SM Vascular Medicine

Research Article

Effect of Gualou Xiebai Banxia Decoction on activin receptor-like kinase 1 mediated low-density lipoprotein uptake of vascular endothelial cells

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Abstract

Excessive low-density lipoprotein (LDL) uptake in vascular endothelial cells is closely related to atherosclerosis (AS) and coronary artery disease (CAD). Our animal study shows that Gualou Xiebai Banxia Decoction (GXBD) could reduce the amount of lipid deposition on the inner wall of blood vessels and relieve symptoms caused by AS and CAD. However, the underlying mechanism of GXBD on cells has not been elucidated yet. In this study, we study the effect of medicated serum, drawn from GXBD-intervened rats, on the human umbilical vein endothelial cells (HUVECs). Our findings suggest that GXBD-medicated rat serum can effectively reduce LDL uptake through downregulating the activin receptor-like kinase 1 (ALK1), therefore decreasing the apoptosis level of HUVECs. As a result, GXBD intervention might be an accountable approach to prevent AS-related diseases.

Keywords: Gualou Xiebai Banxia Decoction; human umbilical vein endothelial cells; activin receptor-like kinase 1; low-density lipoprotein; atherosclerosis

INTRODUCTION

Atherosclerosis (AS) is a precursor of most cardiovascular diseases, including coronary atherosclerotic heart disease and coronary artery disease (CAD). In these diseases, the deposition of AS plaque(s) on a coronary artery often gives rise to coronary artery insufficiency and causes consequences [1,2]. It is known that the apoptosis of vascular endothelial cells (VECs) can be used to accurately predict the AS's emergence; hence, blocking VECs' apoptosis should effectively avoid AS and the related diseases [3,4]. Since excessive low-density lipoprotein (LDL) tempt to react with reactive oxygen species (ROS) to induce the mitochondria-mediated apoptosis [5,6], it is believed that reducing the LDL uptake in VECs is an accountable strategy for prevention and treatment of AS and consequent CAD [7,8].

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Patients with CAD often have the symptom of turbid phlegm obstruction [9, 10], which is classified into the category of chest stuffiness and precordial pain in Traditional Chinese Medicine [11]. Classically, Gualou Xiebai Banxia Decoction (GXBD) is a standard prescription to treat symptoms in this category [12]. Experimental studies have proven that GXBD intervention can play a significant role in regulating abnormal lipid metabolism [13]. However, the underlying mechanism for this intervention is still unclear.

Meanwhile, it is also known that Activin receptor-like kinase 1 (ALK1) is involved in the courses of AS development [14-17]. ALK1 is a transmembrane serine/threonine kinase receptor that specifically binds to extracellular LDL and mediates the LDL uptake for VECs [18, 19]. Hence, it is of interest to explore whether GXBD intervention can relieve CAD-related symptoms through protecting VECs injury from high blood fat. Thus, in this study we fed ApoE-/- mice with high-fat diet to establish an AS model and verified the effect of GXBD on AS. Consequently, we infiltrated human umbilical vein endothelial cells (HUVECs) with oxidized low-density lipoprotein (ox-LDL) to establish *in vitro* VECs' injury model, mimicking pathology seen in patients [20, 21], and explored the effect of GXBD-medicated rat serum on this cell model to understand the underlying molecular mechanism of GXBD intervention in VECs injury through ALK1.

MATERIALS AND METHODS

Experimental animals and human umbilical vein endothelial cells (HUVECs)

All animal operations in this study strictly abide by relevant regulations of National Animal Welfare Ethics and Protection and ARRIVE guidelines [22].

Thirty-three healthy male ApoE-/- mice and 10 healthy male C57BL/6J mice, aged 6

- 8 weeks and weighing 20 ± 2 g, and 10 healthy female Sprague-Dawley rats, aged 6 - 8 weeks and weighing 200 ± 20 g, were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China).

High-fat (15-% fat + 0.25-% cholesterol) feed was purchased from Beijing Keao Xieli Feed Co., Ltd (Beijing, China). Human umbilical vein endothelial cells (HUVECs) were purchased from the Cell Resource Center of the Institute of Basic Medicine, Chinese Academy of Medical Sciences (Beijing, China).

Simvastatin and Gualou Xiebai Banxia Decoction (GXBD) preparation

Simvastatin was purchased from Tianjin Ruyi Shimao Pharmaceutical Co., Ltd (Tianjin, China) and administrated to mice by the does calculated according to the body surface coefficient of 12.3 [23-25]. All herbal medicines were purchased from Jianlian Traditional Chinese Medicine Store (Jinan, Shandong, China). Glutinous rice wine (8-% alcohol) was purchased from RT-Mart Supermarket (Jinan, Shandong, China).

