



Effects of imbalance of lipid metabolism through NF-KB pathway on atherosclerosis and vascular aging in rats

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ABSTRACT

Cardiovascular diseases have become a mainstream disease by intensifying the country's population aging. The purpose of this article is to explore the specific effect and mechanism of lipid metabolism imbalance through the NF-KB pathway on atherosclerosis and vascular aging in rats. Twenty healthy adult rats were randomly divided into two groups, control in the observation group and the observation group. The rats in the observation group were fed a high-fat diet to imbalance the lipid metabolism of the rats. Immunohistochemistry and transmission electron microscope detectors were used to observe the NF-KB pathway in rats and study atherosclerosis-specific conditions of sclerosis and vascular aging. The results show that the imbalance of lipid metabolism through the NF-KB pathway can increase the rate of apoptosis in rat blood vessels by 24% and the proliferation rate by 18%. The number of vascular endothelial cell damage increased by 33%, which promoted atherosclerosis in rats and increased the rate of vascular aging in rats by 27%.

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Introduction

Atherosclerosis (AS) is a manifestation of arteriosclerosis. It is caused by many factors and is one of the diseases that seriously endanger human health, especially in the elderly. Jing pointed out that the main manifestations of atherosclerosis are large and middle arteries with lipid accumulation, hemorrhage, thrombosis, fibrous tissue deposition, calcification, which lead to thickening and hardening of the arterial wall and narrowing of the blood vessels (1). Xiao emphasized in the article that in recent years, due to the severe aging population, huge social pressure, people's general lack of exercise, hypertension, diabetes, and changes in diet structure, the incidence and mortality of atherosclerosis have been increasing year by year (2). Da Motta believes that atherosclerosis is a common and frequent disease of cardiovascular and cerebrovascular diseases, and it is also the main factor leading to coronary heart disease, myocardial infarction, cerebral infarction, cerebral thrombosis and other cerebral cardiovascular diseases (3). Litwin pointed out that how to prevent and treat atherosclerosis is a current problem in the medical community, and exploring the pathogenesis

of atherosclerosis is an important way to treat cardiovascular and cerebrovascular diseases (4). Therefore, it is very important to discuss the treatment and research of AS.

The imbalance of lipid metabolism is an important signal of body dysfunction. Xiaorong emphasized in the article that the imbalance of lipid metabolism refers to the loss of balance when lipid substances are synthesized, decomposed and operated in the body (5). Wu confirmed that the main manifestations of imbalance of lipid metabolism are the increase of TG, LDL, VLDL, TC and the decrease of HDL in the body (6). Huang pointed out that lipid toxicity can occur when imbalanced lipid metabolism. Lipid toxicity refers to the increase in the level of free fatty acids (FFA) in the blood or the increase in intracellular fat content, which exceeds the oxidative ability of various tissue cells to FFA and the fat tissue to fat qualitative storage capacity (7). Park research found that excessive FFA in the form of TG is excessively deposited in the target tissues and organs of insulin action, such as adipose tissue, blood vessels and liver, causing IR, and excessive deposition in the pancreatic islets can make the islet β cells secrete obstacles

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appear (8). In summary, insulin resistance and pancreatic β -cell secretory dysfunction will have an effect on intracellular cell proliferation, migration, and apoptosis through the NF-KB pathway, which may eventually lead to atherosclerosis and vascular aging. This study is mainly to solve some problems in the detection technology and observation methods in the study of the effect of lipid metabolism imbalance through the NF-KB pathway on atherosclerosis and vascular aging in rats, so this article is carried out on the research content and detection methods. In order to improve and innovate, this article is the first to study the aging of vascular tissue and the expression of the NF-KB pathway in atherosclerosis animal models by immunohistochemistry and RT-PCR technology. At the same time, it also studies the imbalance of lipid metabolism and atherosclerosis. Analysis of the relationship between the results showed that the detection and observation by immunohistochemistry and RT-PCR technology can improve the observation effect by 15% and the accuracy of the data by 19%. Generally, this paper uses a new detection method to improve detection efficiency and accuracy, which is very helpful for subsequent research and analysis.

Materials and methods

Selection of experimental objects

In order to investigate the specific effect of lipid metabolism imbalance on atherosclerosis and vascular aging in rats through the NF-KB pathway, this paper designed an experiment. In the experiment, 20 male rats (provided by the Department of Animal Science of Nanjing Medical University) were selected. They were similar in age, weighing between 20g and 30g, with good health and normal ability to transport. The white mice are housed in cages, the feeding temperature is 25 ± 1 °C, the laboratory temperature and humidity are 35% to 40%, and the daytime cycle is 7:00 to 18:00. The other group is the control group.

