

# Preventive effect of Tongxinluo on endothelial survival and vascular integrity, together with inhibition of inflammatory reaction in rats model of intestine ischemia/reperfusion injury

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**Abstract:** This study was design to investigate preventive function of Tongxinluo (TXL) capsule on micro vascular function and endothelial survival in rats model of intestine ischemia/reperfusion (I/R) injury. We randomly divided fifty male Sprague-Dawley rats into Sham group, I/R group, TXL0.4+I/R group, TXL0.8+I/R group, TXL1.6+I/R group (10 rats each). Rat intestine I/R injury was carried out using a model of acute superior mesenteric artery occlusion with 30 min ischemia followed by 60 min reperfusion. The distribution of endothelial apoptosis in intestine was determined by CD31+TUNEL immunofluorescent double staining analysis. VE-Cadherin, ANGPTL4, HMGB1 and NF- $\kappa$ B were determined by immunohistochemical analysis. I/R induced massively endothelial cell apoptosis, accompanied with reduced expression of adherens junction protein VE-Cadherin and up regulation of inflammatory mediator HMGB1 and NF- $\kappa$ B. TXL pretreatment groups (TXL0.4+I/R, TXL0.8+I/R and TXL1.6+I/R group) significantly attenuated endothelial cell apoptosis with a dose-dependent effect. TXL pretreatment could maintain the expression of VE-Cadherin and promote the expression of ANGPTL4 which help to maintain endothelial integrity. TXL pretreatment also exert great influence in inhibiting HMGB1 expression and NF- $\kappa$ B expression induced by I/R. It could be concluded from this study that micro vascular dysfunction and endothelial damage play a causal role in rat intestine I/R injury. TXL pretreatment could significantly prevent the I/R induced pathology of endothelial apoptosis, micro vascular integrity disruption and inflammatory reaction.

**Keywords:** Intestine I/R injury, endothelial cell, endothelial barrier, traditional Chinese medicine, Tongxinluo.

## INTRODUCTION

The intestine dysfunction after ischemia/reperfusion injury is a major and common problem in hospital. Intestine ischemia is associated with different clinical syndromes featured by inadequate blood perfusion to the bowel. It is an important medical condition because of its high mortality rate: Intestinal ischemia accounts for 1 of every 1000 hospital admissions and approximately 1 to 2 of every 100 admissions for abdominal pain. The mortality rates of intestinal ischemia range between 30% and 90% based on the etiology (Herbert *et al.*, 2007; Martinez *et al.*, 2004). Mesenteric venous thrombosis is responsible for 5% to 15% of intestine ischemia. Ischemic colitis, the most common form of intestine ischemia, accounts for 50% to 60% of all the patients of gastrointestinal ischemia (Aterno *et al.*, 2008). Besides, patients suffered from surgical injury or trauma events are more possible to develop intestine I/R injury, which contribute to a high mortality (Koike *et al.*, 1993). It is important in situations that interrupt bloodstream of intestine in many surgeries of cardiovascular or transplantation and *et al.* (Collard *et al.*, 2001). Intestine I/R injury also associated with the septicemia and hypovolemic shock (Moore *et al.*, 1994; Swank *et al.*, 1996).

Studies indicate that intestine is preferentially susceptible to post I/R injury (Kurtel *et al.*, 1991). Intestine I/R injury lead to various inflammatory reaction destroyed the intestine mucosal (Grisham *et al.*, 1988; Arndt *et al.*, 1991). Study also found the process of I/R injury severely harmed intestine microcirculation (Müller *et al.*, 1991). Nevertheless, the clinical data are developing suggesting that there may well be a common "vascular" theme in gastrointestinal inflammation. The vascular endothelium is the key for this symposium on gastrointestinal inflammation (MacCannell, 1993). There is more evidence that microcirculation dysfunction is the key injury factor of intestine I/R injury (Vollmar *et al.*, 2011).

