EPT PROTECTS AGAINST LOW-DENSITY LIPOPROTEIN-INDUCED ENDOTHELIAL DYSFUNCTION

TAN JUNMING[1,2], ZHOU JIE[1], TAN JINGYI[1], HU HUAMING[1], PI YIYUAN[1], TAN BIN[1]
[1]School of Pharmacy, Xiangnan University, Chenzhou 423000, China - [2]School of Pharmaceutical Sciences, Zhengzhou University, Zhengzhou 450001, China

ABSTRACT

Objective*: The protective effects of extractive of pericarpium trichosanthis (EPT)on vascular endothelial cells of rat and human umbilical vein were studied, and low density lipoprotein (LDL) or oxide low-density lipoprotein (ox-LDL) was used to damage vascular endothelium or endothelial cells. The endogenous related substances in blood plasma or cell culture medium were detected to prove that EPT has protective effect on vascular endothelium damaged by LDL.*

Methods*: 1) Aortic ring model: the endoehilal injuried aortic ring was maded by a single dose of LDL (4 mg/kg) intravenously injected into the sublingual vein of male rat for 48 h, and physiological saline injection served as a control. Blood samples were collected from carotid artery in the state of anaesthetization. Vasodilator responses to acetylcholine (Ach) in the isolated aortic rings were determined, and serum concentrations of asymmetric dimethylarginine (ADMA), malondialdehyde (MDA), tumour necrosis factor-α (TNF-α), Nitric oxide (NO). 2) Cell culture and treatment: Incubation human umbilical vein endothelial cells (HUVECs) with ox-LDL (100 μg/ml) for 24h, the medium levels of lactate dehydrogenase (LDH), ADMA, TNF-a, NO, MDA and the intracellular activity of DDAH were measured.*

Results*: A single injection of LDL (4 mg/kg) significantly decreased vasodilator responses to Ach, increased the serum level of ADMA, MDA and TNF-α, and decreased the serum level of NO. EPT (4ml/kg) with 70% ethanol elution markedly reduced the inhibition of vasodilator responses to Ach by LDL, and the protective effect of EPT with 70% ethanol elution was greater compared with the another ethanol elution group. EPT with 70% ethanol elution inhibited the increased level of ADMA, MDA and TNF-α induced by LDL, and increased the serum level of NO. Incubation of HUVECs with ox-LDL (100 μg/ml) for 24h and treated with EPT (0.1mg/ml) markedly decreased the medium levels of LDH, ADMA, MDA and TNF-a, and increased the level of NO in the medium and the intracellular activity of DDAH.*

Conclusions*: EPT can protect the endothelium against damages elicited by LDL in vivo and in culture, the mechanism of protective effect of EPT on endothelium may be related to the DDAH/ADMA pathway.*

Keywords: *extractive of pericarpium trichosanthis, low-density lipoprotein, endothelium, asymmetric dimethylarginine, dimethylarginine dimethy laminohy drolase.*

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Introduction

Endothelial dysfunction has been thought to play an essential role in initiation and progression of atherosclerotic diseases(1). Nitric oxide (NO), which is synthesized from L-arginine by NO synthase (NOS) in endothelial cells, plays a pivotal role in maintain of vascular structure and function, and it is generally described as an 'endogenous anti-atherosclerotic molecule'. Recently, it was reported that L-arginine analogue asymmetric dimethylarginine (ADMA), a major endogenous inhibitor of NOS, could reduce NO production and decrease acetylcholine (ACh)-induced vasodilator responses in vitro and in vivo(2). There is growing evidence that the elevation of circulating ADMA level is involved in endothelial dysfunction in some risk factors of atherosclerosis including

hypercholesterolemia[3] Most of ADMA is degraded by dimethylarginine dimethylaminohydrolase (DDAH) in endothelial cell both in vivo and in culture, which hydrolyzes ADMA to L-citrulline and dimethylamine[3]. It has been suggested that decrease of DDAH activity induced by oxidative stress and/or inflammatory factors is a key factor contributing to the elevation of ADMA level in patients and animals with hypercholesterolemia and in endothelial cells treated with low-density lipoprotein (LDL) or oxidative LDL (ox-LDL)[4].

