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Anakinra in Experimental Acute Myocardial Infarction—Does Dosage or Duration of Treatment Matter?

Fadi N. Salloum · Vinh Chau · Amit Varma · Nicholas N. Hoke · Stefano Toldo · Giuseppe G. L. Biondi-Zoccai · Filippo Crea · George W. Vetrovec · Antonio Abbate

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Abstract
Purpose Interleukin-1 (IL-1) receptor antagonist (Ra) is a naturally occurring IL-1 blocker with a cardioprotective effect during acute myocardial infarction (AMI). Anakinra, recombinant-human IL-1Ra, has been used to prevent heart failure in a mouse model of AMI. The aim of this study was to determine the optimal therapeutic regimen for anakinra in AMI.
Methods We performed dose–response experiments comparing anakinra 1 mg/kg with 100 mg/kg doses, and duration–response experiments comparing 1-week to 2-week treatment. Echocardiography was used to assess cardiac remodeling and systolic function. Histopathology was used to detect apoptotic cardiomyocytes.
Results A higher dose of anakinra was not associated with additional improvement in cardiac remodeling or function. The 2-week anakinra treatment had sustained and more favorable remodeling and systolic function compared to 1-week treatment with significantly smaller left ventricular end-systolic diameter and greater fractional shortening 4 weeks after AMI.
Conclusion Anakinra inhibits apoptosis and ameliorates cardiac remodeling up to 4 weeks after infarction. A 2-week regimen is superior to a 1-week regimen, whereas a higher dose did not provide any further benefit over standard doses.

Key words Interleukin-1 · Cytokine · Apoptosis · Remodelling · Myocardial infarction · Anakinra

F. N. Salloum · V. Chau · A. Varma · N. N. Hoke · S. Toldo · G. W. Vetrovec · A. Abbate
VCU Pauley Heart Center, Virginia Commonwealth University, Medical College of Virginia, Richmond, VA, USA

S. Toldo · F. Crea · A. Abbate
Institute of Cardiology, Catholic University, Rome, Italy

G. G. L. Biondi-Zoccai
Division of Cardiology, University of Turin, Turin, Italy

A. Abbate (✉)
Division of Cardiology/VCU Pauley Heart Center, Virginia Commonwealth University, 1200 East Broad Street-West Hospital, 10th Floor, East Wing, Room 1041, P.O. Box 980281, Richmond, VA 23298-0281, USA
e-mail: aabbate@mcvh-vcu.edu

Introduction

During acute myocardial infarction (AMI), damage occurs in the myocardium and a cascade of pro- and anti-inflammatory mediators are released. An imbalance between pro- and anti-inflammatory mechanisms may be in part responsible for adverse cardiac remodelling after AMI and the subsequent left ventricular dysfunction (LVD) and HF that can ensue [1]. The interleukin-1 (IL-1) system plays a significant role in the myocardial response to ischemia and infarct healing in a delicate balance between pro- and anti-inflammatory mediators [1–10]. Interleukin-1 receptor antagonist (IL-1Ra) is a naturally occurring anti-inflammatory protein, member of the IL-1 family, released during AMI [6, 11–14]. We have recently shown that administration of the drug anakinra, a recombinant human IL-1 receptor antagonist, within 24 h of experimental AMI in an animal model significantly attenuates cardiac remodelling 7 days after AMI, mainly by inhibition of cardiomyocyte apoptosis [15]. A major limitation of that study, however, was the relatively short follow up time frame. Accordingly, the aims of the current study were (a) to

investigate the acute and subacute effects of a higher than standard dose of anakinra and (b) to determine if longer treatment duration with anakinra in the experimental mouse AMI model is associated with additional benefit in terms of cardiac remodelling, systolic function and cardiomyocyte apoptosis.

Methods

Dose–response experiments

Thirty mice were divided into three groups of ten animals per group and all underwent permanent coronary artery ligation according to the protocol described below. The first group, Group A_{low} received low dose anakinra at 1 mg/kg, intraperitoneally (i.p.), immediately after surgery and then daily for a total of 7 days. The second group, Group A_{high} received high dose anakinra at 100 mg/kg, given i.p. immediately after surgery and then daily for a total of 7 days. The final group of ten animals, Group B, received volume-matched normal saline (NaCl 0.9%) given i.p. for 7 days. All animals underwent transthoracic echocardiography (TTE) [as described below] prior to surgery, at 24 h and at day 7. An additional set of five animals underwent sham operation and served as sham controls (Fig. 1a).

