Tiaozhi Tongmai Granules reduce atherogenesis and promote the expression of ATP-binding cassette transporter A1 in rabbit atherosclerotic plaque macrophages and the liver

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Abstract

Objective: ATP-binding cassette transporter A1 (ABCA1) is an integral membrane protein that plays a key role in cellular lipid metabolism, preventing the accumulation of lipids that contribute to the initiation and progression of atherosclerosis. Tiaozhi Tongmai Granules are a Chinese herbal compound that is capable of treating atherosclerosis. This study was designed to explore the potential pharmacological mechanism by which Tiaozhi Tongmai Granules protect against atherosclerosis.

Methods: Forty-nine male New Zealand rabbits were randomly divided into seven groups: normal control group, normal diet; model groups 1 and 2: balloon injury and high-fat diet for 6 or 12 weeks; statin groups 1 and 2: balloon injury and high-fat diet plus atorvastatin for 6 or 12 weeks; and Chinese herb groups 1 and 2: balloon injury and high-fat diet plus Tiaozhi Tongmai Granules.

Keywords

Atherosclerosis; ABCA1; Chinese medicine; Macrophages; Liver

Abbreviations: ABCA1, ATP-binding cassette transporter A1; ALP, alkaline phosphatase; ALT, alanine transaminase; CCA, common carotid artery; CV, central vein; ECA, external carotid artery; HDL-C, high density lipoprotein cholesterol; ICA, internal carotid artery; IEF, internal elastic lamina; LDL-C, low density lipoprotein cholesterol; OD, optical density; TA, thoracic aortae; TC, total cholesterol; TCM, traditional Chinese medicine; TG, triglyceride; WT, weight.

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for 6 or 12 weeks. The granules were administered at a dose of 1.14 g/kg/d, with atorvastatin (1.14 mg/kg/d) serving as positive control. Serum lipid profiles and liver function indices were measured. Atherogenesis was viewed after H&E staining and quantified by thickened intimal area percentage and maximal intimal thickness percentage. The ABCA1 protein expression in atherosclerotic plaque macrophages of the common carotid arteries (CCA), thoracic aortae (TA), and liver tissues were observed by immunohistochemical staining and evaluated using mean optical density (OD) value in macrophages and ABCA1-positive hepatocyte number.

Results: Compared with model group 1 at week 6, Chinese herb group 1 and statin group 1 displayed significant reductions in total cholesterol (TC) (P = 0.027, 0.012) and low-density lipoprotein cholesterol (LDL-C) (P = 0.039, 0.028) levels, as well as marked increases in ABCA1-positive hepatocyte numbers (P all <0.001), and only statin group 1 displayed a markedly reduced maximal intimal thickness percentage in the CCA (P = 0.018). Compared with model group 2 at week 12, Chinese herb group 2 and statin group 2 all presented significant reductions in TC (P = 0.011, 0.003), LDL-C (P = 0.017, 0.010) and thickened intimal area percentage in the CCA (P = 0.001, 0.022), as well as prominent increases in the ABCA1 OD value of both the CCA (P = 0.001, 0.039) and TA (P = 0.001, 0.025) and positive hepatocyte number (P all <0.001). Chinese herb group 2 had a markedly reduced maximal intimal thickness percentage compared with model group 2 (P = 0.006) and a higher positive hepatocytes number than statin group 2 (P = 0.001).

Conclusions: Tiaozhi Tongmai Granules appear to have an anti-atherogenic effect that is most likely mediated by simultaneously upregulating the protein expression of ABCA1 in rabbit atherosclerotic plaque macrophages and in the liver.

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Drugs

Tiaozhi Tongmai Granules, based on a TCM empirical formula, is comprised of seven ingredients: *Radix Astragali* 20 g, *Radix Angelicae Sinensis* 15 g, *Semen Cassiae* 30 g, *Rhizoma Alismatis* 12 g, *Fructus Crataegi* 30 g, *Lumbricus* 15 g, and *Radix Notoginseng* 6 g. The herbal granule compound was prepared according to standard procedure. The seven herbs were decocted together twice in water, and the decoction was filtered. The filtrate was collected, concentrated into the appropriate amount and spray-dried. Adjunct ingredients were added and after blending evenly, the granules were prepared with each dose ultimately weighing 20 gram. The granules were provided by the Beijing University of Chinese Medicine Third Affiliated Hospital. Atorvastatin calcium tablets (Pfizer Pharmaceuticals, Approval No. H20051408, Batch No. 1237304) were provided by the China-Japan Friendship Hospital, Beijing.