EPT is an extractive of pericarpium trichosanthis that is not utilized in protein synthesis. It has been shown that EPT improves endothelial function and inhibits apoptosis of endothelial cell as well as decreases plasma level of LDL, which prevents initiation and progression of atherosclerosis (5). EPT exhibits cardiovascular protective properties through its actions as an antioxidant, anti-inflammatory factor, osmoregulator, and intracellular Ca2+ flux regulator. However, the precise mechanism that accounts for such effects of EPT is not completely defined yet.

Some previous reports and our recent studies showed that some antioxidant and anti-inflammatory drugs could improve endothelial function via reduction of ADMA level by improving DDAH activity in patients and animals with hypercholesterolemia and in LDL-treated rats (6), suggesting the DDAH/ADMA pathway may be a novel therapeutic target for endothelial dysfunction and atherosclerosis. Based on both antioxidant and anti-inflammatory properties of EPT, we hypothesized that EPT can modulate the DDAH/ADMA pathway, which is involved in it protective effect on endothelial function.

In the present study we examined the effect of EPT on endothelial dysfunction induced by a single injection of native LDL in rats and by ox-LDL in cultured endothelial cells, and the involvement of DDAH/ADMA pathway in such effect of EPT.

Materials and methods

Experimental grouping

Grouping of animal experiments

Forty SD rats weighing (210 ±30) g (provided by the Department of Experimental Zoology, Central South University) were randomly divided into a blank group, a model group, a low, medium, and high-dose extract of Trichoderma spp. + LDL group. Group of 8 rats. The treatment methods of each group are as follows: 1) Control group (blank group): intragastric administration of normal saline 0.4ml/100g once a day for five consecutive days, and intravenous injection of normal saline on the fourth day; 2) LDL group (model group): normal saline 0.4 Gavage ml/100g once a day for five consecutive days. On the fourth day, LDL (4 mg/kg) was injected into the tail vein to induce vascular endothelial injury; 3) EPT(L) group (Gualou peel extract low dose + LDL group): With 20 mg/kg of Gualou peel extract, gavage once a day for five consecutive days, inject LDL (4 mg/kg) into the tail vein on the 4th day; 4) EPT(M) group (Dose of Gualou peel extract + LDL group: Gavage with Fructus Trichosanthes skin extract 60 mg/kg once a day for five consecutive days, inject LDL (4 mg/kg) into the tail vein on the 4th day; 5) EPT(H) group (Fructus Fructus cortex extracts High-dose + LDL group): Rhizoma Fructus cortex extract 180 mg/kg was given by gavage once a day for five consecutive days, and LDL (4 mg/kg) was injected into the tail vein on the fourth day.

Cell grouping

The human umbilical cord vein endothelial cells in a 6-well plate were randomly divided into 5 groups: 1) Blank control group: incubate the cells with DMEM medium for 24 hours; 2) ox-LDL treatment group: incubate endothelial cells with 100 mg/ml ox-LDL medium for 24 hours; 3) Low-dose drug + ox-LDL group: Use 5μg/ml fennel peel extract and 100mg/ml ox-LDL into the culture medium to incubate endothelial cells for 24 h; 4) Medium-dose drug+ox-LDL group: use 15μg/ml ml Trichosanthes cortex extract and 100mg/ml ox-LDL were added to the culture medium to incubate endothelial cells for 24 hours; 5) High dose drug + ox-LDL group: 45μg/ml Trichosanthes cortex extract plus 100mg/ml ox-LDL Add culture medium and incubate endothelial cells for 24 h;

Reagents

ADMA, phenylephrine and acetylcholine were purchased from Sigma. DMEM, penicillin and streptomycin were obtained from Gibco. Fetal bovine serum (FBS) was obtained from Sijiqing Biological Engineering Materials (Hangzhou, China). Vitamin E was obtained from Xiamen Fish Liver Oil Factory (Xiamen, China). Lactate dehydrogenase (LDH), malondialdehyde (MDA) and nitric oxide (NO) assay kits were obtained from Ju-Li Biological Medical Engineering Institute (Nanjing, China). Tumor necrosis factor-❑(TNF-❑)

radioimmunoassay kits were obtained from Dongya Immunity Technology Institution (Beijing, China).

