Decreased level of the anti-inflammatory adipokines, secreted frizzled-related protein 5 and adiponectin, in high cholesterol diet-induced atherosclerotic rats

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Abstract

Introduction: The involvement of secreted frizzled-related protein 5 (SFRP5) and adiponectin, two important adipokines produced by adipocytes, in cardiovascular diseases demands further assessment. Therefore, in this study the relation of the adipokines and atherosclerosis was evaluated in Rat.
Materials and methods: For the study, thirty male Wistar rats were divided into 2 groups (each group contain 15 rats): Control group, received a normal diet and the high cholesterol diet (HCD) group which received an additional 2% cholesterol and 0.5% cholic acid for 15 weeks. At the end of treatment, HCD-induced atherosclerotic plaques were observed by hematoxylin and eosin staining of aortic tissue sections. Furthermore, serum levels of SFRP5 and adiponectin in the two groups of rats were measured by immunoassay and their relationships with the development of atherosclerotic plaques in the animals were analyzed.
Results: The serum level of SFRP5 and adiponectin was significantly decreased in HCD rats compared with the control group ($P<0.05$). There was also an inverse relation between the serum level of the two adipokines and atherosclerotic plaque formation ($P<0.05$).
Conclusion: Serum levels of SFRP5 and adiponectin are decreased in rats fed with high cholesterol diet, highlighting the involvement of the two adipokines in atherosclerosis.

Keywords: High cholesterol diet, Atherosclerotic plaque, Adiponectin, SFRP5, Rat

Introduction

Cardiovascular diseases (CVDs) are known as the first cause of death in the world. It has been estimated that in 2012, 17.5 million individuals, that is about 31% of all the global deaths, died from CVDs (1). The main cause of cardiovascular diseases is atherosclerosis (2). Adipose tissue through excreting a number of adipokines that exhibit pro-inflammatory or anti-inflammatory activity, works as a major endocrine organ (3). So, it can provide a novel therapeutic strategy for the treatment of inflammation related metabolic disorders and cardiovascular disease through targeting the molecular mechanisms that lead to dysregulation of adipokines (4). Adiponectin is a plasma protein derived from adipocyte that is accumulated in the injured artery and has potentially anti-inflammatory properties (5).

As a family of soluble proteins, the secreted Frizzled-related proteins (SFRPs) are structurally related to Frizzled (Fz) proteins, the serpentine receptors that mediate the extensively used cell-cell communication pathway involving Wnt signaling. SFRPs were immediately characterized as antagonists bind to Wnt proteins to prevent signal activation because of their homology with the Wnt-binding domain on the Fz receptors (6).

Recently, a protein called the SFRP5 has been discovered that is secreted by adipocytes, is involved in inflammation and insulin resistance in mouse models of obesity and type 2 diabetes mellitus (7). SFRP is expressed also in pericardium, epicardium and endocardium, as well as in all chamber myocardium except for the right ventricle (8). The function of Wnt signaling in cardiac development has been reported frequently (9). Enhanced proliferation and inhibition of further differentiation is a result of the hyper activation of Wnt/β-catenin signaling in cardiac precursors (10). Specifically, a finding reported that coronary artery disease (CAD) patients the serum SFRP5 level was significantly lower than those in non-CAD subjects and the low SFRP5 levels was contributed to CAD (11).

A few Studies about the circulating levels of SFRP5 in coronary artery disease (CAD) have been done. In a research about individuals with and without CAD, Miyoshi et al (12), suggested that low serum SFRP5 levels were independently associated with CAD. Moreover, also in subjects with obesity and type 2 diabetes, it has been shown that levels of plasma SERP5 are decreased (13).

There is some controversy regarding the contribution of adiponectin in CAD. It has been apparently proposed as an inverse predictor for prognosis in CAD patients, however, the high levels of adiponectin have been associated with low CAD risk in asymptomatic subjects (14). And also, adiponectin is considered as a linkage between obesity and atherosclerosis (15).

Given the mentioned data on the cardiovascular effects of SFRP5 and adiponectin, we hypothesize that the two adipokine, SFRP5 and adiponectin, may have a potential role in the development of atherosclerosis and CAD.

