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Basic Study

Regulatory effect of herbal cake-partitioned moxibustion on cholesterol reverse transport nuclear receptor LXRα in rabbits with atherosclerosis

隔药饼灸对动脉粥样硬化兔胆固醇逆转运核受体 LXRα 的调节作用

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Abstract

Objective: To observe the effect of herbal cake-partitioned moxibustion on liver X receptor alpha (LXRα) in rabbits with atherosclerosis.

Methods: Thirty-six male New Zealand rabbits were randomly divided into a normal group, a model group, a herbal cake-partitioned moxibustion group and a simvastatin group according to the random number table method, with 9 rabbits in each group. Rabbits in the model group, the herbal cake-partitioned moxibustion group and the simvastatin group were modeled by high fat feeding method which took 12 weeks. After verification of the successful model, rabbits in the normal group were not treated, in the model group were bundled, in the herbal cake-partitioned moxibustion group were treated with herbal cake-partitioned moxibustion, and those in the simvastatin group were treated with simvastatin, all for a total of 4 weeks. At the end of the experiment, the aorta and liver were observed for pathological changes; serum and liver were used to detect lipid levels; Western blot (WB) and real-time quantitative polymerase chain reaction (RT-qPCR) were used to detect LXRα protein and mRNA expression levels, respectively.

Results: Compared with the normal group, the structure of aorta was disordered, the wall was rough and thick, the intima was unsmooth, and the vascular smooth muscle cells were arranged closely and disorderly in the model group, which was consistent with the characteristics of the rabbit model of aortic atherosclerosis. Compared with the model group, the aortic structure was clear, the degree of hepatocyte degeneration was reduced, the serum total cholesterol and low-density lipoprotein levels were significantly decreased (all $P<0.01$), the high-density lipoprotein level was elevated ($P<0.01$), and the total liver cholesterol was decreased significantly ($P<0.01$) in the rabbits of the herbal cake-partitioned moxibustion group and the simvastatin group; compared with the model group, the protein ($P<0.01$ or $P<0.05$) and mRNA ($P<0.01$) expressions of rabbit LXRα in the herbal cake-partitioned moxibustion group and the simvastatin group were increased.

Conclusion: Herbal cake-partitioned moxibustion can improve the aortic and hepatic lesions, regulate blood lipid and liver lipid levels, increase the expression of liver cholesterol reverse transport nuclear receptor LXRα, promote reverse cholesterol transport in the rabbits with aortic atherosclerosis, therefore produces an antiatherogenic effect.

Keywords: Moxibustion Therapy; Indirect Moxibustion; Medicinal Cake-partitioned Moxibustion; Atherosclerosis; Cholesterol; Liver X Receptors; Rabbits

【摘要】目的：观察隔药饼灸对动脉粥样硬化兔胆固醇逆转运肝X受体α (LXRα)的影响。**方法：**将36只雄性新西兰兔按随机数字表法随机分为正常组、模型组、隔药饼灸组和辛伐他汀组，每组9只。模型组、隔药饼灸组和辛伐他汀组采用高脂饲养法造模，共12周。验证造模成功后，正常组不予治疗，模型组予以捆绑，隔药饼灸组予以隔药饼灸治疗，辛伐他汀组予以辛伐他汀治疗，共治疗4周。实验结束后取主动脉及肝脏观察其病理形态变化，取血清及肝脏检测脂质水平，以蛋白质印迹法(WB)和实时荧光定量聚合酶链反应(RT-qPCR)法分别检测LXRα蛋白及mRNA表达水平。**结果：**与正常组比较，模型组主动脉结构紊乱，管壁粗糙增厚，内膜不光滑，血管平滑肌细胞排列紧密无序，符合主动脉粥样硬化兔模型的特点。与模型组比较，隔药饼灸组和辛伐他汀组兔主动脉结构清晰，肝细胞变性程度减轻，血清总胆固醇、低密度脂蛋白明显降低(均$P<0.01$)，高密度脂蛋白升高($P<0.01$)，肝脏总胆固醇明显降低($P<0.01$)；隔药饼灸组和辛伐他汀组兔与模型组比较，LXRα蛋白($P<0.01$或$P<0.05$)及

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LXRα mRNA ($P<0.01$)表达增加。**结论：**隔药饼灸可改善主动脉粥样硬化兔主动脉及肝脏的病变情况，调节血脂和肝脏脂质水平，提高肝脏胆固醇逆转运核受体LXRα的表达，促进胆固醇逆转运从而发挥抗动脉粥样硬化的作用。