Tongxinluo (TXL), a compound prescription in dried superfine powder form, is prescribed based on the collateral disease doctrine from Traditional Chinese Medicine. The 12 ingredients and it's proportion of TXL are exhibited in table 1. TXL has been permitted for clinical application by Chinese government since 1996 (state medical license NO. Z20060322). Component analysis experiments found that the primary chemical constituents of TXL were ginsenoside Rg1, ginsenoside Rb1, peoniflorin, jujuboside A, jujuboside B, isoborneol, and borneol (Su *et al.*, 2010; Chen *et al.*, 2009; Zhang *et al.*, 2009). Previous studies have reported that TXL contributes to protection of microcirculation against ischemia/reperfusion injury (Liu *et al.*, 2013). However, there is few study reported the treatment of TXL on

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intestine I/R injury. We focus on the microcirculation dysfunction in intestine induced by I/R injury. Conducted experiments aim at illustrating possible function of TXL in terms of intestine I/R injury, especially on endothelial survival and vascular integrity, and related inflammatory reaction.

## **MATERIALS AND METHODS**

### ***Drug preparation***

TXL ultra fine powder ( $\leq 10\mu\text{m}$ ) made of 12 traditional Chinese medicine (see table 1.) was purchased from Yiling Pharmaceutical co. (Shijiazhuang, Hebei, China). The quality of TXL ultra fine powder was strictly keep quality management as described above. We used physiological saline as solvent for dissolution of TXL ultra fine powder.

### ***Animal preparation***

Fifty male Sprague-Dawley rats of specific pathogen free (SPF) grade, weighing ( $200\pm 20$ )g, were supplied by Animal Center of PLA General Hospital (Beijing, SCXK 2012-0001). Rats were raised in laboratory animal room and feed by professional breeder in Animal Center of PLA General Hospital. All animals were handled according to the criterions of Animal Research Committee of PLA General Hospital. Operative steps and experimental protocol were permitted by Animal Ethics Committee of PLA General Hospital.

### ***Intestine ischemia/reperfusion injury***

Rats were anesthetized with 0.3% (1ml/100g) pentobarbital sodium (Sigma, St. Louis, MO, USA) by intramuscular injection. Making a 2-3cm length incision in the middle of rats abdomen. At the fourth mesentery windows ahead ileocecus, bluntly separate superior mesenteric artery. Place an artery clamp right ahead the fourth mesentery windows to block bloodstream for 30 min, and then loosen the clamp to induce reperfusion for 60 min (Zhang *et al.*, 2015). At the end of reperfusion, rats were sacrificed to collect organ tissue immediately.

### ***Experimental protocol***

Rats of Sham group and I/R group, saline solution was given through intragastric at volume of 4 ml/kg/d for 7days. In the TXL+ I/R groups, Saline solution in which dissolved TXL ultra fine powder was given through intragastric at volume of 4ml/kg/d, equal to TXL ultra fine powder dosage of 0.4, 0.8 and 1.6g/kg/d for 7 days' administration. The implementation of intestine I/R injury was performed 2 hours right after last intragastric administration. Random number table method was used to divide rats into 5 groups, Sham, I/R, TXL (0.4g/kg) + I/R, TXL (0.8g/kg) + I/R, and TXL (1.6g/kg) + I/R groups (10 rats each). Fresh I/R injury intestine sections were fixed in 4% buffered formalin (Beijing, Dingguochangsheng Biotech Inc).

### ***Immunofluorescent double staining***

Anti-CD31 antibodies (Abcam Ab64543) were combined with TUNEL (Roche 11684817910). After deparaffinization, pretreated intestine material using citric acid buffer in microwave for 1 min. Using 10% donkey to block intestine material for 30min, then reacted with primary antibody overnight at 4°C. The next step antibody, anti-goat gG-Cy3 antibody (Thermo A10521) was diluted to 1:200 and incubated for 60min. Stained nucleus with 1 mg/ml DAPI and sustained for 3min. Eventually, washing the sides with TBS, then using Fluorescent Mounting Medium to mount. Using Fluorescence Microscope (Olympus BX43) to determine co-expression of CD31 and TUNEL. Activated green light of TUNEL at 494nm, activated red light of CD31 at 550nm, activated blue light of DAPI at 340nm. Image analysis using Image-Pro Plus, calculating IOD value.

