JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

Effects and mechanisms of total *Panax notoginseng* saponins on proliferation of vascular smooth muscle cells with plasma pharmacology method

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Keywords
cyclinD1; cyclin-dependent kinase 4; mitogen-activated protein kinase; total Panax notoginseng saponin; vascular smooth muscle cell

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Received February 24, 2011
Accepted September 22, 2011

doi: 10.1111/j.2042-7158.2011.01379.x

Abstract

Objectives Total *Panax notoginseng* saponin (TPNS) is extracted from *Panax notoginseng*. Our previous studies suggested that TPNS could inhibit intimal hyperplasia. This study discussed the impact of TPNS on the proliferation of vascular smooth muscle cells (VSMCs) and revealed the associated mechanisms through cell cycle-related factors and extracellular regulated protein kinase (ERK) signal transduction pathway.

Methods A VSMC proliferation model induced by platelet-derived growth factor (PDGF) was established to observe the effects of rat drug-containing plasma on VSMC proliferation.

Key findings After being stimulated by PDGF, the proliferating cell nuclear antigen (PCNA) and c-fos content increased, while up-regulation of cyclinD1, cyclin-dependent kinase-4 (CDK4) and down-regulation of p21 protein were observed. These changes were inhibited by atorvastatin and TSPN drug-containing plasma, and the inhibitive activity in both groups was not significant. Furthermore, both atorvastatin and TSPN could obviously inhibit the activation of PDGF-induced P-ERK1/2 and increase the content of MKP-1, there were also no significant differences.

Conclusions These results suggested that atorvastatin and TPNS could inhibit VSMC proliferation by inhibiting the activation of ERK signalling pathway.

Introduction

Because of the complex chemical composition and metabolic processes of Traditional Chinese Medicine (TCM), authenticity and reliability of experiments *in vitro* are affected when crude extracts are added into an experimental system directly. Serum pharmacological method is an approach to performing in-vitro experiments by taking animals' serum after administering herbal extracts to them for a certain time.[1] This method excludes the effects of crude extracts on in-vitro experiments directly, and reflects more closely the actual process in the body. Although the serum pharmacological method is a great improvement in the pharmacological study of TCM, its limitations have also been noted increasingly. During serum preparation, the activated enzymes involved in coagulation may degrade some components of TCM entering the blood. Meanwhile, the endogenous bioactive substances may be changed in the coagulation process. Therefore the efficacy of substances in drug-containing serum can not accurately reflect the active ingredients of TCM, so the study of Chinese medicine *in vitro* by serum pharmacological method may lead to wrong conclusions. Unlike serum, plasma as a liquid part of blood may conserve most components of TCM, so the study of Chinese medicine *in vitro* with drug-containing plasma could accurately reflect the true process of TCM *in vivo*. An experiment using drug-containing plasma *in vitro* is named 'plasma pharmacological method'. Fingerprint analysis with high-performance liquid chromatography (HPLC)-mass spectrometry (MS) showed that drug-containing serum lost most polar components of TCM, while drug-containing plasma retained them.[2] Experiments using VSMCs cultured *in vitro* indicated that drug-containing plasma could reflect better the role of TCM.[3] So the plasma pharmacological method may minimize the interference of factors activated in coagulation and may be a useful means of research for TCM.

