The cerebro-cardiovascular diseases caused by atherosclerosis (AS) has become one of the principal fatal diseases in human being. The most important aspect of the genesis and development process of AS is the transportation of monocytes and vascular smooth muscle cells (VSMCs) to the arterial endothelia, where they phagocytize lipids and transmute to foam cells. The lipid ingestion of macrophage and VSMCs is a self-protective function in human, nevertheless, could the lipids be metabolized and effluxed outside body, namely, the reverse cholesterol transport (RCT) process is the decisive crux for progression and outcome of AS. Nowadays, to promote RCT and reduce cholesterol deposition on vascular wall have become an essential direction for AS research, also the next hot-spot for reducing blood-lipid after Statins have been applied widely in clinical practice.

Huxin Formula (护心方, HXF) is formulated by modifying the Deng's Guanxin Formula (冠心方), which is an experienced Chinese medicine (CM) formula created by well-known CM doctor Prof. DENG Tie-tao, and has been used in clinical practice for more than 30 years, showing marked effects in treating coronary heart disease and angina pectoris. This study was carried out to explore the effect of HXF on RCT in ApoE-gene knockout [ApoE (-/-)] mice.

ABSTRACT Objective: To observe the effect of Huxin Formula (护心方, HXF) on expressions of the chief reverse cholesterol transport (RCT) associated genes, caveolin-1 and scavenger receptor-B I (SR-B I) in ApoE-gene knockout [ApoE (-/-)] mice. Methods: Thirty ApoE (-/-) mice of 4–6 weeks old were randomly divided into three groups (A–C). After being fed with high-fat diet for 16 weeks, they were treated with HXF (1 mL/100 g), pravachol (0.3 mg/100 g), and saline in equal volume respectively; in addition, a blank group was set up with 10 C57BL/6J mice of 6-week old received 16-week high-fat feeding and saline treatment. Animals were sacrificed at the termination of the experiment, their paraffin sections of aortic tissue were used to measure the size of plaque, expressions of caveolin-1 and SR-B I were detected by immunological histochemical method. Results: As compared with the blank group, levels of caveolin-1 and SR-B I were increased in Groups A and B (P<0.01); but the increase in Group A was more significant than that in Group B (P<0.05). The plaque/aorta area ratio decreased significantly in Groups A and B, but showed insignificant difference between the two groups. Conclusion: HXF could obviously increase the expressions of RCT associated genes, caveolin-1 and SR-B I, promote the RCT process, so as to reduce the formation of aorta atherosclerotic plaque in ApoE (-/-) mice.

KEYWORDS Huxin formula, ApoE-gene knockout mice, reverse cholesterol transport, caveolin-1, scavenger receptor-B I
adaptation, weighed, then turned to high-fat feeding contained fat 21% (wt/wt), cholesterol 0.15%, with $^{60}$Co $\gamma$ radiation for disinfection.

**Testing Drugs**

HXF consisted of Radix Ginseng, Radix Notoginseng, Rhizoma Pinelliae Fructus Aurantii, etc. The crude herbal drugs were purchased from Kangmei Pharmaceutical Company (Guangdong, China), all their species were fitting to the provision of Pharmacopoeia of People's Republic of China (Part 1). The preparation of HXF: Reflux distillating twice Radix Ginseng and Radix Notoginseng with 8-fold water for 1 h at 100 $^\circ$C, the two parts reflux liquids were mixed, filtered, ready for use. Other drugs were boiled with 8-fold water for three times, 45 min for the first time and each 30 min for the second and third times, combined the three parts of decoction (passing filtration) and the extracts of Radix Ginseng and Radix notoginseng, concentrated to HXF containing 1.0 g crude drugs/mL, 4 $^\circ$C preserved.

Pravastatin (trade name Pravachol, 10 mg/tablet) was supplied by Zhong-Mei Shanghai Shiguibao Pharmaceitical Co., Ltd., China. It was dissolved in saline to make 50% suspension before use.

**Reagents and Apparatus**

Mouse caveolin-1 immunohistochemical test kit was product of Epitomics; mouse scavenger receptor-B I (SR-B I) immuno-histo-chemical test kit was product of Abcam (UK); DAB developer, product of Maixin-Bio (Fuzhou, China); immunohistochemical test kit (second antibody), product of Jingmei Bioproduct (Shanghai, China).

The following apparatus were applied: Beckman CS-15R high-speed refrigerated centrifuge (USA); Sanyo medical low temperature refrigerator (Japan); LEICA ASP 300 full automatic tissue dehydrating machine (Germany); LEICA EG 1160 paraffin embedding set (Germany); LEICA RM 2135 rotary microtome (Geramny); OLYMPUS CX41 light microscope (Japan).

**Animal Model Establishment**

Thirty ApoE(-/-) mice 4–6 week old and 10 common C57BL/6J mice were entered in experiment after one week adaptative feeding in poultry house of clean grade at room temperature (22–25 $^\circ$C), 50% relative humidity, lighting time 7:00-18:00. C57BL/6J mice were fed with normal forage, ApoE (-/-) mice were fed with high-fat forage separately, 6–7 mice/cage. Twelve weeks later, 2 ApoE(-/-) mice and 2 C57BL/6J mice were randomly selected for confirming the AS plaque formation on aortic root through microscopic observation with HE stain. At that time, uneven thickness of arterial wall with partial endothelial hyperplasia endometria or fibrous cap covered lesion of fibro-plaque stage could be seen in ApoE (-/-) mice.

**Grouping and Treatment**

After animals were fed with high-fat forage for 16 weeks, the ApoE (-/-) mice were divided in random into 3 groups (A–C), treated respectively with HXF (1 mL/100 g, group A), Pravachol (0.3 mg/100 g, group B), and saline in equal volume (group C) respectively for 16 weeks successively once a day via gastric infusion, the dosage of testing drugs were equivalent to 10-fold of the dosage used for human adult weighing 60 kg. The 8 C57BL/6J mice were set as a normal control group, treated with normal saline 1 mL/100 g. At the same time, animals were accessed freely to clean grade high-fat forage and common drinking water.

**Sample Collection**

Samples were gathered at the termination of 16-week treatment, through 24 h of fasting after final administering of testing drug, animals were fixed in consciousness manner, anesthetized with 10% chloral hydrate 0.3 mL/100 g via peritoneal injection, exposed their heart and abdominal aorta in aseptic condition, collected blood in direct vision, which was low temperature centrifuged 3,000 r/min to get serum. Then drawn out the aorta aseptically, peeled off the adventitia, 2 pieces of tissue were taken from appointed part of aortic root along horizontal and longitudinal axis respectively, which was formalin fixed, paraffin sectioned, HE stained, observed and took photo with OPTIMAS researching microscope.

**Items and Methods of Observation**

**Morphologic Observation and Image Analysis**

HE stained aortic tissue slide was observed under a light microscope; areas of AS plaques in various sections were analyzed using computerized IPP image analyzing software; total areas of arterial membrane and plaque, as well as the ratio between them were calculated.

**Immunohistochemical Assay**

Cavilin-1 and SR-BI protein expressions were