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ORIGINAL ARTICLE

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Metformin inhibits nuclear factor κB activation and decreases serum high-sensitivity C-reactive protein level in experimental atherogenesis of rabbits

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Abstract Previous studies demonstrated that metformin has obvious antiatherogenic properties, but the exact mechanism remains unclear. Therefore, we established an atherosclerotic rabbit model in order to investigate the potential effects of metformin on transcription factor nuclear factor κB (NF-κB) and serum high-sensitivity C-reactive protein (hs-CRP) level, which had been regarded as proatherogenic factors. New Zealand rabbits were randomly divided into three groups: a control group ($n = 8$), an atherosclerotic group (AS group, $n = 8$), and a metformin treatment group (Met group, $n = 8$). The experimental atherosclerotic rabbit model was successfully established at the end of the 8th week. From the 9th week, rabbits in the Met group were administered with 150 mg/kg metformin daily by gavage. Blood samples were collected at days 0 and 8, and at 16 weeks to detect the level of blood lipid and serum glucose. At the end of the experiment, blood samples were withdrawn for determining serum hs-CRP. Aortic samples were harvested for histomorphometric analysis. Immunohistochemistry and Western blotting were used to detect the expression of NF-κB subunit p65 in nuclear extracts and phosphorylation of inhibitor of nuclear factor κB (IκB) in cytoplasmic extracts. An experimental atherosclerotic rabbit model was successfully established. The expression of nuclear NF-κB subunit p65 and cytoplasmic phosphorylation of IκB protein in the vessel wall was enhanced ($P < 0.01$, respectively) in the AS group, and serum hs-CRP level was significantly increased in the AS group compared with the control group (3.90 ± 0.25 mg/l versus 1.36 ± 0.14 mg/l, $P < 0.01$). Treatment with metformin significantly attenuated the progression of aortic atherosclerosis. In the Met group, there was a marked reduction in nuclear NF-κB subunit p65 and cytoplasmic phosphorylation of IκB protein expression ($P < 0.01$). Serum hs-CRP concentration was also significantly decreased (3.20 ± 0.20 mg/l versus 3.90 ± 0.25 mg/l, $P < 0.05$). Metformin inhibits the phosphorylation of IκB and the activation of NF-κB in the vessel wall of experimental atherogenesis of rabbits, as well as decreasing the serum level of hs-CRP, thus suggesting that metformin has vascular anti-inflammatory properties, which may be one of its antiatherogenic mechanisms.

Key words Metformin · Atherosclerosis · Nuclear factor-κB · High-sensitivity C-reactive protein · Inflammation

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Introduction

Nowadays, atherosclerosis is considered a slowly progressive inflammatory disease and does not result simply from the accumulation of lipids.[1,2] Numerous inflammatory mediators are involved in the initiation and development of atherosclerosis.[3,4] Among them, the nuclear transcription factor-κB (NF-κB) plays a central role in the process of atherosclerosis. The activated NF-κB signal-transduction pathway can regulate the transcription of a large number of genes, especially those related to inflammation, immunity, apoptosis, and cell proliferation, such as interleukin-6 (IL-6), interleukin-1β (IL-1β), vascular cell adhesion molecule-1 (VCAM-1), and tumor necrosis factor-α (TNF-α).[5] Existing research studies revealed that NF-κB was widely distributed in vascular smooth muscle cells, endothelial cells, and mononuclear macrophages located in atherosclerotic lesions.[6,7] Activation of NF-κB was considered as one of the priming mechanisms of atherosclerosis, and many researchers regarded NF-κB as a major therapeutic target in atherosclerosis, especially for preventive measures.[8,9] The acute-phase protein C-reactive protein (CRP) was originally identified as a clinical marker of the state of

inflammation. Recent clinical studies found that CRP was an independent risk factor of coronary heart disease[10] and increasing evidence has supported CRP playing an important role in atherogenesis.[11]

Metformin, a member of the biguanide family, enjoys wide use for the treatment of type 2 diabetes. It has been shown that metformin has significant antiatherogenic properties in experimental animals.[12] The United Kingdom Prospective Diabetes Study (UKPDS) also demonstrated that administration of metformin reduced macrovascular morbidity and mortality (mainly atherosclerosis disease), which was independent of improvements in glycemic control.[13]

This study aimed at investigating the effect of metformin on the expression of NF-κB and its inhibitory protein IκB in the arterial wall of atherosclerotic rabbits, as well as the changes in serum level of high-sensitivity C-reactive protein (hs-CRP), so as to investigate its possible antiatherogenic mechanisms.