### ***Immunohistochemical staining***

Immunohistochemistry for ANGPTL4, VE-Cadherin, HMGB1 and NF- $\kappa$ B, Antigens were unmasked by microwaving section in 10mmol/L citrate buffer, pH 6.0 for 15 min. Intestine material were placed in processing cassettes, dehydrated through a serial alcohol gradient. Then, pre-treated intestine material with 0.03% hydrogen peroxide methanol solution followed by immersing in a 10mM citric acid buffer under 6.0 pH situation, after that, place the intestine material in autoclave at 121°C for 5min. Anti mouse-HRP (Beijing Kangweishiji Inc, Cw0102) which was diluted to 1:1000 reacted at 4°C overnight. The next step antibody, anti-rabbit-HRP (Beijing Kangweishiji Inc, Cw0103) was allow to react, then washed with PBS followed by color development with DAB. Image analysis using Image-Pro Plus for image analysis, calculated the IOD value of ANGPTL4 (bcam Ab196746), VE-Cadherin (Abcam AB151282), HMGB1 (Abcam Ab78923), NF- $\kappa$ B (Abcam Ab32536) in intestine I/R injury tissue.

## **STATISTICAL ANALYSIS**

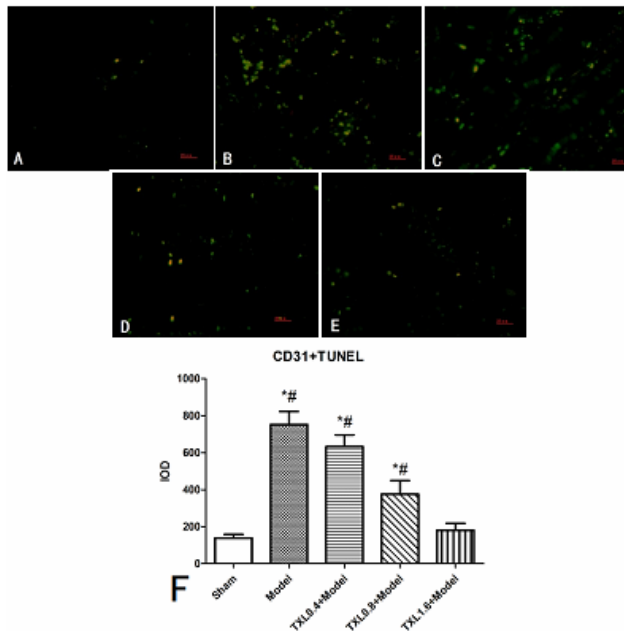
One-way ANOVA was used to analyses differences between groups. Multiple comparisons were analyzed by LSD method. Fisher's exact test was used to analyses qualitative data. A P value that less than 0.05 was regarded as statistically significant. SPSS software was used to analysis all of the data in this article (SPSS 13.0 USA)

## **RESULTS**

### ***TXL pretreatment attenuated I/R induced endothelial apoptosis***

In order to get a better overview of endothelial apoptosis in I/R injury, we performed CD31 and TUNEL immunofluorescent double staining. Immunofluorescent analysis displayed a strong presence after I/R injury (I/R

group) versus Sham group ( $752.68 \pm 69.99$  vs  $139.15 \pm 19.65$ ,  $P < 0.01$ ). TXL pretreatment strongly attenuated the presence of immunofluorescent for CD31 and TUNEL. The difference between TXL0.4+I/R, TXL0.8+I/R and TXL1.6+I/R groups ( $633.04 \pm 62.85$ ,  $376.18 \pm 22.97$ ,  $181.34 \pm 17.52$ ) and I/R group was significantly lowered ( $P < 0.01$ ). Also significantly lowered were the presence of immunofluorescent for CD31 and TUNEL in TXL0.8+I/R group versus TXL0.4+I/R group ( $P < 0.01$ ), and TXL1.6+I/R group versus TXL0.8+I/R group ( $P < 0.01$ ). TXL1.6+I/R group exerted strongest effect against endothelial apoptosis. (fig.1)

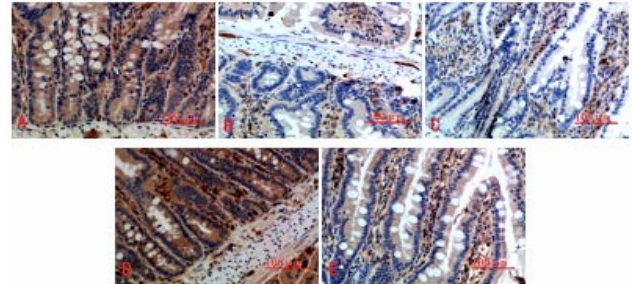


**Fig. 1:** Intestine Immunofluorescent double staining for CD31 and TUNEL in each group. A: Sham group; B: I/R group; C: TXL0.4+I/R group; D: TXL0.8+I/R group; E: TXL1.6+I/R group. Red fluorescence represent for CD31 staining; Green fluorescence represent for TUNEL staining; Yellow fluorescence represent for double staining for CD31 and TUNEL (Scale bar =  $100\mu\text{m}$ ). F: Quantitative analysis of intestine immunofluorescent double staining for CD31 and TUNEL. Data are expressed as means  $\pm$  SD from 10 rats. \* $P < 0.01$  vs. Sham group; # $P < 0.01$  vs. TXL1.6+I/R group.

