

Pravastatin attenuates atherosclerosis after myocardial infarction by inhibiting inflammatory Ly6C^{high} monocytosis in apolipoprotein E knockout mice

Yufei Chen^{1,#} , Hongqi Zhang^{2,#}, Liang Hu^{3,#},
Haiming Shi¹, Xiaojin Liu¹, Jianguo Jia⁴,
Shengjia Sun¹ , Yang Ou¹, Xiping Luo¹,
Guomin Zhou² and Wei Shen¹ 

Abstract

Objective: To evaluate the protective effect of pravastatin on atherosclerotic development and inflammatory monocyte subset in atherosclerotic apolipoprotein E (ApoE)^{-/-} mice after myocardial infarction (MI).

Methods: Male ApoE^{-/-} mice (8 weeks old) were fed a high-fat diet for 14 weeks throughout the experiment. A MI model was produced using 18-week-old ApoE^{-/-} mice. They were randomly divided into three groups: sham group, MI group, and MI+Pra group (40 mg/kg/day pravastatin). After 4 weeks (at the end of the study period), the mice were sacrificed and cardiac function was evaluated by echocardiography. Aortic lesion areas were evaluated using oil red O staining. Plaque macrophage in aortic sinus was analyzed by immunofluorescence staining. Flow cytometry was used to explore the proportions of monocyte subsets in the blood, spleen, and bone marrow.

¹Department of Cardiology, Huashan Hospital, Fudan University, Shanghai, China

²Department of Anatomy, Histology and Embryology, Shanghai Medical College, Fudan University, Shanghai, China

³Cardiovascular Institute of Zhengzhou University, Department of Cardiology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

⁴Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai, China

#These authors contributed equally to this work.

Corresponding author:

Wei Shen, MD, PhD, Department of Cardiology, Huashan Hospital, Fudan University, 12 Middle Wulumuqi Road, Shanghai, 200040, China.

Email: drshenwei@163.com



Results: Pravastatin improved cardiac function and reduced lesion areas. It also attenuated the supply of monocytes in spleen, especially the inflammatory Ly6C^{high} monocyte subset. Pravastatin also subsequently reduced macrophage accumulation in atherosclerotic lesions.

Conclusions: MI accelerated chronic atherosclerosis progress. Pravastatin suppressed atherosclerotic development and inhibited inflammatory monocytosis after MI in ApoE^{-/-} mice.

Keywords

Pravastatin, inflammation, monocyte subsets, atherosclerosis, myocardial infarction, monocytosis

Date received: 21 November 2019; accepted: 18 May 2020

Introduction

Myocardial infarction (MI) can cause a significant increase in the risk of recurrent cardiovascular events.¹ After MI, the risk of recurrent MI or stroke from non-culprit atherosclerotic lesions is elevated by several-fold over the following 6 to 12 months.²⁻⁴ Clinical research showed that patients with ST segment elevation myocardial infarction (STEMI) with angiography evidence had a faster progression of non-culprit coronary artery lesions compared with a cohort without MI.⁵ These findings suggest that an acute ischemic event can accelerate chronic atherosclerosis. Animal studies have further demonstrated that acute MI triggers Ly6C^{high} monocyte accumulation in the spleen and accelerates infiltration of these inflammatory immune cells into a pre-existing arterial plaque, which persists for 12 weeks after the initial event.⁶ Once they are inside the plaque, inflammatory monocytes differentiate into macrophages and produce proteases, such as metalloproteinases and cysteinyl cathepsins, which can erode the extracellular matrix of the fibrous cap in the plaque and render atherosclerotic plaques unstable resulting in MI.⁷

Numerous clinical studies have shown that statins, which are a standard treatment

for MI, have markedly improved the prognosis after MI.^{8,9} However, the influence of statins on inflammation beyond a direct lipid-lowering effect, such as reducing low-density lipoprotein cholesterol (LDL-C), remains controversial.¹⁰ Previous research has shown that carotid atherosclerotic plaques from patients who have taken statins demonstrate a significant reduction in macrophage infiltration.¹¹ In addition, nanoparticle-mediated delivery of pitavastatin could inhibit monocyte mobilization from the spleen and attenuate left ventricle remodeling in mice after MI.¹² We, therefore, hypothesized that statins might inhibit the progression of atherosclerosis and improve cardiac function after MI through inhibiting inflammatory Ly6C^{high} monocytosis. In this study, we evaluated the protective effects of pravastatin on atherosclerotic plaques and monocyte infiltration after MI using an apolipoprotein E (ApoE)^{-/-} mouse model.

Materials and methods

Reagents

Pravastatin sodium was purchased from Sigma-Aldrich (St. Louis, MO, USA). BV421-conjugated anti-mouse Ly6C (clone AL-21), PE-conjugated anti-mouse CD11b

(clone M1/70), APC-conjugated anti-mouse Ly6G (clone 1A8), BV605-conjugated anti-mouse CD115 (clone T38-320), and anti-mouse CD16/32 (clone 2.4G2) were purchased from BD Pharmingen (Franklin Lakes, NJ, USA). Monoclonal antibodies against mouse F4/80 were obtained from Abcam (Cambridge, UK). Mounting medium with DAPI, hematoxylin–eosin (H&E) and Masson's trichrome staining reagents were purchased from Wuhan Servicebio Technology (Wuhan, China). Oil Red O (ORO) was obtained from Sangon Biotech (Shanghai, China).

Animal model

ApoE^{-/-} male mice (8 weeks old) with a C57BL/6 background were purchased from Cavens Laboratory Animal Co., Ltd (Changzhou, China). Mice that were fed a high-fat diet (1.25% cholesterol and 20% fat) were maintained under the specific pathogen-free conditions and housed in the animal facility at Fudan University (Shanghai, China), in compliance with the guidelines for the care and use of laboratory animals and in accordance with to institutional animal care and use committee (IACUC) of Fudan University. The MI model was produced on ApoE^{-/-} mice after 10 weeks of feeding a high-fat diet. They were randomly divided into the following three groups: sham group (sham surgery and carboxymethylcellulose sodium [CMC-Na]), MI group (MI and CMC-Na), and MI+Pra group (MI and pravastatin). Pravastatin was dissolved in 0.5% CMC-Na before administration. Pravastatin (40 mg/kg/day) or 0.5% CMC-Na was intragastrically administered to mice for 4 weeks after MI. Body weight changes were recorded weekly. At the end of the treatment period, mice were euthanized and blood samples were obtained from the portal vein. Plasma lipid profiles, including total cholesterol (TC), LDL-C,

and triglyceride (TG) levels, were measured using an automatic analyzer (Hitachi, Tokyo, Japan). The study scheme is shown in Figure 1a.