【关键词】灸法；间接灸；药饼灸疗法；动脉粥样硬化；胆固醇；肝 X 受体；兔

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Atherosclerosis (AS) is the pathological basis of cardiovascular and cerebrovascular diseases. Cardiovascular and cerebrovascular diseases caused by AS have become the leading cause of death worldwide. Lipid metabolism disorder is the basis of AS lesions. It is characterized by the affected arterial lesions starting from the intima, usually with accumulation of lipids and complex carbohydrates, hemorrhage and thrombosis, and then fibrous tissue hyperplasia and calcinosis, leading to thickened and hard arterial wall, and narrowed vascular lumen[1]. Reverse cholesterol transport (RCT) is the most important way for the body to eliminate excess cholesterol[2], and plays an important role in preventing the occurrence and development of AS[3]. Liver X receptor alpha (LXRα) is a member of the nuclear hormone receptor protein superfamily and acts as a sensor in maintaining cholesterol balance in the body. Recent studies have shown that LXRα can control RCT by regulating the expression of its target gene adenosine triphosphate (ATP) binding cassette transporter G1 (ABCG1)[4]. Herbal cake-partitioned moxibustion has the effect of improving the disorder of lipid metabolism during interfering the progress of AS[5-6]. In this experiment, the effect of herbal cake-partitioned moxibustion on LXRα in AS rabbits was observed, and the mechanism of herbal cake-partitioned moxibustion in the regulation of cholesterol and lipid disorders in AS rabbits was explored.

1 Materials and Methods

1.1 Animals and grouping

Thirty-six healthy male New Zealand rabbits of three months old, weighing 1.5-2.5 kg, were provided by the Animal Experimental Center of Hunan University of Chinese Medicine [animal certificate number: SCXK (Xiang) 2015-0004]. After 7 d of adaptive feeding, the rabbits were divided into a normal group, a model group, a herbal cake-partitioned moxibustion group and a simvastatin group according to the random number table method, with 9 rabbits in each group. One rabbit in the simvastatin group died of diarrhea. The operation and treatment of experimental animals during the experiment complied with the *Instructive Notions with Respect to Caring for Laboratory Animals* promulgated by the Ministry of Science and Technology of China.

1.2 Main reagents and instruments

Ulatan (Shanghai Chemical Reagent Company, China); blood lipid kit (Shanghai Jingdu Biotechnology Co., Ltd., China); rabbit SDF-1 ELISA kit (Wuhan Huamei Bioengineering Co., Ltd., China); simvastatin (approval number: H20066221, Guangdong Pidi Pharmaceutical Co., Ltd., China); trizol (Invitrogen, USA); deoxyribonucleotide triphosphate (dNTP, Beijing Genstar, China); SYBGREEN PCR Mix (Invitrogen, USA); tetramethylethylenediamine (TEMED, Sigma, USA); LXRα rabbit polyclonal antibody and HRP sheep anti-rabbit immunoglobulin G (Proteintech, USA); Super ECL Plus hypersensitive luminescent liquid (Thermo Pierce, USA).

DNP-9162 electrothermal constant temperature incubator (Shanghai Yiheng Scientific Instrument Co., Ltd., China); Motic BA410 research microscope (Motic China Group Co., Ltd., China); TGL-16 desktop refrigerated centrifuge (Changsha Xiangyi Centrifuge Instrument Co., Ltd., China); 164-5050 electrophoresis instrument (Bio-rad, USA); DYCZ-24EN electrophoresis tank and DYCZ-40D film transfer instrument (Beijing Liuyi Instrument Factory, China); PIKO REAL 96 real-time polymerase chain reaction (RT-PCR) instrument and SPL0960 fluorescent PCR plate (Thermo, USA).

1.3 Model preparation

Model was prepared with high-fat feeding method[7], which was to mix cholesterol, lard, egg yolk powder, basic feed (1:5:10:84) and propylthiouracil [10 mg/(kg·bw) per day] and process it into small strips of high fat feed. Rabbits were fed every morning.