Duration–response experiments

Thirty mice were divided into one of three treatment groups and all mice underwent coronary artery ligation as described below. The first group, Group A_{1W} received 1 mg/kg of anakinra given i.p. immediately following surgery and then daily for a total of 7 days. The second group, Group A_{2W}, also received 1 mg/kg of anakinra following surgery but then daily for a total of 14 days. The third group (Group B) received matching volume of normal saline (NaCl 0.9%) after surgery and then daily for 14 days. An additional set of 5 animals underwent a sham operation and served as sham controls. All animals had a TTE prior to surgery and at day 7, 14 and 28 (Fig. 1b).

Surgical procedure

Adult male out-bred Institute of Cancer Research (ICR) mice (10 weeks of age, 26–38 g of weight) were supplied by Harlan Sprague Dawley (Indianapolis, IN). All animal experiments were conducted in accordance to the guidelines on humane use and care of laboratory animals for biomedical research published by National Institutes of Health (No. 85-23, revised 1996). Ischemia was induced by permanent left coronary artery ligation as described previously [15, 16]. The surgical procedures were performed under sterile conditions on day 1 by two skilled operators (FNS and VC). In brief, the animals were anesthetized with the injection of pentobarbital (70 mg/kg i.p.), orotracheally intubated and ventilated on a positive-pressure ventilator. The tidal volume was set at 0.2 ml, and the respiratory rate was adjusted to 133 cycles per minute. A left thoracotomy was performed at the fourth intercostal space, and the heart was exposed by stripping the pericardium. The left coronary artery was then identified and permanently occluded by a 7.0 silk ligature that was placed around the vessel. After successful coronary artery occlusion, all air was expelled from the chest. The animals were extubated and then received intramuscular doses of analgesia (buprenex; 0.02 mg/kg) and antibiotic (gentamicin; 0.7 mg/kg; for 3 days). A total of ten mice underwent a sham operation including every step except coronary ligation. The Institutional Animal Care and Use Committee of Virginia Commonwealth University approved the study.

Echocardiography

TTE was performed under light anaesthesia (pentobarbital 30–50 mg/kg) just prior to surgery and again at the aforementioned intervals. Doppler echocardiography was performed using the Vevo770^{TM} imaging system (VisualSonics Inc., Toronto, Canada) and a 30-MHz probe with the transducer positioned on the left anterior side of the chest wall. The heart was first imaged in the 2-D mode in the short-axis view of the left ventricle (LV). The M-mode cursor was positioned perpendicular to the anterior and posterior wall in order to measure left ventricular end-diastolic- and end-systolic-diameters (LVEDD and LVESD, respectively), according to the American Society of Echocardiography recommendations [17]. M-mode images were then obtained at the level of the papillary muscles below the mitral valve tip. Left ventricular fractional-shortening (FS) was calculated as $(LVEDD - LVESD)/LVEDD \times 100$. Ejection fraction was calculated using the Teichholz formula [18]. Allocation to different treatments was random, and the investigator performing and reading the echocardiogram was blinded to the treatment. The parameters from TTE used for analysis between treatment groups include: LVEDD, LVESD, anterior wall diastolic thickness (AWDT), posterior wall diastolic thickness (PWDT), anterior wall systolic thickness (AWST), posterior wall systolic thickness (PWST) and FS.

