis

After haemodynamic assessment, animals were killed by exsanguination caused by removal of the transducer-attached catheter. The heart was removed and fixed in fresh 4% paraformaldehyde, pH 7.3. The tissue was processed and embedded in paraffin using routine histological procedures. Five micrometre transverse step sections were collected every 200  $\mu$ m through the entire ventricle (approximately 10–12 sections per animal), and stained with Haematoxylin and Eosin (H&E). At 7 days after occlusion, it is too early to differentiate the final results of the cardiac insult and subsequent repair process. Therefore the total lesion area, expressed as a percentage of the total left ventricular wall, was calculated using MetaMorph software (Universal Imaging Corporation, West Chester,



**Figure 1. Schematic representation of suture placement sites**

'High' Sutures were placed at the atrioventricular junction, while 'Middle' and 'Low' Sutures were placed 0.3 and 0.6 mm, respectively, distal to the atrioventricular junction. Adapted from Popesko *et al.* (1992)

PA, USA), and a mean value calculated for each heart. Mid-papillary sections were then divided into anterior, lateral, posterior and septal regions, and lesion size in each of these regions was scored semiquantitatively. Each region was divided into four equal quadrants, and each quadrant scored 1 (lesion present) or 0 (no lesion). The sum of these quadrant scores was used to give each region an overall score from 0 to 4. All analysis was done in a blinded manner.

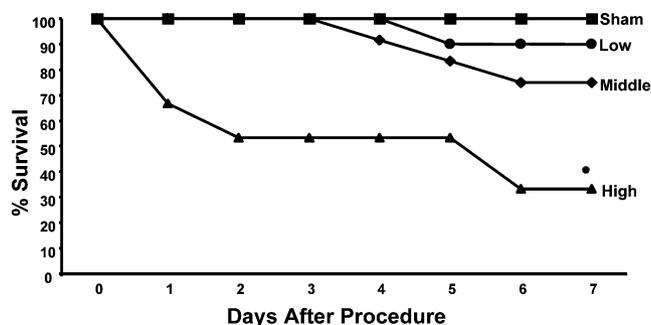
### Statistical analysis

All values are expressed as the mean  $\pm$  s.e.m. or the median (range), as appropriate. Except for survival analysis, data between groups were compared using the Kruskal–Wallis analysis of variance for multiple unpaired, non-parametric groups of variables, followed (where permitted by the Kruskal–Wallis analysis) by the Mann–Whitney  $U$  test, with a Bonferroni correction for multiple comparisons. Survival data were analysed by constructing a Kaplan–Meier table to derive a  $\chi^2$  value. Correlation between selected variables was analysed using the least-squares method for linear regression. The null hypothesis was rejected at  $P < 0.05$ .

## Results

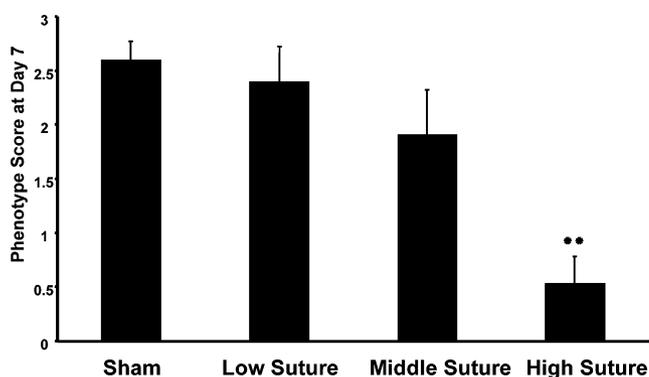
### Effect of occlusion on survival and phenotype

Figure 2 shows survival for sham, Low, Middle and High Suture groups, up to 7 days after the procedure. There was no difference in survival among the sham, Low Suture and Middle Suture groups (100, 90 and 73% survival at day 7, respectively). However, in the High Suture group survival was only 33%, which was significantly ( $P < 0.05$ ) lower than in the sham-operated control group. Thus, the number of animals that completed the protocol was



