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Original Article

Danlou Tablet Improves Chronic Intermittent Hypoxia-Induced Dyslipidemia and Arteriosclerosis by HIF-1α-Angptl4 mRNA Signaling Pathway*

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ABSTRACT **Objective**: To detect whether Danlou Tablet (DLT) regulates the hypoxia-induced factor (HIF)-1α-angiopoietin-like 4 (Angptl4) mRNA signaling pathway and explore the role of DLT in treating chronic intermittent hypoxia (CIH)-induced dyslipidemia and arteriosclerosis. **Methods**: The mature adipocytes were obtained from 3T3-L1 cell culturation and allocated into 8 groups including control groups (Groups 1 and 5, 0.1 mL of cell culture grade water); DLT groups (Groups 2 and 6, 0.1 mL of 1,000 μg/mL DLT submicron powder solution); dimethyloxalylglycine (DMOG) groups (Groups 3 and 7, DMOG and 0.1 mL of cell culture grade water); DMOG plus DLT groups (Groups 4 and 8, DMOG and 0.1 mL of 1,000 μg/mL DLT submicron powder solution). Groups 1–4 used mature adipocytes and groups 5–8 used HIF-1α-siRNA lentivirus-transfected mature adipocytes. After 24-h treatment, real-time polymerase chain reaction and Western blot were employed to determine the mRNA and protein expression levels of HIF-1α and Angptl4. In animal experiments, the CIH model in ApoE$^{-/-}$ mice was established. Sixteen mice were complete randomly divided into 4 groups including sham group, CIH model group [intermittent hypoxia and normal saline (2 mL/time) gavage once a day], Angptl4 Ab group [intermittent hypoxia and Angptl4 antibody (30 mg/kg) intraperitoneally injected every week], DLT group [intermittent hypoxia and DLT (250 mg/kg) once a day], 4 mice in each group. After 4-week treatment, enzyme linked immunosorbent assay was used to detect the levels of serum total cholesterol (TC) and triglyceride (TG). Hematoxylin-eosin and CD68 staining were used to observe the morphological properties of arterial plaques. **Results**: Angptl4 expression was dependent on HIF-1α, with a reduction in mRNA expression and no response in protein level to DMOG or DLT treatment in relation to siHIF-1α-transfected cells. DLT inhibited HIF-1α and Angptl4 mRNA expression (P<0.05 or P<0.01) and reduced HIF-1α and Angptl4 protein expressions with DMOG in mature adipocytes (all P<0.01), as the effect on HIF-1α protein also existed in the presence of siHIF-1α (P<0.01). ApoE$^{-/-}$ mice treated with CIH had increased TG and TC levels (all P<0.01) and atherosclerotic plaque. Angptl4 antibody and DLT both reduced TG and TC levels (all P<0.01), as well as reducing atherosclerotic plaque areas, narrowing arterial wall thickness and alleviating atherosclerotic lesion symptoms to some extent. **Conclusion**: DLT had positive effects in improving dyslipidemia and arteriosclerosis by inhibiting Angptl4 protein level through HIF-1α-Angptl4 mRNA signaling pathway.

KEYWORDS Danlou Tablet, Chinese medicine, hypoxia-induced factor, angiopoietin-like 4, dyslipidemia, arteriosclerosis

Obstructive sleep apnea (OSA) is a common disorder characterized by repeated narrowing or collapse of the upper airway (UA) during sleep, causing a recurrent reduction or cessation of airflow, followed by various physio-pathological syndromes such as body hypoxemia and hypercapnia. Sufferers

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experience loud snoring and sudden body movement as a result.[1] OSA is highly prevalent worldwide, affecting an estimated 3%–9% of adult female and 10%–17% of adult male.[2,3] OSA typically results in chronic intermittent hypoxia (CIH), which contributes to arteriosclerosis,[4] oxidative stress,[5] proatherogenic dyslipidemia and hypertension,[6,7] conferring risk for myocardial infarction, ischemic heart disease,[8] stroke, and death.[9,10] At the same time, OSA treatment, e.g., continuous positive airway pressure (CPAP) can markedly reduce dyslipidemia.[11,12] This may be because CIH inhibits lipoprotein lipase (LPL), a key enzyme of triglyceride-rich lipoprotein clearance, and also up-regulates the expression of a potent LPL inhibitor, adipose angiopoietin-like protein 4 (Angptl4).[13] However, the role of Angptl4 in CIH-induced metallic dysfunction has not yet been determined.

