Biochemical and Biophysical Research Communications 428 (2012) 86–92

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Acipimox attenuates atherosclerosis and enhances plaque stability in ApoE-deficient mice fed a palmitate-rich diet

Feipeng Jin [a,b], Sihua Jiang [a,b], Dachun Yang [a], Xingmei Zhang [c], Yun Yang [c], Yan Zhang [a], Kun Li [c], Yongjian Yang [a,*], Shuangtao Ma [a,*]

[a] Department of Cardiology, General Hospital of PLA Chengdu Military Area Command, Chengdu, Sichuan 610083, PR China
[b] Luzhou Medical College, Luzhou, Sichuan 646000, PR China
[c] Basic Laboratory for PLA Medical Center, General Hospital of PLA Chengdu Military Area Command, Chengdu, Sichuan 610083, PR China

ARTICLE INFO

Article history:
Received 26 September 2012
Available online 8 October 2012

Keywords:
Fatty acids
Palmitate
Palmitic acid
Atherosclerosis
Plaque stability

ABSTRACT

Saturated fatty acids (FA) have been linked to an increased risk of cardiovascular disease. The effects of acipimox, a FA-lowering agent, on palmitate- (an important saturated fatty acid) stimulated atherosclerosis remains to be elucidated. We investigated the effects of acipimox on atherosclerosis in ApoE$^{-/-}$ mice fed a palmitate-rich diet. Male ApoE$^{-/-}$ mice, 6–8 weeks of age, were randomized into three groups. The animals were fed a normal chow diet in the control group, a diet containing 5% palmitic acid in the palmitate group, and a diet containing 5% palmitic acid and 0.02% acipimox in the acipimox group. The plasma lipid profiles, aortic lesions, plaque collagen content and the expression of matrix metalloproteinase (MMP)-2, MMP-3, MMP-9, and MMP-14 and the tissue inhibitor of MMP (TIMP)-1, and TIMP-2 were determined after a 12-week treatment. The palmitate-rich diet significantly increased plasma FA concentrations ($P < 0.01$), enhanced atherosclerotic lesions ($P < 0.01$), decreased plaque collagen content ($P < 0.01$) and upregulated MMP-2 ($P < 0.05$) in the aorta. Additionally, all of these harmful effects were significantly attenuated by co-treatment with acipimox ($P < 0.05$ or $P < 0.01$). The present study suggests that acipimox attenuates atherosclerosis and enhances plaque stability in ApoE$^{-/-}$ mice fed a palmitate-rich diet.

1. Introduction

There are many different types of fatty acids (FAs) [1]. Although some of them, such as omega-3 long chain polyunsaturated FA, have been viewed in recent years as beneficial nutrients with cardioprotective effects [2], saturated FAs have been linked to an increased risk of cardiovascular disease [3]. It has been demonstrated that nonesterified free FAs contribute to overall mortality, specifically cardiovascular death [4,5]. However, the role of saturated FA in the development of atherosclerosis has not been clearly understood.

Monounsaturated FAs were considered protective because they did not result in a proatherosclerotic lipid profile [6–8]. However, our recent study has demonstrated that oleic acid, a major type of dietary monounsaturated FA, induces the development of vascular smooth muscle cells derived from cells and the growth of atherosclerotic plaques in ApoE-deficient (ApoE$^{-/-}$) mice [9]. Palmitate, a saturated long chain FA, is abundant in the diet and associated with oxidative stress and inflammation [10,11]. However, the role of palmitate in the growth of atherosclerotic plaques and plaque stability remains to be elucidated.

Acipimox, 5-methyl-pyrazine-2-carboxylic acid-4-oxide, is a nicotinic acid derivative that inhibits lipolysis and attenuates the release of free FA from adipose tissue [12]. It has been reported that acipimox might ameliorate atherosclerosis in ritonavir-treated LDLR-null mice, an animal model with lipoatrophy and elevated plasma FA concentrations [13]. Additionally, our previous study demonstrated that acipimox might inhibit the growth of atherosclerotic plaques in ApoE$^{-/-}$ mice fed an oleate-rich diet. However, the effects of acipimox on the growth of atherosclerotic plaque and plaque stability in ApoE$^{-/-}$ mice fed a palmitate-rich diet are unknown.

Taken together, we hypothesized that a palmitate-rich diet could accelerate atherosclerosis and promote instability that can be blocked by treatment with acipimox. As a proof-of-concept study, we tested how acipimox affected the growth of atherosclerotic lesions and plaque stability in ApoE$^{-/-}$ mice fed a palmitate-rich diet.