**Figure 2. Effect of suture placement on survival**

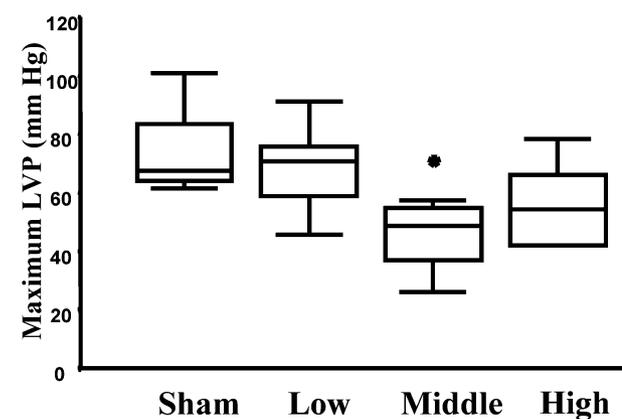
Survival was significantly reduced in the High Suture group compared to sham-operated control animals ( $n = 10$ – $15$ ,  $*P < 0.05$ ).



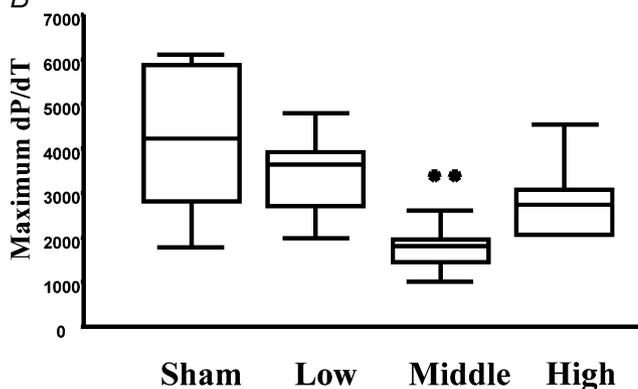
**Figure 3. Effect of suture placement on phenotype**

Phenotype was significantly reduced in the High Suture group compared to all other groups ( $n = 10$ – $15$ ,  $**P < 0.01$ ).

A



B



**Figure 4. Effect of suture placement on maximum LVP (A) and  $dP/dT_{max}$  (B)**

Box plot shows the first quartile about the median (box), and three-quarter range (bars). Only the Middle Suture group had significantly lower maximum LVP and  $dP/dT_{max}$  compared to sham-operated control animals ( $n = 10$ , 9, 8 and 5 for sham, Low, Middle and High Suture groups, respectively,  $*P < 0.05$ ,  $**P < 0.01$ ).

$n = 9$ ,  $n = 8$  and  $n = 5$  in the Low, Middle and High Suture groups, respectively. Phenotype was assessed 7 days after the procedure using a semiquantitative scoring system (Fig. 3). There was no difference among the sham, Low Suture and Middle Suture groups, but the High Suture group had a significantly ( $P < 0.01$ ) lower phenotypic score compared to sham-operated controls.

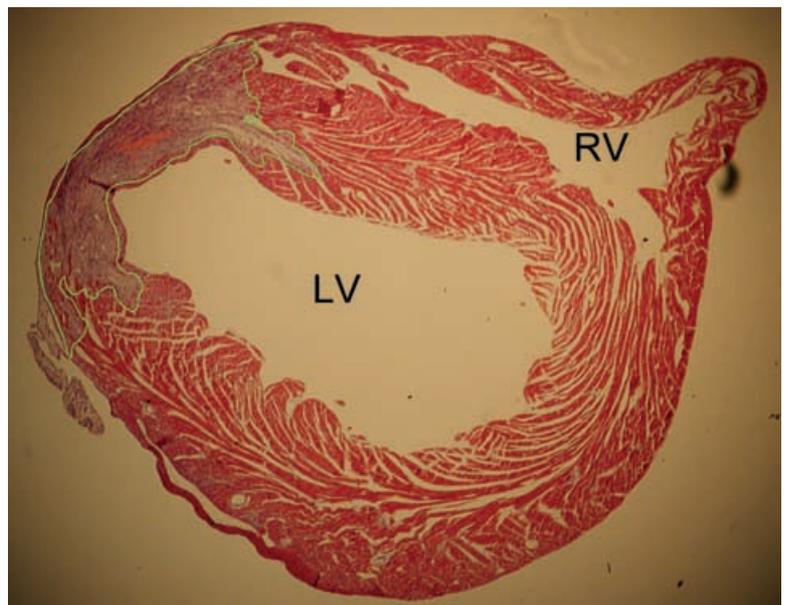
### Effect of occlusion on cardiac function

