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3-(4′-Hydroxyl-3′,5′-dimethoxyphenyl)propionic Acid, an Active Principle of Kimchi, Inhibits Development of Atherosclerosis in Rabbits

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The effects of 3-(4′-hydroxyl-3′,5′-dimethoxyphenyl)propionic acid (HDMPPA) originating from Korean cabbage kimchi were investigated, showing an antioxidant effect on the prevention of atherosclerosis in hypercholesterolemic rabbits. Twenty-one 3-month-old rabbits were fed an atherogenic diet containing 0.5% (w/w) cholesterol and 10% (w/w) coconut oil, whereas another two groups were given an atherogenic diet with intravenous injection of either HDMPPA or simvastatin (0.33 mg/kg/day) for 4 weeks. HDMPPA inhibited the oxidative modification of low-density lipoprotein (IC_{50} = 1.4 μg/mL) and increased 2,2′-diphenyl-1-picrylhydrazyl radical scavenging activity (IC_{50} = 0.78 μg/mL) in a dose-dependent manner. In hypercholesterolemic rabbits, the thickness of intima of aorta of the HDMPPA group was significantly reduced (control versus HDMPPA, 42%; simvastatin, 38%) without a plasma cholesterol-lowering effect. Thiobarbituric acid reactive substance formation in the plasma of the HDMPPA group was significantly decreased compared to that of the control group. Furthermore, the generation of vascular reactive oxygen species in HDMPPA group was suppressed as the cyclooxygenase-2 protein level decreased. These findings suggest that HDMPPA prevents the development of aortic atherosclerosis in high-cholesterol-fed rabbits. The antiatherosclerotic effect of HDMPPA may be due to an antioxidative effect at a low dose without cholesterol-lowering effects.

KEYWORDS: Kimchi; 3-(4′-hydroxyl-3′,5′-dimethoxyphenyl)propionic acid (HDMPPA); antioxidative effect; atherosclerosis; aorta

INTRODUCTION

Cardiovascular disease, currently the leading cause of death and illness in developed countries, will soon become the preeminent health problem worldwide (*1, 2*). Atherosclerosis, a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries, constitutes the single most important contributor to this growing burden of cardiovascular disease. Atherosclerosis involves three processes: oxidation, inflammation, and hypercholesterolemia (*3–6*). This led to a gamut of studies using antioxidants, anti-inflammatory compounds, and cholesterol-lowering agents to reduce atherosclerosis in both human and animal models. Antioxidants such as vitamin E and β-carotene were successful in animal studies but remain to be proven in human trials (*7, 8*). Moreover, several cholesterol-lowering drugs were successful in decreasing atherosclerosis in animal and human models, but are still controversial (*9–13*).

Oxidative stress has been implicated as an important etiologic factor in atherosclerosis and vascular dysfunction, whereas antioxidants present in the cells of the vascular wall decrease the intracellular generation of reactive oxygen species (ROS), inhibit expression of adhesion molecules and monocyte chemoattractants, and improve the biological activity of endothelium-derived nitric oxide (EDNO) (*14*). Also, the increases of ROS generation by hypercholesterolemia are known to produce endothelial dysfunction and modified low-density lipoprotein (LDL), accepted as pivotal risk factors (*15*). Statins increase nitric oxide (NO) bioavailability by stimulating and up-regulating the endothelial nitric oxide synthase (eNOS) or by decreasing O_2^- production in vascular endothelial cells and inhibit plaque formation and preserve endothelial function without lipid-lowering effects in rabbits fed a high-cholesterol diet (*16–18*).