Apoptosis

At the end of the aforementioned procedures, the animal hearts were removed and stored in 10% formalin for at least 48 h. Subsequently, transverse sections of the median third of the left ventricle were taken for analysis of cardiomyo-

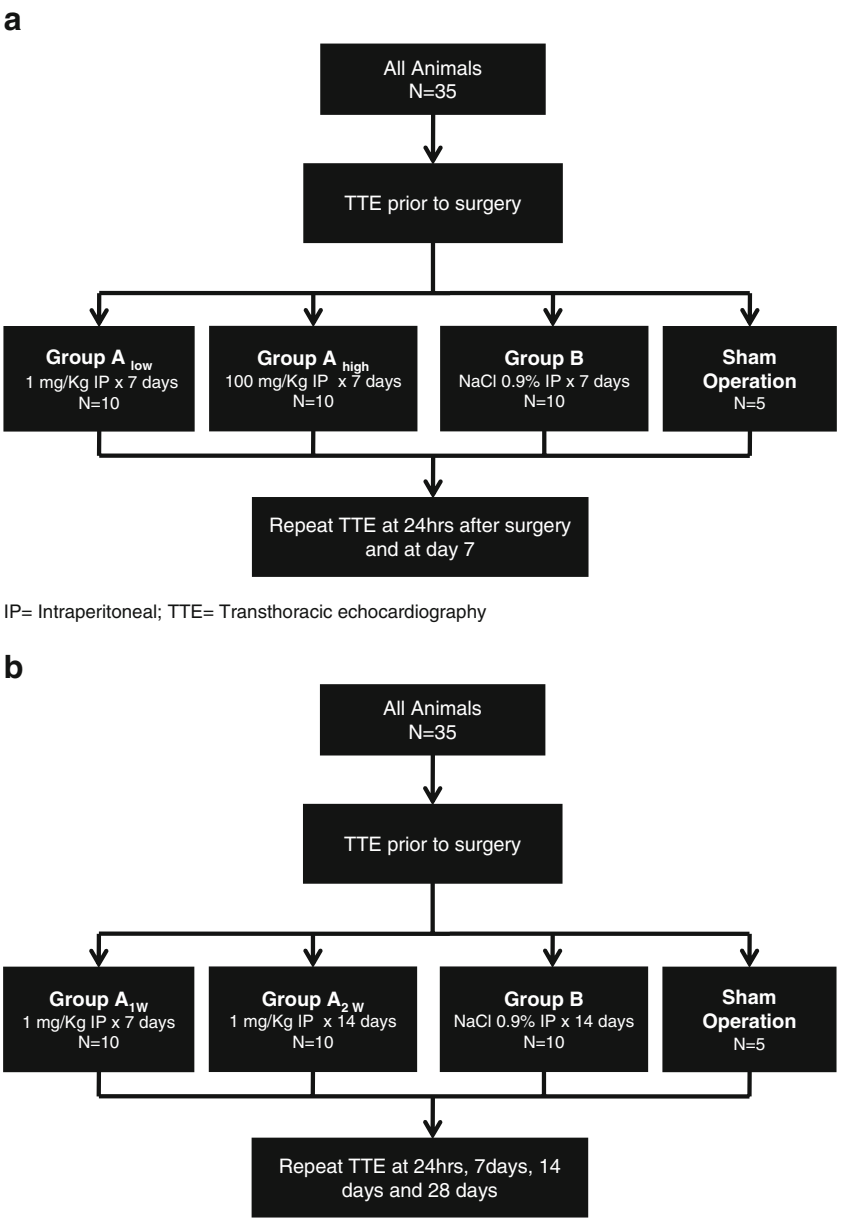

Fig. 1 **a** The process by which the dose–response experiments were performed. All animals underwent trans-thoracic echocardiography (TTE) before surgery and then were divided into one of four groups—low dose anakinra (1 mg/kg), high dose anakinra (100 mg/kg), saline treatment (equivalent volume of NaCl 0.9%) or sham. All underwent repeat TTE 24 h after surgery and again at day 7. **b** The process by which the duration–response experiments were performed. All animals underwent trans-thoracic echocardiography (TTE) before surgery and then were divided into one of four groups—anakinra (1 mg/kg) for 1 week, anakinra (1 mg/kg) for 2 weeks, saline treatment (NaCl 0.9%) for 2 weeks or sham. All animals underwent repeat TTE 24 h after surgery, and again at days 7, 14 and 28

cytes apoptosis. In-situ end labeling of DNA fragmentation (TUNEL) was performed using the Apoptag kit (Oncor, Gaithersburg, MD). The detailed protocol has been published elsewhere [19]. The peri-infarct area was defined as the zone bordering the infarct where viable myocardium was prevalent. The apoptotic rate (AR) was expressed as the number of apoptotic cardiomyocytes on all cardiomyocytes per field. The apoptotic rate in the peri-infarct regions was calculated using at least five random fields, which virtually cover the entire peri-infarct area. The allocation to different treatments was random, and those performing the AR determination (AV, NNH) were blinded to the treatment.

