Double Regulation of Supplement-Detoxification on Diabetic Atherosclerosis by Inhibiting Excessive Autophagy

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Abstract: Objective To explore the mechanism of Huoxue Jiedu Jiangtang recipe (HJJR) in regulating the hyperactivation of endoplasmic reticulum stress (ERS) autophagy PERK-eIF2α-LC3 pathway, and reveal its prevention for diabetic atherosclerosis. Methods First, the rats were injected with streptozotocin intraperitoneally to make the diabetic models, and then the aortic balloon injury combined with high-fat feeding were used to make the models of atherosclerosis who were randomly divided into model group, western medicine group (Gliquidone + Benazepril), low-dose HJJR group (HJJR1), high-dose HJJR group (HJJR2), and accepted corresponding drugs for 8 weeks respectively. Another blank group was used as control. The changes of serum glycosylated hemoglobin (GHb), homocysteine (Hcy) and angiotensinII (Ang1I) were compared. HE staining was used to detect aortic pathology. The mRNA transcription of PRK-like endoplasmic reticulum kinase (PERK), eucaryotic translation initiation factor 2α (eIF2α) and CCAAT / Enhance-Binding Protein Homologous Protein (CHOP) were tested by reverse-transcription polymerase chain reaction (RT-PCR). Western blot were used to detect the expression of autophagy activating protein ATG6 (Beclin-1), autophagy protein microtubule-associated protein light chain LC3-1, LC3-II and LC3-II/LC3-1. Results Compared with the model group, after the drug intervention, the aortic tissue injury in each drug group were reduced, and the improvement in the Chinese medicine group was significantly better than that of the western medicine group (P < 0.05). All treatment groups could significantly lowered the levels of serum GHb, Hcy, and Ang1I (P < 0.05), could down regulate the transcription level of PERK, eIF2α and CHOP mRNA (P < 0.05); and decreased the amount of Beclin 1, LC3-II and LC3-II/LC3-1 autophagy protein in aortic cells (P < 0.05). The effect of HJJF group was better, and it was more significant with the increase of HJJF dose. Conclusion HJJR can protect arteries by inhibiting ERS caused by metabolic disorder in diabetes, correcting the homeostasis affecting ER, reducing the overreaction of PERK-eIF2-LC3, and alleviating autophagy effect. Keywords: Diabetes Mellitus, Atherosclerosis, Huoxue Jiedu Jiangtang Recipe, Endoplasmic Reticulum Stress, Autophagy

1. Introduction

Atherosclerosis (AS) is an important initiating factor for cardiovascular and cerebrovascular diseases, such as coronary heart disease (CHD) and stroke, so it is very important to study the prevention and treatment for AS. In the past, the AS preventing mainly focused on inflammation theory and vascular endothelial injury. [1] Recently, it was found that excessive autophagy can lead to increased autophagic cell death, while the death of vascular smooth muscle cells and endothelial cells can reduce the synthesis of collagen fibers and thin the fibrous cap of plaque, reduce the stability of plaque, and then cause acute clinical events [2]. Therefore, during the occurrence and development of AS, exploring the effect of regulating autophagy mechanism on AS will provide a new strategy for the AS preventing and treating. According to the theory of traditional Chinese medicine (TCM), “deficiency of Qi and Yin, accumulation of stasis and toxin” are the main pathogenesis of diabetic AS, so “supplementing Qi, nourishing Yin, promoting blood circulation, detoxifying and eliminating accumulation” are its main therapeutic principle [3]. The preliminary clinical research found that
2. Methods

2.1. Animal Modeling

2.1.1. Diabetes Modeling

100 male SD rats, weighing 220-250g, with certificate number SCXK (Gui) 2014-002, provided from the Laboratory Animal Center of Guangxi Medical University, were randomly divided into normal group, model group, western medicine group (gliquidone+benazepril group), low dose HJJR group (HJJR1) and high dose HJJR group (HJJR2). At room temperature of 22±2°C, relative humidity of 40-50%, and 7 days after being fed with common feed, model group, western medicine group, HJJR1 and HJJR2 were given equal volume distilled water. The normal group were given the same amount of citric acid buffer by intraperitoneal injection, and fed common feed.