Preparation of LDL and LDL oxidation by copper

Native LDL was isolated from freshly prepared normal human plasma though sequential density gradient ultracentrifugation in sodium bromide density solutions in the density range 1.019-1.063 g/ml as previously described[6]. Then LDL was dialyzed against 0.01 PBS (pH 7.4) containing 0.01% EDTA and stored at 4°C in the dark. Protein concentration was measured using Lowry's method[7].

Oxidation of LDL was induced by adding 10 μM CuSO4 for 24 h at 37°C. The content of thiobarbituric acid reactive substance (TBARS), reflecting the amount of lipid peroxidation, were measured by previously described methods(8). The levels of TBARS were 5.26 ± 0.96 and 26.03 ± 5.26 μmol/g protein for LDL and ox-LDL, respectively.

Animal treatment

Male Sprague-Dawley rats weighing 180 - 220 g were obtained from Laboratory Animal Center, Central South University, China. Rats were pretreated with LDL (4 mg/kg, i.v.) at 48 h before the experiment to induce endothelial dysfunction, the rats were treated with EPT (4 ml/kg) once a day for 5 days before the experiment and then treated with LDL (4 mg/kg) for 48 h in the presence of drugs. At the end of experiment, the blood samples of the rats were collected for measures of plasma levels of ADMA, NO, MDA and TNF-❑, and the thoracic aortas were isolated for determination of Ach-induced vasodilator response. All procedures were carried out in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health.

Cell culture and treatment

Human umbilical vein endothelial cells (HUVECs) were obtained from Tumor Research Institute of Peking Union Medical College (Beijing, China). HUVECs were cultured in DMEM containing 10% (v/v) FBS, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a humidified atmosphere of 5% CO2. When the cells had reached subconfluence, cells were passaged into 6-well culture dishes and the conditioned medium was replaced by the serum-free medium for 24 h. Then cells were treated with ox-LDL (100 mg/ml) for 24 h to induce cell injure[9]. For EPT, endothelial cells were prior exposed to EPT (0.1mg/ml) for 1 h, and then exposed to ox-LDL for 24 h in the presence of drugs. At the end of experiment, the cultured medium were collected for determinations of levels of ADMA, NO, MDA and TNF-❑❑ as well as activity of LDH, and cells were lysed for measure of DDAH activity.

Determination of endothelium-dependent vasodilator responses

The rats were anesthetized with sodium pentobarbital (60 mg/kg, i.p.). After blood samples were collected from artery, the thoracic aorta was rapidly isolated and cut into rings of 3 ~ 4 mm length. The rings were suspended horizontally between two stainless steel wires and mounted in a 5 ml organ chamber filled with warmed (37°C) and oxygenated (95% O_2 and 5% CO_2) Krebs' solution. The Krebs' solution had the following composition (mM): NaCl, 119.0; NaHCO3, 25.0; KCl, 4.7; KH2PO4, 1.2; MgSO4·7H2O, 1.2; CaCl2, 2.5; and Glucose, 11.0. One of ring ends was connected to a force transducer. The aortic ring was stretched with 2 g resting force and equilibrated for 60 min, and then precontracted with KCl (60 mM). After a maximal response to KCl was obtained, the rings were washed repeatedly with Krebs' solution and equilibrated again for 30 min. In order to measure vasodilator responses, rings were contracted with phenylephrine to 40% - 50% of their maximal contraction. After the contraction stabilized, an accumulative concentration-response curve to acetylcholine (3 × 10-9 - 10-6 M) was observed.

Determination of LDH activity

The activity of LDH in the conditioned medium, as an indicator of cell cytotoxicity, was measured spectrophotometrically using a commercially available assay kit.