The preparation of Gualou Xiebai Banxia Decoction (GXBD) followed the protocol in the "Herbal Prescription Science" [26]. Briefly, 24 g Gualou (dried and mature fruit of *Trichosanthes kiilowii* Maxim.), 9 g Xiebai (the dry bulb of *Allium macrostemon* Bge.), and 12 g Banxia (the dry tuber of *Pinellia ternata* (Thunb) Briet.) were crushed together before decocted by glutinous rice wine for 1 hour thrice with the respective volume 1000, 600, and 400 mL. After each decoction, four layers of gauze were used to filter the decoction. Then, three batches of filtrates were combined in water bath at 55 ℃ until the final volume was concentrated to 90 mL (*i.e.*, 0.5 g filtrate/mL). The final product was stored at 4 ℃ for later use.

Aorta sample preparation

Six hours after the final intervention, the mice were anesthetized by a 1 ml/100 g intraperitoneal injection of 10-% chloral hydrate. The thoracic cavities were opened and the sternums were cut out to expose the heart. The aorta samples were completely separated from the root of the aortic artery to the branch of the iliac artery and fixed in 4-% paraformaldehyde for 3 days after excess blood was rinsed off.

Consequently, aorta samples were washed twice with PBS, immersed in oil red O staining solution (Solarbio, Beijing, China) for 60 min, differentiated with 75-% ethanol until the lipid plaques in the lumens displayed orange-red or bright-red color, while other parts of samples were nearly colorless. Then, the samples were washed twice by distilled water.

Medicated and non-medicated serum preparation

After adaptive feeding for 1 week, 5 rats were randomly selected as the intervention group, administered an intragastric dose of 4.65 g/kg of the decoction (calculated according to the body surface coefficient of 6.2, to convert the required dose for rats) twice a day for 5 consecutive days [27]. One hour after the final administration, the rats were anesthetized by a 1 ml/100 g intraperitoneal injection of 10-% chloral hydrate. Blood was collected from the abdominal aorta, incubated at 37 ℃ for 30 min to promote blood coagulation, and kept at 4 ℃ for 2 hours. Afterwards, the sample were spun down at 3000 rpm for 15 min to remove the precipitation. Then, the serum was incubated at 56 ℃ for 30 min to inactivate the complement. Before being transferred into a cryopreservation tube and stored at -20 ℃ for later use, the serum was also filtered through a 0.22 μm microporous cartridge to keep sterile [28].

The remaining rats were considered as the control group, which did not receive any intervention. Likewise, non-medicated serum was extracted from the control group using the same procedure as the intervention group did and stored at -20 ℃ for later use.

HUVECs culture and ALK1 knockdown

HUVECs were maintained by DMEM/F12 (Solarbio) with 10-% fetal bovine serum (Solarbio) at 37 ℃ and 5-% CO2 with saturated humidity [29].

When the culture reached a fusion degree of 60% - 70%, the ALK1 siRNA (Tsingke, Wuhan, China) was transfected into the HUVECs for ALK1 knockdown using Lipofectamine 3000 (ThermoFisher, Shanghai, China). The success of ALK1 reduction was examined 24 hours after transfection through LightCycler® 480 II fluorescence quantitative PCR instrument (Roche, Shanghai, China) with FastStart Universal SYBR Green Master (Roche). The sample preparation for qPCR is listed in the mRNA Preparation section. Primers used for qPCR are listed in **Table 1**.

HUVECs injury model construction

When HUVECs culture reached a fusion degree of 80% - 90%, the media was removed and the culture was rinsed by PBS twice. Then, l mL trypsin (0.25%) was added to the culture for 5 - 10 min to detach the cells from the substrate before adding DMEM complete culture media to suspend the cells. Afterwards, the cells were spun down from the media by 1000 rpm centrifugation for 5 min, and then re-suspended in DMEM at 1x106 cells/mL seeding concentration, measured by Gallios flow cell meter (Beckman Coulter, Suzhou, China). Consequently, cells were implanted into a 6-well plate, 1 mL per well, for 24 hours before infiltrated by l ml of 100 μg/mL ox-LDL (Solarbio) for another 24 hours to construct the injury model [30].

Injury model evaluation through cell apoptosis assessment

The success of HUVECs injury model was evaluated through the occurrence of cell apoptosis. If the level of apoptosis appears significantly higher in the model than in the control, the model is successfully constructed [31].

To assess cell apoptosis, the culture was subjected to 0.25-% trypsin (without EDTA). Then, the cells were collected by centrifugation, washed with PBS, re-suspended in binding buffer (Absin, Shanghai, China), and sequentially added 5 μL

FITC Annexin V (Absin) and 5 μL Propidium iodide (Absin). After adding the fluorescent markers, the samples were avoided exposing to the light and assessed by NovoCyte 1030 flow cytometry (Agilent Technologies, Beijing, China) within 60 min.

