Protective effects and potential mechanism of 
Tongxinluo on mouse with thromboangiitis obliterans 
induced by sodium laurate

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Abstract:
Background: In China, Tongxinluo (TXL) has been widely used for the 
treatment of cardio-cerebrovascular diseases. Studies have shown that TXL has anti-inflammatory and antithrombotic properties.

Purpose: This study aimed to investigate the inhibitory effects of TXL on sodium laurate-induced thromboangiitis obliterans (TAO) and the underlying mechanisms.

Methods: A TAO mouse model was established by injecting sodium laurate into the femoral artery. After treatment with compound danshen tablet (CDT, 0.52 g/kg) and various doses of TXL (0.38, 0.75, 1.5 g/kg), the recovery of blood flow in the lower limbs of mice was detected by laser Doppler imaging.

Pathological changes and thrombosis of the femoral artery were observed by hematoxylin and eosin (H&E) staining. Levels of thromboxane B2 (TXB2), 6-keto-prostaglandin F1a (6-keto-PGF1a), endothelin-1 (ET-1), IL (interleukin)
-1β and IL-6 were measured using enzyme-linked immunosorbent assay (ELISA). Levels of activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT) and fibrinogen (FIB) were detected by a fully automated biochemical analyzer. The expression of tumor necrosis factor (TNF-α) and inducible nitric oxide synthase (iNOS) in the femoral artery wall was determined by immunohistochemical analysis.

Results: TXL promoted the restoration of blood flow in the lower limbs, reduced the area of thrombosis in the femoral artery, and alleviated the pathological changes in the femoral artery wall. TXL also decreased the expression of TXB$_2$ and ET-1 and increased 6-keto-PGF$_{1α}$. TXL reduced the levels of IL-6 and IL-1β and significantly inhibited the overexpression of TNF-α and iNOS in the femoral artery wall. Moreover, TXL prolonged APTT, PT, TT, and reduced FIB levels.

Conclusion: TXL has a protective effect on TAO mice, and the mechanism may involve in the inhibition of thrombosis and TAO-related inflammatory responses. Therefore, TXL may be a potential drug for the treatment of TAO in conventional medicine.

Key Words: Tongxinluo; thromboangiitis obliterans; thrombosis; inflammation

Abbreviations: TAO, thromboangiitis obliterans; TNF-α, tumor necrosis factor; IL, interleukin; TXL, Tongxinluo; iNOS, inducible nitric oxide; HRP, horseradish peroxidase; TXB$_2$, thromboxane B2; 6-keto-PGF$_{1α}$. 
1. Introduction

Thromboangiitis obliterans (TAO), also known as Buerger's disease, is a nonatherosclerotic, nonaneurysmal, occlusive and progressive vascular inflammatory disease that mainly involves the blood vessels and nerves of the patient’s lower limbs [1]. The early stages of the disease are characterized by ischemic symptoms such as coldness, numbness and pale skin at the end of the toes. In the late stages, patients may develop symptoms such as limb ulceration and gangrene, which may eventually lead to amputation. The pathogenesis mechanism of TAO has not yet been fully elucidated. It is currently believed that smoking is closely related to the development of TAO, and studies have confirmed that smoking cessation can significantly reduce the incidence of adverse events such as amputation, but smoking cessation alone does not alleviate symptoms such as rest pain, intermittent claudication, and limb ulcers [2].

The pathological manifestations of TAO are mainly the aggregation of leukocytes and inflammatory thrombosis in the diseased vessel wall, often accompanied by fibrotic mature thrombosis in the final stage [3]. Therefore,
thrombosis and inflammatory vascular injury have been shown to be the main pathological changes in patients with TAO, which has been confirmed by several studies. An analysis of cytokine activity in TAO patients showed upregulated expression of several inflammatory factors such as tumor necrosis factor (TNF-α), interleukin (IL)-1β, IL-6, IL-4, IL-17 and IL-23 [4,5]. In addition, a hypercoagulable state of blood was observed in both TAO patients and rat models [6,7]. Current treatment for TAO mainly includes pharmacological and surgical therapies. Drug treatment is represented by thrombosis inhibitors such as aspirin and cilostazol, while surgical treatment is focused on angioplasty or bypass surgery. The bleeding complication of drugs and the high recurrence rate of surgery limit their use, and there is no effective treatment for TAO patients because of the wide distribution of the lesion in the blood vessels and the complexity of the disease progression. Improving and maintaining the blood flow of the affected limb is the key to solving these problems [8].