* Corresponding authors. Address: Department of Cardiology, General Hospital of PLA Chengdu Military Area Command, 270 Rongdu Rd., Tianhui Town, Jinniu District, Chengdu, Sichuan 610083, PR China. Fax: +86 028 8657 0419.
E-mail addresses: yongjiany@yahoo.cn (Y. Yang), shuangtaoma@yahoo.cn (S. Ma).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.10.011

2. Materials and methods

2.1. Animal care

Experimental procedures were approved by the Hospital Animal Care and Use Committee. Thirty male ApoE$^{-/-}$ mice, 6–8 weeks of age, were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). Animals were housed under a 12 h/12 h day/night cycle with *ad libitum* access to food and water. The mice were randomized into three groups: the control group ($n = 10$), the palmitate group ($n = 10$) and the acipimox group ($n = 10$). Mice in the palmitate group were fed a diet containing 5% palmitic acid, and mice in the acipimox group were fed a diet containing 5% palmitic acid and 0.02% acipimox. The diets were maintained for 12 weeks.

2.2. Serum lipids

Fasting blood samples were obtained, and triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and free FA levels were measured by colorimetric assays using a commercially available kit (Jiancheng Bioengineering Institute, Nanjing, China) in accordance with the manufacturer's instructions.

2.3. Atherosclerosis analysis

Atherosclerotic lesions of the thoraco-abdominal aorta were analyzed by Oil-red-O staining. The stained area was measured using computer-assisted morphometric analysis. Atherosclerotic lesions in the aortic roots were examined in cross-sections of the aortic origin. The sections (5 μm) were stained with hematoxylin and eosin, and images were taken on an Olympus BX41 microscope (Olympus Corp., Tokyo, Japan). The atherosclerotic lesion area was quantified using Nikon NIS-Elements Research Software.

2.4. Collagen analysis

Paraffin-embedded aortic sinus sections were stained with Masson's Trichrome using a fast Masson dye kit (Jiancheng Bioengineering Institute, Nanjing, China) in accordance with the manufacturer's instructions. The percentage of positively stained area was calculated using Nikon NIS-Elements Research Software.

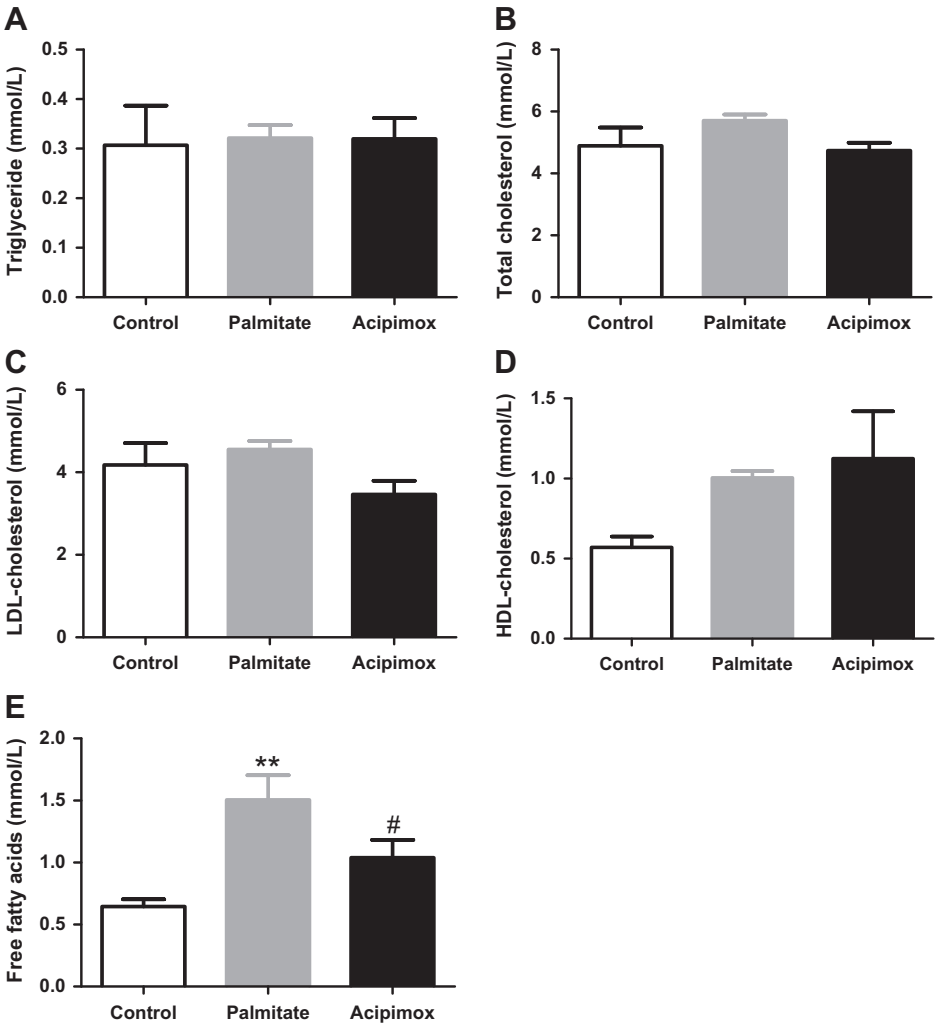

Fig. 1. Acipimox attenuates palmitate-induced hyper-free fatty acidemia. The plasma triglyceride (A), total cholesterol (B), LDL-cholesterol (C), HDL-cholesterol (D) and free fatty acids (E) concentrations in ApoE$^{-/-}$ mice in the control (open bars), palmitate (shaded bar) and acipimox (solid bars) group after a 12-week treatment. The data are expressed as the mean ± SEM; $n = 10$ mice per group. $^{**}P < 0.01$ compared to the control group, $^{\#}P < 0.05$ compared to the palmitate group. LDL, low-density lipoprotein; HDL, high-density lipoprotein.