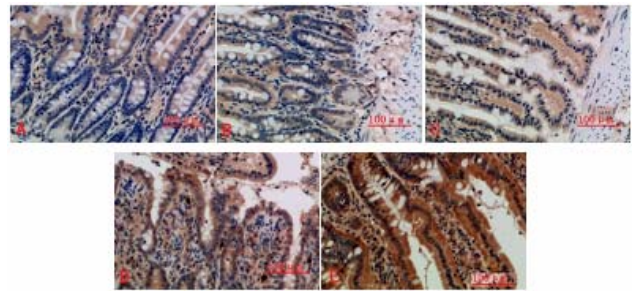
#### ***TXL pretreatment protects microvascular integrity and endothelial barrier against I/R injury***

Expression of VE-cadherin (vascular endothelial cadherin) and endothelial barrier guardian ANGPTL4 in intestine I/R tissue. Immunohistochemistry analysis displayed significantly lowered expression of VE-cadherin in rats of I/R group versus Sham group ( $P < 0.01$ ). TXL pretreatment strongly maintained the expression of immunohistochemistry for VE-cadherin. We observed an upregulated VE-cadherin expression in rats of TXL0.8+I/R group and TXL1.6+I/R group versus I/R group ( $P < 0.01$ ). Besides, VE-cadherin expression in rats

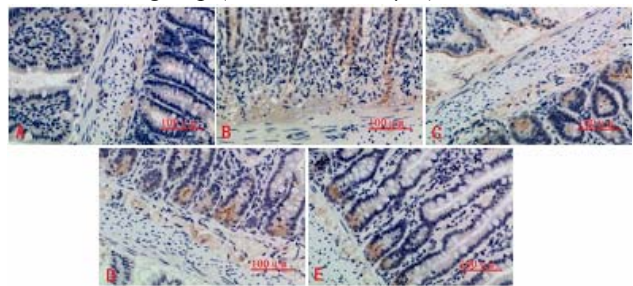
of TXL0.8+I/R group was highly prominent versus TXL1.6+I/R group ( $P < 0.01$ ). I/R injury didn't activated ANGPTL4 expression in rats intestine versus Sham group ( $P > 0.05$ ). While, TXL pretreatment strongly activated the ANGPTL4 expression. The difference between TXL0.4+I/R, TXL0.8+I/R and TXL1.6+I/R groups and I/R group was highly prominent ( $P < 0.01$ ). Also highly significant were the ANGPTL4 expression in TXL0.8+I/R group versus TXL0.4+I/R group ( $P < 0.01$ ), and TXL1.6+I/R group compared to TXL0.8+I/R group ( $P < 0.01$ ). TXL1.6+I/R group exerted strongest effect of promoting ANGPTL4 expression. (table 1, fig. 2.& 3.)



**Fig. 2:** Intestine Immunohistochemistry staining for VE-cadherin in each group. A: Sham group; B: I/R group; C: TXL0.4+I/R group; D: TXL0.8+I/R group; E: TXL1.6+I/R group (Scale bar =  $100\mu\text{m}$ ).



**Fig. 3:** Intestine Immunohistochemistry staining for ANGPTL4 in each group. A: Sham group; B: I/R group; C: TXL0.4+I/R group; D: TXL0.8+I/R group; E: TXL1.6+I/R group (Scale bar =  $100\mu\text{m}$ ).



**Fig. 4:** Intestine Immunohistochemistry staining for HMGB1 in each group. A: Sham group; B: I/R group; C: TXL0.4+I/R group; D: TXL0.8+I/R group; E: TXL1.6+I/R group (Scale bar =  $100\mu\text{m}$ ).