The protocols for animal handling were previously approved by our institutional animal ethics committee in the Department of Laboratory Animal Science of Fudan University (approval number 20160835A214).

Myocardial infarction procedure

Mice were intubated and ventilated with 2% isoflurane and additional oxygen. After thoracotomy that was performed in the fourth left intercostal space, the left anterior descending (LAD) branch of coronary artery was identified and permanently ligated with a monofilament nylon 8-0 suture. Myocardium below the ligation turned pale and local myocardial movement was weakened. Mice in the sham group received tracheotomy without performing LAD ligation. The thorax was closed with nylon 6-0 suture. The success of the MI procedure was confirmed by an increase in the ST-segment on an electrocardiogram (ECG) recording (Figure 1b).

Echocardiography

Echocardiographic studies were performed 4 weeks after MI using a Vevo 2100 ultrasound system (Visual Sonics, Toronto, Canada). Mice were anesthetized by inhalation of 2% isoflurane. The left parasternal short-axis view was recorded at the level of papillary muscles. Simultaneous transversal M-mode tracings were recorded in the middle of left ventricular (LV) cavity. LV diameter at end diastole/systole (LVDd/s) was determined by M-mode measurement and LV volumes at end diastole/systole (LVEDV and LVESV) were calculated. Ejection fraction (EF)

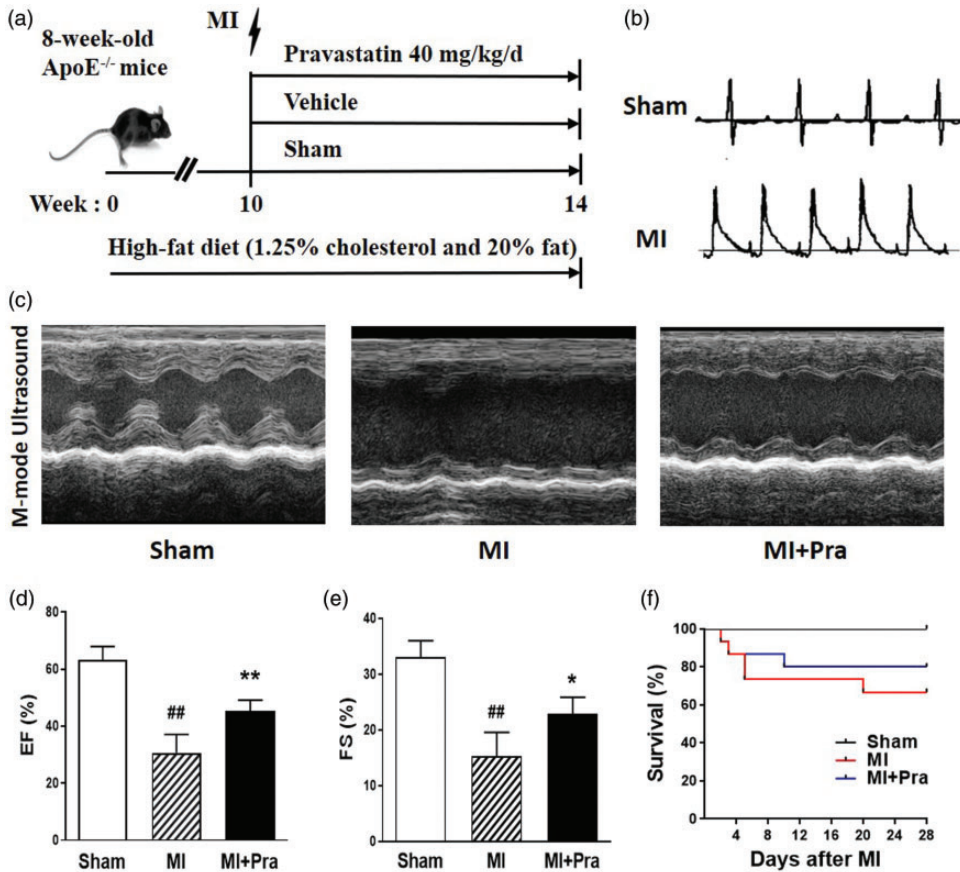


Figure 1. Cardioprotective effects of pravastatin in ApoE^{-/-} mice after MI. (a) Experiment design scheme. Eight-week-old male ApoE^{-/-} mice were fed a high-fat diet (1.25% cholesterol and 20% fat) for 14 weeks as an animal model of *in vivo* atherosclerosis. MI was induced when they were fed the high-fat diet for 10 weeks. Mice were randomly assigned to one of three groups including the Sham group (sham surgery and CMC-Na), MI group (MI surgery and CMC-Na), or MI+Pra group (MI surgery and 40 mg/kg/day pravastatin). All animals received the assigned study drug via intragastric gavage for an additional 4 weeks after MI. (b) Representative electrocardiogram showing ST segment elevation after LAD ligation. (c) Cardiac function was determined by echocardiography 4 weeks after surgery. Both EF% (d) and FS% (e) were evaluated 4 weeks after surgery. (f) The survival rates in the three groups (Sham: n = 6, MI: n = 10, MI+Pra: n = 12). Data are shown as the mean \pm SEM. ^{##}*P* < 0.01 versus Sham group; ^{*}*P* < 0.05, ^{**}*P* < 0.01 versus MI group. ApoE, apolipoprotein E; MI, myocardial infarction; CMC-Na, carboxymethylcellulose sodium; Pra, pravastatin; LAD, left anterior descending; EF, ejection fraction; FS, fractional shortening; SEM, standard error of the mean.

and fractional shortening (FS) were calculated using the following formulas: EF (%) = (LVEDV - LVESV) / LVEDV \times 100; and FS (%) = (LVDD - LVDs) / LVDD \times 100.