Chinese compound preparation Tongxinluo (TXL) has the effects of activating blood circulation and relieving pain, benefiting Qi and activating blood circulation. Previous studies have found that it has excellent effects in inhibiting thrombosis, protecting vascular endothelial cells, reducing the inflammatory response, and maintaining vasodilation [9,10,11]. A recent study found that TXL reduced inflammation and enhanced the stability of atherosclerotic plaques by regulating intestinal flora [12]. Other studies found
that TXL significantly reduced plasma viscosity and improve the indexes of hemorheology in patients\textsuperscript{[13]}. These studies all further confirm the anti-inflammatory and antithrombotic properties of TXL. In recent years, there have been a large number of experimental studies on the treatment of cardio-cerebrovascular diseases with TXL\textsuperscript{[14,15]}, but to the best of our knowledge, there are currently no relevant studies on the effects of TXL on TAO mice. In the current study, we hypothesized that TXL plays an inhibitory role in the progression of TAO. We established an animal model by injecting sodium laurate into the femoral artery of mice to observe the effects of TXL on TAO mice and explore the underlying mechanisms.

2. Materials and methods

2.1 Drugs, reagents and instruments


Primary antibodies TNF-\(\alpha\) (Abcam, ab183218), inducible nitric oxide synthase (iNOS, Abcam, ab15323), horseradish peroxidase (HRP)-labeled sheep anti-rabbit IgG secondary antibody (Abcam, ab150077), mouse IL-6,
IL-1β, thromboxane B2 (TXB2), 6-keto-prostaglandin F1α (6-keto-PGF1α), and endothelin-1 (ET-1) enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Enzyme Link Biotechnology Co., Ltd., ml063160, ml063132-J, ml601808, ml002160, ml001965) were used.

A fully automatic biochemical analyzer (Siemens ADVIA 2400, Germany), fully automatic inverted microscope (ZEISS, Germany), multifunctional enzyme marker (TECAN, Switzerland), tissue embedding machine (LEICA, Germany), and paraffin sectioning machine (LEICA, Germany) were used.

2.2 Animal model preparation and grouping

A total of ninety male SPF-grade C57/BL6J mice, 6 weeks old, weight 25±3g, were purchased from Beijing Weitong Lihua Experimental Animal Co., Ltd. (production license No. SCXK (Beijing) 2016-0006). This experiment was approved by the Experimental Animal Ethics Committee of Hebei Yiling Pharmaceutical Co., Ltd. (No. N2021-060).