2.1.2. Diabetic Atherosclerosis Modeling

Atherosclerosis model was established by aortic balloon injury combined with high-fat diet. The model group, western medicine group, HJJR1 and HJJR2; group diabetic rats were fasted for 8 hours, then, were anesthetized by injected with 3% pentobarbital sodium 10 ml/kg intraperitoneally. Under sterile conditions, make central cervical incision, separate the left external carotid artery, ligate the distal end, insert a 1.5F balloon toward the heart, fill the balloon after entering about 8cm, slowly pull back about 5cm after feeling resistance, empty the balloon, enter 5cm again, fill and pull back, repeat 3 times, exit the balloon, ligate the proximal end, suture layer by layer, and inject penicillin sodium for 3 days after surgery. Then continue to feed with high-fat diet for 1 month.

2.2. Animal Administration

HJJR (composed of Ophiopogon japonicus, Ginseng, Schisandra chinensis, Cornus officinalis, Astragalus membranaceus, Rehmannia glutinosa, Yam, Turtle shell, Peach kernel, Danpi, Coptis chinensis, Rhubarb, Salvia miltiorrhiza) purchased from the Affiliated Hospital of Youjiang National Medical College, were decocted in stainless steel bucket, concentrated to 130% in high dose and 80% in low dose respectively, and stored in refrigerator for standby. In HJJR1 group, 3ml/100g of 80% solution was administered to the stomach, while in HJJR2 group, 3ml/100g of 130% solution was administered. The western medicine group was fed with benazepril 2.50 g/kg and Gliquidone 7.50 mg/kg (Beijing Wanhui Shuanghe Pharmaceutical Co., Ltd.) daily, reaped for same intragastric volume as HJJR group, continued to feed high-fat diet for 2 months. The normal group and model group were given equal volume distilled water. The food and water intake were recorded daily.

2.3. Sampling

Half an hour after the last administration, blood were taken from jugular vein, serum were separated, and stored in -20°C refrigerator. Then the rats were killed, the thoracic aorta were quickly taken out on ice, washed twice with normal saline, divided into three parts, one part were fixed with 4% paraformaldehyde for HE staining to examine artery pathology, and the remaining two parts were put into -80°C liquid nitrogen for Western blot and RT-PCR experiments to detect the expression of Beclin-1, LC3-I, LC3-II autophagic proteins, and endoplasmic stress signal molecules PERK, eIF2α, CHOP mRNA transcription.

2.4. Index Detection

2.4.1. Detection of Serum Glycosylated Hemoglobin (GHb), Homocysteine (Hcy) and Angiotensin II (Ang1I)

The levels of GHb, Hcy and Ang1I were measured by enzyme-linked immunosorbent assay (ELISA). Their kits were purchased from Beijing Huayeyang Biotechnology Co., Ltd. Their contents were measured according to the standard curve, which experimental procedures were strictly carried out according to the instructions. Take out the serum sample from the -20°C refrigerator and melt it at room temperature. The specific detection steps are as follows: (i) Add samples to the enzyme standard plate, and set blank holes, standard holes, and sample holes respectively. In addition to blank holes, add 100ul of standard solution or sample, mix gently, reacting at 37°C for 120min. (ii) Discard the liquid, add 100ul of testing solution A, react at 37°C for 60min, and wash the plate three times. (iii) Add 100ul of testing solution B to each hole, reacting at 37°C for 60min, wash the plate for 5 times, and shake it dry. (iv) Add 90ul of substrate (TMB) solution into each hole, color rendering in dark for 30min. (v) Add 50 ul of termination solution to each hole to terminate the reaction. Measure the absorbance (OD data) of each hole at 450 nm wavelength with an enzyme-linked instrument. (vi) Calculate GHb, Hcy and Ang1I in the sample according to the OD data of the standard and sample.

