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Effect of high fluoride and high fat on serum lipid levels and oxidative stress in rabbits

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ABSTRACT

The purpose of this study was to explore the effects of high fluoride and high fat on triglyceride (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total antioxidant capacity (T-AOC), lipid peroxide (LPO) and malondialdehyde (MDA) in rabbits. A factorial experimental design was used, with two factors (fluoride and fat) and three levels. Seventy-two male rabbits were randomly assigned into nine groups according to initial weight and serum lipid levels. The rabbits were fed with basic feed, moderate fat feed or high fat feed and drank tap water, fluoridated water at levels of 50 and 100 mg fluorion/L freely. Biological materials were collected after 5 months, and serum lipid, T-AOC, LPO, and MDA levels were then measured. Using these data, the separate and interactive effects of high fluoride and high fat were analyzed. High fluoride and high fat both increased serum levels of TC, HDL-C and LDL-C significantly ($P < 0.05$), and there was also a synergistic effect between high fluoride and high fat ($P < 0.05$). High fluoride and high fat had different effects on TG levels: high fat significantly increased TG levels ($P < 0.01$) whereas high fluoride had nothing to do with TG levels ($P > 0.05$). High fat significantly elevated LPO and MDA levels and lowered T-AOC levels in serum ($P < 0.05$). Similarly, high fluoride significantly increased LPO and MDA levels in serum ($P < 0.05$). However, there was no interactive effect between high fat and high fluoride on these indexes. In summary, high fluoride and high fat increased serum TC and LDL-C levels individually and synergistically, and this would cause and aggravate hypercholesterolemia in rabbits. At the same time, high fluoride and high fat both made the accumulation of product of oxidative stress in experimental animals.

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1. Introduction

Endemic fluorosis seriously harms the health of rural residents in China. The main features of the disease are dental fluorosis and skeletal fluorosis, which affect young children and adults. Besides skeletal and dental damage, excessive exposure to fluoride can also cause other non-phrenological hazard, such as metabolic, structural and functional damage to the nervous system (Wu et al., 2006), kidneys, liver (Chandrajith et al., 2011; Chattopadhyay et al., 2011), cardiovascular system (Amini et al., 2011; Sun et al., 2013; Liu et al., 2014), decreasing intellectual ability in children (Ding et al., 2011; Han et al., 2014). The non-phrenological effects of fluorosis often go unnoticed due to the slow onset of symptoms and the non-specific injuries.

In recent years, there has raised public concern about damage to the cardiovascular system, especially atherosclerosis and hypertension, which caused by excessive exposure to fluoride. Atherosclerosis is an inflammatory process of the vascular wall, characterized by the accumulation of lipids and fibrous elements in the large- and medium-sized elastic and muscular arteries (Lusis, 2000). Studies have found several risk factors for the development of atherosclerosis, including hyperlipidemia (Talayero and Sacks, 2011), hypertension (Nieto et al., 1995), diabetes (Gruden et al., 2012), oxidative stress (Hansson, 2005), and smoking (Ansari et al., 2012), etc. Previous research has shown that chronic exposure to high levels of fluoride can not only influence lipid metabolism, but also affect oxidative stress. Some experimental studies in animals have demonstrated that excess intake of fluoride can cause disorder of lipid metabolism that promote the formation of atherosclerosis. However, other study reported that excess intake of fluoride can lower the concentration of very low density lipoprotein (VLDL) in plasma and so hinder the progression of atherosclerosis (Birkner et al., 2008).

The main aim of this experiment was to observe the effects of high fluoride and high fat on serum lipid levels and oxidative stress in experimental animals, and then to analyze separate and interactive effects of these factors. Based on these findings, a preliminary hypothesis for the mechanism by which excess intake of fluoride induce atherosclerosis will be proposed.

2. Materials and methods

2.1. Instruments and reagents

Assay kits for triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total antioxidant capacity (T-AOC), lipid peroxidation (LPO), and malondialdehyde (MDA) were purchased from Nanjing Jian Cheng Bioengineering Institute, China.

2.2. Animals and treatment

Seventy-two healthy, male Japanese big eared rabbits in clean grade (2400–2600 g weight) were selected as our experimental animals. They were housed in an air-conditioned room with a humidity level of 45–65% and a temperature of 20 ± 2 °C. In accordance with our factorial design with two factors (lipid and fluoride) and three levels, the rabbits were randomly assigned to nine groups of eight animals according to their initial weight and serum lipid levels. Based on previous research (Zhang et al., 2009), the high fat feed (89% basic feed +1% cholesterol +10% lard) and the moderate fat feed (94.5% basic feed +0.5% cholesterol +5% lard) were adopted. Based on an earlier report (Zhu et al., 2008), the highly fluoridated water contained 100 mg fluorion/L and the slightly fluoridated water contained 50 mg fluorion/L were determined. Rabbits in the different groups were fed basic, moderate fat or high fat feed and drank tap water or fluoridated water (50 or 100 mg fluorion/L, the water was fluoridated with sodium fluoride). The experimental design is shown in Table 1. The assigned feed and drinking water were available at all times throughout the experiment. The rabbits were exposed to fluoride and fat for 5 months and then sacrificed under chloral hydrate anesthesia. All experiments were approved by the Animal Ethics Committee of Harbin Medical University.

Table 1 – Diet and drinking water in different groups.

Group	Diet	Drinking water
A[1]	Basic feed[2]	Tap water[3]
B	Basic feed	The slightly fluoridated water
C	Basic feed	The highly fluoridated water
D	Moderate fat feed	Tap water
E	Moderate fat feed	The slightly fluoridated water
F	Moderate fat feed	The highly fluoridated water
G	High fat feed	Tap water
H	High fat feed	The slightly fluoridated water
I	High fat feed	The highly fluoridated water

[1] Group A is the control group.
[2] The formula of basic feed included soybean meal (10%), milled barley (38%), bran (50%), salt (1%) and fish meal (1%).
[3] The concentration of fluorion in tap water was 0.38 mg/L.

2.3. Detection of fluorion in serum

All analytical measurements for fluorion in serum were carried out according to a standardized method used in China (Health Industry Standard of the People's Republic of China, 2001). For the analysis, 0.4 mL serum and 0.4 mL total ionic strength adjustment buffer (TISAB) were mixed at pH 5, and the fluorion selective electrode (Yingke Crystal Materials Company) was used to measure the fluorion concentrations in the samples. All samples were detected thrice (independent aliquots) and the mean of the three determinations was used as the value for the fluorion concentrations in serum. The detection limit of this method is 0.012 μg/mL.

2.4. Serum lipid and oxidant/antioxidant index assays

Blood samples (not less than 15 mL/animal) were drawn from the femoral artery and stand at room temperature for 2 h, and then were centrifuged at 3000 rpm for 20 min at room temperature to separate the serum. Aliquots of serum (150 μL)