Angptl4 is transcriptionally regulated by hypoxia-induced factor 1 (HIF-1),[14,15] as CIH-induced up-regulation of Angptl4 in visceral fat is abolished by heterozygous deficiency of HIF-1α and constitutive overexpression of HIF-1α increases Angptl4 levels.[16] HIF-1 is composed of 2 protein subunits including an oxygen sensitive α subunit (HIF-1α) and a constitutively expressed β subunit (HIF-1β).[17,18] HIF-1α regulates the expression of genes in response to hypoxia to maintain physiological oxygen homeostasis. Under normoxic conditions, HIF-1α becomes prolyl and is then degraded in proteasomes, whereas, under low oxygen stress, HIF-1α accumulates rapidly and stabilizes.[19,20] HIF-1α plays an indispensable role in the regulation of lipid metabolism and hypoxia-induced lipid accumulation, and its overexpression leads to increased levels of serum triglycerides.[21,22] HIF-1α protein content is regulated at the mRNA translation level or by changes in its rate of degradation.[23] To date, however, the signals involved in stabilization by hypoxia are unknown. HIF-1α levels can be increased by the suppression of prolyl hydroxylase (PHD) activity. DMOG is a cell permeable, competitive inhibitor of PHD and can stabilize HIF-1α both *in vitro* and *in vivo*.[24-27]

Chinese patent medicine Danlou Tablet (DLT,丹蒌片) has been widely used in China in angina pectoris and acute coronary syndrome.[28] It has similar effects as statins and has been demonstrated to significantly of phlegm and stasis, decrease the serum level of molecular inflammation, reduce plaque area, and attenuate the pathological degree of arteriosclerosis.[29] The involved mechanism may be via the reduction of serum lipids and release of platelet-derived growth factor, inhibition of the extracellular signal-regulated kinase signal pathway and vascular smooth muscle cells[30] and underlying mechanisms remain elusive.[31] Based on high performance liquid chromatography (HPLC), Wu, et al[32] analyzed 15 quality-control markers of DLT and found good consistency in active markers among 12 different batches.

In this study, we investigated whether DLT regulates the HIF-1α-Angptl4 mRNA signaling pathway in response to dimethyloxalylglycine (DMOG)-induced hypoxia. We also tested whether DLT can improve CIH-accelerated atherosclerosis by exposing $ApoE^{-/-}$ mice to a high fat and cholesterol diet, as well as to DLT and CIH, for 4 weeks. We selected this animal model as it is one of the most relevant models for atherosclerosis.[33]

METHODS

Cell Experiments

Cell Preparation

The 3T3-L1 cell was obtained from Shanghai Cell Bank (China) and cultured at 37 ℃ in a humidified atmosphere (95% air, 5% CO_2) using Dulbecco's modified Eagle's medium (DMEM, Batch No. 8158297, Gibco, ThermoFisher Scientific, USA) in 10% fetal bovine serum (FBS, Batch No. 1715753, Gibco, ThermoFisher Scientific, USA). Isobutylmethylxanthine (IBMX), insulin, and dexamethasone were purchased from Sigma (Merck KGaA, Germany) and were used to promote cellular differentiation. After 8 days, mature adipocytes were obtained. For transfection studies, mature adipocyte cells were exposed to either vehicle or DMOG for 24 h after infection with HIF-1α-siRNA transfected lentivirus. According to the HIF-1α gene information in GenBank, the best lentivirus siRNA interference sequence was compounded and selected: 5'-[TGCTGTTGGCAAGCATCCTGTACTGTGTTTTGGCCACTGACTGACACAGTACAATGCTTGCCAA]-3'.