The rabbits in the normal group were fed with normal diet at 150 g/d, and the other three groups were fed with high-fat diet (about 100 g) plus normal feed (about 50 g) for 12 weeks. After 12 weeks, random sampling was performed. One rabbit in each group received blood collection and aorta dissection. The serum blood lipid level was significantly increased. The atherosclerotic plaque was found on the inner wall of aorta, indicating that the AS rabbit model was successfully prepared.

1.4 Intervention method

1.4.1 Acupoint location

Acupoint location was referred to the *Experimental Acupuncture Science*[8] combined with anthropomorphic comparison.

Ganshu (BL 18): 1.5 cm away from the middle of the 9th and the 10th thoracic vertebra spinous process.

Xinshu (BL 15): 1.5 cm away from the middle of the 5th and the 6th thoracic vertebra spinous process.

Pishu (BL 20): 1.5 cm away from the middle of the 11th and the 12th thoracic vertebra spinous process.

Tianshu (ST 25): 3 cm away from the umbilicus.

Fenglong (ST 40): The lateral crest of fibula at the midpoint of the calf.

Juque (CV 14): At the intersection of the upper 1/4 and lower 3/4 of the line between the umbilicus and the xiphoid cartilages, and on the ventrimeson.

1.4.2 Interventions

Rabbits in the normal group did not receive any intervention.

Rabbits in the model group were bundled without intervention.

Rabbits in the simvastatin group were treated with oral simvastatin [crushed with clean glass bottles and mixed into the feed at a dose of 1.96 mg/(kg·bw)].

Rabbits in the herbal cake-partitioned moxibustion group received herbal cake-partitioned moxibustion. A specific moxa cone was used (0.5 cm in diameter and 1.5 cm in height with a paper pad of 3.0 mm in thickness and 1 cm in diameter under the moxa cone). The herbal cake consisted of equal proportions of *Dan Shen* (*Radix Salviae Miltiorrhizae*), *Da Huang* (*Radix et Rhizoma Rhei*), *Shan Zha* (*Fructus Crataegi*), *Yu Jin* (*Radix Curcumae*) and *Ze Xie* (*Rhizoma Alismatis*). The herbal medicines were grinded into powder and filled into a bottle for later use after mixture. The herbal cake was prepared just before use. Each time, an appropriate amount of powder was made into a paste with vinegar to produce a cake with a thickness of about 3.0 mm and a diameter of about 1.0 cm using a 10 mL syringe without needle.

The rabbit was fixed on the rabbit table to remove hair around the acupoints. The herbal cakes were directly placed on the acupoints, and then the moxa cones were placed on the herbal cakes. The moxa cones were ignited for moxibustion with 4 moxa cones successively used for each acupoint (about 30 min).

The first group of acupoints (prone position) included bilateral Ganshu (BL 18), Xinshu (BL 15) and Pishu (BL 20); the second group of acupoints (supine position) included bilateral Tianshu (ST 25), Fenglong (ST 40) and Juque (CV 14). Moxibustion was performed once a day for 4 weeks with the two groups of acupoints used alternately.

1.5 Investigation items and methods

1.5.1 Pathological observation of rabbit aorta and liver tissue.

The rabbits were anesthetized by ear vein injection of 20% urethane [4 mL/(kg·bw)]. The bifurcation blood vessel from aorta to the abdominal aorta was dissected, rinsed with saline, and soaked in a neutral formaldehyde solution to fix. The samples were dehydrated with a series of graded ethanol followed by xylene transparency, and then embedded in paraffin. The sections were sliced at 4 μm and stained with hematoxylin-eosin (HE). The histopathological morphologies of the aorta and liver were observed under optical microscope (×400).

1.5.2 Determination of lipid levels in serum and liver tissues

The total cholesterol (TC) level was determined enzymatically according to the kit instructions. The levels of low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were determined by colorimetry. The liver tissues were cut into pieces after weighed and then homogenized by adding 9 mL of chloroform and methanol mixture (chloroform and methanol at 1:1) for per gram of tissue. The mixture was thoroughly shaken in a test tube for lipid extract after 24 h. The supernatant was collected by centrifuge. Liver TC level was detected using the same method as that in serum[9].