Experimental materials

The main equipment used in this experiment: NP1640 medium, NF-KB monoclonal antibody non-immune serum, Y1008 electronic balance, flow cytometer, pathological image analyzer, arterial cell detector, BX230 transmission electron-optical microscope, 735B type Spectrophotometer (Jiangsu

Zhongdu Analytical Instrument Co, Ltd.), 786S type ultraviolet spectrophotometer (Jiangsu Zhongdu Analytical Instrument Co, Ltd.), fluorescence display, PSCX imaging instrument, lipid metabolism analyzer, etc. Other auxiliary equipment and reagents (Table 1) are needed for this experiment.

Table 1. Other auxiliary equipment and reagents

Group	Usage amount	Source
Automatic biochemical analyzer	1	Sony Group of Japan
Vascular Transmission Microscope	1	Xin He Hospital
Absolute ethanol	300ml	Shanghai Analytical Instruments

Detection method

The index of lipid metabolism imbalance in rats adopts the SYBT detection method, the application of the lipid metabolism detection kit, the expression formula of TLR-4 is calculated by relative quantification, and the standardized ratio is 2CT method. The specific expression of lipid metabolism was determined by the rat lipid metabolism gene. Serum NF-KB levels were detected by immunohistochemical analysis. The thickness of the rat aortic blood vessel was measured by the PLS5.0 pathological image analysis system, and the maximum vertical thickness of the aortic vascular adventitia basement membrane to the vascular intima surface was taken as the measurement value.

Results and discussion

Analysis of the effect of lipid metabolism imbalance on atherosclerosis

The study found that the imbalance of lipid metabolism can promote the occurrence and development of vascular complications by activating the NF-KB pathway, namely: increased activity of the NF-KB pathway, increased formation of end-products of advanced lipid metabolism, and nuclear factor-(NF-KB). The number increases and the activity of the hexosamine pathway increases. In addition, the changes in atherosclerosis index (Table 2) under the imbalance of lipid metabolism can also directly inhibit the proliferation of vascular endothelial cells (VEC), induce VEC apoptosis, and at the same time promote

the expression of adhesion molecules, so that monocytes adhere to the arterial VEC. It leads to thickening of the intima-media of the artery, which promotes the occurrence of atherosclerosis, a steady-state imbalance of lipid metabolism. Compared with patients with non-lipid metabolic imbalance, the incidence of arterial ischemic stroke is 2 to 3 times higher, and the incidence of ischemic stroke in patients with cardiovascular disease is twice that of patients without cardiovascular disease. In addition to the imbalance of lipid metabolism, another major factor that triggers atherosclerosis due to changes in NF-KB levels is damage to vascular cell proliferation, autophagy, and apoptosis systems.

Table 2. Comparison of atherosclerosis indexes under the imbalance of lipid metabolism; (A) Project Team, (B) Before imbalance of lipid metabolism, (C) After imbalance of lipid metabolism, (D) After the experiment

A	B	C	D
Monocyte activity	67±0.584	178±0.235	320±2.36
VEC apoptosis	72±0.448	166±0.485	219±3.24
Vascular wall thickness	93±1.435	190±0.628	423±5.13

Analysis of the effect of lipid metabolism imbalance on vascular aging

Vascular calcification mainly occurs in the intima and middle layer of the aorta and medium-sized arteries (Figure 1). From the data in Figure 1, it can be seen that the imbalance of lipid metabolism has caused the acceleration of vascular aging, which has increased the rate of vascular aging in rats by 27%. Arterial calcification often occurs with vascular aging. Although vascular calcification is often accompanied by hypertension and atherosclerotic plaques, the researchers found that calcification of the medial elastic layer does not depend on the appearance of atherosclerosis. At present, it is believed that the main mechanism of calcification of the blood vessel wall is caused by the abnormal metabolism of calcium and phosphorus.

The study found that the imbalance of lipid metabolism is the main reason for the induction of cardiovascular and cerebrovascular diseases, greatly

increasing the probability of cardiovascular and cerebrovascular diseases (Figure 2).

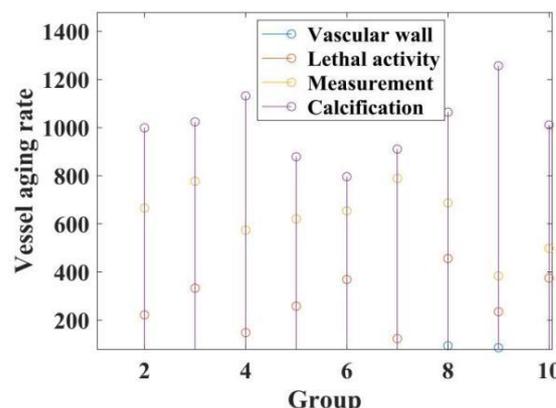


Figure 1. The effect of lipid metabolism imbalance on the speed of vascular aging