Quality control of Tiaozhi Tongmai Granules

The monarch herb, the leading ingredient in Tiaozhi Tongmai Granules, is *Radix Astragali*, in which astragaloside IV acts as one of the main bioactive components, and thus is the marker of quality control for the herb. The content of astragaloside IV, detected using previously reported method, in the prepared granules was 0.046%, which met the requirements of the Pharmacopoeia of People’s Republic of China, 2010 Edition. In addition, we performed a component identification test of another main ingredient in the granules, *Semen Cassiae*, according to a previously described method and confirmed that the granules contained chrysophanol and aurantio-obtusin, the two representative markers of *Semen Cassiae* prescribed by the Pharmacopoeia. The aforementioned components are described in more details in Supplemental files 1 and 2. All herbs were reliably provided by the Beijing University of Chinese Medicine Third Affiliated Hospital.

Animal groups

After being fed adaptively normal diets for 1 week, all rabbits were randomly divided into 7 groups (7 rabbits per group). The normal control group was fed with a normal diet for 2 weeks and sacrificed. The remaining six groups were all fed with high-fat diets for 2 weeks and then received a balloon injury procedure on the left common carotid arteries. In these six groups, model groups 1 and 2 continued to receive high-fat diets for 6 and 12 weeks before sacrifice, respectively. Statin groups 1 and 2 received atorvastatin therapy plus high-fat diets for 6 and 12 weeks, respectively, before sacrifice. Chinese herb groups 1 and 2 received Tiaozhi Tongmai Granules plus high-fat diets for 6 and 12 weeks, respectively, before sacrifice.

Establishment of atherosclerotic model in rabbits

ABC1 overexpression in normal C57BL/6 mice on a proatherogenic diet may play an anti-atherogenic role and significantly reduce aortic atherosclerosis. However, apoE gene knock-out (apoE-KO) mice overexpression of ABCA1 (i.e., ABCA1 transgene, ABCA1-Tg) has produced conflicting data. Singaraja et al. reported a marked reduction in atherosclerotic lesions in ABCA1-Tg×apoE-KO mice, whereas Joyce et al. demonstrated a significant increase in atherosclerosis. Reasons behind these phenomena are not clear. Most likely the proatherogenic effect induced by the absence of the apoE gene may counteract the protective effect of ABCA1, leading to difficulty and uncertainty in the interpretation of the results. To avoid interference related to the gene knock-out, we chose New Zealand rabbits with a normal genetic background. Proatherogenic serum lipid profiles on a high-fat diet are achieved easily in these animals, and when combined with a balloon injury operation, the formation of atherosclerotic plaques can be accelerated.

The balloon injury procedure was performed as follows: fasting rabbits were anesthetized using 25% urethane (4 ml/kg) intravenously combined with Sumianxin (0.05 ml/kg) intramuscularly and placed on the back (face-up). Systemic heparinization was performed by intravenously injecting heparin sodium at a dosage of 200 IU/kg. After the cervical skin was depilated, sterilized, and incised along the median line, the left common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were freed and exposed by blunt dissection at the level of the superior border of the thyroid cartilage. The distal ECA was ligated, and the CCA and ICA were occluded temporarily with two vascular clamps. At the site 0.3 cm away from the carotid bifurcation in the left ECA, a tiny v-shaped opening was cut with a pair of ophthalmic microscopic scissors. A heparinized hollow catheter conveying device was then inserted into the CCA from that opening. After releasing the vascular clamp on the CCA, a catheter with a tiny inflatable balloon (2.0 mm × 15 mm or 2.5 mm × 15 mm) at its tip, a balloon catheter (SeQuent, B. Braun Medical, Inc., USA), was threaded through the conveying device into the CCA. When the tip was placed at the proximal end of the CCA, the balloon was inflated to a low atmospheric pressure (2.0–3.5 atm) with a manual pressure pump. The inflated balloon was then retracted at an appropriate speed to the distal end of the CCA and deflated to produce a vascular injury. After repeating the injury procedure thrice, the balloon catheter was withdrawn, followed by ligation of the ECA at the position between the opening and carotid bifurcation. Finally, the remaining vascular clamp was removed to restore blood flow in the left CCA-ICA passage. The wound was sutured and bandaged.

