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SHORT REPORT

Interleukin-1β modulation using a genetically engineered antibody prevents adverse cardiac remodelling following acute myocardial infarction in the mouse

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Background

Acute myocardial infarction (AMI) is the result of myocardial necrosis secondary to ischaemia and of local and systemic inflammation that further enhances tissue damage and promotes cardiac enlargement and failure (adverse cardiac remodelling).[1] Interleukin-1 (IL-1) is the prototypic inflammatory cytokine produced at the site of injury and is responsible for endothelial activation, leucocyte recruitment, and amplification of the response.[2] During AMI, IL-1 leads to further myocardial damage.[3,4] Recent developments in the treatment of autoinflammatory diseases provide sufficient clinical evidence that modulating IL-1 activity halts systemic as well as local disease.[2] Two ongoing clinical trials are testing the effects of IL-1 antagonism in AMI using a recombinant human IL-1 receptor antagonist, anakinra.[5,6] Anakinra prevents binding to the receptor of both IL-1 isoforms (α and β). A novel strategy for IL-1 modulation is the use of genetically engineered antibodies that bind IL-1β with ultra high affinity.[7] This approach, however, is faced with uncertainties related to the existence of the two IL-1 isoforms[2] and the prior failure of a similar approach using a hamster antibody to IL-1β in a mouse model of AMI.[8]

Aims

The aim of the current study was therefore to test the effects of IL-1β modulation using a novel genetically engineered mouse antibody that binds to IL-1β with high affinity in a mouse model of AMI.

Methods

Adult male outbred ICR mice were purchased from Harlan Laboratories (Indianapolis, IN, USA). All animal experiments were conducted under the guidelines on Humane Use and Care of Laboratory Animals for biomedical research published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study protocol was approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee. Thirty-six mice underwent experimental myocardial infarction as previously described.[4] Briefly, mice were anaesthetized with intraperitoneal (IP) pentobarbital (70 mg/kg), intubated orotracheally, and ventilated on a positive-pressure ventilator. Left thoracotomy was performed at the fourth intercostal space and the heart was exposed by stripping the pericardium. The left descending coronary artery was then identified with a surgical microscope (Leica F40) and ligated with a 7.0 silk ligature. A group of four mice underwent sham operation as previously described.[4] After surgery, mice were randomly assigned to treatment with XMA 052 MG1K, a genetically engineered antibody which consists of Human Engineered™ heavy and light chain variable regions fused to mouse Gamma-1 and Kappa constant regions that bind murine IL-1β with a high affinity, administered by IP injection at three different doses (0.05, 0.5, or 5 mg/kg) or a control murine IgG1 kappa antibody. Treatments were administered immediately after surgery and then again 7 days later ($n = 6$ per group). One additional group of mice ($n = 6$) was used to test the effects of pretreatment with an additional dose of XMA 052 MG1K (0.5 mg/kg), administered 48 h prior to surgery. All of the anti-IL-1β antibodies were provided by XOMA (US) LLC (Berkeley, CA, USA) at no cost. All mice underwent transthoracic echocardiography before surgery and at 7, 14, and 28 days after coronary ligation. Mice which died before Day 7 were replaced to keep the number of observations at six or more per group. Doppler echocardiography was performed with the Vevo770

