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Effect of nonpeptide NK1 receptor antagonist L-703,606 on the edema formation in rats at early stage after deep partial-thickness skin scalding

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ABSTRACT

Objective: To investigate the effect and the relevant potential mechanism of nonpeptide neurokinin 1 (NK1) receptor antagonist L-703,606 in the edema formation after burn injury. **Method:** L-703,606 treatment was performed in Sprague-Dawley (SD) rats at early stage after deep partial-thickness skin scalding. One hundred and fifty two adult male SD rats were used in the study and randomly divided into sham scald (SS, n=8), scald control (SC, n=48), and L-703,606 treatment (LT, n=48) groups. The rats in SC and LT groups were subjected to 20% total body surface area (TBSA) deep partial-thickness skin scalding. Modified Evans blue extravasation, tracing electron microscopy by lanthanum nitrate and mean water content assay were employed to observe and detect the changes of vascular permeability, ultrastructure and edema formation in adjacent tissue to the wounds and in the jejuna of rats at early stage (72 h) after scald. **Results:** The pathological increase of vascular permeability in the periwound tissue and jejunum of rats in LT group were significantly lower than that in SC group (P<0.01), and recuperated earlier. Meanwhile, the changes of water contents of corresponding tissues in LT group were lighter than those in SC group (P<0.01). The ultrastructural changes of the microvessels in the peri-wound tissue of LT group showed that the junctions between microvascular endothelium cells were more narrow than those of SC group, moreover, and the number of opening and the engorgement and cavitation of the vascular endothelium cells decreased, the areosis and edema in perivascular tissue lightened, and the precipitation of the high eletron density lanthanum tracing agent in the interspace of the tissue decreased significantly in LT group. **Conclusions:** It is concluded that nonpeptide NK1-receptor antagonist L-703,606 could lighten the vascular permeability and edema formation in the periwound tissue and jejunum, and accelerate the normalization process of pathological changes in the tissues of rats after scald.

1. Introduction

The local and systematic vascular permeability and intravascular fluid extravasation which were remarkably increased after thermal injury usually result in tissue edema formation in severe burn patients. Besides the direct injury of heat to the vascular structure, the chemical meditors such as vasoactive amines (histamine, 5-HT), prostaglandin, and free radicals play an important role in increasing vascular permeability[1,2]. For the last twenty years with the deep research on substance P (SP), one member of the tachykinin family, the mediator effects of SP which mediates vasodilatation and increases vascular permeability caused by inflammation have been attached more and more importance[3,4]. Studies have shown that the response of tissue edema caused by SP or electric stimulation, and the increased capillary permeability in both of which were obviously related to the activity of tachykinin nerves[5]. In the present study, after nonpeptide NK1-receptor antagonist L-703,606 given intravenously we used modified Evans blue extravasation method, electron microscopy and mean water content assay to observe and detect the changes of vascular permeability and edema formation in the adjacent tissue area to the wound and in the jejuna of rats at early stage (72 h)

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after scald, and so to identify the effect of NK1 receptor in the increased capillary permeability and the edema formation postscald.

2. Materials and methods

2.1. Animals and treatment groups

Experimental protocols were approved in advance by the Animal Protocol Review Committee of the Fourth Military Medical University for animal program. One hundred and fifty two male Sprague-Dawley rats with body weight 160-180 g, supplied by the animal center of the Fourth Military Medical University, were randomly divided into sham scald (SS, n=8), scald control (SC, n=48), and L-703,606 treatment (LT, n=48) groups. The rats in SC and LT groups were subjected to skin scalding (to 20% TBSA deep partial-thickness, certified by pathological biopsy). Then tissue specimens were taken at 1, 4, 8, 24, 48, 72 h time points postscald (8 rats at one time point from each of group SC and LT). The rats in group LT were injected intravenously with 250 nmol/kg body weight of L-703,606 (RBI Corp, USA) 30 min before scalding.

