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# Inhibition of aortic intimal hyperplasia and cell cycle protein and extracellular matrix protein expressions by BuYang HuanWu Decoction

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ABSTRACT

*Aim of the study:* The inhibitive effect of BuYang HuanWu Decoction (BYHWD) and its major components on vascular intimal hyperplasia and the expressions of cell cycle protein and extracellular matrix protein.
*Materials and methods:* Sprague-Dawley rats were randomly divided into sham-operated, control, alkaloid, glycoside, BYHWD and atorvastatin groups. Rat aorta intima in all groups were injured by insesion of domestic balloon catheter into the aortae except sham-operated rats. Drugs were administrated orally from the second day after vascular injury and continued for 14 days. The injured segments of aortae were collected on the sixteenth day after operation to observe the morphological changes of vascular structure and to examine the expressions of proteins in vascular cells associated with cell cycle including proliferating cell nuclear antigen(PCNA), cyclinD$_1$ and cyclinE, and extracellular matrix(ECM) proteins including collagen I (Col-I) and fibronectin (FN), further to discover the involved biologically active substances and the potential mechanisms.
*Results:* Alkaloid and glycosid isolated from BYHWD were more effective than BYHWD in the inhibition of intimal hyperplasia and the expressions of PCNA, cyclinD$_1$, cyclinE, Col-I and FN, suggesting that alkaloid and glycoside may be the main components of BYHWD responsible for the observed inhibition of excessive hyperplasia of vascular intima.
*Conclusions:* The mechanism associated with the anti-hyperplasia activity of BYHWD and its effective components may be related to the blockage of cell cycles of VSMC, and the inhibition of the ECM protein synthesis, even the increased degradation of ECM proteins.


## 1. Introduction

Restenosis incidence rate after percutaneous transluminal coronary angioplasty (PTCA) remains as high as 30–40% in coronary heart disease (Williams et al., 2000), which seriously affected the long-term curative effects of the therapy. The basic pathological process of restenosis after PTCA involves the tearing and denudation of coronary intima, platelet accumulation and adhesion to injury sites, the proliferation and migration of VSMC from the media to the intima, and synthesis of ECM. The excessive proliferation of VSMC and synthesis increases of ECM were the key factors in restenosis occurred after PTCA, and therefore inhibiting the proliferation of VSMC and promoting the clearance of ECM became important measures to attenuate the restenosis happened after PTCA.

BYHWD, a formula of TCM, had been used to prevent and treat the ischemic cardio-cerebral vascular diseases since Ming Dynasty in China. Our previous study (Yang et al., 2006; Tang et al., 2007; Wu et al., 2008a,b) showed BYHWD and its major components, alkaloids and glycosides, were able to inhibit the formation of arterial thrombosis, and even the aortic vascular intimal hyperplasia after balloon catheter injury in rats. In this study the anti-hyperplasia effects of BYHWD and alkaloids and glycosides extracted from BYHWD after aortic intimal injury by balloon catheter, and the potential mechanisms involved from the perspective of cell cycle regulation and the ECM protein expression were investigated. This study provided

*Abbreviations:* BYHWD, BuYang HuanWu Decoction; CDK, cyclin-dependent kinase; Col-I, collagenI; ECM, extracellular matrix; FN, fibronectin; HPLC, high performance liquid chromatograph; PCNA, proliferating cell nuclear antigen; PTCA, percutaneous transluminal coronary angioplasty; TCM, traditional Chinese medicine; VSMC, vascular smooth muscle cell.

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**Table 1**
The location, collection and voucher specimen of materials.

| Materials | Original plants or animals | Location | Collection date | Voucher specimen |
|---|---|---|---|---|
| *Radix Astragali* | *Astragalus membranaceus* (Fisch.) Bunge, Leguminosae | Fanzhi, Shanxi | October 2004 | Zhou R.B. 1874 |
| *Radix Paeoniae Rubra* | *Paeonia lactiflora* Pall., Paeoniaceae | Ganzi, Sichuan | September 2004 | Zhou R.B. 1853 |
| *Rhizoma Chiuanxiong* | *Ligusticum chuanxiong* Hort., Umbelliferae | Dujiangyan, Sichuan | May 2004 | Zhou R.B. 1668 |
| *Radix Angelicae Sinensis* | *Angelica sinensis* (Oliv.) Diels, Umbelliferae | Minxian, Gansu | September 2004 | Zhou R.B. 1862 |
| *Flos Carthami* | *Carthamus tincforius* L., Compositae | Yanjin, Henan | June 2004 | Zhou R.B. 1790 |
| *Semen Persicae* | *Prunus persica* (L.) Batsch, Rosaceae | Beichuan, Sichuan | July 2004 | Zhou R.B. 1823 |
| *Lumbricus* | *Pheretima aspergillum* (E. Perrier), Megascolecidae | Guangdong | October 2004 | Zhou R.B. 1875 |

useful information for developing medicine used in the treatment of vascular hyperplasia diseases from TCM.

## 2. Materials

### 2.1. Experimental animals

Male adult Sprague–Dawley rats, weighed from 300 to 350 g, were provided by Experimental Animal Center, Epidemic Prevention Station of Hunan Province, PRC. Animals were allowed to drink and eat freely, caged in an environment of 18–20 °C and 65–70% relative humidity.