1.5.3 Liver LXRα protein expression

The protein of liver tissues was extracted by adding an appropriate volume of lysate, and then the protein concentration was determined by bicinchoninic acid (BCA) protein quantification method. The protein was separated by 6% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred to a polyvinylidene fluoride (PVDF) membrane (300 mA, 60 min). The membrane was blocked with 5% skim milk for 60 min. The primary antibody was diluted at 1:1 000 with 1× tris buffered saline with tween (TBST) and incubated with the membrane overnight at 4 ℃. At the end of the incubation, the membrane was washed with 1× TBST for 3 times, 15 min each time. The HRP-labeled secondary antibody was diluted with 1× TBST (1:3 000) and incubated with the membrane for 45-60 min. At the end of the incubation, the membrane was washed with 1× TBST for 3 times, 15 min each time. The membrane was incubated with ECL chemiluminescence solution for 3 min and wrapped with preservation film after the liquid was absorbed by absorbent paper, and then exposed to the X-film in the dark box for several seconds to several minutes and developed. The image gray value of the obtained protein bands was analyzed by the Quantity One analysis software.

1.5.4 Liver LXRα mRNA expression

Primers for LXRα mRNA detection are shown in Table 1.

Table 1. Primers for LXRα mRNA detection

Name	Amplification length (bp)	Upstream primer sequence	Downstream primer sequence
LXRα	289	5'-ACCGAAGATGCTGGGGAACGAG-3'	5'-GCTCCTCCTCCTGTCGCTTCA-3'
β-actin	107	5'-CATCCTGCGTCTGGACCTGG-3'	5'-TAATGTCACGCACGATTTCC-3'

Liquid nitrogen frozen tissues about 200 mg grinded in 1 mL trizol, and the total RNA was extracted after fully lysing, and the purity and concentration of RNA were determined using the kits. The cDNA was obtained by reverse transcription reaction using a reverse transcription kit. The reaction system was as follows: dNTP mix (2.5 mmol/L) 6 μL, primer mix 3 μL, RNA template 6 μL, 5× RT buffer 6 μL, DTT (0.1 mol/L) 3 μL, HiFiScript, 200 U/μL 1.5 μL, RNase-free water 4.5 μL; incubated at 42 ℃ for 30-50 min, 85 ℃ for 5 min. When the reaction was completed, the cDNA was briefly centrifuged, cooled on ice and stored at –20 ℃. The reaction system was configured in proportion (30 μL in total): template (reverse transcript) 1 μL, primer A (10 μmol/L) 0.5 μL, primer B (10 μmol/L) 0.5 μL, PCR H_2O 13 μL, 2× SYBGREEN PCR master mix 15 μL. The set procedure was: 50 ℃ for 2 min, 95 ℃ for 10 min, 95 ℃ for 5 s, 60 ℃ for 30 s, for 40 cycles. The fluorescence value was read in the extension phase each time. Relative quantification of each gene expression was performed using the $2^{-\Delta\Delta Ct}$ method.

1.6 Statistical methods

All data were input into the computer and processed using SPSS 20.0 software. The measurement data were expressed as mean ± standard deviation ($\bar{x}$ ±s). Comparison between groups was performed with the least significant difference (LSD) method for data with homogeneity of variance, and Tamhane method for data with heterogeneity of variance. The rank sum test was used when the normality was not satisfied. The difference was statistically significant at P<0.05.

2 Results

2.1 Pathological observation of rabbit aorta in each group

Rabbits in the normal group showed a clear aortic structure, the luminal endometrium was smooth, and the vascular smooth muscle cells were arranged closely and orderly without significant lipid infiltration. Of the rabbits in the model group, the aortic structure was disordered, the wall was rough and thick, the intima was not smooth, the vascular smooth muscle cells were arranged closely and disorderly, and there were a lot of foam cells. The aortic structure and arrangement of rabbits in the model group were in accordance with the characteristics of the AS model. In the herbal cake-partitioned moxibustion group, the aortic structure was clear, the thickness of smooth muscle cells was uniform, and the foam cells were significantly reduced. In the simvastatin group, the aortic structure was clear, the intima was thickened, and a small amount of lipid infiltrabbition was observed. The details are shown in Figure 1.