Drug Administration

The DLT (Batch No. 20181112) was manufactured by Jilin Connell Pharmaceutical Co. Ltd., China, in accordance with the Chinese Pharmacopoeia 2015.[34] DLT were examined at 3 concentration gradients at 12, 24, and 48 h adding to the mature adipocytes, and then inhibition ratio of cells were measured by cell counting kit-8 (CCK-8) assay

(Batch No. DV0652, Dojindo Laboratories, Japan). The maximal inhibitory concentration of DLT on cells was 1,000 μg/mL at a 24-h treatment time. Thus, we selected 1,000 μg/mL and 24 h as the optimal concentration and treatment time of DLT for treating mature adipocytes.

Grouping

The mature adipocytes were allocated into 8 groups: control groups (Groups 1 and 5, 0.1 mL of cell culture grade water for 24 h); DLT groups (Groups 2 and 6, 0.1 mL of 1,000 μg/mL DLT submicron powder solution for 24 h); DMOG groups (Groups 3 and 7, DMOG and 0.1 mL of cell culture grade water for 24 h); DMOG plus DLT groups (Groups 4 and 8, DMOG and 0.1 mL of 1, 000 μg/mL DLT submicron powder solution for 24 h). Groups 1–4 used mature adipocytes and groups 5–8 used HIF-1α-siRNA lentivirus-transfected mature adipocytes.

Analysis of Gene Expression by Quantitative Polymerase Chain Reaction

A SYBR FAST quantitative polymerase chain reaction (qPCR) kit master mix (Batch No. 006300-3-1, KAPA Biosystems, USA) was used for real-time PCR analyses. Real-time PCR primers were designed for the genes of interest. Thermal cycling conditions consisted of 45 3-step cycles including denaturation for 10 s at 95 ℃, annealing for 60 s at 59 ℃, and extension for 15 s at 72 ℃. All reactions were performed in triplicate. The relative expression levels of HIF-1α and Angptl4 genes were calculated by the $2^{-\triangle\triangle Ct}$ comparative method.

Analysis of Protein Expression by Western Blot

Western blot was performed to determine the levels of HIF-1α and Angptl4 protein expression. The cells were lysed in cold RAPI buffer (Batch No. 20170612, Solarbio, China). A bicinchoninic acid (BCA) protein assay kit (Batch No. A81911125, Multiscience, China) was used to determine the HIF-1α and Angptl4 protein concentrations following the manufacturer's instructions.

The proteins were subsequently separated on 10% sodium dodecyl sulphate-polyacrylamide gels and transferred to polyvinylidene fluoride (PVDF) membranes, which were blocked with 5% bovine serum albumin (BSA)-Tris-buffered saline-Tween-20 (TBST) for 1 h. The membranes were incubated with specific primary antibodies (mouse anti-HIF-1α, rabbit anti-angiopoietin-like 4, rabbit anti-glyceraldehyde phosphate dehydrogenase antibodies) overnight at 4 ℃. After washing 3 times with TBST (10 min each time), the membranes were incubated with secondary antibodies (goat anti-mouse and rabbit anti-goat, Jackson) for 40 min under indoor temperature. After again washing 3 times with TBST (10 min each time), an ECL kit (Batch No. WBKLS0500, Millipore, Germany) was used to detect the immune complexes present on the membranes.

Animal Experimental Protocols

Animal Modeling

Sixteen male ApoE$^{-/-}$ mice (C57BL/6J, clean class, Batch No. SCXK (Jing) 2016-0010; Vital River Laboratory Animal Technology Co., China) with age of 12 week-old and a body weight of 30±0.5 g were used for the experiment. The mice were exposed to CIH or intermittent air (IA) and were fed with a high fat and high cholesterol diet. The CIH treatment involved FiO_2 cycling from 21% to 6.5%, 60 times/h from 9:00 am to 9:00 pm.[35] Feed was restricted, with each mouse initially fed 13 g in the first week, followed by 2 g in the following week until the fourth week, twice a day. Feed was not allowed during the intermittent hypoxia process, and mice were fed in the cage at the rest of the time. The mice in the non-CIH group was placed in a same sized animal container as the CIH group at the same time, with air pulses supplied instead of intermittent hypoxia daily. All mice were maintained in a temperature-controlled room (20–26 ℃, 40%–70% relative humidity) with a 12:12 light-dark cycle.