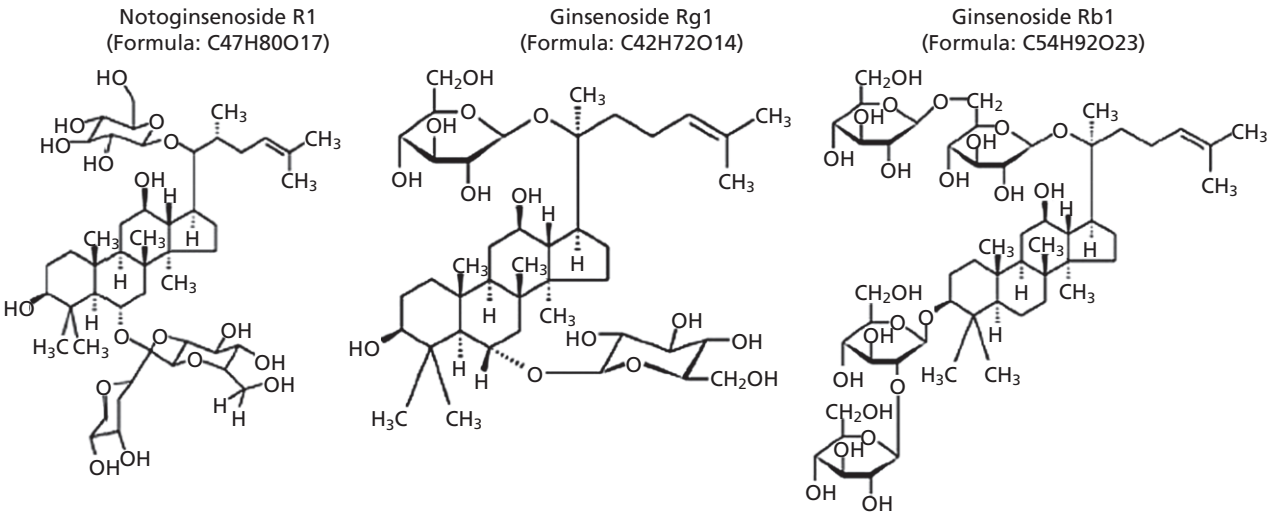

Figure 1 Chemical structures of the main effective components of total *Panax notoginseng* saponin.

The VSMC is one of the major cellular components that maintains vascular tension and functions. Study has proved that when the intima has been injured, the abnormal proliferation of VSMCs is an important factor that induces atherosclerosis, a vascular proliferative disease.[4] Therefore it is crucial to understand the molecular mechanism of VSMC proliferation and search for suitable medicines for the prevention and treatment of vascular proliferative disease, such as atherosclerosis and restenosis after percutaneous coronary interventions.

Total *Panax notoginseng* saponin (TPNS) is the main pharmacologically active ingredient of *Panax notoginseng* with a content reaching 8–12%, and is made up of ginsenoside Rg1, ginsenoside Rb1, notoginsenoside R1, etc. (Figure 1). Our previous studies showed that TPNS could inhibit intimal hyperplasia and resist VSMC proliferation after balloon injury in rats, but the mechanism is not clear.[5] So the aim of this study is to investigate the mechanism of TPNS inhibiting VSMC proliferation via cell cycle regulation and mitogen-activated protein kinase (MAPK) signal transduction with a platelet-derived growth factor (PDGF)-induced proliferation model and plasma pharmacology method. Meanwhile, we will not only verify the feasibility of plasma pharmacology, but also clarify the mechanism of action of TPNS and provide experimental evidence for its application.

Materials and Methods

Reagents

Dulbecco's modified Eagle's medium (DMEM medium) and trypsin (1:250) were obtained from Gibco Co., Ltd (New York, USA). DMEM medium was prepared with ultra-pure water, bacteria were filtered and removed, and then it was preserved at −20°C. Fetal bovine serum (FBS) was purchased from Sijiqing reagent Co., Ltd (Hangzhou, PRC), complement was inactivated for 30 min at 56°C, and separately packed after being filtered and bacteria removed. MAPK/ERK kinase specific inhibitor PD098059 was provided by Sigma Co., Ltd (St Louis, USA). RIPA cell lysis buffer (containing 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 0.1% SDS) and phosphatase inhibitor were provided by Dingguo Co., Ltd (Beijing, PRC).

Proliferating cell nuclear antigen (PCNA) antibody, c-fos antibody, cyclinD1 antibody, cyclin-dependent kinase inhibitor P21 antibody, phosphor-extracellular regulated protein kinases1/2 (P-ERK1/2) antibody (phosphorylation sites tyr 204) and mitogen-activated protein kinase phosphatase-1 (MKP-1) antibody, were first antibodies, and were mouse anti-rat monoclonal antibodies provided by Santa Cruz Biotechnology Inc (Santa Cruz, USA). Cyclin-dependent kinase 4 (CDK4) antibody, β-actin antibody, were first antibodies and were mouse anti-rat polyclonal antibodies provided by Bios Co., Ltd (Beijing, PRC). The second antibody labelled by fluorescein isothiocyanate (FITC) was goat anti-mouse to assay PCNA, c-fos, cyclinD1, P21, CDK4 antibody. The second antibody labelled by horseradish peroxidase (HRP) was rabbit anti-mouse to assay P-ERK1/2, MKP-1, β-actin antibody; these second antibodies were all provided by Amersham Co., Ltd (Amersham, UK). ECL Western Blotting kit was provided by Dingguo Co., Ltd (Beijing, PRC).