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Recently, several naturally occurring constituents have received considerable attention because of their potential antioxidant activity, and it has been associated with the prevention and/or treatment of atherosclerosis (*19*). Koreans may experience lower morbidity and mortality due to cardiovascular disease than populations of Western countries because they normally consume about 150 g of kimchi everyday, corresponding to 5% of the daily food intake. Major ingredients of baechu kimchi are baechu (Korean cabbage), red pepper, garlic, and ginger, which have high levels of dietary fiber, vitamin C, β-carotene, lactic acid bacteria, β-sitosterol, minerals, and other health-promoting components (*20*, *21*). Studies from our laboratory using optimally ripened kimchi or its ingredients showed a beneficial effect on atherosclerosis in human and animal models. Furthermore, the study of solvent fractions of kimchi on LDL oxidation and plasma cholesterol concentration in rabbits fed an atherogenic diet showed the greatest effects in the dichloromethane fraction (*21*). Therefore, the 3-(4′-hydroxyl-3′,5′-dimethoxyphenyl)propionic acid, molecular weight 226, isolated and identified from the dichloromethane fraction of freeze-dried kimchi showed antioxidant activity on LDL oxidation and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging effect (*22*). On the basis of previous results, we designed this study to test the hypothesis that 3-(4′-hydroxyl-3′,5′-dimethoxyphenyl)propionic acid (HDMPPA) attenuates atherosclerosis by its antioxidative effects in rabbits fed a high-cholesterol diet.

MATERIALS AND METHODS

Chemicals. Chemically synthesized HDMPPA originally isolated from Korean cabbage kimchi was used in this study after biological identification (*22*). HDMPPA was prepared and provided by the Department of Chemistry (Pusan National University, Korea), and simvastatin was kindly provided by Dong-wha Pharmaceutical Co. Ltd. (Anyang, Korea). All other reagents and buffers were purchased from Sigma Chemical Co. (St. Louis, MO).

DPPH Radical Scavenging Activity. DPPH radical scavenging activity was determinded according to the method of Blois (*23*). In brief, HDMPPA, simvastatin, and vitamin C were incubated with 60 μM DPPH in ethanol solution at room temperature. The absorbance of the tested sample solution was measured at 517 nm with a spectrophotometer (UV-2401PC, Shimadzu Ltd.).

Preparation of LDL and Copper Ion-Mediated Oxidation of LDL. Human LDL (Sigma L-5402) was dialyzed against phosphate-buffered saline (PBS) for 20 h at 4 °C to remove the EDTA, and protein concentration was determined according to the method of Lowry et al. (*24*). Oxidation of LDL was carried out according to the methods of Schuh et al. (*25*) and Yagi (*26*). Briefly, the oxidation of LDL (100 μg of LDL protein/mL) was induced at 40 °C by 5 μM $CuSO_4$ in PBS in the presence or absence of sample. One milliliter of thiobarbituric acid (TBA)−trichloroacetic acid (TCA)−HCl solution (0.4% TBA, 15% TCA, and 2.5% HCl) was added to the mixture, and it was heated at 95–100 °C for 20 min. TBA-reactive substance (TBARS) was determined by measuring the absorbance at 532 nm. The value of TBARS was expressed in nanomoles of malondialdehyde (MDA) per milligram of LDL protein by a calibration curve constructed from MDA (0–25 nmol/mL) in tetramethoxypropane.

Animals and Diets. Male New Zealand White rabbits aged 3 months (1.9–2.0 kg) were purchased from Daehan Animal Center Co. Ltd. (Daejeon, Korea). The animals were housed individually in stainless steel cages in a room at a 23 ± 2 °C controlled temperature, relative humidity of 55 ± 15%, and 12 h light−dark cycle. Care of the animals and all experimental procedures were conducted in accordance with the institutional guidelines for animal research. After completion of a 2 week acclimation period, rabbits were divided by body weight into three groups of seven animals each. The control group was fed a chow diet (Purina) containing 0.5% cholesterol (w/w) and 10% coconut oil (w/w), whereas the other two groups were given a control diet with HDMPPA or simvastatin administered at 0.66 mg/kg by intravenous injection via the marginal ear vein every other day for 4 weeks, respectively. With regard to the employed drug doses, it has been confirmed that it is equipollent to treat the patients with hypercholesterolemia (*11*). Diet and drinking water were given ad libitum during the experimental periods. The body weights of the animals were monitored during the experiment. At the end of the experiment, rabbits were anesthetized with intravenously administered ketamine HCl (50 mg/mL, Korea United Pharmaceuticals, Inc.) after 12 h of fasting. Blood samples were collected from arteries, and then the liver and aorta were removed for biochemical analysis.