Oil red O staining

After blood samples were drawn from the portal vein, the aortic root and aorta were excised. For *en face* analysis, aortas were

fixed in 4% formalin before oil red O (ORO) staining for 40 minutes. Images were obtained using Image-Pro Plus 8 (Media Cybernetics, Silver Spring, MA, USA) to quantify the lesion area, as described previously.¹³

Histology and immunofluorescence study

For aortic sinus analysis, hearts were fixed in 4% formalin, dehydrated, embedded with paraffin, and sliced into 5- μ m-thick sections from the aortic region towards the apex of the heart. H&E staining (Servicebio) was then performed. For plaque analysis, fibrous caps and necrotic cores were examined using Masson's trichrome staining (Servicebio). To measure fibrous cap thickness, at least three measurements of the thinnest fibrous cap within one atherosclerotic plaque were taken and averaged, as described previously.¹⁴ The necrotic core area was analyzed by measuring the total acellular area in atherosclerotic plaque, as described previously.¹⁴ F4/80 staining was performed to determine the macrophage content in the atherosclerotic plaque. Sections were stained with anti-F4/80 antibody (1:500, rat anti-mouse monoclonal antibody) followed by incubation with goat anti-rabbit (1:300) antibody. Slides were mounted with mounting medium containing DAPI (Servicebio). Images were analyzed in the area of atherosclerotic lesions using a confocal fluorescence microscope (Olympus, Tokyo, Japan) and Image-Pro Plus software (Media Cybernetics).

Flow cytometry

Blood was obtained via the portal vein using 50 mmol/L EDTA. The erythrocytes were lysed using fluorescence-activated cell sorting (FACS) lysing solution (BD Biosciences, Franklin Lakes, NJ, USA). Spleen and bone marrow were processed in phosphate buffer saline (PBS) with 2% fetal bovine serum (FACS buffer), and red blood

cells were then removed. The mixture was then filtered through a 70- μ m cell container. After incubation with anti-CD16/32 monoclonal antibodies for 5 minutes at 4°C, cell suspensions were incubated with CD11b, CD115, Ly6G, and Ly6C. Samples were analyzed using a BD FACSCelesta Flow Cytometer (BD Biosciences) and FlowJo software (Treestar, Ashland, OR, USA). Monocytes were identified as CD11b⁺, Ly6G⁻, CD115⁺ and Ly6C^{high/low}.

Statistical analysis

All results are presented as the mean \pm standard error of the mean (SEM). Differences between two groups were assessed using an unpaired Student's *t*-test, and comparison among multiple groups was evaluated using a one-way analysis of variance (ANOVA). Statistical analysis was performed using GraphPad Prism 8 (San Diego, CA, USA). A *P* value of 0.05 or less was considered statistically significant.

Results

Effects of pravastatin on body weight and plasma lipids in ApoE^{-/-} mice after MI

Body weight did not show significant differences among the groups. However, after 4 weeks of pravastatin treatment, plasma TC in ApoE^{-/-} mice was significantly lower compared with the MI group (*P*<0.05). Pravastatin also significantly reduced the LDL-C level compared with the MI group (*P*<0.05), but there was no significant effect on the TG level (Table 1).

Pravastatin improves cardiac function in ApoE^{-/-} mice after MI

After MI, visualization of wall movement was weakened and the ventricle was enlarged. After pravastatin treatment, cardiac function was improved after MI by

Table 1. Body weight and plasma lipids at 4 weeks after MI.

Groups	Body weight (g)	TC (mmol/L)	LDL-C (mmol/L)	TG (mmol/L)
Sham (n=6)	31.4±0.5	23.1±1.8	20.2±1.4	1.9±0.2
MI (n=10)	30.1±0.4	25.7±1.1	22.9±1.3	2.0±0.1
MI+Pra (n=12)	29.2±0.3	20.2±1.1*	17.4±0.9*	1.8±0.1

Data are shown as the mean ± SEM.

* $P < 0.05$ versus MI group.

MI, myocardial infarction; Pra, pravastatin; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; SEM, standard error of the mean.

echocardiography (Figure 1c). EF or FS was significantly decreased after MI compared with sham ($P < 0.01$); however, pravastatin treatment reversed the cardiac dysfunction compared with the MI group ($P < 0.01$ and $P < 0.05$, respectively; Figure 1d and e). At day 28 after MI, the survival rates of the MI group and MI+Pra group were 66.6% (n=10) and 80.0% (n=12), respectively. However, all mice in the sham group (n=6) survived throughout the observation period (Figure 1f). Postmortem examination indicated that the main cause of death was cardiac rupture.

Pravastatin inhibits atherosclerotic plaques in $ApoE^{-/-}$ mice after MI

Atherosclerosis developed slowly in the sham group. MI can facilitate atherosclerotic plaque development, but pravastatin inhibited the process in *en face* aortas (Figure 2a). We analyzed different parts of the aorta and there were similar findings (Figure 2b). In particular, atherosclerotic plaques in aortic sinuses were shown with H&E staining (Figure 2c). Pravastatin treatment significantly reduced atherosclerotic plaques in aortic sinuses after MI compared with the MI group ($P < 0.001$; Figure 2d).

Pravastatin improves atherosclerotic plaque stability in $ApoE^{-/-}$ mice after MI

Using Masson's trichrome staining in the aortic sinus, we found that the fibrous cap

thickness of the plaque was decreased and the necrotic core area was enlarged after MI compared with sham (Figure 3a), but that pravastatin can reverse the modulation ($P < 0.05$ and $P < 0.01$, respectively; Figure 3b and c).

Pravastatin suppresses macrophage accumulation in aortic sinus in $ApoE^{-/-}$ mice after MI

Macrophages in the atherosclerotic plaque were examined via immunofluorescence using F4/80 antibody (Figure 4a). F4/80-positive areas were significantly increased after MI ($P < 0.001$ compared with sham), but this increase was markedly inhibited by pravastatin treatment ($P < 0.001$ compared with the MI group; Figure 4b).