Protein expression examination through Western blotting

The cell samples were lysed, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transfer electrophoresis (Tanon, Shanghai, China) to attach the target proteins to the PVDF membrane. After rinsed, the membrane was subjected to antibodies against individual target proteins, including ALK1, LDL, Bax, Caspase-9, and Caspase-3 (Bioss, Beijing, China) before secondary antibody was applied to develop the signals.

All the primary antibodies are rabbit raised and the secondary antibody is the goat-raised anti-rabbit IgG (Bioss). Chemiluminescence Imaging System (Tanon) was used to detect the protein signals.

mRNA preparation for qPCR

Total mRNAs were extracted from cells using TRIzol reagent (ThermoFisher), and reverse transcription (RT) reactions were performed using specific primers, designed for ALK1, Bax, Caspase-9, and Caspase-3 accordingly. The sequences of these RT primers are listed in **Table 1**. RT reactions for mRNAs were carried out using the Oligo dT primer (ThermoFisher). qPCR was conducted using the Revert Aid First Strand cDNA Synthesis Kit (ThermoFisher) and performed on the LightCycler® 480 II fluorescence quantitative PCR instrument (Roche). The mRNAs of GAPDH were used as the references for cross comparison.

Statistical processing

All data were statistically processed using SPSS 22.0 software (IBM, Armonk, New York, USA), and expressed as mean ± standard deviation. One-way analysis of variance was used to compare data among groups, and the Tukey's multiple comparison test was used to compare pairwise data. When $p < 0.05$, it is considered statistically significant.

RESULTS

GXBD reduces aortic plaque area of AS model mice

The animal study procedure is schematically presented in **Figure 1.** After adaptive feeding for one week, all ApoE-/- mice were fed with high-fat feed for 12 weeks to build an atherosclerosis (AS) model. Then, the aortas of 3 randomly picked ApoE-/- mice were subjected to Oil Red O staining to confirm the success of the AS model construction (Figure 1A).

Consequently, 30 ApoE-/- mice were randomly and equally divided into three groups: the model group, the GXBD group, and the positive control (PC) group. Then the mice in the GXBD group and the PC group were separately administered an intragastric dose of 9.225 g/kg of decoction and 4.1 mg/kg of simvastatin, respectively, twice a day for six consecutive weeks. Meanwhile, ten C57BL/6J mice also joined the remaining ten ApoE-/- mice, which did not receive any intervention, as the control group.

After the final administration, we used Oil Red O to stain the mouse aortas for plaque observation. The images showed that the AS conditions were improved after the GXBD and simvastatin administration for the GXBD and PC group, respectively (Figure 1B). When the plaque area in the aorta were quantified, it was showed that the plaques increased significantly (p □ 0.01) in the model group, compared to the control group; however, they decreased significantly after GXBD and simvastatin intervention in the GXBD group and the PC group, respectively, compared with the model group (Figure 1C and Supplementary Information).

These results are in agreement with our previous study, in which we tested the mouse model to determine the effects of GXBD intervention on the condition of mouse serum and blood fat [32]. Besides GXBD can effectively inhibit the development of AS, it seems to also alleviate the pathological process of AS. Hence, how GXBD affects the activities of VECs should be further investigated.

Oxidized-LDL infiltration induces HUVECs apoptosis

To establish the injury model, we used ox-LDL infiltration to induce apoptosis of HUVECs **(Figure 2)**. HUVECs without treatment (Figure 2A; control group) or infiltrated with 100 μg/

Table 1: Sequences of the applied primers.

The name of primer	Sequence of primer (5' →3')
GAPDH-F	GGAGCGAGATCCCTCCAAAAT
GAPDH-R	GGCTGTTGTCATACTTCTCATGG
Hu-ALK1-F	CATCGCCTCAGACATGACCTC
Hu-ALK1-R	GTTTGCCCTGTGTACCGAAGA
Hu-Bax-F	CCCGAGAGGTCTTTTTCCGAG
Hu-Bax-R	CCAGCCCATGATGGTTCTGAT
Hu-caspase-9-F	CTCAGACCAGAGATTCGCAAAC
Hu-caspase-9-R	GCATTTCCCCTCAAACTCTCAA
Hu-caspase-3-F	CATGGAAGCGAATCAATGGACT
Hu-caspase-3-R	CTGTACCAGACCGAGATGTCA

