Am J Physiol Heart Circ Physiol 297: H2004–H2014, 2009.
First published October 2, 2009; doi:10.1152/ajpheart.00208.2009.

Traditional Chinese medication Tongxinluo dose-dependently enhances stability of vulnerable plaques: a comparison with a high-dose simvastatin therapy

Lei Zhang,[1]* Yan Liu,[2]* Xiao Ting Lu,[2]* Yi Ling Wu,[4] Cheng Zhang,[1] Xiao Ping Ji,[1] Rong Wang,[1] Chun Xi Liu,[1] Jin Bo Feng,[1] Hong Jiang,[1] Xin Sheng Xu,[3] Yu Xia Zhao,[2] and Yun Zhang[1]

[1]*The Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Health and* [2]*Department of Traditional Chinese Medicine, Shandong University Qilu Hospital, Jinan;* [3]*Department of Cardiology, Dongying People's Hospital, Dongying, Shandong; and* [4]*Institute of Integrated Traditional Chinese and Western Medicine Research, Shijiazhuang, Hebei, P. R. China*

Submitted 4 March 2009; accepted in final form 18 September 2009

Zhang L, Liu Y, Lu XT, Wu YL, Zhang C, Ji XP, Wang R, Liu CX, Feng JB, Jiang H, Xu XS, Zhao YX, Zhang Y. Traditional Chinese medication Tongxinluo dose-dependently enhances stability of vulnerable plaques: a comparison with a high-dose simvastatin therapy. *Am J Physiol Heart Circ Physiol* 297: H2004–H2014, 2009. First published October 2, 2009; doi:10.1152/ajpheart.00208.2009.—This study was carried out to test the hypothesis that Tongxinluo (TXL) as a Chinese herbal medicine enhances stability of vulnerable plaque dose dependently via lipid-lowering and anti-inflammation effects, similar to a high-dose simvastatin therapy. After abdominal aortic balloon injury, 75 rabbits were fed a 1% cholesterol diet for 10 wk and were then divided into five groups for 8-wk treatment: control group, low-dose TXL group, moderate-dose TXL group, high-dose TXL group, and high-dose simvastatin group. At the end of *week 16*, an adenovirus containing p53 was injected into the abdominal aortic plaques. Two weeks later, plaque rupture was induced by pharmacological triggering. The incidence of plaque rupture in all treatment groups (14.3%, 7.1%, 7.7%, and 7.1%) was significantly lower than that in control group (73.3%; $P > 0.01$). TXL dose-dependently lowered serum lipid levels and inhibited systemic inflammation. Corrected acoustic intensity and fibrous cap thickness of the aortic plaques were significantly increased, whereas plaque area, plaque burden, vulnerable index, and expression of oxidized low-density lipoprotein (ox-LDL) receptor 1, matrix metalloproteinase 1 (MMP-1), MMP-3, tissue inhibitor of MMP 1, and NF-κB in plaques were markedly reduced in all treatment groups when compared with the control group. Similar to high-dose simvastatin group, high-dose TXL group exhibited a low serum level of low-density lipoprotein cholesterol and ox-LDL, a low expression level of systemic and local inflammatory factors and a low plaque vulnerability index, with no differences in the incidence of plaque rupture among all treatment groups. TXL dose-dependently enhances the stability of vulnerable plaques and prevents plaques from rupture. Simvastatin and TXL offer similar protection in terms of lipid-lowering, anti-inflammation, and antioxidation effects.

Tongxinluo capsule; atherosclerosis; inflammation

RECENT STUDIES SHOW THAT PLAQUE rupture and subsequent intraluminal thrombosis is the most common cause of acute coronary syndrome (ACS) (3, 10). Early prevention of plaque rupture might be an effective way to reduce the risk of this catastrophic life-threatening event. Although several approaches including angiogenesis and arteriogenesis have been proposed to mitigate myocardial ischemia secondary to ACS (5, 6, 26), an effective strategy to prevent plaque rupture is still lacking. Studies of factors promoting plaque instability and changing plaque characteristics from unstable to stable may help develop a useful preventive strategy.

Pathological studies have demonstrated that vulnerable plaques are characterized by a large lipid core, a thin fibrous cap depleted of extracellular matrix (collagen and proteoglycans) and smooth muscle cells (SMCs), and infiltration by macrophages and T cells with outward positive remodeling and increased plaque vascularity (33). In most patients with ACS, more than one vulnerable plaque is present in their coronary arteries, and thus systemic drug therapy should be the treatment of choice. Unfortunately, an ideal drug for stabilizing vulnerable plaques is still lacking. Although many studies demonstrated the capability of statins in stabilizing vulnerable plaques via their pleiotropic effects (1, 22), the PROVE-IT trial found that 22.4% of enrolled patients experienced an acute coronary event despite an intensive statin therapy for 2 years (30). Moreover, liver dysfunction and myopathy as side effects of statin administration caused some patients to withdraw from statin treatment. Therefore, exploration of new drugs with high efficacy and low side effects for stabilizing vulnerable plaques is clearly warranted.

Pharmaceutics of herbal medicine is undergoing rapid development in China. With the progression of modern technology, more and more herbal compound extracts are being authenticated, standardized, and administered successfully in clinical practice. Tongxinluo (TXL), developed two decades ago, was registered in the State Food and Drug Administration of China for treatment of angina pectoris in 1996. TXL is extracted, concentrated, and freeze-dried from a group of herbal medicine, such as ginseng, radix paeoniae rubra, borneol, and spiny jujuba seed, which contains multiple active components that may be responsible for its antianginal effects. However, it remains unclear whether TXL can stabilize vulnerable plaques dose dependently and what mechanisms are involved. This study was carried out to test the hypothesis that TXL enhances stability of vulnerable plaque dose dependently via lipid-lowering and anti-inflammation effects, similar to a high-dose simvastatin therapy. Since recent clinical trials revealed a better outcome in patients with ACS under intensive statin treatment (7, 13), a high-dose simvastatin was chosen as

* L. Zhang, Y. Liu, and X. T. Lu contributed equally to this work.

Address for reprint requests and other correspondence: Address for Correspondence: Y. Zhang or Y. X. Zhao, Shandong Univ. Qilu Hospital, Jinan, No. 107, Wen Hua Xi Road, Jinan, Shandong, 250012, P.R. China, (e-mail: zhangyun@sdu.edu.cn).

a therapeutic standard to compare with different doses of TXL in a rabbit model of vulnerable plaques and the potential mechanisms involved in the TXL treatment were examined.

MATERIALS AND METHODS

Components and preparation of TXL. TXL powder was provided by Shijiazhuang Yiling Pharmaceutic (Hebei, China). The herbal drugs were authenticated and standardized on marker compounds according to the Chinese Pharmacopoeia 2005. TXL contains 12 medicinal components, which were ground to superfine powder with the diameter $\leqq$10 μm by a micronizer and prepared as capsules. To reduce the dose variability of TXL capsule among different batches, the species, origin, harvest time, medicinal parts, and concocted methods for each component were strictly standardized. Moreover, high performance liquid chromatography (HPLC), high performance capillary electrophoresis, and gas chromatography were applied to quantitate the components of the TXL capsule.

Dosing reproducibility of TXL. To assess the dosing reproducibility of TXL capsule, 10 different batches were randomly selected from all batches produced from January 2008 to July 2009 by Shijiazhuang Yiling Pharmaceutic (Hebei, China), and HPLC was applied to record fingerprint chromatograms of the aqueous extracts of the 10 batches for similarity analysis. Detailed methods and results are described in the supplemental data.

Experimental protocol. A total of 75 male New Zealand White rabbits weighing 1.7–2.1 kg were randomly selected and housed at the Animal Care Center of Shandong University Qilu Hospital. The experiment complied with the Animal Management Rule of the Ministry of Public Health, People's Republic of China (documentation 55, 2001), and the experimental protocol was approved by the Animal Care Committee of Shandong University.

A rabbit model of vulnerable plaques was produced by modification of a previous method reported from our laboratory (8, 9, 45). All rabbits underwent balloon-induced endothelial injury in the abdominal aorta after being anesthetized with 3% pentobarbital sodium (30 mg/kg iv) and were then fed a high-cholesterol diet (1% cholesterol) for 10 wk. Thereafter, the atherogenic diet was replaced with a regular diet, and rabbits were randomly divided into five groups for treatment: control group that received no treatment ($n = 15$), low-dose TXL group that received oral TXL of 0.15 $g \cdot kg^{-1} \cdot day^{-1}$ ($n = 15$), moderate-dose TXL group that received oral TXL of 0.15 $g \cdot kg^{-1} \cdot day^{-1}$ ($n = 15$), high-dose TXL group that received oral TXL of 0.6 $g \cdot kg^{-1} \cdot day^{-1}$ ($n = 15$), and high-dose simvastatin group that received oral simvastatin (Merck, Hangzhou, China) of 5 $mg \cdot kg^{-1} \cdot day^{-1}$ ($n = 15$). At the end of *week 16*, after the largest plaque in the abdominal aorta was identified with intravascular ultrasound imaging (IVUS), an incision was made in the abdomen medially, and the abdominal aortic segment with the largest plaque was isolated. Under IVUS guidance, a 50-μl suspension of adenovirus containing p53 (Ad5-CMV.p53; 8×10^9 plaque-forming units/ml) was injected from the aortic adventitia into the largest plaque in the abdominal aorta in all five groups. The position of the transfected plaque was marked by an iliopsoas stitch, and its distance to the bifurcation of the iliac artery was recorded. Two weeks after transfection, plaque disruption was triggered by intraperitoneal administration of Russell's viper venom (Sigma, St. Louis, MO; 0.15 mg/kg) and, 30 min later, intravenous injection of histamine (0.02 mg/kg). The rabbits were euthanized, and vessels 1 cm long were harvested from the Ad5-CMV.p53-injected segments. Body weight was monitored throughout the experiment.