Determination of nitrite/nitrate concentration

The level of nitric oxide in the conditioned medium and in the serum was determined indirectly as the content of nitrite and nitrate. The level of nitrite/nitrate was measured as previously described[10]. Briefly, nitrate was converted to nitrite with aspergillus nitrite reductase, and the total nitrite was measured with the Griess reagent. The absorbance was determined at 540 nm with a spectrophotometer.

Determination of ADMA concentration

The proteins in the conditioned medium and in the serum were removed using 5-sulfosalicylic

acid[11]. The concentration of ADMA was measured by high-performance liquid chromatography (HPLC) as described previously with some modification[12]. HPLC was carried out using a Shimadzu LC-6A liquid chromatograph with Shmadzu SCL-6A system controller and Shimadzu SIC-6A autosampler. O-Phthaldiadehyde adducts of methylated amino acids and internal standard ADMA produced by precolumn mixing were monitored using a model RF 530 fluorescence detector set at λ^{ex} = 338 and λ^{em} = 425 nm on a Resolve C18 column. Samples were eluted from the column using a linear gradient containing mobile phase A composed of 0.05 M (pH 6.8) sodium acetate-methanol-tetrahydrofuran (81: 18: 1 v:v:v) and mobile phase B composed of 0.05 mM sodium acetate-methanol-tetrahydrofuran (22: 77: 1 v:v:v) at a flow-rate of 1 ml/min.

DDAH activity assay

The activity of DDAH in endothelial cells was estimated by directly measuring the amount of ADMA metabolized by the enzyme (13). In an ice bath, cell lysates were divided into 2 groups, and ADMA was added (final concentration 500 ❑M). To inactivate DDAH, 30% sulfurosalicylic acid was immediately added to 1 experimental group. This group provided a baseline of 0% DDAH activity. The other lysate was incubated at 37°C for 2 h before the addition of 30% sulfurosalicylic acid. The ADMA level in each group was measured by HPLC as described above. The difference in ADMA concentration between two groups reflected the DDAH activity. For every experiment, DDAH activity of cells exposed to normal conditioned medium is defined as 100%, and DDAH activity in other conditions was expressed as percentages of the ADMA metabolized compared with the control.

Determination of TNF-❑❑ concentration

The level of TNF-α in the conditioned medium and in the serum was determined by radioimmunoassay kits using antisera raised against rat TNF-α, 125I-labeled TNF-α and rat TNF-α standard[14]. The standard curve for TNF-❑ measured by radioimmunoassay kits was linear from 0.3 mg/l to 24.3 mg/l; the detection limit was 0.3 mg/l.

Determination of MDA concentration

The concentration of thiobarbituric acid reactive substance in the conditioned medium and in the serum, reflecting the level of lipid peroxide, was measured spectrophotometrically as previously described[15] and expressed as the amount of MDA.

Statistic analysis

Results are expressed as means ± S.E.M. The data were analyzed by one-way ANOVA followed by the Newmann-Keuls-Student t test for multiple comparisons. The significance level was chosen as $P< 0.05$.

Results

Vasoconstrictor and vasodilator responses

Phenylephrine was added to increase smooth muscle tone in the rat aortic rings. Vasoconstrictor responses to phenylephrine (1 ❑M) were significantly increased in the rats treated with LDL. The tension was 1.47±0.09 and 1.07±0.06 g for LDL-treated group and control, respectively ($n = 8$, $P < 0.01$). As shown in Fig 1, in the presence of phenylephrine, ACh (3×10^{-9} -10^{-6} ❑M) caused a concentration-dependent relaxation in the isolated rat aorta, and treatment with LDL (4 mg/kg) for 48 h significantly decreased vasodilator responses to ACh. EPT (30 or 60 or 180 mg/kg) significantly attenuated the inhibition of vasodilator responses to ACh in the rats pretreated with LDL. EPT itself had no effect on vasodilator responses to acetylcholine (Figure 1).