2.2. Modified Evans blue extravasation method

The modified Evans blue extravasation method was performed as described by Lu[3]. The absorbances of serial Evans blue diluents from 0.005 to 50 mg/L dissolved with dimethyl formamide were measured at 620 nm wavelength with spectrophotometer (DH-580, Beckman Corp., Germany). Then the standard curve was plotted between the Evans blue concentrations and the corresponding absorbances. After deep anaesthesia by pentobarbital sodium (30 mg/kg, intraperitoneal injection), the rats were given with 2 mg/kg body weight of Evans blue (Serva Corp., USA) solution 30 min before observation time point via the tail vein. Normal saline of 300 mL was perfused through the left ventrical to wash out the blood immediately before tissue specimens collection and Evans blue remained in the vascular system. A 0.5 cm × 0.5 cm piece of whole thickness skin and two 1.0 cm long segments of jejuna were taken immediately following perfusion from the adjacement area to the scald wound and the inferior third part of small intestine respectively. After being weighed, the tissues were put into 2 mL dimethyl formamide and kept at 50 ℃ for 24 h with temperature thermostat water bath. At 620 nm wavelength, the absorbances of tissue extractions were measured with DH-580 spectrophotometer, and the relevant contents of Evans blue were achieved according to the standard curve. The ratio between the contents of Evans blue in the adjacent area to the wound or those in the jejuna and the tissue weights were calculated, and the results were denotated as the Evans blue contents of those tissues (μg/g), which represented the changes of vascular permeability in the above tissues.

2.3. Tissue water content assay

Animals of each group were executed by decapitation at different time points after scald. The skin samples of about 1 cm × 1 cm were cut and obtained immediately along the margin of wound. Meanwhile, two 5 cm long intestinal canals were harvested at distal end and proximal end from the one third of the inferior segment of small intestine, and the separated intestinal canals were split lengthways. The tissue samples from skin and jejunum were agitated in physiological saline to wash out adhesion, and blotted with absorbent paper, then were put into oven to be desiccated continually at 90 ℃ for 72 h after being weighed with an analytic balance. The weights of dried samples were measured again. The mean water contents of the tissue were calculated as follow: (wet weight-dry weight)/ wet weight = mean water content (mg/mg).

2.4. Tracing electron microscopy by using lanthanum nitrate

The tracing electron microscopy by lanthanum nitrate was performed as described by Pederson[6]. After deep anaesthesia by pentobarbital sodium (30 mg/kg, intraperitoneal injection) at 1, 24, 48, 72 h time points postscald, the rats were perfused to fix on constant pressure of 150 mmHg. They were firstly perfused for 5 min with physiological saline to flush out blood, then for 15 min with 1% glutaraldehyde, 0.1 mol/L sodium dimethylarsenate buffering fixation fluid, and finally for 15 min with 3% lanthanum nitrate, 3% glutaraldehyde, 0.1 mol/L sodium dimethylarsenate buffering fixation fluid. About 2 mm × 5 mm skin sample beside the wound was cut immediately along the margin of wound. The tissue samples from skin were put into 3% lanthanum nitrate, 3% glutaraldehyde, 0.1 mol/L sodium dimethylarsenate buffering fixation fluid to fix for 2 h, then were cleaned for 10 minutes by 2 times with 2% lanthanum nitrate, 0.1 mol/L sodium dimethylarsenate buffering fixation fluid, and finally they were fixed with 2% lanthanum nitrate, 1% osmium tetroxide, 0.1 mol/L sodium dimethylarsenate buffering fixation fluid for 2 h. The samples were embedded with resin and sectioned by semithin section, and stained with plumb and uranium routinely. Transmission electron microscope was used to observe the ultramicrostructure and the lanthanum nitrate deposition outside the micrangium, and the results were

assessed to indicated vascular permeability by classification as follows: little (+), small amounts (++), medium amounts (+++) and a great amounts (++++).

2.5. Statistical analysis

All the data were analyzed by using SPSS10.0 software. Each group of data was expressed as mean ± standard error ($\bar{x}\pm$ s), and t test was performed in the analysis among groups in order to compare the significance of differences among group LT, group SS and group SC. The result analysis of tracing electron microscopy by applying lanthanum nitrate was performed by nonparametric rank sum test between groups.

3. Results

3.1. Changes of vascular permeability

The changes of vascular permeability beside the wounds and in the jejuna of rats postscald are showed in Table 1 and Table 2.

