Histol Histopathol (2014) 29: 1593-1600
DOI: 10.14670/HH-29.1593

**http://www.hh.um.es**

**Histology and Histopathology**
*Cellular and Molecular Biology*

# Demonstration of an add-on effect of probucol and cilostazol on the statin-induced anti-atherogenic effects

**Yanli Wang[1,2], Liang Bai[1,2], Yan Lin[1,2], Hua Guan[1,2], Ninghong Zhu[1,2], Yulong Chen[1,2], Yafeng Li[1,2], Shoucui Gao[1,2], Sihai Zhao[1,2], Jianglin Fan[3] and Enqi Liu[1,2]**

[1]Research Institute of Atherosclerotic Disease, Xi'an Jiaotong University Cardiovascular Research Center, Xi'an, Shaanxi, China, [2]Laboratory Animal Center, Xi'an Jiaotong University School of Medicine, Xi'an, Shaanxi, China and [3]Department of Molecular Pathology, Interdisciplinary Graduate School of Medicine and Engineering, University of Yamanashi, Yamanashi, Japan

**Summary.** Statins are often prescribed for treatment of cardiovascular diseases, although there are still many patients who cannot be effectively treated by statins alone. Both probucol and cilostazol exhibit anti-atherogenic effects. In the current study, we attempted to investigate whether a probucol and cilostazol combination had any add-on effects on atorvastatin. To examine this hypothesis, we fed Japanese white rabbits with a cholesterol-rich diet supplemented with atorvastatin alone (Statin group), probucol and cilostazol (PC group), atorvastatin, probucol and cilostazol (APC group), and compared their effects on plasma lipids and aortic atherosclerosis. All three drug-treated groups had lowered total cholesterol levels compared with the vehicle group but high-density lipoproteins cholesterol levels of the atorvastatin group were higher than other groups. Although aortic atherosclerosis was significantly reduced in all drug-treated groups, the most prominent atheroprotective effect was seen in APC group (APC: 67% reduction> PC: 43% reduction> Statin group: 42% reduction over the vehicle). Morphometric analysis revealed that the reduced aortic atherosclerosis in all three groups was mainly attributed to the reduction of intimal macrophages and smooth muscle cells. These results suggest that a combination of probucol and cilostazol with statin enhances statin's anti-atherogenic functions, which may be beneficial for those patients who are less responsive to statin therapy alone.

*Offprint requests to:* Enqi Liu, PhD, Professor and Director, Research Institute of Atherosclerotic Disease, Xi'an Jiaotong University School of Medicine, China. e-mail: liuenqi@mail.xjtu.edu.cn

**Key words:** Atorvastatin, Probucol, Cilostazol, Atherosclerosis, Hypercholesterolemia

## Introduction

Atherosclerosis is the foremost cause of mortality in both developed and developing countries (Mathers and Loncar, 2006; Rosamond et al., 2008; Reiner et al., 2011). Atherosclerosis is a multifactorial disease and many factors are involved in its pathogenesis and progression. Hypercholesterolemia is one of the main risk factors of atherosclerosis, therefore treatment of hypercholesterolemia is still the major task for preventing the development of atherosclerosis. In this regard, statins, a 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitor is the first choice for treating hyperlipidemic patients because statins can not only reduce the plasma low-density lipoproteins cholesterol (LDL-C) through inhibition of cholesterol synthesis but also show anti-inflammatory effects, "so-called pleiotropic functions". In spite of this, there are still many atherosclerotic patients who cannot be effectively treated by statins alone. According the report by Maron et al., statin therapy alone can lead to the

**Abbreviations.** APC, atorvastatin, probucol and cilostazol; AS, atherosclerosis; CRP, C-reactive protein; CVD, cardiovascular disease; CZ, cilostazol; HDL-C, high-density lipoproteins cholesterol; LDL-C, low-density lipoprotein cholesterol; MDA, malondialdehyde; MΦ, macrophage; MMPs, matrix metalloprotainases; non-HDL-C, non-high-density lipoprotein cholesterol; ox-LDL, oxidized low-density lipoprotein ; PB, probucol; PC, probucol and cilostazol; SMC, smooth muscle cell; SOD, superoxide dismutase; TC, total cholesterol; TG, triglycerides

reduction of cardiovascular event risk by 30% (Maron et al., 2000), suggesting that many atherosclerosis patients need alternative therapy. In addition, the European Society of Cardiology and the European Atherosclerosis Society (ESC/EAS) (Reiner et al., 2011), and the American Diabetes Association and the American College of Cardiology (ADA/ACC) (Brunzell et al., 2008) recommend that LDL-C should be lowered to < 70 mg/dl in patients at high risk. When statin treatment cannot achieve this goal, alternative strategies are required (Yamazaki et al., 2013).

Probucol (PB) is a diphenolic compound with anti-oxidative, anti-inflammatory and lipid-lowering property. Until now, many studies revealed that PB exhibited potent anti-atherogenic effects in both experimental animals and humans (Yamamoto, 2008; Yamashita et al., 2008; Yamashita and Matsuzawa, 2009). Recent studies revealed that PB improved the functions of high-density lipoproteins cholesterol (HDL-C) thereby enhancing reverse cholesterol transport (Hirano et al., 2005; Miida, et al., 2008). Cilostazol (CZ), an inhibitor of type 3 phosphodiesterase exerts antiplatelet aggregation through suppression of cyclic adenosine monophosphate degradation (Weintraub, 2006) and is often used for treating thrombotic vascular disease (Lugnier, 2006). Several lines of evidence revealed that CZ suppressed intracellular reactive oxygen species production (Kim et al., 2002) and increased nitric oxide production (Ota et al., 2008). Furthermore, CZ inhibited proliferation of smooth muscle cells (SMCs) and foam cell formation (Okutsu et al., 2009; Hattori et al., 2009; Nakaya et al., 2010). Therefore, CZ exerts anti-atherogenic effects in addition to its anti-platelet aggregation.