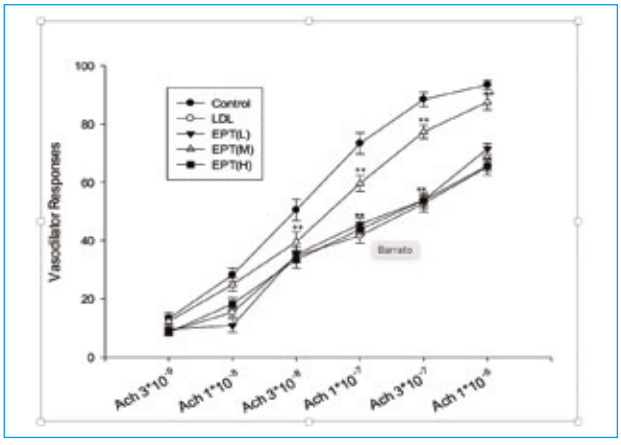

Fig. 1: Effect of EPT on vasodilator responses to acetylcholine in the isolated rat thoracic aorta in the rats pretreated with native LDL. Values are means±S.E.M. (n = 8). ***P<0.01 vs CON; +P<0.05, ++P<0.01 vs LDL.*

Concentrations of nitrite/nitrate

After treatment with LDL (4 mg/kg) for 48 h, serum concentrations of nitrite/nitrate were significantly decreased. EPT (4ml/kg) significantly inhibited the reduced concentration of nitrite/nitrate by LDL. The decreased level of nitrite/nitrate by LDL was also inhibited by pretreatment with vitamin E (100 mg/kg) (Figure 2).

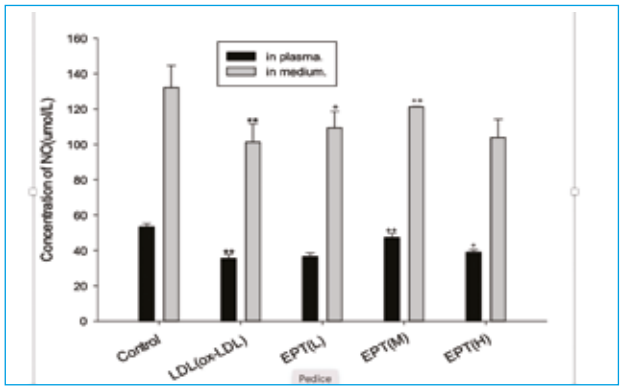

Fig. 2: Effects of EPT on concentrations of NO in the plasma and the conditioned medium. Values are means±S.E.M. (n =6-8).
***P<0.01 vs CON; +P<0.05, ++P<0.01 vs LDL or ox-LDL*

Exposure of endothelial cells to ox-LDL (100 μg/ml) for 24 h caused a significant decrease in concentration of nitrite/nitrate in the medium (P < 0.01). Treatment with EPT (0.1mg/ml) significantly attenuated the decreased level of nitrite/nitrate by ox-LDL (P < 0.01)(Figure 2).

Activity of LDH

Exposure of endothelial cells to ox-LDL (100 mg/ml) for 24 h caused a significant increase in the activity of LDH in the medium (P < 0.01). EPT (0.1 mg/ml) significantly inhibited the elevated activity of LDH by ox-LDL (P < 0.01) (Figure 3).

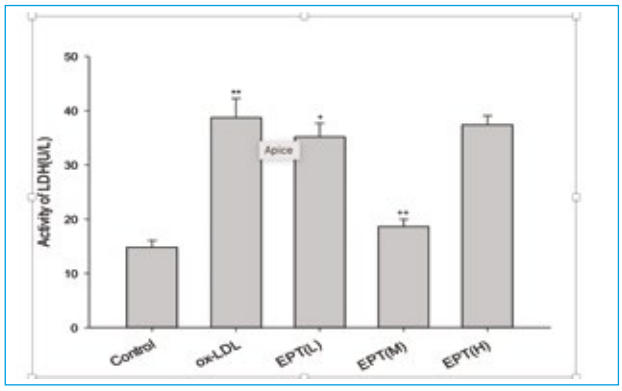

Fig. 3: Effect of EPT on activity of LDH in the conditioned medium. Endothelial cells were treated with ox-LDL (100 μg/ml) for 24 h. Values are means±S.E.M. (n = 6).
***P<0.01 vs CON; +P<0.05, ++P<0.01 vs ox-LDL.*

