Anti-Oxidative Stress and Anti-Apoptosis Effects of He Ying An Xin-Formula

He Wei1#, An Yating2#, Li Yongmin1*, Zhang Haixia1, Wang Lei1, Chen Xilong1 and Su Zhiyuan1

1Hebei North University, Zhangjiakou, China
2Tianjin Academy of Traditional Chinese Medicine Affiliated Hospital, Tianjin, China

Abstract

Objective: To investigate anti-oxidative protective effects and potential anti-apoptosis mechanisms of He Ying An Xin-Formula (HYAX-F).

Method: VSMC were incubated by H$_2$O$_2$ (200µmol/L) for 2 h as oxidative stress control group, same dosage of H$_2$O$_2$ was administrated 2 hours after treatment with HYAX-F (100µg/mL, 50µg/mL, 25µg/mL) as therapeutic group and incubated for another 24 hours, cell supernatant content of GSH and MDA were measured. Sub-cutaneous injection in back with D-galactose (125 mg/kg) to establish ageing rat model. Control group: D-galactose 125 mg/kg, therapeutic groups: HYAX-F 500 mg/kg+D-galactose 125 mg/kg, HYAX-F 250 mg/kg+D-galactose 125 mg/kg, HYAX-F 125 mg/kg+D-galactose 125 mg/kg, normal group and young group. The RT-PCR technique was used to measure the expression of relevant genes, such as tumor necrosis factor- alpha (TNF-α) and B-cell lymphoma-2(Bcl-2) in the tissues of brain and liver.

Results: Compared with the control group, HYAX-F can significantly enhance the content of GSH (P<0.05) and decrease the content of MDA (P<0.01). Also HYAX-F can significantly decrease TNF-α expression and increase Bcl-2 expression in rats brain and liver tissues (P<0.05, P<0.01).

Conclusion: HYAX-F has greatly protective effects on anti-oxidative of rat VSMC and anti-apoptosis of ageing rats.

Keywords: He Ying An Xin-Formula (HYAX-F); Vascular smooth muscle cells; Anti-oxidative stress; Ageing rats; Anti-apoptosis

Introduction

He Ying An Xin-formula (HYAX-F) originated from "Nan Jing", which is a classical treatment principle to treat cardiovascular diseases. Clinically HYAX-F was used to treat treatment for chronic heart failure and ischemic heart disease, previous studies have demonstrated that it has the effect of improving cardiac function, slowing down the cardiac muscle remodelling, improving the neuroendocrine and hemodynamics [1], however the anti-oxidative stress and anti-apoptosis effects on ageing rats were not clear. In this study, we studied the effects of the HYAX-F on oxidative stress in rat vascular smooth muscle cells, and its mechanism of the anti-apoptosis effect of ageing rats.

Materials and Method

HYAX-formula extraction

HYAX-Formula was composed of the following herbs: Cinnamomi ramulus, Paconiae radix alba, Poria, Salviae miltiorrhizae radix et rhizoma, Panaci quinquefolii radix, Polygonati odorati rhizoma, Salviae miltiorrhizae radix et ramulus, Paeoniae radix alba, Poria, Cinnamomi ramulus, Paconiae radix alba, Poria. All herbs (500 g) were extracted with 70% EtOH (3000 ml) for 1.5 h, then collected extractions of three times, evaporating the solvent to get residue 93.5 g (yield of 18.7%). These extracts were stored at 4℃ before use.