Because the pathogenesis of atherosclerosis is involved in many factors such as LDL oxidization, inflammatory reaction and platelet activation under hypercholesterolemia, we envisioned that combinational therapy using different agents targeting the different factors may be more efficient than a single agent at high doses. Our recent studies showed that combined PB with CZ exerted synergist anti-atherogenic effects (Chen et al., 2013) and PB exhibited strong inhibition of the early stage of atherosclerosis of cholesterol-fed rabbits (Niimi et al., 2013), which are well suited for studying cardiovascular diseases (CVD) because rabbits are sensitive to cholesterol diet and rapidly develop atherosclerosis (Fan and Watanabe, 2000, 2003). These findings allowed us to hypothesize whether combined PB and CZ with statin would enhance the anti-atherogenic effects of statins. In this study, we found that PB and CZ indeed exerted add-on effects on anti-atherogenic functions of statin in cholesterol-fed rabbits.

## Materials and methods

### *Animals and diets*

Fifty Japanese white rabbits (male, 4-mon) were supplied by Wuhan Institute of Biological Products (Wuhan, China) and were fed a diet containing 0.5% cholesterol for one week. Plasma levels of total cholesterol (TC) were measured and 40 rabbits were selected for the following experiment based on the TC levels (ranging from 300 to 500 mg/dl). Those rabbits with TC levels either higher or lower than the range were excluded. Rabbits were randomly divided into 4 groups (n=10 for each group) and fed a diet containing 0.3% cholesterol and 3% soybean oil for 16 weeks. (1) atorvastatin (Statin) group, supplemented with 0.005% atorvastatin in the diet; (2) PB and CZ (PC) group, supplemented with 0.3% PB and 0.3% CZ in the diet; (3) APC group, fed with the same diet supplemented with 0.005% atorvastatin, 0.3% PB and 0.3% CZ; (4) Vehicle group was fed the same cholesterol diet only. All doses were chosen according to the previous studies (Chen et al. 2013; Niimi et al. 2013).

PB and CZ were provided by Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). Atorvastatin calcium was purchased from Sequoia Research Products Ltd., (Pangbourne, UK). The diets were prepared by Vital River Laboratories (Beijing, China). All rabbits were fed with restricted diet intake (100 g/rabbit per day) and given free access to water. The experiment protocols were approved by the Animal Administration Committee of Xi'an Jiaotong University and performed according to the Xi'an Jiaotong University Guidelines for Animal Experimentation, and the Guide for the Care and Use of Laboratory Animals Published by the US National Institutes of Health (NIH Publication NO. 85-23, revised 1996).

### *Determination of plasma lipids levels and other biochemical parameters*

The plasma TC and triglyceride (TG) levels were measured biweekly and plasma HDL-C and LDL-C levels were measured every 4 weeks. Rabbits were fasted for 16h before blood collection. Blood samples were collected by the ear artery into tubes containing EDTA and then plasma was separated by centrifugation at 2000 rpm/min (20 min, 4°C). The plasma levels of TC, TG, HDL-C and LDL-C were measured using standard commercial assay kits (Biosino Bio-technology and Science Inc., Beijing, China). We also measured the plasma inflammatory marker, C-reactive protein (CRP) using an ELISA kit (Immunology Consultants Laboratory, Inc. Portland, OR). The plasma superoxide dismutase (SOD) and malondialdehyde (MDA) levels were measured by xanthine oxidase assay and thibabituric acid assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) respectively. Plasma levels of oxidized low-density lipoprotein (ox-LDL) were measured by an ELISA kit (R&D Systems, Minneapolis, MN).

### *Quantification of gross atherosclerotic lesions*

At the end of the experiment, all rabbits were sacrificed by intravenous injection of an overdose of

sodium pentobarbital solution. Rabbit aortas were collected for analysis of the aortic lesions as described previously (Zhao et al., 2008). Aortic en face atherosclerosis was evaluated using image analysis software (WinROOF Ver.6.5, Mitani Co., Ltd. Fukui, Japan) after aortic trees were stained with Sudan IV.

### *Histology and immunohistochemistry*

For the microscopic quantification of lesions, the aorta was cut into 8 to 10 sections (4 μm) as described previously (Zhang et al., 2010). All sections were stained with hematoxylin and eosin (H&E) and intimal