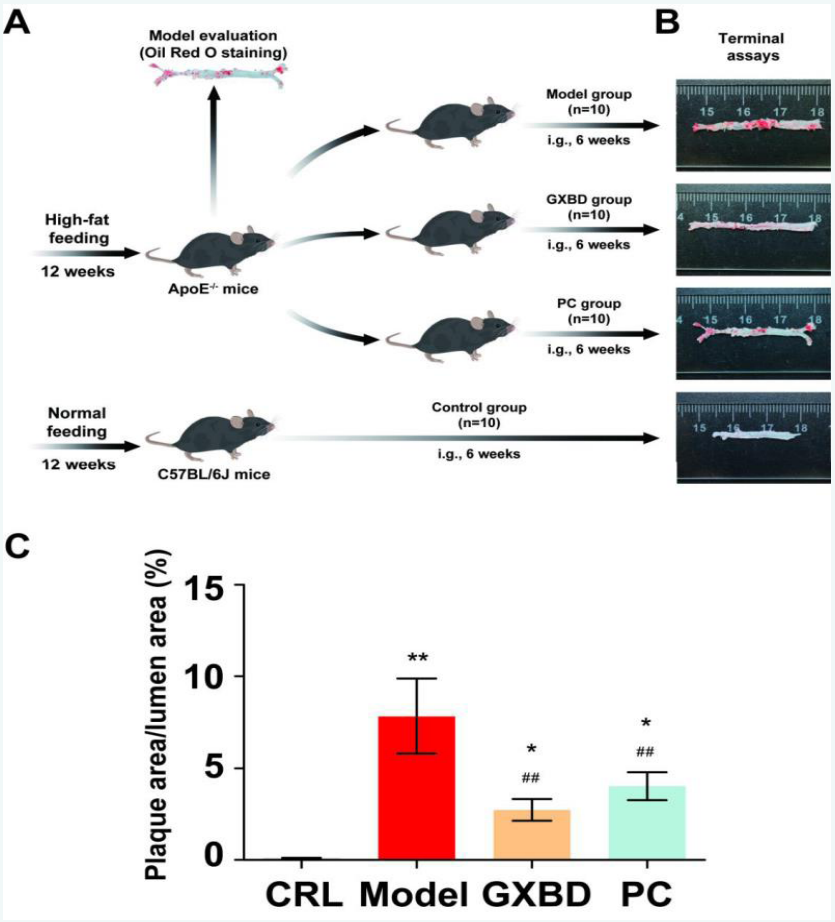

Figure 1 The scheme illustrates the procedure of mice experiments and GXBD effects on aortas. (A) Procedure of mice experiments; (top) the AS plaques on the aorta shown as red stains. (B) Aortas, obtained from different mice groups, stained by Oil O Red. Samples from top to bottom: control group (CRL), injury model group (Model), GXBD group (GXBD), positive control group (PC). (C) Bar plot presenting the percentages of plaque area to the lumen area for these groups. * denotes $P < 0.05$ and ** denotes $P < 0.01$ when a group is compared with the control group, and ## denotes $P < 0.01$ when a group is compared with the model group.

ml ox-LDL for 24 hours (Figure 2B; experimental group) were subjected to the flow cytometry to detect their apoptotic levels (Figure 2C and D). The results are significantly different ($P < 0.01$), where the apoptotic level of ox-LDL-treated HUVECs increases 5.6 folds from the control group (Figure 1E and Supplementary Information).

Hence, the HUVECs injury model is built successfully through ox-LDL infiltration.

Medicated serum alleviates HUVECs injury

GXBD takes effects on the body cells only after it has been digested through the digestive system. To mimic this process, we intragastrically administered GXBD to rats for 5 days and collected the rats' medicated serum (see Materials and Methods Section) for this study.

We treated the cells using six different conditions. First, the control group: the cells are left without intervention. Secondly, the model group: the cells are infiltrated with ox-LDL for 24 hours. Thirdly, the non-medicated serum group: the cells are infiltrated with ox-LDL as well as non-medicated serum for 24 hours. Fourthly, the medicated serum group: the cells are infiltrated with ox-LDL as well as the medicated serum for 24 hours. Fifthly, the ALK1-knockdown group: the ALK1-knockdown cells are infiltrated with ox-LDL for 24 hours. Sixthly and the last: the medicated-serum/ALK1-knockdown group: the ALK1-knockdown cells are infiltrated with ox-LDL as well as medicated serum for 24 hours (**Figure 3**). The comparison of the levels of apoptosis from these six groups would reveal the GXBD's effects on the prevention of HUVECs injury caused by excessive LDL uptake.