2.5. Immunohistochemistry

Thoracic aortas were fixed in 4% paraformaldehyde for 12 h, embedded in paraffin and then cut into sections (5 μm). The sections were incubated with anti-matrix metalloproteinase (MMP)-2, anti-MMP-3, anti-MMP-9, anti-MMP-14, anti-tissue inhibitor of MMP (TIMP)-1 and anti-TIMP-2 antibodies (diluted 1:50, Boster Bioengineering Co., Wuhan, China). Specific binding was detected with complexes of biotinylated goat anti-rabbit IgG secondary antibody and horseradish peroxidase using ABC kits (Boster Bioengineering Co., Wuhan, China). The antigen–antibody complex was subsequently visualized with a DAB solution. The sections were viewed under a light microscope. The percentage of positively stained area was calculated using Nikon NIS-Elements Research Software.

2.6. Statistical analysis

Continuous data are presented as the mean ± SEM. Comparisons between the groups were determined by one-way ANOVA with the Tukey–Kramer Post-hoc multiple comparison test (SPSS Inc., Chicago, IL). Probabilities of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Acipimox attenuates palmitate-induced hyper-free fatty acidemia

The plasma TG, TC, LDL-C and HDL-C concentrations were not affected by the palmitate-rich diet or acipimox treatment (Fig. 1A–D). The palmitate-rich diet induced hyper-free fatty acidemia in the ApoE$^{-/-}$ mice ($P < 0.01$, Fig. 1E). In addition, the increased plasma free FA concentration was significantly attenuated by co-treatment with acipimox ($P < 0.05$, Fig. 1E).

3.2. Acipimox attenuates palmitate-induced acceleration of atherosclerosis

After a 12-week treatment, the atherosclerotic plaque size in the thoraco-abdominal aorta was significantly increased in the palmitate group compared to the control group ($P < 0.01$, Fig. 2A and C). Furthermore, the mice in the palmitate group showed a slight but not significant increase in the size of aortic root plaques compared to the aortic root plaques in the control mice (Fig. 2B and D). As expected, the plaque sizes in both the thoraco-abdominal aorta and aortic root were significantly attenuated by co-treatment with acipimox (both $P < 0.01$, Fig. 2A–D).