2.2 Pathological observation of rabbit liver tissues

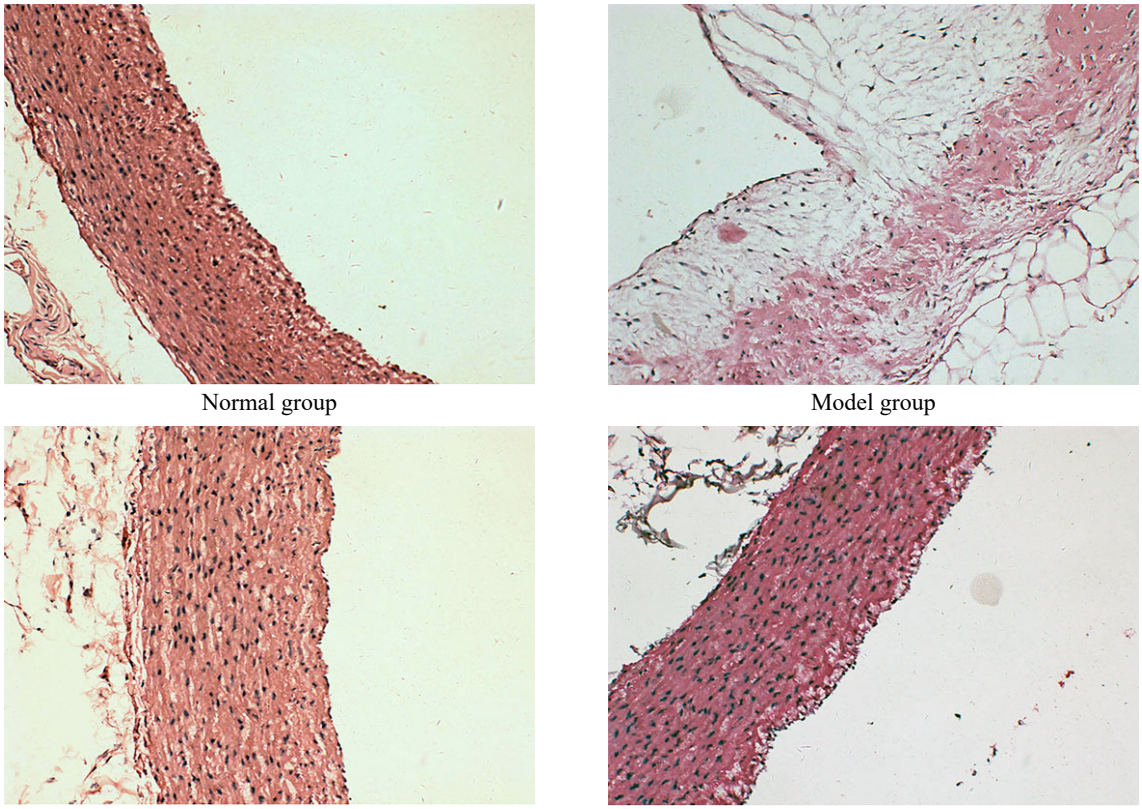
Rabbits in the normal group showed normal liver cell morphology and no obvious lipid droplets; rabbits in the model group showed unclear structure, narrowed hepatic sinusoids, swollen hepatocytes, transparent cytoplasm, traits change of nucleus due to compression, and dispersed fat vacuoles; rabbits in the herbal cake-partitioned moxibustion group showed mild degeneration of hepatocytes, and decreased lipid droplet deposition; rabbits in the simvastatin group showed a small amount of hepatocytes, water-like degeneration and a small amount of vacuolated lipid droplets. The details are shown in Figure 2.

2.3 Changes in blood lipids

Compared with the normal group, the serum TC and LDL-C levels in the model group were higher, and the differences between the two groups were statistically significant (P<0.01); the serum HDL-C level in the model group was lower, and the difference between the two groups was statistically significant (P<0.01). Compared with the model group, the serum TC and LDL-C levels of the rabbits in the herbal cake-partitioned moxibustion group and the simvastatin group were significantly lower, and the serum HDL-C level was increased, and the between-group differences were statistically significant (P<0.01). There were no significant differences in the levels of TC, LDL-C and HDL-C between the herbal cake-partitioned moxibustion group and the simvastatin group, indicating that herbal cake-partitioned moxibustion had a regulating effect on blood lipids and the effect was similar to that of simvastatin. The details are shown in Table 2.