Pravastatin inhibits inflammatory $Ly6C^{high}$ monocytes in circulation and spleen

The flow cytometric gating strategy for total monocytes ($CD11b^{+}$, $Ly6G^{-}$, and $CD115^{+}$), in addition to $Ly6C^{high}$ and $Ly6C^{low}$ monocyte subsets, is shown in Figure 5a. Circulating monocytes and inflammatory $Ly6C^{high}$ monocytes were increased after MI (both $P < 0.001$ compared with sham), and this increase was inhibited by pravastatin ($P < 0.05$ and $P < 0.01$, respectively; Figure 5b and c). Because the circulating monocytes have migrated from the spleen and bone

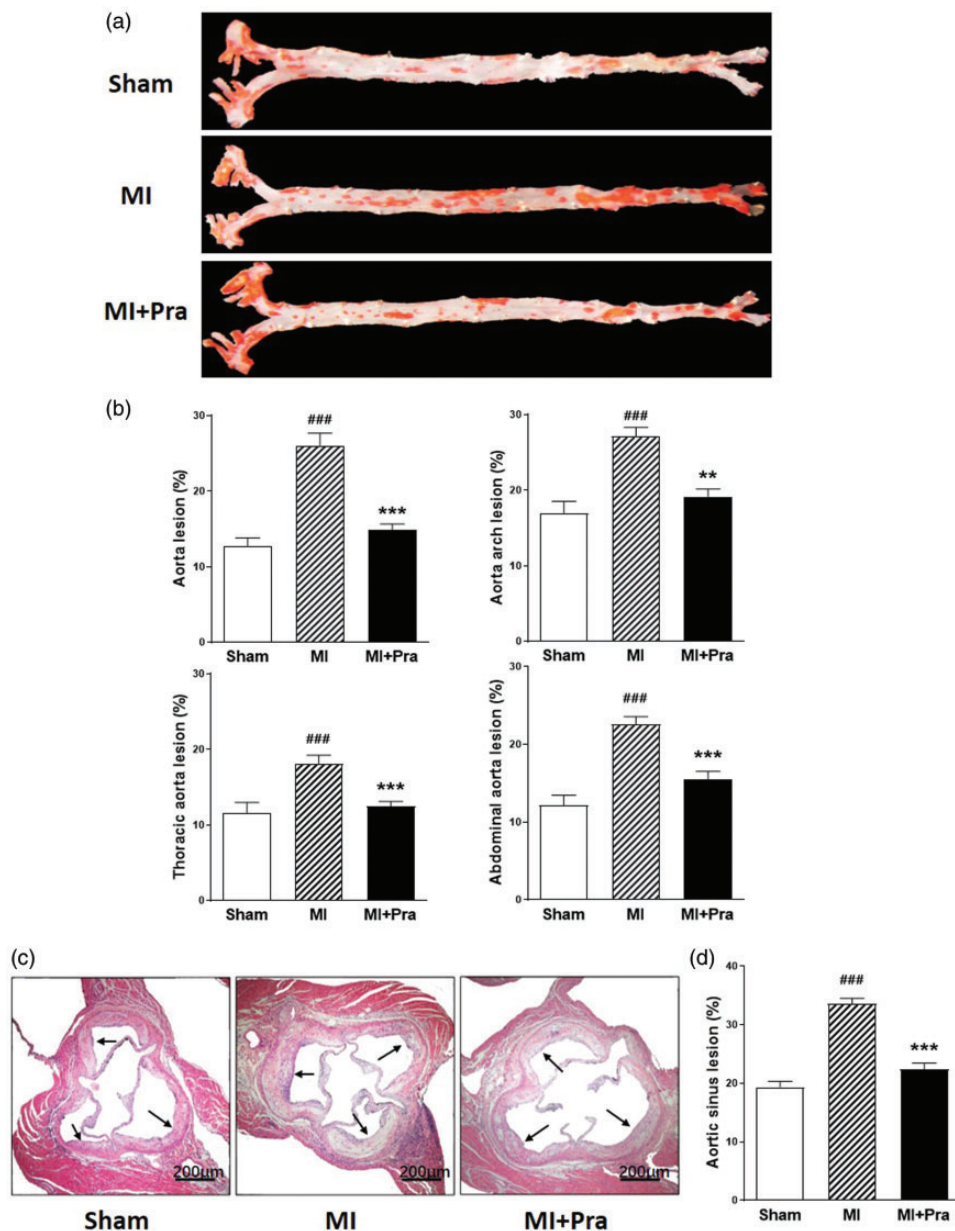


Figure 2. Pravastatin suppresses atherosclerotic plaque growth in ApoE^{-/-} mice after MI. (a) ORO staining in aorta from ApoE^{-/-} mice in the Sham group, MI group, or MI+Pra group. (b) Percentage of atherosclerotic area in the aorta, aortic arch, thoracic aorta, or abdominal aorta (Sham: n = 6, MI: n = 10, MI+Pra: n = 10). (c) Hematoxylin and eosin staining of aortic sinuses in the Sham group, MI group, or MI+Pra group. The black arrows indicate the atherosclerotic area. Scale bar: 200 μm; magnification ×40. (d) Percentage of the atherosclerotic area in the aortic sinus (Sham: n = 6, MI: n = 10, MI+Pra: n = 10). Data are shown as the mean ± SEM. ^{###}*P* < 0.001 versus Sham group, ^{**}*P* < 0.01, ^{***}*P* < 0.001 versus MI group. ApoE, apolipoprotein E; MI, myocardial infarction; ORO, oil red O; Pra, pravastatin; SEM, standard error of the mean.