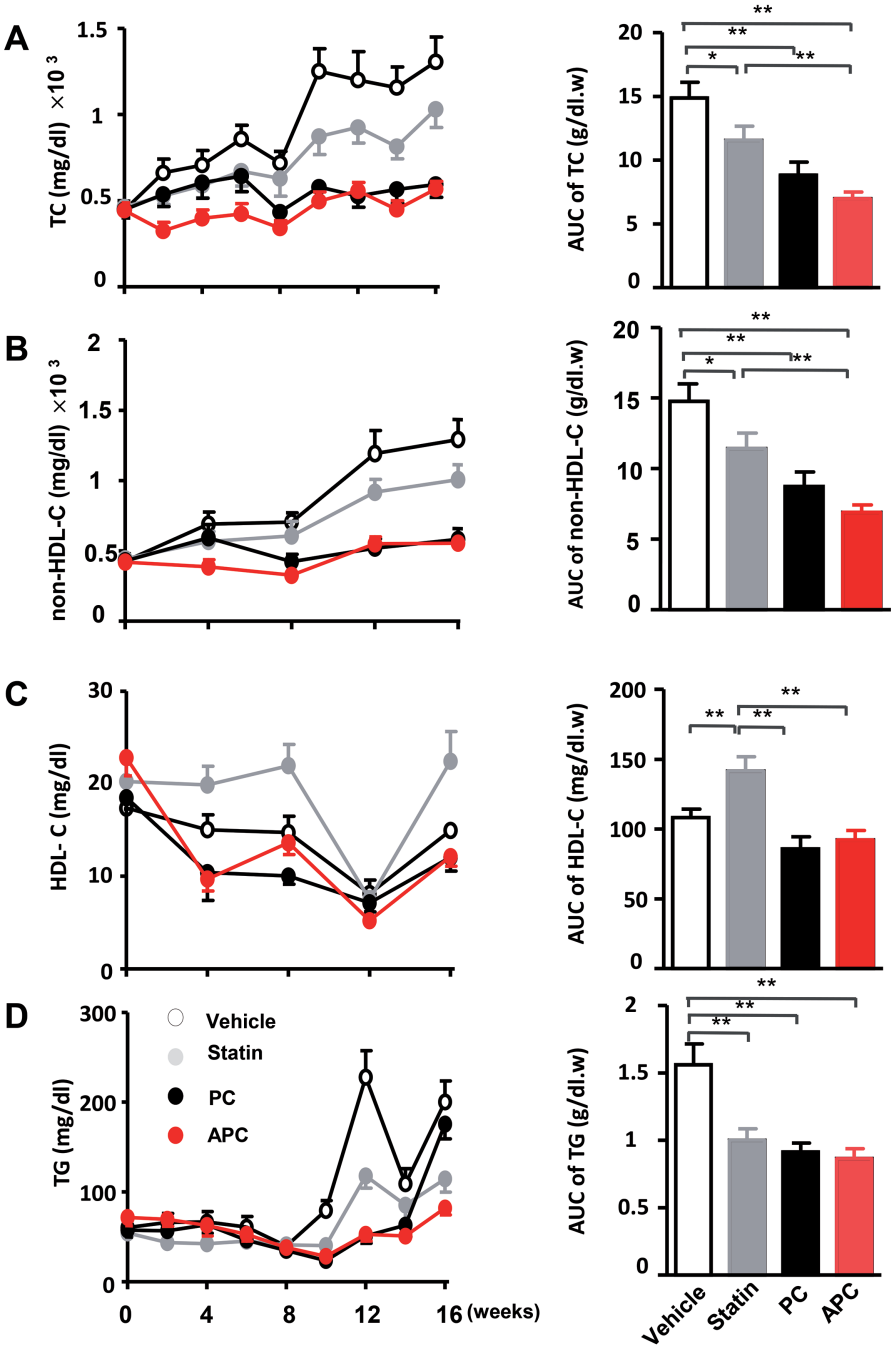


**Fig. 1.** Plasma lipids levels. The plasma total cholesterol (TC) **(A)**, non-high-density lipoprotein cholesterol (non-HDL-C) **(B)**, high-density lipoprotein cholesterol (HDL-C) **(C)**, and triglyceride (TG) **(D)** levels were measured, and the area under the curve (AUC) was calculated (right). Data are expressed as the mean ± SEM. *P<0.05, ** P<0.01. n=10 for each group.

lesion area was measured by an image analysis system described above. For microscopic evaluation of cellular components in the lesions, serial paraffin sections were immunohistochemically stained with monoclonal antibodies against rabbit macrophage (MΦ) (RAM11, Dako, Carpinteria, CA, USA) and SMCs (α-smooth muscle actin, Thermo Fisher Scientific Pierce, Rockford, IL, USA). Immunostaining was visualized using an AEC kit (Zhongshan Biotechnology, Beijing, China).

### *Statistical analysis*

The statistical analysis was carried out by one-way ANOVA followed by Dunnett's test and was performed using the SPSS 13.0 software. In all cases, P values <0.05 were considered as significant difference.

## Results

### *Plasma lipids levels*

Analysis of plasma lipids revealed that all three drug-treated groups had low levels of TC compared to the vehicle group throughout the experiment periods (Fig. 1A). To quantify the hypercholesterolemia duration extent, we measured the incremental area under the curve and found that the lipid-lowering effect was the strongest in APC group, followed by PC and Statin group (Fig. 1A right). Lipid-lowering effects were basically attributed to the reduction of non-HDLs in all groups (Fig. 1B). However, HDL-C levels were higher in the Statin group than the other three groups (Fig. 1C). In addition to cholesterol-lowering effect, three drug therapies reduced the levels of TG at the later period of cholesterol diet feeding (Fig.1D). During the experiment, there was no difference in food intake and body weight among the 4 groups (data not shown).