2.4.2. The Experiment

Thoracic aorta was dehydrated step by step by ethanol → paraffin embedded → cut 44um slice→baking at 60°C in oven→xylene dewaxing→dehydration step by step with...
ethanol→dyeing for 5min with hematoxylin→water washing for 5min→hydrochloric acid ethanol differentiation for 30s→water soaking for 15min→Add eosin solution for 2min→water washing →dehydration step by step with ethanol→image collection and analysis with 400 times microscope. Semi quantitative analysis of HE: Grade 0: vascular endothelial structure is smooth, cells are arranged orderly, surface is dense, and intima of aortic tissue is complete; Grade 1: vascular endothelial structure is smooth, cells are arranged orderly, and aortic intima is complete; Grade 2: swelling of cells, thickening of intima, with atherosclerotic plaques in vessels; Grade 3: the cells were obviously swollen, arranged unevenly, the intima was thickened, and there were many atherosclerotic plaques in vessels; Grade 4: vascular endothelial cells fall off and disorder, cells swell significantly, intima thickens significantly, and there are concentrated atherosclerotic plaques in vessels.

2.4.3. Detection of PERK, eIF2α and CHOP mRNA
Transcription in Aortic Tissue by RT-PCR
Take out the aorta from the -80°C refrigerator, add Trizol solution to grind for decomposition →centrifuge at 12000 rpm for 10 min→take the supernatant, add 200µL chloroform, 12000 rpm, centrifuge for 10 min→Add isopropanol to supernatant, 12000 rpm, centrifugation for 15 min, then discard the supernatant→add 1 mL 75% ethanol to rinse the sediment, 4°C, 12000 rpm, centrifuge for 5 min, then discard the supernatant→add 1 mL anhydrous ethanol, 12000 rpm, centrifuge for 5 min, discard the supernatant, dry for 10 min at room temperature→add 40µL DEPC water to dissolve RNA. According to the instructions of the reverse transcription kit: 42°C, 15 min; 85°C, 5 min; reverse transcription into cDNA. Amplify the obtained cDNA to the target gene (according to the operating instructions of the PCR kit), steps and circulation parameters: Denaturation at 95°C for 10 min; 95°C, 15s; 60°C, 60s; 40 cycles. Record the number of cycles to reach the setting threshold, CT data. Using the dissolution curve to detect the specificity of the primer, and using 2^(-ΔΔCt) to calculate the mRNA transcriptions. The primers for the experiment were designed and synthesized by Kangwei Century Biotechnology Co., Ltd. Table 1 for primers.

Table 1. Primer List.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Upstream primer</th>
<th>Downstream primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>PERK</td>
<td>5′-CTTGGACCTGGTCTGCT-3′</td>
<td>5′-GGGGAGGCTTCGACAGCA-3′</td>
</tr>
<tr>
<td>eIF2a</td>
<td>5′-ACAGCCCTTCCAAGA-3′</td>
<td>5′-ACCAGGGATTACAAAGC-3′</td>
</tr>
<tr>
<td>CHOP</td>
<td>5′-TCAACCAAGAAGACAC-3′</td>
<td>5′-GGCGCTTGTAGAGCCAC-3′</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-GATTGGTAGGAGTCTTGA-3′</td>
<td>5′-TGAACCTGCGGTTTAGAG-3′</td>
</tr>
</tbody>
</table>