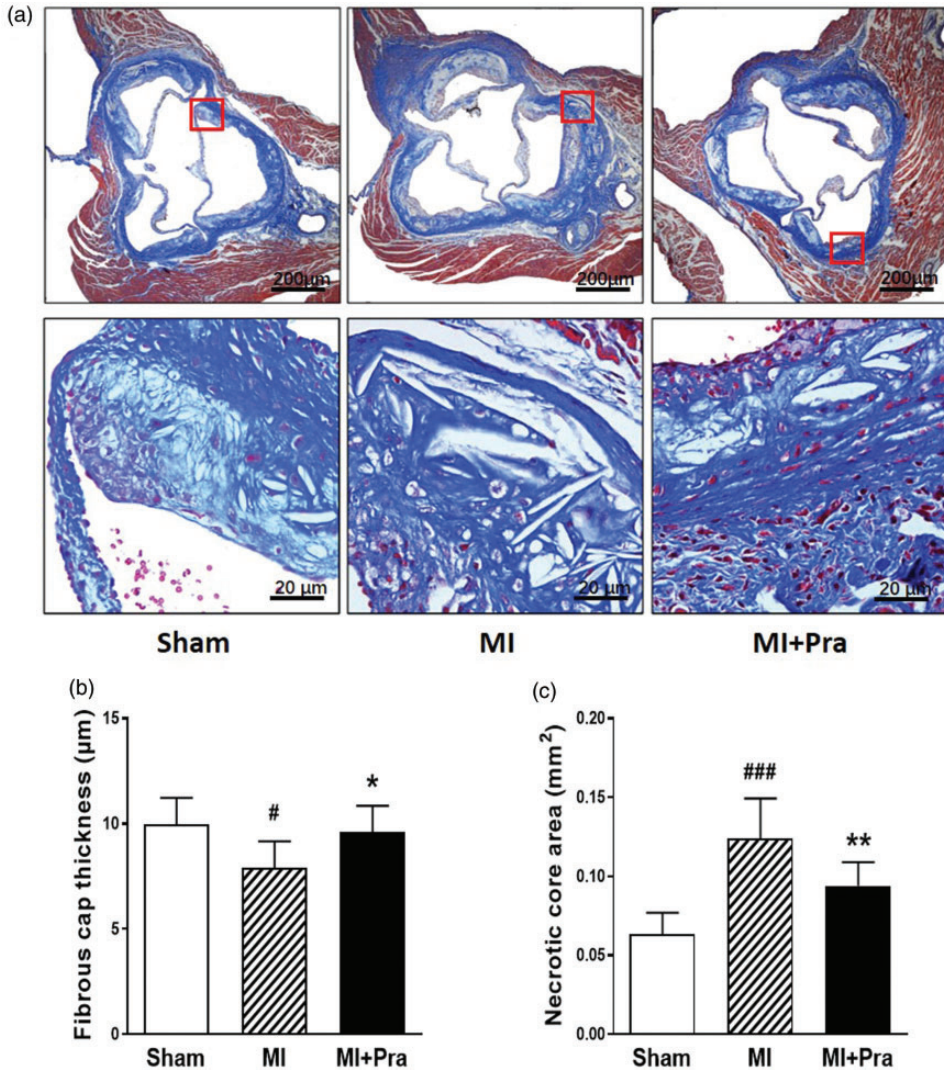


Figure 3. Effect of pravastatin on characteristics of atherosclerotic plaques in ApoE^{-/-} mice after MI. (a) Masson's trichrome staining of aortic sinuses in the Sham group, MI group, or MI+Pra group. Upper panel: scale bar, 200 μm; magnification, ×40; lower panel: scale bar, 20 μm; magnification, ×400. (b) Quantification of the fibrous cap thickness or necrotic core area in the aortic sinus sections (n=6 in each group). Data are shown as the mean ± SEM. [#]*P* < 0.05, ^{###}*P* < 0.001 versus sham group, ^{*}*P* < 0.05, ^{**}*P* < 0.01 versus MI group. ApoE, apolipoprotein E; MI, myocardial infarction; Pra, pravastatin; SEM, standard error of the mean.

marrow, we investigated whether pravastatin inhibited monocyte subset levels in the spleen or bone marrow after MI. After MI, monocytes and inflammatory Ly6C^{high} monocytes levels in spleen were

higher compared with sham (*P* < 0.01 and *P* < 0.001, respectively). Pravastatin treatment significantly reduced total monocytes and Ly6C^{high} monocytes in the spleen compared with the MI group (*P* < 0.05 and

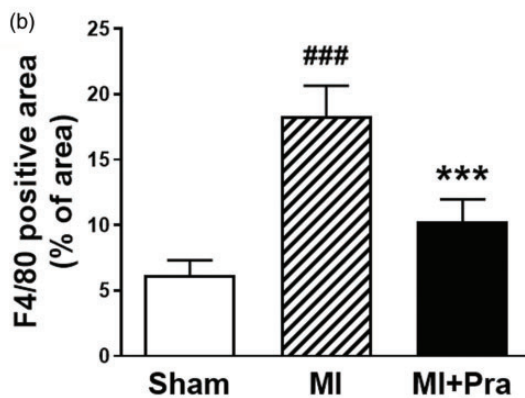
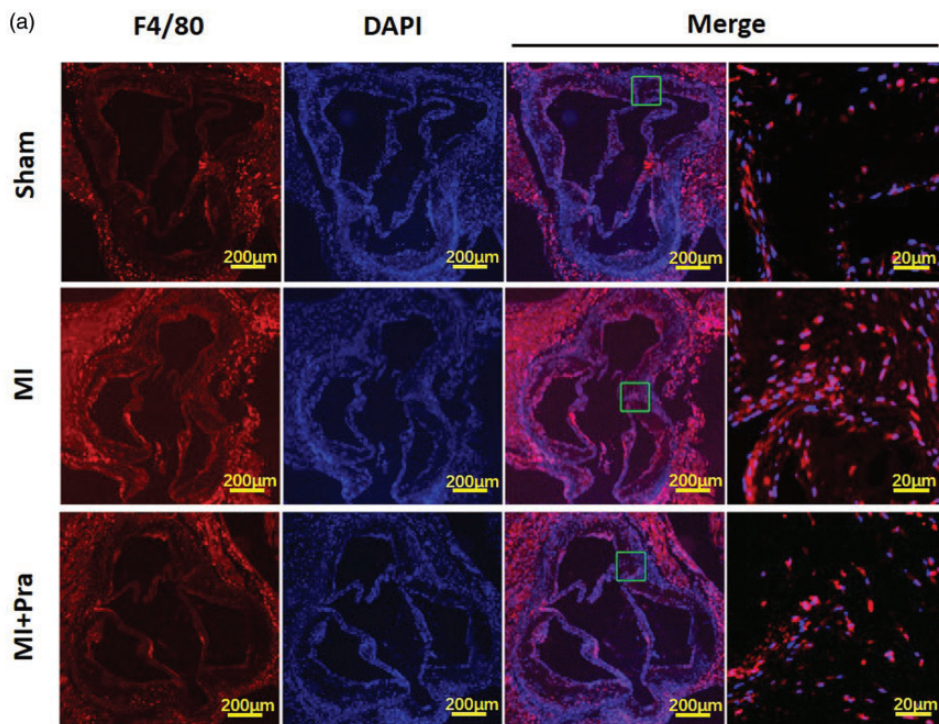