Materials and methods

Establishment of rabbit AS model

Animal experiments were conducted according to the Principles of Laboratory Animal Care and Use (NIH Publication No. 85-23). Twenty-four male New Zealand white rabbits, 2.5 months old, were purchased from the Center of Experimental Animals, Tongji Medical School. They were housed in individual cages and divided into three groups using a random digits table after 1 week's acclimation: control group ($n = 8$), atherosclerotic group (AS group, $n = 8$) and metformin treatment group (Met group, $n = 8$). Rabbits in the control group were fed a normal diet (150 g/day). As described in previous reports,[14] the endothelial damage was induced by bringing about an immune response in the vessel by injecting bovine serum albumin via marginal ear vein of rabbits in the AS group and Met group. Then animals in the AS group and Met group received a high-cholesterol diet (1% cholesterol and 5% lard, Alpha Biotechnologies, Wuhan, China) 150 g/day. All animals in three groups were allowed to drink water ad libitum. Ascending aorta high-frequency ultrasonography (GE Vivid color Doppler ultrasonic apparatus; GE Healthcare, Milwaukee, WI, USA) was performed at the 8th week. The intimal-medial thicknesses (IMT) of ascending aorta in the AS group and Met group were significantly increased, compared with that of the control group. In addition, atherosclerotic plaques were observed, suggesting the successful establishment of experimental atherosclerosis model (Fig. 1). Rabbits in the AS group were fed with a high-cholesterol diet continuously, while rabbits in the Met group were administered with 150 mg/kg/day metformin (Metformin hydrochloride tablets, purchased from Bristol–Myers Squibb [New York, NY, USA], were dissolved in normal sodium to obtain a 5% solution, and given by gavage at a fixed time once a day.) in addition to the high-cholesterol diet. The rabbits were weighed once a week, and the dosage was adjusted according to their weight. All the rabbits were euthanized at the end of 16th week.

Biochemistry analysis

At day 0, the end of the 8th week, and the end of the experiment, 2 ml of blood samples were withdrawn via marginal ear vein 12 h post meal. Serum total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) were determined by spectrophotometry. Low-density lipoprotein cholesterol (LDL-C) was calculated according to the Friedwald formula.[15] Glucose serum levels were measured by the glucose oxidase–peroxidase method.

Histomorphometry

At the end of the experiment, all the animals were anesthetized with sodium pentobarbital (30 mg/kg i.v.), and aortas distal to the renal artery were harvested. Ascending aorta rings about 5 mm wide, just below the aortic arch, were sectioned and fixed in 10% formalin for 24 h, paraffin-embedded. Serial 4 μm slices were cut for hematoxylin–eosin staining or used for immunohistochemical analysis. The left vascular tissues were snap-frozen in liquid nitrogen and stored at −70°C for Western blot analysis.

Sections of ascending aorta (hematoxylin–eosin stained) were analyzed with an automated computerized image analyzer (ImagePro Plus, Cybernetics, Bethesda, MD, USA). The intimal-medial thickness (IMT, mm), intimal cross-sectional area (Intima, mm^2) and medial cross-sectional area (Media, mm^2) were measured for each group. The intimal cross-sectional area was determined by subtracting the lumen area from the area enclosed by the internal elastic lamina. The medial area was determined by subtracting the area enclosed by the internal elastic lamina from that enclosed by the external elastic lamina. Mean areas were calculated to deduce Intima/Media (I/M) ratios. Four sections from each animal were analyzed and the values were averaged.