2.4.4. Detection of Beclin-1, LC3-I and LC3-II Functional Proteins in Aortic Tissues by Western Blot
Take out the aorta from the -80°C refrigerator, add 1mL Lysis Buffer, lyse the homogenate for 15min, centrifuge for 5min, take the supernatant, measure its concentration with BCA kit. After SDS-PAGE electrophoresis and membrane transformation, put PVDF membrane into incubation box, add sealing solution containing 5% skimmed milk powder, and shake it for 1.5 h. After sealing, wash the membrane with TBST for 3 times. Put the membrane into the diluent containing the first antibody and incubate it overnight in a shaking table at 4°C. Shake at room temperature for 30 min, suck out the first antibody, and wash 3 times with TBST. Dilute the secondary antibody with 5% skimmed milk solution, and shake it for 1h. Retrieve the secondary antibody, and then wash the membrane with TBST for three times. Add mixed liquid luminous reagent on PVDF, react for 3min, use ECL imager to take and store the pictures, and use Image J software to measure and analyze the gray data.

2.5. Statistical Treatment
All data were processed with SPSS 19.0 software, and expressed with mean ± standard deviation (±S). The data were tested for homogeneity of variance, and if the variance were homogeneous, LSD statistical method were to use; otherwise, Tamhane’s T2 method were used. Analyze intergroup differences with one-way ANOVA. P <0.05 indicate the statistically significant difference.

3. Result
3.1. Analysis of the Staining Results of Aortic Tissue
In the blank group, the vascular endothelial structure were smooth, the cells arranged orderly, the surface were dense, and the intima of aortic tissue were intact; while in model group, vascular endothelial cells shed, arranged disorderly, swelled significantly, intima thickened obviously, and there were concentrated atherosclerotic plaques in vessels. Compared with normal group, the scores in model group increased significantly (P<0.01); Compared with model group, the scores of each administration group decreased (P<0.05); Compared with western medicine group, HJJR1 and HJJR2 groups had lower scores (P<0.05), and the effect was significant with the increase of dose. The results are shown in Table 2 and Figure 1.

Table 2. Comparison of pathological change scores of rat aorta (±S, n=20).

<table>
<thead>
<tr>
<th>Group</th>
<th>normal</th>
<th>model</th>
<th>western medicine</th>
<th>HJJR1</th>
<th>HJJR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Score</td>
<td>2.12±0.04</td>
<td>3.22±0.08</td>
<td>2.34±0.09</td>
<td>1.85±0.08</td>
<td>1.52±0.07</td>
</tr>
</tbody>
</table>

compared with the normal group, aP<0.01; Compared with the model group, bP<0.05; Compared with western medicine group, cP<0.05.
3.2. Effect of HJJR on the Levels of GHb, Hcy and Ang1I in Rats

Compared with normal group, the contents of GHb, Hcy and Ang1I in model group were significantly increased (P<0.01); Compared with the rats in model group, after administration, the GHb, Hcy and Ang1I in each administration group decreased, with P<0.05; Compared with western medicine group, HJJR1 and HJJR2 were more effective (P<0.05); HJJR has better effect with increasing dose. The results are shown in Table 3.

Table 3. Effect of HJJR on serum levels of GHb, Hcy and Ang1I (±S, n=20).

<table>
<thead>
<tr>
<th>Group</th>
<th>GHb (mmol/L)</th>
<th>Hcy (mmol/L)</th>
<th>Ang1I (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>6.43±1.16</td>
<td>12.12±1.62</td>
<td>2.98±0.08</td>
</tr>
<tr>
<td>model</td>
<td>21.87±4.52</td>
<td>20.21±2.81</td>
<td>8.51±0.07</td>
</tr>
<tr>
<td>western medicine</td>
<td>14.76±4.08</td>
<td>18.81±0.24</td>
<td>3.77±0.08</td>
</tr>
<tr>
<td>HJJR1</td>
<td>12.97±3.78</td>
<td>16.68±0.22</td>
<td>2.83±0.08</td>
</tr>
<tr>
<td>HJJR2</td>
<td>15.76±4.51</td>
<td>17.79±0.21</td>
<td>3.74±0.09</td>
</tr>
</tbody>
</table>

compared with the normal group, *P<0.01; Compared with the model group, #P<0.05; Compared with western medicine group, △P<0.05.