The effect of occlusion on cardiac function was determined by measuring maximum LVP and  $dP/dT_{\max}$  7 days after the procedure (Fig. 4). Although both the Low and Middle Suture groups had low intragroup variability (coefficient of variance (%c.v.): 20 and 17%, respectively, for maximum LVP; 27 and 23%, respectively, for  $dP/dT_{\max}$ ), only the Middle Suture group showed a significant reduction in maximum LVP (Fig. 4A) and  $dP/dT_{\max}$  (Fig. 4B) compared to the sham control group (maximum LVP: sham group 73.9 mmHg (45.9–101.3 mmHg), Middle Suture group 58.5 mmHg (40.2–65.6 mmHg);  $dP/dT_{\max}$ : sham group 4337 (2016–6129), Middle Suture group 2045 (1294–2803);  $n = 8$ –10,  $P < 0.05$  and  $P < 0.01$  for maximum LVP and  $dP/dT_{\max}$ , respectively). The High Suture group, with low surviving  $n$  numbers and high variability (%c.v.: 43 and 53% for maximum LVP and  $dP/dT_{\max}$ , respectively), was not different from any other group. For all groups, heart rate during cardiac function measurement was 400–500 beats  $\text{min}^{-1}$ .

### Quantification of lesion size

A representative H&E-stained cross-section from a Middle Suture group animal 7 days after myocardial infarction is shown in Fig. 5. Among the Suture groups, there were generally no histologic changes proximal to the suture. Lesion size as a proportion of the left ventricular wall varied considerably through the myocardium; however, there was no consistent pattern of lesion size change in any Suture group. Thus, in 43% of animals the lesion increased from base to apex; in 31% of animals the lesion increased and then decreased; in 22% of animals the lesion remained approximately the same; and in 4% of animals the lesion reduced from base to apex. The septum and right ventricular wall were rarely affected, and if affected, less than 1% of the total septal or right ventricular area was diseased. No histologic changes were seen in any sham-operated animal.

Lesion size was quantified as a percentage of the total left ventricular wall in 10–12 transverse step sections per animal, and a mean value obtained for each animal. Lesions were 1.8-fold larger in the Middle Suture group compared to the Low Suture group (Fig. 6,  $n = 8$ ,  $P < 0.05$ ). In the High Suture group, lesion size did not increase compared to the Middle and Low Suture groups. Variability was high (59–99%c.v.) for this end-point in all occluded groups. We also analysed left ventricular lesion size in the region of maximum lesion for each animal; results were similar to those obtained by calculating average lesion size ( $r = 0.91$ ,  $P < 10^{-8}$ ).



**Figure 5. Histologic analysis of infarcted myocardium**

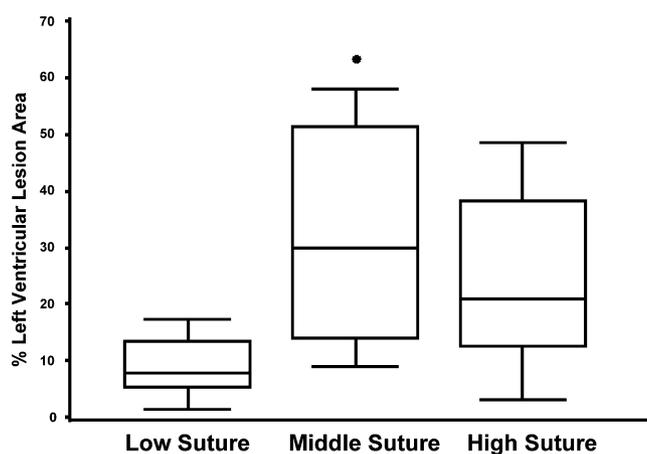
Representative cross-section from the Middle Suture group, stained with H&E (original magnification 40 $\times$ ). Lesion area outlined in green. (RV, right ventricle; LV, left ventricle)