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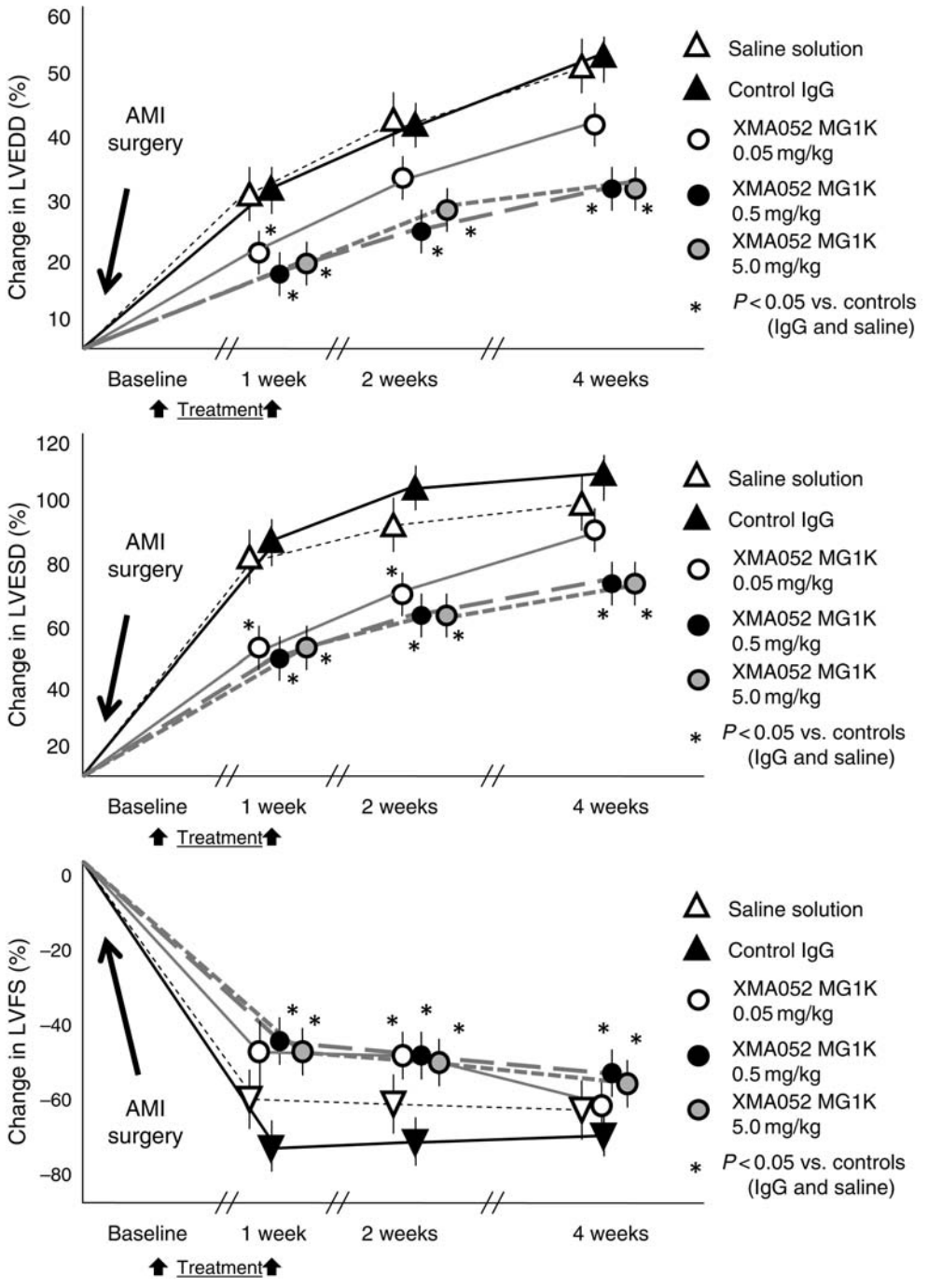

Figure 1 Serial changes in left ventricular end-diastolic diameter (LVEDD) (top panel), left ventricular end-systolic diameter (LVESD) (mid panel), and left ventricular fractional shortening (LVFS) (bottom panel) after AMI over a 4-week period in the five treatment arms.

imaging system (VisualSonics, Inc., Toronto, Canada). The heart was first imaged in the two-dimensional mode in the parasternal and apical views and measurements were performed according to the to the American Society of Echocardiography recommendations.[9] The left ventricular (LV) end-diastolic diameter (LVEDD), LV end-systolic diameter (LVESD), anterior wall diastolic thickness, anterior wall systolic thickness, posterior wall diastolic thickness, and posterior wall systolic thickness were measured at M-mode. Left ventricular fractional shortening (LVFS) was calculated as follows: FS = (LVEDD − LVESD)/LVEDD × 100. The number of segments with abnormal kinesis (which correlates with infarct size) was determined using a 17-segment map. An apical view was used to measure the ejection time (ET), and the time interval between the end of the transmitral A wave and the following E wave (AE). The myocardial performance index (MPI or Tei index) was then computed (MPI = [AE − ET]/ET). The tricuspid annular plane systolic excursion (TAPSE) was also measured as a marker of right ventricular function. The investigator performing and reading the echocardiograms was blinded to the treatment allocation.