Cells culture and treatment

Rat vascular smooth muscle cells(VSMC) (Beijing dingguochangsheng Biotechnology Co., Ltd.) were maintained in high-glucose Dulbecco’s modified Eagle’s medium(Hyclone scientific, USA) supplemented with 10% calf serum(Difco International, Netherlands) at 37℃ in a sterile 5% CO$_2$ incubator. When VSMC cultured to 80%-90% confluent monolayer cells, trysin-EDTA (Difco International, Netherlands) was used to dissociation cells and sub-cultured as a ratio of 1:3. Prior to treatment, VSMC were plated into 48-well plates (Costar, USA) at a density of 6 × 10$^4$ cells/ml. VSMC were induced by 200 µmol/L H$_2$O$_2$ for 2 hours as control group [2], while HYAX-Formula was treated as different dosages(100 µg/mL, 50 µg/mL, 25 µg/mL) for 24 hours as treatment groups before induced by 200 µmol/L H$_2$O$_2$ for 2 hours, then cultural supernatants were collected for GSH and MDA detections.

Measurement of GSH and MDA

VSMCs in 48-well plates were induced as previously described. The amount of supernatant GSH and MDA was determined with the GSH and MDA kits (Nanjing Jiancheng Bioengineering Institute, China).

Animals

The experiment was carried out in 40 ageing rats and 8 youth rats (male, weighing 220-240 g, Vital River Laboratory Animal Technology Co., Ltd.) was administered 2 hours after treatment with HYAX-F (100µg/mL, 50µg/mL, 25µg/mL) as therapeutic group and incubated for another 24 hours as treatment group, while HYAX-Formula was treated as different dosages(100 µg/mL, 50 µg/mL, 25 µg/mL) for 24 hours as treatment groups before induced by 200 µmol/L H$_2$O$_2$ for 2 hours, then cultural supernatants were collected for GSH and MDA detections.

*Corresponding author: Li Yongmin, Hebei North University, 11 Zuanishi South Road, Jingkai District, Hebei, 075000, China, Tel: 0086-313-4029228; E-mail: li-yongmin2001@sina.com

*Co-Author: An Yating and He wei contributed equally to this work.

Received January 18, 2016; Accepted February 06, 2016; Published February 12, 2016

Citation: Wei H, Yating A, Yongmin L, Haixia Z, Lei W, et al (2016) Anti-Oxidative Stress and Anti-Apoptosis Effects of He Ying An Xin-Formula. J Pharmacogn Nat Prod 2: 115. doi:10.4172/2472-0992.1000115

Copyright: © 2016 Wei H, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Co. Ltd., Beijing China) acclimated for 1 week before the experiments. All animals were fed with a standard diet and drink ad libitum, and adapted to the experimental conditions at 22 ± 2°C, humidity 60 ± 5% with a fixed 12-h artificial light period. The experimental mice were overseen and all protocols were approved by the Committee of the Animal Use and Care Committee of HeBei North University.

After 1 week, 40 ageing rats were divided into 5 groups, except normal ageing rats group (group A), other ageing rats were subcutaneous injected 125 mg/kg D- galactose to induce aged rats model [3], such as control ageing rats group (group B), high dosage of HYAX-F group (500 mg/kg+125 mg/kg D- galactose, group D), medium dosage of HYAX-F group (250 mg/kg+125 kg D-galactose, group E), and low dosage of HYAX-F group (125 mg/kg+125 mg/kg D-galactose, group F), 8 youth rats as normal group (group C). HYAX-F extractions suspended in 5% acacia solution (500 mg/kg, 250 mg/kg and 125 mg/kg) and vehicle (5% acacia solution) were administrated orally to ageing rats. After administration of 42 days, brain and liver were collected under anesthesia and immediately frozen in liquid N2 or orally to ageing rats. After administration of 42 days, brain and liver were extracted suspended in 5% acacia solution (500 mg/kg, 250 mg/kg and 125 mg/kg) and vehicle (5% acacia solution) were administrated orally to ageing rats. After administration of 42 days, brain and liver were collected under anesthesia and immediately frozen in liquid N2.