Statistical analysis

Statistical analysis was performed using the SPSS 11.0 package for Windows. Continuous variables are expressed as mean and standard error. The *T* test for unpaired data was used when comparing means between two groups only. Random-effects ANOVA for repeated measures were used to compare pre- and post-intervention echocardiographic parameters between the groups with the post-hoc two-sided Dunnett's test to specifically compare the between-subjects effects when comparisons between multiple groups are made. Unadjusted two-tailed *P* values are reported throughout, with statistical significance set at the 0.05 level.

Results

Dose–response experiments

No significant differences in LVEDD, LVESD, AWDT, PWDT, AWST, PWST, and FS were found between treatment groups at baseline (prior to surgery) and 24 h after surgery. At 7 days however, both Group A_{low} (anakinra 1 mg/kg) and Group A_{high} (anakinra 100 mg/kg) had significantly lower LVEDD [4.4±0.1 mm and 4.3±0.1 mm, respectively] versus saline treated mice, LVEDD [4.9±0.1 mm] (P=0.005 and P=0.040, respectively). Similarly, a significant difference in LVESD and FS was found between the anakinra treated and saline treated mice (Group A_{low} LVESD [3.3±0.1 mm], Group A_{high} [3.2±0.1 mm] vs. Group B [4.2±0.1 mm], P=0.001 and P=0.021, respectively) (Fig. 2a,b). FS was significantly higher in the anakinra treated mice, in Group A_{low} [24.7±0.1%], Group A_{high} [25.2±0.1%] compared to reduced FS in saline treated mice, [15.8±0.1%], (P=0.005 and P=0.013, respectively). No significant differences were found when comparing Group A_{low} and Group A_{high} at 24 h or at 7 days.

Duration–response experiments

No significant difference was found between treatment arms at baseline and 24 h after surgery. At 7 and 14 days, Group A_{1W} and Group A_{2W} had significantly lower LVEDD, LVESD and greater FS compared to the saline treated group, Group B, all P values<0.05 (Fig. 3a–c). No significant differences were found between Group A_{1W} and Group A_{2W} at day 7 and day 14; however Group A_{1W} had a trend towards greater LVESD and lower FS at 14 days when compared to Group A_{2W}. At day 28, both Group A_{1W} and Group A_{2W} continued to have significantly lower LVEDD, LVESD and greater FS vs. Group B. Also at the 4-week time-point, Group A_{2W} had significantly smaller LVESD compared to Group A_{1W} [3.0±0.1 mm vs. 3.8±0.1 mm, P=0.044] and significantly greater FS when compared to animals only treated for 1-week, Group A_{1W} [32.5±0.1% vs. 22.3±0.1%, P=0.012]. Accordingly, both Group A_{1W} and Group A_{2W} had significantly lower AR compared to saline (P<0.001 for both groups) with an approximate 40% reduction in Group A_{2w} when compared to Group A_{1W}, although this did not reach statistical significance (Fig. 4).