Drug intervention and animal administration

Atorvastatin was administered at a clinical equivalent dosage of 1.14 mg per kilogram per day (equivalent to 20 mg per 70 kilogram adult per day), and Tiaozhi Tongmai Granules were administered at a clinical equivalent dosage of 1.14 g per kilogram per day (equivalent to one dose of 20 gram per 70 kilogram adult per day) all via intragastric injection. The two types of drugs were dissolved in an appropriate amount of distilled water to ensure the drug delivery volume was maintained at 4 ml per kilogram per day. Model groups were intragastrically administered the same volume of distilled water alone.
without any drugs. All rabbits remained in individual cages, with temperature-controlled air-conditioning in 12 hour cycles of light and dark. Animals were each given ad libitum access to distilled water and a high-fat diet of 120 g per day.

**Specimen collection**

At the end of experiment, blood samples were collected via the right common carotid arteries and centrifuged for 10 min at 3000 rpm at 4 °C to obtain serum. Levels of serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alanine transaminase (ALT) and alkaline phosphatase (ALP) were measured with a Hitachi 7600 biochemistry autoanalyzer (Japan). After sacrificing the animals by air embolization, 1 cm long vascular tissues were cut from the left CCA at its bifurcation and from the thoracic aorta at the start of the descending aorta. The left middle lobe of the liver was selected for collection of tissue blocks, and a 1 cm × 1 cm × 0.5 cm volume was considered suitable. After rinsing gently with normal saline, the tissue was fixed in 10% neutral buffered formalin and embedded in paraffin. Four micron thick serial sections were used.

**Morphological analysis**

Sections were stained with Harris hematoxylin and eosin (H&E) using Leica ST 5020 Multistainer (Germany). Images were viewed and captured with an Olympus BX53 microscope attached to the Olympus cellSens imaging software platform (Japan). For vascular tissues, the measurement of (1) the thickened intimal area (i.e., plaque area) and (2) the maximal intimal thickness and the circumference of internal elastic lamina (IEF), by which the circular area surrounded by IEF and its radius can be calculated, were performed using Image-Pro Plus software (version 6.0, Media Cybernetics, USA). Finally, the thickened intimal area per circular area surrounded by IEF (%) and the maximal intimal thickness per radius of circular area surrounded by IEF (%) were quantified to evaluate the degree of plaque progression by taking the average of 3 sections spaced 20 microns apart.

**Immunohistochemical staining**

Tissue sections were mounted on positively charged slides (CITOGLAS, 188105W), deparaffinized and rehydrated. Heat-induced antigen retrieval was performed using a pressure cooker in sodium citrate buffer (10 mM sodium citrate in distilled water, pH 6.0) for 3 minutes as soon as the cooker had reached full pressure. Sections on the slides were treated with 3% H2O2 in PBS for 15 min to inactivate the endogenous peroxidase, followed by incubation in 10% normal goat serum in PBS for 30 min at room temperature to block non-specific protein-protein interaction. The primary mouse monoclonal anti-ABCA1 antibody (Abcam, ab18180) was applied to the slides diluted to the concentration of 10 μg/ml (for blood vessels) or 5 μg/ml (for liver) in antibody diluent (Invitrogen, 00-3118) and then the ready-to-use polymer-HRP anti-mouse secondary antibody (ZSGB-BIO, PV-9002) was used according to the manufacturer’s protocol. Afterward, the slides were stained with DAB solution (ZSGB-BIO, ZLI-9018) for the appropriate time control under a microscope and finally dehydrated, cleared, and mounted.