Concentrations of ADMA

Treatment with LDL (4 mg/kg) for 48 h significantly increased the concentration of ADMA in the plasma. EPT (4ml/kg) significantly inhibited the elevated concentration of ADMA by LDL (Figure 4).

Incubation of endothelial cells with ox-LDL (100 μg/ml) for 24 h significantly increased the level of ADMA in the medium (P < 0.01). Treatment with EPT (0.1mg/ml) significantly inhibited the elevated concentration of ADMA by ox-LDL (P < 0.01) (Figure 4).

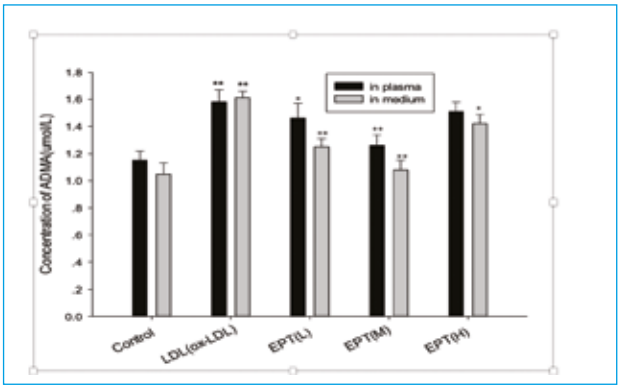

Fig. 4: Effects of EPT on concentrations of ADMA in the plasma and the conditioned medium. Values are means±S.E.M. (n =6-8).
***P<0.01 vs CON; +P<0.05, ++P<0.01 vs LDL or ox-LDL*

Activity of DDAH

After incubation with ox-LDL (100 μg/ml) for 24 h, the activity of DDAH in endothelial cells was significantly decreased. Treatment with EPT (0.1mg/ml) significantly attenuated the inhibition of endothelial DDAH activity by ox-LDL (P < 0.01)(Figure 5).

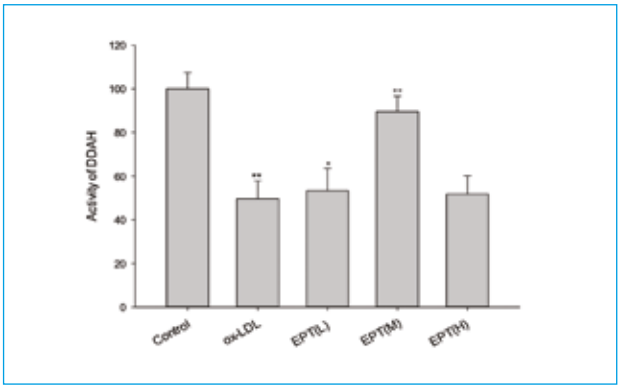

Fig. 5: Effect of EPT on activity of DDAH in the conditioned medium. Endothelial cells were treated with ox-LDL (100 μg/ml) for 24 h. Values are means±S.E.M. (n = 6).
***P<0.01 vs CON; +P<0.05, ++P<0.01 vs LDL.*

Concentration of MDA

After treatment with LDL (4 mg/kg) for 48 h, serum concentrations of MDA were significantly increased. EPT (20,60 or 180 mg/kg) significantly inhibited the elevated concentration of MDA by LDL (Figure 6).

Exposure of endothelial cells to ox-LDL (100 μg/ml) for 24 h significantly increased the concentration of MDA in the medium. EPT (0.1mg/

ml) significantly inhibited the elevated concentration of MDA by ox-LDL (Figure 6).