**RT-PCR for relative genes expression in ageing rat brain and liver**

Total RNA was extracted from tissue using TRIzol (Invitrogen life technologies, USA) according to the manufacturer’s protocol. Samples (1 µg of RNA) were reverse-transcribed using a first-strand cDNA synthesis kit (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, USA) according to the manufacturer’s instructions. Briefly, the total reaction volume was 20 µL with the reaction incubated as follows in an PE-480 HYBAID (Perkin Elmer, USA): 10 min at 25°C, 120 min at 37°C, 5 min at 85°C, and hold at 4°C.

**RT-PCR measurement of RNA expression**

Real-time PCR was performed with an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, USA) using Power SYBR® Green PCR master mix (Applied Biosystems, USA) according to the protocols provided by the manufacturer. Briefly, PCR was performed in a final volume of 20 µl including 10 ng sample cDNA, 5 µM specific forward and reverse primers, and 10 µl Power SYBR® green PCR Master Mix. PCR reactions consisted of an initial denaturing cycle at 95°C for 10 min, followed by 40 amplification cycles: 15 s at 95°C and 1 min at 60°C. The primers used were as Table 1. Results were presented as levels of expression relative to those of controls after normalization to GADPH using the 2-∆∆CT methods. Analysis was carried out in triplicates.

**Statistical analysis**

Values are expressed as mean ± S.D. All the grouped data were statistically performed with SPSS 11.0. Significant differences between means were evaluated by one-way analysis of variance (ANOVA) and Tukey’s Studentized range tests were used for post hoc evaluations. P<0.05 was considered to indicate statistical significance.

**Results**

**Effects of HYAX-F on secretion of GSH and MDA of VSMCs in supernatant**

VSMCs were treated with the dosage of 100 mg/ml and 25 mg/ml of HYAX-F can promote GSH content in supernatant compared with control group (P<0.05). Moreover, each dosage of HYAX-F can significantly decrease MDA content in supernatant compared with control group (P<0.01). In control group, cells were merely induced by H2O2 to apoptosis, showed lower GSH content and higher MDA content in supernatant compared with normal group (Figure 1).

**Effects of HYAX-F on expression of TNF-α and Bcl-2 genes in brain and liver of ageing rats**

HYAX-F can significantly down-regulate TNF-α expression in brain and liver of ageing rats compared with control group (P<0.05, P<0.01). High-dosage and low-dosage of HYAX-F can significantly up-regulate Bcl-2 expression in brain and liver of ageing rats compared with control group as well (P<0.05, P<0.01) (Figures 2 and 3).

**Discussion**

Vascular ageing has been implicated in the progression of age-related cardiovascular disorders. Epidemiological discover ageing is associated with an increased prevalence of cardiovascular disease, vascular smooth muscle cells (VSMC) comprise the major arterial cell population, and changes in VSMC contribute to alterations in vascular remodelling and cell signalling. Cellular senescence is a permanent
non-replicating state characterized by growth arrest, increased oxidative stress, telomere and mitochondrial dysfunctions. In cultured cells, critical DNA damage triggered by a variety of chemical agents and stresses including oxidative stress and activation of oncogenes induces rapid “stress-induced premature senescence”. VSMC senescence in atherosclerotic plaques is a characteristic feature of atherosclerosis and is associated with increased levels of reactive oxygen species (ROS). ROS increases intracellular (DNA) damage and ultimately can elicit the onset of apoptosis or the induction of cellular senescence. H$_2$O$_2$ can stimulates NAD(P)H oxidase to generate O$_2$· and develops a thickened intima consisting of infiltrating vascular smooth muscle cells(VSMCs) and resulting in local inflammation [6,7]. Ageing increases oxidative stress and inflammation [8]. Apoptosis is regulated by apoptosis modulating proteins, which are divided into two major categories: pro-apoptotic proteins and anti-apoptotic proteins. Apoptosis is the result of the loss of balance between these two kinds of proteins. TNF-α is known to be one of the cytokines that can induce apoptosis by its receptor pathway, which can initiate the cascade reaction related to apoptosis and nerve injury. TNF-α transfer into intracellular by combine with its receptor, uptake by target cell lysosomal resulted in reduced lysosomal stability and leakage of enzyme, causing cell lysis, it can also change metabolism of glucose in target cells, causes a decrease in intracellular pH, leading to cell death [9,10]. Bcl-2 plays an important role in the regulation of apoptosis as well. Except grow factors caused apoptosis, ROS is the main cause of cell death, while Bcl-2 acts to inhibit apoptosis by suppressing ROS production [11]. In this study, the gene expression of TNF-α and Bcl-2 were used to evaluate the anti-apoptosis effect of HYAX-F. Results showed that compared with D-galactose induced ageing senescent rat, gene expression of TNF-α both in liver and brain magnificently down-regulated. Whereas HYAX-F (500 mg/kg and 125 mg/kg) could significantly up-regulate Bcl-2 gene expression both in liver and brain.

