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Effect of Ginsenosides on Malondialdehyde, Nitric Oxide and Endothelin-1 Production in Vascular Endothelial Cells Suffering from Lipid Peroxidation Injury

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Abstract: Ginsenosides are the main effective substance in *Panax ginseng* and have bioactivity to improve cardiovascular function. In this study, effect of ginsenosides on malondialdehyde (MDA), Nitric Oxide (NO) and endothelin-1 (ET-1) production in human vascular endothelial cells strain VEC304 treated with diamide was studied. The treatment of VEC304 with 0.01 μL $\cdot L^{-1}$ diamide significantly increased MDA production ($p<0.01$), significantly decreased NO production ($p<0.05$) and slightly increased ET-1 production in cells, indicating that diamide induced lipid Peroxidation injury for VEC304. While after VEC304 injured by lipid peroxidation were treated with ginsenosides, MDA production and ET-1 production in cells were decreased significantly ($p<0.01$) by 29.81 and 38.18%, respectively and NO production in cells was increased significantly ($p<0.01$) by 6.04 times. The results implied that *Panax ginseng* and ginsenosides work effectively on cardiovascular diseases probably by anti-oxidation and increasing NO production and decreasing ET-1 production in VEC.

Key words: Ginsenosides, lipid peroxidation, nitric oxide, endothelin-1, cardiovascular diseases

INTRODUCTION

Ginseng the root of medicinal plant: *Panax ginseng*, is precious Chinese traditional medicine. It contains many chemical active constituents (e.g., ginsenosides, polysacchoride, panaxynol, amino acid, trace elements) and has a variety of bioactivities such as improving cardiovascular function, anti-tumor, anti-aging and so on (Li and Zhang, 1997; Nakata *et al*., 1998; Bai *et al*., 2003).

Vascular Endothelial Cell (VEC) is monolayer cell locating under vascular endothelium and among tissues. It is the largest incretion gland (Lerman *et al*., 1991) and plays an important role in pathology and physiology of cardiovascular diseases by secreting vasoactive substances, which modulates vasomotor tone, stanches, inhibits thrombus formation and maintains normal function of vascular. Nitric Oxide (NO) is the important vasodilatation substance secreted by vascular endothelium, namely Endothelium-Derived Relaxing Factor (EDRF), which is the main factor modulating blood stream and vascular smooth muscle tension (Moncada *et al*., 1991). After NO is released from vascular endothelium, it permeates into vascellum to restrain smooth muscle cell (SMC), activates guanylate cyclase in SMC, up-regulates c-GMP production in SMC and then affects Ca^{2+}-Na^{+} channel activity in SMC membrane, increases Ca^{2+} concentration and leads to vascular relaxation (Radomski *et al*., 1990; Murad, 1994). Additionally, NO could inhibit vascular smooth muscle proliferation, prevent blood platelet from aggregation and inhibit thrombus formation (Radomski *et al*., 1987; Willoughby *et al*., 2003). Endothelin (ET), isolated and purified from the culture solution of porcine main artery endothelial cells firstly by Yangisawa in 1988 (Itoh *et al*., 1988), is a potent vasoconstrictive substance comprising 21 amino acid residues, which is endothelium-derived constricting factor (EDCF). So far, ET is the most strong and lasting vasoconstrictor. ET plays role in vasoconstriction and promoting smooth muscle proliferation mainly.

VEC dysfunction is triggering things of the occurrence and the development of diversified cardiovascular diseases (Stern *et al*., 1985; Gibbons, 1997). And lipid peroxidation injury is one of the main

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factors causing VEC dysfunction. Lipid peroxidation involves a series of radical reactions of covalent bonds in unsaturated fatty acid. Malondialdehyde (MDA) is known as a universal biomarker of lipid peroxidation and can be measured upon reaction with thiobarbituric acid (TBA) (Janero, 1990; Halliwell and Chirico, 1993).