Plasma and Aortic Lipid Contents. Concentrations of TC, TG, and HDL-C in plasma were determined by enzymatic colorimetric methods using commercial kits (Cholesterol E kit BC 108-E and Triglyceride E kit BC 118, Asan Pharmaceutical Co., Ltd., Korea). LDL-C was accomplished according to the procedures described by Friedwald and Levy (*27*). Also, the plasma was analyzed for lipid profile by using an automatic autoanalyzer (Aeroset, Abbott Laboratory) to compare the results obtained from commercial kits. A 3 cm segment of iliac−femoral artery was used to determine the lipid contents of the aorta according to the Folch method (*28*).

Plasma TBARS Concentration. TBARS concentration of the plasma was measured by using the methods of Schuh et al. (*25*) and Yagi (*26*) as previously described in oxidation of LDL.

Histopathological Analysis. The aortic fragments from aortic arch to iliac−femoral artery were isolated and fixed with 10% formalin neutrally buffered solution after the blood had been washed out with saline. The vascular segment (about 3 mm) at 5 mm distance from the furcation of the left subclavian artery was embedded in paraffin, and 5 μm thick cross sections from each paraffin block were cut and stained with hematoxylin and eosin to determine the thickening of the aorta. Images of the aortas were captured with a Nikon digital camera, and the thickness of aorta was quantified by computer image analysis, using OPTIMAS 6.2 software.

Quantification of ROS in Aorta. Aorta was homogenized with a polytron homognizer in 7 volumes (v/w) of ice-cold homogenization solution containing 50 mM phosphate buffer (pH 7.4), 0.5 mM phenylmethanesulfonyl fluoride, 1 mM EDTA, 80 mg/L trypsin inhibitor, and 1 μM leupeptin. The homogenate was centrifuged at 900*g* at 4 °C for 15 min, and supernatants were used for biochemical assays and Western blotting analysis.

ROS generation was measured using a fluorescence probe (*29*, *30*). Briefly, 2′,7′-dichlorofluorescin diacetate (DCFH-DA, Molecular Probes, Inc., Eugene, OR) was added to homogenates for a final concentration of 25 μM. The change in fluorescence intensity was measured every 5 min for 30 min on a fluorescence reader (Bio-TEK Instruments, Inc., Winooski, VT) at excitation of 485 nm and emission of 530 nm. ROS status was calculated as fluorescence per minute.

Western Blot Analysis of Cyclooxygenase (COX)-2 in Aorta. Western blot was carried out as described previously (*31*, *32*). Total protein equivalents of each sample was separated by a SDS−polyacrylamide mini-gel as described by Laemmli (*33*). Antibodies against COX-2, β-actin, and HRP-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Antibody labeling was detected using enhanced chemiluminescence (Amersham Life Science, Inc., Arlington Heights, IL) and exposed to radiographic film. Prestained blue protein marker was used for molecular weight determination. The concentration of total protein in samples was measured with Sigma protein assay reagent kit containing bicinchoninic acid.

Statistical Analysis. Statistical analyses were performed using Statistical Analysis Software (SAS Institute). The results are presented the means and standard deviation. The significance of the difference between group means was analyzed by one-way ANOVA followed by Duncan's multiple-range tests. Values of $p < 0.05$ were considered to be significant.

RESULTS

DPPH Radical Scavenging Activity and Copper Ion-Mediated Oxidation of LDL. As shown in **Table 1**, HDMPPA was found to be stronger than vitamin C in free radical scavenging activity, whereas simvastatin did not show such an

Table 1. Antioxidant Activity of HDMPPA[a] on DPPH Radical Scavenging and LDL Oxidation

	IC_{50}[b]	
sample	LDL oxidation	DPPH radical scavenging
HDMPPA	1.4 ± 0.1	0.8 ± 0.1
simvastatin	2.9 ± 0.1	inactive
vitamin C	12.9 ± 0.3	5.6 ± 0.2

[a] Values are means of triplicates. HDMPPA, molecular weight 226, was originally identified from Chinese cabbage kimchi. The HDMPPA used in this study was chemically synthesized. [b] Fifty percent inhibition concentration (μg/mL).

effect. The increase of TBARS during incubation of LDL with Cu^{2+} was dose-dependently inhibited by HDMPPA. IC_{50} values for LDL oxidation inhibition by HDMPPA, simvastatin, and vitamin C were 1.4, 2.9, and 12.9 μg/mL respectively.