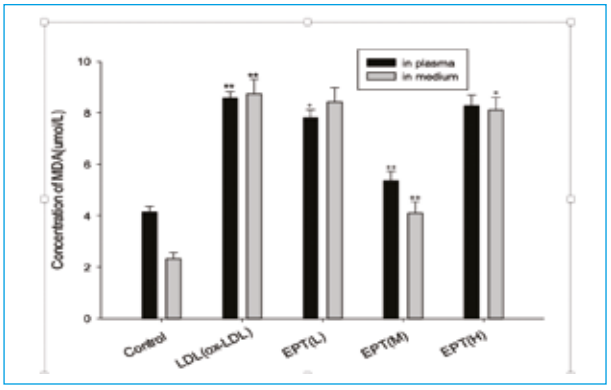

Fig. 6:Effects of EPT on concentrations of MDA in the plasma and the conditioned medium. Values are means±S.E.M. (n =6-8).
***P<0.01 vs CON; +P<0.05, ++P<0.01 vs LDL or ox-LDL.*

Concentrations of TNF-❑

After pretreatment with LDL (4 mg/kg) for 48 h, the serum concentration of TNF-❑❑was significantly increased. EPT (4 ml/kg) significantly inhibited the elevated concentration of TNF-❑ by LDL (Figure 7).

Culture of endothelial cells with ox-LDL (100 μg/ml) for 24 h caused a significant increase in the concentration of TNF-❑ in the medium. EPT (0.1mg/ml) significantly inhibited the elevated concentration of TNF-❑ by ox-LDL (Figure 7).

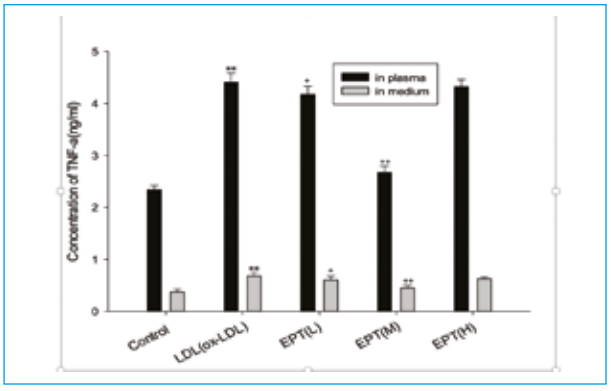

Fig. 7: Effect of EPT on activity of TNF-αin the plasma and the conditioned medium. Values are means±S.E.M. (n =6-8).
***P<0.01 vs CON; +P<0.05, ++P<0.01 vs LDL or ox-LDL.*

Discussion

The major findings of the present study were: (1) EPT significantly attenuated the impairment of endothelium-dependent vasodilatation in isolated rat aortic rings induced by a single injection of native LDL concomitantly with an elevation of NO release and a decrease in plasma level of ADMA; (2) EPT significantly attenuated ox-LDL-induced cell injury concomitantly with elevations of NO level and a decrease in ADMA level in the conditioned medium as well as an increase in activity of DDAH in endothelial cells.

Recently, much attention focused on the effects of ADMA, a major endogenous inhibitor of NOS, on endothelial dysfunction and atherogenesis. ADMA could competitively and nonselectively inhibit three isoforms of NOS activity and decrease NO production in vitro and in vivo. In isolated aortic rings and in vivo experiment, exogenous ADMA concentration-dependently caused vasoconstriction and decreased ACh-induced vasodilator responses[13]. Moreover, ADMA has been found to increase oxidative stress in endothelial cell, induce vascular inflammatory responses and accelerate formation of foam cells, and it is thought as a novel pro-atherogenic molecular[14].

It has been shown that the substantive accumulation of LDL in the arterial wall where it becomes ox-LDL, which impairs endothelial function in the hypercholesterolemic animals and patients, has been suggested to be a key event in early atherogenesis[15]. Previous observations have reported that plasma level of ADMA was markedly elevated associated with endothelial dysfunction in hypercholesterolemic animals and patients[14]. Furthermore, our recent works showed that a single injection of native LDL caused a rapid accumulation and oxidation of LDL in the arterial wall, which results in endothelial dysfunction concomitantly with an elevation of ADMA level[15].