### Correlation between lesion area and cardiac function

There was no correlation between lesion area and maximum LVP or  $dP/dT_{\max}$  (data not shown). However, the effect of MI on cardiac function in humans depends greatly on which region of the left ventricular wall is affected. We therefore analysed lesion area semiquantitatively in each of four regions: the anterior, lateral and posterior walls and the septum. There was no difference between the Suture groups in regional distribution of injury. In all groups, the anterior wall was most affected, followed by the lateral wall, while the posterior wall and septum were minimally affected, if at all. Both anterior (Fig. 7A) and lateral wall (Fig. 7B) lesion scores showed a significant, albeit weak, negative correlation with  $dP/dT_{\max}$  ( $r = -0.46$  for both,  $P < 0.05$ ). There was also a similar correlation between maximum LVP and anterior wall infarct ( $r = -0.44$ ,  $P < 0.05$ ), but not with lateral wall infarct. Neither the posterior wall nor the septum showed any correlation with cardiac function.

### Discussion

In this study we have shown that, with precise placement of the occluding suture, the mouse MI model may provide useful assays for future therapeutic studies. In this study survival, phenotype, cardiac function and degree of infarct were highly dependent on the position of the occluding suture. Occlusion approximately 0.3 mm distal of the atrioventricular junction (the Middle Suture)

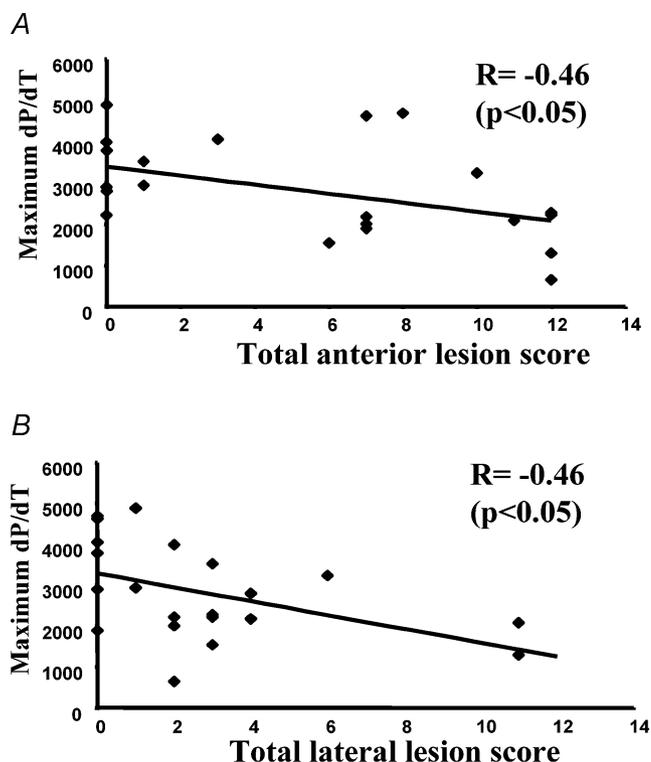


**Figure 6. Effect of suture placement on lesion size**

Box plot shows the first quartile about the median (box), and three-quarter range (bars). The Middle Suture group had significantly larger lesions than the Low Suture group ( $n = 10, 9, 8$  and  $5$  for sham, Low, Middle and High Suture groups, respectively,  $*P < 0.05$ ). No lesion was seen in any sham-operated animal.

led to significant alterations in haemodynamic and histologic end-points, without any significant reduction in survival or phenotype, compared to sham-operated control animals. This suggests that the Middle Suture is the most useful group for further therapeutic studies.