After one week of acclimatization to the laboratory conditions, all mice were randomly divided into six groups (15 per group): the Sham group, compound danshen tablet group (CDT), TAO model group, high-dose TXL group (TXL-H), medium-dose TXL group (TXL-M), and low-dose TXL group (TXL-L). All mice except the sham group were injected with sodium laurate in the femoral artery as previously described [16]. Specific operations were as follows: mice were anesthetized with 20 mg/kg pentobarbital sodium
intraperitoneally and fixed in the supine position. The line between the midpoint of the right groin and the knee was the surgical site. The skin was incised, and the femoral artery was isolated from the femoral artery and femoral nerve. An arterial clamp was used to block blood flow from the proximal femoral artery, and a suture was placed under the femoral artery (to secure the syringe needle in the femoral artery and ensure that the sodium laurate solution did not leak out). A 1-ml syringe (with a 32-gauge needle) was used to draw 0.1 ml of sodium laurate solution dissolved in pure water (5 mg/ml, pH=8), and then the femoral artery was stabbed approximately 0.5 cm from the proximal to the distal end. After fixing the needle with suture, sodium laurate was slowly injected into the femoral artery, while the sham group was injected with saline. Alpha-cyanoacrylate fast medical adhesive was then applied to the puncture site and the arterial clip was withdrawn when it solidified. When there was no active bleeding, the wound was sutured, and 0.1 ml of penicillin was injected intraperitoneally to prevent infection. Immediately after the operation, the modeling was considered successful when the blood flow of the surgical lower limb was reduced by more than 80% using laser Doppler imaging. After 3 days of modeling, mice in the sham and TAO model groups were intragastrically administered 0.5% (w/v) sodium carboxymethylcellulose, mice in the CDT group were intragastrically administered 0.52 g/kg CDT, and mice in the TXL-H, TXL-M, and TXL-L groups were intragastrically administered 1.5 g/kg, 0.75
g/kg, and 0.38 g/kg TXL, respectively. Once a day for 4 weeks. After treatment, all mice were euthanized, and the femoral arteries were collected for homogenization and histological examination. Blood samples were collected from the ophthalmic vein for molecular analyses.

2.3 Laser Doppler imaging

The ratio of blood flow in the surgical (right) limb to the contralateral non-surgical limb was measured with a Laser Doppler perfusion imaging system (Moor Instruments Limited, Millwey, Axminster, Devon, EX135HU, UK). Images were obtained before and immediately after arterial ligation and on postoperative days 7, 14 and 21.

2.4 Hematoxylin and eosin (H&E) staining kits

The collected femoral arteries were soaked in 10% (v/v) neutral buffered formalin for 24 h at room temperature, paraffin embedded, and sectioned to 4-μm thickness. All arterial specimens were dehydrated with different dilutions of ethanol and xylene, and the dehydrated sections were stained with H&E and then cemented with neutral resin. The conventional morphology of the stained sections was observed with a fully automated inverted microscope and photographed at 40× magnification.

2.5 ELISA analysis

The collected blood was centrifuged at 4500 r/min for 10 min, and the serum and plasma were stored in nonanticoagulated and anticoagulated tubes, respectively, at -80°C. The levels of TXB₂, 6-keto-PGF₁α, and ET-1 in
plasma and IL-1β and IL-6 in serum were measured using ELISA kits according to the manufacturers’ instructions.

2.6 Fully automated biochemical analyzer for activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT) and fibrinogen (FIB) levels

Collected plasma was processed as described for the ELISA experiments, and plasma APTT, PT, TT and FIB levels were measured using a fully automatic biochemical analyzer.

2.7 Immunohistochemical analysis of TNF-α and iNOS in femoral arterial walls

Collected femoral artery samples were processed as described for the H&E staining assay. The specimens were incubated overnight at 4°C with TNF-α (1:100 dilution) and iNOS (1:400 dilution) primary antibodies. HRP-labeled sheep anti-rabbit IgG was used as a secondary antibody and incubated with the samples. After visualization with DAB for 3 min, the slices were restained with hematoxylin. All images were taken using a fully automated inverted microscope. Quantitative analysis was performed using ImageJ software.

2.8 Statistical analysis

Data were presented as the mean ± standard deviation (SD) and were analyzed using one-way ANOVA and post-hoc Bonferroni test. All statistical
analyses were performed using the statistical software SPSS 23.0. P <0.05 was considered statistically significant.