Others have reported that ox-LDL also significantly increases levels of ADMA in cultured endothelial cells[16]. Increasing evidence shows that lower-ADMA therapy can significantly improve endothelial dysfunction and prevent progression of atherosclerosis in patients and animals with hypercholesterolemia or LDL-treated rats[17]. The conditionally semiessential amino acid EPT has been shown to possess endothelial protective and anti-atherogenic effects, such as inhibiting high glucose-induced apoptosis of endothelial cells and improving smoking-induced endothelial dysfunction[18].

In the present study, EPT at its pharmacological dose significantly attenuated the inhibition of endothelial function induced by LDL in vivo and prevented damage of endothelial cells in vitro, concomitantly with a decrease in ADMA levels and

an elevation of NO release. These results suggest that EPT can protect endothelial cells against ox-LDL-induced damage, which may involve reduction of ADMA level and increase in NO release.

It is known that ADMA is synthesized by protein arginine methyltransferases (PRMTs) utilizing S-adenosylmethionine as methyl group donor, and it is degraded by DDAH, an oxidant-sensitive enzyme that has sulfhydryl groups in structure[19]. ADMA and DDAH are widely distributed in tissues including endothelial cells (20). The lipid-induced deregulation of DDAH is thought to play an important role in the elevation of ADMA in hypercholesterolaemia[20]. It is revealed that the elevated ADMA level is attributed to the increased activity of PRMTs and the decreased activity of DDAH in cultured endothelial cells[21], and that a decrease in DDAH activity is believed to be related to oxidative stress[18]. Some antioxidants such as vitamin E, probucol and xanthones have been shown to attenuate the elevation of ADMA via increasing DDAH activity[22]. EPT has been shown to possess potent antioxidant actions, and it could inhibit lipid peroxidation and lower production of oxidant free radical[23]. In the present study, EPT, similar to vitamin E as a positive control drug, significantly decreased the level of ADMA concomitantly with an increased activity of DDAH and a reduction of lipid peroxidation (as shown by the decreased level of MDA). These observations support the notion that the decreased level of ADMA by EPT is related to the increased activity of DDAH by reduction of lipid peroxidation.

Another possibility responsible for EPT to reduce the level of ADMA is involved in some inflammatory cytokines, such as TNF-a. An increase in TNF-a level was observed in animals and patients with hypercholesterolaemia, which upregulates the expression of adhesion molecule and increases the adhesion of monocyte to endothelium[24]. Our recent work also showed that LDL or ox-LDL caused an increase in level of TNF-a both in vivo and in vitro[25]. It was reported that TNF-a elevated level of ADMA via decreasing the activity of DDAH in cultured endothelial cells[26], and some anti-inflammation drugs, such as aspirin and fenofibrate, decreased the elevated level of ADMA induced by LDL via reduction of TNF-α[27]. EPT as an anti-inflammation factor is recently described. It at high concentration can inhibit mRNA expression of TNF-❑ and decrease its release from some inflammatory cells[28].

Furthermore, our present results found that EPT also inhibited the release of TNF-a from endothelial cells induced by ox-LDL, which may contribute to the decreased plasma level of TNF-a in LDL-treated rats. Taken together, these results suggest that the reduction of ADMA by EPT may be related to the decreased TNF-a level.

In conclusion, the present study suggests that EPT protects endothelial dysfunction induced by LDL in vivo or by ox-LDL in endothelial cells, and the protective effect of EPT on the endothelium is related to decrease in ADMA level via improvement of DDAH activity by inhibition of lipid peroxidation and reduction of inflammatory cytokine TNF-a level. Therefore, our finding that the beneficial effect of EPT on the prevention of ox-LDL-induced endothelial dysfunction suggests that this amino acid may have important implications for the pharmacological manipulation of high-lipid-associated atherosclerosis.

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Corresponding Author:
Prof Tan Bin
Email: tanbin1661@126.com
(China)