To gain the information regarding the effect of GXBD on HUVECs injury, cells in these conditions were separately subjected to the cell cytometry to test their apoptosis levels (Figure 3A). The comparison show that the (negative) control group has the lowest apoptosis level, which is significantly different from those of other groups (Figure 3B and Supplementary Information). On the other hand, the model group and the non-medicated-serum group have are the two groups sharing the highest apoptosis level amongst these groups. The study also suggests there is no

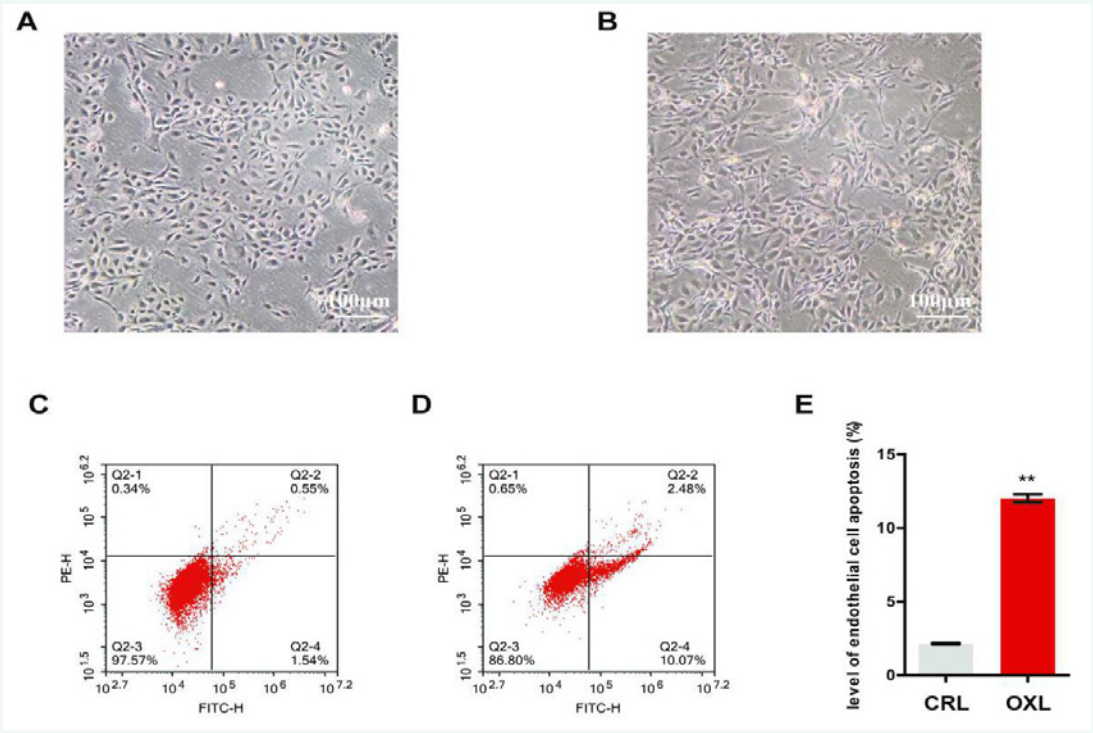

Figure 2 ox-LDL infiltration increases the apoptotic level of HUVECs. The cell images of HUVECs (A) without treatment (control; CRL) and (B) with ox-LDL infiltration for 24 hours (experimental group; OXL). Scale bar: 100 μm. The scatter plots presenting the percentage of apoptosis in the (C) CRL and (D) OXL HUVECs group. (E) Bar plot presenting the percentage of apoptosis for these two groups. ** denotes $P < 0.01$ between the two samples.