Grouping

After modelling, 16 mice were complete randomly divided into different groups as follows: sham group, model group [intermittent hypoxia and NS (2 mL/time) gavage once a day], Angptl4 Ab group [intermittent hypoxia and Angptl4 antibody (30 mg/kg) intraperitoneally injected every week[36]] and DLT group [intermittent hypoxia and DLT (250 mg/kg) once a day], 4 mice in each group.

After treatment for 4 weeks, blood samples, tissue specimens and blood vessel specimens were collected after intervention. After fasting for 5 h, posterior orbital blood was drawn under 2% isoflurane anesthesia and centrifuged at 5,000 r/min for 10 min at 4 ℃. Subsequently, the fat pad of the epididymis and cardiac tissue specimens were taken (tissue specimens) and the aortic arch were taken (blood

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vessel specimens) separated by phosphate buffer solution (PBS) slowly irrigating from the left ventricle, after animals were euthanized by cervical dislocation method. Blood samples of mice were collected. Serum and tissue samples were frozen at –80℃ until used for the measurement of biochemical parameters.

Serum Lipid Levels Detection

Serum total cholesterol (TC) and triglyceride (TG) levels were detected by enzyme-linked immunosorbent assay (ELISA, Nanjing Jiancheng Biology, China (Batch No. A111-1 and No. A110-2).

Aortic Hematoxylin and Eosin Staining

Vascular specimens were fixed with 4% paraformaldehyde, embedded in paraffin sections, and stained with hematoxylin-eosin (HE) to observe the atherosclerotic plaque in the aortic arch.[37] The cross-sectional area and necrotic area of total plaque were analyzed.

Determination of Aortic CD68 Expression by Immunohistochemistry

The expression of CD68 in mouse aortic plaque (macrophages) was detected by immunohistochemistry.[38] After the endogenous peroxidase activity had been inhibited by hydrogen peroxide (H_2O_2) for 20 min, sections were incubated overnight at 4 ℃ with primary antibody of CD68 (Batch No. ab201340, Abcam, China), followed by the secondary antibody of goat anti-mouse IgG (HRP, Batch No. A0216, Beyotime Biotechnology, China). The sections were viewed under a light microscope (Zeiss, Germany).

Statistical Analysis

Statistical analysis was performed using Microsoft Office and SPSS 23.0. All values were reported as means ± SEM after confirming that all continuous variables were normally distributed using the Kolmogorov-Smirnov test. Statistical significance for comparisons was determined by One-way ANOVA test for multiple comparisons. All tests were two-sided, and the significance level was set at $P<0.05$.

RESULTS

Cell Experiment Results

DLT Inhibits Angptl4 mRNA by Down-Regulating HIF-1α RNA Expression

To evaluate the genetic influence of DLT and gene regulatory pathway of HIF-1α on Angptl4, we examined the HIF-1α and Angptl4 mRNA expressions with and without specific HIF-1α knockdown. Angptl4 expression was dependent on HIF-1α, with a reduction in expression observed in relation to siHIF-1α-transfected cells ($P<0.01$). Furthermore, DLT inhibited HIF-1α and Angptl4 mRNA expression with and without DMOG addition ($P<0.05$ or $P<0.01$). Changes in Angptl4 mRNA were not observed in response to DMOG or DLT treatment in the presence of siHIF-1α in groups 5–8 (Figure 1).

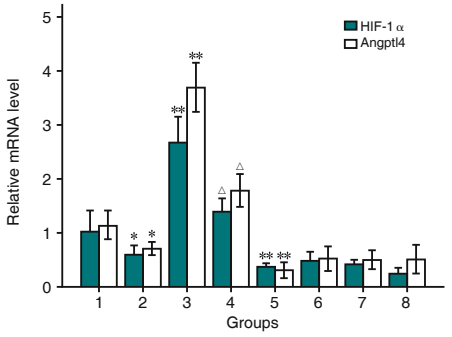

Figure 1. Changes in HIF-1α and Angptl4 mRNA Levels in Mature Adipocytes Groups by qRCR ($\bar{x} \pm$ SEM)

Notes: Groups 1–4 used mature adipocytes and groups 5–8 used HIF-1α-siRNA lentivirus-transfected mature adipocytes. Control group: Groups 1 and 5; DLT group: Groups 2 and 6; DMOG group: Groups 3 and 7; DMOG plus DLT group: Groups 4 and 8; the same below. $^{*}P<0.05$, $^{**}P<0.01$ *vs*. Group 1; $^{\triangle}P<0.01$ *vs*. Group 2

DLT Promotes HIF-1α Protein Degradation

DLT reduced HIF-1α and Angptl4 protein in mature adipocytes ($P<0.01$), as the effect on HIF-1α protein also was found in the presence of siHIF-1α ($P<0.01$). When infected with siHIF-1α, Angptl4 protein levels did not change in response to DMOG or DLT treatment (Figure 2).