Experimental animals

Male adult Sprague–Dawley rats, 120–150 g, were provided by Experimental Animal Center, Epidemic Prevention

Station of Hunan Province, PRC (Certificate of Conformity: No. 20-010). The experimental protocol was approved by the Institutional Animal Care and Ethical Committee at the Hunan University of TCM. All animals used were handled in accordance with Guidance Suggestions for the Care and Use of Laboratory Animals (The Ministry of Science and Technology of PRC, 2006). Rats were allowed to drink and eat freely, caged under an environment of 18 ~ 20°C and 65~70% relative humidity with a 12-h light–dark cycle.

Drugs and content analysis

TPNS was extracted from the main root or rhizome of the Araliaceae plant, *Panax notoginseng* (Burk.) F.H.Chen. TPNS used in this study was purchased from Guangxi Wuzhou Pharmaceutical (Group) Co., Ltd (Guangxi PRC). The content was determined by HPLC analysis (Type 2487; Breeze work station in Waters Company, USA). The characteristic chromatography peaks of notoginsenoside R1, ginsenoside Rb1 and ginsenoside Rg1 could be seen clearly, with the content being: ginsenosides Rg1 50.4%, ginsenosides Rb1 30.9% and notoginsenoside R1 12.5%, through the chromatographic peak area of standard preparation and the sample (Figure 2). Atorvastatin (specifications: 10 mg/tablet) was purchased from Godecke GmbH (Visselhovede, Germany) and packaged by Pfizer pharmaceuticals Co., Ltd (batch number: 080117; New York, USA), which was made into a suspension by distilled water (concentration: 2 mg/ml) for use.

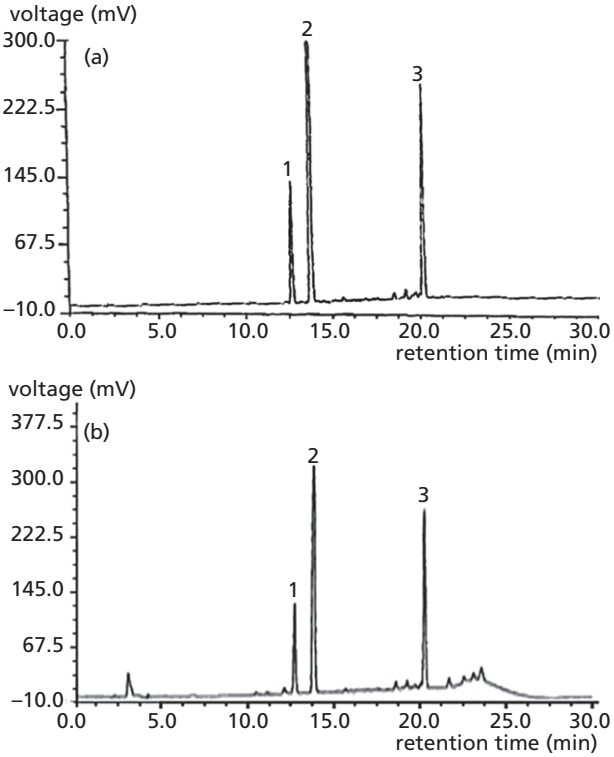

Figure 2 The chromatogram of notoginsenoside R1, ginsenoside Rb1 and ginsenoside Rg1 of TPNS. 1. Notoginsenoside R1 2. Ginsenoside Rg1 3. Ginsenoside Rb1. (a) Notoginsenoside R1, Ginsenoside Rb1 and Ginsenoside Rg1 standard substance (b) TPNS sample.

Culture and identification of vascular smooth muscle cells

VSMCs were cultured and passaged with the explant method.[6] The α-smooth muscle actin (SMα-actin) expression in VSMCs was determined by immunocytochemistry. The positive lots of SMα-actin could be seen in VSMC cytoplasm. Cells from passage 4–6 were used for the following experiments.

Preparation of drug-containing plasma

The drug-containing plasma was prepared according to the method described in the literature.[7] Rats were randomly divided into blank control, TPNS (200 mg/kg) and atorvastatin (20 mg/kg) groups. Each group consisted of 10–12 rats. The rats in the blank control group were fed with distilled water (10 ml/kg) and the rats in other groups were fed with the respective drugs. The rats were administered with water or drug at 0900 h and 1600 h each day. After seven consecutive gavages, the rats were anaesthetized by peritoneal injection of 10% chloralhydratein 2 h after the last medicine administration. Blood was drawn from the common carotid artery, anti-coagulated with 1.5% EDTA-Na_2 (blood : anticoagulant = 9 : 1) and centrifuged at 4°C 3000 rpm for 15 min. The plasma in the same group was pooled, filtered and stored at −70°C.