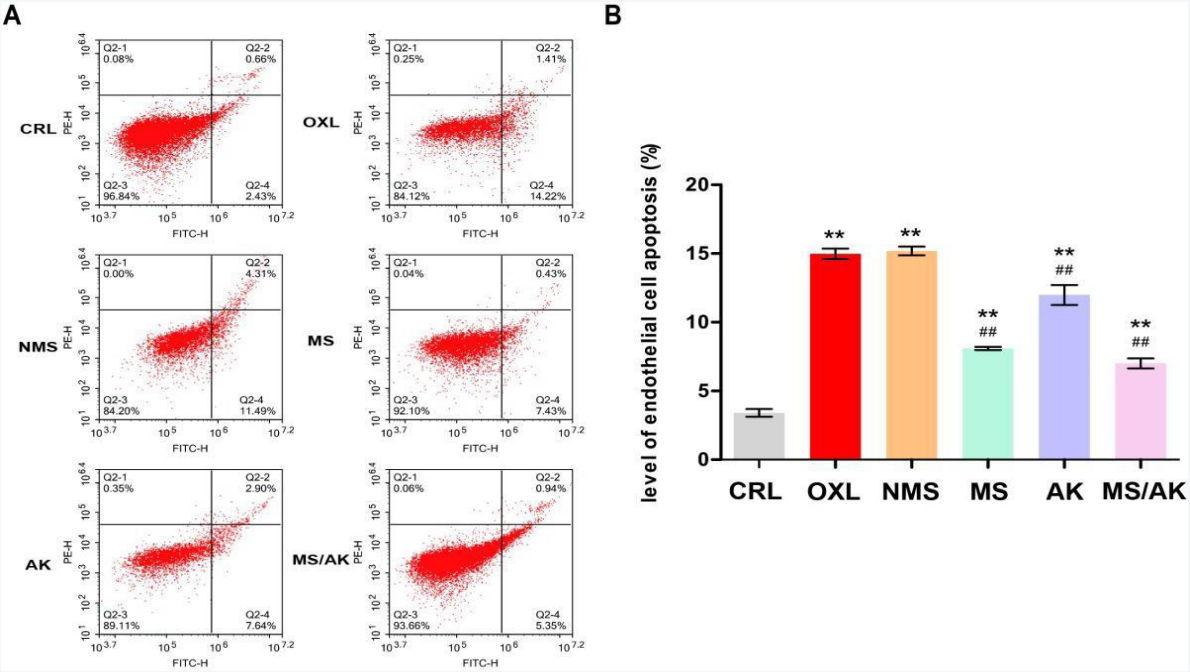

Figure 3 Medicated serum prevents HUVECs apoptosis. Scatter plots presenting the apoptosis level of (A) control group (CRL), ox-LDL-infiltrated model group (OXL), non-medicated-serum group (NMS), medicated-serum group (MS), ALK1-knockdown group (AK), medicated-serum/ALK1-knockdown group (MS/AK). (B) Bar plot presenting the percentage of apoptosis of HUVECs in different conditions. ** denotes $P < 0.01$ when a group is compared with the control (CRL) group, and ## denotes $P < 0.01$ when a group is compared with the model (OXL) group.

extra effect on HUVECs when adding the rat (non-medicated) serum to the culture. Besides, the low apoptosis level in the ALK1-knockdown group, compared to the positive controls (both the model and non-medicated-serum group), also confirms that the ALK1 pathway is involved in the LDL uptake of the HUVECs. Finally and most importantly, the two groups with the presence of medicated serum have a significantly lower apoptosis level than the positive controls. This comparison shows that medicated serum has a "protective effect" on HUVECs from LDL damage.

Medicated serum downregulates Bax, Caspase-9 and Caspase-3 expressions

Since the mitochondrial-mediated apoptosis pathway in injured HUVECs shall be suppressed upon the presence of medicated serum, we probed the expression levels of proteins in the apoptosis pathway, Bax, Caspase-9 and Caspase-3, under these conditions **(Figure 4)**.

Both Western blotting (Figure 4B) and qPCR (Figure 4C and supplementary information) assessments show that medicated serum of GXBD has the same trends in lowering the mRNA levels of those proteins as the ALK1 knockdown does. These assessments further consolidate the conclusion that medicated serum of GXBD can alleviate HUVECs injury due to excessive LDL.

LDL triggers ALK1 expression but medicated serum reduces LDL uptake

The medicated serum of GXBD can alleviate HUVECs injury from excessive LDL; however, it is of interest whether this effect is directly related to the ALK1. Hence, we probed the ALK1 expression and LDL uptake under these six conditions **(Figure 5)**.

First, we examined whether the ox-LDL infiltration has effect on the ALK1 expression in the HUVECs. The levels of ALK1 expression are compared between cells with and without ox-LDL infiltration. Both the Western blot (Figure 5B; first two columns) and mRNA quantity (Figure 5C; first two columns) assessments show that the presence of ox-LDL in the culture can induce the expression of ALK1 in the HUVECs. In addition, the Western blot results of the lipoprotein (Figure 5D) suggest that the amounts of LDL uptake follow the same trends in the ALK1 expression, supporting that ALK1 is involved in the LDL uptake. More importantly, this study shows that medicated serum of GXBD seems to have the effects on both reducing the ALK1 expression and lowering LDL uptake (Figure 5B and C; 4 and 6 columns).

In addition, the phenomenon that the application of medicated serum to HUVECs subjected to ALK1-knockdown further reduces the ALK1 expression strongly suggests that medicated serum either suppress the expression of ALK1 during the gene regulation stage or interfere the ALK1-LDL binding before ALK1 uptake.

Medicated serum of GXBD downregulates ALK1 expression of HUVECs

To explore whether medicated serum of GXBD can downregulate the expression of ALK1 in HUVECs, we applied medicated serum to the HUVECs culture without ox-LDL infiltration and test the ALK1 expression **(Figure 6)**.