For vascular tissues, the plaque area and the integrated optical density of positively expressed ABCA1 protein within the plaque were measured using Image-Pro Plus software. The ratio of the latter to the former was the mean optical density that was needed. For the liver, all hepatocytes and the hepatocytes expressing ABCA1 protein, designated positive cells, within the individual hepatic lobules were counted, and the positive cells per all hepatocytes (%) value was then calculated. The quantifications were performed by taking the average of 3 sections (for the blood vessels) and 5 independent fields of view in 3 sections (for the liver).

**Statistical analysis**

SPSS statistics software (version 18.0, SPSS Inc., USA) was used for statistical analysis. All data were presented as mean ± standard deviation (SD). The comparison of the mean values between two groups was evaluated with a two-tailed Student’s t-test, and multiple group comparisons were performed with one-way analysis of variance (ANOVA) followed by the LSD or S-N-K method. P-values less than 0.05 were considered significant.

**Results**

**General conditions and serum biomarkers**

Two rabbits died of diarrhea, 1 of flatulence, and 3 of drugs entering the lungs during intragastric injection. The remaining animals remained alive and well. The surgical wounds healed without incident. Serum biomarkers and body weight of rabbits in all groups are listed in Table 1. Among serum lipid biomarkers, TC level decreased significantly in statin group 1 and Chinese herb group 1 compared with model group 1 at week 6 (F = 11.987, P = 0.012 and P = 0.027) as well as in statin group 2 and Chinese herb group 2 compared with model group 2 at the week 12, respectively (P = 0.003 and P = 0.011). Similarly, LDL-C level also presented significant reductions in statin group 1 (F = 10.421, P = 0.028) and Chinese herb group 1 (P = 0.039) compared with model group 1 as well as in statin group 2 (P = 0.010) and Chinese herb group 2 (P = 0.017) compared with model group 2. However, TG and HDL-C levels failed to display any significant differences among all groups. Between the two drug groups, no significant difference in TC and LDL-C was observed at either time points. Liver function indices, such as ALT and ALP, were non-statistically (F = 0.732 and F = 0.358, P all > 0.05) different between the model group and two drug groups at either time points.

**Evaluation of atherosclerotic plaque**

In the present study, the size of atherosclerotic plaques evaluated was represented by two types of percentages:
the thickened intimal area per circular area surrounded by IEF and the maximal intimal thickness per radius of circular area surrounded by IEF. For the CCA, at week 6 after balloon injury, the two percentage values were the largest in model group 1 and lower in the two drug groups, with only the maximal intimal thickness percentage in statin group 1 decreasing significantly (P = 0.018, Fig. 1B) compared with model group 1. At week 12 after balloon injury, the thickened intimal area percentage increased to the top in model group 2 and decreased significantly in statin group 2 and Chinese herb group 2 (F = 21.146, P = 0.022 and P = 0.001) compared with model group 2 (Fig. 1A), of which Chinese herb group 2 presented a slightly greater reduction than statin group 2, although the trend was not significant (P = 0.546). The maximal intimal thickness percentage decreased significantly in Chinese herb group 2 (F = 12.461, P = 0.006) but not in statin group 2 (P = 0.351) compared with model group 2 (Fig. 1B).

As thoracic aortae (TA) had just formed a minimal lesion after 6 weeks due to the only impact of a high level of blood lipids, the present study only evaluated the more significant lesion formed after 12 weeks. At this point in time, neither of the two percentages for the TA in model group 2 was comparable to those of the CCA after 6 weeks in model group 1. The two drug groups both displayed reducing percentages compared with model group 2, yet these reductions were not significant (P all >0.05).

**ABCA1 expression in macrophages within atherosclerotic plaques of common carotid arteries**

At week 6 post-procedure, ABAC1 protein was mostly expressed focally in the superficial layer of plaques in the model group 1 (Fig. 2A – d, g). In statin group 1, the protein had an increasing region of expression still at the superficial layer of plaques, primarily with a banded structure (Fig. 2A – e, h). There was a broader distribution of ABCA1 protein in Chinese herb group 1, some of which even reached the middle layer of plaques in addition to the superficial layer (Fig. 2A – f, i). At the cellular level, ABCA1 protein was expressed not only in the plasma membrane but also within the cytoplasm of individual macrophages within plaques. In statin group 1, ABCA1 protein was commonly expressed in the peripheral region of the cytoplasm near the plasma membrane (Fig. 2A – h). In Chinese herb group 1, the protein filled nearly the whole cell in addition to its membrana expression, which was most likely due to the absence of large lipid droplets in the cytoplasm (Fig. 2A – i). These results suggest that ABCA1 has a broader cytoplasmic localization for achieving its shuttling