Determination of vascular smooth muscle cell proliferation and cell cycle related protein

The previous studies[3] showed that the half-inhibitory concentration (IC50) of atorvastatin drug-containing plasma and TPNS drug-containing plasma were both 13% ± 3%, which can significantly inhibit platelet-derived growth factor-bb (PDGF-BB) induced VSMC proliferation, increase the numbers of G_0/G_1 phase cells, and decrease G_2/M phase and S phase cells at a final concentration of 10%. So the final concentration of blank plasma and drug-containing plasma was 10%. The cultured cells were divided into plasma-free control group, PDGF-induced group, blank control plasma group, TSPN drug-containing plasma group and atorvastatin drug-containing plasma group. VSMCs were cultured in 96-well culture plates followed by 24 h culture in serum-free DMEM medium to synchronize cells in G_0 phase. The experiment included a plasma-free control group, PDGF-induced

group, blank control plasma group, TSPN drug-containing plasma group and atorvastatin drug-containing plasma group. The plasma-free control group had 200 μl culture medium added per well. For the PDGF-induced group, 180 μl culture solution was added in each well and 20 μl of PDGF-BB was then added to each well to get a final concentration of 10 ng/ml. The following experiments were performed after 24 h culture and repeated five times for each group. The contents of cell cycle related proteins P21, cyclinD1 and CDK4 and proliferation related proteins PCNA and c-fos were detected by FACS analysis as follows: 1 ml of 1×10^6/ml cell suspension mixed with 1 ml of 80% ethanol and set at −20°C for 2 h. The cells were washed with phosphate-buffered saline (PBS) and placed in an ice bath for 5 min after addition of 0.25% Triton X-100. Twenty microlitres of first antibody (1 : 100) was added into the cell suspension and the suspension was kept at 4°C overnight in the dark, after being washed with PBS. FITC-labelled goat anti-mouse polyclonal antibody (l : 40) was then added and incubated at 37°C for 30 min. One millilitre of PBS containing Propidium iodide (10 g/L) and 0.1% RNase A were added and incubated at 37°C for another 30 min in the dark. A FACSCalibur (Becton Dickinson) cytometer with excitation wavelength of 506 nm was used to detect the protein content. The average mean fluorescence intensity (MFI) was used to reflect the relative amount of detected protein content.

Assay of vascular smooth muscle cell P-ERK1/2 and MKP-1

P-ERK1/2 and MKP-1 contents were detected with Western-blot. Cultured cells were divided into: plasma-free control group, PDGF-induced group (terminal concentration 10 ng/ml), PD098059 group, 10% blank control plasma group, 10% TSPN drug-containing plasma group and 10% atorvastatin drug-containing plasma group. Serum-free medium was used to culture the cells for 24 h and synchronize growth in the G_0 phase, then PD098059 (terminal concentration 50 μM) was added to the PD098059 group. The corresponding drug-containing plasma (terminal concentration 10%) was added to other groups. Three hours later, PDGF-BB (terminal concentration: 10 ng/L) was added to all groups for 24 h-culture. The procedure was repeated five times for each group. First 1×10^6 VSMCs were detached by RIPA cell lysis buffer, and their protein concentration was detected by bicinchonininc acid (BCA) method. After the protein sample was boiled and denatured for 5 min, electrophoresis separation was carried out using 10% SDS-PAGE. Electrophoresed proteins were wetly transferred to polyvinylidene fluoride membrane, the membrane was then incubated with anti-P-ERK1/2, monoclonal antibody (1 : 1500), polyclonal antibody of β-actin (1 : 1000), MKP-1 monoclonal antibody (1 : 1500) at room temperature and dyed with enhanced chemiluminescence (ECL) after membrane being washed. Development and exposure was carried out using X-ray film which was placed in the image analysis system of Gel Doc2000 to determine the integral optical density (IOD) of objective band, taking β-actin as an internal reference and taking the ratio of the IOD values of protein objective band and the IOD values of β-actin protein objective band as relative content of target protein.