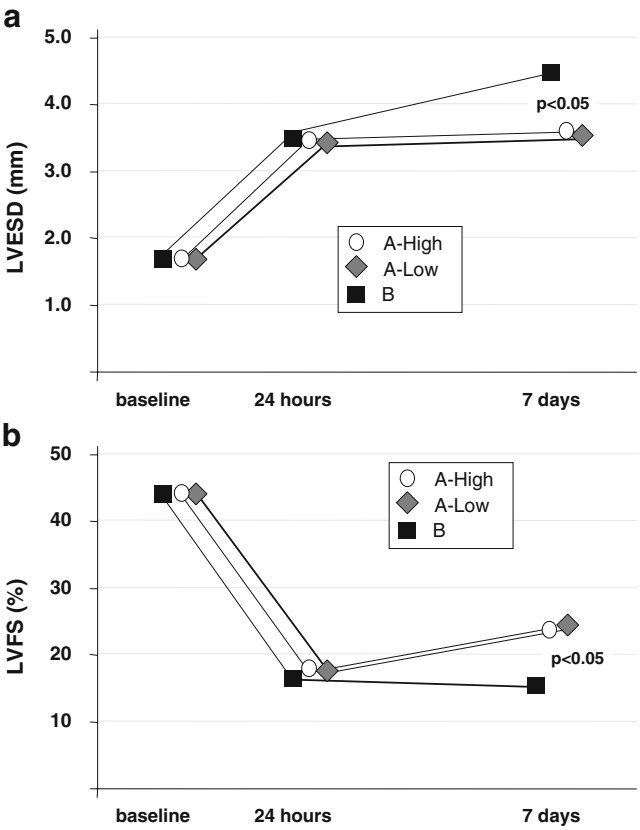

Fig. 2 **a** No difference in left ventricular end-systolic diameter (LVESD) was found between high dose anakinra (100 mg/kg), low dose anakinra (1 mg/kg) and saline groups at baseline and at 24 h after surgery. However by day 7, both anakinra treatment groups had lower LVESD when compared to the saline treated group (P<0.05), without any significant difference between the low and high anakinra doses. **b** No difference in left ventricular fractional shortening (FS) was found between high dose anakinra (100 mg/kg), low dose anakinra (1 mg/kg) and saline groups at baseline and at 24 h after surgery. However at day 7, both high and low dose treatment groups had significantly higher FS when compared to the saline treated group (P<0.05), without difference between the low and high anakinra doses

Discussion

IL-1 blockade by anakinra in experimental AMI is associated with a reduction in cardiomyocyte apoptosis and accordingly, prevents cardiac remodeling up to at least 4 weeks after surgery. These results also show that a 2-week treatment regimen with anakinra is superior to 1-week regimen, and that minimal to no late loss in cardiac remodeling is observed with the 2-week treatment. We found, however, no additional benefit with higher than usual doses of anakinra.

Anakinra is a recombinant human IL-1Ra that modulates IL-1 activity by competitive antagonism of the IL-1 type I cell membrane receptor. IL-1 mediates the inflammatory response through production of nitric oxide and prostaglandins and promotes tissue remodeling by upregulating the synthesis of chemokines and adhesion molecules [20]. By preventing IL-1 from binding to its membrane receptor, anakinra inhibits pro-inflammatory signal transduction [21].

