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Atherosclerosis lesion is accelerated by persistent systemic inflammation but attenuated by saponins from *Panax Notoginseng* in rabbits ☆

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Abstract

Objective: To explore the roles of persistent systemic inflammation in atherosclerosis and the effects of saponins of *Panax Notoginseng* (PNS) on this process in rabbits. **Methods:** Thirty rabbits were divided randomly and equally into 6 groups, *i.e.*, control, high-fat diet, inflammation, aspirin, PNS and simple-inflammation group. All the animals except that in control group and simple-inflammation group were fed with high-fat diet for 8 weeks. Based on that, rabbits in inflammation, aspirin and PNS groups were treated with zymosan injection (10 mg/kg, i.p.). Normal saline was given to rabbits in control group. Besides zymosan injection, animals in aspirin and PNS group were administrated with aspirin (12 mg/kg, i.g.) and PNS (120 mg/kg, i.g.) respectively. The animals in simple-inflammation group were treated with zymosan injection (10mg/kg, i.p.) and fed with normal diet. The atherosclerosis lesion in aortas was observed by Sudan IV staining. Serum total cholesterol, triglyceride (TG), TNF-α and activity of post-heparin lipoprotein lipase (LPL) were measured at the end of the 4th and 8th week after an overnight fast. **Results:** Compared with high-fat diet group, the area of atherosclerosis lesion, serum TG and TNF-α were markedly increased in rabbits of inflammation group, and the activity of LPL was decreased remarkably. Serum TNF-α level was negatively correlated with the activity of post-heparin LPL (r=−0.708, P<0.01). The area of atherosclerosis, serum TG and TNF-α were decreased in aspirin and PNS group compared with that in inflammation group, and the activity of LPL was increased remarkably. Compared with control group, serum TG and TNF-α were markedly increased in simple-inflammation group, while LPL activity was decreased. Atherosclerotic lesion did not occur in simple-inflammation group. **Conclusion:** Persistent systemic inflammation could accelerate the formation of atherosclerosis lesion in aortas, which partly depend on the decreasing of the activity of post-heparin lipoprotein lipase. PNS could improve the changes caused by inflammation.

Keywords: Atherosclerosis; Saponins of panax notoginseng; Lipoprotein lipase; Inflammation

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1. Introduction

Cardiovascular disease is one of the major health care problems and the most common cause of death among individuals from developed and developing nations. Atherosclerosis is known to be a disease which results in a host of complications of cardiovascular disease, including ischemia, acute coronary syndromes and stroke. It has been demonstrated that AS is not merely the passive accumulation of lipids within artery walls. It seems that AS should be a complex result of extensive factors, although the acceptable explanation for the mechanism of the formation of AS has not been presented.

Epidemiological studies found that patients with infected diseases had higher risk of influencing with atherosclerosis [1], which indicated that inflammatory factor, may played an important role in the initiation and progression of AS. The inflammatory component of atherogenesis has been increasingly recognized over the last decade. Inflammation participates in all stages of atherosclerosis, not only during initiation and evolution of lesions, but also with precipitation of acute thrombotic complications [2]. Previous clinical studies showed the strong and consistent relationships between the initiation and progression of AS and some markers of inflammation. For example, Sbarsi's study on 248 patients with coronary artery disease indicated the important role of TNF and TNF receptors in atherosclerosis [3], and Schillinger found the evidence for a close temporal correlation between inflammatory biomarkers hs-CRP and morphological features of rapidly progressive carotid atherosclerosis [4]. Although some studies showed several inflammatory markers had been linked to coronary heart disease and to hyperlipidaemia and several risk factors [5], and the correlation of inflammation and lipid metabolism has been studied in diabetes and the other metabolic disease [6], the relationship between inflammation and hyperlipidaemia in atherosclerosis has not been investigated extremely. Hence, the present study was designed to reveal the interaction between inflammation and lipid metabolism during the procession of atherosclerosis.