#### ***TXL pretreatment inhibits inflammatory reaction induced by I/R***

Expression of HMGB1 and NF- $\kappa$ B, two centre inflammatory mediators in rats intestine. Immuno-

**Table 1:** Composition of Tongxinluo (TXL)

Ingredients (Latin name)	Ingredients (Chinese name)	Family	Part used	Voucher specimen number	Ratio (%)
Insects					
<i>Hirudo nipponica</i> Whitman	Shui zhi	Hirudinidae	Dried body	12,004	27.330
<i>Cryptotympana pustulata</i> Fabricius	Can tui	Cicadidae	Skin	12,005	18.111
<i>Steleophaga plancyi</i> (Bolony)	Tu biechong	Corydiidar	Female dried body	12,003	18.111
<i>Buthus martensii</i> Karsch	Quan xie	Buthidae	Dried body	12,002	18.111
<i>Scolopendra subspomopes mutilans</i> L. Koch	Wu gong	Psittacidae	Dried body	12,001	3.623
Plants					
<i>Boswellia carteri</i> Birdw	Ru xiang	Burseraceae	Resin	11,006	5.927
<i>Dalbergia odorifera</i> T. Chen	Jiang xiang	Leguminosae	Heartwood of stem and root	11,005	4.000
<i>Bomeolum syntheticum</i>	Bing pian	Dipterocarpaceae	Resin	11,007	3.626
<i>Panax ginseng</i> C.A.Mey	Ren shen	Araliaceae	Root and rhizome	11,001	1.667
<i>Paeonia lactiflora</i> Pall	Chi shao	Ranunculaceae	Root	11,003	1.558
<i>Ziziphus jujube</i> Mill. Var. <i>spinosa</i> (Bunge)	Suan zaoren	Rhamnaceae	Seed	11,002	1.173
<i>Santalum album</i> L.	Tan xiang	Santalaceae	Heartwood of stem	11,004	0.354

**Table 2:** Immunohistochemistry analysis for VE-cadherin and ANGPTL4 ( $\bar{x} \pm s$ , IOD)

Treatment	Sham	I/R	TXL0.4+I/R	TXL0.8+I/R	TXL1.6+I/R
VE-Cadherin	374.49 $\pm$ 31.14	122.29 $\pm$ 1.46*	116.22 $\pm$ 10.50*	461.06 $\pm$ 38.86*#	288.95 $\pm$ 25.08*#
ANGPTL-4	22.17 $\pm$ 2.54	38.98 $\pm$ 3.65	72.43 $\pm$ 7.78*#	113.45 $\pm$ 10.78*#	221.24 $\pm$ 17.96*#

**Table 3:** Immunohistochemistry analysis for HMGB1 and NF- $\kappa$ B ( $\bar{x} \pm s$ , IOD)

Treatment	Sham	I/R	TXL0.4+I/R	TXL0.8+I/R	TXL1.6+I/R
HMGB1	13.71 $\pm$ 5.94	476.77 $\pm$ 46.76*	354.15 $\pm$ 37.37*#	194.90 $\pm$ 35.25*#	80.83 $\pm$ 21.70*#
NF- $\kappa$ B	104.51 $\pm$ 15.88	765.72 $\pm$ 76.85*	514.72 $\pm$ 41.62*#	335.30 $\pm$ 29.64*#	165.71 $\pm$ 12.18#

Quantitative analysis of Intestine Immunohistochemistry staining for HMGB1 and NF- $\kappa$ B in each group. Data are expressed as means  $\pm$  SD from 10 rats. \*P < 0.01 vs. Sham group; #P < 0.01 vs. I/R group.

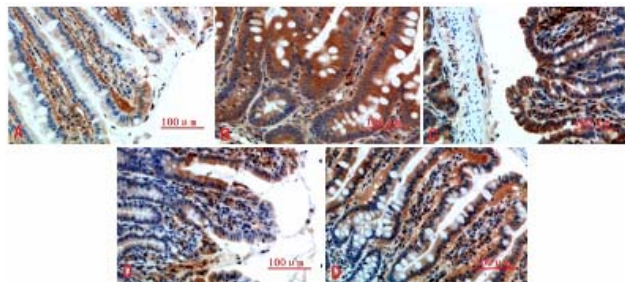
histochemistry analysis displayed highly significant upregulation of the two centre inflammatory mediators in rats of I/R group versus Sham group (P<0.01). TXL pretreatment displayed strongly significant down regulation of immunohistochemistry for the two centre inflammatory mediators. The difference between TXL0.4+I/R, TXL0.8+I/R and TXL1.6+I/R groups and I/R group was prominently lower (P<0.01). Also significant lower were the presence of immunofluorescent for the two centre inflammatory mediators in rats of TXL0.8+I/R group compared to TXL0.4+I/R group (P<0.01), and TXL1.6+I/R group versus TXL0.8+I/R group (P<0.01). TXL1.6+I/R group significantly inhibited inflammatory reaction (table 2, figs. 4 & 5).