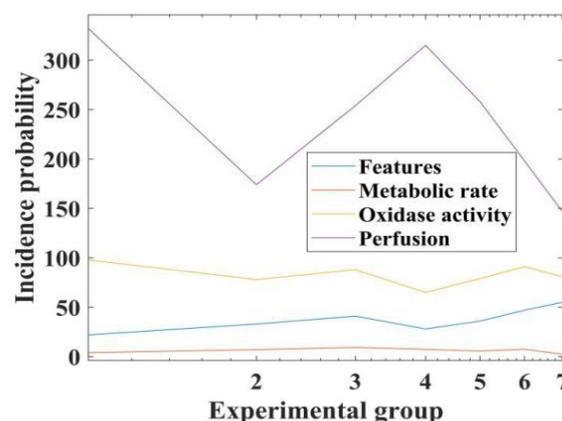


Figure 2. Unbalanced lipid metabolism greatly increases the probability of cardiovascular and cerebrovascular diseases

Comprehensive analysis of the effect of lipid metabolism imbalance through NF-KB pathway on atherosclerosis and vascular aging in rats

The study found that the imbalance of lipid metabolism through the NF-KB pathway will have a certain effect on the rate of cell proliferation, apoptosis, and the number of cell damage in rat blood vessels (Figure 3). The experiments in this paper show that after multiple passages, rat coronary endothelial cells show reduced dysfunction and aging-related proliferative capacity, increased oxidative stress, and activation of NF-KB and p53 signaling pathways. Endothelial cell aging includes telomere-dependent and telomere-independent aging.

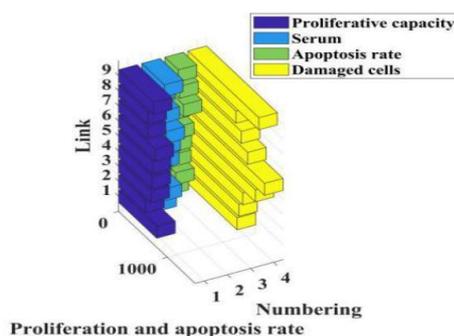


Figure 3. The rate of cell proliferation, apoptosis, and the number of cell damage in blood vessels with a certain effect

The results show that the imbalance of lipid metabolism through the NF-KB pathway can promote atherosclerosis in rats (Figure 4), which increases the thickness of the blood vessel wall and reduces the elasticity of the blood vessel wall. After analysis, the aortic endothelium of the normal group of mice was continuous, intact, and the structure was clear. There was no stenosis of the blood vessel lumen, no atherosclerotic plaque formation, and no obvious inflammatory cell infiltration. In the lipid metabolism imbalance group, the local aortic endothelial structure was destroyed, and obvious plaque formation was observed, the lumen was obviously narrow, and there were a lot of cholesterol crystals and inflammatory cell infiltration in the plaque.

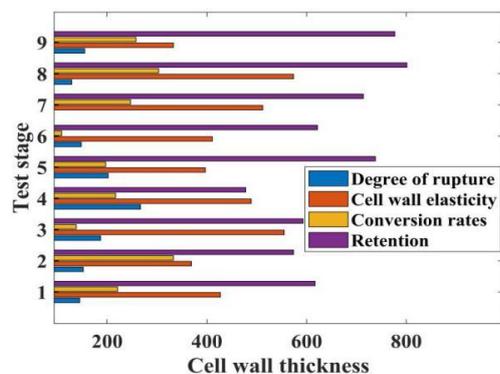


Figure 4. Comprehensive analysis of the effect of lipid metabolism imbalance through NF-KB pathway on atherosclerosis

Atherosclerosis (As) and vascular aging are key causative factors of cardiovascular and cerebrovascular diseases. Atherosclerosis and vascular aging are currently considered to be a multi-factor and multi-step disease involving an imbalance of lipid metabolism and vascular adhesion molecules, cytokines, growth factors, inflammatory cells, vascular endothelial cells (ECs) and vascular smooth

muscle cells (VSMCs) and many other factors. The NF-KB signaling pathway is a classic signal transduction pathway mediated by cytokines. Not only participate in the inflammatory response but also regulate endothelial cell damage, oxidative stress, smooth muscle proliferation and migration, vascular cell apoptosis and other processes. This article focused on the effects and mechanisms of the imbalance of lipid metabolism through the NF-KB signaling pathway and its inhibitors on atherosclerosis and vascular aging, and its mechanism, which is very important for solving cardiovascular and cerebrovascular problems.