### *Aortic atherosclerosis analysis*

To analyze the aortic lesions of atherosclerosis, we compared the lesion size of the *en face* atherosclerosis visualized by Sudan IV staining. Although all drug treatment protected against the diet-induced aortic

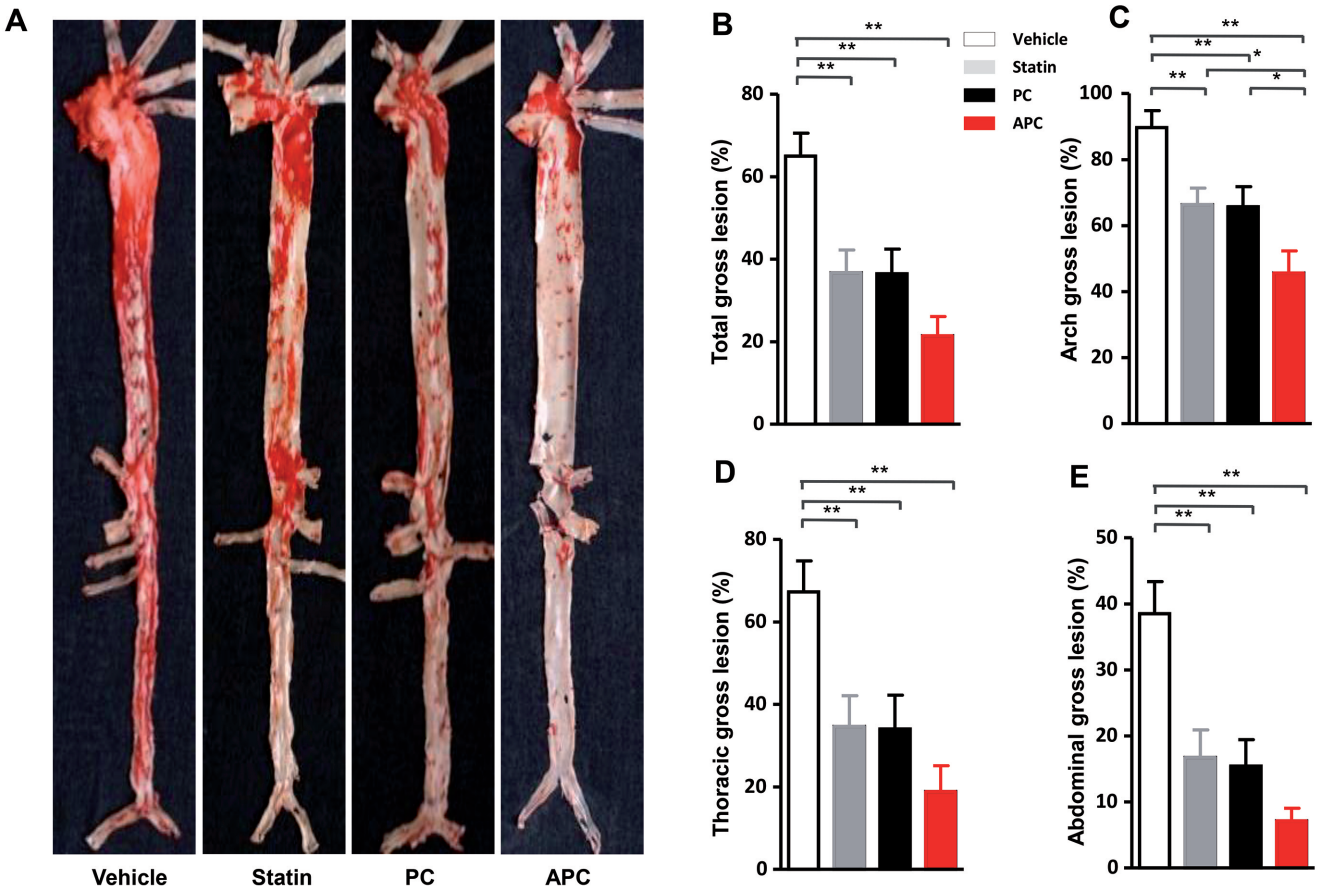


**Fig. 2.** Aortic atherosclerotic lesion area. Aortic trees were stained with Sudan IV **(A)**, and total **(B)**, arch **(C)**, thoracic **(D)** and abdominal **(E)** gross lesion areas were measured. Data are expressed as the mean ± SEM. *P<0.05, ** P<0.01. n=10 for each group.

atherosclerosis, the APC group showed more prominent inhibitory effects on the total aortic lesions (67% reduction vs. vehicle) than PC (43% reduction vs. vehicle) and Statin group (42% reduction vs. vehicle) (Fig. 2A-B). It seems that the reduction of atherosclerotic lesions occurred evenly in all parts of aortic trees because the lesion size of aortic arch, abdominal and thoracic aortas was significantly reduced in all three groups compared to vehicle group (Fig. 2C-E).