3.1.1. Periwound tissue

In periwound tissue, the vascular permeability of SC group was obviously higher than that of SS group. The vascular permeability increased and reached to the highest at 1 h postscald with SC:SS = 24.21 (P<0.01). Then it gradually decreased but kept higher than SS group to 72 h postscald with SC:SS=13.13 (P<0.01).

Also in periwound tissue during the 72 h postscald, the vascular permeability of LT group increased first and then decreased, and gradually recovered. In the whole process, the general increase of vascular permeability in LT group was less than that in SC group. At 1 h postscald, it was about 48.83% of that of SC group with LT:SS=11.82 (P<0.01), and at 4 h postscald, it reached the lowest with 42.24% of that of SC group and LT:SS= 8.52 (P<0.01). Then it increased slowly to the highest at 48 h postscald with 86.56% of that of SC group (P<0.01). At 72 h postscald, it decreased again to 75.31% of that of SC group (P<0.01).

3.1.2. Jejunum tissue

In SC group, the trend of vascular permeability in jejunum tissue was rising quickly first, slowly descending then, and rising again. At 1 h postscald, the vascular permeability rose obviously with SC: SS=4.37 (P<0.01). The vascular permeability ratioes of SC to SS group were 2.74, 1.59, 1.16 at 4 h, 8 h and 24 h postscald respectively. It increased again at 48 h postscald with SC:SS=2.42 (P<0.01). But at 72 h postscald, the vascular permeability of SC group was a little different from that of SS group without statistical difference (P>0.05).

In LT group, the trend of vascular permeability in jejunum tissue was nearly similar to that of SC group, but the change extents of it smaller in comparison with SS group. At 1 h postscald, the vascular permeability of LT group was 67.12% that of SC group (P<0.01). At 24 h postscald, the vascular permeability was 85.38% with LT: SS=1.16 (P<0.01) of SC group. In the time of 48 h to 72 h postscald, the differences of vascular permeability between LT and SC group were not remarkable (P>0.05).

3.2. Changes of tissue water contents

The changes of tissue water contents beside the wound and in the jejuna of rats postscald are showed in Table 3 and

Table 1
Postscald changes of Evans Blue contents in periwound tissue in rats ($\bar{x}$ ± s, μg/g).

Group	Animal number	The time postscald					
		1 h	4 h	8 h	24 h	48 h	72 h
SS	8	15.7±6.7					
SC	48	380.1±31.2**	316.5±25.7**	271.3±22.1**	300.4±28.9**	253.7±24.6**	206.2±20.5**
LT	48	185.6±20.4$^{**\triangle\triangle}$	133.7±15.4$^{**\triangle\triangle}$	170.1±17.8$^{**\triangle\triangle}$	203.2±23.7$^{**\triangle\triangle}$	219.6±29.9$^{**\triangle\triangle}$	155.3±18.7$^{**\triangle\triangle}$

Note: vs SS, $^{**}P$<0.01; vs SC, $^{\triangle\triangle}P$<0.01.

Table 2
Postscald changes of Evans Blue contents in jejunum tissues in rats ($\bar{x}$ ± s, μg/g).

Group	Animal number	The time postscald					
		1 h	4 h	8 h	24 h	48 h	72 h
SS	8	47.3±8.5					
SC	48	206.7±12.8**	129.6±6.4**	75.2±7.1**	54.3±7.5*	114.5±8.6**	59.1±6.1*
LT	48	138.7±9.7$^{**\triangle\triangle}$	102.4±5.5$^{**\triangle\triangle}$	60.1±4.8$^{**\triangle\triangle}$	64.9±7.9$^{**\triangle\triangle}$	108.5±8.4**	55.3±7.1*

Note: vs SS, $^{*}P$<0.05, $^{**}P$<0.01; vs SC $^{\triangle}P$<0.05, $^{\triangle\triangle}P$<0.01.

Table 3

Postburn changes of water content percents in periwound tissues in rats ($\bar{x}$ ± s, %).