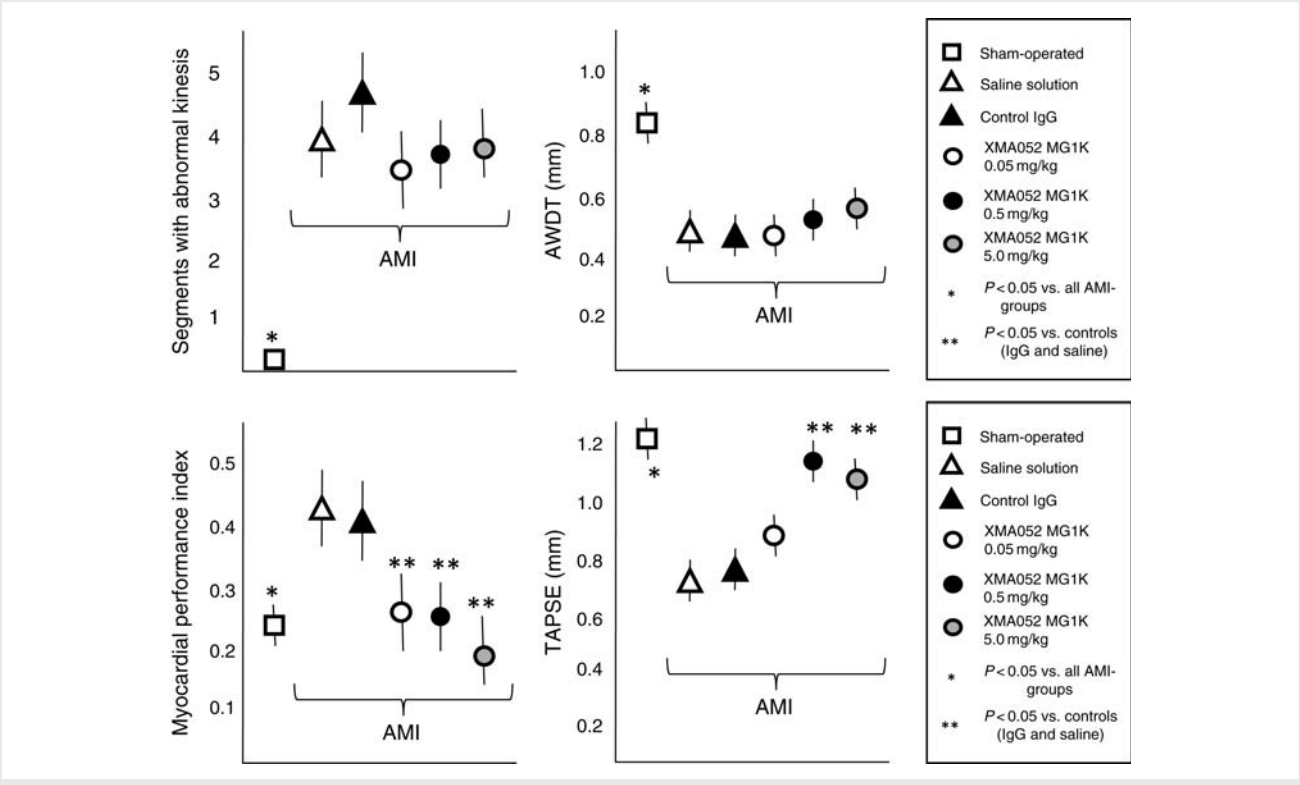

Figure 2 Number of segments with abnormal kinesis (a surrogate for infarct size) (top left panel), the anterior (infarct) wall diastolic thickness (AWDT) (top right panel), the myocardial performance index (bottom left panel), and the tricuspid annular plane systolic excursion (TAPSE) (bottom right panel) 1 week after surgery in the sham-operated mice and in the five AMI treatment arms.