Biochemical assay. Blood was drawn from rabbits fasting overnight at baseline and at the end of *weeks 10*, *16*, and *18*. Serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were obtained by enzymatic assays with use of an automated biochemical analyzer (Roche Hitachi 917, Japan). At the same time, serum levels of highly sensitive CRP (hs-CRP) were assayed by use of a highly sensitive enzyme-linked immunosorbent assay (ELISA) kit (Adlitteram Diagnostic, San Diego, CA), and serum levels of monocyte chemoattractant protein-1 (MCP-1), soluble intercellular adhesion molecular-1 (sICAM-1), and oxidized LDL cholesterol (ox-LDL) were measured by use of ELISA kits (Adlitteram Diagnostic).

Intravascular ultrasound imaging. IVUS was performed in rabbits under deep anesthesia by use of a commercially available system (Galaxy; Boston Scientific, Boston, MA). The 40-MHz IVUS catheter was advanced into the thoracic aorta through the left femoral artery, and ultrasound images were recorded during automatic pullback of the catheter to the iliofemoral artery at a speed of 0.5 mm/s. Each IVUS study was carried out according to a standard procedure (28). IVUS was performed at the end of *week 16* to guide adventitial injection into the largest aortic plaques and was repeated at the end of *week 18* before pharmacological triggering to measure the external elastic membrane area (EEMA) and the lumen area (LA) at the same site by referring to the recorded distance from the bifurcation of the iliac artery to the adventitial injection. Plaque area (PA) was calculated as PA = EEMA − LA, and plaque burden (PB%) was calculated as PB% = PA/EEMA × 100%.

High-frequency ultrasonography. At the end of *week 18* before pharmacological triggering, a high-frequency duplex ultrasonographic system (HP SONOS 7500) and a 7.5-MHz transducer were used to acquire images of the abdominal aorta from all rabbits starved overnight. The transducer, placed directly on the shaved skin of the rabbit abdomen, was carefully manipulated to image the largest plaque at the level of gene transfection by referring to the recorded distance from the bifurcation of the iliac artery to the adventitial injection. After the aortic longitudinal and transversal axis views were obtained, the aortic diameter at end-diastole (Dd) and the maximal intima-media thickness (IMT) were measured by two-dimensional echocardiography, and the aortic peak velocity (*V*p) was recorded by the pulsed Doppler technique. Thereafter, ultrasound-integrated backscatters from the aortic wall and the atherosclerotic plaques were analyzed by acoustic densitometry. Ultrasound signal intensity (AII) of the aortic intima and adventitia in the largest atherosclerotic plaques was recorded and averaged, and the corrected AII (AII%) was derived by calculating the ratio of AII of the intima to the AII of the adventitia.

Histological and immunohistochemical staining. The gene-transfected segments of the abdominal aorta were removed, fixed overnight in 4% paraformaldehyde, and then cut into two equal segments. One segment, dehydrated and embedded in paraffin, was cut into serial 5-μm cross-sections for general histological staining with hematoxylin & eosin (H&E), picrosirius red, and specific immunohistochemical staining. The other segment was used for cryosections and cut into 6-μm-thick sections for H&E and Oil-red O staining. Immunohistochemical staining involved standard techniques as described previously (36). In brief, endogenous peroxidase activity was inhibited by incubation with 3% H_2O_2. Sections were blocked with 5% goat serum in phosphate-buffered saline (PBS) and incubated overnight at 4°C with primary antibodies. After a PBS wash, the sections were incubated with secondary antibody at 37°C for 30 min. Immunohistochemical staining was visualized by use of a diaminobenzidine kit (Zhongshan Goldenbridge Biotechnology, Beijing, China) according to the manufacturer's instructions. Nucleus counterstaining involved hematoxylin (blue) or methyl green (green). The primary antibodies included monoclonal antibodies against rabbit macrophages (RAM-11; Lab Vision NeoMarkers, Fremont, CA) and α-smooth muscle cell actin (Chemicon, Boston, MA), mouse anti-human lectin-like ox-LDL receptor 1 (LOX-1) (R&D, R&D systems), mouse anti-human matrix metalloproteinase 1 (MMP) (Chemicon), mouse anti-rabbit MMP-3 (Chemicon), mouse anti-human tissue inhibitor of metalloproteinase 1 (TIMP-1; Santa Cruz Biotechnology), and mouse anti-human mouse anti-NF-κB P65 subunit. Monoclonal antibodies were diluted to 1:800, 1:200, 1:50, 1:3,000, 1:800, 1:50, and 1:100 to detect RAM-11, α-actin, LOX-1, MMP-1, MMP-3, TIMP-1, and NF-κB, respectively.

Histological analysis. Slides were scanned by microscopy (Olympus BX51; Olympus, Tokyo, Japan), and histopathological parameters were analyzed by use of a computer-assisted morphometric analysis system (Image-Pro Plus 5.0; Media Cybernetics, Bethesda, MD). The fibrous cap thickness was measured at 10 equidistant points around the cap in each slice; three slices per sample were measured, and values were averaged. The area of positive staining of lipids, collagen, smooth muscle cells (SMCs), macrophages, LOX-1, MMP-1, MMP-3, TIMP-1, and NF-κB was expressed as a percentage of stained area divided by plaque area in at least 10 high-power fields (×400). The vulnerability index was calculated as (macrophage staining % + lipid staining %) / (SMCs % + collagen fiber %) (37). Sections stained with H&E were analyzed for disrupted plaques, histologically defined as fibrous cap discontinuity connected with overlying intraluminal thrombus (34).

Molecular biological studies. Tissue samples were frozen with use of liquid nitrogen. Total RNA was extracted by use of TRIZol reagent (Invitrogen). Total RNA was quantified by spectrophotometry and reverse transcribed with use of the M-MLV Reverse Transcriptase System (Promega, Madison, WI) with oligo(dT) primers. mRNA expression of LOX-1, MCP-1, ICAM-1, MMP-1, MMP-3, and TIMP-1 in plaques was examined by quantitative RT-PCR with use of LightCycler (Roche Applied Science, Indianapolis, IN) following the manufacturer's instructions. The mRNA sequences were obtained from GenBank (Bethesda, MD). Quantitative values were obtained from the threshold cycle value (Ct), the point at which a significant increase in fluorescence was first detected. The transcript number of glyceraldehydes 3-phosphate dehydrogenase was quantified as an internal control. Experiments were performed in triplicate for each data point, and the data were analyzed with the $2^{-\Delta\Delta CT}$ method (25). The results of RT-PCR were confirmed by gel electrophoresis. The primers are in Table 1.

Statistical analysis. All numeric data were expressed as means ± SD and shown by one-sample Kolmogorov-Smirnov test to be in normal distribution. Differences in continuous variables between two groups were assessed by unpaired Student's *t*-test, and comparison among multiple groups involved use of ANOVA. Categorical variables were analyzed by Fisher's exact test. All data analyses were performed with SPSS v13.0 (SPSS, Chicago, IL). A P value of <0.05 was considered statistically significant.

RESULTS

During the experiment, five rabbits died of diarrhea. Data were available for analysis for 15 rabbits in the control group, 14 in low-dose TXL group, 14 in moderate-dose TXL group, 13 in high-dose TXL group, and 14 in high-dose simvastatin group.

Biochemical studies. Serum levels of TC, TG, LDL-C and HDL-C at baseline in all rabbits were 1.91 ± 0.31 mmol/l, 0.55 ± 0.14 mmol/l, 0.96 ± 0.24 mmol/l, and 0.95 ± 0.16 mmol/l, respectively, with no significant differences among the five groups of rabbits. TC, TG, and LDL-C were elevated in all five groups of rabbits after a high cholesterol diet for 10 wk but no significant differences in these levels was noted among the five groups at *week 10* (Fig. 1). In the control group, withdrawal of a high-cholesterol diet alone resulted in significantly reduced serum levels of TC, TG, and LDL-C at *weeks 16* and *18* compared with the corresponding values at *week 10* (all $P < 0.01$), although the changes of the HDL-C levels were insignificant. At the end of *weeks 16* and *18*, TXL administration with low, moderate, and high doses all significantly lowered serum levels of TC, TG, and LDL-C compared with the corresponding values at *week 10* (all $P < 0.01$). The serum levels of TC and LDL-C in high-dose TXL group were lower than those in low-dose and moderate-dose TXL groups (both $P < 0.01$), but did not differ significantly from those in the high-dose simvastatin group. Likewise, the serum levels of TG in moderate-dose and high-dose TXL groups were lower than those in low-dose TXL group (both $P < 0.05$) but was similar to those in high-dose simvastatin group. At the end of *weeks 16* and *18*, the serum HDL-C levels were increased in all treatment groups compared with those in the control group ($P < 0.01 \sim 0.05$), and these effects were more significant in the moderate-dose and high-dose TXL groups than the low-dose TXL group. However, there was no significant difference in the serum levels of HDL between high-dose TXL group and high-dose simvastatin group. Thus TXL lowered serum TC, TG, and LDL-C levels but increased serum HDL-C levels dose-dependently and these therapeutic effects were similar between high-dose TXL group and high-dose simvastatin group.