3. Results

3.1 Effect of TXL on sodium laurate-induced lower limb thrombosis and its promotion of blood flow recovery in the lower limb

The results of the H&E staining of femoral artery sections are shown in Fig. 1A. In the sham group, there was almost no thrombus formation in the arterial lumen, the morphology of the vessels was normal, and the smooth muscle cells were neatly arranged. In the TAO model group, the lumen was almost completely occluded with thrombi, and vascular smooth muscle cells were disordered. Compared with the model group, the TXL-L and TXL-M groups had a smaller thrombi and alleviated smooth muscle disorder. In the CDT and TXL-H groups, there was only a small amount of erythrocyte aggregation and an insignificant smooth muscle disorder. Laser Doppler images were acquired before and immediately after arterial injection of sodium laurate and on days 7, 14 and 21 postoperatively (Fig. 1B). The images showed a significant decrease in lower limb perfusion in all experimental groups immediately after femoral artery injection of sodium laurate, followed by an increase in a time-dependent manner. Quantification of the percentage of tissue perfusion in the surgical limb versus the non-surgical limb. As shown in Fig. 1C, on the 14th day after surgery, the CDT, TXL-M, and TXL-H groups had significantly higher blood flow ratios.
in the surgical lower limb than the TAO model group. On day 21 of surgery, the blood flow ratio of the surgical lower limb was significantly higher in all treatment groups than in the TAO model group, and TXL restored blood flow in a dose-dependent manner.
A. Sham, TAO, CDT, TXL-L, TXL-M, TXL-H

B. Before, Immediately, After 7 days, After 14 days, After 21 days

C. Blood flow ratio (%) vs. Time

Legend:
- **TAO**
- **CDT**
- **TXL-L**
- **TXL-M**
- **TXL-H**
Fig. 1. Visualization of femoral artery thrombosis in TAO mice and the effect of TXL on blood flow recovery in the lower limbs of TAO mice. (A) The area of femoral artery thrombi and the morphology of the vessel wall in all experimental groups were observed using a fully automatic inverted microscope (H&E staining, 400×). The black arrows represent thrombi. Scale bar = 50 μm. (B) Laser Doppler perfusion imaging was performed before and immediately after arterial ligation and on postoperative days 7, 14 and 21. (C) Quantification of tissue perfusion in the surgical limb is expressed as a percentage of perfusion in the nonoperated limb. The data are presented as the means ± SD. * p < 0.05, ** p < 0.01 compared with the sham group, # p < 0.05, ## p < 0.01 compared with the CDT group, & p < 0.05, && p < 0.01 compared between the TXL groups. n=3.

3.2 Effect of TXL on plasma TXB$_2$, ET-1 and 6-keto-PGF$_{1α}$ and serum IL-6 and IL-1β

Plasma TXB$_2$, ET-1 and 6-keto-PGF$_{1α}$ and serum IL-6 and IL-1β levels in mice were evaluated by ELISA. As shown in Fig 2A-2C, the TAO model group exhibited significantly higher levels of TXB$_2$ and ET-1, and lower 6-keto-PGF$_{1α}$ levels than the sham group. Compared with the TAO model group, TXB$_2$ and ET-1 levels were significantly decreased in all treatment groups, with more pronounced decreases in the CDT and TXL-H groups. The levels of 6-keto-PGF$_{1α}$ were elevated in the CDT and TXL-H groups compared with the TAO model group, while no significant changes were observed in the TXL-L and TXL-M groups. As shown in Fig. 2D and 2E, the
levels of IL-6 and IL-1β were significantly higher in the TAO model group than in the sham group and decreased to varying degrees in all treatment groups, except for the TXL-L group, which showed no marked change. Furthermore, the effect of high-dose TXL in reducing IL-6 was close to that of CDT and better than that of medium-dose TXL. These results suggested that TXL inhibited thrombosis by downregulating TXB2 and ET-1 and upregulating 6-keto-PGF₁α in plasma, and also reduces the inflammatory response by downregulating IL-6 and IL-1β levels.