Immunohistochemical staining

The expression of NF-κB subunit p65 in the vessel wall of the aorta was detected by immunohistochemical staining strictly according to the instructions. Prior to immunostaining, the 4-μm ascending aorta sections were pretreated by microwave hydrolytic method in 0.01 mol/l citrate buffer (0.01 mol/l, pH 6.0) for 5 min at 100°C to enhance the immunoreactivity, and washed with phosphate-buffered saline (PBS: 0.01mol/l, pH 7.35). After blocking nonspecific immunoglobulin-binding sites by Tris-buffered saline (TBS: 50 mmol/l Tris–HCl, 150 mmol/l NaCl, pH 7.6) containing 4% bovine serum albumin (BSA), sections were incubated with primary antibody (anti-NF-κB p65 polyclonal antibody, Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h at room temperature, followed by incubation with

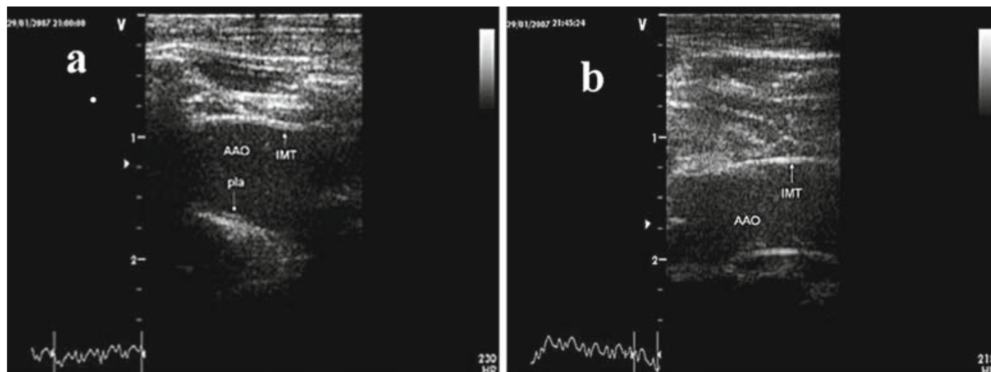

Fig. 1. Sonogram images of the ascending aorta of rabbits fed with high cholesterol diet (atherosclerotic [AS] AS group and metformin treatment (Met) group, **a**) and control group (**b**) at the end of the 8th week. Intimal plaque and increased intimal-medial thickness (*IMT*) were detected in all rabbits fed a high-cholesterol diet. *AAO*, ascending aorta; *pla*, atherosclerotic plaque

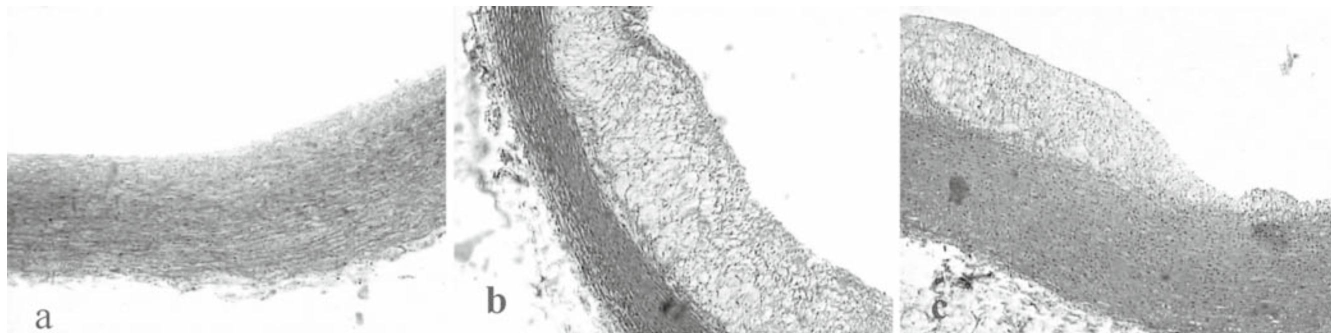

Fig. 2a–c. Photomicrographs of representative cross sections of ascending aorta from the three groups (H&E stain, ×40)