3.3. Effect of HJJR on PERK, eIF2α and CHOP mRNA Transcription

Compared with normal group, the PERK, eIF2αand CHOP mRNA transcription in model group increased significantly (P<0.01); Compared with model group, the PERK, eIF2αand CHOP mRNA transcription decreased (P<0.05); Compared with western medicine group, the PERK, eIF2αand CHOP mRNA transcription decreased more significantly (P<0.05); The effect of HJJR is better with increase dose, with dose dependent. The results are shown in Table 3 and Figure 2.

Table 4. Comparison of mRNA transcription levels of PERK, eIF2αand CHOP in rat aorta of each group (2^ΔΔCt, ±S, n=20).

<table>
<thead>
<tr>
<th>Group</th>
<th>CHOP</th>
<th>PERK</th>
<th>eIF2α</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>1.09±0.27</td>
<td>1.05±0.20</td>
<td>1.08±0.29</td>
</tr>
<tr>
<td>model</td>
<td>7.45±0.35*</td>
<td>7.82±0.28*</td>
<td>7.66±0.31*</td>
</tr>
<tr>
<td>western medicine</td>
<td>4.38±0.41*</td>
<td>4.87±0.46*</td>
<td>4.88±0.45*</td>
</tr>
<tr>
<td>HJJR1</td>
<td>4.32±0.55*</td>
<td>4.81±0.87*</td>
<td>4.69±0.87*</td>
</tr>
<tr>
<td>HJJR2</td>
<td>3.14±0.37*</td>
<td>3.33±0.28*</td>
<td>3.19±0.28*</td>
</tr>
</tbody>
</table>

compared with the normal group, *P<0.01; Compared with the model group, #P<0.05; Compared with western medicine group, △P<0.05.
Figure 2. Amplification and dissolution curve of PERK, eIF2α and CHOP.
3.4. Effect of HJJR on Beclin 1, LC3-1, LC3-II Functional Protein and LC3-II/LC3-1 Ratio

Compared with the normal group, the Beclin 1, LC3-II and LC3-II/LC3-1 in the model group, western medicine group, HJJR1 and HJJR2 group were significantly higher than those in the normal group (P<0.01). Compared with the model group, the Beclin 1, LC3-II and the ratio of LC3-II/LC3-1 in the western medicine group, HJJR1 and HJJR2 group decreased significantly (P<0.05). Compared with the western medicine group, the HJJR1 and HJJR2 group expressed less Beclin 1 and LC3-II, decreased LC3-II/LC3-1 ratios (P<0.05), with dose dependent. The results are shown in Table 5 and Figure 3.

Table 5. Effect of HJJR on the Expression of Autophagy Proteins Beclin 1, LC3-1, LC3-II and LC3-II/LC3-1 in Aortic Cells of Rats (x̅±S, n=20).

<table>
<thead>
<tr>
<th>Group</th>
<th>Beclin 1</th>
<th>LC3-1</th>
<th>LC3-II</th>
<th>LC3-II/LC3-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td>0.554±0.020</td>
<td>1.943±0.120</td>
<td>0.958±0.076</td>
<td>0.493±0.08</td>
</tr>
<tr>
<td>model</td>
<td>2.223±0.052</td>
<td>2.011±0.066</td>
<td>2.921±0.082</td>
<td>1.453±0.11</td>
</tr>
<tr>
<td>western medicine</td>
<td>1.581±0.028a</td>
<td>2.082±0.027</td>
<td>2.589±0.038a</td>
<td>1.244±0.13a</td>
</tr>
<tr>
<td>HJJR1</td>
<td>1.616±0.053a</td>
<td>2.051±0.051</td>
<td>1.316±0.068a</td>
<td>0.642±0.12a</td>
</tr>
<tr>
<td>HJJR2</td>
<td>1.443±0.017a</td>
<td>2.044±0.021</td>
<td>1.041±0.027a</td>
<td>0.509±0.10a</td>
</tr>
</tbody>
</table>

compared with the normal group, *P<0.01; Compared with the model group, *P<0.05; Compared with western medicine group, #P<0.05.