2.4 TC level changes in liver tissues

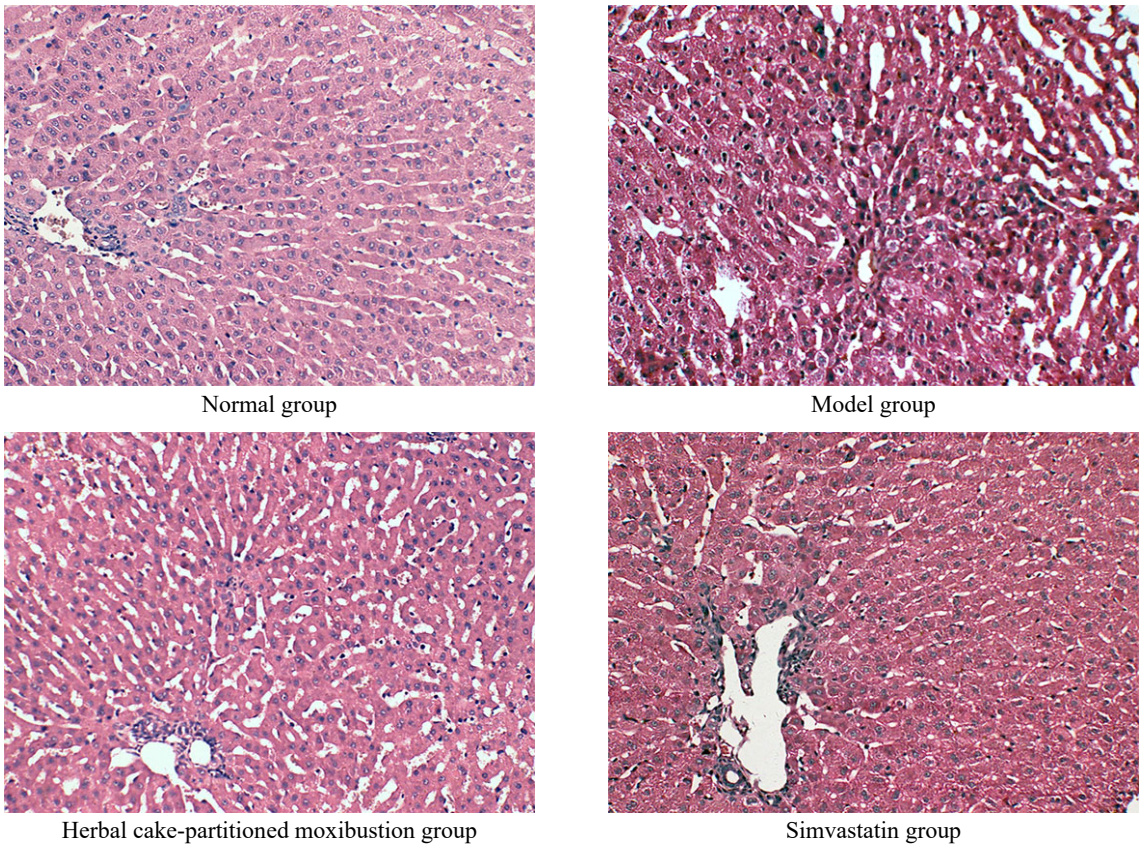
Compared with the normal group, the cholesterol level in the rabbit liver tissues of the model group was increased, and the difference was statistically significant (P<0.01). Compared with the model group, the cholesterol levels of the rabbit liver tissues in the herbal cake-partitioned moxibustion group and the simvastatin group were significantly lower (P<0.01). There was no significant difference between the herbal cake-partitioned moxibustion group and the simvastatin group (P>0.05). The details are shown in Table 3.



Normal group　　Model group

Herbal cake-partitioned moxibustion group　　Simvastatin group

Figure 1. Rabbit aorta in each group (HE, ×400)



Normal group　　Model group

Herbal cake-partitioned moxibustion group　　Simvastatin group

Figure 2. Liver status of rabbits in each group (HE, ×400)

Table 2. Comparison of blood lipid levels in rabbits ($\bar{x}$ ±*s*, mmol/L)

Group	*n*	TC	LDL-C	HDL-C
Normal	8	4.09±0.61	1.00±0.19	0.37±0.07
Model	8	9.17±0.98[1)]	1.52±0.13[1)]	0.20±0.04[1)]
Herbal cake-partitioned moxibustion	8	4.92±0.85[2)]	1.01±0.16[2)]	0.43±0.10[2)]
Simvastatin	7	4.29±0.98[2)]	1.10±0.13[2)]	0.39±0.09[2)]

Note: Compared with the normal group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$

Table 3. Comparison of TC level in rabbit liver tissues ($\bar{x}$ ±*s*, mmol/L)

Group	*n*	TC
Normal	8	1.45±0.32
Model	8	5.08±1.19[1)]
Herbal cake-partitioned moxibustion	8	1.27±0.94[2)]
Simvastatin group	7	2.01±0.33[2)]