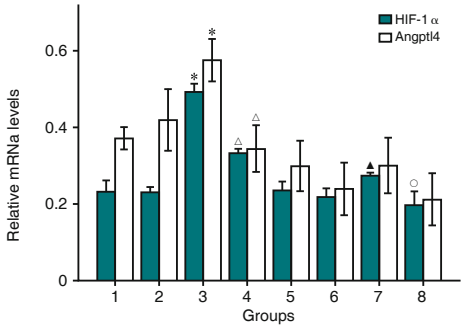

Figure 2. Changes in HIF-1α and Angptl4 Protein Levels in Mature Adipocytes Groups by Western Blot ($\bar{x} \pm$ SEM)

Notes: $^{*}P<0.01$ *vs*. Group 1; $^{\triangle}P<0.01$ *vs*. Group 2; $^{\blacktriangle}P<0.05$ *vs*. Group 5; $^{\circ}P<0.01$ *vs*. Group 7

Animal Experiment Results

Serum Lipid and Lipoprotein Assays

Compared with the sham group, TG and TC

levels in the model group were significantly increased (P<0.01). Compared with the model group, TG and TC levels in the serum of the Angptl4 Ab and DLT groups were significantly reduced (P<0.01; Figure 3).

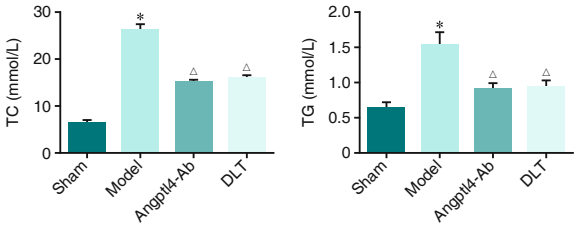

Figure 3. Determination of Serum Lipids and Lipoproteins in Mice by ELISA (n=4, $\bar{x} \pm$ SEM)

Notes: *P<0.01 *vs*. sham group; △P<0.01 *vs*. model group

Observation of Morphological Properties of Arterial Plaque

Compared with the sham group, the area of atherosclerotic plaque in the model group increased significantly, with a thickened artery wall and narrowed lumen. Compared with the model group, the atherosclerotic plaque areas in the Angptl4 Ab and DLT groups were significantly reduced, the artery wall thickness was somewhat narrowed, and the atherosclerotic lesion symptoms were alleviated to some extent (Figure 4).

Compared with the model group, the Angptl4 Ab and DLT groups showed significantly reduced atherosclerotic plaque area stained for CD68, abatement of staining intensity, narrowing of arterial wall thickness, and reduction in symptoms of atherosclerotic lesions (Figure 4).

DISCUSSION

OSA is a common cause of dyslipidemia due to CIH.[39] Risk factors of OSA include obesity, aging, male and so on.[40] OSA-associated gene abnormalities, such as those related to leptin, insulin-like growth factor-1, glucokinase, adenine nucleotide deaminase, melatonin-3 receptor, glucose-regulating protein, adrenergic receptor, orexin, and glucose regulating proteins, are known to negatively affect blood lipid distribution.[41] There is currently no reliable medical treatment to prevent dyslipidemia caused by OSA. Topical treatment like CPAP may provide comfort and symptomatic relief, but is not always accepted by patients.[42] The main novel finding of our present study was that DLT exhibited similar effects as Angptl4 protein antibodies on improving dyslipidemia and arteriosclerosis by inhibiting Angptl4 protein expression via HIF-1 α -Angptl4 mRNA signaling pathway.