Statistical analysis

All values were expressed as mean ± SD. The mean comparison among each group was analysed with one-way analysis of variance if the data showed normal distribution and homogeneity of variance. Log transformation was used in the analysis if the data did not accord with variance homogeneity. The homogeneity square deviation between every two groups was tested by LSD test. $P < 0.05$ was considered statistically significant.

Results

The impact of each drug-containing plasma on vascular smooth muscle cell proliferation and cell cycle-related protein content

The contents of cyclinD1, CDK4, PCNA and c-fos in VSMCs of the PDGF group were significantly increased (all $P < 0.01$), while the content of P21 protein was significantly decreased ($P < 0.01$) compared with the plasma-free control group. The contents of cyclinD1, CDK4, PCNA and c-fos in the blank-plasma group were also significantly higher than those in the plasma-free control group (all $P < 0.01$), but were slightly lower than those of the PDGF-induced group (all $P < 0.05$). The P21 content in the blank-plasma group was also significantly lower than that in the plasma-free control group ($P < 0.01$), but was slightly higher than that in the PDGF-induced group ($P < 0.05$). The TPNS-plasma and atorvastatin-plasma groups showed significantly decreased contents of cyclinD1, CDK4, PCNA and c-fos ($P < 0.05$), but increased content of P21 compared with the blank-plasma group ($P < 0.05$). There were higher cyclinD1, CDK4, PCNA and c-fos contents (all $P < 0.05$), but lower P21 content ($P < 0.05$) in drug-containing groups compared with the plasma-free control group. There was no significant difference among the drug-containing plasma groups ($P > 0.05$) (Table 1).

Effect of each drug-containing plasma on the content of P-ERK1/2 and MKP-1

P-ERK1/2 includes Tyr-204 phosphorylated ERK1 and ERK2 (MW 42 KDa and 44 KDa, respectively). The p-ERK1/2

Table 1 The comparison of each group on PDGF-induced vascular smooth muscle cell cycle-related protein content

Group	N	CyclinD1	CDK4	P21	PCNA	c-fos
Plasma-free control	3	22.12 ± 0.38	13.01 ± 0.33	20.88 ± 0.52	40.02 ± 0.12	22.88 ± 0.26
PDGF-induced	3	39.97 ± 0.21△	30.66 ± 0.15△	9.21 ± 0.22△	62.05 ± 0.11△	39.38 ± 0.17△
Blank control plasma	3	36.04 ± 0.18△	26.98 ± 0.25△	13.22 ± 0.33△☆	57.88 ± 0.08△☆	36.77 ± 0.12△☆
Atorvastatin-containing plasma	3	24.73 ± 0.11△△	18.32 ± 0.14△△*	18.15 ± 0.32△△*	43.08 ± 0.33△△*	24.34 ± 0.22△△*
TSPN-containing plasma	3	25.01 ± 0.25△△	17.38 ± 0.14△△*	16.28 ± 0.31△△*	45.58 ± 0.21△△*	25.02 ± 0.25△△*

Data are presented as $\bar{x} \pm s$, $n = 3$. △$P < 0.01$, △△$P < 0.05$ vs plasma-free control group, ☆ $P < 0.05$ vs PDGF-induced group, *$P < 0.05$ vs blank control plasma group.

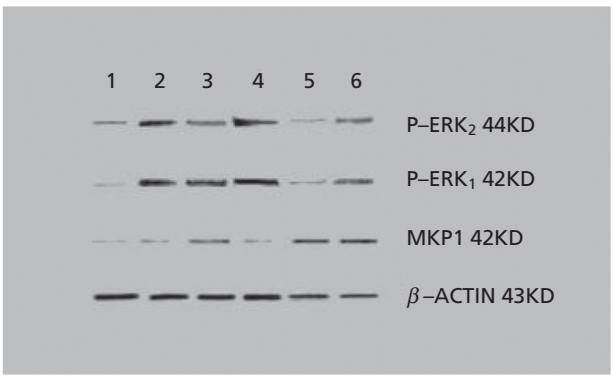

Figure 3 Western blotting analysis of P-ERK1/2 and MKP-1 proteins. 1. Plasma-free control group. 2. PDGF-induced group. 3. PD098059 group. 4. Blank control plasma group. 5. TSPN-containing plasma group. 6. Atorvastatin-containing plasma group.