Figure 4. Effect of pravastatin on F4/80 immunoreactivity in macrophages in ApoE^{-/-} mice after MI. (a) F4/80 immunofluorescence staining in the aortic sinus in the Sham group, MI group, or MI+Pra group. Scale bar, 200 μ m; magnification, $\times 40$. Magnified picture in last column: scale bar, 20 μ m; magnification, $\times 400$. (b) Percentage of F4/80-positive area in the aortic sinus sections ($n=6$ in each group). Data are shown as the mean \pm SEM. ^{###} $P < 0.001$ versus Sham group, ^{***} $P < 0.001$ versus MI group. ApoE, apolipoprotein E; MI, myocardial infarction; Pra, pravastatin; SEM, standard error of the mean.

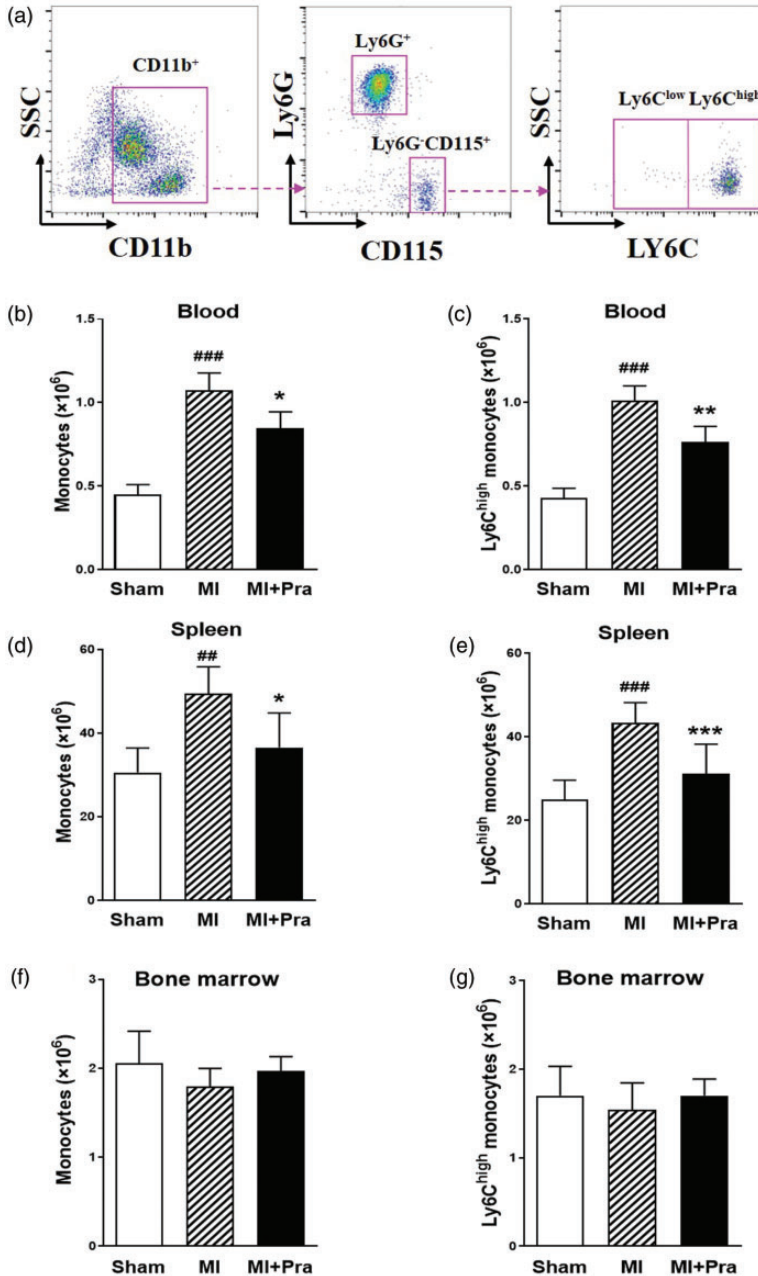


Figure 5. Pravastatin reduces blood and splenic monocytosis in ApoE^{-/-} mice after MI. (a) Flow cytometric gating strategy for monocytes and Ly6C^{high} monocytes. Quantification of monocytes (b) or Ly6C^{high} monocytes (c) in blood samples 4 weeks after MI. Quantification of monocytes (d) or Ly6C^{high} monocytes (e) in the spleen 4 weeks after MI. Quantification of monocytes (f) or Ly6C^{high} monocytes (g) in bone marrow 4 weeks after MI. Bar graphs are from the Sham group (n=6), MI group (n=10), or MI+Pra group (n=12). Data are shown as the mean \pm SEM. ##*P* < 0.01, ###*P* < 0.001 versus Sham group, **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus MI group.

ApoE, apolipoprotein E; MI, myocardial infarction; Pra, pravastatin; SSC, side-scattered light; SEM, standard error of the mean.

<0.001, respectively; Figure 5d and e), but not in the bone marrow (Figure 5f and g).