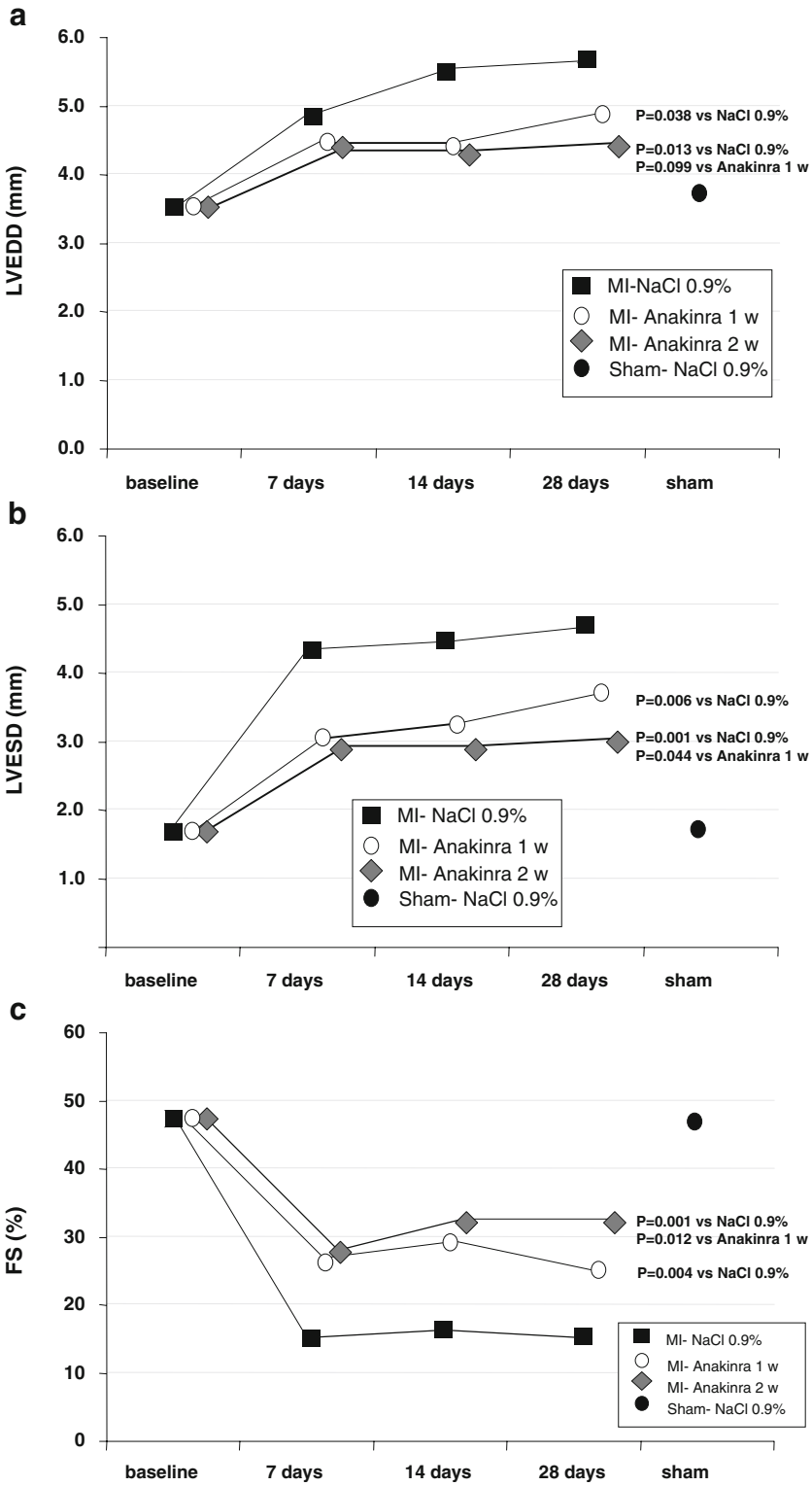

Fig. 3 **a** There was no significant difference in left ventricular end-diastolic diameter (LVEDD) between anakinra for 1 week, anakinra for 2 weeks, and saline treatment groups at baseline or at day 7. However by day 28, both 1 and 2 week anakinra treatment arms had lower LVEDD when compared to the saline group (P=0.038 and P=0.013, respectively), with a trend toward smaller LVEDD in the 2-week anakinra treatment vs. 1-week treatment. Similarly, **b** shows that by day 28, both the 1-week and 2-week anakinra treatment groups had lower LVESD compared to saline (P=0.006 and P=0.001, respectively) and the 2-week treatment group had a significantly lower LVESD when compared to the 1-week group (P=0.044). **c** Accordingly, that by day 28, the two anakinra treatment arms had significantly higher FS than the saline treated group (P=0.004 and P=0.001, respectively), and also that the 2-week treatment group had a significantly higher FS when compared to the 1-week group (P=0.012)

It has also been shown that during myocardial ischemia, IL-1 is produced by the cardiomyocyte and is responsible for the acute inflammatory response within the myocardium [22]. Thus not surprisingly, in an experimental animal model of forced overexpression of IL-1Ra in the myocardium, there was less inflammation in response to ischemic injury and a reduced number of cardiomyocytes undergoing apoptosis [23]. In the mouse AMI model, due to permanent

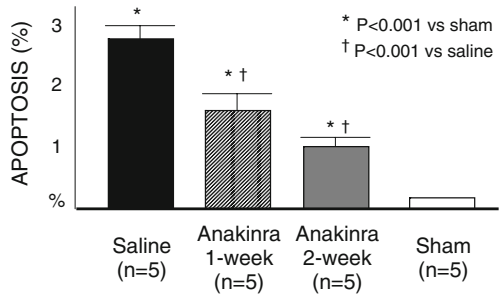

Fig. 4 This figure illustrates the percent of apoptotic cardiomyocytes at day 28 in the saline, 1-week anakinra, 2-week anakinra treatment groups and sham treated mice. One-week and 2-week treatment arms had a significant reduction in apoptosis when compared to the saline treated mice ($P<0.001$ for both). However, no significant difference in apoptosis was found when comparing the two anakinra treatment groups