Fig. 2. Effect of TXL on plasma TXB₂, ET-1 and 6-keto-PGF₁α and serum IL-6 and IL-1β levels. (A) TXB₂ expression; (B) ET-1 expression; (C) 6-keto-PGF₁α expression; (D) IL-6 expression; (E) IL-1β expression. The data are presented as the means ± SD. *p < 0.05, **p < 0.01 compared with the sham group, #p < 0.05, ##p < 0.01 compared with the CDT group, &p < 0.05, &&p < 0.01 compared between the TXL groups. n=3
3.3 Effect of TXL on APTT, PT, TT and FIB

Plasma APTT, PT, TT and FIB levels were measured by a fully automated biochemical analyzer. As shown in Fig. 3A-3D, compared to the sham group, the TAO model group had a shorter APTT, PT and TT and elevated levels of FIB, which were reversed in the treatment group. Moreover, the effects of TXL prolonging APTT and reducing FIB expression displayed a dose-dependent pattern. These results indicated that TXL treatment attenuated impaired coagulation to varying degrees and that high-dose treatment provided better reversal than low-dose treatment.
Fig. 3. Effect of TXL on plasma APT, PT, TT and FIB. (A) APTT expression; (B) PT expression; (C) FIB expression; (D) TT expression. The data are presented as the means ± SD. * p < 0.05, ** p < 0.01 compared with the sham group, # p < 0.05, ## p < 0.01 compared with the CDT group, & p < 0.05, && p < 0.01 compared between the TXL groups. n=3

3.4 Effect of TXL on the expression of TNF-α in the femoral arteries

TNF-α is a proinflammatory cytokine produced by monocytes/macrophages, fibroblasts, T lymphocytes, osteoblasts and
endothelial cells \cite{17}, and is highly associated with thrombosis \cite{18}. Its expression in the femoral arterial walls was determined by immunohistochemical staining (Fig. 4A). After staining, positive expression of TNF-\(\alpha\) manifested as yellow or brown granules. Few TNF-\(\alpha\) expression was observed in the sham group, while the TAO model group showed the most TNF-\(\alpha\) expression among all groups. The TXL-L and TXL-M groups had less TNF-\(\alpha\) immunolabeling than the TAO model group, while the CDT and TXL-H groups had the least immunolabeling of all treatment groups.

The quantitative immunohistochemical analysis of TNF-\(\alpha\) is shown in Fig. 4B. The positive-stained area of TNF-\(\alpha\) in the TAO model group was higher than that in the sham group. Compared with the TAO model group, the levels of TNF-\(\alpha\) were significantly decreased in all treatment groups except the TXL-L group, and the TXL-H group had lower TNF-\(\alpha\) expression than the TXL-M group. These results indicated that TNF-\(\alpha\) expression was significantly increased in the femoral arteries of TAO mice, but could be significantly reduced by medium and high doses of TXL.
Fig. 4. Effect of TXL on TNF-α expression in the arterial wall according to immunohistochemistry (400×). The black arrows represent positive expression of TNF-α in the femoral artery (A). Quantitative analysis of the levels of TNF-α expression in the arterial wall using immunohistochemistry (B). Scale bar = 50 μm. The data are presented as the means ± SD. * p < 0.05, ** p < 0.01 compared with the sham group, # p < 0.05, ## p < 0.01 compared with the CDT group, & p < 0.05, && p < 0.01 compared between the TXL groups. n=3-5.

3.5 Effect of TXL on the expression of iNOS in the femoral arteries

iNOS, as the main factor for high nitric oxide (NO) production in
pathological states, plays an important role in the inflammatory thrombosis process. The expression of iNOS in femoral arterial walls was identified by immunohistochemical staining, which also appeared as brown granules (Fig. 5A). The TAO model group showed intense punctate iNOS staining in the vessel wall, whereas the sham group had very low levels of iNOS expression. The TXL-L and TXL-M treatment groups had a lower number of iNOS particles than the TAO model group, while the TXL-H and CDT groups had lowest expression levels.