### *Morphometric analysis of the lesions*

To analyze the microscopic lesion size under a light microscope and investigate which cellular components were affected by the drug treatment, we measured the intimal lesions of the aortic arch in which the lesions were the most severe. As shown in Fig. 3, all three drug treatments significantly reduced the intimal lesion size similar to the gross lesion quantitative results: the APC group showed the strongest reduction (85%) followed by

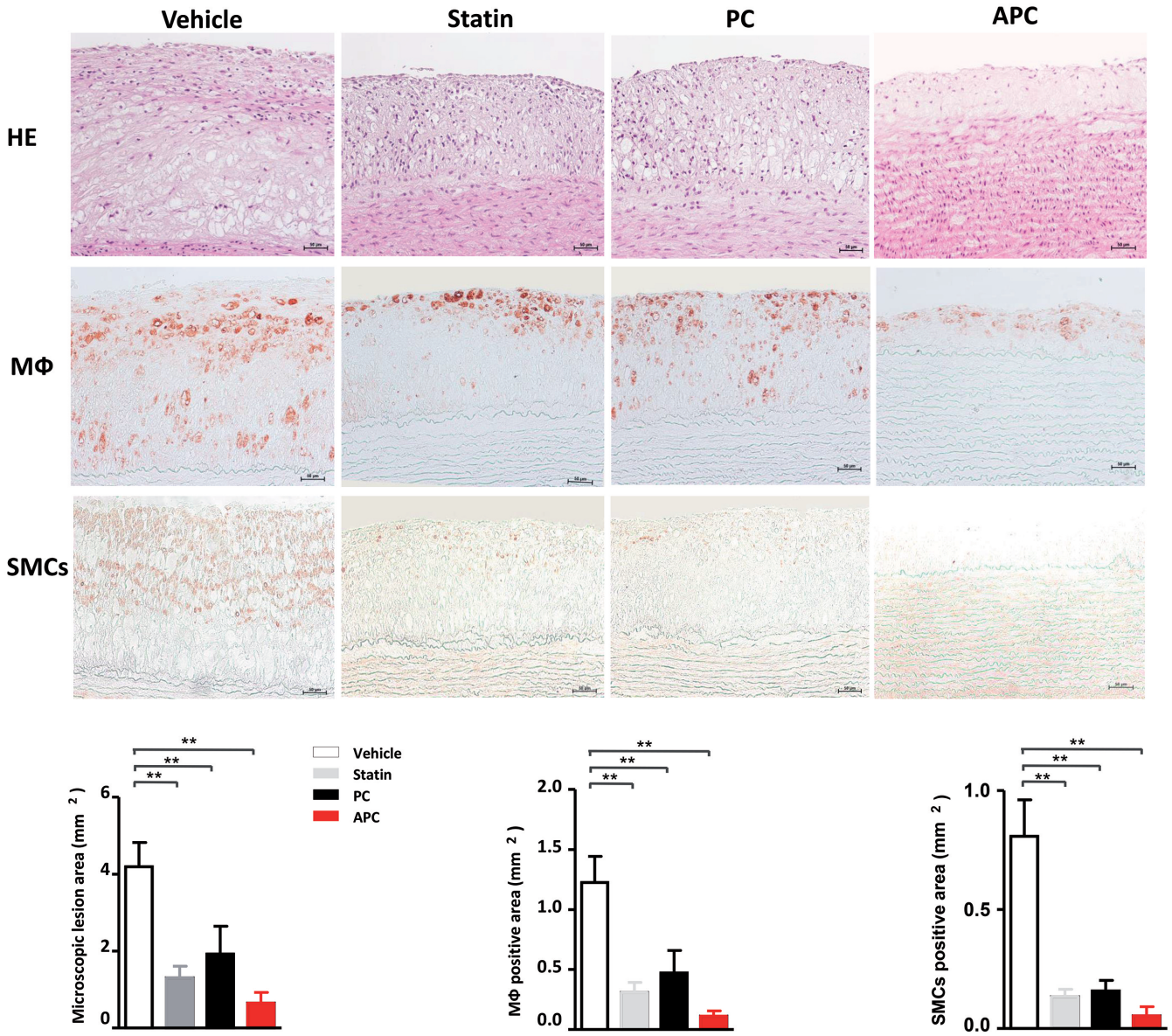


**Fig. 3.** Histological analysis of aortic atherosclerosis. Representative micrographs are shown in the upper panel and microscopically quantitative analysis of the intimal lesions, lesion positive area of macrophage (MΦ) and smooth muscle cells (SMCs) are shown in the bottom panel. Data are expressed as the mean ± SEM. ** P<0.01. n=7 for each group.

PC group (54%) and Statin group (69%) compared to the vehicle group. Immunohistochemical staining showed that the reduction of the intimal lesion size was caused by a decreased number of both MΦ and SMCs. This tendency (reduced MΦ and SMCs) was similar to the intimal size reduction, namely, APC> Statin >PC groups in terms of inhibitory potency of the lesion size.

### *Oxidative stress and inflammation marker in plasma*

To evaluate whether there were any changes in plasma lipid peroxidation and inflammatory markers, we measured plasma levels of MDA, SOD, ox-LDL and CRP at the end of experiment. As shown in Fig. 4, all three drug treatments led to a significant reduction of plasma MDA, ox-LDL, CRP levels but an increase of plasma SOD levels compared to the vehicle. These beneficial effects of ABC were apparently stronger than those of PC and Statin group.