4. Discussion

Autophagy is a highly conserved mechanism of eukaryotic cells. Under the regulation of related genes, autophagy uses lysosomes to degrade damaged organelles and senescent proteins in cells, and maintain cell homeostasis, which is an indispensable metabolic pathway [5]. The endoplasmic reticulum is the main organelle in cell. When unfolded or misfolded proteins accumulate in the cell, the unfolded protein reaction (UPR) will be activated and endoplasmic reticulum stress (ERS) occur [6]. If the homeostasis of the intracellular environment is not restored for a long time, the excessive activation of ERS- autophagy will aggravate the cell damage, leading to autophagic cell death [7]. Generally, ERS induces autophagy through UPR three pathways: protein kinase like endoplasmic reticulum response kinase (PERK), inositol demanding enzyme 1 (IRE-1) and activated transcription factor 6 (ATF6) [8]. In the PERK signaling pathway, PERK is the activation of eukaryotic translation initiation factor 2α (eIF2α) and closely related to autophagic reaction in cells, as a result, PERK/eIF2α pathway is an important regulator mediating the interaction between ERS and autophagy. Activated ATF4 and CCAAT enhancer binding protein homologous protein (CHOP) in PERK signal pathway can up-regulate the transcription of microtubule associated protein light chain 3 (LC3) and autophagy associated gene ATG5, and regulates the autophagy protein ATG12 by pass of eIF2α-ATF4-CHOP, promotes the transformation of LC3-I into LC-III (autophagy marker), and upregulates the expression of autophagy activating protein Beclin-1 (ATG6) by inhibiting the transcription of anti-apoptotic protein B cell lymphoma protein 2 (Bcl-2) gene, thus starting autophagy [9, 10].

The state of diabetes causes damage to the normal pathway of glucose metabolism, increases oxidative stress, produces excessive reactive oxygen species (ROS), blocks the folding of protein spatial structure, causes the accumulation of unfolded proteins, and then induces ERS, affecting the homeostasis of endoplasmic reticulum [11]. Accumulation of cholesterol, advanced glycosylation end products (AGEs), free fatty acids, homocysteine (Hcy), etc. in vascular smooth muscle and monocyte macrophages can continuously over activate UPR, leading to activation of PERK. ATF6 and IRE1 signal pathways, which not only promotes ERS response, but also enhances autophagy effect [12, 13]. Hyperhomocysteine (Hcy) is a common concomitant symptom of diabetic patients, which cooperates with AGEs to produce disulfide bond reaction with proteins, disturbs the normal protein folding in endoplasmic reticulum, increases the synthesis, uptake and accumulation of cholesterol and triglycerides, upregulates UPR effectors GRP78, JRE1 and ATF6, and triggers vascular endothelial cell ERS [14]. Hyperglycemia can also activate the renin-angiotensin system (RASS) system, increase AngII, and activate protein kinase C (PKC) through AT1 receptor, thus increasing vascular permeability [14]. Persistent ERS activation and enhanced autophagy effect lead to the interaction between ERS and autophagy from adaptive and protective to persistent and destructive, in which excessive autophagy of smooth muscle cells will lead to autophagic impotence and even autophagic death (type II programmed death) [15].