Group	Animal number	The time postscald					
		1 h	4 h	8 h	24 h	48 h	72 h
SS	8	0.748±0.068					
SC	48	0.629±0.038**	0.782±0.061**	0.883±0.046**	0.815±0.055**	0.784±0.082**	0.756±0.088**
LT	48	0.708±0.067$^{**\triangle\triangle}$	0.751±0.072$^{*\triangle\triangle}$	0.800±0.078$^{**\triangle\triangle}$	0.782±0.053$^{*\triangle}$	0.769±0.089$^{*\triangle}$	0.738±0.091*

Note: vs SS, *P<0.05, **P<0.01; vs SC $^{\triangle}P$<0.05, $^{\triangle\triangle}P$<0.01.

Table 4

Postscald changes of water content percents in jejunum tissues in rats ($\bar{x}$ ± s, %).

Group	Animal number	The time postscald					
		1 h	4 h	8 h	24 h	48 h	72 h
SS	8	0.804±0.052					
SC	48	0.784±0.055*	0.779±0.067**	0.763±0.071**	0.826±0.045**	0.860±0.053**	0.837±0.049**
LT	48	0.793±0.038$^{*\triangle}$	0.789±0.062$^{*\triangle}$	0.780±0.045$^{*\triangle}$	0.815±0.069$^{*\triangle}$	0.828±0.035$^{**\triangle}$	0.821±0.057$^{**\triangle}$

Note: vs SS, *P<0.05, **P<0.01; vs SC $^{\triangle}P$<0.05, $^{\triangle\triangle}P$<0.01.

Table 4.

3.2.1. Periwound tissue

Above all, the change trends of tissue water contents of periwound tissue were basically paralled in both SC and LT groups. At 1 h postscald, both groups presented a dehydration state with water contents in SC and LT groups as 84.09% and 94.65% of that in SS group. The tissue water contents of SC and LT increased after and reached the highest at 8 h postscald, showing edema state with SC:SS=1.18 and LT:SS=1.07; then decreased tardily until 72 h postscald with SC:SS=1.01 (P>0.05) and LT:SS=98.67% (P>0.05). At the early stage from 1 h to 24 h postscald, the changes of water contents in LT group were much smaller than those in SC group(P<0.01).

3.2.2 Jejunum tissue

At 72 h postscald, the tissue water contents of jejuna in SC and LT groups were nearly the same. At the early scald stage, the jejuna of both SC and LT groups showed exsiccosis, which reached the lowest with SC:SS=94.90% less than LT:SS=97.01% (P<0.05) by 8 h postscald. The jejuna presented a certain edema state till 24 h postscald, and became severely injured showing the edema worse at 48h postscald with SC:SS=1.07 higher than LT:SS=1.03 (P<0.01) and recovered gradually later.

3.2.3. Ultrastructural changes of the microvessels in periwound tissue

In SS group, the integrated intercellular space of microvascular endothelium cells of periwound tissue could be seen. The lanthanum nitrate was confined to the lumen and the inner wall of micrangium, and did not permeate the intercellular space of microvascular endotheliocytes. There was no lanthanum nitrate in tissue space (Figure 1).

Figure 1. Transmission electron microscope observation of periwound tissue of SD rats in SS group.
The lanthanum nitrate was confined to the lumen and the inner wall of micrangium, and did not permeate through the intercellular space of microvascular endotheliocytes. There was no lanthanum nitrate in tissue space outside the micrangium. (×15 000).

In SC group, the permeability of the junctions between microvascular endothelium cells of periwound tissue increased at 1 h postscald, and the lanthanum nitrate could be seen clearly entering into tissue along the open intercellular space of microvascular endothelium cells. The vacant space was limted because of edema (Figure 2). At 24 h postscald, the number of intercellular space openings of microvascular endothelium cells decreased, and the tissue space outside the microvessels augmentated obviously. The tissue edema was obviously remarkable, and there was a great quantity of lanthanum nitrate deposition in tissue outside the microvessels (Figure 3). At 48 h postscald, the tissue edema was still severe, which was demonstrated by much deposition of lanthanum nitrate. The number of intercellular space opening of microvascular endothelium cells was less than that of 24 h postscald. At 72 h postscald, the number of intercellular space opening of microvascular endothelium cells obviously decreased, and the edema could still be seen but were lightened than that of 48 h postscald.