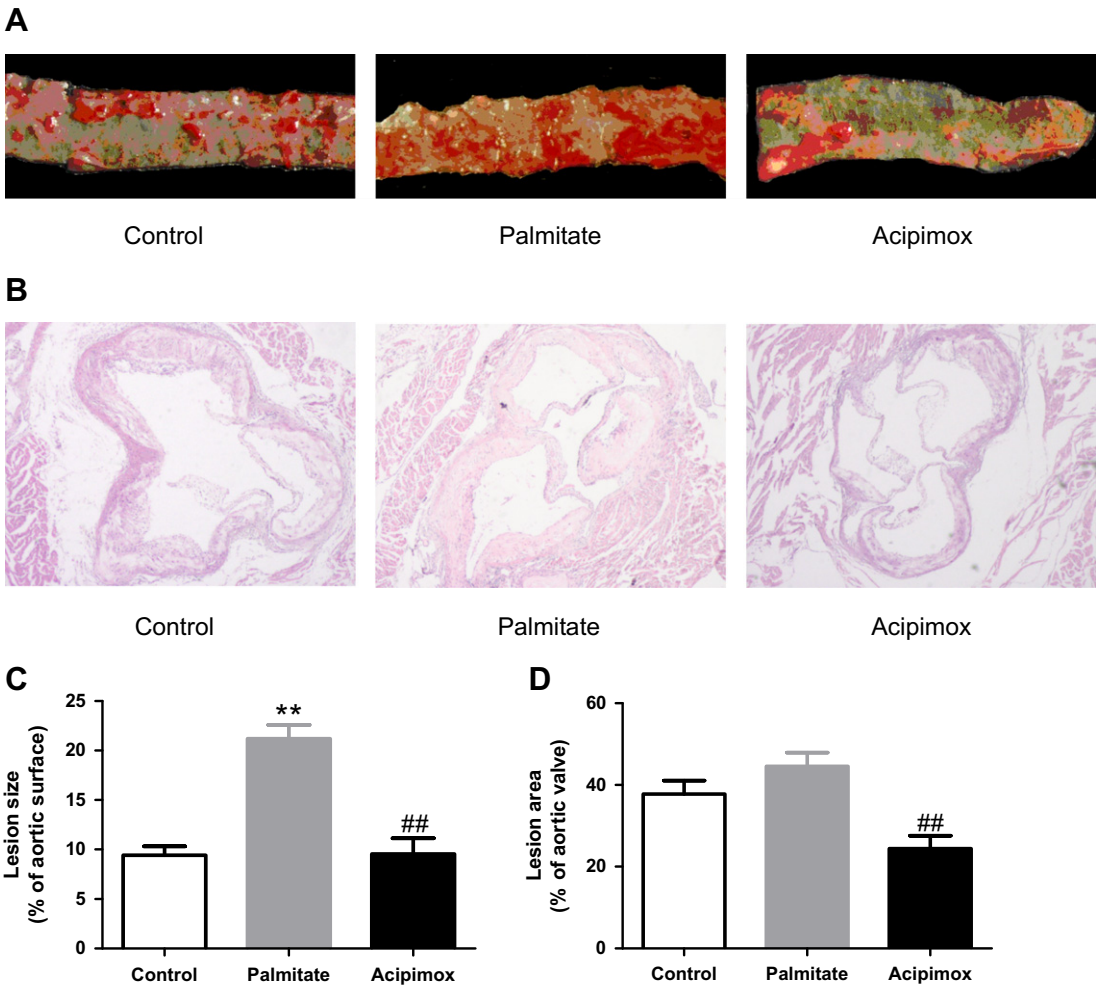

Fig. 2. Acipimox attenuates palmitate-induced acceleration of atherosclerosis. (A) Representative images of thoraco-abdominal lesions in ApoE$^{-/-}$ mice from the control, palmitate and acipimox group. Lesions are stained with Oil Red O. (B) Representative images of aortic root lesions from the three groups stained with hematoxylin and eosin. (C) The plaque size was measured as the percentage of the total aortic surface. (D) The plaque size of the aortic root was expressed as the percentage of the aortic valvular area. The data are expressed as the mean ± SEM; $n = 6$ mice per group. $^{**}P < 0.01$ compared to the control group, $^{\#\#}P < 0.01$ compared to the palmitate group.

