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Progression of atherosclerosis in ApoE-knockout mice fed on a high-fat diet

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Abstract. – OBJECTIVE: To construct the atherosclerosis model of ApoE-knockout (ApoE$^{-/-}$) mouse after deposition of high-fat diet at brachiocephalic artery and coronary artery.

MATERIALS AND METHODS: 40 healthy 8-week SPF-level male ApoE$^{-/-}$ mice of body weigh 16 to 23 g were averagely divided into a high-fat diet group and an ordinary diet group. Mice were sacrificed in a time course study by 3 months, 6 months, 9 months and 12 months. The brachiocephalic artery and coronary artery were extracted for pathological examination, and enzyme-linked immunosorbent assay (ELISA) was used for the examination of serum high-sensitivity C-reactive protein (hs-CRP) content and the relative expression level of liver peroxisome proliferator activated receptor (PPAR).

RESULTS: Plaque ratio in lumen area, lipid ratio in plaque area and plaque spontaneous rupture rate of brachiocephalic artery and coronary artery in high-fat diet group are all significantly higher than those in ordinary diet group during the same period (p<0.05). In the high-fat diet group, those indexes mentioned above in brachiocephalic artery are all significantly higher than those in coronary artery during the same period (p<0.05). Hs-CRP content in high-fat diet group is significantly higher than that in ordinary diet group during the same period, while PPAR relative expression level is significantly lower than that in ordinary diet group during the same period (p<0.05).

CONCLUSIONS: It was found that ApoE$^{-/-}$ mice after high-fat diet depict the phenotype of the atherosclerotic mouse model, and the brachiocephalic artery is better than coronary artery in terms of material selection, with inflammatory responses also playing an important role.

Key Words:
ApoE-knockout mice, High-fat diet, Brachiocephalic artery, Coronary artery, High-sensitivity C-reactive protein, Liver peroxisome proliferator activated receptor.

Introduction

ApoE-knockout (ApoE$^{-/-}$) mouse model are considered as an ideal model for researching atherosclerosis. As a kind of polymorphic protein, ApoE is the important ligand of apolipoprotein to eliminate receptor mediation. In the absence of ApoE, cholesterol gets accumulated at vascular walls, which makes vascular walls thickened and hardened, with less elasticity and narrow lumen, it leads to atherosclerosis[1]. Several studies demonstrated that after high-fat diet, the levels of triglyceride, total cholesterol and low density lipoprotein cholesterol (LDL) of ApoE$^{-/-}$ mice increase significantly. All of those parameters are in close relation with development of atherosclerosis[2].

Different researchers studied ApoE$^{-/-}$ mice for different feeding time, with brachiocephalic artery and aortic sinus are most in material selection, to observe the progression of atherosclerosis[3,4]. There are also some researches about inflammatory response in the progress of atherosclerosis, such as high-sensitivity C-reactive protein (hs-CRP) and interleukin[5]. Some scholars propose that there are differences between atherosclerosis of different artery parts of mice and corresponding parts of human bodies, especially the coronary artery[6]. Based on this, our study further analyzes the development of atherosclerosis, by studying changes at brachiocephalic artery and coronary artery, and role played by inflammatory response.

Materials and Methods

Experiment Materials

8-week old SPF-level male ApoE$^{-/-}$ mice (n=40) were selected for this study. The body

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weights of animals ranged from 16 to 23 g. Mice were raised in Laboratory Animal Center of Peking University Health Science Center, with animal license number SCXK (Beijing) 2012-0001. Rearing temperature was between 22-25 °C, relative humidity was 60%, and light-dark cycle was 12 hours. Mice in each group were raised in different cages with 1 mouse in 1 cage. Animals were given continuous supply of drinking water and feed. In all cages, drinking bottles and paddings were disinfected under high pressure.

Experiment Methods

Mice were divided into high-fat diet group and normal diet group. High-fat diet includes 21% fat and 0.25% cholesterol, purchased from Beijing Keao Xieli Feed Company Ltd., and certification number was SCXK (Beijing) 2009-0012. Mice were sacrificed after every 3 months for up to 12 months. The brachiocephalic artery and coronary artery were extracted for pathological examination. The extraction method of coronary artery is directly extracting the whole heart.