## DISCUSSION

Microcirculation is the circulation of blood in the smallest blood vessels. Microcirculation serves as an important part of circulation and constituent of organ and tissues. Terminal arterioles, capillaries, and venules are the main composition of microcirculation. Those micro

vascular is mainly constituted of endothelial cells, embedded within a specific basal membrane. Endothelial barrier serves an important role in vascular integrity, in the perspective of molecular, adhesion molecules closely connected endothelial cells to form endothelial barrier, among those molecules VE-cadherin plays the centre role (Gavard J, 2014). The expression of VE-cadherin was specific for the endothelium in almost all types of vessels. In endothelial cells, VE-cadherin is intensively expressed and situated at adheren junctions (Gavard J, 2009). VE-cadherin is benefit for the protection of endothelial barrier and vascular integrity. Attenuated VE-cadherin function is associated with decomposition of vascular wall (Crosby *et al.*, 2005), and to regulate the macromolecules passage through the endothelium in vitro (Gavard J *et al.*, 2006; Fukuhara *et al.*, 2005). Besides, disturbing VE-cadherin in animal experiments resulted a broken vascular integrity (Crosby *et al.*, 2005; Corada *et al.*, 1999). Evidence has verified that VE-cadherin is necessary for forming adheren junctions and keeping endothelium barrier function. (Gavard J *et al.*, 2006; Taddei *et al.*, 2008; Heupel *et al.*, 2008; Hebda *et al.*, 2013). Accordingly,

VE-cadherin could be the leader in endothelium adhesion molecules, because VE-cadherin regulates the expression and/or the localization of other adhesion molecules, such as claudin-5 and N-cadherin (Gavard *et al.*, 2008; Taddei *et al.*, 2008; Giampietro *et al.*, 2012).



**Fig. 5:** Intestine Immunohistochemistry staining for NF- $\kappa$ B in each group. A: Sham group; B: I/R group; C: TXL0.4+I/R group; D: TXL0.8+I/R group; E: TXL1.6+I/R group (Scale bar = 100  $\mu$ m).

ANGPTL4 belongs to angiogenin like protein family, is a kind of lipoprotein lipase inhibitor, plentifully expressed in adipose tissue, liver, intestine, skeletal muscle and ischemic tissues. ANGPTL4 regulates survival and adhesion of endothelial, coordinates vascular permeability in particular (Galaup *et al.*, 2012; Li *et al.*, 2011; Perdiguero *et al.*, 2011). Over expression of ANGPTL4 enhanced endothelial barrier function and promoted cell-cell junction protein vinculin location in cell membrane in a tumor study (Galaup *et al.*, 2006). ANGPTL4 secreted by ischemia tissues could also enhanced endothelial barrier function (Cazes *et al.*, 2006). Besides, in the high glucose environment, exogenous application or enhanced endogenous expression of ANGPTL4 could up regulate the HCMEC (Human Cardiac Micro vascular Endothelial Cells) adherens junction protein expression and help to locate the expression in cell mesenchyme, such as JAM-A, VE-cadherin and Integrin- $\alpha$ 5/ $\beta$ 1 (Qi, 2015). Tissue suffered from ischemia or hypoxia could trigger endothelial cell transcription factor to activate ANGPTL4 gene (Lee *et al.*, 2013; Larter *et al.*, 2012). In addition, ANGPTL4 was not only exhibited the ability to modulate endothelial adhesion, but also regulate endothelial survival in vitro (Kim *et al.*, 2000).

We observed significantly lowered VE-cadherin expression in intestine I/R injury, while I/R injury exerted no significant changes in ANGPTL4 expression. TXL pretreatment effectively maintained VE-cadherin expression and up regulated ANGPTL4 expression, which indicated TXL's protective effect on endothelial cadherin and endothelial barrier. Meanwhile, high dose administration of TXL presented greater protective effect. TXL could reverse I/R induced endothelial barrier injury and vascular integrity damage, by up regulating ANGPTL4 expression and maintaining VE-cadherin expression.