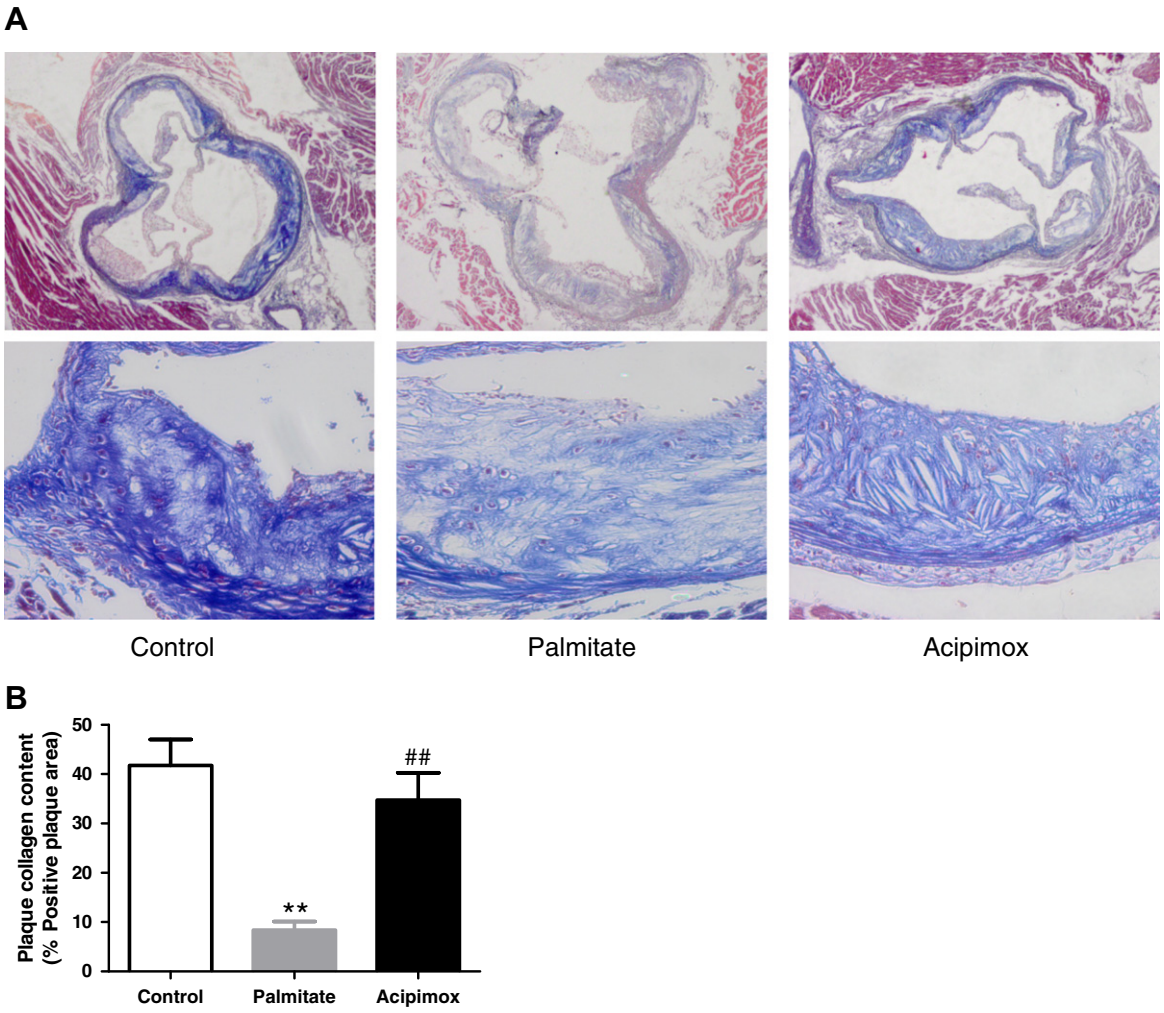

Fig. 3. Acipimox attenuates palmitate-induced decrease in collagen content. (A) Representative images of aortic lesions stained with Masson's Trichrome. Upper panel, 100×; lower panel, 400×. (B) Collagen deposition was expressed as the percentage of the positively stained area. The data are expressed as the mean ± SEM; $n = 6$ mice per group. $^{**}P < 0.01$ compared to the control group, $^{\#\#}P < 0.01$ compared to the palmitate group.

3.3. Acipimox attenuates palmitate-induced decrease of collagen content

Collagen plays a critical structural role in stabilizing plaques and is an indicator of plaque vulnerability. The collagen content in atherosclerotic plaques in the aortic root was significantly decreased in the palmitate-fed mice relative to the plaques in the control mice ($P < 0.01$, Fig. 3A and B). Moreover, the palmitate-induced decrease in collagen content was significantly reduced by treatment with acipimox ($P < 0.01$, Fig. 3A and B).

3.4. Acipimox attenuates palmitate-induced upregulation of MMP-2

The balance between MMP and TIMP expression plays an important role in collagen deposition in the aorta. The protein expression levels of MMP-2, MMP-3, MMP-9, MMP-14, TIMP-1 and TIMP-2 were detected by immunohistochemistrical staining. There was no distinct staining for MMP-3 or TIMP-1 in the aortic sections (data not shown). MMP-2 was significantly upregulated by the palmitate-rich diet, whereas this effect was remarkably attenuated by co-treatment with acipimox ($P < 0.05$ and $P < 0.01$, Fig. 4A and E). The palmitate-rich diet significantly decreased the expression of MMP-9 ($P < 0.01$), which was not affected by co-treatment with acipimox (Fig. 4B and F). The protein expression levels of MMP-14 and TIMP-2 were not affected by palmitate or acipimox (Fig. 4C, D, G and H).

4. Discussion

Palmitate is the major type of saturated FA in a high-fat diet. An early study analyzing the composition of FA in avian atherosclerotic plaques confirmed that palmitic acid accounted for approximately 50% of FA in the aortic lesions [14]. These findings suggest that dietary palmitate could play an important role in the development of atherosclerosis. Previous studies mostly focused on the role of palmitate in metabolic disorders and insulin resistance [15]. The present study provides compelling evidence supporting the concept that a palmitate-rich diet not only significantly enhances the growth of atherosclerotic plaques but also promotes plaque instability. Therefore, efforts to reduce the concentration of plasma FA might be an effective strategy for the treatment of atherosclerosis induced by a palmitate-rich diet.

Hyper-free fatty acidemia usually results from an increased intake of dietary FA or FA release from lipolysis. Although a healthy diet is crucial for preventing atherosclerosis, adherence to a healthy diet is usually poor [16]. Acipimox, a lipolysis inhibitor, has been demonstrated to reduce the plasma FA concentration in obese patients [17], and it also attenuates the level of proathero-