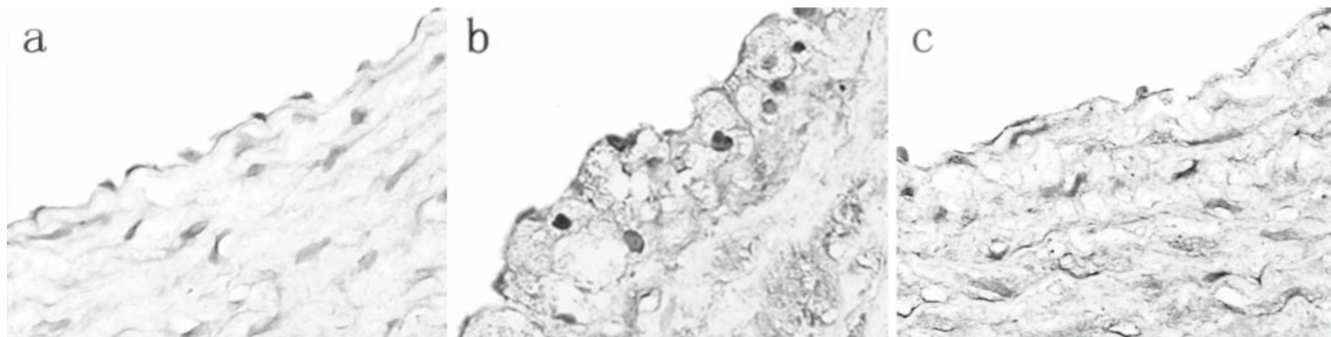

Fig. 3a–c. Immunohistochemistry staining of nuclear factor (NF)-κB p65 on cross sections of ascending aortas from the three study groups. The sections with the anti-NF-κB p65 showed a strong nuclear positive signal in the AS group (**b**). Metformin significantly reduced the NF-κB p65 positive nuclear number (**c**) (×400)

horseradish peroxidase-coupled goat antirabbit immunoglobulin (HRP-IgG, Santa Cruz Biotechnology) for 1 h at room temperature.

Western blot

Cytoplasmic proteins and nuclear proteins were extracted from pooled vascular tissues with RIPA Lysis Buffer (Beyotime Biotechnology, Haimen, China).The concentrations of proteins were determined by the Bradford method. Cytoplasmic or nuclear protein samples (20 μg) were boiled for 5 min in sample buffer (including 250 mM Tris–HCl [pH 6.8], 4% sodium dodecyl sulfate, 10% glycerol, 2% β-mercaptoethanol, and 0.003% bromophenol blue), separated on 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and then electroblotted onto ECL-nitrocellulose membrane (Invitrogen, Carlsbad, CA, USA). The nonspecific sites were blocked by incubation with 5% nonfat dry milk in TBS with 0.05% Tween-20 (TBS-T) for 1 h at room temperature. After being washed for three times in TBS-T, membranes were incubated at 4°C overnight with primary antibody. These primary antibodies include anti-NF-κB p65, anti-inhibitors of κB (IκB), anti-phospho-inhibitors of kB (p-IkB) (1:1000, Santa Cruz Biotechnology), and rabbit polyclonal anti-histone H3 (Bio-Legend). Membranes were washed three times in TBS-T and incubated with the secondary antibody (HRP-IgG, Santa Cruz Biotechnology) for 1 h. The immunoreactive protein complexes were detected using an enhanced

chemiluminescence detection system (ECL; Pierce, Rockford, IL, USA) according to the manufacturer's protocol. The results were quantified by a scanning densitometer using image-analysis systems and expressed as relative densitometric units.

Enzyme-linked immunosorbent assay (ELISA) test

Two milliliters of blood were drawn from marginal ear vein at sacrifice. Serum hs-CRP level was measured by ELISA according to the instruction of hs-CRP ELISA kit (USCN Sciences, Wuhan, China).

Statistical analysis

All data were given as mean ± SEM, and analyzed by standard SPSS computer programs (Statistical Program for Social Sciences, Chicago, IL, USA; software version 13.0). Significant differences were evaluated by one-way analysis of variance (ANOVA). A value of $P < 0.05$ was considered statistically significant.

Results

General condition

At the beginning of the study, the mean weights of the rabbits in different groups had no significant difference. All rabbits gained weight during the study, but no significant difference was observed between the groups.

Blood lipid and serum glucose

At week 0, there was no significant difference in blood lipid level and serum glucose level between each group. At 8 and 16 weeks, blood lipid level of rabbits in AS and Met groups was significantly increased, compared with the control group ($P < 0.01$). Especially, the TC and LDL levels of the Met group were decreased compared with those of the AS group. However, there was no statistical difference ($P > 0.05$). A trend of increased serum glucose level was observed in all three groups during the study, but there was no significant difference between them at the end of study (Table 1).