HMGB1 (high mobility group box-1) is a key inflammatory mediator in inflammatory response found in 1999 (Wang *et al.*, 1999). Subsequent studies (Andersson *et al.*, 2010; Fink, 2007; Yang *et al.*, 2010) confirmed the fact that HMGB1 was an important inflammatory mediator and inflammatory cytokine, which served as a centre factor to initiate and sustain inflammatory cascade reactions in diseases such as hemorrhagic shock, sepsis and I/R injury. However, NF- $\kappa$ B was a well established inflammatory mediator in I/R injury (Spehlmann *et al.*, 2009). Activated NF- $\kappa$ B extensively participated in immune and inflammatory reactions and activated many kinds of cytokines and inflammatory mediators (Shi *et al.*, 2014). HMGB1 could activate one or several signal paths in endothelial cell, enterocyte and neutrophil, lead to the expression of ERK1/2, Akt and NF- $\kappa$ B (Park *et al.*, 2006). Researchers used anti-HMGB1 antibody to interrupt HMGB1 expression resulting in NF- $\kappa$ B activation reduction in hemorrhage induced inflammatory reaction (Kim *et al.*, 2005). We studied the two co-related centre mediators in inflammatory cascade reactions, HMGB1 and NF- $\kappa$ B, to investigate I/R induced inflammatory reaction.

We observed highly significant HMGB1 and NF- $\kappa$ B expression in intestine I/R injury. TXL pretreatment effectively lowered HMGB1 and NF- $\kappa$ B expression, which indicated TXL's protective effect against inflammatory reaction. Meanwhile, high dose administration of TXL presented greater protective effect. TXL could attenuate I/R induced inflammatory reaction by lowering centre inflammatory mediators HMGB1 and NF- $\kappa$ B.

On the other hand, we performed Immunofluorescent double staining for CD31 and TUNEL to get a better overview of endothelial apoptosis in intestine I/R injury. Results indicated that intestine I/R injury lead to tremendous endothelial apoptosis in intestine tissue. While TXL pretreatment could significantly attenuate I/R induced endothelial apoptosis and high dose administration of TXL presented greater protective effect.

However, the major chemical ingredients of TXL, which played a vital role in protecting against intestine I/R injury in rats, is still unclear in the present study. According to previous studies, ginsenoside Rg1, ginsenoside Rb1, peoniflorin, jujuboside A, jujuboside B, isoborneol, and borneol were the major chemical ingredients of TXL. Studies have reported that ginsenoside Rg1 and ginsenoside Rb1 alleviated I/R injury in heart and brain (Li *et al.*, 2016; Deng *et al.*, 2015; Wang *et al.*, 2013). Data provided evidence that ginsenoside Rg1 had protective effects on A $\beta$ 25-35-induced endothelial cells apoptosis (Yan *et al.*, 2013) and paeoniflorin had protective effects on hypoxia-induced endothelial cells apoptosis (Ji *et al.*, 2012).

Besides, ginsenoside Rg1, ginsenoside Rb1 and borneol presented anti-inflammatory effect in I/R injury by inhibiting NF- $\kappa$ B expression (Wang *et al.*, 2013; Liu *et al.*, 2011). Nonetheless, other major chemical ingredients of TXL have not been reported to participate in the regulation of endothelial apoptosis, endothelial function and inflammatory. More attentions should be paid to these chemical ingredients of TXL in further study.

## CONCLUSION

I/R injury in rat intestine presented with significant endothelial apoptosis, disruption of endothelial integrity and endothelial barrier, severe inflammatory reaction. TXL pretreatment protects intestine from I/R injury, at least in part, attenuating endothelial apoptosis, protecting endothelial integrity by maintaining VE-cadherin expression, attenuating micro vascular permeability by increasing ANGPTL4 expression, inhibiting HMGB1 and NF- $\kappa$ B mediated inflammatory reaction. Inhibition of inflammatory reaction may be associated with the protection of endothelial integrity and endothelial barrier. However, decreased endothelial apoptosis is supposed to be the core of TXL's protective effect among all its function against intestine I/R injury in rats intestine.

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