Brachiocephalic artery and coronary artery were separated under the microscope, stained by oil red O frozen section. The tissues were fixed in 40 g/L paraformaldehyde, cleaned and dehydrated in PBS, frozen section was made, stained by oil red O staining liquor, washed, cell nucleus was re-stained by hematoxylin, washed, aired and sealed. Oil red O staining liquor was provided by American company AMERSCO, CM 1950 frozen section machine and DM 3000 optical microscope were provided by Leica (Wetzlar, Germany).

Observation Indexes

Plaque ratio in lumen area, lipid ratio in plaque area and plaque spontaneous rupture rate of brachiocephalic artery and coronary artery in both groups were compared during the same period, among which plaque spontaneous rupture rate is the average embedding fiber cap number/the age of each mouse. ImageJ software was used to analyze indexes, inverted microscope was provided by Japanese company CKZ Olympus, and microscopic imaging system was provided by the Cold Spring Harbor Laboratory (Cold Spring Harbor, NY, USA).

Hs-CRP content and the relative expression level of liver peroxisome proliferator activated receptor (PPAR) were compared. Enzyme-linked immuno sorbent assay (ELISA) was adopted, kits were all purchased from American Company RB, and kit reference manual was strictly followed to carry out the experiment. OD_{450} nm wave length was measured by ELISA, and finally, sample concentration was calculated by drawing log-logit double logarithmic standard curve.

Statistical Analysis

All data were processed by SPSS 20.0 software (SPSS Inc., Chicago, IL, USA), measurement data was expressed by mean ± standard deviation (±S). The *t*-test was adopted for comparison between groups; enumeration data was expressed by (%), and X^2-test was adopted for comparison between groups; $p<0.05$ was considered that the difference is of statistical significance.

Results

Comparison of Microscopic Atheromatous Plaques Condition between Two Groups

Plaque ratio in lumen area, lipid ratio in plaque area and plaque spontaneous rupture rate of brachiocephalic artery and coronary artery in high-fat diet group were found to be significantly higher than those in ordinary diet group during the same period ($p<0.05$). In high-fat diet group, those indexes mentioned above in brachiocephalic artery were all significantly higher than those in coronary artery during the same period ($p<0.05$) (Tables I-IV).

Comparison of hs-CRP and PPAR Expression Level between Two Groups

Hs-CRP content in high-fat diet group was significantly higher than that in ordinary diet group during the same period, while PPAR relative expression level is significantly lower than that in ordinary diet group during the same period ($p<0.05$) (Table V).

Discussion

The pathological atherosclerotic plaques performances of $ApoE^{-/-}$ mice varies with different high-fat feed compositions, animals age, which made most of the studies inconclusive and even contradictory to each other[7,10]. We suggested that if brachiocephalic artery represents the formation of brain plaques of human bodies, and if coronary artery is similar to that of human bodies,

Table I. Comparison of microscopic atheromatous plaques condition between two groups in 3 months.

	Brachiocephalic artery			Coronary artery		
Groups	Plaque ratio in lumen area	Lipid ratio in plaque area	Plaque spontaneous rupture rate	Plaque ratio in lumen area	Lipid ratio in plaque area	Plaque spontaneous rupture rate
Normal diet	2.5±0.3	6.3±1.2	1.5±0.2	0.7±0.1	2.2±0.6	0.7±0.2
High fat diet	16.4±3.3	28.7±7.4	9.7±2.1	7.2±1.9	13.4±5.5	3.6±0.7
t	5.627	5.578	6.301	6.327	6.698	5.847
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table II. Comparison of microscopic atheromatous plaques condition between two groups in 6 months.

	Brachiocephalic artery			Coronary artery		
Groups	Plaque ratio in lumen area	Lipid ratio in plaque area	Plaque spontaneous rupture rate	Plaque ratio in lumen area	Lipid ratio in plaque area	Plaque spontaneous rupture rate
Normal diet	5.7±1.2	9.3±2.4	6.7±1.3	3.3±0.8	4.2±1.9	3.7±0.6
High fat diet	35.9±7.2	38.7±9.3	26.6±10.2	18.6±4.7	20.7±8.3	14.2±6.4
t	6.328	6.745	6.932	7.201	7.632	7.712
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

