International Journal of Pharmacology

ISSN 1811-7775
Effect of Ginsenosides on Malondialdehyde, Nitric Oxide and Endothelin-1 Production in Vascular Endothelial Cells Suffering from Lipid Peroxidation Injury

Tan Jun, Zhu Liancai and Wang Bochu
1Key Lab for Biomechanics and Tissue Engineering Under the State Ministry of Education, Bioengineering College, Chongqing University, Chongqing, 400030, People’s Republic of China
2Department of Life Science and Chemistry, Chongqing Education College, Chongqing, 400067, People’s Republic of China

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 diame was studied. The treatment of VEC304 with 0.01 μM 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 is the root of medicinal plant: Panax ginseng, is precious Chinese traditional medicine. It contains many chemical active constituents (e.g., ginsenosides, polysaccharide, 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 excretion 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 vassellum to restrain smooth muscle cell (SMC), activates guanylate cyclase in SMC, up-regulates c-GMP production in SMC and then affects Ca2+-Na+ channel activity in SMC membrane, increases Ca2+ 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 (Itch 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
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 Reisent, 1998).

**MATERIALS AND METHODS**

**Materials:** Ginsenosides with purity over 98% was a generous gift from Technmate 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 surgery university (Chongqing, China). Each rabbit in the blank serum group was administrated orally with 30 mL saline every day for three days; 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 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 \( \mu m \) cellulose acetate membrane for twice, calibrated 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% \( \text{CO}_2 \) 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 \( \times 10^6 \) 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% \( \text{CO}_2 \) 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 \( \mu L^{-1} \) 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:** No in this experimental, Griess Method was adopted to detect NO with 50 \( \mu L^{-1} \) incubated culture medium from each well according to the indication on the NO assay kit, which was commercially available.

**ET-1 content assay:** Radiommunucassay (RIA) method was used to measure the ET-1 production in cells with 100 \( \mu L^{-1} \) 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 mean±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, Re, Rd, Rg3, Rg2 and Rg1); 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 have the main drug effective substance in ginseng and have many pharmacological activities on cardiovascular system such as blocking Ca⁺⁺ channels, dilating 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 abnormality 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

![Graph 1: MDA production in VEC304. *significantly different compared with model group, t-test, p<0.01.](image)

![Graph 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.](image)

![Graph 3: ET-1 production in VEC304. *significantly different compared with model group, t-test, p<0.01.](image)
the triggering thing of atherosclerosis and atherosclerosis obliterans (Roes, 1993; Gibbons, 1997) and is also the key factor resulting in dysfunction of hemostasis and anti-thrombus (Gong and Chatterjee, 2003). When VECs are injured, NO production will decrease and ET-1 secretion will increase, which will result in the following sequentially: vascular smooth muscle contraction, 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 infarction) are related to abnormality 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 μL -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.

ACKNOWLEDGMENTS

This research was financially supported by the Science and Technology Project of Education Commission of Chongqing (Grant No. KJ051503). We are grateful to the Chongqing Education College Science Research Project for support of this research.

REFERENCES