Therefore, the aim of this study was to evaluate the role of the adipokines, adiponectin and SFRP5, in high cholesterol diet (HCD)-induced atherosclerotic rats.

Materials and methods

Animal: Adult male Wistar rats, weighting 200-250 g, were maintained at the temperature 23 ± 2°C, 50-55% humidity, and a circadian cycle of 12 hours lightness and 12 hours darkness. Commercial rat food pellets and water were available ad libitum. The animals were randomly divided into two groups (in each groups, n=15). Control group received a normal diet and the HCD group received a high cholesterol diet (2 % cholesterol and 0.5% cholic acid added to normal diet) for 15 weeks.

The total 2% supplementary cholesterol received daily by rats on the HCD group consisted of 1% cholesterol mixed with normal food (standard pellet) and 1% cholesterol mixed with sunflower oil, which was given by oral gavage. The animals received humane care, since this study adheres to for animal research rules that reviewed and approved by institutional appointed committee and it was also approved by Ethics Committee of university of Ilam medical sciences.

Histological and biochemical evaluation: After 15 weeks, blood samples were collected through the heart of animals.

Serum SFRP5 and adiponectin concentrations were determined using commercial immunoassay kits (USCN Life Science Inc, Wuhan, China) based on the instruction of their manufacturers.
Animals were anesthetized by inhalation of diethyl ether, after which terminal blood sample withdrawal from the cardiac ventricles was performed with the use of 2.5-ml syringes. Then, the aorta was resected and fixed in fixator for 24 hours, embedded in paraffin, after which 6 μm sections were cut and hematoxylin and eosin staining procedure was performed. The sections were observed under light microscope (Lica-1100) for qualitative and quantitative changes in the aorta structure.

Statistical analysis

Data were presented as mean ± SD and the differences between the 2 groups were evaluated with an independent t-test. A p value < 0.05 was set as significant.

Results

The serum levels of adiponectin and SFRP5 were significantly decreased in the HCD compared with the control group (Table 1). Hematoxylin and eosin -stained sections of aortic vessels are shown in Figure1 (panels A and C). In the control group, normal aortic vessels were observed. The lumen was large without any narrowing by atherosclerotic plaque (Figure 1, panel A), whereas in the rats receiving cholesterol, numerous foam cells and occasional cholesterol cleft were observed in the HCD group, the proliferation of smooth muscle cells and thickening of the intima could be seen (Figure1, panels B and C).

Table1. Comparison of serum levels of adiponectin and SFRP5 in the studied rats.

Variable	Control group	HCD group	P value
SFRP5 (ng/ml)	75.8±0.97	99.8±6.9	0.001
Adiponectin (pg/ml)	68.2±1.9	78±6.1	0.001

Data are expressed as means ± SD; SFRP5, secreted frizzled-related protein5; HCD, high cholesterol diet.

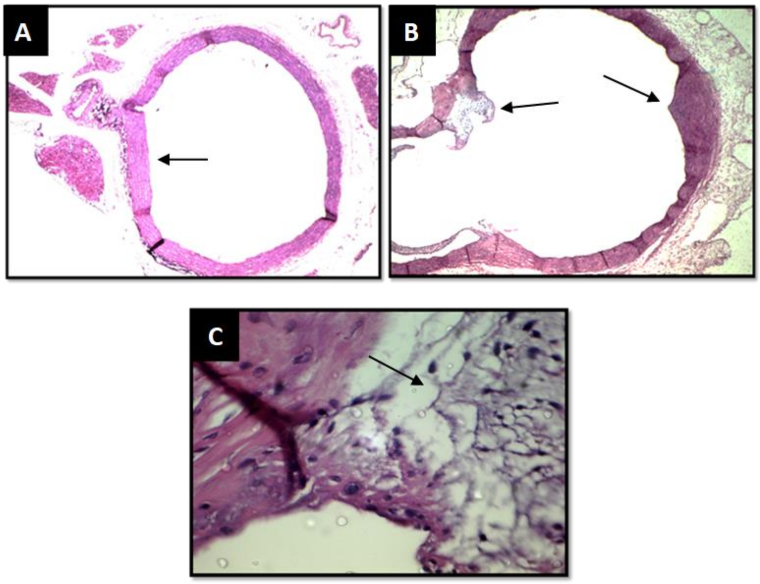

Figure 1. Histopathological evaluation of atherosclerotic plaques in rats fed a high cholesterol diet. Aortic tissue sections were stained by hematoxylin and eosin. (A) Aortic vessel in the control group after 15 weeks; (B, C), Aortic vessel after 15 weeks in the high cholesterol diet group (plaques in inner layer are observed).