We prepared five batches of HUVECs culture; each contained 5 mL of culture media. Consequently, we sequentially added 1 mL of rat's serum, in which the medicated serum were increased 25-% stepwise, to the five batches. After 24 hours, we conducted Western blot against ALK1 (Figure 6A). The normalized results (using GAPDH as the housekeeping protein) reveal that GXBD-

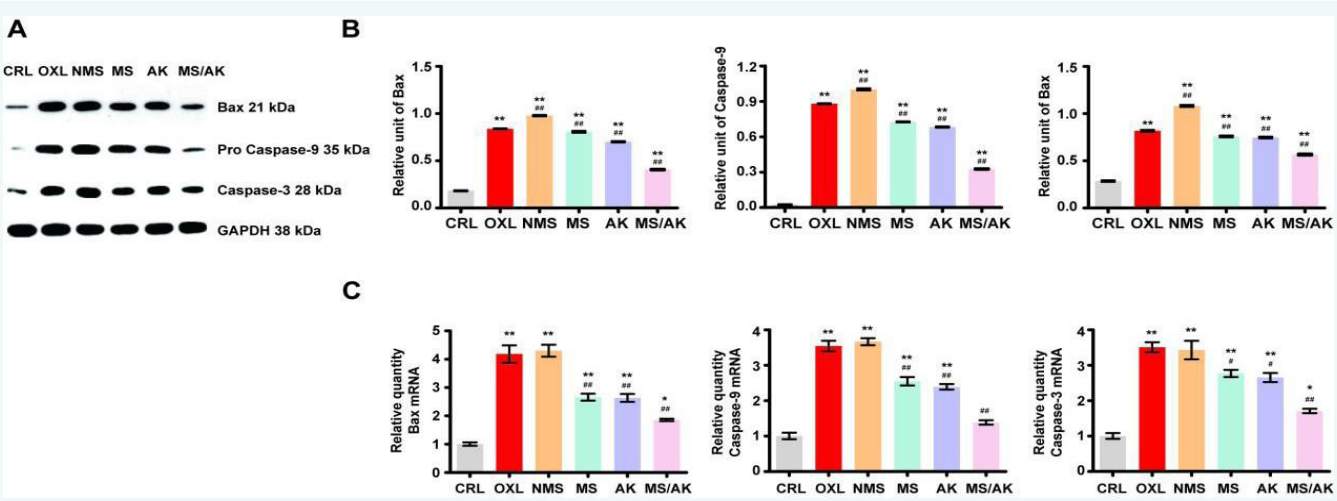

Figure 4 Medicated serum lowers the Bax, Caspase-9 and Caspase-3 expression. (A) Western blots against Bax, Caspase-9, Caspase-3 and GAPDH (housekeeping protein). Sample from left to right: control group (CRL), ox-LDL-infiltrated model group (OXL), non-medicated-serum group (NMS), medicated-serum group (MS), ALK1-knockdown group (AK), medicated-serum/ALK1-knockdown group (MS/AK). (B) Bar plot presenting the relative quantity of Bax, Caspase-9 and Caspase-3 expression (left to right), normalized by GAPDH. (C) Bar plot presenting the mRNA quantity of Bax, Caspase-9 and Caspase-3 (left to right) in these conditions. * denotes $P < 0.05$ and ** denotes $P < 0.01$ when a group is compared with the control group, and # denotes $P < 0.05$ and ## denotes $P < 0.01$ when a group is compared with the model group.

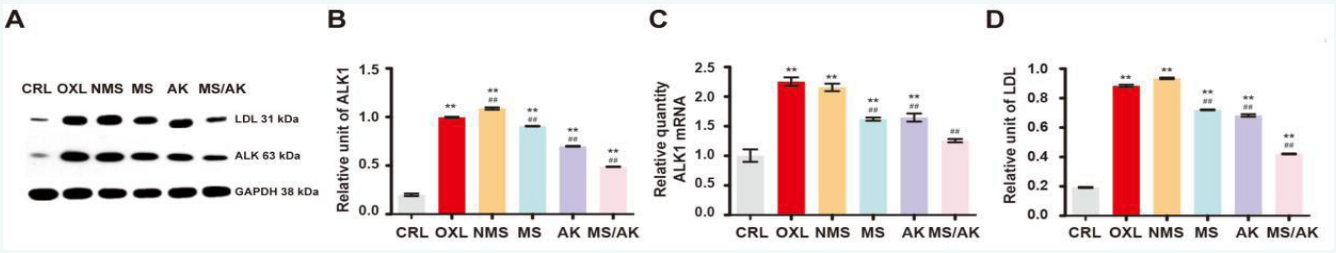

Figure 5 Medicated serum lowers LDL uptake and ALK1 expression of HUVECs. (A) Western blots against LDL, ALK1, and GAPDH (housekeeping protein). Samples from left to right: control group (CRL), ox-LDL-infiltrated model group (OXL), non-medicated-serum group (NMS), medicated-serum group (MS), ALK1-knockdown group (AK), medicated-serum/ALK1-knockdown group (MS/AK). (B) Bar plot presenting the expression levels of ALK1, normalized by GAPDH amount. (C) Bar plot presenting the mRNA quantities of ALK1 among these conditions. (D) Bar plot presenting the amount of LDL uptake, normalized by GAPDH amount. ** denotes $P < 0.01$ when a group is compared with the control group, and ## denotes $P < 0.01$ when a group is compared with the model group.