DLT significantly decreased HIF-1 α protein levels in mature adipocytes under DMOG-stimulating hypoxia by inhibiting HIF-1 α mRNA expression and promoting HIF-1 α protein degradation, which, in turn, likely inhibited Angptl4 mRNA expression, resulting in Angptl4 protein level reduction. DLT administration showed no direct effect on Angptl4 mRNA and protein regulation. These results highlight the potential of DLT treatment for OSA-induced dyslipidemia.

The HIF family of transcription factors are principal mediators of the cell response to hypoxia.[43] HIF-1 consists of a constitutively expressed β subunit and an O_2-regulated α subunit. HIF-1 α activation by sustained hypoxia occurs due to inhibition of O_2-dependent prolyl hydroxylation.[44] CIH activates HIF-1 α not only through decreased degradation but also via increased HIF-1 α biosynthesis as it increases generation of reactive oxygen species through NADPH oxidase.[45] HIF-1 α overexpression in adipose tissue increases the levels of serum triglycerides, whereas HIF-1 α deficiency abolishes

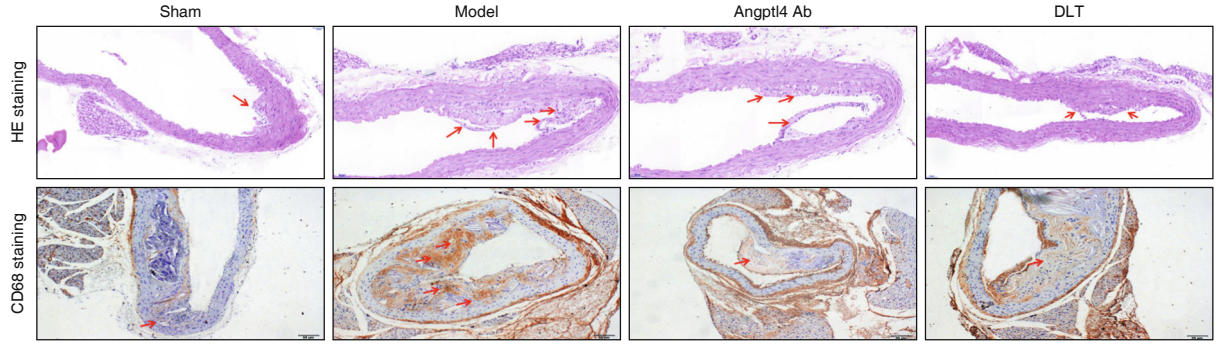

Figure 4. Observation of Morphological Properties of Arterial Plaques in Mice (× 200)

Notes: HE staining and CD68 staining results of aortic arch artery tissue in ApoE$^{-/-}$ mice. The red arrows in the image indicate atherosclerotic plaques

the CIH-induced increase in serum triglycerides. CIH induces severe hypoxia in adipose tissue, thus we used adipocytes and DMOG to up-regulate HIF-1α expression in the current study.[46]

DLT was initially approved by the China Food and Drug Administration for patients with CHD and angina pectoris in 2005. Unlike chemical drugs with a single active pharmaceutical ingredient, botanical drugs usually consist of complex mixtures of phytochemical constituents. Two most important Chinese herbal medicines of DLT compound preparation are *Salviae Miltiorrhizae* and *Trichosanthes kirilowii* Maxim. *Salviae Miltiorrhizae*, including the main bioactive ingredients such as Danshensu,[47] salvianolic acid-A[48] and -B,[49] has pharmacological activities including anti-oxidation, suppressing platelet adhesion and aggregation,[50] antioxidation,[51] promoting microcirculation, improving hemorheology, and regulating blood lipids.[52] *Trichosanthes kirilowii* Maxim, the main bioactive ingredients of which is 3,29-dibenzoyl rarounitriol,[53] is clinically used to treat thoracic obstruction, angina, cardiac failure, myocardial infarction, pulmonary heart disease, some cerebral ischemic diseases, etc (Appendixes 1 and 2).[54]