Discussion

In this study, 15 weeks of administration of a HCD was used to induce atherosclerotic plaque formation in Wistar rats. After this period, plaque formation was clearly observed in aortic sections and the levels of SFRP5 and adiponectin were significantly decreased. Development of atherosclerosis, obesity, insulin resistance and fatty liver disease is protected by adiponectin (17). Inhibition of inflammatory factors, such as TNF-α in an NF-kB-dependent manner protects the anti-atherosclerotic effects of adiponectin (18, 19). Adiponectin levels could also represent an important marker for patients at higher risk of cardiovascular disease. Clinically, adiponectin is served as a biomarker for cardiovascular disease. However, as a biologically active molecule, it protects the vasculature at all stages of atherogenesis. Atherosclerosis is considered largely as an inflammatory disease (20). A recent study showed that the serum adiponectin level was reduced through the use of high fat diets in rats, along with a decrease in serum levels of HDL-C and increased plasma lipid levels and weight gain (21). It was appeared that patients with CAD were taking antiplatelet agents, angiotensinogen converting enzyme inhibitors or angiotensin receptor blocker as well as statin, but the concentration of their adiponectin was significantly lower than that of the control group (22). In the present study, the serum adiponectin level in HCD fed rats showed a significant decrease compared to control group. The results of this study are consistent with previous studies.

The implication of SFRP5 were recently demonstrated, as an anti-inflammatory biomarker, in patients with CAD in comparison with non-CAD patients (12). For the first time in this study, the level of SFRP5 serum in male Wistar rats compared to control group was demonstrated.

The mechanisms by which low SFRP5 levels are associated with increased risk of CAD are not clearly determined. One SFRP5 mechanistic link with CAD may be related the inhibitory effects of inflammatory mediators produced by macrophages and/or adipocytes (7). Furthermore, a potent relationship between serum SFRP5 levels and oxidative stress was explore in human (11). SFRP5 lead to the suppression of inflammatory response in rheumatoid arthritis fibroblast-like synoviocytes (23). Decreased level of SFRP5 is accompanied by a significant high level of the pro-inflammatory risk factor, Wnt5a in chronic inflammatory diseases such as obesity and type 2 diabetes **(**24, 25). It should be regarded that Wnt signaling contributes in atherosclerosis and cardiovascular diseases (26). Furthermore, some studies showed that SFRP5 represses inflammatory reaction following ischemia/reperfusion damage in the heart of mice via antagonizing the non-canonical wnt5a signaling (27**)**. Thus, it can be surmised that in reduced SFRP5 state the inflammatory mediators, oxidative stress and Wnt5a signaling are promoted. Production of inflammatory mediators that are expressed by macrophages and/or adipocytes by antagonizing c-Jun N-terminal kinase 1 phosphorylation, a downstream target of non-canonical WNT5a signaling may be inhibited by SFRP5 (7). It has been shown that WNT5a secreted from inflammatory cells repress the differentiation of preadipocytes into mature fat cells, highlighting that WNT5a behaves as a pro-inflammatory factor (24). It should be noted that the serum SFRP5 level in the subjects with CAD is significantly decreased compared with those in the non-CAD subjects (12).

Additionally, the serum SFRP5 levels in CAD patients elevated significantly among individuals over 65 years of age (28). In the present study, it was first shown that the serum SFRP5 levels in rats fed with HCD was significantly decreased. Due to the restricted studies on the serum SFRP5 levels, further studies are needed.

Conclusion

According to the results of this study, in HCD-induced atherosclerotic rats, the serum levels of the adipokine, SFRP5 and adiponectin, showed a significant decrease compared to that in control rats, therefore, it seems that the adipokines have a fundamental role in atherosclerosis development.

Acknowledgments

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