Meantime, the deposition of lanthanum nitrate in tissue space was remarkablely decreased (Figure 4). Compared with those of SS group, the assessment results of other corresponding from SC group time points were all of obvious difference (P<0.05).

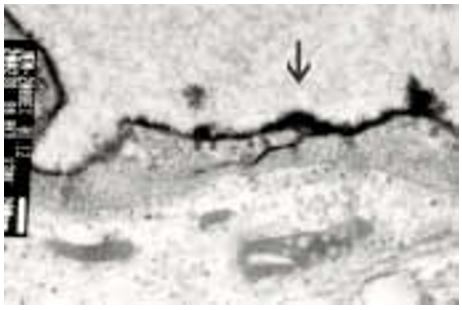

Figure 2. Transmission electron microscope observation of periwound tissue of SD rats in SC group at 1 h postscald.
The intercellular space between microvascular endothelial cells had opened (↑), and the lanthanum nitrate entered into tissue along the opening intercellular space opening of microvascular endothelial cells. (×30 000)

Figure 3. Transmission electron microscope observation of periwound tissue of SD rats in SC group at 24 h postscald. The intercellular space opening (↑) of microvascular endothelial cells.
The tissue space augmentated obviously. There was a great quantity of lanthanum nitrate depositing in the tissue outside the micrangium. (×30 000)

Figure 4. Transmission electron microscope observation of periwound tissue of SD rats in SC group at 72 h postscald. The tissue edema could still be seen clearly and the deposition of lanthanum nitrate was remarkablely decreased in tissue space. (×5 000)

In LT group, a few of the intercellular spaces of microvascular endothelium cells had opened at 1 h postscald, and the lanthanum nitrate could be seen permeating along them, but were less than that of 1 h postscald in SC group. The edema was not obviously remarkable, and the deposition of lanthanum nitrate in tissue space was parum (Figure 5). At 24 h postscald, the number of intercellular space opening of microvascular endothelium cells was less than that of 24 h postscald in SC group. The edema was obvious but lightened than that of 24 h postscald in SC group, and the deposition of lanthanum nitrate in tissue space was obviously less than that of 24 h postscald in SC group (Figure 6). At 48 h and 72 h postscald, the number of intercellular space opening of microvascular endothelium cells and the deposition of lanthanum nitrate in tissue space were also remarkablely decreased, and the edema was lightened in comparison with that of SC group (Figure 7). As compared with those of SS group, the assessment results of other time points from LT group were all of obvious difference (P<0.05).

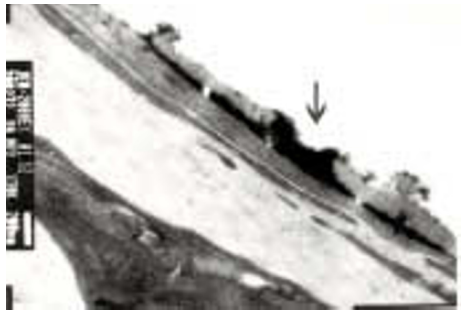

Figure 5. Transmission electron microscope observation of periwound tissue of SD rats in LT group at 1 h postscald.
Some of the intercellular space of microvascular endothelial cells had opened at 1 h postscald (↑), and the lanthanum nitrate could be seen permeating along the openings, which were less than that of 1 h postscald in SC group. The tissue edema was not obviously remarkable, and the deposition of lanthanum nitrate was little in tissue space outside the micrangium. (×30 000)

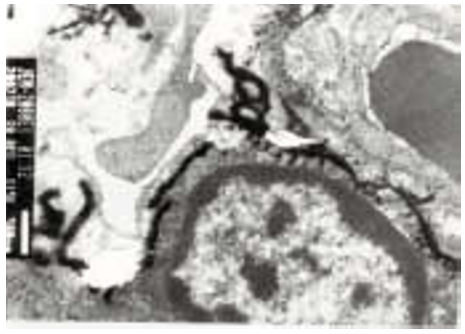

Figure 6. Transmission electron microscope observation of periwound tissue of SD rats in LT group at 24 h postscald. The number of intercellular space opening of microvascular endothelial cells was less than that of 24 h postscald in SC group.
The edema was obvious but much lightened than that of 24 h postscald in SC group, and the deposition of lanthanum nitrate in tissue space was obviously less than that of 24 h postscald in SC group. (×15 000)