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**Table 1**  Serum lipid profiles, liver function, and body weight of rabbits among groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>TC (mmol/L)</th>
<th>TG (mmol/L)</th>
<th>LDL-C (mmol/L)</th>
<th>HCL-C (mmol/L)</th>
<th>ALT (U/L)</th>
<th>ALP (U/L)</th>
<th>WT (kg)</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>1.05 ± 0.23</td>
<td>0.78 ± 0.46</td>
<td>0.34 ± 0.06</td>
<td>3.26 ± 0.23</td>
<td>23.00 ± 1.44</td>
<td>49.00 ± 8.49</td>
<td>3.00 ± 0.15</td>
</tr>
<tr>
<td>Model 1</td>
<td>7</td>
<td>23.08 ± 3.07</td>
<td>0.90 ± 0.58</td>
<td>24.20 ± 2.49</td>
<td>4.19 ± 1.38</td>
<td>22.00 ± 4.66</td>
<td>69.15 ± 9.28</td>
<td>3.32 ± 0.19</td>
</tr>
<tr>
<td>Statin 1</td>
<td>6</td>
<td>12.10 ± 6.47</td>
<td>1.12 ± 1.35</td>
<td>13.32 ± 4.12</td>
<td>3.74 ± 2.83</td>
<td>36.50 ± 6.36</td>
<td>68.75 ± 6.55</td>
<td>3.28 ± 0.13</td>
</tr>
<tr>
<td>Chinese herb 1</td>
<td>6</td>
<td>13.63 ± 4.95</td>
<td>0.66 ± 0.57</td>
<td>14.03 ± 4.92</td>
<td>5.08 ± 1.42</td>
<td>32.00 ± 5.64</td>
<td>65.60 ± 5.20</td>
<td>3.10 ± 0.24</td>
</tr>
<tr>
<td>Model 2</td>
<td>6</td>
<td>32.03 ± 4.21</td>
<td>0.83 ± 0.76</td>
<td>33.15 ± 2.45</td>
<td>7.22 ± 0.95</td>
<td>37.29 ± 6.02</td>
<td>58.25 ± 8.52</td>
<td>4.35 ± 0.78</td>
</tr>
<tr>
<td>Statin 2</td>
<td>6</td>
<td>16.95 ± 4.31</td>
<td>0.98 ± 0.48</td>
<td>19.16 ± 6.24</td>
<td>6.83 ± 0.78</td>
<td>50.50 ± 7.78</td>
<td>66.00 ± 5.66</td>
<td>4.25 ± 0.87</td>
</tr>
<tr>
<td>Chinese herb 2</td>
<td>5</td>
<td>19.45 ± 7.42</td>
<td>0.89 ± 0.63</td>
<td>20.30 ± 5.87</td>
<td>6.88 ± 0.66</td>
<td>40.75 ± 3.42</td>
<td>54.00 ± 7.07</td>
<td>4.30 ± 0.81</td>
</tr>
</tbody>
</table>

Data are the presented as the mean ± SD. *P < 0.05, vs. model group 1; **P < 0.05, vs. model group 2.

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**Figure 1**  Sizes of atherosclerotic plaques in the CCA and TA. (A) Ratio of thickened intimal area to circular area surrounded by IEF in the CCA at weeks 6 and 12 and the TA at week 12. (B) The ratio of maximal intimal thickness to radius of circular area surrounded by IEF in the CCA at both time points and the TA at week 12. *P < 0.05, vs. model groups.
between the plasma membrane and the cytoplasm, although it acts as an integrated membrane protein and performs its function mainly in the plasma membrane.