Table 1. Change of rabbit's blood lipid concentrations and glucose serum levels in the three groups (mmol/l)

Group	Week 0	8th week	16th week
Control *n* = 8			
TC	1.66 ± 0.06	1.58 ± 0.16	3.53 ± 0.60
TG	0.72 ± 0.09	0.75 ± 0.22	0.82 ± 0.17
HDL-C	0.57 ± 0.13	0.58 ± 0.09	0.66 ± 0.07
LDL-C	0.60 ± 0.15	0.63 ± 0.12	2.52 ± 0.61
GLU	4.80 ± 0.90	5.33 ± 0.98	5.26 ± 0.50
AS *n* = 8			
TC	1.55 ± 0.11	24.01 ± 2.79*	47.17 ± 7.22*
TG	0.77 ± 0.16	3.21 ± 0.56	2.64 ± 1.05
HDL-C	0.61 ± 0.10	1.13 ± 0.34	0.70 ± 0.18
LDL-C	0.54 ± 0.21	21.42 ± 2.54*	45.28 ± 6.92*
GLU	4.81 ± 0.82	5.05 ± 0.92	5.54 ± 0.76
Met *n* = 8			
TC	1.67 ± 0.09	23.84 ± 2.19*/#	41.38 ± 11.73*/#
TG	0.76 ± 0.13	3.26 ± 0.61	2.27 ± 1.61
HDL-C	0.58 ± 0.12	1.08 ± 0.19	0.65 ± 0.13
LDL-C	0.57 ± 0.17	21.01 ± 1.76*/#	39.70 ± 11.14*/#
GLU	4.78 ± 0.79	5.16 ± 0.77	5.28 ± 0.52

Values are represented as mean ± SEM
AS, atherosclerotic group; Met, metformin treatment group; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol; GLU, glucose
* $P < 0.01$ versus control group at the same time, # $P > 0.05$ versus AS group at the same time

Histomorphometric analysis

The aortic intima in the control group was generally normal, as only the endothelial cell monolayer was present. After 8 weeks of daily high-cholesterol feeding, the aortic intima in the AS group was significantly thickened as evidenced by injured and fallen endothelial cells, and proliferating smooth muscle cells. Typical atherosclerotic plaques constituting many foam cells and inflammatory cells were observed. The proliferative degree of intima in the Met group was significantly weakened compared with the AS group. In addition, plaque area was also significantly decreased in this group (Fig. 2).

Compared with the control group, intimal-medial thickness (IMT), intimal cross-sectional area (Intima), and the ratio of intimal area to medial area (I/M) were significantly increased in the AS group (both $P < 0.01$), treatment with metformin 150 mg/kg/day prominently attenuated these increases (IMT: 0.55 ± 0.04 mm versus 0.73 ± 0.05 mm, $P < 0.05$; Intima: 4.14 ± 0.18 mm^2 versus 5.47 ± 0.36 mm^2, $P < 0.05$; I/M ratio: 1.27 ± 0.07 versus 1.59 ± 0.15, $P < 0.01$). The cross-sectional media was slightly larger in the AS group animals as compared to the control group, but it was not significantly different between each group ($P > 0.05$) (Table 2).

Immunohistochemical analysis for NF-κB

Vessel wall cells (endothelial cells [EC], smooth muscle cells [SMC], and mononuclear cells) with stained yellow brown nucleus were considered positive for NF-κB expression. In the control group, there was very weak nuclear expression of NF-κB p65. NF-κB was strongly activated in the AS group, and large quantities of yellow brown granular precipitates were observed in the nucleus of most cells. Some NF-κB positive cells could be found on the vessel wall in the Met group; however, the expression was significantly decreased compared with AS group (Fig. 3).