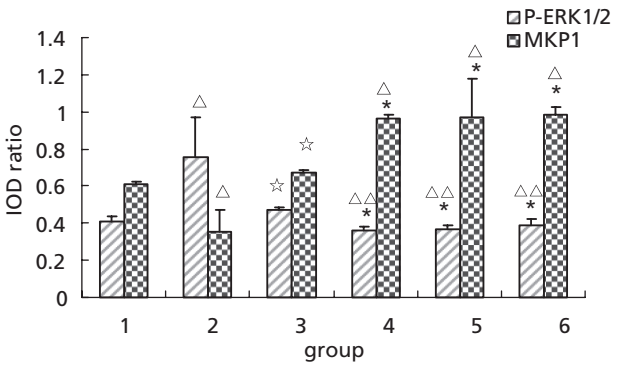

Figure 4 The comparison of each group on PDGF-induced VSMC MPAK signal transduction pathway-related protein content ($\bar{x} \pm s$, $n = 3$). 1. Plasma-free control group. 2. PDGF-induced group. 3. Blank control plasma group. 4. PD098059 group. 5. TSPN-containing plasma group. 6. Atorvastatin-containing plasma group. △$P < 0.01$, △△$P < 0.05$ vs plasma-free control group, ☆$P < 0.05$ vs PDGF-induced group, *$P < 0.05$ vs blank control plasma group.

content in the PDGF-induced group was significantly increased ($P < 0.01$) compared with that in the plasma-free control group. PD098059 was shown to inhibit the content of P-ERK1/2 stimulated by PDGF ($P < 0.05$). The p-ERK1/2 content in the blank control plasma group was lower than that in the PDGF-induced group, too ($P < 0.05$). Compared with the blank-plasma group and plasma-free group, the p-ERK1/2 contents of TPNS-plasma and atorvastatin-plasma group were significant reduced (all $P < 0.05$), and there was no significant difference among TPNS, PD098059 and atorvastatin groups (Figures 3 and 4).

The MKP-1 content in the PDGF-induced group was markedly reduced ($P < 0.01$) compared with the content in the plasma-free control group. PD098059 could inhibit the reduction of MKP-1 content induced by PDGF ($P < 0.05$). The MKP-1 content in the blank control plasma group was higher than that in the PDGF-induced group ($P < 0.05$). The MKP-1 contents in all drug-containing groups were similar to that in the PD098059 group, but were significantly higher than in the blank-plasma group (all $P < 0.05$) and plasma-free control group ($P < 0.01$). The difference in MKP-1 content among each drug-containing group was not significant ($P > 0.05$) (Figures 3 and 4).

Discussion

Growth factors act on the corresponding receptors of the VSMC membrane, activate intracellular signalling pathways and result in intranuclear gene expression and VSMC proliferation. PDGF-BB was shown to stimulate DNA synthesis, division and proliferation in a variety of cells including VSMCs, fibroblasts and spongiocytes.[8] In this study, PDGF-BB was used to stimulate VSMC proliferation as a model.

PCNA is a cell-proliferation-related nuclear protein involved in DNA synthesis and is only synthesized in proliferating cells. PCNA is a sensitive marker of cells in proliferation. Previous studies by others showed that the content of PCNA was directly proportional with the degree of cell proliferation.[9] Proto-oncogene c-fos is involved in the process of cell growth and differentiation.[10] The activations of c-myc and c-fos genes initiate VSMC proliferation. The content of c-fos in cells may reflect the status of VSMC proliferation. Therefore, the contents of PCNA and c-fos were used to reflect VSMC proliferation in this study.

This study shows that PCNA and c-fos increased after being stimulated by PDGF, which enhanced VSMC proliferation. Compared with the PDGF group, the blank plasma group had reduced contents of PCNA and c-fos. These changes may be caused by EDTA-Na_2 in the plasma. The atorvastatin drug-containing plasma was shown to reduce PCNA and c-fos contents, which suggested that atorvastatin could inhibit PDGF-induced VSMC proliferation. These results were consistent with earlier reports that atorvastatin can inhibit PDGF-induced VSMC proliferation.[11] Similarly, TPNS-plasma can also inhibit the increase in PCNA and c-fos contents, which suggested that TPNS could inhibit PDGF-induced VSMC proliferation. It is possible that the anti-proliferative effect of TPNS was the main mechanism that delayed the occurrence of restenosis, and TPNS had the same effect with atorvastatin in inhibiting the VSMC proliferation.