After 12 weeks, model group 2 presented an unsharp distribution of ABCA1 protein in plaques and faint membranal and cytoplasmic expression in individual macrophages (Fig. 2A e, m, p). In statin group 2, ABCA1 was expressed strongly in the middle layer of plaques and weakly in the superficial layer. Protein expression was maintained in the plasma membrane of macrophages and in the peripheral region of cytoplasm near the plasma membrane (Fig. 2A e, n, q). In Chinese herb group 2, ABCA1 was expressed vigorously in all layers of plaques and distributed in larger areas within the macrophage cytoplasm in addition to their membranal expression (Fig. 2A e, o, r) compared with the other two groups.

In regard to the mean optical density (OD) value of ABCA1 protein within plaques (Fig. 2B), statin group 2 after 12 weeks, the OD of ABCA1 increased significantly in statin group 2 and Chinese herb group 2 (P < 0.039 and P < 0.001). Chinese herb group 2 displayed a slightly greater increase trend than statin group 2, although this trend was not significant (P = 0.156).

**ABCA1 expression in macrophages within thoracic aortae atherosclerotic plaques**

ABCA1 protein was located mostly in the middle plaque layer and was at low levels in the superficial and deep layer in model group 2 after 12 weeks (Fig. 3A a, g). In comparison, statin group 2 displayed an even distribution of ABCA1 protein in the superficial and even the deep layer of plaques in addition to the middle layer (Fig. 3A b, h). The expression of ABCA1 in Chinese herb group 2 extended to all layers (Fig. 3A c, i). The OD of ABCA1 within plaques was significantly increased in statin group 2 and Chinese herb...
group 2 (F = 10.853, P = 0.025 and P = 0.001) compared with model group 2 (Fig. 3B), but there was no significant difference between the two drug groups (P = 0.211).

**ABCA1 expression in the liver**

Hepatocytes that expressed ABCA1 protein in their plasma membrane (hereafter called positive cells) were located mostly in the peripheral region within the hepatic lobule and rarely in the centrilobular area adjacent to the central vein (CV) in model group 1 after 6 weeks (Fig. 4A–a, d). Compared with model group 1, the amount of positive cells within individual hepatic lobules was significantly increased in statin group 1 and Chinese herb group 1 (F = 9.093, P all <0.001, Fig. 4B). In addition, increased positive cells were observed mainly in the perilobular region in statin group 1.
However, the difference in positive cell number between these two drug groups was non-significant ($P = 0.075$). The amount of positive cells within individual hepatic lobules in model group 2 was decreased significantly compared with model group 1 ($P < 0.001$), and such cells were hardly observed in the perilobular region after 12 weeks (Fig. 4A – g, j). Statin group 2 still displayed a perilobular distribution of positive cells (Fig. 4A – h, k), whereas Chinese herb group 2 demonstrated an extensive distribution including the centrilobular area near the CV (Fig. 4A – i, l). The positive cell numbers in these two drug groups ($P$ all <0.001) increased significantly compared with model group 2, and the increase rate was significantly higher in Chinese herb group 2 than in statin group 2 ($P = 0.001$).

![Figure 4](image)

**Figure 4** ABCA1 protein expression patterns in the liver. (A) Immunohistochemical staining of ABCA1 in liver tissue sections in each group. Images a, b, c, g, h, i, and m demonstrate a representative hepatic lobule. The letter V refers to the central vein, and the brown rings indicate the ABCA1-positive hepatocytes. Images d, e, f, j, k, l, and n (bars = 50 μm) are the higher magnifications of dotted boxes in images a, b, c, g, h, i, and m, respectively. (B) Evaluation of the percentage of ABCA1-positive hepatocytes in one hepatic lobule among all groups. *$P < 0.05$, vs. model group 1; **$P < 0.05$, vs. model group 2; ***$P < 0.05$, vs. statin group 2.

**Discussion**

The results demonstrated that Tiaozhi Tongmai Granules, which embody the TCM therapeutic principles of replenishing qi, activating blood, strengthening spleen, and resolving phlegm, can have an anti-atherogenic effect in rabbits. Thus, the potential pharmacologic mechanisms by which this therapeutic principle works will be the main content discussed in this paper.