SPSS 11.0 (Chicago, IL, USA) was used for the statistical analysis. ANOVA was used for multiple comparisons with *post hoc t*-test to explore between group differences. For comparisons of interval changes between multiple-group random effects, ANOVA for repeated measures was used to determine the main effect of time, group, and time-by-group interaction. Statistical differences were considered significant, if the two-sided *P*-value was <0.05.

Results

Baseline echocardiographic values were similar in all groups. Ten mice died within 7 days and were replaced (three in the normal saline group, two in the Ig control, two in the 0.05 mg/kg, and one each in the 0.5 and 5 mg/kg XMA 052 MG1K groups). On echocardiography, as expected, we observed significant increases in LV diameters (LVEDD and LVESD) and a significant decrease in LVFS as early as 7 days after surgery compared with baseline in all groups (except sham-operated mice). Mice receiving XMA 052 MG1K had a smaller increase in LVEDD and LVESD and a smaller decrease in LVFS compared with mice treated with murine IgG1 control or mice treated with normal saline (*Figure 1*). The effects of treatment were numerically superior with the 0.5 and 5 mg/kg dose compared with the 0.05 mg/kg dose of XMA 052 MG1K (*Figure 1*). At 4 weeks, 3 weeks after the last dose, the mice treated with the 0.05 mg/kg showed no significant difference in cardiac dimensions and size compared with the control groups, whereas the mice treated with 0.5 and 5 mg/kg had a significantly smaller increase in LVEDD and LVESD and a smaller decrease in LVFS (*Figure 1*).

The number of segments with abnormal kinesis (hypokinesis akinesis or dyskinesis), a surrogate for infarct size, was 3.9 ± 0.4 in the saline-treated mice, and it was not significantly affected by treatment (*Figure 2*). Accordingly, the anterior wall (infarct) thickness was 0.52 ± 0.05 mm in the saline-treated group and was unaffected by treatment (*Figure 2*).

On the other hand, the MPI or Tei index, a marker of combined systolic and diastolic dysfunction and a surrogate marker for heart failure related mortality, was significantly increased after AMI (reflecting poor function) but was preserved in the mice treated with XMA 052 MG1K (*Figure 2*).

Similarly, TAPSE, a marker of right ventricular function and a surrogate marker for AMI-related mortality, was significantly decreased after AMI (reflecting poor function) but was partially preserved in the mice treated with XMA 052 MG1K (*Figure 2*).

Pre-treatment with an additional dose of XMA 052 MG1K 48 h prior to surgery offered no advantage over treatment after surgery (data not shown).

Conclusions

Modulation of IL-1 activity using a genetically engineered antibody to IL-1β ameliorates cardiac enlargement and dysfunction following AMI in the mouse without affecting the infarct size.

The results are comparable to those observed with anakinra and IL-1Trap which both block IL-1β and IL-1α.[4,10] This confirms that modulation of the IL-1β isoform is sufficient to reduce IL-1 activity as IL-1β represents the isoform involved in the intercellular and systemic effects, whereas IL-1α has intracellular and autocrine effects.[2]

These results contrast with the lack of benefit and actual harm using a heterologous hamster anti-mouse antibody at a similar dose in a similar AMI model, although using a different strain of mouse,[8] and are potentially related to differences in the structure and function of the anti-IL-1β antibody used, suggesting a role of the effector system and antibody clearance.[11,12] A regulatory function has also been proposed for the XOMA antibody which binds IL-1β to reduce but not completely block all activity,[13] which is potentially important in maintaining a physiological role of IL-1β in compensatory hypertrophy.[14]

In conclusion, IL-1 modulation using genetically engineered antibodies specifically targeted at safely modulating IL-1β activity represents a promising translational approach to prevent heart failure following AMI.

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Conflict of interest: none declared.

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