Saponins of *Panax Notoginseng* (PNS) is the effective extracted ingredient from *Panax Notoginseng*, a traditional Chinese herb. The studies on PNS showed that it had many positive effects on cardiovascular system and lipid metabolism, such as inhibitory effect on vascular smooth muscle cell proliferation [7], anti-hyperlipemia [8], protection of artery injury [9], *etc*. Besides the effects above, its anti-inflammation role had been interested too [10]. In this research, we tried to approach the mechanism of the effects of PNS on cardiovascular system.

2. Materials and methods

2.1. Materials

Aspirin, cholesterol and zymosan A were obtained from Sigma Chemical Co. PNS powder (purity 99%) was gifted by Kunming Research Institute of Botany, Chinese Academy of Science. TNF-α ELISA kit was obtained from Jingmei Biothec Co. TC and TG kits were obtained from Rongsheng biotech Co. LPL activity kit was purchased from Jiancheng Bioengineering Co. Nanjing, China.

2.2. Animals and experimental protocol

Male Japanese rabbits weighting 2.2–2.5 kg, obtained from the Third Military Medical University Experimental Animal Center. Animals were maintained in accordance with guidelines of the committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW publication No. [NIH] FS-23) on Animal Care.

Thirty male Japanese rabbits were divided into six groups randomly. All of the rabbits except that in control and simple-inflammation were fed with high fat diet (0.5% cholesterol, 5% yolk powder, and 5% lard). Based on that, rabbits in inflammation group were injected each two days with 10 mg/ml zymosan suspension (10 mg/kg, i.p.). Normal saline was given to rabbits as control. Besides zymosan injection, animals in aspirin and PNS group were administrated with aspirin (12 mg/kg, i.g.) and PNS (120 mg/kg, i.g.). The animals in simple inflammation group were treated with zymosan injection (10 mg/kg, i.p.) and fed with normal diet. The animals in control group were administrated with standard chows and normal sodium injection i.p..

2.3. Lesion assessment

Anaesthetic rabbits were killed by air-embolism at the end of the 8th week. The aortas of animals were isolated from aortic arch to the end piece of thoracic aorta and opened longitudinally. To identify lipid-rich intraluminal lesions, the aortas were stained with Sudan IV. Image analysis was performed using Image Pro Plus (Version 4.5). The amount of lesion formation in each animal was expressed as percent lesion area per total area of the aorta.

2.4. Analysis of serum total cholesterol, triglyceride and TNF-α

After an overnight fast, venous blood from ears was sampled at the end of the 4th and 8th week for measurement. Serum was separated by spin for 10 min (2 000 r/min). Concentrations of serum total cholesterol and triglyceride were determined enzymatically using commercially available kits. Serum TNF-α concentration was quantitated by ELISA kit.

2.5. Activity of post-heparin LPL

At the end of the 4th and 8th week, after an overnight fast, blood samples were drawn 15 min after intravenous injection with heparin (150 IU/kg). The samples were immediately chilled to 4 °C and stored at −80 °C until assayed. Post-heparin LPL activity was determined by following the manufacturer's instruction.

2.6. Statistical analyses

All data were expressed as the *mean*±*SD*. Comparisons among the groups were made by an ANOVA analysis with SPSS 8.0 for Windows. Pearson correlation coefficients were used to describe the association between LPL and serum TNF-α. $P<0.05$ was considered statistically significant.

3. Results

3.1. Atherosclerotic area ratio in aortas

Severe atherosclerotic lesions were found in all groups except control group after 8 weeks feeding. In inflammation group, treatment with zymosan caused a noticeable increase ($P<0.05$) in atherosclerotic area ratio compared with which in high-fat diet group. Lesion area in aspirin and PNS groups was lower than that in inflammation group ($P<0.05$) (Fig. 1, 2).

3.2. Animal plasma total cholesterol and triglyceride levels

Hypertriglyceridemia and hypercholesteraemia occurred in animals of all groups with high-fat diet. Treatment of Zymosan remarkably accelerated the increase in plasma TG caused by high fat feeding. Compared with control group, TG level was remarkable increased in simple-inflammation group. Noticeable reduction of TG level was observed in the aspirin and PNS group. There was no statistical significance of TC level among all groups except control and simple-inflammation group (Table 1).