through comparatively long feeding of high-fat diet (e.g., 12 months), the plaque ratio in lumen area, lipid ratio in plaque area and plaque spontaneous rupture rate of brachiocephalic artery and coronary artery are significantly higher compared to normal diet group. We observed that in high-fat diet group, those indexes in brachiocephalic artery were significantly higher than those in coronary artery during the same period. The observation indexes reflects the plaque structure and stability characteristics better. In the past, a few researches noted that during the comparatively long feeding period (e.g. 18 months), atheromatous plaques of coronary artery of ApoE$^{-/-}$ mice are still not obvious, which is considered to be related with strains of mice, feed formula and parts of materials selection. This research concludes that plaque ratio in lumen area at brachiocephalic artery was 16.4% after 3 months' feeding, while plaque ratio in lumen area at coronary artery was only 7.2%; both numbers increase with the extension of feeding time, and after 12 months' feeding, plaque ratio in lumen area at brachiocephalic artery was 76.9%, while plaque ratio in lumen area at coronary artery was 49.7%, which reach an ideal atheromatous plaques model (Tables I-IV). As for the theory that the formation of atheromatous plaques at coronary artery was not better than at brachiocephalic artery, it still needs to be ex-

Table III. Comparison of microscopic atheromatous plaques condition between two groups in 9 months.

	Brachiocephalic artery			Coronary artery		
Groups	Plaque ratio in lumen area	Lipid ratio in plaque area	Plaque spontaneous rupture rate	Plaque ratio in lumen area	Lipid ratio in plaque area	Plaque spontaneous rupture rate
Ordinary diet	9.3±2.4	12.1±5.2	9.3±1.7	5.2±1.7	6.9±2.3	5.7±1.3
High fat diet	56.4±13.6	49.7±16.4	40.9±15.6	36.6±10.2	34.7±15.2	32.1±13.6
t	7.524	7.928	8.301	8.427	8.624	8.847
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table IV. Comparion of microscopic atheromatous plaques condition between two groups in 12 months.

	Brachiocephalic artery			Coronary artery		
Groups	Plaque ratio in lumen area	Lipid ratio in plaque area	Plaque spontaneous rupture rate	Plaque ratio in lumen area	Lipid ratio in plaque area	Plaque spontaneous rupture rate
Normal diet	16.7±5.3	19.3±6.1	13.2±4.9	10.3±2.9	13.8±4.7	8.3±1.7
High fat diet	76.9±17.8	58.4±21.3	59.7±18.4	49.7±15.4	49.6±16.7	43.2±15.2
t	9.524	9.327	9.467	9.675	9.728	9.832
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Table V. Comparison of hs-CRP and PPAR expression level between two groups.

	3 months		6 months		9 months		12 months	
Groups	hs-CRP (mg/L)	PPAR	hs-CRP (mg /L)	PPAR	hs-CRP (mg/L)	PPAR	hs-CRP (mg/L)	PPAR
Normal diet	0.9±0.3	105.4±26.8	1.6±0.5	243.8±54.6	1.9±0.6	397.5±79.8	2.3±0.8	510.7±103.5
High fat diet	4.3±0.9	62.7±21.5	7.2±1.3	96.4±35.9	10.4±4.6	131.7±64.8	12.6±5.2	162.6±79.5
t	4.629	7.518	4.927	7.938	5.617	8.624	6.237	8.752
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

plored further. Alike humans, compared with large arteries such as aorta, the formation speed of atheromatous plaques at coronary artery is not that high and the severity is also not that high.

We found that hs-CRP content in high-fat diet group was significantly higher than that in ordinary diet group during the same period, while PPAR relative expression level was significantly lower than that in ordinary diet group (Table V). It has been verified by many researches that inflammatory reaction is the important component of atheromatous plaques formation theory[11,12]. The process of atherosclerosis includes the participation of multiple cells, including vascular wall cells (such as endothelial cells and vascular smooth muscle cells) and blood-borne cells (monocytes, macrophages and lymphocytes). Each type of cell more or less expresses PPAR, and becomes potential point of PPAR ligand[13]. An increasing number of evidences indicate that PPAR ligand can adjust multiple stages in the process of atherosclerosis, e.g., dysfunction of endothelial cells, aggregation of subendothelial LDL and modified LDL, differentiation of monocytes to foam cells, migration of vascular smooth muscle cells migration and proliferation, etc.[14-18].

Conclusions

The reliable atherosclerosis model of ApoE$^{-/-}$ mice after high-fat diet can be constructed, brachiocephalic artery is better than coronary artery in terms of material selection, and inflammatory response plays an important role as well.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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