The imbalance of lipid metabolism mainly affects the intravascular cells through the NF-KB pathway. The nuclear factor (NF-KB) is a transcription factor with multiple regulatory effects on gene transcription. It can specifically bind to specific sites of various cell gene promoters or enhancer sequences to promote transcription and expression. It can react with inflammation; immune responses are closely related to important pathophysiological processes such as cell proliferation, transformation and apoptosis (9). Studies have shown that the genes involved in the inflammatory response in the formation of atherosclerotic plaques are mostly NF-KB target genes, whose transcriptional expression is regulated by the NF-KB pathway conduction pathway (Figure 5) (10). NF-KB is a nuclear protein factor that can specifically bind to the immunoglobulin chain gene enhancer JCB sequence (GGGACTTTCC), and is a dimeric protein composed of the Rel family (11). There are five members of this family, namely: NF-xB1 (p50), NF-xB2 (p52), RELA (p65), RELB and C-Rel (12). The p65 /C-Rel heterodimer can be different from tissue factor and granulocyte-macrophage colony-stimulating factor (GM-CSF) gene promoter p65/c-Rel the binding site of the source dimer binds, thereby exerting the NF-KB / Rel family's role in regulating the expression of different target genes (13).

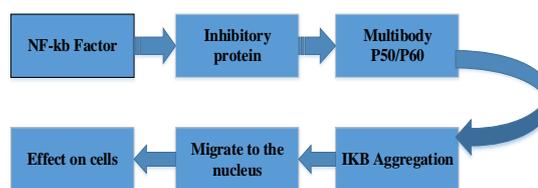


Figure 5. NF-KB pathway conduction path

The study found that when the intravascular cells are at rest, NF- κ B and its inhibitor protein I κ B family members form a hetero multimer P50-P60-I κ B α or I κ B β /3, in the form of an inactive complex, exist in the cytoplasm (14). IKK further causes the phosphorylation and degradation of I κ B molecules, and NF- κ B dissociates from I κ B trimer release NF- κ B and activates the NF- κ B signaling pathway (15). NF- κ B translocate into the cell nucleus, combines with target genes in the nucleus, and initiates the transcription of related genes. Therefore, BRB phosphorylation degradation and dissociation from NF- κ B is the key to this activation pathway, so it is called the IBSE-dependent phosphorylation classic pathway (16). Another non-classical pathway is the activation of NF- κ B-induced kinase leading to phosphorylation and proteolytic processing of cytoplasmic precursors, thereby activating NF- κ B2, and the activated NF- κ B and RELB, NIK to form a complex into vascular cells to regulate genes transcription (17).

Aging refers to the process of degenerative changes in the organizational structure, physiological function and psychological behavior of the body with age. As part of global aging, the vascular aging process (Figure 6) in turn has an important effect on overall aging (18). The mechanism of vascular aging includes intricate network regulation from the tissue to the cell and molecular level. Understanding at any level alone is one-sided and limited. Factors affecting vascular aging include external factors (such as living environment, lifestyle habits) and internal factors (such as genetic factors) (19). The imbalance of the body's steady-state brought about by aging causes the vascular system to be easily damaged and difficult to repair, that is, the blood vessels enter the "aging" state. Among the various diseases related to aging, the incidence of cardiovascular system diseases ranks first, which also shows that vascular aging plays an important role from another perspective (20). With the increase of age, the structure and function of blood vessels will change accordingly (21). In addition, it can be seen that collagen increased, elastic fibers decreased, fractured, and matrix mucopolysaccharide deposition increased (22).



Figure 6. Vascular aging process

The study found that the active expression of the NF- κ B factor in blood vessels can promote the transformation of cells to secreted phenotypes. NF- κ B factor is regulated by Msx2, NF- κ B and β -catenin signaling system (23). Factors that initiate the "ossification cascade" response include bone morphogenetic proteins, chronic traumatic stimuli, and metabolic products including reactive oxygen species (ROS). These factors cause the activation of NF- κ B and inflammatory factors (such as TNF α , IL-1/6) (24, 25). The NF- κ B pathway is an important factor affecting the changes of vascular cells (26, 27).

The current study indicated that the endothelial cell apoptosis induced by an imbalance of lipid metabolism is related to the activation of NF- κ B, which leads to the upregulation of p53 protein expression and the downregulation of Bcl-2 protein expression. From this preliminary speculation that many Chinese medicines have the role of regulating lipid metabolism imbalance, anti-atherosclerosis and cardiovascular protection. The protective mechanism of the active ingredients of traditional Chinese medicine against vascular endothelial injury may be related to inhibiting the activation of NF- κ B, reducing the expression of inflammatory molecules in endothelial cells, and resisting apoptosis.

In summary, NF- κ B, as a key factor in gene transcription, plays an important regulatory role in the formation of atherosclerosis. Therefore, the mechanism of anti-AS of drugs through the NF κ B signal transduction pathway is deeply studied, and the target cell specificity and selective NF- κ B activation blockers in endothelial cells that are selective for different NF- κ B members, carry out prospective basic experimental research at the cellular and molecular level, which is expected to be related to atherosclerosis and vascular aging. The medical treatment of sexual diseases has brought breakthrough progress.

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Interest conflict

The authors declare no conflict of interest.

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