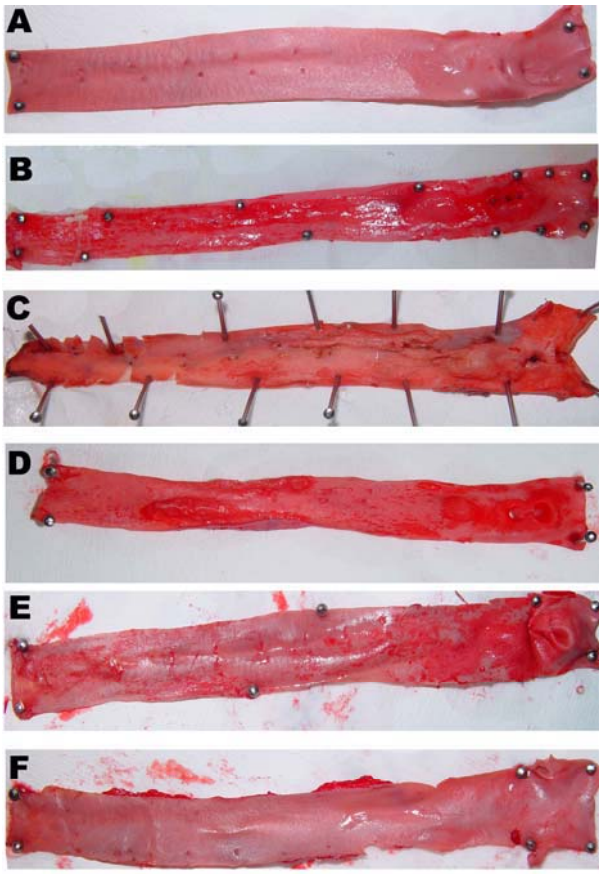

Fig. 1. Photographs show the atherosclerosis area in aortas (sudan IV staining). A: Control group; B: High-fat group; C: Inflammation group; D: Aspirin group; E: PNS group; F: Simple-inflammation group.

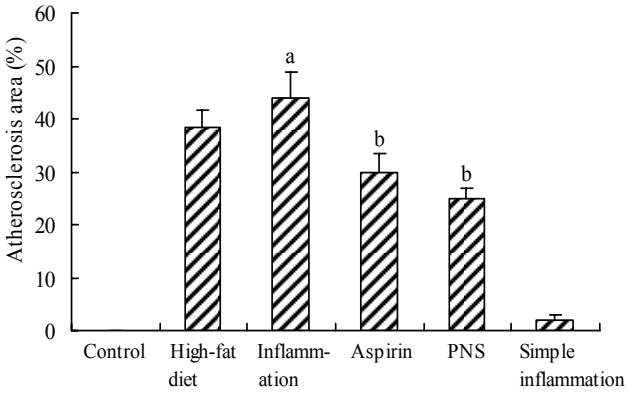

Fig. 2. Graph shows the mean values of atherosclerosis area ratio. Treatment with zymosan revealed remarkable lower lesion area than high-fat diet group. Lesion area in aspirin and PNS treatment groups are lower than zymosan group. n=5. *mean±SD*. [a] $P<0.05$ *vs* high-fat diet group, [b] $P<0.05$ *vs* inflammation group.

3.3. Serum TNF-α level

The treatment of Zymosan simply noticeably increased serum TNF-α level. The treatments with zymosan and high-fat diet together caused remarkable increase of TNF-α level compared with high-fat diet group. The level of serum TNF-α of aspirin and PNS group was remarkable reduced compared with inflammation group (Table 2).

3.4. Post-heparin LPL activity

At the end of the 4th and 8th week, post-heparin LPL activity of animals in inflammation group was reduced compared with that in high-fat diet group (*P<0.05*). Treatment of zymosan simple could decrease the activity of post-heparin LPL. The treatment of aspirin and PNS could recover the activity of post-heparin LPL partly (Table 3).