In this study effect of ginsenosides on MDA, NO and ET-1 production in VEC304 suffering from lipid peroxidation injury induced by diamide, was studied to reveal the mechanism of ginsenosides working on cardiovascular diseases. Diamide with 0.01 $\mu L \cdot L^{-1}$ concentration in culture medium was adopted as the triggering reagent of radical and a sulfhydryl reagent, which is known to oxidize sulfhydryl groups to the disulfide form (Kosower *et al*., 1969) and is often used to model lipid peroxidation injury (Dafre and Reisenl, 1998).

MATERIALS AND METHODS

Materials: Ginsenosides with purity over 98% was a generous gift from Tecthmate Co. Beijing China. Diamide was purchased from Eastern Regent Co. Chongqing China; F-12 DMEM culture medium was purchased from Hyclone Co. America; Newborn Calf serum was purchased from Biotechnology Development Center. China; MDA assay kit was purchased from Nanjing Jiancheng Institution of Biological Engineering (Jiangsu, China); NO assay kit was purchased from Beyotime Biotechnology Co. (Jiangsu, China); ET-1 assay kit was purchased from Dongyakemei Biotechnology Development Center (Beijing, China).

Preparation of serum with ginsenosides: Two groups (blank serum group and ginsenosides serum group) of rabbits were used, each having three adult female Japanese flap-eared rabbits which were purchased from the third military surgeon university (Chongqing, China). Each rabbit in the blank serum group was administrated orally with 30 mL saline one time each day for three days in succession; and that in the ginsenosides serum groups with ginsenosides (100 $mg \cdot kg^{-1} \cdot day^{-1}$) instead. At 2 h after the last administration on the third day, the blood was obtained germ freely from main ventral artery of the rabbit. The sampled blood was allowed to stand at 25°C for about 5 h and centrifuged at 2500 $r \cdot min^{-1}$ for 20 min to separate the serum. The serum was filtrated with 0.22 μm cellulose acetate membrane for twice, calefied with 56°C water for 30 min and then stored at -20°C. And the ginsenosides concentration in serum is 69.5 $mg \cdot L^{-1}$ measured by applying colorimetry.

Cell culture: Human vascular endothelial cells strain VEC304 was purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China). VEC304 were stored in liquid nitrogen were restored to life and put into culture flask with DMEM culture medium, which include 10% newborn calf serum and then were incubated in 5% CO_2 culture tank under the condition of 37°C and 80% relative humidity. When cells came into logarithmic growth, they were prepared into cell suspend solution with culture medium. And then the concentration of cells was adjusted to about 1×10^5 mL^{-1}.

Experimental groups: Cells suspension aforementioned was inoculated into 96-well culture plate with 0.1 mL^{-1} each well. After incubated in 5% CO_2 culture tank for 24 h under the condition of 37°C and 80% relative humidity, the liquid supernatant was thrown away. Experiments were carried out in three groups of different procedures: (I) control group: the culture medium were thrown away and the culture medium containing 20% blank serum were added into culture system after cells were cultured normally for 4 h; (ii) model group: the culture medium containing 0.01 $\mu L \cdot L^{-1}$ diamide were previously added into culture system, after 4 h the culture medium containing diamide were thrown away and the culture medium containing 20% blank serum were added into culture system; (iii) ginsenosides group: the culture medium containing 0.01 $\mu L \cdot L^{-1}$ diamide were previously added into culture system, after 4 h the culture medium containing diamide were thrown away and the culture medium containing 20% ginsenosides serum were added into culture system. Each group had six parallel samples. MDA, NO and ET-1 production in VEC were detected for each group after cells were incubated for 24 h as above.

MDA content assay: From each well, 100 μL^{-1} incubated culture medium were collected and two samples were mixed into one. Then MDA production in cells was measured by applying thiobarbituric acid method and according to the indication on the MDA assay kit.