### 2.2. Balloon catheters

Balloon catheters were bought from Tianjin Zhongtuo Latex Technology Development Limited Company. Catheters were retrofited by the needle, No. 51/2. A domestic 2.0F balloon catheter was assembled with balloon and catheter in the same way as described before (Wu et al., 2008a,b).

### 2.3. Composition identification, vouchers specimens, active ingredients detection and the positive control drug option of BYHWD

#### 2.3.1. Composition identification and vouchers specimens

BYHWD was composed of 60 g *Radix Astragali*, 9 g *Radix Paeoniae Rubra*, 6 g *Rhizoma Chiuanxiong*, 9 g *Radix Angelicae Sinensis*, 9 g *Flos Carthami*, 9 g *Semen Persicae*, and 9 g *Lumbricus*. The location and time of collection and the voucher specimens of the plants were listed in Table 1. All the materials were identified by TCM professionals. The voucher specimens were deposited at the Drugs Museum of the School of Chinese Pharmacy, Hunan University of TCM, Changsha, Hunan, China.

#### 2.3.2. Standard preparation and control drug

The chemicals used as standards including astragaloside IV (batch number:0781-200311, purity ≥98%), paeoniflorin (batch number: 110736-200424, purity ≥98%), amygdalin (batch number: 110820-200403, purity ≥98%), and tetramethylpyrazine hydrochloride (batch number: 110817-200305, purity ≥98%) were bought from Biological Products Analysis Bureau of Ministry of Public Health of China.

#### 2.3.3. The basis on choosing the active ingredients

*Radix astragali* mainly contained the components such as saponins, flavone, polysaccharide and amino acid. Saponins component included astragaloside I, II, III, IV, V, VI, VII, VIII, etc. (State Administration of Traditional Chinese Medicine, 1999). Astragaloside IV was the marking composition of *Radix astragali* for quality identification. The Pharmacopoeia of the People's Republic of China, in 2005 edition, regulated that the content of astragaloside IV in *Radix astragali* could not be less than 0.040% by HPLC determination. In addition, astragaloside IV inhibited vessel contraction through blocking calcium influx and intracellular calcium release. The endothelium-dependent vessel dilation of astragaloside IV was attributed mainly to the endothelium-dependent NO-cGMP pathway (Zhang et al., 2007). Astragaloside IV can increase the fibrinolytic potential of cultured human umbilical vein endothelial cells (HUVECs) not only by upregulating the expression of tissue-type plasminogen activator, but also by downregulating the expression of plasminogen activator inhibitor-1 (Zhang et al., 1997).

*Radix Paeoniae Rubra* mainly contained the effective glycoside components as paeoniflorin, oxypaeoniflorin, benzoylpaeoniflorin, etc. (State Administration of Traditional Chinese Medicine, 1999). Paeoniflorin was the marking composition of *Radix Paeoniae Rubra* for quality identification. The Pharmacopoeia of the People's Republic of China, in 2005 edition, regulated that the content of paeoniflorin in *Radix Paeoniae Rubra* could not be less than 1.80% by HPLC determination. According to the references, VSMC proliferation and the expressions of platelet-derived growth factor, c-myc mRNA were inhibited by Qixue Bingzhi Recioe with paeoniflorin as the main component. The effects were related to the inhibition of fluorescent intensity of intracellular $Ca^{2+}$ and the activities of mitogen-activated protein kinase and protein kinase C (Ji et al., 2005, 2006).

*Semen Persicae* mainly contained amygdalin, prunasin, sterol, organic acid, etc. (State Administration of Traditional Chinese Medicine, 1999). Amygdalin was the marking composition of *Semen Persicae* for quality identification. It is reported that Silso-San-Gami (SSG) containing *Semen Persicae* has inhibitory effects on the development of atheromatous plaques in spontaneous familial hypercholesterolemia model rabbits. The antioxidative effects of SSG on LDL appear to be the source of the beneficial effects observed in this study (Park et al., 2004).

*Rhizomaligusticichuanxiong* mainly contained tetramethylpyrazine, perlolyrine, ligustilide, ferulic acid, protocatechuic acid, etc. (State Administration of Traditional Chinese Medicine, 1999). Tetramethylpyrazine was the marking composition of *Rhizomaligusticichuanxiong* for quality identification. It is also reported that angiotensin II could activate nuclear factor-kappaB expression and decrease the expression of bone morphogenetic protein-2, which were probably the participation mechanism of atherosclerosis. Tetramethylpyrazine played its important roles in anti-atherosclerosis probably by inhibiting the above courses (Ren et al., 2007). Tetramethylpyrazine at a lower concentration is shown to inhibit phosphoinositide breakdown and thromboxane $A_2$ formation, and at a higher concentration, it leads to the inhibition of platelet aggregation through binding to the glycoprotein IIb/IIIa complex (Sheu et al., 1997).

Therefore, the determinations of astragaloside IV, paeoniflorin, amygdalin and tetramethylpyrazine were selected for quality control of the extracts in our study.

#### 2.3.4. The option of positive control drug

Atorvastatin packaged by Pfizer Pharmaceuticals Ltd. (USA) was purchased from Godecke GmbH (Germany). Atorvastatin as the positive control drug was suspended in distilled water (concentration: 1 mg $mL^{-1}$) before use.