Serum levels of hs-CRP, MCP-1, sICAM-1, and ox-LDL at baseline in all rabbits were 7.92 ± 2.41 mmol/l, 114.55 ± 23.98 mmol/l, 94.63 ± 13.55 mmol/l, and 1.07 ± 0.34 mmol/l, respectively, with no significant differences among the five groups of rabbits. At the end of *week 10*, the five groups of

Table 1. *Primers for RT-PCR*

Molecules	MW	Locus	Primer Sequence	T_m, °C
GAPDH	475	NM001082253	S: GAGCTGAACGGGAAACTCAC A: GGTCTGGGATGGAAACTGTG	62
LOX-1	218	NM_001082633	S: TGGAAATGGCTGTTGACGAC A: CTTGCTGTTGCTTTAGGAGGT	62
ICAM-1	178	AB128157	S: GGCGGCTCAGTGTCTCATTCCC A: GTCCTCGGCTTCTGCCACCATC	62
MCP-1	251	NM001082294	S: CAGCCAGATGCCGTGAA A: TTGGGTTGTGGAATAAGAGGT	54
MMP-1	177	NM_001082793	S: GAGGAGGAGACGGAGGTGAT A: GGGAACGCTGGCAGTAGAG	62
MMP-3	258	NM_001082280	S: CGCTTTGCTCAGCCTATCCAC A: CCAACATCAGGAACGCCACA	70
TIMP-1	96	AY829731	S: AGGATTTGACGCCTTGGGG A: GCGGTTCTGGGATTTGTGG	60

LOX-1, lectin-like ox-LDL receptor 1; ICAM-1, intercellular adhesion molecular-1; MCP-1, monocyte chemoattractant protein-1; MMP-1, matrix metalloproteinase 1; MMP-3, matrix metalloproteinase 3; TIMP-1, tissue inhibitor of metalloproteinase 1; MW, molecular weight; T_m, melting temperature.

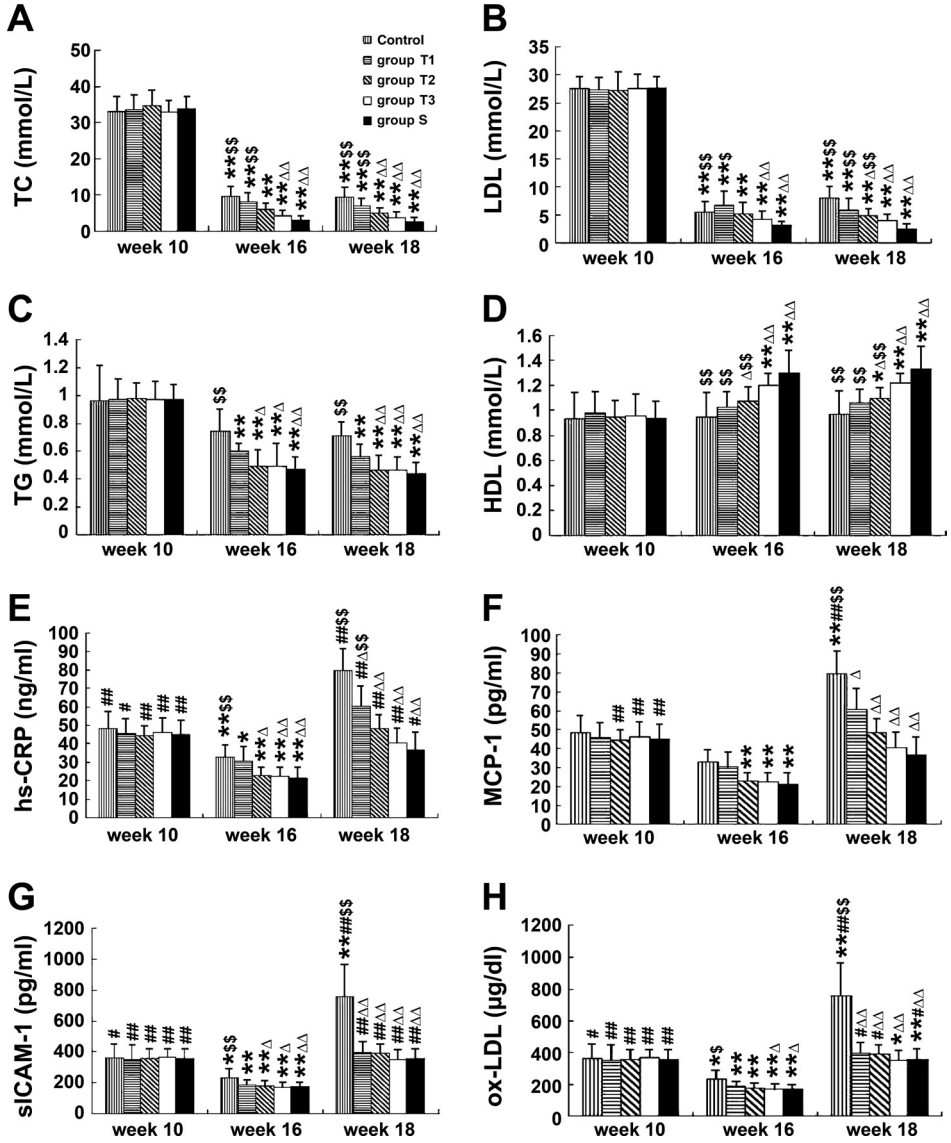

Fig. 1. Biochemical measurements in 5 groups of rabbits. Control, control group; T1, low-dose Tongxinlo (TXL) group; T2, moderate-dose TXL group; T3, high-dose TXL group; S, high-dose simvastatin group. *A–H*: serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), highly sensitive CRP (hs-CRP), monocyte chemoattractant protein-1 (MCP-1), soluble intercellular adhesion molecular-1 (sICAM-1), and oxidized LDL cholesterol (ox-LDL) in 5 groups at *weeks 10*, *16*, and *18*, respectively. $*P < 0.05$, $**P < 0.01$ vs. *week 10*; $\#P < 0.05$, $\#\#P < 0.01$ vs. *week 16*; $\Delta P < 0.05$, $\Delta\Delta P < 0.01$ vs. control group; $\$P < 0.01$, $\$\$P < 0.01$ vs. simvastatin group.

rabbits did not differ significantly in serum levels of hs-CRP, MCP-1, sICAM-1, and ox-LDL (Fig. 1). However, at *week 18*, these inflammatory marker levels were significantly lower in all treatment groups than in the control group ($P < 0.01 \sim 0.05$). The serum levels of hs-CRP and MCP-1 were lower in the moderate-dose and high-dose TXL groups than in the low-dose TXL group (both $P < 0.01$) but were similar to those in the high-dose simvastatin group. In contrast, the serum levels of sICAM-1 and ox-LDL did not differ significantly among all treatment groups at *week 18*. Thus TXL lowered serum hs-CRP and MCP-1 levels dose dependently, whereas a low dose of TXL was as effective as a high dose of TXL in lowering serum sICAM-1 and ox-LDL levels.

High-frequency ultrasonography. The values of IMT, Dd, and *V*p did not differ among all five groups at *week 18* (Table 2). In contrast, values of AIIc% were significantly higher in all treatment groups than in the control group ($P < 0.01 \sim 0.05$), with no difference in AIIc% among four treatment groups.

IVUS measurements. LA did not differ significantly among all five groups (Table 3). However, EEMA, PA, and PB% in the four treatment groups were significantly lower than those in the control group ($P < 0.01 \sim 0.05$), with no difference among four treatment groups.

Incidence of plaque rupture. Histological analysis indicated that after pharmacological triggering, of 15 rabbits in the

Table 2. *Ultrasonographic measurements in five groups at week 18*

Groups	IMT, mm	*D*, cm	*V*p, cm/s	AIIc, %
Control	0.11±0.02	0.34±0.06	116.8±18.38	65.14±5.25
TXL				
Low dose	0.10±0.02	0.30±0.04	108.11±16.78	74.38±5.57*
Moderate dose	0.11±0.01	0.30±0.07	113.51±21.73	75.60±6.55*
High dose	0.11±0.02	0.29±0.07	106.10±20.69	76.83±5.29*
High-dose simvastatin	0.10±0.02	0.31±0.05	114.98±17.99	77.62±4.58*

Values are means ± SD. TXL, Tongxinluo; IMT, maximal intima-media thickness; *D*, aortic diameter at end diastole; *V*p, aortic peak velocity; AIIc, corrected ultrasound intensity of the aortic intima and adventitia. $*P < 0.01$ vs. control group.