Figure 7. Transmission electron microscope observation of periwound tissue of SD rats in LT group at 72 h postscald.
The number of intercellular space opening of microvascular endothelial cells and the deposition of lanthanum nitrate in tissue space was also remarkablely decreased, and the tissue edema was lightened than that of SC group. (×20 000)

4. Discussion

SP, one of early found neuropeptides, constitutes the mammalian tachykinin family together with NKA (neurokinin A), NKB (neurokinin B), NPK (neuropeptide K), NP γ (neuropeptide γ), physalaemin, eledoisin, kassinin and others. They widely distribute in the central and peripheral nervous system, skin, respiratory tract, genitourinary tract and endocrine glands[7]. SP positive nerve fibres also distribute along the stratum basale epidermidis, and around the capillaries of dermic papillar layer and subcutaneous tissue in human. It interlaces into nets and extends to mucosas and veins of intestines, then links to peripheral branches of primary sensory nerve[8].

Studies have shown that there are three types of tachykinin family receptors: NK1, NK2, NK3, NK1 receptor, which has the highest selectivity and strongest affinity with SP, is especially called SP receptor[9]. The sequence study of it indicates that NK1 receptor belongs to G-protein-coupled receptor family. After combining with NK1 receptor in central and peripheral nervous system, SP gets in touch with the second meditor of phosphatidylinositol diphosphate through G-protein, and affects the calcium channel on membranes through inositol triphosphare, which causes the depolarization of membrane potential and the changes of activation of protein kinase to exert its complicated physiological function[10]. Studies on SP antagonists mainly are about the competitive antagonists which are competitively combining with NK1 receptor. The early studies of the competitive antagonists to SP included changing the framework of SP peptide, i.e. peptide antagonists. As their low affinity and selectivity, organic compounds (nonpeptide antagonists) with higher affinity and selectivity became the hotspot of researches about SP antagonists step by step, and have taken a more and more important part in researches about SP in many fields. Among them, the representative ones are CP-96345, CP-99994, RP-67580. It was reported that CP-96345 had a strong affinity with NK1 receptor, which was equal to the natural agonists of SP, but to NK2 and NK3 receptors, its affinity reduce to 1/1 000, expresseing a strong selectivity. This is the remarkable precedent of studies about nonpeptide antagonist of NK1 receptors[11]. Afterwards, a great quantity of nonpeptide antagonists of NK1 receptor with high affinity was found, including RP-67580, L-703,606, etc. L-703,606 could inhibit the combination of 125I-Tyr8-SP and NK1 receptors of human (IC_{50}= 2 nM). The difference of composition between L-703,606 and CP-96345 is that a methoxyl ($-OCH_3$) in one of benzene rings in the structure of CP-96345 is substituted by a iodine group (-I)[12]. As the effectively competitive antagonist of NK1 receptor, L-703,606 could competitively inhibit the combination of SP and NK1 receptor leading up to inhibiting the function of SP[13]. One study on NK1 receptor-mediated plasma extravasation in the rat trachea showed that using L-703,606 beforehand could inhibit the edema formation in the rat trachea caused by capsaicin[14]. L-703,606 also has an obvious inhibiting action in plasma exudation and edema caused by TFLLR-NH (2), the antagonist of proteinase-activated receptor 1(PAR1)[15]. The affinity of SP, CP-96345, RP-67580, L-703,606 to NK1 receptor was as follows: SP ≈ CP-96345 ≈ L-703,606 > RP-67580[16].

Heat injury such as sacld could activate the nocuity afferent nerve, then the end of peripheral nerve releases neuropeptide, which accelerate inflammatory reaction. Tachykinin family, including SP, does an important part in this reaction. SP and NKA mediated capillary reaction which accelerated the edema formation such as vasodialation, plasma exudation and so on[17]. The metalegs of rat were subjected to heat injury by being put into hot water at 60 ℃ for 20 s. At 45 min after heat injury, L-703,606 (100 nmol) was injected in sole of the foot, and the other foot was used as control. The volume changes were observed 7 h after heat injury and before scalding with plethysmograph, and were used as indexes of edema formation. The increase of volume of the foot which was injected with L-703,606 was significantly not so much as that of the control.