4. Discussion

There was increasing evidences to support the role of inflammation in the initiation and development of atherosclerosis [11]. Jongstra *et al* [12] found local chronic inflammation of arterial intima could predispose to atherosclerosis in mice, which provided a solid evidence for the importance of inflammation in atherosclerosis. On the base of classic atherosclerosis model, we treated the animals with zymosan injection to induce a persistent systemic inflammation in rabbits. Zymosan was used to induce multiple organ failure in the animal models with injection i.p. *via* tightly non-septic systemic inflammation [13]. We adjusted the dosage of zymosan in order to cause a persistent systemic inflammation in rabbits. The results showed more atherosclerotic area occurred in aortas of zymosan-treated animals compared with that in high-fat diet group, which indicated that the persistent systemic inflammation could accelerate the formation of atherosclerosis lesion.

Table 1

Effects of persistent inflammation in high-fat diet rabbits and the effects of aspirin(12 mg/kg) and Saponins of *Panax notoginseng* (PNS, 120 mg/kg) on serum TC and TG level. (n=5, *mean±SD*)

Group	TC (mmol/L)			TG (mmol/L)		
	0 week	4th week	8th week	0 week	4th week	8th week
Control	3.5±1.2	4.0±1.4	4.1±1.5	0.59±0.18	0.56±0.18	0.67±0.27
High-fat diet	2.9±0.7	22.9±3.7	22.2±3.2	0.62±0.14	2.32±0.93	2.28±1.00
Inflammation	3.4±0.6	23.0±4.1	22.5±4.2	0.58±0.18	4.42±0.94[b]	4.74±0.99[b]
Aspirin	3.3±0.7	20.2±4.3	21.36±3.7	0.57±0.19	2.58±0.79[c]	2.50±0.89[c]
PNS	3.3±0.8	21.0±3.4	21.9±3.5	0.58±0.16	2.82±0.93[c]	2.33±0.92[c]
Simple-inflammation	3.0±0.8	3.9±0.8	4.3±1.1	0.72±0.12	1.01±0.21[a]	1.13±0.26[a]

[a] $P<0.05$ *vs* control group, [b] $P<0.05$ *vs* high-fat diet group, [c] $P<0.05$ *vs* inflammation group.

Table 2

Effects of persistent inflammation in high-fat diet rabbits and the effects of aspirin (12 mg/kg) and Saponins of *Panax notoginseng* (PNS, 120 mg/kg) on serum TNF-α level. (*n*=5, *mean*±*SD*)

Group	TNF-α (pg/ml)		
	0 week	4th week	8th week
Control	108±22	114±20	121±25
High-fat diet	112±27	215±55	208±54
Inflammation	105±20	384±56[b]	348±60[b]
Aspirin	104±20	264±35[c]	257±47[c]
PNS	108±24	276±55[c]	252±60[c]
Simple-inflammation	104±25	265±48[a]	272±58[a]

[a] $P<0.05$ *vs* control group, [b] $P<0.05$ *vs* high-fat diet group, [c] $P<0.05$ *vs* inflammation group.

Table 3

Effects of persistent inflammation in high-fat diet rabbits and the effects aspirin (12 mg/kg) and Saponins of *Panax notoginseng* (PNS, 120 mg/kg) on serum post-heparin LPL activity to FFA. (*n*=5, *mean*±*SD*)

Group	LPL activity ($\mu mol \cdot ml^{-1} \cdot h^{-1}$)		
	0 week	4th week	8th week
Control	95.3±18.1	91.9±10.4	92.1±11.3
High-fat diet	96.5±8.7	83.2±9.9	83.0±7.6
Inflammation	98.3±10.5	63.1±9.5[b]	66.6±9.4[b]
Aspirin	97.5±9.9	83.9±10.0[c]	80.3±11.3[c]
PNS	109.5±7.4	84.6±9.0[c]	79.9±9.0[c]
Simple-inflammation	97.1±8.4	85.9±7.2[a]	82.3±8.2[a]

[a] $P<0.05$ *vs* control group, [b] $P<0.05$ *vs* high-fat diet group, [c] $P<0.05$ *vs* inflammation group.