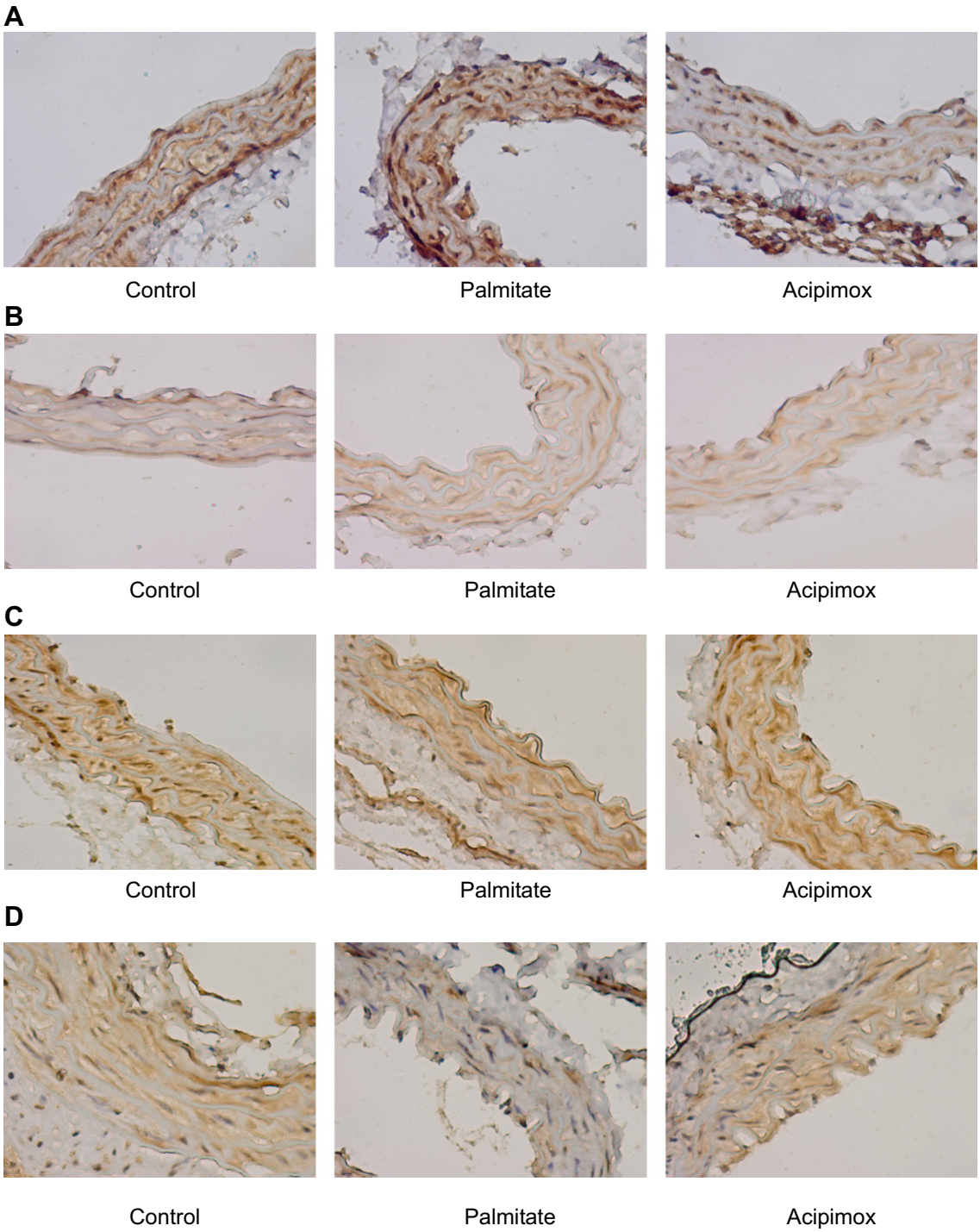

Fig. 4. Acipimox attenuates palmitate-induced upregulation of MMP-2. Representative images of immunohistochemistrical staining showing the expression of MMP-2 (A), MMP-9 (B), MMP-14 (C) and TIMP-1 (D) in the aorta. The protein expression levels of MMP-2 (E), MMP-9 (F), MMP-14 (G) and TIMP-1 (H) were expressed as the percentage of the positively stained area. The data are expressed as the mean ± SEM; $n = 4$ mice per group. $^{*}P < 0.05$, $^{**}P < 0.01$ compared to the control group, $^{\#\#}P < 0.01$ compared to the palmitate group. MMP, matrix metalloproteinase; TIMP, tissue inhibitors of metalloproteinase.

genic plasma lipoproteins and atherosclerosis caused by ritonavir-induced lipolysis [13]. We have previously used acipimox in ApoE$^{-/-}$ mice fed an oleate-rich diet and observed it significantly reduced atherosclerotic plaques [9]. However, previous studies did not address plaque instability, which plays a critical role in the rupture of plaques and the onset of cardiovascular events. The present study demonstrated that treatment with acipimox significantly ameliorates atherosclerotic lesion development and enhances plaque stability by increasing collagen deposition in mice with hyper-free fatty acidemia. This finding indicates that acipimox may be practical treatment for patients with atherosclerosis concomitant with hyper-free fatty acidemia.

The precise mechanism by which palmitate stimulates atherosclerosis remains to be elucidated [18]. Our previous study demonstrated that oleic acid increased lipid deposition in vascular smooth muscle cells and induced foam cell formation [9].