Body and Liver Weights and Plasma Lipids. Body and liver weights and plasma lipid concentrations are shown in **Table 2**. Body weights of rabbits at the start of the experiment were similar among three groups, and body weight gained was slightly lower in the HDMPPA and simvastatin groups, but there were no significant differences among the groups. Although the TC and LDL-C concentrations of plasma in the 0.5% cholesterol-fed control group were markedly elevated compared with those in the HDMPPA and simvastatin groups at 4 weeks, there were no significant differences among the groups. Similarly, the TG concentration of the HDMPPA group was slightly decreased compared with those in the control groups, but no significant differences were found among the groups. The HDL-C concentration was not changed during the experiment.

Histopathologies of the Aorta. Figure 1 shows the changes of thickness of the aortic walls in 0.5% cholesterol diet fed rabbits at 4 weeks. The intimal thickness of the aorta in the control group was significantly increased due to the 0.5% cholesterol diet (**Figure 1A**). However, the HDMPPA and simvastatin groups showed significantly reduced intimal thickening, compared to the control group, by 42 and 38%, respectively (**Figure 1B−D**). The total thickness of the aorta was slightly reduced in the HDMPPA group (**Figure 1D**), and the thickness of media and externa in the aorta was not significantly different among the groups (data not shown).

TBARS of Plasma and Aortic Lipid Contents. As shown **Figure 2**, the level of plasma TBARS was significantly lower in the HDMPPA and simvastatin groups than in the control group by 35 and 26%, respectively (**Figure 2A**). The TG content in the aorta was significantly reduced in the HDMPPA and simvastatin groups compared with the control group, whereas TC content was slightly lower in the HDMPPA group compared with the control group, but the differences were not statistically significant (**Figure 2B**).

ROS Generation and COX-2 Protein Levels in the Aorta. Figure 3 shows the ROS generation and COX-2 protein levels of the aorta in 0.5% cholesterol diet fed rabbits at 4 weeks. ROS generation in the aorta was markedly increased by 3.3 times in the control group compared with those in the normal group (data not shown, $p < 0.05$). In contrast, the simvastatin and HDMPPA groups inhibited the increase of ROS generation by 35 and 38% compared with the control group, respectively (**Figure 3A**, $p < 0.05$). COX-2 protein expression in the aorta was markedly increased by 19 times in the control group compared with those in normal group (data not shown, $p < 0.05$). In contrast, treatment with simvastatin and HDMPPA decreased the COX-2 protein leveld by 74 and 59% compared with the control group, respectively (**Figure 3B**).

DISCUSSION

A number of animal and human studies on kimchi have shown antioxidant and lipid-lowering effects (*20, 21*). Also, 3-(4′-hydroxy-3′,5′-dimethoxyphenyl)propionic acid (HDMPPA), an active principle of optimally ripened kimchi, might be produced by microbial action during its fermentation, and kimchi lactic acid showed weight reduction and lipid-lowering effects in rats fed high-fat diets (*34*).

Our results showed that in vitro, the inhibitory effects on copper ion-mediated oxidation of LDL and free radical scavenging activity of HDMPPA were greater than those of simvastatin and vitamin C, respectively. Probucol and fluvastatin showed a strong free radical scavenging activity, whereas lovastatin, pravastatin, and simvastatin yielded only very weak effects (*16*). Although HMG-CoA reductase inhibitors, simvastatin, have inhibitory effects on LDL oxidation, it was shown to have no effect on free radical scavenging in vitro. On the basis of these results, the antioxidant activity of HDMPPA was thought to be due to the phenolic O−H bond in its structure, and antioxidants have been reported to have free radical scavenging activity implicated in the phenolic O−H bond, which may be attributable to its hydrogen-donating ability (*35, 36*). Therefore, in the present study, we investigated the effects of HDMPPA by intravenous administration on atherosclerotic lesions in 0.5% cholesterol diet fed rabbits for 4 weeks. Also, to examine the pharmacological effect of HDMPPA, we administered it intravenously, which allows it to be metabolized more readily in the blood rather than in the liver and a target organ.