No content assay: In this experimental, Griess Method was adopted to detect NO with 50 μL^{-1} incubated culture medium from each well according to the indication on the NO assay kit, which was commercially available.

ET-1 content assay: Radioimmunoassay (RIA) method was used to measure the ET-1 production in cells with 100 μL^{-1} incubated culture medium from each well according to the indication on the ET-1 assay kit, which was commercially available.

Statistical analysis: All values are represented as the means±SD for six samples. The differences of datum in mean values were analyzed by the two-tail Student t-test by using Statistical Program for Social Sciences and a p-value of less than 0.05 or 0.01 was considered significant.

RESULTS AND DISCUSSION

MDA, NO and ET-1 production results are illustrated, respectively in Fig. 1, 2 and 3 for all the groups. In comparison with control group, NO production in VEC304 of model group decreased significantly ($p<0.05$) by 40.05%, MDA and ET-1 production in VEC304 of model group increased by 22.68 and 8.14%, respectively (Fig. 1) In comparison with model group, NO production in VEC304 of ginsenosides group increased significantly ($p<0.01$) by 6.04 times, MDA and ET-1 production in VEC304 of ginsenosides group decreased significantly ($p<0.01$) by 29.81 and 38.18%, respectively. (Fig. 2). In comparison with control group, NO production in VEC304 of ginsenosides group increased significantly ($p<0.01$) by 3.64 times, MDA and ET-1 production in VEC304 of ginsenosides group decreased significantly ($p<0.01$) by 13.42 and 33.15%, respectively (Fig. 3).

So far, over 30 kinds of ginsenosides have been purified from ginseng and were classified into the panaxadiol group (e.g., Rb1, Rb2, Rb3, Rc, Rd, Rg3, Rh2 and Rs1); the panaxatriol group (e.g., Re, Rf, Rg1, Rg2 and Rh1) and the oleanolic acid group (e.g., Ro) on the basis of the chemical structure of their sapogenins. Modern pharmacological studies have shown that ginsenosides were the main drug effective substance in ginseng and have many pharmacological activities on cardiovascular system such as blocking Ca^{2+} channels, dilatating vas, inhibiting blood platelet aggregation and clearing free radical and so on (Kim *et al.*, 1999; Yook *et al.*, 2002).

Once VEC is injured, it would result in VEC dysfunction and abnormity of active substances (e.g., NO, ET, thromboxane A2 TXA2, prostacyclin PGI2) secretion. The functional injury of VEC is known to induce VEC's secreting some adhesion molecules which could result in leukocyte or platelet adhering to VEC and going in clearance under VEC to form foam cells, which is

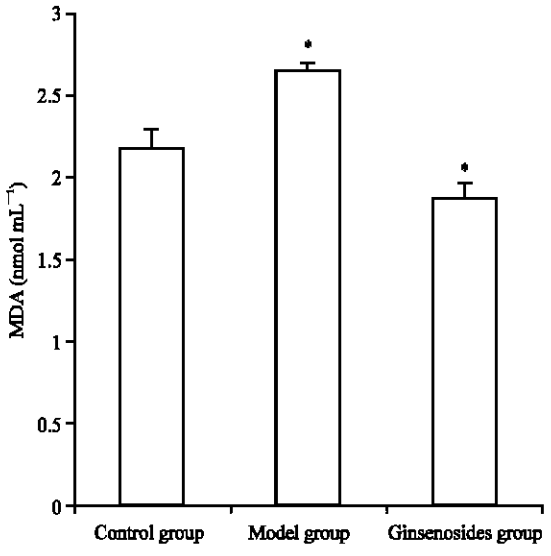

Fig. 1: MDA production in VEC304, *significantly different compared with model group, t-test, $p<0.01$,