## Discussion

In the current study, we demonstrated that addition of PB and CZ enhanced the anti-atherogenic effects of atorvastatin in cholesterol-fed rabbits. While statins are still the first choice for the treatment of hypercholesterolemia and atherosclerosis, there are about 70% CVD patients who cannot be treated properly by statins alone and alternative therapies are urgently required (Maron et al., 2000; Cooney et al., 2010; Arca et al., 2012). For those patients, high doses of statins are sometimes administered to reduce the plasma cholesterol levels. However, high doses of statins are not always effective and at the same time, can increase the risks of side-effects. Roberts showed that even doubling the doses of each statin drug led to the reduction of serum LDL-C by only 5-7% (Roberts, 1997). In other words, many CVD patients at high risk cannot successfully reach LDL-C levels to 70 mg/dl simply by increasing

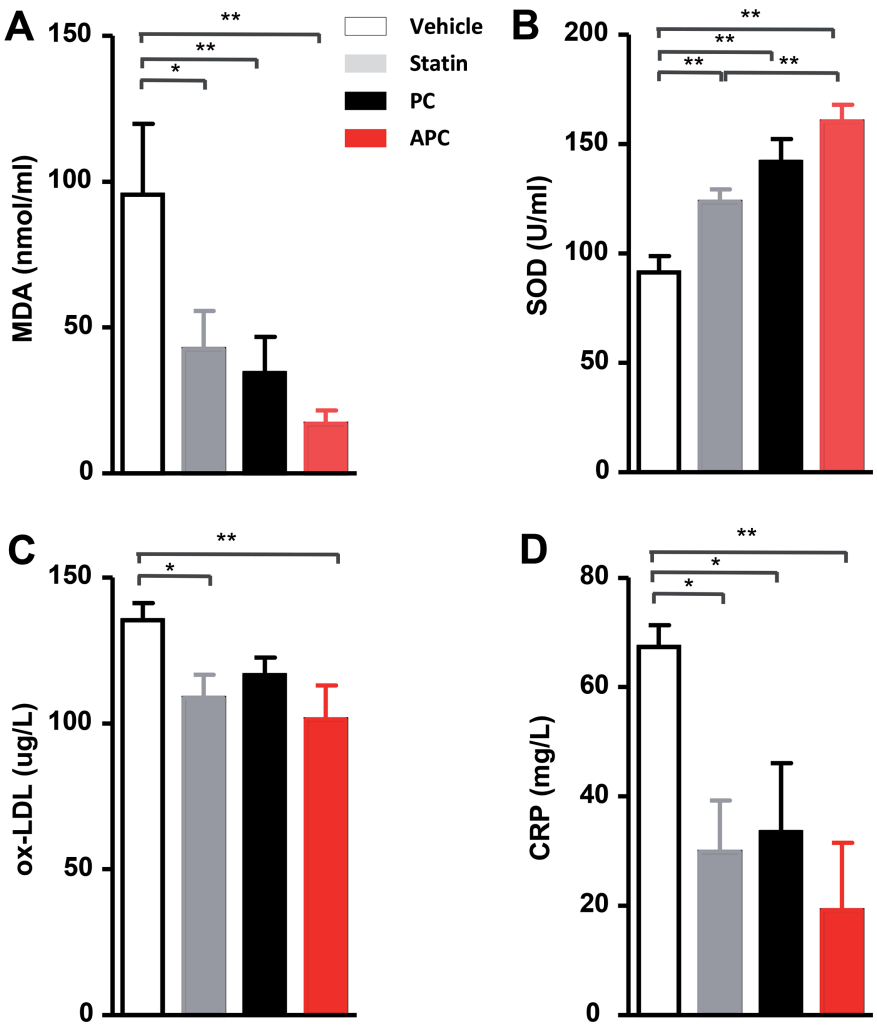


**Fig. 4.** Oxidative stress and inflammation marker. The plasma malonaldehyde (MDA) **(A)**, superoxide dismutase (SOD) **(B)**, oxidized low-density lipoprotein (ox-LDL) **(C)** and C-reactive protein (CRP) **(D)** levels were measured after cholesterol-fed 16 weeks. Data are expressed as mean ± SEM. *P<0.05, ** P<0.01. n=10 for each group.

doses of statins. Alternatively, combining statins with other therapies may be required. Towards this point, we performed a series of studies to test different anti-atherogenic drugs, anti-oxidant agent PB and anti-platelet aggregation agent CZ using cholesterol-fed rabbits. We found that combined PB and CZ exhibited potent anti-atherogenic effects compared to PB or CZ alone (Chen et al., 2013). Furthermore, the current study showed that PB and CZ had an add-on effect on the statin.

There are several implications from the current study. While it remains to be verified clinically, combining three drugs (statin, PB and CZ) at low doses may be appropriate for those patients who are not responsive to statin for the treatment of hypercholesterolemia and prevention of cardiovascular events. It is well known that statins reduce cholesterol synthesis by inhibition of HMG-CoA reductase and increasing hepatic LDL receptor activity. Statin also has anti-inflammatory effects. Combined use of PB and CZ not only led to the enhancement of statin's lipid-lowering effect, but also to the reduction of aortic atherosclerosis. In such a circumstance, three drugs may have synergistic effects because multiple pharmacologic functions are present. This notion was supported by the observation that the lesions of aortic atherosclerosis in APC group were characterized by reduced MΦ and SMCs. It should be pointed out, the HDL-C levels in triple drug group was reduced than that in statin treatment group alone, and therefore HDL-C levels may not be directly involved in the modulation of lesion formation in cholesterol-fed rabbits. On the other hand, it is clear that low levels of HDL-C induced by PB treatment enhance reverse cholesterol transport (Yamamoto et al., 2011). PB may offset the HDL-C elevating functions of atorvastatin because the HDL-C levels in APC group were not significantly changed compared to the vehicle group.