Table 3. *Intravascular ultrasound measurements in five groups at week 18*

Groups	LA, mm²	EEMA, mm²	PA, mm²	PB, %
Control	6.50±0.61	10.18±1.31	3.69±1.43	0.35±0.11
TXL				
Low dose	6.32±0.89	8.94±1.08†	2.62±0.73†	0.29±0.07*
Moderate dose	6.24±0.73	8.77±0.99†	2.53±0.84†	0.28±0.08*
High dose	6.18±0.89	8.56±0.98†	2.37±0.67†	0.28±0.06*
High-dose simvastatin	6.70±0.79	8.82±1.07†	2.12±0.66†	0.24±0.06†

Values are means ± SD. LA, lumen area; EEMA, external elastic membrane area; PA, plaque area; PB, plaque burden. *$P < 0.05$; †$P < 0.01$ vs. control group.

control group, 11 showed plaque rupture (73.3%) compared with only two of 14 rabbits in low-dose TXL group (14.3%), one of 14 rabbits in moderate-dose TXL group (7.1%), one of 13 rabbits in high-dose TXL group (7.7%), and one of 14 rabbits in high-dose simvastatin group (7.1%) (all $P < 0.01$). In contrast, no significant difference in plaque rupture rates was found among four treatment groups (Fig. 2).

Histological and immunohistochemical staining. When compared with that of the control group, fibrous cap thickness was significantly increased in four treatment groups (all $P < 0.01$; Table 4). However, the fibrous cap thickness was higher in the high-dose TXL group than that in the low- and moderate-dose TXL group but was similar to that in the high-dose simvastatin group ($P < 0.01 \sim 0.05$). Thus TXL dose-dependently increased the thickness of fibrous cap.

As revealed by RAM11 immunohistochemistry, the relative content of macrophages in plaques was higher in the control group than that in four treatment groups ($P < 0.01 \sim 0.05$), with no significant difference detected among the four treatment groups (Fig. 3 and Table 4). The plaque content of lipids in the control group was higher than that in high-dose TXL and simvastatin groups (both $P < 0.01$) but did not differ from that in the low- and moderate-dose TXL groups (Fig. 4 and Table 4). The plaque content of SMCs was lower in the control group than that in all four treatment groups ($P < 0.01 \sim 0.05$) and was lower in the low-dose TXL group than that in high-dose TXL and simvastatin groups ($P < 0.05$; Fig. 4 and Table 4). The plaque content of collagen was lower in the control group than that in four treatment groups (all $P < 0.01$) and was lower in the low-dose TXL groups than in the high-dose TXL group ($P < 0.05$; Fig. 3 and Table 4). As a result, the vulnerability index in the control group was lower than that in all four treatment groups (all $P < 0.01$) and was lower in the low-dose TXL group than in the high-dose TXL group ($P < 0.05$; Table 4). However, this parameter did not show significant difference between high-dose TXL and simvastatin groups (Table 4).

Similar to macrophage staining, immunostaining showed that the expression levels of LOX-1, MMP-1, MMP-3, and NF-κB were lower in plaques of all treatment groups than in the control group (all $P < 0.01$; Fig. 4 and Fig. 5). TXL dose-dependently lowered the protein expression levels of LOX-1, MMP-1, MMP-3, and NF-κB, which were similar in high-dose TXL group and high-dose simvastatin group. In contrast, the protein expression levels of TIMP-1 were higher in all treatment groups than in the control group but did not differ among three TXL groups (all $P < 0.01$; Fig. 4 and Fig. 6).

mRNA expression of the target genes. The mRNA expression levels of MCP-1 and LOX-1 were significantly higher in the control group than in all four treatment groups (all $P < 0.01$; Fig. 5) but not significantly different among the four treatment groups. The mRNA expression levels of ICAM-1 were higher in the control and low-dose TXL groups than in the moderate-dose TXL, high-dose TXL, and high-dose simvastatin groups (all $P < 0.01$; Fig. 5). The mRNA expression levels of MMP-1 and MMP-3 were higher in the control group than in all four treatment groups ($P < 0.01 \sim 0.05$) and were lower in high-dose TXL and simvastatin groups than that in low- and moderate-dose TXL groups ($P < 0.01 \sim 0.05$). In contrast, TXL dose-dependently increased the mRNA expression levels of TIMP-1 ($P < 0.01 \sim 0.05$), with the level in high-dose TXL group being similar to that in high-dose simvastatin group (Fig. 5).

DISCUSSION

The major finding of the present study was that in an established rabbit model of vulnerable plaques, TXL dose-dependently lowered serum lipid levels and effectively inhibited local and systemic inflammation. Corrected acoustic intensity and fibrous cap thickness of the aortic plaques were significantly increased, whereas plaque area, plaque burden, plaque vulnerability index, and expression levels of LOX-1, MMP-1, MMP-3, TIMP-1, and NF-κB in plaques were markedly reduced in all treatment groups compared with the control group. Similar to high-dose simvastatin group, high-dose TXL group exhibited a low serum level of LDL-C and ox-LDL, a low expression level of systemic and local inflammatory factors, and a low plaque vulnerability index, with no differences in the incidence of plaque rupture among all treatment groups. To the best of our knowledge, our study is the first to show that TXL dose-dependently enhances the stability of vulnerable plaques and that simvastatin and TXL offer similar protection in terms of lipid-lowering, anti-inflammation, and antioxidation effects.

Recent clinical trials demonstrated that when compared with moderate-dose statin therapy, high-dose statin therapy reduced cardiovascular mortality and other clinical endpoints to a greater extent. In the A-Z trial involving 4,500 patients with acute coronary syndrome, 80 mg simvastatin, which is the

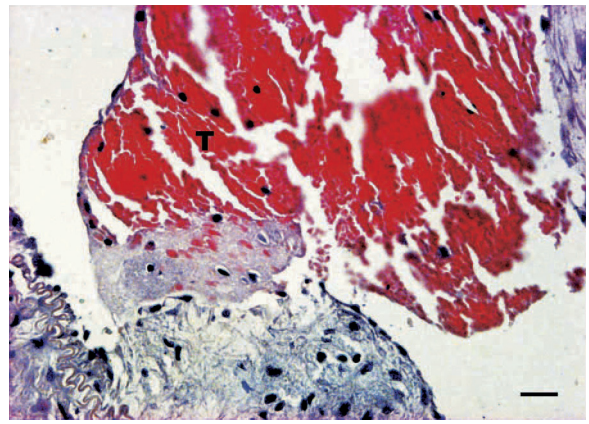

Fig. 2. Hematoxylin & eosin staining of an intraluminal thrombus overlying a fissured plaque (scale bar = 50 μm). Scattered blue staining by hematoxylin represents nuclei. T, thrombus.

Table 4. *Pathological measurements in five groups at week 18*

Groups	Macrophages, %	Lipids, %	SMCs, %	Collagen, %	Vulnerability Index	Fibrous Cap Thickness, μm
Control	21.96±5.87[d]	36.22±4.30[d]	10.83±1.67[d,e]	13.37±2.36[d,f]	2.47±0.52[d,f]	71.26±7.88[d]
T1	16.48±2.99[a]	32.75±5.48[d]	14.44±3.30[a,e]	19.21±3.91[b,d]	1.53±0.43[b,d]	101.33±13.18[b,d]
T2	15.29±3.04[b]	30.54±6.76	16.13±3.57[b]	18.99±2.84[b,d]	1.33±0.30[b,d]	105.91±10.74[b,c]
T3	15.46±1.87[b]	27.46±5.57[b]	20.05±5.50[b,e]	22.08±6.05[b,d,e]	1.08±0.30[b,e]	111.18±11.53[b,e]
S	13.39±2.73[b]	25.14±1.81[b,f]	21.71±6.41[b,e]	26.74±4.04[b,f]	0.82±0.13[b,f]	114.34±9.13[b,f]

Values are means ± SD. SMCs, smooth muscle cells; control, control group; T1, low-dose TXL group; T2, moderate-dose TXL group; T3, high-dose TXL group; S, high-dose simvastatin group. [a]$P < 0.05$, [b]$P < 0.01$ vs. control group; [c]$P < 0.05$, [d]$P < 0.01$ vs. high-dose simvastatin group; [e]$P < 0.05$, [f]$P < 0.01$ vs. low-dose TXL group.

highest recommended dose for clinical application, was superior to a routine dose of 40 mg simvastatin in reducing the cardiovascular events (38). A meta-analysis of high-dose statin trials including the TNT, IDEAL, PROVE-IT, and A-Z trials demonstrated a 16% reduction in cardiovascular death or myocardial infarction with high-dose statin therapy compared with moderate-dose statin therapy (4, 29).The mechanisms underlying these therapeutic effects involve so-called pleiotropic effects (29) of statins including lipid lowering, anti-inflammation, and anti-oxidation effects, which may contribute to enhanced plaque stability in these patients. For these reasons, a high-dose simvastatin was chosen as a positive control therapy for stabilization of vulnerable plaques in this study.