Our results showed that hypercholesterolemia was induced by a 0.5% cholesterol diet for 4 weeks, and plasma TC and LDL-C levels were lower in HDMPPA and simvastatin groups than in the control group, although the levels were not significantly different among the groups. The dose of HDMPPA in this protocol (0.33 mg/kg/day) was not effective in reducing plasma lipids. HMG-CoA reductase inhibitor (statins) is a potent inhibitor of cholesterol biosynthesis in the liver by blocking the conversion of HMG-CoA to mevalonate, the rate-limiting step in the mevalonate pathway. Their common mechanism is the inhibition of endogenous cholesterol synthesis and the subsequent increased expression of the LDL receptor, resulting in an up-regulated catabolic rate for plasma LDL. Consequently, it has resulted in the beneficial treatment of hyperlipidemia and coronary artery disease (*11*). However, several studies have demonstrated that independent of lipid-lowering effects, statin has pleiotropic effects such as the reduction of endothelial dysfunction, inhibition of inflammatory responses, stabilization of atherosclerotic plaque, and modulation of procoagulant activity and platelet function (*16–18*). Furthermore, several clinical studies have suggested that the beneficial effects of statin treatment begin earlier than the cholesterol-lowering effect (*11*). Our present study demonstrated that the HDMPPA group inhibited atherosclerotic lesions through the decrease of TBARS levels in plasma, aortic ROS generation, and COX-2 protein expression at least in part in hypercholesterolemic rabbits. These beneficial effects of HDMPPA were not associated with its cholesterol-lowering effects. Therefore, our findings suggest that HDMPPA directly reduces atherosclerosis through its antioxidant effect. Several studies using rabbits have shown that hypocholesterolemic agents possessing antioxidative activity, such as probucol (*37*), fluvastatin (HMG-CoA inhibitor) (*38*), and glimepiride (hypoglycemic drug) (*39*), showed antiatherosclerotic effects at high doses due to lowering of plasma cholesterol levels, whereas they suppressed the development of atherosclerotic lesions at low doses due to their antioxidative

Table 2. Effect of HDMPPA[a] on Body and Liver Weights and Plasma Lipid Concentrations of Rabbits Fed a 0.5% Cholesterol Diet for 4 Weeks

group[b]	body wt gained (kg)	liver wt (g)	TC (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)
control	0.65 ± 0.19	106.7 ± 9.5	1706.7 ± 321.7	1354.1 ± 285.8	49.2 ± 3.8	334.3 ± 37.3
simvastatin	0.61 ± 0.17	103.8 ± 6.4	1493.3 ± 441.1	1166.1 ± 373.8	57.0 ± 10.4	313.3 ± 66.7
HDMPPA	0.56 ± 0.17	97.7 ± 16.2	1381.7 ± 388.0	1098.57 ± 343.6	49.7 ± 7.1	266.3 ± 50.3

[a] Values are presented as mean ± SD ($n = 7$). Data in the columns are not significantly different among groups at the 0.05 level of significance. See the legend of **Table 1**. [b] Control group was fed 0.5% cholesterol and 10% coconut oil and injected with 0.33 mL/kg/day. HDMPPA group was fed 0.5% cholesterol and 10% coconut oil, and HDMPPA was injected at 0.33 mg/kg/day. Simvastatin group was fed 0.5% cholesterol and 10% coconut oil, and simvastatin was injected at 0.33 mg/kg/day.