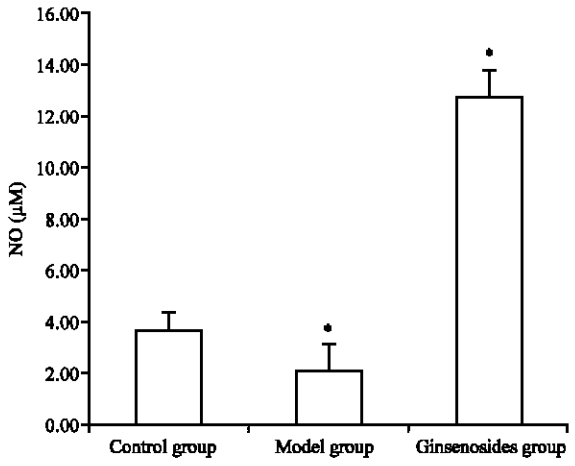

Fig. 2: NO production in VEC304 *significantly different compared with model group, t-test, $p<0.05$, *significantly different compared with control group, t-test, $p<0.01$

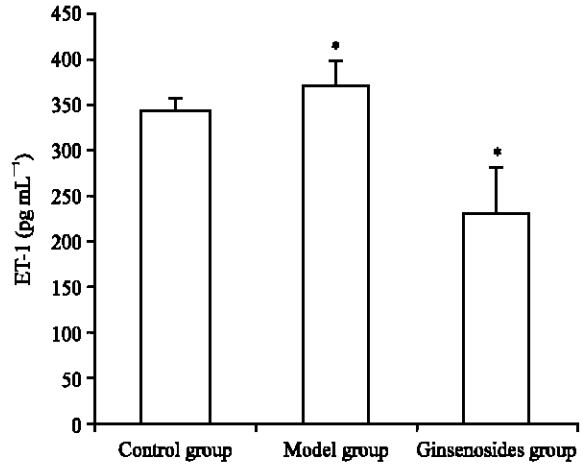

Fig. 3: ET-1 production in VEC304, *significantly different compared with model group, t-test, $p<0.01$

the triggering thing of atherosclerosis and atherosclerosis obliterans (Ross, 1993; Gibbons, 1997) and is also the key factor resulting in dysfunction of hemostasis and anti-thrombus (Gong and Chatterjee, 2003). When VEC are injured, NO production will decrease and ET-1 secretion will increase, which will result in the follows sequentially: vascular smooth muscle constriction, blood platelet aggregation, expression of leukocyte adhesion molecules and smooth muscle cells proliferation (Lerman *et al*., 1995; Deckert *et al*., 1998). A number of cardiovascular diseases (e.g., hypertension, atherosclerosis, hyperlipidemia, myocardial injury) are related to abnormity of NO and ET-1 production (Zolk *et al*., 1999; Duchman *et al*., 2000). Among the many factors causing VEC injury, lipid peroxidation injury is the main inducement (Hennig and Chow, 1988). As shown in Fig. 1, MDA production in cells treated with diamide (model group) increased significantly ($p<0.01$) compared with that of control group. MDA is a credible marker of lipid peroxidation and high level MDA showed that lipid peroxidation and oxidative stress was aggravated after cells were treated with diamide. Meanwhile NO production decreased significantly ($p<0.05$) and ET-1 production increased slightly in VEC304 (Fig. 2 and Fig. 3). So it could be concluded that VEC304 were submitted to lipid peroxidation injury after treated with 0.01 $\mu L \cdot L^{-1}$ diamide. While ginsenosides could up-regulate NO production and down-regulate MDA and ET-1 production in VEC304 submitted to lipid peroxidation injury significantly ($p<0.01$) by 6.04 times, 29.81 and 38.18%, respectively. Above results showed that ginsenosides could alleviate lipid peroxidation injury of VEC304 and regulate active substances (e.g., NO, ET) secretion in VEC304. This implied that ginseng and ginsenosides could work on cardiovascular diseases effectively by anti-oxidation and activating Nitric Oxide Synthases (NOS) to increase NO production in VEC, then decrease ET-1 production, dilate vas and inhibit blood platelet aggregation.

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