Discussion

In this study, we demonstrated that pravastatin improved cardiac function and attenuated atherosclerotic plaque growth after MI in ApoE^{-/-} mice. This effect can be associated with inhibition of monocyte delivery to the blood (a process called monocytoysis) and macrophage infiltration, especially targeting inflammatory Ly6C^{high} monocyte levels in the blood and spleen.

We successfully established an animal model for post-MI atherosclerosis using an ApoE^{-/-} mouse on a high-fat diet. ApoE^{-/-} mice develop advanced atherosclerosis after several weeks on a high-fat diet, making them ideal for atherosclerosis research.¹⁵ However, even this robust model itself does not spontaneously show acute thrombosis or MI. In our study, we induced MI by ligating LAD, which mimics a clinically common location of MI. This model can be easily reproduced, which provides a useful tool to study atherosclerosis, especially after MI.

Our study demonstrated that pravastatin suppressed atherosclerotic plaque growth and enhanced plaque stability with restored fibrous cap thickness and reduced necrotic core areas after MI. Numerous clinical studies have shown that statin treatment improves outcomes in post-MI patients and reduces the risks for recurrent cardiovascular events.^{16,17} Although pravastatin exhibited modest reductions in TC and LDL-C in ApoE^{-/-} mice after MI, which is similar to previous reports,¹⁸⁻²¹ this might be not the only mechanism to cause 50% reduction of atherosclerotic lesions in our study. Therefore, additional mechanisms are possible, such as an anti-inflammatory effect, to work synergistically with the lipid-lowering effect on atherosclerosis.

In patients with coronary artery disease but well-regulated lipid levels, inflammatory monocyte-derived macrophages are present in the atherosclerotic plaque, and they are associated with coronary plaque vulnerability.²² Additionally, inflammatory monocytes are an independent predictor of cardiovascular events in patients with atherosclerosis.²³ Enhanced monocyte endothelial adhesion can increase plaque inflammation after MI.²⁴ Therefore, we explored the anti-inflammatory effects of statins after MI in ApoE^{-/-} mice.

Pravastatin, a HMG-CoA reductase inhibitor, is widely used to prevent and treat atherosclerotic heart disease, including secondary prevention after acute MI.²² In addition to its lipid-lowering effect, pravastatin also has other pleiotropic effects, such as anti-inflammatory or immunomodulatory effects.¹⁰ Patients with MI who have taken statins exhibited lower levels of pro-inflammatory mediators, including high sensitivity C-reactive protein and interleukin-6.²⁵ The underlying mechanism remains unknown. Our study illustrated that pravastatin inhibits monocytoysis in the blood and spleen, especially in the subset of inflammatory Ly6C^{high} monocytes. This effect can contribute to slowing the progression of atherosclerosis after MI.

Although monocytes and macrophages are essential for MI recovery, significant enhancement of inflammatory monocytes and macrophages can deteriorate cardiac function.²⁶ Optimal range of the monocyte supply can achieve the best outcome.²⁷ It has been shown that most of the increased monocytes originate from the spleen, which is a reservoir for monocytes, after MI in mice.²⁸ MI triggers additional inflammatory monocytes in the spleen to migrate to the ischemic myocardium and atherosclerotic plaques.²⁹ In our study, reduction of inflammatory monocytoysis in the spleen by pravastatin suggests that pravastatin

suppresses post-MI inflammation to attenuate atherosclerotic development. Bone marrow is another source of circulating monocytes; however, we did not observe a significant change in monocytes in bone marrow after MI. We speculate that MI triggers multifactorial hematopoiesis activation pathways, including pro-inflammatory and anti-inflammatory effects.⁶ Previous reports showed that MI triggers sympathetic outflow from the central nervous system. This β_3 adrenergic stimulation mobilizes monocyte progenitors from bone marrow to migrate to the spleen, where they can multiply in response to hematopoietic growth factors. Finally, the inflammatory monocytes can leave the spleen and enter into the plaque.^{6,30} Another possibility is that there are physiologically far fewer monocytes in bone compared with the spleen,³¹ and these changes are difficult to observe after MI. Our results suggest that the spleen serves as a reservoir for monocytes,³² which can be activated after MI, and pravastatin can inhibit this process.

There are several approaches for future studies. First, we are not sure if other statins can exhibit similar effects. Second, detailed mechanisms of signaling pathways and downstream target genes for anti-inflammation modulated by pravastatin need to be explored. Third, how ApoE is involved in inflammation after MI is unknown.

Conclusion

Pravastatin suppressed atherosclerotic plaque development and inhibited inflammatory monocytes in ApoE^{-/-} mice after MI. This further confirms that pravastatin can be used as secondary prevention approach after MI, including in patients with a normal lipid level.


Declaration of conflicting interest

The authors declare that there is no conflict of interest.


Funding

This research was funded by a grant from the National Natural Science Foundation of China (Grant No. 81673701 and 81573710).

ORCID iDs

Yufei Chen  <https://orcid.org/0000-0002-4346-6391>

Shengjia Sun  <https://orcid.org/0000-0002-4253-3046>

Wei Shen  <https://orcid.org/0000-0003-3732-989X>

References

1. Jernberg T, Hasvold P, Henriksson M, et al. Cardiovascular risk in post-myocardial infarction patients: nationwide real world data demonstrate the importance of a long-term perspective. *Eur Heart J* 2015; 36: 1163–1170.
2. Stone GW, Maehara A, Lansky AJ, et al. A prospective natural-history study of coronary atherosclerosis. *N Engl J Med* 2011; 364: 226–235.
3. Witt BJ, Brown RJ, Jacobsen SJ, et al. A community-based study of stroke incidence after myocardial infarction. *Ann Intern Med* 2005; 143: 785–792.
4. Milonas C, Jernberg T, Lindback J, et al. Effect of angiotensin-converting enzyme inhibition on one-year mortality and frequency of repeat acute myocardial infarction in patients with acute myocardial infarction. *Am J Cardiol* 2010; 105: 1229–1234.
5. Han Y, Jing J, Tu S, et al. ST elevation acute myocardial infarction accelerates non-culprit coronary lesion atherosclerosis. *Int J Cardiovasc Imaging* 2014; 30: 253–261.
6. Dutta P, Courties G, Wei Y, et al. Myocardial infarction accelerates atherosclerosis. *Nature* 2012; 487: 325–329.
7. Libby P. Inflammation in atherosclerosis. *Arterioscler Thromb Vasc Biol* 2012; 32: 2045–2051.