Western blot analysis of NF-κB p65 and phosphorylation of IκB

As shown in Fig. 4, the activation of NF-κB was determined by its nuclear extracts. In the AS group, the expression of

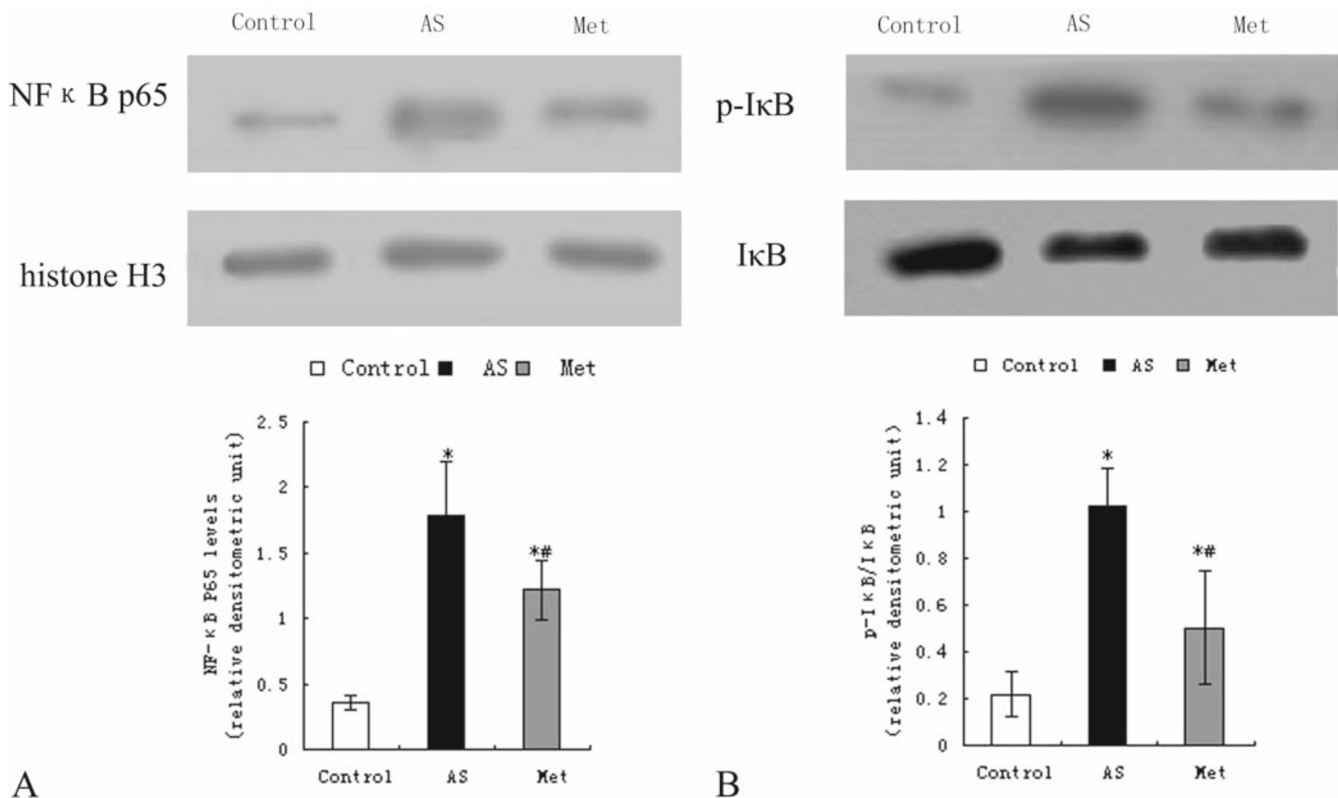

Fig. 4A,B. Activation of nuclear factor-κB (*NF-κB*) subunit in the aorta of rabbits from each group. **A** The protein levels of NF-κB subunit p65 in nuclear extracts was measured by Western blot, as described in Materials and methods. **B** The protein levels of phosphorylation of inhibitor of NF-κB (*IκB*) in cytoplasmic extracts was measured by Western blot, as described in Materials and methods. Values are given as mean ± SEM. *$P < 0.01$ versus control group, #$P < 0.05$ versus AS group

Table 2. Histomorphometric measurements of rabbit's ascending aorta from each group

Group	IMT (mm)	Intima (mm^2)	Media (mm^2)	I/M ratio
Control	0.22 ± 0.02	0.00 ± 0.00	3.03 ± 0.11	0.00 ± 0.00
AS	0.73 ± 0.05*	5.47 ± 0.36*	3.48 ± 0.12	1.59 ± 0.15*
Met	0.55 ± 0.04*/#	4.14 ± 0.18*/#	3.28 ± 0.14	1.27 ± 0.07**