In our previous clinical report, DLT relieved the clinical symptoms of angina in patients with coronary artery disease (CAD) and improved inflammatory (hs-CRP, HCY, IL-6) and plaque factors (matrix metalloprotein-9, myeloperoxidase, vascular cell adhesion molecule-1).[24] Wang, et al[55] reported that DLT accelerates blood circulation and eliminates intravascular phlegm, playing critical roles in managing dyslipidemia and atherosclerosis based on CM theory. Liu, et al[56] conducted experiments to identify the cardio-protective effects of DLT, showing that DLT can significantly improve cardiac function and histopathology, inhibit the incidence of fatal and nonfatal ventricular fibrillation, and accelerate the severity of risk-region myocardial ischemia and reperfusion arrhythmia *in vivo*. Moreover, previous studies have revealed that puerarin, the main component of DLT, can significantly protect cardiomyocytes from anoxia/reoxygenation injury *in vitro* by ameliorating mitochondria dysfunction,[57,58] and regulating NF-κB signal pathway.[59] In addition to cardiac function, DLT can also relieve arterial endothelial injury, improve formation of new capillaries, and alleviate inflammatory reactions from atherosclerotic plaque.[60]

To date, however, the beneficial effects of DLT have not been mechanistically confirmed by adequate randomized, prospective, double-blind, placebo-controlled clinical trials. We aimed to investigate the mechanism of DLT benefit to vessel function under hypoxia conditions and found that DLT can reduce HIF-1α mRNA and protein expression levels. Therefore, the benefits of DLT in improving dyslipidemia may be explained by mechanisms related to decreased HIF-1α levels.

LPL, which can modulate lipid levels, nutrient partitioning, and coronary atherosclerosis risk,[61,62] is endogenously inhibited by Angptl4.[63] Further research has found that the inactivating variants in Angptl4 reduce risk of CAD in humans, suggesting that modulating Angptl4 may be a target for reducing risk of dyslipidemia.[64] As mentioned above, HIF-1α played a role in Angptl4-mediated dyslipidemia and HIF-1α mRNA deficiency likely abolished Angptl4 protein up-regulation directly at the adipocyte level without effecting on Angptl4 mRNA. These results are consistent with an earlier report, which showed that Angptl4 mRNA levels are influenced by HIF-1α mRNA.[46] Further results also found that DLT could reduce Angptl4 protein levels by inhibiting Angptl4 mRNA expression through the HIF-1α-Angptl4 regulation pathway, thereby playing an important role in the treatment of hyperlipidemia and arteriosclerosis caused by intermittent hypoxia.

Several limitations in this study should be acknowledged. Firstly, we only found DLT effects on HIF-1α-Angptl4 regulation, and the interaction between molecular substances in other signaling pathways should be fully considered. Secondly, similar to most Chinese herbs, DLT contains many components proven to have positive effects. We used DLT submicron powder solution in this study, rather than isolated effective constituents, which needs further examination.

Despite the above-mentioned limitations, we hope that further studies testing various DLT components and CIH-treated animals will ultimately justify the beneficial impact of DLT on post-OSA adverse vessel functions.

In summary, we identified CIH-induced dyslipidemia and arteriosclerosis, as well as the regulatory mechanism of the HIF-1α-Angptl4 mRNA signaling pathway in regard to DLT treatment. We observed that ApoE$^{-/-}$ mice acquired dyslipidemia and arteriosclerosis when exposed to CIH, and Angptl4 protein antibodies relieved the adverse outcomes. Interestingly, DLT demonstrated the same effects as Angptl4 protein antibodies on improving dyslipidemia and arteriosclerosis by inhibition of Angptl4 protein levels via the HIF-1α-Angptl4 mRNA signaling pathway. However, DLT had no direct effect on Angptl4 mRNA or protein regulation. Our findings support DLT as an effective treatment for CIH-induced dyslipidemia and arteriosclerosis and revealed the HIF-1α-Angptl4 mRNA signaling pathway mechanism.

Conflict of Interest

The authors declare that they have no conflict of interests.

Author Contributions

Tang JJ drafted this manuscript, Wang SH designed the study; Liu ZG, Yi R and Zhang YB completed the statistical analysis; Tang JJ, Li GX, Liu ZG, Yi R, Yu D, Zhang YB and Wang SH performed this study; Wang SH and Li GX made critical revision of the manuscript and contributed to the rationalization of the study. All authors read and approved the final manuscript for publication.

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