The quantitative immunohistochemical analysis of iNOS is shown in Fig. 5B. There was substantial positive expression of iNOS in the TAO model group compared to the sham group. The area of positive iNOS staining was significantly lower in all treatment group than in the TAO model group, with the TXL-H and CDT groups having better effects in reducing iNOS. These results suggested that TXL could significantly reverse the elevated iNOS expression in TAO mice.
**Fig. 5.** Effect of TXL on iNOS expressions in the arterial wall according to immunohistochemistry (400×). The black arrows represent positive expression of iNOS in the femoral artery (A). Quantitative analysis of the levels of iNOS in the arterial wall using immunohistochemistry (B). Scale bar = 50 μm. The data are presented as the means ± SD. * p < 0.05, ** p < 0.01 compared with the sham group, # p <0.05, ## p < 0.01 compared with the CDT group, & p <0.05, && p < 0.01 compared between the TXL groups. n=3-5

4. **Discussion**

According to the Chinese medicine theory of collateral disease, TAO belongs to the category of “collateral-vascular system disease”. It is believed
that the system of blood vessels throughout the body is divided into
Qi-collateral, which runs the meridians, and Mai-collateral, which runs the
blood. The latter Mai-collateral is morphologically consistent with the
microscopic blood vessels in Western medicine and plays the role of
infiltrating qi and blood, moisturizing the limbs, promoting metabolism, and
exchanging fluid and blood. The unobstructed flow of the Mai-collateral and
the normal flow of qi and blood are the basis for the vascular system to
maintain normal physiological functions. However, because of the tortuous
and narrow morphology of the Mai-collateral, qi and blood are diffusely
distributed in the lower limbs of the body and run slowly, and Mai-collateral
is most likely to be “blocked” when various pathogenic factors injure it.
Therefore, the principle of treating “collateral-vascular system diseases” is to
“unblocking the Mai-collateral”, so as to restore the unobstructed flow of the
Mai-collateral \[20\].

In response to the pathogenetic characteristics of “collateral-vascular
system diseases”, and according to the general principle of “benefitting Qi
and unblocking the Mai-collateral, activating blood circulation and resolving
blood stasis”, Dr. Wu combined different treatment methods and selected
medicines to form the TXL formula, which is the representative formula of
the theory of collateral disease. The formula includes radix ginseng, leech,
whole scorpion, periostracum cicadae and ice chips et. In the formula, radix
ginseng is the ruling herb, which tonifies the heart energy and strengthens the
blood flow so that the Mai-collateral of heart will flow naturally. Leech can resolve blood stasis and open the collateral, and centipede can search the wind and open the collateral, which are the ministering medicines. The whole scorpion and periostracum cicadae can open the collateral and stop spasms and can relieve the spasm of heart collateral. Ice chips can make all medicines reach the place of illness smoothly. The combined effect of all the medicines is to invigorating the qi, moving the blood and unblocking the collateral [21]. Therefore, TXL has a theoretical basis for the treatment of TAO. Together with the anti-inflammatory and anti-thrombotic properties of TXL found in previous pharmacological studies. We hypothesized that TXL inhibits the development of TAO.

To test this hypothesis, we successfully established a mouse TAO model with sodium laurate injection into the femoral artery, in which the underlying pathological change is inflammatory vascular endothelial injury in the lower limbs. It is more consistent with the pathological manifestation of human thrombo-occlusive vasculitis compared to femoral artery ligation. As expected, typical pathological changes of thrombosis and inflammation were observed in the model group of mice.