Data are represented as mean ± SEM. A significant decrease of intimal-medial thickness (IMT), intimal cross-sectional area (Intima) and the ratio of intimal area to medial area (I/M) were noted in Met group
* $P < 0.01$ versus control group, ** $P < 0.01$ versus AS group, # $P < 0.05$ versus AS group

nuclear NF-κB subunit p65 was significantly higher than that of the control group ($P < 0.01$). Metformin decreased the expression of subunit p65 in nuclear extracts (Met group versus AS group: 1.21 ± 0.22 versus 1.79 ± 0.40, $P < 0.01$; Fig. 4A).The phosphorylation of IκB in cytoplasmic proteins of ascending aortas was also measured by Western blot analysis. We found that there was an increased expression of phosphorylation of IκB in the AS group (AS group versus control group: 1.02 ± 0.16 versus 0.21 ± 0.09) and metformin inhibited phosphorylation of IκB (Met group versus AS group: 0.50 ± 0.24 versus 1.02 ± 0.16, $P < 0.01$; Fig. 4B).

Serum hs-CRP concentration

The level of hs-CRP in the AS group and Met group were significantly higher than that of the control group (3.96 ± 0.63 mg/l, 2.79 ± 0. mg/l versus 1.36 ± 0.14 mg/l, both $P < 0.01$). Supplementation with metformin significantly decreased serum hs-CRP concentration ($P < 0.05$) (Table 3).

Table 3. Serum high-sensitivity C-reactive protein level in each group (mg/l)

Control	AS	Met
1.36 ± 0.14	3.90 ± 0.25*	3.2 ± 0.20*/#

Data are represented as mean ± SEM
* $P < 0.01$ versus control group, # $P < 0.05$ versus AS group

Discussion

This study demonstrated that chronic treatment with metformin attenuated the progression of pre-existing aortic

atherosclerosis in hypercholesterolemic rabbits. Furthermore, it is the first in vivo study to address that metformin inhibits IκB phosphorylation and NF-κB activation in the aortic vessel wall and decreases hs-CRP level in the serum of rabbits with atherosclerosis induced by a high-cholesterol diet, suggesting that metformin had anti-inflammatory properties, which might be one of its antiatherogenic mechanisms.

Atherosclerosis is a complex disease in which many factors are involved. The injury-reaction theory together with recent pathophysiological studies demonstrated that AS is a chronic disease with low grade of inflammation.[1,2,16] Nuclear factor κB is a group of structurally related transcriptional proteins that forms dimers composed of various combinations of members of the NF-κB/Rel family proteins. The most predominant form of NF-κB is the p50/p65 heterodimer. When in a resting state, it binds with the inhibitory protein IκB and locates in the cytoplasm in a form of a trimer. When activated by activators (such as oxidant stress, modified proteins, proinflammatory cytokines, etc.), IκB is phosphorylated by IκB kinase (IKK) and ultimately degraded via ubiquitination.[17–23] Activated NF-κB translocates into the nucleus and binds with a specific sequence in the promoter region of the target genes, initiating the transcription of corresponding genes and the expression of proteins. Nuclear factor κB plays important roles in regulating innate immune and inflammatory responses. The products of these target genes (such as interleukin-6, interleukin-8, interferon, CRP, matrix metalloproteinases, intercellular adhesion molecule-1, vascular cell-adhesion molecule-1, etc.) are involved in formation and progression of plaques in atherosclerosis.[5] Previous studies demonstrated that NF-κB was obviously activated at the site of plaques in atherosclerosis.[6,7] In addition, the degree of NF-κB activation was positively correlated with the extent of pathological changes in atherosclerosis. The activation of NF-κB in patients with acute coronary syndrome was more obvious than in patients with stable angina pectoris.[24] Nuclear factor κB had been considered as a good therapeutic target in atherosclerosis, especially for preventive measures.[9] C-reactive protein (CRP), an acute-phase protein, was originally considered as a nonspecific marker of inflammation. However, recent studies have suggested that CRP is a strong independent clinical marker of increased coronary heart disease risk,[10,25] which may play an important pathophysiological role in the development and progression of atherosclerosis. The potential mechanisms include induction of endothelial dysfunction,[26] promotion of lipid plaque formation,[27,28] inhibition of endothelial progenitor cell survival and differentiation,[29] and activation of complement in atherosclerotic plaque intima.[30] It has been demonstrated that overexpressed human CRP accelerated the progress of AS in transgenic apoE–/– mice.[31]