Recent studies revealed that both PB and CZ have been shown to inhibit proliferation of SMCs and foam cell formation (Deng et al., 2004; Kim et al., 2005). Our previous study revealed that the combinatorial anti-atherogenic effect of PB and CZ is partly mediated through an improvement in the vascular functions by increasing the levels of NO and endothelial protein S-nitrosylation (Chen et al., 2013). In the current study, MDA and ox-LDL levels were decreased while SOD was increased in the APC group compared to other groups. Consistently, inflammatory marker, such as plasma CRP, was reduced in APC group compared to the other three groups.

It should be pointed out, however, there are several limitations in the current study. First, it is not known whether APC treatment has any beneficial effects on the plaque stability such as matrix metalloprotainases (MMPs) and cathepsins (Liang et al. 2006, Cheng et al. 2012). In a preliminary study, we compared the mRNA expression of MMP-2 and MMP-12 along with tissue inhibitor of metalloproteinases 1-3 but did not find any significant difference between APC and other groups (data not shown). In spite of this, one can envision that the reduction of total number of lesional MΦ induced by APC would certainly lead to less production of these enzymes which eventually stabilizes the plaques. Second, although PB is widely used in Asia, it is not common in many other countries such as Europe, which may limit the use of PB in these areas. This is mainly due to the fact that PB reduces plasma levels of HDL-C as a notorious side-effect. Nevertheless, recent studies have revealed that PB improves the functions of HDLs by enhancing reverse cholesterol transport while HDL-C levels in the plasma are low (Yamashita and Matsuzawa, 2009). Therefore, it is still unknown whether PB-induced low HDL-C is "good or bad" or whether it is just a consequence of improvement of HDL functions. CZ can inhibit platelet aggregation but has less side-effects (bleeding) than aspirin. Although these two old drugs have many therapeutic effects, it needs to be verified in future whether it can be widely accepted in those countries where they are not popular.

In conclusion, our study revealed that addition of PB and CZ enhances the statin's anti-atherogenic effects in cholesterol-fed rabbits. While it remains to be verified in future, these results suggest that multiple drug therapy acting at different atherogenic targets in the vascular wall may be beneficial for those CVD patients who are not responsive to statin alone.

*Acknowledgements.* This work was partly supported by the National Natural Science Foundation of China (81070250, 81270348), by National Science and Technology Support Program (No. 2012BAI39B02), by a Public Service Platform Grant of Shaanxi Province (2012FWPT-03), and by Otsuka Pharmaceutical Co., Ltd.
*Conflict of interest.* None declared.

## References

Arca M., Pigna G. and Favoccia C. (2012). Management of statin-intolerant patient. Panminerva. Med. 54, 105-118.

Brunzell J.D., Davidson M., Furberg C.D., Goldberg R.B., Howard B.V., Stein J.H. and Witztum J.L. (2008). Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. J. Am. Coll. Cardiol. 51, 1512–1524.

Chen Y., Zhao S., Huang B., Wang Y., Li Y., Waqar A.B., Liu R., Bai L., Fan J. and Liu E. (2013). Probucol and cilostazol exert a combinatorial anti-atherogenic effect in cholesterol-fed rabbits. Thromb. Res. 132, 565-571.

Cheng X., Shi G., Kuzuya M., Sasaki T., Okumura K. and Murohara T. (2012). Role for cysteine protease cathepsins in heart disease: focus on biology and mechanisms with clinical implication. Circulation 125, 1551-1562.

Cooney M.T., Dudina A., D'Agostino R. and Graham I.M. (2010). Cardiovascular risk-estimation systems in primary prevention: do they differ? Do they make a difference? Can we see the future? Circulation 122, 300-310.

Deng Y.M., Wu B.J., Witting P.K. and Stocker R. (2004). Probucol

protects against smooth muscle cell proliferation by upregulating heme oxygenase-1. Circulation 110, 1855-1860.

Fan J. and Watanabe T. (2000). Cholesterol-fed and transgenic rabbit models for the study of atherosclerosis. J. Atheroscler. Thromb. 7, 26-32.

Fan J. and Watanabe T. (2003). Transgenic rabbits as therapeutic protein bioreactors and human disease models. Pharmacol. Ther. 99, 261–282.

Hattori Y., Suzuki K., Tomizawa A., Hirama N., Okayasu T., Hattori S., Satoh H., Akimoto K. and Kasai K. (2009). Cilostazol inhibits cytokine-induced nuclear factor-kappaB activation via AMP-activated protein kinase activation in vascular endothelial cells. Cardiovasc. Res. 81,133-139.

Hirano K., Ikegami C., Tsujii K., Zhang Z., Matsuura F., Nakagawa-Toyama Y., Koseki M., Masuda D., Maruyama T., Shimomura I., Ueda Y. and Yamashita S. (2005). Probucol enhances the expression of human hepatic scavenger receptor class B type I, possibly through a species-specific mechanism. Arterioscler. Thromb. Vasc. Biol. 25, 2422-2427.

Kim K.Y., Shin H.K., Choi J.M. and Hong K.W. (2002). Inhibition of lipopolysaccharide induced apoptosis by cilostazol in human umbilical vein endothelial cells. J. Pharmacol. Exp. Ther. 300, 709-715.

Kim M.J., Park K.G., Lee K.M., Kim H.S., Kim S.Y., Kim C.S., Lee S.L., Chang Y.C., Park J.Y., Lee K.U. and Lee I.K. (2005). Cilostazol inhibits vascular smooth muscle cell growth by downregulation of the transcription factor E2F. Hypertension 45, 552-556.