The spleen in TCM is primarily in charge of transformation and transportation of grain or food, that is, the spleen transforms grain into food essence and blood and transports the body fluid for the stomach. This mechanism of blood formation is described in various TCM classics as a process in which the transformed food essence from grain is discharged into the blood vessels, designated meridians or
collaterals, and is further transformed into red blood, which is refined essence, followed by circulation for the nourishment of proximal and distal tissues, all via the spleen. The actions of discharging and circulating are actually an alternative embodiment of transportation. Thus, the general pathway of “essence metabolism” includes the continuous processes of essence transformation to transportation and from transportation to transformation again. In addition, these processes are also the ones that transfer from non-selective to selective essence disposal in which essence is continuously subdivided into components, such as cholesterol and other lipids, with different functions. The refined essence within blood vessels can be further utilized by the cells in the vascular wall, transported within the cells, or removed from the cells depending on cellular metabolic needs. This then is a multi-level, step-by-step refined and microcosmic metabolic pathway of essence, every level of which involves the functioning of the spleen.

According to TCM theory, pathologically, the spleen is the source of phlegm. If the spleen’s function is impaired, the grain (food) is not completely transformed, and the untransformed portion converts into phlegm. Similarly, if the ABCA1-mediated cholesterol efflux is impaired, large amounts of cholesterol that is deposited within vascular macrophages lead to an increasing number and size of intracellular lipid droplets and eventually foam cell formation, thus threatening overall cellular lipid metabolism or even energy homeostasis. The excess cholesterol accumulated in macrophages loses its physiologic functions and form the pathological substances required for the generation of atherosclerotic plaques. These substances resemble those that have a thick and dense nature and stagnate in the meridians, tissues, and viscera-phlegm as defined by TCM. Actually, such lipid plaques are generally known as phlegm by a number of TCM scholars. Thus, it is reasonable to speculate that the dysfunction of ABCA1 in macrophages is the determining factor for phlegm formation. ABCA1 in macrophages plays a role that is very similar to the spleen in TCM, and its function may be regarded as the same as that of the spleen’s function. In addition, the up- and down-regulation of ABCA1 protein expression level would represent the strength of the spleen’s function. It follows that the abstract spleen at the macro level in TCM is microcosmically subdivided into individual figurative vascular macrophage. Therefore, if macrophages maintain an active cholesterol efflux mediated by ABCA1, that is, maintaining the robust TCM function of the spleen, cholesterol could be completely transformed following ingestion by macrophages, just as Ji Sheng Fang (prescriptions for succoring the sick) described, so that there is limited opportunity for them to form phlegm, that is, lipid plaques. The present study appears to confirm our hypothesis that Chinese herbs function by strengthening the spleen and resolving phlegm. The herbs in Tiaozi Tongmai Granules include raw Huangqi (Radix Astragali), Danggui (Radix Angelicae Sinensis), Dilong (Lumbricus) and Sanqi powder (Radix Notoginseng), are used. In our study, this formula was found to improve hepatocellular expression of ABCA1 at weeks 6 and 12, setting the stage for mobilizing the spleen functionality in macrophages.

Chinese herbs appear to play a lipid-lowering effect in the early lesion stage. With increasing duration of treatment, this effect was more significant although milder than that observed with atorvastatin. The atherosclerotic lesions of the CCA were mild in the early stage and became advanced in the later stage, whereas the lesions of the TA in the later stage were still mild and not yet comparable to those of the CCA in the early stage. The therapeutic effects of Chinese herbs and statin for reducing atherogenesis and up-regulating ABCA1 protein expression in vascular macrophages were not prominent for the mild lesions of the CCA in the early stage; only atorvastatin reduced the maximal intimal thickness percentage. When the advanced lesions of the CCA formed in the later stage, these effects were significant, and Chinese herbs played a more marked role in