In this study, survival was significantly lower in the High Suture group; this has previously been observed. Du *et al.* (1998) reported that a 'too-high' occlusion site generated an ischaemic zone comprising up to 70% of the left ventricle, which the mice did not survive. Here, similarly, the High Suture group showed low survival and poor phenotype. Assessment of overall phenotype in this way does not necessarily indicate a specific aetiology; however, as suture placement was the only experimental variable, these results suggest that phenotypic changes were related to the High suture placement, and that this resulted in too severe an infarction. With 67% mortality and high morbidity, the surviving animals are not likely to be a representative sample. This is shown by the highly variable cardiac function among the survivors. Although low surviving  $n$  numbers made reliable statistical analysis difficult, the High Suture group was not different from sham-operated control animals. Thus the surviving



**Figure 7. Correlation between lesion area and cardiac function**

There was a significant, albeit weak, correlation between anterior lesion score and  $dP/dT_{\max}$  (A), and lateral infarct score and  $dP/dT_{\max}$  (B;  $r = -0.46$  for both,  $P < 0.05$ ).

animals in the High Suture group are likely to be a selected population with better endogenous collateralization or greater reliance on other coronary arteries. In addition, it is ethically difficult to justify the use of a model with such severe outcomes, especially when the Middle Suture group provides a reproducible model without such severe effects on morbidity and mortality.

Survival in the Middle and Low Suture groups was similar to previously published studies, which have reported 50–76% survival 7 days after induction of MI (Lutgens *et al.* 1999). Intra-group variability was lowest in the Middle and Low Suture groups. However, the Low Suture group was not different from sham-operated control animals in haemodynamic end-point assays. Due to the 'low' occlusion, a substantial proportion of the left ventricle remained perfused and therefore functional. In contrast, in the Middle Suture group a reduction of 20 mmHg in maximum LVP and 2400 in  $dP/dT_{\max}$  compared favourably with previous published studies (Lutgens *et al.* 1999; Du *et al.* 2000; Gao *et al.* 2000). Moreover, the use of a catheter-based system provided global cardiac functional data in a practical, accessible and timely form, which is desirable for relatively high-throughput screening studies.

Specific measurements of intragroup variability have not yet been reported. In this study we determined the coefficients of variance (%c.v.) to characterize the variability of haemodynamic and histologic measurements within each group. Variability was also used to evaluate the usefulness of each end-point for future therapeutic experiments. Low variability (17–23%) in maximum LVP and  $dP/dT_{\max}$  in the Middle Suture group, in conjunction with significant reductions compared to sham-operated controls, suggests that these end-points may be robust enough to assess potential treatments. Power calculations, based on intragroup variability, predict that  $n = 12$  mice per group would be required in order to measure accurately a 20% improvement in maximum LVP and a 30% improvement in  $dP/dT_{\max}$ . In contrast, higher variability in our histologic data means that  $n = 14$  mice per group would be required to demonstrate a 40% reduction in overall lesion size. The increased experimental 'noise' in this end-point may also contribute to the fact that we did not observe a significant correlation between total left ventricular lesion area and cardiac function. This is in contrast to a previous study which showed a significant negative correlation between overall infarct size and echocardiographic parameters (Gao *et al.* 2000).

When we analysed four different regions (anterior, lateral and posterior wall and the septum) separately, however, cardiac function correlated most strongly with

lesion size in the anterior and lateral regions. These are the regions whose supply is most dependent on the LAD coronary artery, and which have the largest influence on myocardial contractions. Not surprisingly therefore, as in humans, injury to these regions has the greatest negative effect on cardiac function. However, the strength of the correlations suggests that other factors also contribute, which probably include endogenous collateralization and/or reliance on coronary vessels other than the LAD coronary artery. It therefore appears that lesion size may be a useful secondary end-point for future treatment studies, as long as the limitations of the assay are acknowledged.

In summary, we have shown that occlusion of the LAD coronary artery 0.3 mm distal to the atrioventricular junction produces a significant and reproducible reduction in cardiac function, and a significant increase in histologic changes in the left ventricle. Although some, more sophisticated, measurements, such as functional assessment of collateralization using coloured microspheres, are not yet technically feasible in the mouse, the model as described here may provide a useful preclinical tool. Further, we provide power calculations which may facilitate the development of studies to assess potential new treatments in this model.

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