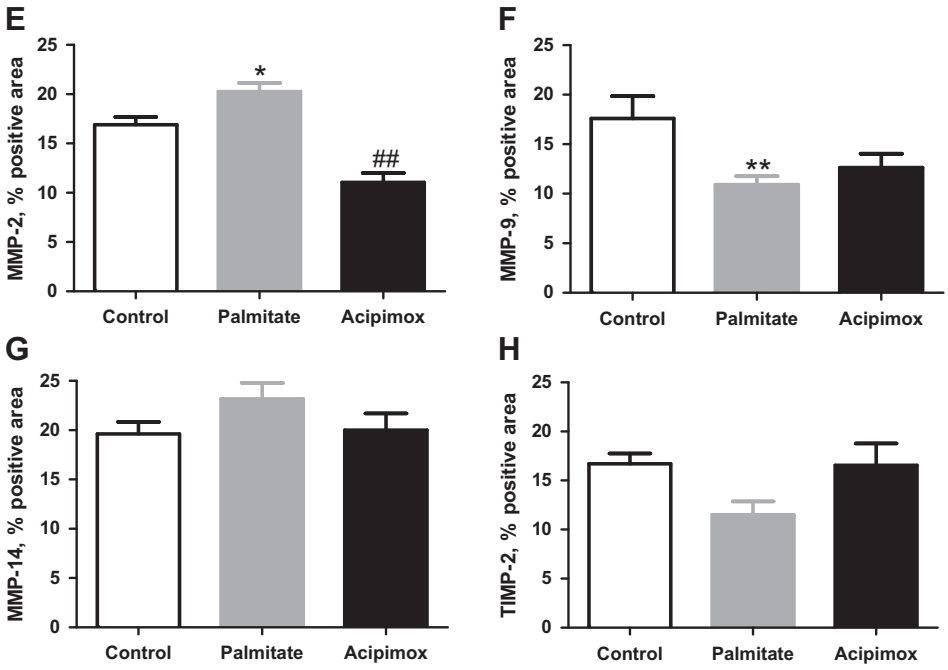

Fig. 4. (*continued*)

Additionally, the current study has shown a cause-and-effect relationship between hyper-free fatty acidemia and atherosclerosis. Thus it is likely that palmitate-induced plaque growth is mediated directly by increasing the FA uptake by vascular smooth muscle cells or infiltrated macrophages and inducing the foam cell formation.

Plaque collagen content plays an important role in preventing plaque rupture and is decreased in unstable atherosclerotic lesions observed in patients with acute coronary syndrome [19]. MMP/TIMP is the major enzyme system responsible for the collagen content in several tissues including the aorta [20–22]. According to the results from this study, one mechanism by which acipimox could attenuate palmitate-induced plaque instability is to reverse the upregulation of MMP-2. However, this hypothesis needs to be confirmed in animals that are deficient in MMP-2 expression.

In summary, a palmitate-rich diet increased plasma FA concentrations, enhanced atherosclerotic lesions, promoted plaque instability and upregulated MMP-2 in the aorta. All of these harmful effects were significantly blocked by co-treatment with acipimox. If these results were applied to clinical settings, treating patients with acute coronary syndrome or other unstable atherosclerotic lesions with acipimox could be a novel and potent strategy for inhibiting atherosclerosis and stabilizing plaque.

Acknowledgments

This work was supported by grant 2011YG-B22 from the General Hospital of PLA Chengdu Military Area Command to Dr. Shuangtao Ma. We thank Yan Luo and Yihua Chen (Department of Pathology) for skillful technical assistance. We acknowledge Song Hu (Chengdu Medical College) for animal care.