TXL, derived from a group of herbal medicine including ginseng, radix paeoniae rubra, borneol, and spiny jujuba seed, has been used to treat patients with angina pectoris for more than one decade in clinical practice (40). Recent studies have demonstrated that TXL has pleiotropic effects including improvement of endothelial function, lipid lowering, antioxidation, vasodilatation, antithrombosis, anti-inflammation, anti-apoptosis, and enhancement of angiogenesis (21, 24, 39, 44), which may be due to the cumulative or synergistic effects of multiple compounds present in the herbal extract. For example, ginseng is the major ingredient of TXL and contains a group of triterpene glycosides called ginsenosides. It has been reported that ginsenoside Rb1 effectively blocks homocysteine-induced endothelial dysfunction, superoxide anion production, and endothelial nitric oxide synthase downregulation in porcine coronary arteries (46). Similarly, ginsenoside Rb2 is able to lower levels of TC and TG in 3T3-L1 adipocytes treated with high amounts of cholesterol and fetal bovine serum, which is mediated by stimulating the expression of the sterol regulated element binding protein and leptin mRNA (17). Several studies found that paeonia has antioxidative, vasodilatory, antiplatele, lipid-lowering, and anti-inflammatory capacities (12, 20, 41). Spiny jujuba seed showed potent immunological adjuvant activity (27) and lipid-lowering effects (42). All these pharmacological effects may contribute to the antianginal efficacy of TXL in clinical practice. Nonetheless, it remains unclear whether TXL can stabilize vulnerable plaques dose dependently and what mechanisms are involved. In published experimental studies in rabbits, the highest dose of simvastatin was 5 $mg \cdot kg^{-1} \cdot day^{-1}$ (14), which corresponds to a daily dose of 17.5 mg in rabbits with an average body weight of 3.5 kg after high-cholesterol feeding. Therefore, the ratio of the highest clinical dose (80 mg) to the highest experimental dose (17.5 mg) of simvastatin is 4.57, which was then used to define the ratio of the clinical dose to the experimental dose of TXL. The routine clinical dose range of TXL capsule (2–4 capsules, 3 times per day) approximates to 2.40–4.80g/day of TXL powder, which may correspond to the dose range of 0.15–0.3 $g \cdot kg^{-1} \cdot day^{-1}$ in rabbits with an average body weight of 3.5 kg by taking account of the ratio of 4.57. The highest dose of 0.6 $g \cdot kg^{-1} \cdot day^{-1}$ used in this study corresponds to 9.60 g/day of TXL powder, a dose twice as high as the routine dose of 4.80 g/day for patients with angina pectoris, similar to the relation between 80 mg and 40 mg simvastatin. Using three different doses, we found that TXL capsule dose-dependently increased the thickness of fibrous caps and plaque contents of SMCs and collagen and reduced the serum levels of TC, TG, LDL-C, HDL-C, hs-CRP, and MCP-1; protein expression levels of LOX-1, MMP-1, MMP-3, and NF-κB in plaques; and mRNA expression levels of MMP-1 and MMP-3 in plaques as well as vulnerability index. TXL treatment also dose-independently increased AIIc% and decreased the serum levels of sICAM-1 and ox-LDL, EEMA, PA, PB%, plaque rupture rate, and plaque contents of macrophages and lipids, as well as mRNA expression levels of MCP-1 and LOX-1, because for these effects, a low dose of TXL was as effective as a high dose of TXL. These results indicate that TXL capsule has three major effects on atherosclerosis: anti-inflammation, antioxidation, and lipid-lowering, and the first two seem to be more significant than the last one.

The risk of plaque rupture is related to both intrinsic plaque vulnerability and extrinsic hemodynamic triggers (7, 11). Histological characteristics of plaques vulnerable to rupture include an atrophic fibrous cap, a lipid-rich necrotic core, an accumulation of inflammatory cells, and imbalance between extracellular matrix synthesis and degradation (3, 10). Any therapeutic strategies aimed at stabilizing vulnerable plaque should be able to reduce the incidence of plaque rupture. In this study, plaque instability and rupture were induced in rabbits by a high-cholesterol diet, balloon-induced endothelial injury, intraplaque injection of p53, and pharmacological triggering, and the major mechanisms involved in this animal model are apoptosis of SMCs and apoptosis-enhanced inflammation (8, 9, 23, 43). Our results showed that a high rate of plaque rupture (73.3%) was induced in the control group, which was similar to a previous report from our laboratory (8, 43), whereas a high dose of simvastatin reduced the plaque rupture rate by 90% (7.1%). It is interesting that the moderate-dose TXL group, high-dose TXL group, and high-dose simvastatin group exhibited a similar low rate of plaque rupture (7.1%-7.7%), suggesting that moderate-dose TXL capsule is as effective as high-dose simvastatin in reducing the rate of plaque rupture in these animals.

In the present study, a high cholesterol diet for 10 wk significantly increased, whereas resuming a normal diet for 6

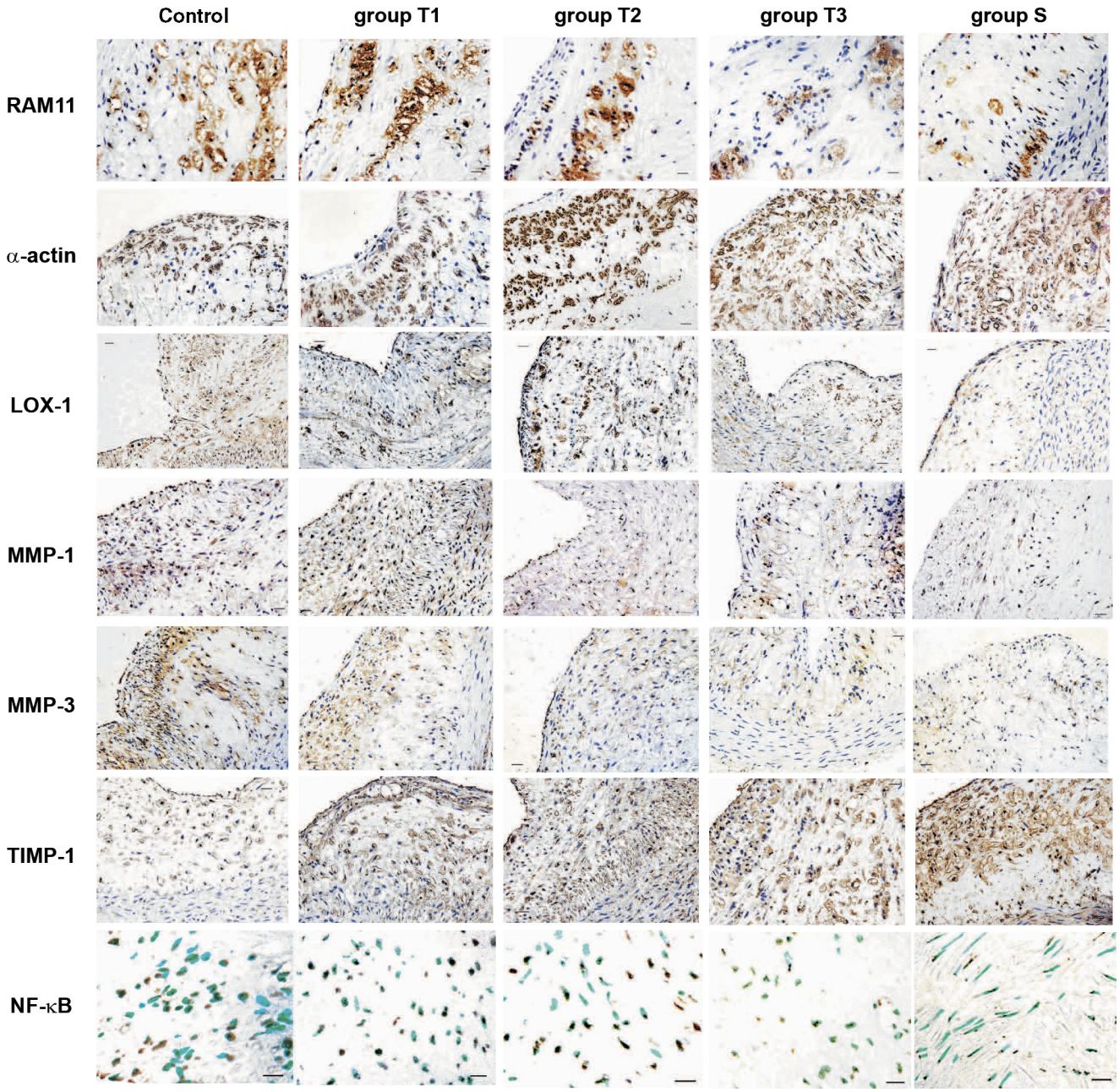

Fig. 3. Immunohistochemical staining for macrophages (RAM11), α-actin, lectin-like ox-LDL receptor 1 (LOX-1), MMP-1, MMP-3, tissue inhibitor of MMP 1 (TIMP-1), and NF-κB in 5 groups of rabbits. Control, control group; T1, low-dose Tongxinluo (TXL) group; T2, moderate-dose TXL group; T3, high-dose TXL group; S, high-dose simvastatin group. From *top* to *bottom*, brown staining represents macrophages (scale bar = 100 μm), α-actin (scale bar = 100 μm), LOX-1 (scale bar = 100 μm), MMP-1 (scale bar = 100 μm), MMP-3 (scale bar = 100 μm), TIMP-1 (scale bar = 100 μm), and NF-κB (scale bar = 50 μm), respectively. Blue staining by hematoxylin or green staining by methyl green indicates nuclei.

wk significantly decreased, the serum levels of TC, TG, and LDL-C in rabbits. Nevertheless, administration of a high-dose simvastatin for 8 wk significantly reduced the serum levels of TC, TG, and LDL-C to a lower level. When compared with the serum LDL-C levels in the control group, TXL treatment with small, moderate, and high doses lowered serum LDL-C levels by 26.7%, 38.3%, and 53.1, respectively. Although a low dose of TXL was less effective in lipid lowering than higher doses of TXL, the high-dose TXL group was as effective as the high-dose simvastatin group. Thus TXL capsule offers an effective drug for lipid lowering although such an effect is dose dependent.