According to the theory of traditional Chinese medicine (TCM), diabetes belongs to the category of “Xiao Ke”, Qi-Yin deficiency and dryness-heat characteristic are the basic diabetic pathogenesis, and also the basic pathogenesis of diabetes AS, Qi deficiency can cause blood stasis, while Yin deficiency can aggravate internal heat; excessive...
accumulation of heat and stasis can develop into toxin, while phlegm and stasis can turn into accumulate over time [16]. According to «Jingyue Quanshu·accumulation» “all tangible illness are accumulated by stagnation, retention of pus and blood stasis, and condensed into disease lumps”, the formation of atherosclerotic plaque is consistent with the mechanism of accumulation in TCM. Therefore, on the basis of nourishing Yin, replenishing Qi and resolving phlegm, the therapeutic effect of detoxification and promoting blood circulation should be emphasized. Just as «The Law of Medical Doors» “Qi incompatible should be unobstructed, depression be opened, blood stasis be dispersed, and fire stagnation be eliminated or dissipated” discussion on Chest pain. HJJR consists of ginseng, Schisandra chinensis, Ophiopogon japonicus, Astragalus membranaceus, Cornus officinalis, Rehmannia glutinosa, Salvia miltiorrhiza, Rhubarb, Coptis chinensis, turtle shell, peony bark and peach kernel, In which, Schisandra chinensis and Ophiopogon japonicus are Li Xiao (Jin-Yuan Dynasty great doctors) famous prescriptions for tonifying Qi and nourishing Yin; Rehmannia glutinosa, cornel and yam are Zhang Jingyue (Ming Dynasty physician) “Zuo Gui Pill” for treating Yin deficiency; Turtle shell, rhubarb, Danpi and peach kernel are Zhang Zhongjing (Eastern Han Dynasty famous doctor) “Turtle shell pills” ingredients, with functions of “activating blood circulation, softening firmness and detoxifying”; in addition, added Astragalus membranaceus to strengthen Qi-invigorating and detoxification, Coptis chinensis to reinforce heat-clearing, detoxification and dampness-drying, and Kadan to intensify stasis-dispersing. It is an innovation from tonifying Qi, nourishing Yin, removing stasis, clearing away toxin, and dispersing knots to deal with diabetic AS.

5. Conclusion

LC3 and Beclin-1 are autophagy marker proteins. LC3 initially synthesized an untreated precursor form LC3, which was then split by ATG4 to generate an active cytosol form LC3-I, next step, LC3-I interacted with membrane phosphatidyl-ethanolamine to generate LC3-II by ATG7 catalysed, while the relative amount of LC3-II reflects the content of autophagosome, and LC3-II/LC3-I ratio can be used as a marker of autophagy level, reflecting autophagy activity [17, 18]. In this study, on the basis of diabetic modeling in rats, through aortic balloon injury, combined with high-fat feeding, the AS model was created, which promoted the over activation of ERS signaling pathway, thus causing autophagy. After successful modeling, the expression of Beclin-1, LC3-II and the ratio of LC3-II/LC3-I in AS aorta increased significantly, indicating over-activated autophagy. After the intervention by HJJR, the expression of Beclin-1, LC3-II and the ratio of LC3-II/LC3-I in aorta decreased significantly, suggesting that excessive autophagy were inhibited. The high-level expression of glycosylated hemoglobin, Hey and Ang1II was inhibited, and the marker molecules eIF2a, PERK and eIF2a of ERS were down regulated, indicating that HJJR reduced the expression of ERS autophagy signal pathway molecules (PERK-eIF2a-LC3) at the protein and gene levels. At the same time, the HJJR can reduce the shedding of vascular endothelial cells, inhibit the cell swelling, reduce the formation of atherosclerotic plaque in the blood vessels, significantly alleviate the intimal hyperplasia, protect the aortic intima, and the effect of the HJJR increases with the dosage.

6. Recommendations

Since the 1980s, atherosclerosis (AS) treatment has made progress in the general population. However, in the diabetic population, the prevalence of AS is increasing. At present, both domestic and foreign countries have established diagnostic and therapeutic guidelines for clinical practice, but the efficacy of diabetic AS is still poor, which is related to the complexity of AS with diabetes. Although AS is a pathological process involving multiple factors, this study has found that, regulating excessive autophagy can help prevent AS progress, and provide new targets and basis for the treatment of diabetic AS for TCM. Multi-link, multi-channel and multi-target integrated regulatory intervention is the direction for AS treatment in the future, which is the full play of the unique advantages of TCM.

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References