In the present experiment, CDT is a proprietary Chinese medicine formula consisting of panax ginseng, danshen and ice chips, which is mainly used in the treatment of coronary heart disease, angina pectoris and other cardiovascular and cerebrovascular diseases [22]. In addition, CDT was found
to have antithrombotic \cite{23} and anti-inflammatory \cite{24} properties, so it was used as a positive control in our study. In the observation of TXL for thrombosis, H&E staining visually revealed that TXL reduced the area of thrombus in the lumen of the femoral artery and attenuated the structural alteration of the vessel wall by sodium laurate. In addition, APTT, PT, TT and FIB are important indicators for the diagnosis of abnormalities in the coagulation system. PT is a general indicator of exogenous coagulation factors; APTT is a major indicator of endogenous coagulation factors; TT is a common pathway for both exogenous and endogenous coagulation systems, which can reflect the level of FIB, and the prolongation of TT indicates the existence of high fibrinolysis in the body \cite{25,26}; FIB is a substrate of thrombin, which is an important indicator for clinical detection of thrombosis. Shortening of APTT, PT, TT and elevated FIB indicate abnormal fibrinolytic activity and a hypercoagulable state of the blood, which facilitates thrombosis \cite{27}. In this study, we found that the blood of TAO mice was hypercoagulable and prone to form thrombus. In contrast, TXL prolonged APTT, PT, and TT and reduced FIB, which indicated that TXL inhibited thrombosis. In addition, the levels of vasoactive substances such as TXB$_2$, 6-keto-PGF$_{1\alpha}$ and ET-1 are also important indicators to observe the effect of TXL on thrombosis. Prostacyclin (PGI$_2$) and thromboxane A2 (TXA$_2$) are important substances that regulate thrombosis and inflammation. However, PGI$_2$ and TXA$_2$ are very unstable, so their stable metabolites, TXB$_2$ and
6-keto-PGF₁α, respectively, are usually used to measure their expression \[28\].

TXB₂ promotes vasoconstriction, platelet aggregation and thrombosis, while 6-keto-PGF₁α promotes vasodilation and inhibits platelet aggregation \[29\]. Moreover, ET-1 is widely present in endothelial cells and is an endogenous long-acting vasoconstriction regulator that works in concert with TXB₂ to maintain vascular tone \[30\]. Our study demonstrated that TXL dose-dependently decreased the expression of plasma TXB₂ and ET-1 and increased the expression of 6-keto-PGF₁α, which indicated its role in promoting vasodilation and inhibiting thrombosis.

Interleukins are key mediators of the systemic anti-inflammatory response. Among the inflammatory molecules associated with thrombosis in TAO patients, IL-1β and IL-6 play an important role in the development of vascular injury \[31\]. One study found that among patients at high vascular risk, inhibition of IL-1β expression reduced IL-6 and C-reactive protein (CRP) by more than 50% and fibrinogen by 20% \[32\]. In addition, TNF-α is a multifunctional cytokine that plays a key role in proinflammatory and prothrombotic responses. Studies have shown that TNF-α may be involved in the pathological process of TAO through the following three pathways: promoting thrombosis by inducing monocyte/macrophage expression; cytotoxic effects that can disrupt the structure of vascular endothelial cells and lead to endothelial dysfunction; and enhancing reactive oxygen production by endothelial NADPH oxidase through NF-κB, exacerbating
oxidative stress to further damage endothelial cells \cite{33,34,35}. Furthermore, TNF-α can induce macrophages to express an inflammatory enzyme, iNOS, which is not expressed in the physiological state and is only induced after injury. Under inflammatory conditions, activation of iNOS can induce the sustained release of large amounts of NO above physiological concentrations, leading to increased production of reactive nitrogen-oxygen end products, which further leads to lipid peroxidation, cellular necrosis, and the release of more inflammatory mediators that predispose to thrombus formation \cite{36}. In this study, TXL was shown to inhibit thrombosis and inflammation by reducing iNOS levels and to attenuate vascular injury by reducing TNF-α, IL-1β, and IL-6. These findings are consistent with the conclusions of previous studies on the mechanism of vascular inflammation-reducing effects of TXL \cite{37,38}.

5. **Conclusion:**

The above results suggest that TXL treats TAO by inhibiting inflammatory responses and thrombosis. This study broadens the therapeutic scope of TXL and demonstrates its potential as an herbal medicine.

**Author contributions**

Huailin Gao designed the present study and revised the paper. Jiaojiao Gu participated in the animal experiments and wrote the paper. All authors agree to be accountable for all aspects of the work, ensuring its integrity and accuracy.
Conflicts of Interest

The authors declare that they have no conflicts of interest.

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