References

[1] F.B. Hu, J.E. Manson, W.C. Willett, Types of dietary fat and risk of coronary heart disease: a critical review, J. Am. Coll. Nutr. 20 (2001) 5–19.
[2] K. He, Fish, long-chain omega-3 polyunsaturated fatty acids and prevention of cardiovascular disease––eat fish or take fish oil supplement?, Prog Cardiovasc. Dis. 52 (2009) 95–114.
[3] P.W. Siri-Tarino, Q. Sun, F.B. Hu, R.M. Krauss, Saturated fatty acids and risk of coronary heart disease: modulation by replacement nutrients, Curr. Atheroscler. Rep. 12 (2010) 384–390.
[4] S. Pilz, H. Scharnagl, B. Tiran, U. Seelhorst, B. Wellnitz, B.O. Boehm, J.R. Schaefer, W. Marz, Free fatty acids are independently associated with all-cause and cardiovascular mortality in subjects with coronary artery disease, J. Clin. Endocrinol. Metab. 91 (2006) 2542–2547.
[5] M. Pirro, P. Mauriege, A. Tchernof, B. Cantin, G.R. Dagenais, J.P. Despres, B. Lamarche, Plasma free fatty acid levels and the risk of ischemic heart disease in men: prospective results from the Quebec cardiovascular study, Atherosclerosis 160 (2002) 377–384.
[6] F. Perez-Jimenez, J. Lopez-Miranda, P. Mata, Protective effect of dietary monounsaturated fat on arteriosclerosis: beyond cholesterol, Atherosclerosis 163 (2002) 385–398.
[7] L.G. Gillingham, S. Harris-Janz, P.J. Jones, Dietary monounsaturated fatty acids are protective against metabolic syndrome and cardiovascular disease risk factors, Lipids 46 (2011) 209–228.
[8] P.M. Kris-Etherton, AHA Science Advisory. Monounsaturated fatty acids and risk of cardiovascular disease. American Heart Association. Nutrition Committee, Circulation 100 (1999) 1253–1258.
[9] S. Ma, D. Yang, D. Li, B. Tang, Y. Yang, Oleic acid induces smooth muscle foam cell formation and enhances atherosclerotic lesion development via CD36, Lipids Health Dis. 10 (2011) 53.
[10] T. Koyama, S. Kume, D. Koya, S. Araki, K. Isshiki, M. Chin-Kanasaki, T. Sugimoto, M. Haneda, T. Sugaya, A. Kashiwagi, H. Maegawa, T. Uzu, SIRT3 attenuates palmitate-induced ROS production and inflammation in proximal tubular cells, Free Radic. Biol. Med. 51 (2011) 1258–1267.
[11] T. Coll, E. Eyre, R. Rodriguez-Calvo, X. Palomer, R.M. Sanchez, M. Merlos, J.C. Laguna, M. Vazquez-Carrera, Oleate reverses palmitate-induced insulin resistance and inflammation in skeletal muscle cells, J. Biol. Chem. 283 (2008) 11107–11116.
[12] M. Flechtner-Mors, C.P. Jenkinson, A. Alt, G. Adler, H.H. Ditschuneit, Effects of acipimox on the lipolysis rate in subcutaneous adipose tissue of obese subjects, Diabetes Metab. Res. Rev. 17 (2001) 387–390.
[13] W. Guo, S. Wong, J. Pudney, R. Jasuja, N. Hua, L. Jiang, A. Miller, P.W. Hruz, J.A. Hamilton, S. Bhasin, Acipimox, an inhibitor of lipolysis, attenuates atherogenesis in LDLR-null mice treated with HIV protease inhibitor ritonavir, Arterioscler. Thromb. Vasc. Biol. 29 (2009) 2028–2032.
[14] R.W. St Clair, H.B. Lofland Jr., T.B. Clarkson, Composition and synthesis of fatty acids in atherosclerotic aortas of the pigeon, J. Lipid Res. 9 (1968) 739–747.
[15] M. Zhao, Z.F. Zhang, Y. Ding, J.B. Wang, Y. Li, Astragalus polysaccharide improves palmitate-induced insulin resistance by inhibiting PTP1B and NF-kappaB in C2C12 myotubes, Molecules 17 (2012) 7083–7092.
[16] T. Pearson, L. Kopin, Bridging the treatment gap: improving compliance with lipid-modifying agents and therapeutic lifestyle changes, Prev. Cardiol. 6 (2003) 204–211.
[17] A.T. Santomauro, G. Boden, M.E. Silva, D.M. Rocha, R.F. Santos, M.J. Ursich, P.G. Strassmann, B.L. Wajchenberg, Overnight lowering of free fatty acids with acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects, Diabetes 48 (1999) 1836–1841.

[18] C.F. Semenkovich, Fatty acid metabolism and vascular disease, Trends Cardiovasc. Med. 14 (2004) 72–76.
[19] V. Neumeister, M. Scheibe, P. Lattke, W. Jaross, Determination of the cholesterol–collagen ratio of arterial atherosclerotic plaques using near infrared spectroscopy as a possible measure of plaque stability, Atherosclerosis 165 (2002) 251–257.
[20] D.C. Yang, S.T. Ma, Y. Tan, Y.H. Chen, D. Li, B. Tang, J.S. Chen, X.H. Su, G. Li, X. Zhang, Y.J. Yang, Imbalance of matrix metalloproteinases/tissue inhibitor of metalloproteinase-1 and loss of fibronectin expression in patients with congestive heart failure, Cardiology 116 (2010) 133–141.
[21] D. Yang, S. Ma, D. Li, B. Tang, Y. Yang, Angiotensin II receptor blockade improves matrix metalloproteinases/tissue inhibitor of matrix metalloproteinase-1 balance and restores fibronectin expression in rat infarcted myocardium, Biochem. Biophys. Res. Commun. 388 (2009) 606–611.
[22] M.M. Steed, N. Tyagi, U. Sen, D.A. Schuschke, I.G. Joshua, S.C. Tyagi, Functional consequences of the collagen/elastin switch in vascular remodeling in hyperhomocysteinemic wild-type, eNOS−/−, and iNOS−/− mice, Am. J. Physiol. Lung Cell. Mol. Physiol. 299 (2010) L301–L311.