coronary ligation, significant LV dysfunction and dilatation are evident as early as 1 week after surgery. This adverse remodeling further proceeds for 4-weeks, with minimal or no further dilatation after that point in time. The optimal duration of IL-1 blockade in experimental AMI remains unclear. In the current study, we described that late loss in terms of increased LVEDD, LVESD and reduced FS occurred at 4-weeks in those animals treated for only 1-week. Benefits of treatment were maintained at 4-weeks in the 2-week regimen and longer than 2-week treatment appeared not to be warranted in this model, as no late loss was seen in these mice. The optimal duration of IL-1 blockade may however vary based on the experimental model. For instance, in a model of vessel wall response to injury, IL-1Ra given for 4-weeks was associated with a greater effect compared to IL-1Ra given for only 2-weeks [24]. Moreover whether these results may be applied to humans remains questionable.

Furthermore, the ideal dose of anakinra that prevents adverse remodeling remains uncertain. In a wide range of in vitro studies, we described that anakinra was effective in extremely low doses but without a clear dose–response correlation. In vivo, however, the 100 mg/kg dose of anakinra was associated with a small yet significant reduction in infarct size that was not observed with the 1 mg/kg dosing regimen [18]. This is in agreement with prior studies in experimental stroke in which infarct sparing effects were noted with 100 mg/kg treatment doses [25]. However in the current study, while we found that the 100 mg/kg is safe, we failed to demonstrate significant functional benefits with the higher dose of anakinra at 24 h after AMI or at day 7 suggesting that the infarct sparing effect may not be essential for the benefit of the drug, although we may not exclude that a longer follow up could have shown that a difference does indeed exist. Moreover, the optimal dose–response relation may vary based on the model studies and the endpoint of interest: i.e. while we found a dose–response relation for infarct size, we could not find a dose–response relation for in vitro apoptosis, or for cardiac remodeling [15].

Anakinra is commercially marketed as Kineret™ by Amgen Corporation, and is an FDA approved drug for the treatment of rheumatoid arthritis (RA) [26–28]. The administration of anakinra has been shown to be quite effective in patients with rheumatoid arthritis and its excellent safety profile makes it an ideal drug for translational research. However, the short half life of the drug and need for prolonged treatment challenge its use in daily clinical practice. Currently in the treatment of RA, longer acting drugs such as infliximab and etanercept are the preferred agents [30]. The possibility of longer and more effective IL-1 receptor blockade is certainly appealing and several studies are currently ongoing to determine efficacy and safety of such agents. Rilonacept (IL-1 trap) is a newly developed recombinant fusion protein designed to bind and neutralize both circulating IL-1α and IL-1β thus preventing their binding to the IL-1 type I cell surface receptor. Although rilonacept has been recently approved for the treatment of Cryopyrin-Associated Periodic Syndromes (CAPS) including Muckel–Wells syndrome and there are ongoing Phase II trials in rheumatoid arthritis, clinical experience with this new drug is quite limited [31]. XOMA-052 is a monoclonal antibody which selectively binds to IL-1β and is currently under investigation for the treatment of rheumatoid arthritis, gout and diabetes mellitus. It is a humanized IgG2 antibody with a long half-life (15 to 21 days) and thus has the potential for sustained IL-1β blockade [32]. Whether the more selective IL-1β blocker XOMA-052 or the anti-IL-1α/β agent, rilonacept will prove more effective or more practical than anakinra, while maintaining a similar safety profile, remains unknown.

In summary, IL-1 receptor blockade by anakinra ameliorates adverse cardiac remodeling in experimental AMI by inhibiting cardiomyocytes apoptosis. The clinical implications of IL-1 blockade to the thousands of patients who suffer from heart failure each year secondary to myocardial remodelling following an AMI is promising and further translational research is warranted. Considering the short half-life of anakinra and the need for prolonged IL-1 blockade, further studies with longer lasting IL-1 antagonists in AMI and HF need to be considered.

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