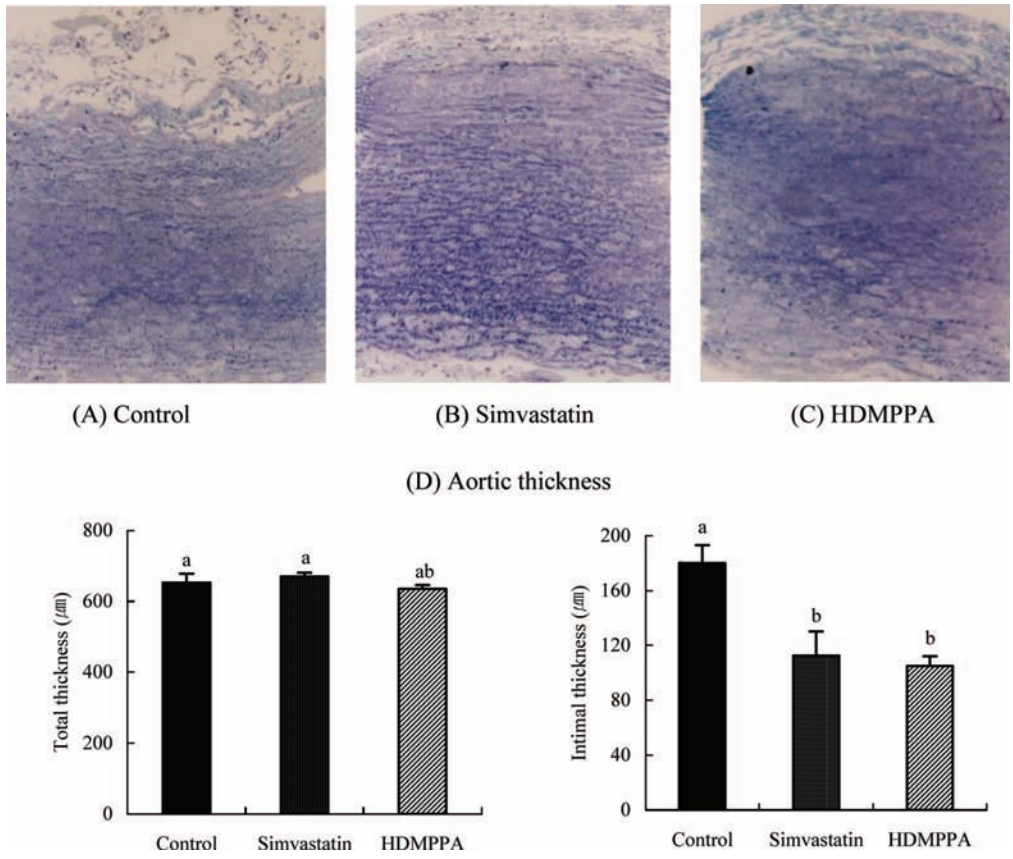

Figure 1. Effect of HDMPPA injected into the ear vein on aorta tissue morphology and aortic thickness of rabbits fed a 0.5% cholesterol diet for 4 weeks: (**A**−**C**) hematoxylin and eosin-stained section (×200); (**D**) aortic thickness evaluated in aorta. Values are mean ± SD ($n = 7$). (a, b) Data represented by the columns are significantly different by one-way ANOVA followed Duncan's multiple-range test at the 0.05 level of significance. See the legends of **Tables 1** and **2**.

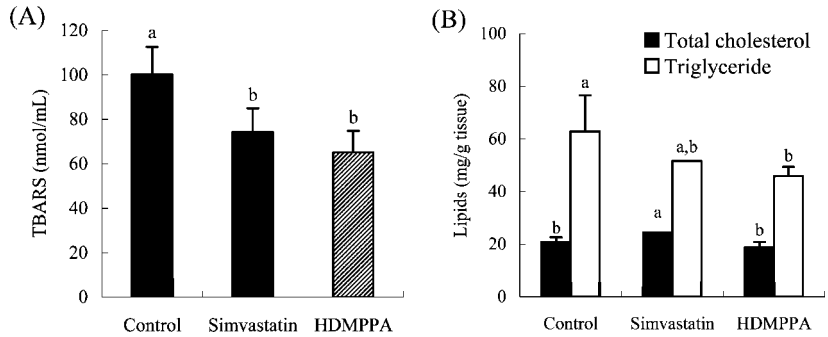

Figure 2. Effect of HDMPPA injected into the ear vein on TBARS levels in the plasma and lipid contents in the aorta of rabbits fed a 0.5% cholesterol diet for 4 weeks: (**A**) TBARS in the plasma; (**B**) lipid contents in the aorta. Values are mean ± SD ($n = 7$). (a, b) Data in the column are significantly different by one-way ANOVA followed by Duncan's multiple-range test at the 0.05 level of significance. See the legends of **Tables 1** and **2**.