8. Schwartz GG, Olsson AG, Ezekowitz MD, et al. Effects of atorvastatin on early recurrent ischemic events in acute coronary syndromes: the MIRACL study: a randomized controlled trial. *JAMA* 2001; 285: 1711–1718.
9. Baigent C, Keech A, Kearney PM, et al. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. *Lancet* 2005; 366: 1267–1278.
10. Tunon J, Badimon L, Bochaton-Piallat ML, et al. Identifying the anti-inflammatory response to lipid lowering therapy: a position paper from the working group on atherosclerosis and vascular biology of the European Society of Cardiology. *Cardiovasc Res* 2019; 115: 10–19.
11. Martin-Ventura JL, Blanco-Colio LM, Gomez-Hernandez A, et al. Intensive treatment with atorvastatin reduces inflammation in mononuclear cells and human atherosclerotic lesions in one month. *Stroke* 2005; 36: 1796–1800.
12. Mao Y, Koga JI, Tokutome M, et al. Nanoparticle-mediated delivery of pitavastatin to monocytes/macrophages inhibits left ventricular remodeling after acute myocardial infarction by inhibiting monocyte-mediated inflammation. *Int Heart J* 2017; 58: 615–623.
13. Higashi Y, Sukhanov S, Shai S, et al. Insulin-like growth factor-1 receptor deficiency in macrophages accelerates atherosclerosis and induces an unstable plaque phenotype in apolipoprotein E-deficient mice. *Circulation* 2016; 133: 2263–2278.
14. Dutta P, Hoyer F, Sun Y, et al. E-selectin inhibition mitigates splenic HSC activation and myelopoiesis in hypercholesterolemic mice with myocardial infarction. *Arterioscler Thromb Vasc Biol* 2016; 36: 1802–1808.
15. Tabas I. Cardiology: bad matters made worse. *Nature* 2012; 487: 306–308.
16. Cannon CP, Steinberg BA, Murphy SA, et al. Meta-analysis of cardiovascular outcomes trials comparing intensive versus moderate statin therapy. *J Am Coll Cardiol* 2006; 48: 438–445.
17. Kasai T, Miyauchi K, Kurata T, et al. Long-term (11-year) statin therapy following percutaneous coronary intervention improves clinical outcome and is not associated with increased malignancy. *Int J Cardiol* 2007; 114: 210–217.
18. Bea F, Blessing E, Bennett B, et al. Simvastatin promotes atherosclerotic plaque stability in apoE-deficient mice independently of lipid lowering. *Arterioscler Thromb Vasc Biol* 2002; 22: 1832–1837.
19. Johnson J, Carson K, Williams H, et al. Plaque rupture after short periods of fat feeding in the apolipoprotein E-knockout mouse: model characterization and effects of pravastatin treatment. *Circulation* 2005; 111: 1422–1430.
20. Zhou XX, Gao PJ and Sun BG. Pravastatin attenuates interferon-gamma action via modulation of STAT1 to prevent aortic atherosclerosis in apolipoprotein E-knockout mice. *Clin Exp Pharmacol Physiol* 2009; 36: 373–379.
21. Zhou F, Tan Y, Chen XH, et al. Atorvastatin improves plaque stability in diabetic atherosclerosis through the RAGE pathway. *Eur Rev Med Pharmacol Sci* 2018; 22: 1142–1149.
22. Yamamoto H, Yoshida N, Shinke T, et al. Impact of CD14(++)CD16(+) monocytes on coronary plaque vulnerability assessed by optical coherence tomography in coronary artery disease patients. *Atherosclerosis* 2018; 269: 245–251.
23. Rogacev KS, Cremers B, Zawada AM, et al. CD14++CD16+ monocytes independently predict cardiovascular events: a cohort study of 951 patients referred for elective coronary angiography. *J Am Coll Cardiol* 2012; 60: 1512–1520.
24. Wright AP, Ohman MK, Hayasaki T, et al. Atherosclerosis and leukocyte-endothelial adhesive interactions are increased following acute myocardial infarction in apolipoprotein E deficient mice. *Atherosclerosis* 2010; 212: 414–417.
25. Gruzdeva O, Uchasova E, Dyleva Y, et al. Early effects of treatment low-dose atorvastatin on markers of insulin resistance and inflammation in patients with myocardial infarction. *Front Pharmacol* 2016; 7: 324.

26. Panizzi P, Swirski FK, Figueiredo JL, et al. Impaired infarct healing in atherosclerotic mice with Ly-6C(hi) monocytosis. *J Am Coll Cardiol* 2010; 55: 1629–1638.
27. Nahrendorf M, Pittet MJ, Swirski FK. Monocytes: protagonists of infarct inflammation and repair after myocardial infarction. *Circulation* 2010; 121: 2437–2445.
28. Swirski FK, Nahrendorf M, Etzrodt M, et al. Identification of splenic reservoir monocytes and their deployment to inflammatory sites. *Science* 2009; 325: 612–616.
29. Leuschner F, Rauch PJ, Ueno T, et al. Rapid monocyte kinetics in acute myocardial infarction are sustained by extramedullary monocytopoiesis. *J Exp Med* 2012; 209: 123–137.
30. Libby P, Nahrendorf M and Swirski FK. Leukocytes link local and systemic inflammation in ischemic cardiovascular disease. *J Am Coll Cardiol* 2016; 67: 1091–1103.
31. Yang M, Büsche G, Ganser A, et al. Morphology and quantitative composition of hematopoietic cells in murine bone marrow and spleen of healthy subjects. *Ann Hematol* 2013; 92: 587–594.
32. Swirski FK and Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. *Science* 2013; 339: 161–166.