Apart from their typical lipid-lowering effects, statins have been recognized to have anti-inflammation and antioxidation properties. Among many inflammatory factors, transcription factor NF-κB is known to regulate a wide variety of genes, including those controlling LOX-1 and MMP expression (15). Ox-LDL plays a key role in the accumulation of foam cells in atherogenesis, and LOX-1, expressed in endothelial cells, macrophages, and SMCs in the intima of atherosclerotic lesions

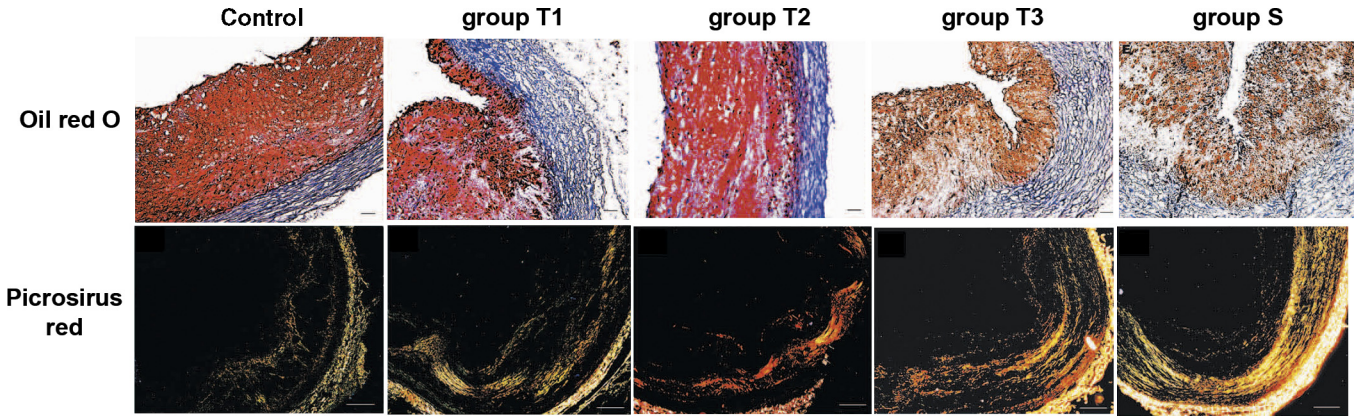

Fig. 4. Oil-red O and picrosirius-red staining in 5 groups of rabbits. The lipid content of plaques was depicted by Oil-red O staining (scale bars = 100 μm), and the collagen content of plaques was exhibited by picrosirius red staining visualized under polarized light (scale bars = 200 μm). Red staining by Oil-red O denotes lipids, and blue staining by hematoxylin depicts nuclei. Green, yellow, and red staining by picrosirius red indicates different collagen fibers viewed under polarized light.

(16, 19), is one of the scavenger receptors mediating cellular uptake of ox-LDL.

Recent studies have shown that statins reduce macrophage growth within atherosclerotic plaques, decrease superoxide production, and increase endothelial nitric oxide production (35). Clinical studies have demonstrated that patients exhibiting lower serum levels of hs-CRP after statin therapy have better clinical outcomes than those with higher CRP levels (2, 31, 32). In this study, both high-dose simvastatin and high-dose TXL markedly lowered the serum levels of hs-CRP and MCP-1 and local expression levels of LOX-1, MMP-1, MMP-3, and NF-κB to a similar extent. However, a low dose

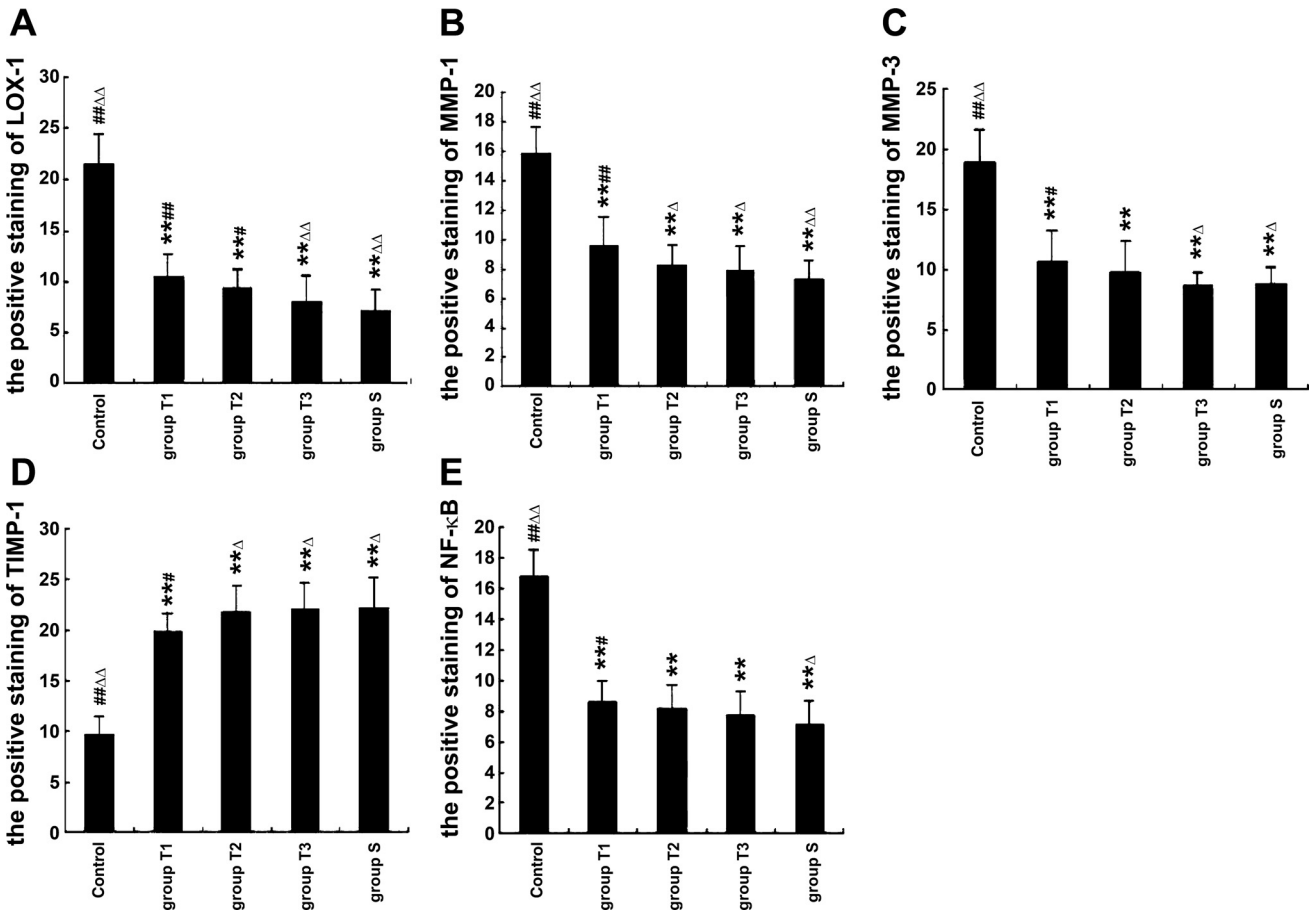

Fig. 5. The relative protein expression in plaques of 5 groups of rabbits. The protein expression levels of LOX-1 (*A*), MMP-1 (*B*), MMP-3 (*C*), TIMP-1 (*D*), and NF-κB (*E*) were shown. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group; $\#P < 0.05$, $\#\#P < 0.01$ vs. high-dose simvastatin group; $\Delta P < 0.0$, $\Delta\Delta P < 0.01$ vs. low-dose TXL group.