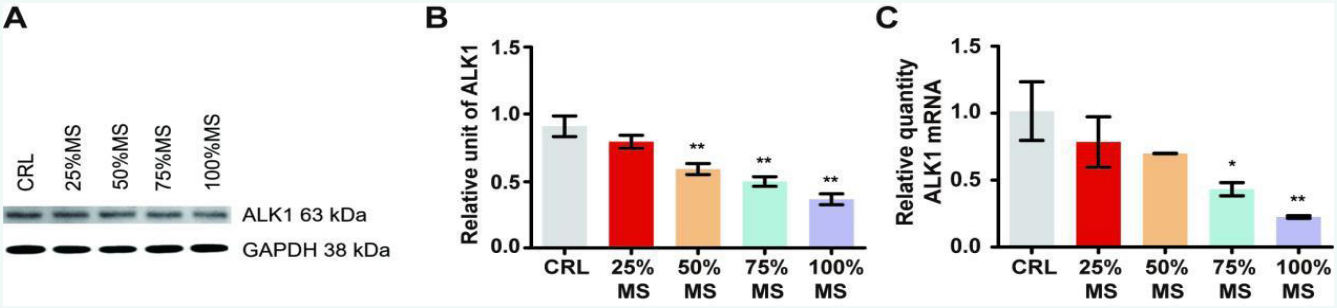

Figure 6 Medicated serum of GXBD downregulates ALK1 expression of HUVECs. (A) Western blots against ALK1 and GAPDH. Samples from left to right: control group (CRL), 75% non-medicated serum and 25% medicated serum (25% MS), 50% non-medicated serum and 50% medicated serum (50% MS), 25% non-medicated serum and 75% medicated serum (75% MS), 100% medicated serum (100% MS). (B) Bar plot presenting the amount of ALK1 expression, normalized by the amount of GAPDH (housekeeping protein). (C) Bar plot presenting the mRNA quantities of ALK1 in different GXBD-medicated-serum addition. * denotes $P < 0.05$ and ** denotes $P < 0.01$ when a group is compared with the control (CRL) group.

medicated serum do have an effect on reducing the level of ALK1 expression in the HUVECs' culture regardless the absence of ox-LDL infiltration (Figure 6B and Supplementary Information).

Using the same setting, we also applied qPCR to test the mRNA levels of ALK1 in HUVECs. Again, the result confirms that GXBD-medicated serum can intervene the expression level of ALK1 in HUVECs (Figure 6C and Supplementary Information).

In summary, medicated serum can intervene the level of ALK1 expression in HUVECs, hence lowering the ALK1-mediated LDL-endocytosis and further preventing the stimulation of mitochondrial-mediated apoptosis pathway that results in the upregulation of Bax, Caspase-9, and Caspase-3. Therefore, GXBD can smoothen the pathological process of AS and CAD through alleviating vascular endothelial cell injury.

DISCUSSION AND CONCLUSION

In view of the fact that apoptosis of VECs is a precursor of CAD, our study suggests that GXBD can alleviate VECs injury through blocking the initiation of AS and prevent CAD. We show that medicated serum of GXBD could downregulate the level of ALK1 expression, hence reducing the LDL uptake to the endothelial cells and consequent reaction between LDL and ROS that would trigger the cytotoxic mitochondrial-mediated apoptosis pathway.

LDL that enters cells through LDL receptors is decomposed in lysosomes for cholesterol release. Following, cholesterol released from lysosomes transfer to the endoplasmic reticulum and inhibit the production of LDL receptors, thereby establishing a negative feedback loop for LDL uptake through LDL receptor [33-35]. However, no similar feedback loop has been found in the cells to regulate the LDL uptake through ALK1, making the downregulation of ALK1 expression more critical in controlling LDL uptake and preventing the apoptosis of endothelial cells. As shown in the study, GXBD could be a natural product that is accountable to prevent endothelial cell injury through exposing to excessive blood fat.

This study sets the foundation for investigating the full cellular responses after HUVECs expose to the medicated serum of GXBD, which might have other effects on HUVECs along with reducing the ALK1 expression. Since the downregulation of ALK1 expression must interfere the transcriptional activities of HUVECs' genome, the changes in the protein expression profile must have carried out other unknown effects on the cells. The extension of this work to study the whole cell behavior changes with GXBD intervention can set a new paradigm to the Chinese Traditional Medicine research at the cellular level and provide new directions to target therapeutic drugs.

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DATA AVAILABILITY STATEMENT

The data supporting this study are available in the **supplementary materials** of this article.

DISCLOSURES

All animal procedures were approved by the Institutional Animal Care and Use Committee at Shandong University of Traditional Chinese Medicine.

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