Note: Compared with the normal group, 1) $P<0.01$; compared with the model group, 2) $P<0.01$

2.5 Comparison of LXRα protein expression in rabbit livers

Compared with the normal group, the LXRα protein expression in the model group was significantly reduced ($P<0.05$); compared with the model group, the LXRα protein expressions in the herbal cake-partitioned moxibustion group and the simvastatin group were increased ($P<0.01$, $P<0.05$). There was no significant difference between the herbal cake-partitioned moxibustion group and the simvastatin group ($P>0.05$). The details are shown in Figure 3 and Table 4.

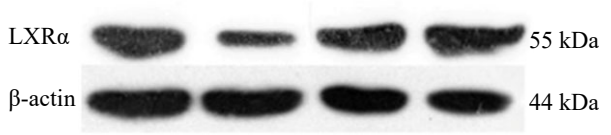


Figure 3. Comparison of LXRα protein expression in rabbit liver

Table 4. Comparison of LXRα protein expression in rabbit livers ($\bar{x}$ ±*s*)

Group	*n*	LXRα protein expression
Normal	8	0.61±0.17
Model	8	0.44±0.14[1)]
Herbal cake-partitioned moxibustion	8	0.64±0.12[2)]
Simvastatin group	7	0.62±0.13[3)]

Note: Compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.01$, 3) $P<0.05$

2.6 Comparison of LXRα mRNA expression in rabbit livers

Compared with the normal group, the LXRα mRNA expression in the model group was decreased, and the difference was statistically significant ($P<0.05$). Compared with the model group, the LXRα mRNA expressions in the rabbit livers of the herbal cake-partitioned moxibustion group and the simvastatin group were increased, and the differences were statistically significant ($P<0.01$). The LXRα mRNA expression was higher in the herbal cake-partitioned moxibustion group than in the simvastain group, and the difference was statistically significant ($P<0.05$). The details are shown in Table 5.

Table 5. Comparison of LXRα mRNA expression in rabbit livers ($\bar{x}$ ±*s*)

Group	*n*	LXRα mRNA expression
Normal	8	6.24±3.37
Model	8	2.56±1.46[1)]
Herbal cake-partitioned moxibustion	8	9.95±3.68[2)3)]
Simvastatin group	7	8.06±3.50[2)]

Note: Compared with the normal group, 1) $P<0.05$; compared with the model group, 2) $P<0.01$; compared with the simvastatin group, 3) $P<0.05$

3 Discussion

AS can be ascribed to chest Bi-impediment, dizziness or stroke in traditional Chinese medicine (TCM) according to the clinical symptoms. It happens mainly because of the blocked meridians due to intermingled phlegm and bloodstasis, resulting in blockage of vessels and dystrophy of heart meridian.

Herbal cake-partitioned moxibustion belongs to indirect moxibustion in moxibustion method. It is performed by putting a herbal cake on the selected acupoints, and then a moxa cone is placed on the herbal cake. The active ingredient of the herbal cake medicine will infiltrate through skin into the acupoints by the thermal effect of moxibustion. Thereby, the

physical factors produced during moxibustion, the pharmacochemical factors of the herbal cake and the specific effects of acupoints produce a comprehensive effect, to unblock the meridians, reconcile qi and blood, and regulate the Zang-fu organs[10].

Abnormal lipid metabolism such as dyslipidemia characterized by elevated LDL-C and TC and imbalance of cholesterol homeostasis are the most important risk factors for AS[11]. Down-regulating LDL-C level can reduce the risk of cardiovascular and cerebrovascular diseases, and can significantly reduce the development and its mortality risk of atherosclerotic cardiovascular disease[12-13]. Effective control of dyslipidemia is of great significance for the prevention and treatment of AS. Our research showed that both herbal cake-partitioned moxibustion and simvastatin can reduce serum LDL-C and TC, and liver TC, indicating that herbal cake-partitioned moxibustion can produce a certain regulatory effect on lipid disorders.