The vascular endothelial cells (VECs) together with the basal lamina constructe the barrier of blood vessel endothelium (BVE), and the permeability of which controlls the exchange of the cells, solute and fluid in exterior and interior of blood vessel. Many toxic, inflammatory and thrombotic mediators could all impaired the function of the barrier of BVE, which leads to the increase of permeability. The conjunctions of VECs consist of complicated, cancellated trarsmembrane proteins which are aligned in cell membrane

and kytoplasm[18]. The dynamic structure of the conjunctions of VECs is very important, and the VECs could change the configuration of the conjunctions fleetly to allow the pass of plasma fractions and circulatory blood cells. The status is reversible, and the disorganization and the new synthesis of the conjunctions are only in a few minutes[19]. There could be some certain changes of density and number in the conjunctions of VECs according to the different function of organs, and they could controll the exudation of blood components and giant molecular substance and stabilize the colloid osmotic pressure of the blood[20,21]. The culture of VECs from umbilical vein and arteriae aorta in vitro had confirmed that under the stimulation of single factor such as thrombase, histamine and bradykinin, the changes of protein kinase A, protein kinase C, PTK, and the concentration of Ca2+ caused the proteins in exterior and interior of VECs to phosphorylation of tyrosine and myosin light chain, which leaded to depolymerization and redistribution of proteins in exterior and interior of VECs, and the changes of cytoskeletal structure, contraction of VECs and the increase of the number of conjunctions of VECs[22].

Several studies have shown the inflammatory mediators, enzymes, endotoxin and Ca^{2+} could increase the number of opening conjunctions after severe scald, which leaded to blood cells and giant molecular substances entering into tissue space and resulted in the changes of colloid osmotic pressure, and caused the edema formation of tissues and organs. In our present study, L-703,606 could lighten the pathological increase of vascular permeability and water contents in the periwound tissue and jejunum, and accelerate the pathological changes return to normalization. We inferred that the underlying mechanism of those L-703,606 effects might be as follows:

Ⅰ: L-703,606 could inhibit the combination of SP and NK1 receptor, and so decrease the NO production from vascular endothelium and lighten its corresponding vasodialation.

Ⅱ: Through inhibiting the combination of SP and NK1 receptor in endothelium, L-703,606 could lessen the extent of activation of second messenger system which was coupled with G-protein and mediated by phosphatidylinositol and Ca^{2+}, so as to decrease the opening of interspace of vascular endothelial cells and transmembrane transport of fluid and plasma protein through microvascular wall to attenuate the rapid decrease of plasma colloid osmotic pressure and rapid increase of interstitial colloid osmotic pressure.

Ⅲ: L-703,606 could decrease the consumptive release of SP from the end of peripheral sensory nerve, so the vasodialative effect of SP in veins could be lightened.

Ⅳ: By suppressing the combination of SP and NK1 receptor in mastocyte, L-703,606 could reduce the release of histamine and limit the increase of vascular permeability caused by histamine[23].

Evans blue used in the experiment is a biological dye agent. It has a strong affinity for serum protein and can stably be combined with albumen in several seconds after being injected. It is usually used for detecting blood capacity, vascular permeability and so on as a marker. Compared the ratio-labelled with marked albumen to detect the radioactivity of tissues, Evans blue method needs a organic solvent to effectively extract the Evans blue filtered into tissues. Dimethyl formamide used in our study, is called "omnipotent solvent" because of its excellent dissolution with many organic and inorganic solvents. In comparision with formamide used in traditional Evans blue method, dimethyl formamide can extract the Evans blue filtered into tissues better in order to reflect the changes of vascular permeability[24].

Above all, SP antagonist, especially nonpeptide antagonist, which possesses stable metabolism, high selectivity and strong affinity, has an important value in the pahtophysiologic study and the treatment investigation after burn injury.

Conflict of interest statement

The authors declare that they have no conflicts of interest.

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