Conclusion

HYAX-F can resist H$_2$O$_2$ caused VSMC from oxidative stress damage, and regulate gene expression of TNF-α and Bcl-2 in ageing senescent rat liver and brain to anti-apoptosis. We also partly confirmed the mechanism of HYAX-F regulated cardiovascular disease by anti-oxidative stress and anti-apoptosis.

Acknowledgement

This research was supported by Program for innovative talents cultivation of HeBei North University (CXRC1319).

References

1. Li YMWB, Xin GQ, Bo AH, Wu YS, Zhou N (2009) Effects of Heyinganxin Fang on Myocardial Apoptosis in Chronic Congestive Heart Failure Rats. LiShiZhen Medicine And Materia Media Research 20: 300-301.
2. Cao YJ, Zhang YM, Qi JP, Liu R, Zhang H, et al. (2015) Ferulic acid inhibits H2O2-induced oxidative stress and inflammation in rat vascular smooth muscle cells via inhibition of the NADPH oxidase and NF-κB pathway. Int Immunopharmacol 28: 1018-1025.
3. Hadzi-Petrushev N, Stojkovski V, Mitrov D, Mladenov M (2015) D-galactose induced changes in enzymatic antioxidant status in rats of different ages. Physiol Res 64: 61-70.
4. Zhao L, Li AQ, Zhou TF, Zhang MQ, Qin XM (2014) Exendin-4 alleviates...
angiotensin II-induced senescence in vascular smooth muscle cells by inhibiting Rac1 activation via a cAMP/PKA-dependent pathway. Am J Physiol Cell Physiol 307: C1130-1141.

5. Li M, Fukagawa NK (2010) Age-related changes in redox signaling and VSMC function. Antioxid Redox Signal 12: 641-655.

6. Monk BA, George SJ (2015) The Effect of Ageing on Vascular Smooth Muscle Cell Behaviour--A Mini-Review. Gerontology 61: 416-426.

7. Wang M, Monticone RE, Lakatta EG (2014) Proinflammation of aging central arteries: a mini-review. Gerontology 60: 519-529.

8. Martins PN, Markmann JF (2012) Age-related differences in hepatic ischemia/reperfusion: gene activation, liver injury, and protective effect of melatonin. J Surg Res 185: e19-21.

9. Shahzad M, Small DM, Morais C, Wojcikowski K, Shabbir A, et al. (2015) Protection against oxidative stress-induced apoptosis in kidney epithelium by Angelica and Astragalus. J Ethnopharmacol.

10. Yan X, Xuerui (2015) Cyanidin-3-O-glucoside Induces Apoptosis and Inhibits Migration of Tumor Necrosis Factor-alpha-Treated Rat Aortic Smooth Muscle Cells. Cardiovasc Toxicol 1-9.

11. Anusha S, Mohan CD, Ananda H, Baburajeev CP, Rangappa S, et al. (2016) Adamantyl-tethered-biphenylic compounds induce apoptosis in cancer cells by targeting Bcl homologs. Bioorg Med Chem Lett 26: 1056-1060.