RCT is the most important way for the body to discharge excess cholesterol, and it is also one of the important mechanisms of the body in anti-AS[14]. RCT is the process that excess cholesterol in the body is transported via a transporter from peripheral cells to HDL, circulated to the liver with blood, bound to receptors of hepatocyte surface, selectively ingested and converted to bile salts, and then discharged with feces. This process includes three component elements of intracellular cholesterol efflux, cholesterol esterification, and *in vivo* clearance[15]. RCT is a complex dynamic equilibrium process involving multiple enzymes, receptors, and protein transporters. Changes in any component elements of cholesterol efflux, transportation, and clearance can affect the rate and efficiency of RCT[16]. Liver X receptor (LXR) is a family of receptors in the nucleus, divided into two subtypes of LXRα and LXRβ. In recent years, it has been found that LXRα is most abundantly expressed in liver and can sense the level of oxidized sterols in hepatocytes. It is an important nuclear transcription factor regulating liver lipid metabolism. It is a key signal transduction pathway of lipid metabolism that is currently of interest to scholars at home and abroad, and it also plays an important role in the regulation of ester metabolism and RCT *in vivo*.

Activated LXRα can increase the RCT to liver and excretion of cholesterol in tissues by up-regulating the expressions of apoli-protein A (ApoE), ATP-binding cassette transporter A1 (ABCA1), ABCG1 and other target genes, which inhibit the formation of AS in animal models[17]. Knockout experiments have also demonstrated that LXRα has strong anti-AS capabilities[18]. Naik SU, *et al*[19] subcutaneously injected ^{3}H-labeled cholesterol into wild-type c57BL/6L mice and then LXR agonist GW3965, finding that ABCA1 expression in liver was increased, blood HDL-C was increased significantly, and fecal 3H bile acid was increased. Zhao C, *et al*[20] found that the expressions of ABCA1, ABCG1 and ApoE were increased after activation of LXRα in macrophages and promoted RCT. Therefore, by up-regulating the expression of LXRα in macrophages, it is possible to inhibit the intracellular deposition of lipids, delay the formation of foam cells, and prevent the progression of AS, thereby maintaining the stability of the cholesterol environment *in vivo*.

The herbal cake in this study mainly contains *Dan Shen* (*Radix Salviae Miltiorrhizae*), *Da Huang* (*Radix et Rhizoma Rhei*), *Shan Zha* (*Fructus Crataegi*), *Yu Jin* (*Radix Curcumae*) and *Ze Xie* (*Rhizoma Alismatis*). Among the ingredients, *Dan Shen* (*Radix Salviae Miltiorrhizae*) acts to circulate blood, resolve stasis, clear heart and alleviate restlessness; *Da Huang* (*Radix et Rhizoma Rhei*) to promote bowel movement and clear the bowel; *Shan Zha* (*Fructus Crataegi*) to move qi, resolve stasis and promote digestion; *Yu Jin* (*Radix Curcumae*) to cool blood and circulate blood, move qi and resolve phlegm; and *Ze Xie* (*Rhizoma Alismatis*) to drain dampness and resolve phlegm. The herbal cake can therefore alleviate symptoms of AS.

Chinese medicine holds that the heart dominates blood and blood vessels. The combination of Back-Shu point Xinshu (BL 15) and Front-Mu point Juque (CV 14) is indicated to activate heart yang, benefit heart qi and thus promote blood circulation. As for other points, Tianshu (ST 25) is the Front-Mu point of the large intestine, Fenglong (ST 40) is a specific point to resolve phlegm, Ganshu (BL 18) and Pishu (BL 20) are the Back-Shu points of the liver and spleen, the four points are used in combination to soothe the liver, strengthen the spleen, regulate the spleen and stomach and thus increase the activity of liver enzymes. This may in turn regulate gastrointestinal function in absorption and discharge and normalize blood fat.

Moxibustion has the effect of warming to dissipate external cold pathogen, warming meridians and dredging collaterals, and activating blood circulation to remove stasis. Applying herbal cake-partitioned moxibustion to the above acupoints could jointly achieve the goal of anti-AS and regulating blood lipids.

The results of this study showed that herbal cake-partitioned moxibustion could improve the aorta and liver lipidation, regulate blood lipid and liver lipid levels, and improve the LXRα expression in the livers of AS rabbits, thereby promoting RCT and playing an anti-AS role. This study partially revealed the mechanism of herbal cake-partitioned moxibustion for AS, and provided a theoretical and experimental evidence for the clinical application of this method to treat AS.

Conflict of Interest

The authors declared that there was no potential conflict of interest in this article.

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Statement of Human and Animal Rights

The treatment of animals conformed to the ethical criteria.

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