ApoA-I, a major protein component of high density lipoprotein in plasma, is produced by the liver and circulates to peripheral tissues such as vascular walls. Interaction of lipid-poor apoA-I with the ABCA1 molecules of macrophages promotes phosphorylation of ABCA1 protein, boosting its kinase activities and enhancing its stability via a variety of signaling pathways, including PCPLC-DG-PKC, AC-cAMP-PKA, and RhoA GTPase as well as thus maximizing the potential of ABCA1 functionally. However, failing to be lipitated, lipid-poor apoA-I suffers a rapid catabolism once produced. Fortunately, the hepatocellular ABCA1 mediates the initial lipidation of apoA-I. Harboring a larger hepatocytes base than macrophages, the liver is the organ that expresses the most ABCA1 proteins. Such large amounts of ABCA1 in hepatocytes aid apoA-I in achieving the fullest potential of ABCA1 in peripheral macrophages functionally, just as the harmony of qi and blood helps mobilize the full potential of the spleen’s function in macrophages. The protein expression of the functional ABCA1 in hepatocytes plays a unique role that is, to some extent, the equivalent of the harmony of qi and blood.

To enhance protein expression of hepatocellular ABCA1, qi and blood need to be harmonized. For this reason ingredients that replenish qi and activate blood in Tiaozi Tongmai Granule, including raw Huangqi (Radix Astragalii), Danggui (Radix Angelicae Sinensis), Dilong (Lumbricus) and Sanqi powder (Radix Notoginseng), are used. In our study, this formula was found to improve hepatocellular expression of ABCA1 at weeks 6 and 12, setting the stage for mobilizing the spleen functionality in macrophages.

Chinese herbs appear to play a lipid-lowering effect in the early lesion stage. With increasing duration of treatment, this effect was more significant although milder than that observed with atorvastatin. The atherosclerotic lesions of the CCA were mild in the early stage and became advanced in the later stage, whereas the lesions of the TA in the later stage were still mild and not yet comparable to those of the CCA in the early stage. The therapeutic effects of Chinese herbs and statin for reducing atherogenesis and up-regulating ABCA1 protein expression in vascular macrophages were not prominent for the mild lesions of the CCA in the early stage; only atorvastatin reduced the maximal intimal thickness percentage. When the advanced lesions of the CCA formed in the later stage, these effects were significant, and Chinese herbs played a more marked role in
lowering the maximal intimal thickness percentage. As this percentage represents the degree of plaque eccentricity, the results indicated that atorvastatin reduced the degree of plaque eccentricity of mild lesions, whereas Chinese herbs diminished that of advanced lesions more obviously. For the mild lesions of the TA in the later stage, the effect of reducing atherogenesis was not fully achieved, whereas the up-regulation of ABCA1 protein expression in macrophages was significant. Thus, the effect of Chinese herbs in reducing atherogenesis may be more related to lesion severity than to lesion stage, and up-regulation of ABCA1 protein expression in macrophages may be more correlated with duration of drug intervention than to the lesion severity.

In the liver, ABCA1 protein expression was activated and up-regulated during the early development stage of atherosclerotic lesions and further enhanced by Chinese herbs and atorvastatin. In the later development stage of lesions, a relatively high level of ABCA1 protein expression was maintained, with Chinese herbs displaying a more significant advantage than atorvastatin. These results suggested that ABCA1 protein in hepatocytes might be more sensitive to the up-regulating effect of drugs compared with vascular macrophages, which thus accounts for the occurrence of their early therapeutic effect.

In sum, though in the short-term, Chinese herbal therapy may fail to present marked improvement of mild atherosclerotic lesions, it may be sensible to under-estimate the power of gradual and quantitative changes and abandon treatment, as reflected in the old saying that good things come to those who wait. Thus, the results from our study indicate that sustained administration of Tiaozhi Tongmai Granules may produce significant therapeutic effects.

Conclusion

The herbs in Tiaozhi Tongmai Granules, a compound that embodies the therapeutic principle of strengthening the spleen and resolving phlegm, work synergistically by replenishing qi and activating blood and improve the function of the spleen in vascular macrophages via the hepatocyte ABCA1/macrophase ABCA1 pathway, thereby producing an anti-atherogenic effect derived from the sustained application of the medication.

Competing interests

The authors declare that they have no competing interests.

Author’s contributions

SWG and MZ conceived the study. QS designed the study. QS, LZ, JL, and JTW carried out the establishment of the animal model, animal administration, drug intervention and H&E staining. QS and JNW performed the immunohistochemical staining. DDY and CLZ analyzed the data. QS wrote the manuscript. All authors read and approved the final manuscript.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jtcms.2014.11.007.

References