Besides the changes of atherosclerotic area ratio, we found the level of TG in animals of inflammation group was noticeable higher than that in high-fat diet group. The similar changes occurred in the level of serum TNF-α. The change of post-heparin lipoprotein lipase activity was contrary to TG and TNF-α. TG is one of the received major risk factors of atherosclerosis [14]. It's obvious that the increased level of TG would cause the lesion getting worse. To observe the reason of the changes of TG, the activity of post-heparin LPL was focused in our study. Lipoprotein lipase (LPL) is expressed in the membrane of blood vessel, muscle tissue and lipid tissue, and plays an important role in metabolism of lipid, especially in TG metabolism. It is now thought to be associated with AS, as well as inflammation. Studies on LPL showed that high activity of LPL seemed to be beneficial to body. In 1989, Eckel demonstrated that the up-regulation of LPL would have patient role of obesity therapy [15]. Jensen found the prevention effects of diet-induced obesity in transgenic mice over-expressing skeletal muscle lipoprotein lipase [16]. Tomonari's research found the over-expression of lipoprotein lipase in transgenic heritable hyperlipidemic rabbits could improves hyperlipidemia and obesity [17]. And the agonist of LPL, NO-1886 could decrease the ectopic lipid deposition in diet-induced diabetic swine [18] and suppresses fat accumulation in high-fat fed rats [19]. On the other side, Henderson's study on 730 coronary artery disease patients found that the activity of post heparin serum LPL of patients were remarkable lower than that of normal [20]. These results above indicated the probable relationship between the activity of post heparin serum LPL and cardiovascular disease, including atherosclerosis. And some studies showed the relationship of inflammation and LPL activity. Eynatten's results demonstrated an association of decreased post- heparin LPL activity with systemic inflammation [21]. The studies all above suggested that LPL might be a linkage between inflammation and atherosclerosis. We observed the changes of activity of LPL and TNF-α level in this study, and tried to analysis the interaction of them.

The result of the relationship analysis between LPL activity and TNF-α showed that there was a negatively relationship between them($r=-0.708$, $P<0.01$). The result was coincidence with the finding of Eynattenl [21]. We proposed that inflammation could aggravate the TG level in high-fat diet rabbits through decreasing the activity of post-heparin LPL, and accelerated the formation of AS lesion at the end.

To verify the hypothesis, aspirin, one of the classic anti-inflammation drugs, was used in our

study as the antagonist of inflammation. Aspirin is a classic non-steroidal anti-inflammatory drug. It represents the anti-inflammation effect through inhibiting cyclooxygenase. Our data showed aspirin reduced TNF-α level in rabbits compared with inflammation group, and the area ratio of AS lesion in aspirin group was remarkably less than which in inflammation group. Besides, aspirin decreased TG level and increased post-heparin LPL activity. The results showed that aspirin could recover the reduced activity of LPL by inflammation, and then reduce the level of TG.

Simple inflammation stimulation caused remarkable increase TG level. There was almost no significant atherosclerosis lesion occurred in simple-inflammation group. It seems that the TG level of simple-inflammation group was not high enough to cause the formation of atherosclerosis. But the changes of TG, TNF-α and LPL activity supported the hypothesis above.

PNS has received beneficial effects on cardiovascular system, but the mechanism of its effects are not clarified. It has the modulatory effect on inflammation besides the effects above [22]. Our data showed PNS could reduce the formation of atherosclerotic lesion. Similarly to aspirin, the changes caused by PNS included increasing the activity of LPL, decreasing TNF-α and TG. The results demonstrated a positive effect of PNS on the interaction between inflammation and TG metabolism in atherosclerosis. It provided solid evidence for the therapeutic effects of PNS on cardiovascular system.

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