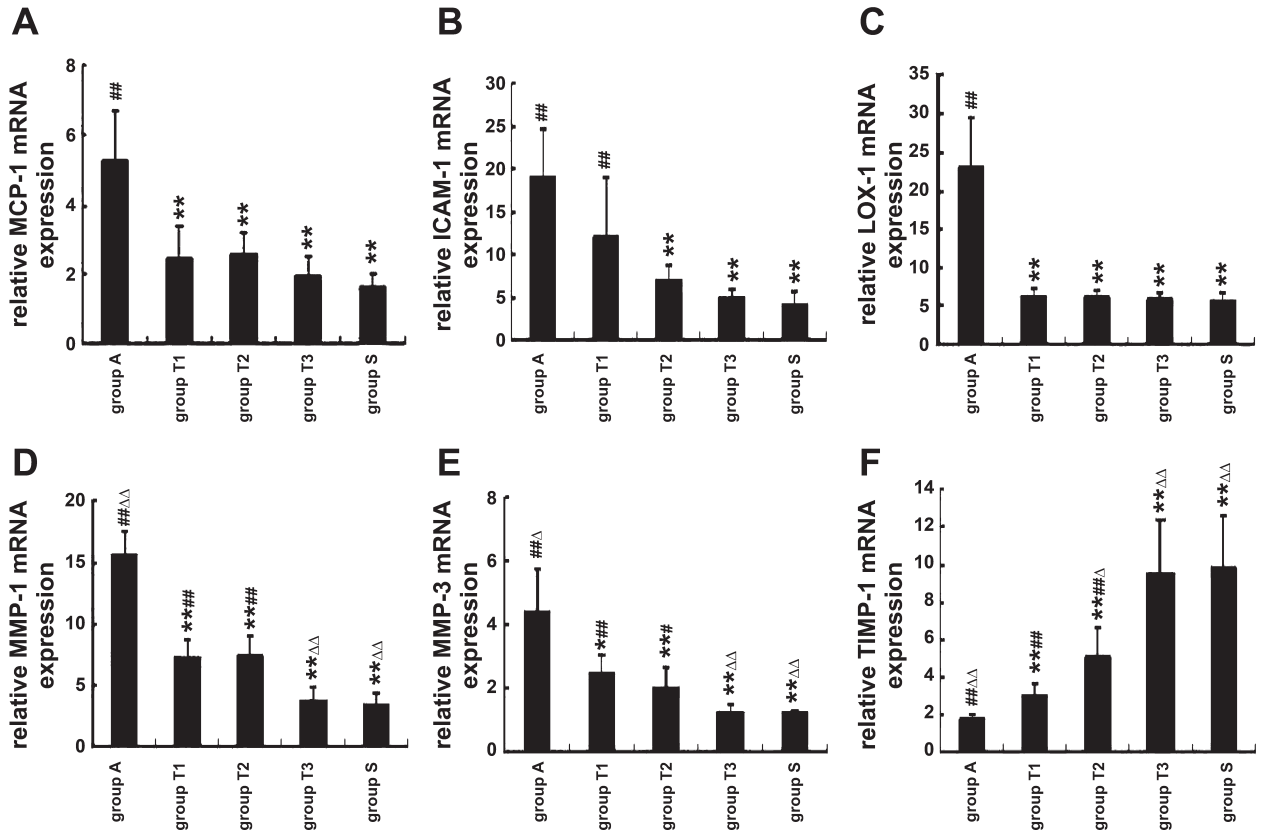

Fig. 6. The relative mRNA expression in plaques of 5 groups of rabbits. The mRNA expression levels of LOX-1 (*A*), MCP-1 (*B*), ICAM-1 (*C*), MMP-1 (*D*), MMP-3 (*E*), and TIMP-1 (*F*) were shown. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control group; $\#P < 0.05$, $\#\#P < 0.01$ vs. high-dose simvastatin group; $\Delta P < 0.05$, $\Delta\Delta P < 0.01$ vs. low-dose TXL group.

of TXL was also found to be as effective as high-dose simvastatin in reducing the serum levels of sICAM-1 and ox-LDL, plaque contents of macrophages, and local expression levels of MCP-1 and LOX-1, indicating that TXL is a potent drug for anti-inflammation and antioxidation. In an in vitro study, Zhang et al. (44) found that TXL extract protected endothelial cells from palmitic acid-induced injury, which was likely mediated by boosting intracellular antioxidant capacity through AMPK pathway. A previous study showed that TXL treatment reduced the plasma concentration of oxidized LDL and attenuated the formation of aortic atherosclerotic lesions (39). In a rabbit model of atherosclerosis, Li et al. also demonstrated that TXL treatment was similar to a small dose of simvastatin (2 $mg \cdot kg^{-1} \cdot day^{-1}$) in stabilizing atherosclerotic plaques. However, rabbits in this study had only moderate hyperlipidemia, the treatment course was short (4 wk), and the incidence of plaque rupture was not reported (21).

A head-to-head comparison between simvastatin and TXL capsule in this study revealed that both drugs had similar pleiotropic effects including lipid lowering, anti-inflammation, and antioxidation. Although high-dose TXL and high-dose simvastatin had similar lipid-lowering effects, low-dose TXL was as effective as high-dose simvastatin in attenuating systemic and local inflammation, indicating that TXL is more potent than simvastatin for anti-inflammation. These pharmacological properties make TXL an effective drug in preventing plaque rupture, even at a low dose. With respect to the side effects of the two drugs, statin administration is well known to result in liver dysfunction and myolysis in some patients, whereas TXL treatment has shown no noticeable side effects except for mild stomach discomfort as reported in pharmacokinetic studies and clinical observations. Although a few patients treated with a maintaining dose of warfarin may exhibit decreased international normalized ratio after ginseng administration, no adverse effects of bleeding or troublesome drug interaction was reported after administration of TXL. In developing countries with a high prevalence of type B hepatitis, clinical administration of statins is often limited by liver dysfunction in patients, and TXL capsule may provide a potential therapeutic alternative. Furthermore, since both TXL capsule and simvastatin have strong anti-inflammation and lipid-lowering effects, the combined application of the two drugs may offer a great potential for enhanced plaque stabilization.

Our study contains several limitations. First, although components of TXL capsule were clear, the dose-effect relationship of these components and their interactions remain to be clarified. Second, although lipid-lowering, anti-inflammation, antioxidation, and plaque stabilization effects of TXL have been clarified, the detailed molecular mechanisms underlying these effects require further investigation. Third, the potential additive effects of simvastatin and TXL on plaque stabilization need to be tested.

In conclusion, both simvastatin and TXL enhance the stability of plaque and prevent plaque rupture in rabbits. Lipid lowering, anti-inflammation and anti-oxidation are the major

mechanisms underlying the beneficial effects of simvastatin and TXL.

GRANTS

This study was supported by the National 973 Basic Research Program of China (No. 2005CB523301), the National High-tech Research and Development Program of China (No.2006AA02A406), the Program of Introducing Talents of Discipline to Universities (No.B07035), the State Key Program of National Natural Science of China (No. 60831003), a grant from National Natural Science of China (No. 60971023), and a grant of Natural Science Foundation of Shandong Province (No. Y2007C064).

DISCLOSURES

No conflicts of interest are declared by the authors.