Metformin, an insulin-sensitizing biguanide which is recommended by the guidelines of the International Diabetes Mellitus Federation,[32] is the foundation stone for the treatment of type 2 diabetes patients and is widely used in clinical practice. Metformin increases liver and peripheral tissue sensitivity to insulin as well as reducing hepatic glucose production.[33] Previous studies shown that, beyond its effects on glucose metabolism, metformin was able to increase fibrolysis,[34] reduce plasminogen activator inhibitor (PAI)-1,[35] regulate lipid metabolism, improve endothelial function, and decrease advanced glycation end products.[36,37] Animal models and clinical studies have shown that metformin has obvious antiatherogenic properties;[12,13] however, its exact mechanisms are still unclear.

This study established a rabbit atherosclerotic model induced by immunologic injury plus high-cholesterol diet. Furthermore, the model was validated by high-frequency ultrasonography at the 8th week. Increased LDL cholesterol in blood infiltrated into the endangium and resulted in endothelial injury, initiating an injury-immune inflammatory reaction. Oxidized LDL cholesterol (ox-LDL) activated NF-κB in vascular endothelial cells and smooth muscle cells via binding to oxidized LDL receptor-1 (LOX-1) on the cell surface,[38] and thus initiating the expression of corresponding immunoinflammatory genes. Obvious degradation of IκBα in the vessel wall of the aorta was observed in the AS group, while the activation of NF-κB in the nucleus was significantly increased. Simultaneously, serum hs-CRP concentration was increased. Treatment with metformin for 8 weeks alleviated the progress of high-cholesterol diet-induced experimental atherogenesis in rabbits, significantly decreased the degradation of inhibitory protein IκBα and the activation of NF-κB in the diseased region, and reduced serum hs-CRP level. The study also found that fasting glucose levels had no significant differences between each group, which was consistent with other reports.[39,40] Although free fatty acid excess impairs β-cell function, high fat feeding in rabbits produces impaired glucose tolerance but not fasting hyperglucemia. Metformin can improve glucose tolerance and insulin sensitivity, but has no significant effect on fasting blood glucose concentration in nondiabetic animals.[41]

Two thirds (even 80%) of the patients diagnosed as coronary artery disease are complicated with hyperglycosemia (including injured glucose regulation and diabetes),[42,43] and more than 75% of type 2 diabetes patients will die of cardiovascular complications (mainly atherosclerosis). Chronic inflammation and insulin resistance are now considered to be common risk factors for both coronary arterial atherosclerosis and type 2 diabetes.[44,45] Intervention in the immune inflammatory mechanism had become a hot topic of current studies.[46] Clinical trials revealed that patients with impaired glucose tolerance and type 2 diabetes had decreased serum levels of inflammation mediators, such as soluble intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1), and E-selectin, after metformin treatment. However, the effect was not associated with the control of blood glucose level.[47] Recently, some investigators found that metformin could decrease the expression of inflammatory cytokines in IL-1β/TNF-α induced vessel wall cells in culture dish via dose-dependent inhibition on NF-κB activation, indicating that it had a direct anti-inflammatory effect. Further study showed that metformin realized its inhibition effect on NF-κB activation by blocking the phosphatidylinositol-3-kinase (PI3K)-Akt signaling

pathway.[48] However, other studies considered that metformin inhibited the activity of IKK by activating AMP-activated protein kinase (AMPK), and thus decreased the phosphorylation and degradation of IκB, resulting in the inhibition on NF-κB activation.[49] Our study found that metformin inhibits activation of NF-κB and decreases serum CRP concentration in vivo, but the exact upstream signaling pathway for these effects was not investigated. AMP-activated protein kinase, a critical signaling molecule for the regulation of multiple metabolic processes, is now considered as a novel anti-inflammatory signaling pathway.[50] The AMPK–NF-κB signaling pathway may play an important role in metformin's antiatherogenic effect by regulating the inflammatory response, although the exact molecular mechanisms responsible for this action need further study.

In conclusion, our study demonstrated that metformin had an antiatherogenic effect. The mechanism may be related to the inhibition of NF-κB activation, thereby preventing the vascular inflammatory response which eventually induces atherogenesis.

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