Liang J., Liu E., Yu Y., Kitajima S., Koike T., Jin Y., Morimoto M., Hatakeyama K., Asada Y., Watanabe T., Sasaguri Y., Watanabe S. and Fan J. (2006). Macrophage metalloelastase accelerates the progression of atherosclerosis in transgenic rabbits. Circulation 113, 1993-2001.

Lugnier C. (2006). Cyclic nucleotide phosphodiesterase (PDE) superfamily: a new target for the development of specific therapeutic agents. Pharmacol. Ther. 109, 366-398.

Maron D.J., Fazio S. and Linton M.F. (2000). Current perspectives on statins. Circulation 101, 207-213.

Mathers C.D. and Loncar D. (2006). Projections of global mortality and burden of disease from 2002 to 2030. PLoS. Med. 3, 2011-2030.

Miida T., Seino U., Miyazaki O., Hanyu O., Hirayama S., Saito T., Ishikawa Y., Akamatsu S., Nakano T., Nakajima K., Okazaki M. and Okada M. (2008). Probucol markedly reduces HDL phospholipids and elevated prebeta1-HDL without delayed conversion into alpha-migrating HDL: putative role of angiopoietin-like protein 3 in probucol-induced HDL remodeling. Atherosclerosis 200, 329-335.

Nakaya K., Ayaori M., Uto-Kondo H., Hisada T., Ogura M., Yakushiji E., Takiguchi S., Terao Y., Ozasa H., Sasaki M., Komatsu T., Ohsuzu F. and Ikewaki K. (2010). Cilostazol enhances macrophage reverse cholesterol transport in vitro and in vivo. Atherosclerosis 213, 135-141.

Niimi M., Keyamura Y., Nozako M., Koyama T., Kohashi M., Yasufuku R., Yoshikawa T. and Fan J. (2013). Probucol inhibits the initiation of atherosclerosis in cholesterol-fed rabbits. Lipids Health Dis.12, 166.

Okutsu R., Yoshikawa T., Nagasawa M., Hirose Y., Takase H., Mitani K., Okada K., Miyakoda G. and Yabuuchi Y. (2009). Cilostazol inhibits modified low-density lipoprotein uptake and foam cell formation in mouse peritoneal macrophages. Atherosclerosis 204, 405-411.

Ota H., Eto M., Kano M.R., Ogawa S., Iijima K., Akishita M. and Ouchi Y. (2008). Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of Sirt1 in human endothelial cells. Arterioscler. Thromb. Vasc. Biol. 28, 1634-1639.

Reiner Z., Catapano A.L., De Backer G., Graham I., Taskinen M.R., Wiklund O., Agewall S., Alegria E., Chapman M.J., Durrington P., Erdine S., Halcox J., Hobbs R., Kjekshus J., Filardi P.P., Riccardi G., Storey R.F. and Wood D. (2011). ESC/EAS Guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). Eur. Heart J. 32, 1769-1818.

Roberts W.C. (1997). The rule of 5 and the rule of 7 in lipid-lowering by statin drugs. Am. J. Cardiol. 80, 106-107.

Rosamond W., Flegal K., Furie K., Go A., Greenlund K., Hailpern S.M., Ho M., Howard V., Kissela B., Kittner S., Lloyd-Jones D., McDermott M., Meigs J., Moy C., Nichol G., O'Donnell C., Roger V., Sorlie P., Steinberger J., Thom T., Wilson M. and Hong Y. (2008). Heart disease and stroke statistics-2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 117, e125-e146.

Weintraub W.S. (2006). The vascular effects of cilostazol. Can. J. Cardiol. 22 (Suppl B), 56B-60B.

Yamamoto A. (2008). A uniqe antilipidemic drug-probucol. J. Atheroscler. Thromb. 15, 304-305.

Yamamoto S., Tanigawa H., Li X., Komaru Y., Billheimer J.T. and Rader D.J. (2011). Pharmacologic suppression of hepatic ATP-binding cassette transporter 1 activity in mice reduces high-density lipoprotein cholesterol levels but promotes reverse cholesterol transport. Circulation 124, 1382-1390.

Yamashita S. and Matsuzawa Y. (2009). Where are we with probucol: a new life for an old drug? Atherosclerosis 207, 16-23.

Yamashita S., Hbujo H., Arai H., Harada-Shiba M., Matsui S., Fukushima M., Saito Y., Kita T. and Matsuzawa Y. (2008). Long-term probucol treatment prevents secondary cardiovascular events: a cohort study of patients with heterozygous familial hypercholesterolemia in Japan. J. Atheroscler. Thromb. 15, 292–303.

Yamazaki D., Ishida M., Watanabe H., Nobori K., Oguma Y., Terata Y., Koyama T., Iino K., Kosaka T. and Ito H. (2013). Comparison of anti-inflammatory effects and high-density lipoprotein cholesterol levels between therapy with quadruple-dose rosuvastatin and rosuvastatin combined with ezetimibe. Lipids Health Dis.12, 9.

Zhang C., Zheng H., Yu Q., Yang P., Li Y., Chen F., Fan J. and Liu E. (2010). A practical method for quantifying atherosclerotic lesions in rabbits. J. Comp. Pathol. 142, 122-128.

Zhao S., Zhang C., Lin Y., Yang P., Yu Q., Yang P., Fan J. and Liu E. (2008). The effects of rosiglitazone on aortic atherosclerosis of cholesterol-fed rabbits. Thromb. Res. 123, 281-287.

Accepted July 31, 2014