effects on LDL oxidaiton. Similarly, HDMPPA at a dose of 0.33 mg/kg/day significantly decreased the concentration of plasma TBARS, which is a marker for lipid peroxidation, and increased the resistance of LDL oxidation in vitro. Also, natural antioxidative substances, such as vitamin E (*40*) and taurine (*41*), and natural foods containing flavonoids (*42*) have been reported to exert antiatherosclerotic effects by suppressing LDL susceptibility to oxidation without decreasing plasma cholesterol

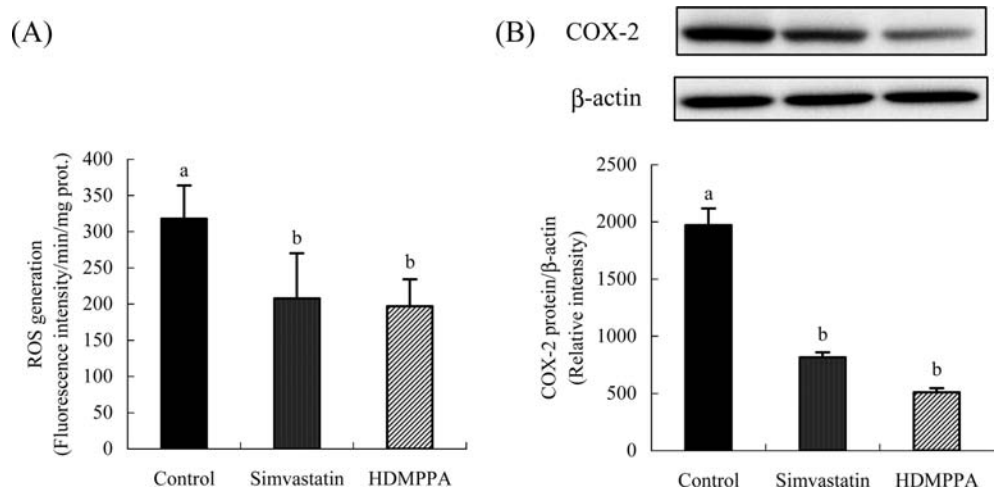

Figure 3. Effect of HDMPPA injection into the ear vein on ROS generation and COX-2 protein levels in the aorta of rabbits fed a 0.5% cholesterol diet for 4 weeks: (**A**) ROS generation estimated by staining with a fluorometer using DCF-DA; (**B**) expression of COX-2 protein determined by Western blot analysis. Values are mean ± SD ($n = 7$). Quantitation of the COX-2 protein expression was performed by densitometric analysis. (a, b) Data are significantly different by one-way ANOVA followed by Duncan's multiple-range test at the 0.05 level of significance. See the legends of **Tables 1** and **2**.