REFERENCES

1. **Aikawa M, Rabkin E, Okada Y, Voglic SJ, Clinton SK, Brinckerhoff CE, Sukhova GK, Libby P.** Lipid lowering by diet reduces matrix metalloproteinase activity and increases collagen content of rabbit atheroma: a potential mechanism of lesion stabilization. *Circulation* 97: 2433–2444, 1998.
2. **Albert MA, Danielson E, Rifai N, Ridker PM.** Effect of statin therapy on C-reactive protein levels: the Pravastatin Inflammation/CRP Evaluation (PRINCE): a randomized trial and cohort study. *JAMA* 286: 64–70, 2001.
3. **Burke AP, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R.** Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. *N Engl J Med* 336: 1276–1282, 1997.
4. **Cannon CP, Steinberg BA, Murphy SA, Mega JL, Braunwald E.** Meta-analysis of cardiovascular outcomes trials comparing intensive versus moderate dose statin therapy. *J Am Coll Cardiol* 48: 438–445, 2006.
5. **Cao R, Bråkenhielm E, Pawliuk R, Wariaro D, Post MJ, Wahlberg E, Leboulch P, Cao Y.** Angiogenic synergism, vascular stability and improvement of hind-limb ischemia by a combination of PDGF-BB and FGF-2. *Nat Med* 9: 604–613, 2003.
6. **Cao Y, Hong A, Schulten H, Post MJ.** Update on therapeutic neovascularization. *Cardiovasc Res* 65: 639–648, 2005.
7. **Chan PS, Nallamothu BK, Gurm HS, Hayward RA, Vijan S.** Incremental benefit and cost-effectiveness of high-dose statin therapy in high-risk patients with coronary artery disease. *Circulation* 115: 2398–2409, 2007.
8. **Chen WQ, Zhang L, Liu YF, Chen L, Ji XP, Zhang M, Zhao YX, Yao GH, Zhang C, Wang XL, Zhang Y.** Prediction of atherosclerotic plaque ruptures with high-frequency ultrasound imaging and serum inflammatory markers. *Am J Physiol Heart Circ Physiol* 293: H2836–H2844, 2007.
9. **Chen WQ, Zhang Y, Zhang M, Ji XP, Yin Y, Zhu YF.** Establishing an animal model of unstable atherosclerotic plaques. *Chin Med J (Engl)* 117: 1293–1298, 2004.
10. **Davies MJ.** The pathophysiology of acute coronary syndromes. *Heart* 83: 361- 366, 2000.
11. **de Korte CL, Pasterkamp G, van der Steen AF, Woutman HA, Bom N.** Characterization of plaque components with intravascular ultrasound elastography in human femoral and coronary arteries in vitro. *Circulation* 102: 617–623, 2000.
12. **Ghayur MN, Gilani AH, Rasheed H, Khan A, Iqbal Z, Ismail M, Saeed SA, Janssen LJ.** Cardiovascular and airway relaxant activities of peony root extract. *Can J Physiol Pharmacol* 86: 793–803, 2008.
13. **Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr, Stone NJ.** Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation* 110: 227–239, 2004.
14. **Hernández-Presa MA, Ortego M, Tuñón J, Martín-Ventura JL, Mas S, Blanco-Colio LM, Aparicio C, Ortega L, Gómez-Gerique J, Vivanco F, Egido J.** Simvastatin reduces NF-kappaB activity in peripheral mononuclear and in plaque cells of rabbit atheroma more markedly than lipid lowering diet. *Cardiovasc Res* 57: 168–177, 2003.
15. **Hofnagel O, Luechtenborg B, Eschert H, Weissen-Plenz G, Severs NJ, Robenek H.** Pravastatin inhibits expression of lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) in Watanabe heritable hyperlipidemic rabbits: a new pleiotropic effect of statins. *Arterioscler Thromb Vasc Biol* 26: 604–610, 2006.
16. **Kataoka H, Kume N, Miyamoto S, Minami M, Moriwaki H, Murase T, Sawamura T, Masaki T, Hashimoto N, Kita T.** Expression of lectin-like oxidized low-density lipoprotein receptor-1 in human atherosclerotic lesions. *Circulation* 99: 3110–3117, 1999.
17. **Kim EJ, Lee HI, Chung KJ, Noh YH, Ro Y, Koo JH.** The ginsenoside-Rb2 lowers cholesterol and triacylglycerol levels in 3T3-L1 adipocytes cultured under high cholesterol or fatty acids conditions. *BMB Rep* 42: 194–199, 2009.
18. **Kong WJ, Zhao YL, Xiao XH, Jin C, Li ZL.** Quantitative and chemical fingerprint analysis for quality control of Rhizoma Coptidischinensis based on UPLC-PAD combined with chemometrics methods. *Phytomedicine* 16: 950–959, 2009.
19. **Li DY, Chen HJ, Mehta JL.** Statins inhibit oxidized-LDL-mediated LOX-1 expression, uptake of oxidized-LDL and reduction in PKB phosphorylation. *Cardiovasc Res* 52: 130–135, 2001.
20. **Li H, Dai M, Jia W.** Paeonol attenuates high-fat-diet-induced atherosclerosis in rabbits by anti-inflammatory activity. *Planta Med* 75: 7–11, 2009.
21. **Li Z, Yang YJ, Qin XW, Ruan YM, Chen X, Meng L, Zhang HD.** Effects of tongxinluo and simvastatin on the stabilization of vulnerable atherosclerotic plaques of aorta in aortic atherosclerosis and molecular mechanism thereof: a comparative study with rabbits. *Zhonghua Yi Xue Za Zhi* 86: 3146–3150, 2006.
22. **Libby P, Aikawa M.** Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. *Nat Med* 8: 1257–1262, 2002.
23. **Lin HL, Xu XS, Lu HX, Zhang L, Li CJ, Tang MX, Sun HW, Liu Y, Zhang Y.** Pathological mechanisms and dose dependency of erythrocyte-induced vulnerability of atherosclerotic plaques. *J Mol Cell Cardiol* 43: 272–280, 2007.
24. **Liu JX, Shang XH, Wang G.** Effect of tongxinluo capsule on experimental myocardial ischemia, arrhythmia and hyperlipidemia. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 17: 425–428, 1997.
25. **Livak KJ, Schmittgen TD.** Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C (T)) Method. *Methods* 25: 402–408, 2001.
26. **Lu H, Xu X, Zhang M, Cao R, Bråkenhielm E, Li C, Lin H, Yao G, Sun H, Qi L, Tang M, Dai H, Zhang Y, Su R, Bi Y, Zhang Y, Cao Y.** Combinatorial protein therapy of angiogenic and arteriogenic factors remarkably improves collaterogenesis and cardiac function in pigs. *Proc Natl Acad Sci USA* 104: 12140–12145, 2007.
27. **Matsuda H, Murakami T, Ikebata A, Yamahara J, Yoshikawa M.** Bioactive saponins and glycosides. XIV. Structure elucidation and immunological adjuvant activity of novel protojujubogenin type triterpene bisdesmosides, protojujubosides A, B, and B1, from the seeds of Zizyphus jujuba var spinosa (Zizyphi Spinosi Semen). *Chem Pharm Bull (Tokyo)* 47: 1744–1748, 1999.
28. **Mintz GS, Nissen SE, Anderson WD, Bailey SR, Erbel R, Fitzgerald PJ, Pinto FJ, Rosenfield K, Siegel RJ, Tuzcu EM, Yock PG.** American College of Cardiology Clinical Expert Consensus Document on Standards for Acquisition, Measurement and Reporting of Intravascular Ultrasound Studies (IVUS). A report of the American College of Cardiology Task Force on Clinical Expert Consensus Documents. *J Am Coll Cardiol* 37: 1478–1492, 2001.
29. **Patel TN, Shishehbor MH, Bhatt DL.** A review of high-dose statin therapy: targeting cholesterol and inflammation in atherosclerosis. *Eur Heart J* 28: 664–672, 2007.
30. **Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA, Braunwald E; Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) Investigators.** C-reactive protein levels and outcomes after statin therapy. *N Engl J Med* 352: 20–28, 2005.
31. **Ridker PM, Rifai N, Clearfield M, Downs JR, Weis SE, Miles JS, Gotto AM Jr; Air Force/Texas Coronary Atherosclerosis Prevention Study Investigators.** Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. *N Engl J Med* 344: 1959–1965, 2001.
32. **Ridker PM, Rifai N, Lowenthal SP.** Rapid reduction in C-reactive protein with cerivastatin among 785 patients with primary hypercholesterolemia. *Circulation* 103: 1191–1193, 2001.
33. **Shah PK.** Mechanisms of plaque vulnerability and rupture. *J Am Coll Cardiol* 41: 15S–22S, 2003.
34. **Shiomi M, Ito T, Hirouchi Y, Enomoto M.** Stability of atheromatous plaque affected by lesional composition: study of WHHL rabbits treated with statins. *Ann N Y Acad Sci* 947: 419–423, 2001.
35. **Shishehbor MH, Brennan ML, Aviles RJ, Fu X, Penn MS, Sprecher DL, Hazen SL.** Statins promote potent systemic antioxidant effects through specific inflammatory pathways. *Circulation* 108: 426–431, 2003.

36. **Torzewski M, Klouche M, Hock J, Messner M, Dorweiler B, Torzewski J, Gabbert HE, Bhakdi S.** Immunohistochemical demonstration of enzymatically modified human LDL and its colocalization with the terminal complement complex in the early atherosclerotic lesion. *Arterioscler Thromb Vasc Biol* 18: 369–378, 1998.
37. **Williams H, Johnson JL, Carson KG, Jackson CL.** Characteristics of intact and ruptured atherosclerotic plaques in brachiocephalic arteries of apolipoprotein E knockout mice. *Arterioscler Thromb Vasc Biol* 22: 788–792, 2002.
38. **Wiviott SD, de Lemos JA, Cannon CP, Blazing M, Murphy SA, McCabe CH, Califf R, Braunwald E.** A tale of two trials: a comparison of the post-acute coronary syndrome lipid-lowering trials A to Z and PROVE IT-TIMI 22. *Circulation* 113: 1406–1414, 2006.
39. **Wu YL, You JH, Yuan GQ, Liang JQ, Jia ZH, Liu KJ, Wei C.** The effects of tongxinluo supermicro powder on nuclear factor-kappaB, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 expression in aorta of rabbits fed with high-lipid diet. *Zhonghua Xin Xue Guan Bing Za Zhi* 35: 271–274, 2007.
40. **Xu GC, Gao RL, Wu YL, Liu JL, Gao XD, Li H.** Clinical study on tongxinluo capsule in treatment of patients with angina pectoris caused by coronary heart disease. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 17: 414–416, 1997.
41. **Xu H, Wen C, Chen KJ.** Study on the effect of rhizoma Chuanxiong, radix paeoniae rubra and the compound of their active ingredients, Xiongshao Capsule, on stability of atherosclerotic plaque in ApoE$^{-/-}$ mice. *Zhongguo Zhong Xi Yi Jie He Za Zhi* 27: 513–518, 2007.
42. **Yuan BX, Li Q.** Effects of saponnins of zizyphus jujubanill seeds on plasma lipids and lipopropotein cholesterins of rats. *Chin Pharm Bull* 6: 34–36, 1990.
43. **Zhang L, Liu Y, Lu XT, Xu XS, Zhao YX, Ji XP, Zhang PF, Liu CX, Tang MX, Chen WQ, Zhang Y.** Intraplaque Injection of Ad5-CMV. p53 aggravates local inflammation and leads to plaque instability in rabbits. *J Cell Mol Med*. In Press.
44. **Zhang L, Wu YL, Jia ZH, Zhang Y, Shen HY, Wang XL.** Protective effects of a compound herbal extract (tong xin luo) on free fatty acid induced endothelial injury: implications of antioxidant system. *BMC Complement Altern Med* 8: 39, 2008.
45. **Zhong L, Chen WQ, Ji XP, Zhang M, Zhao YX, Yao GH, Zhang PF, Zhang C, Zhang Y.** Dominant-negative mutation of monocyte chemoattractant protein-1 prevents vulnerable plaques from rupture in rabbits independent of serum lipid levels. *J Cell Mol Med* 6A: 2362–2371, 2008.
46. **Zhou W, Chai H, Lin PH, Lumsden AB, Yao Q, Chen C.** Ginsenoside Rb1 blocks homocysteine-induced endothelial dysfunction in porcine coronary arteries. *J Vasc Surg* 41: 861–868, 2005.