levels. In the present study, histopathological analysis of the arterial lesions revealed typical characteristic features of early atherosclerosis in the control group, which were mainly caused by the accumulation of foam cells. Accumulation of lipid-laden foam cells was found in the subendothelial space, but no morphological alterations generally seen in more advanced stages of the disease, such as fibrous plaque formation concomitant with proliferation of smooth muscle cells and deposition of extracellular matrix, were observed. The development of fatty streak lesion in aortas was significantly increased in rabbits fed an atherogenic diet containing 1.3% cholesterol and 3% lard for 30 days, whereas *Curcuma longa* extract supplementation reduced oxidative stress and attenuated aortic fatty streak development (*43*). Hence, we speculate that HDMPPA significantly inhibits some process in the early cellular events of atherosclerosis, which may be implicated in the oxidation and inflammation mechanism of atherosclerosis (*2–6*). Also, our results showed that the reduction in intimal thickening in the HDMPPA group resulted from the decrease of ROS generation and COX-2 protein level in the aortas of hypercholesterolemic rabbits. These effects of HDMPPA were stronger than or similar to that of simvastatin. Oxidative stress has been implicated as an important etiologic factor in atherosclerosis and vascular dysfunction, whereas antioxidants may inhibit atherogenesis and improve vascular function (*14*). Superoxide can be produced by a variety of cells, most notably leukocytes and endothelial cells, which use the NAD(P)H oxidase as the main source of ROS in arterial vessels of hypercholesterolemic animals (*44*). An important characteristic of endothelial dysfunction is the impaired synthesis, release, and activity of endothelium-derived nitric oxide, which mediates vascular relaxation and inhibits platelet aggregation, vascular smooth muscle proliferation, endothelium-leukocyte interactions, and even oxidative modification of LDL (*45*). The endothelium-derived vascular relaxing factor, which is now recognized to be either NO or a related compound, is rapidly destroyed by superoxide radical generating systems. In addition, the O_2^- may react with $NO^\bullet$, which is also produced in excess within the endothelium of hypercholesterolemic animals, to produce the highly injurious peroxynitrite radical, and it may further enhance LDL modification and thus lipid accumulation within the vascular wall (*44*). Oxidation of LDL is considered to be an important step and seems to modulate cellular gene expression by an up-regulation pathway of transcription factors in the development of atherosclerosis (*46*). Curiously, additional oxidized LDL (oxLDL) may also form as a result of the action NO or the enzyme responsible for leukotriene formation lipoxygenase, both of which can be released by the endothelial cells, presenting a dilemma. As shown in **Figure 3**, the decrease of ROS generation in the HDMPPA group may lead to the decrease of COX-2 protein in the arterial walls of rabbits fed a 0.5% cholesterol diet for 4 weeks. Our results confirmed that HDMPPA directly inhibits the development of atherosclerosis through, at least in part, the decrease of ROS generation by COX-2 in the aorta. COX-2 isoform is a key mediator of inflammation, up-regulated in activated monocyte/macrophages, suggesting that selective COX-2 inhibition might reduce atherogenesis through its anti-inflammatory effects (*47*). Licofelone reduced the intima/media ratio in injured arteries, macrophage infiltration in the neointimal area, monocyte chemoattractant protein-1 (MCP-1) gene expression, COX-2 protein expression, and the activation of nuclear factor-κB in rabbit atheroma (*48*). In the present study, the inhibition of intima hyperplasia in the HDMPPA group similar to that effected by simvastatin was observed, and it was thought to be caused by the decrease of inflammation in atherosclerosis such as inhibition of macrophage recruitment, proliferation, and migration of SMCs. Also, these observations open the possibility that redox-sensitive transcription factor and members of the nuclear receptor superfamily of ligand-dependent transcriptions factors, key regulators of inflammation and lipid homeostasis in macrophages, may have therapeutic value in atherosclerosis (*49*). The participation of these effects in the antiatherosclerotic property of HDMPPA will be clarified in future studies.

In conclusion, our present data show that HDMPPA exerts an antiatherosclerotic effect by decreasing aortic thickness, ROS generation, and COX-2 protein expression level in the aorta. This study therefore provides important new insights that may contribute to the putative effects of HDMPPA in suppressing the progression of atherosclerosis in the aorta during hypercholesterolemia. These observations suggest that synthetic HDMPPA, as an antioxidant, may have therapeutic applications in human atherosclerosis.

ABBREVIATIONS USED

HDMPPA, 3-(4′-hydroxy-3′,5′-dimethoxyphenyl)propionic acid; ROS, reactive oxygen species; NO, nitric oxide; COX-2, cyclooxygenase-2; TBARS, thiobarbituric acid-reactive substances; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-Co A reductase; SMC, smooth muscle cells; oxLDL, oxidized low-density lipoprotein; eNOS, endothelial nitric oxide synthase; MCP-1, monocyte chemoattractant protein-1; EDNO, endothelium-derived nitric oxide.

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