Cell proliferation depends on cell cycle progression being orderly initiated by stimulating extracellular signals. The cell cycle is successively composed of G_1, S, G_2 and M phases. It has two check-points to determine whether proliferation continues or whether static status is entered: one is the G_1-S check-point at the beginning of DNA synthesis and the G_2-M check-point at the beginning of mitosis. The former seems to be the more important. The G_1-S check-point is mainly regulated by a series of cyclins and cyclin-dependent kinase (CDK), which are predominantly cyclinD/CDK4, CDK6, cyclin E/CDK2, etc.[12] CyclinD and cyclin E are two key regulatory enzymes in cell proliferation and play an important role in the progression from G_0 to G_1 phase and from G_1 to S phase, respectively, in the cell cycle.[13] CyclinD and cyclin E play a critical role in promoting VSMC proliferation via regulating the transformation of cells from differentiation to dedifferentiation. CyclinD includes D1, D2 and D3, and has a role similar to that of sensor of growth factor, which connects extracellular signals with the progression of the cell cycle and is the active assistant factor of CDK. Injection of cyclinD1 antibody into the normal fibroblast in G_1 phase was shown to prevent cells from entering into S phase, which indicates that cyclinD is necessary for the progression from the late phase of G_1 to S phase.[14] P21 and P27 proteins, members of the cyclin-dependent kinase inhibitor (CKI) family, inhibit a variety of cyclin and CDK in the G_1-S check-point and cause cells to stagnate in G_0-G_1 phase. The decreased contents of P21 and P27 proteins allow VSMCs to enter into S phase through G_1 phase to complete the process of cell proliferation. Previous research has shown that P21 and P27 in blood vessels of atherosclerotic lesions are low and exhibits a negative correlation with VSMC proliferation.[15] Thus, the contents of cyclinD1 and CDK4 were measured as positive regulatory factors of VSMC cell cycle in this study, while the content of P21 protein was measured as a negative regulatory factor of the VSMC cell cycle.

The results of this experiment showed that the contents of cyclinD1 and CDK4 of cultured VSMCs increased and the P21 protein content decreased after PDGF stimulation, suggesting that the activity of these factors promoted cell cycle conversion. Blank control plasma gently reduced the contents of cyclinD1 and CDK4, and increased the content of P21, which could be connected with EDTA-Na_2. Atorvastatin drug-containing plasma weakened the changes of VSMC cyclinD1, CDK4 and P21 protein induced by PDGF stimulation, which suggests that the inhibitive activity of atorvastatin on the transformation of cell cycle may be related to reducing the contents of cyclinD1 and CDK4 and increasing P21. TSPN drug-containing plasma could also inhibit the enhancement of VSMC cyclinD1 and CDK4 and the reduction of P21. All of above indicated that TSPN might inhibit the PDGF-induced VSMC proliferation by preventing the enhancement of cyclinD1 and CDK4 and the reduction of P21.

The effect of growth factors role in corresponding receptors can introduce nucleus through multiple signalling pathways. In all signalling pathways, MAPK is the most essential. MAPK is composed of a group of serine threonine protein kinases involved in the process of signal transduction from the cell surface to the intracellular nucleus. MAPK is located at the convergence and common pathway of many signal transduction pathways involved in cell proliferation.[16] MAPK in mammalian cells mainly includes three sub-tribes, namely, ERK, c-jun N-terminal kinase (JNK) and P38 MAPK. ERK is the main signalling pathway for cell proliferation. VSMC proliferation initiated by growth factors and other cellular factors primarily is ERK-mediated.[17] The ERK phosphorylation happens after growth factors influences cells, then the phosphorylated ERK can make gene expression to promote cell proliferation, and leads to cell proliferation. Many experiments showed that ERK activation could be induced by balloon injury.[18] Some studies have shown that antisense oligonucleotide could reduce the activity of ERK1 and ERK2 and inhibit VSMC proliferation.[19] Another important pathway in cells is the mitogen-activated protein kinase phosphatases (MKPs). MKP-1 in the MKP family can inactivate MAPK through dephosphorylation at serine/threonine.[20] It has been reported that MKP-1 mRNA levels in rat carotid artery began to fall just two days after injury by balloon catheter and reached its lowest level five days later. So the reduction of MKP-1 may be directly related to the proliferation of VSMCs in the artery injured by balloon catheter.[21]

This study shows that the stimulation of VSMCs by PDGF initiated enhanced cell proliferative activity, increased p-ERK1/2 content and reduced MKP-1 content after 24 h, which indicated that PDGF might activate the ERK signal pathway and decrease the inhibition of MKP-1 on ERK, and thereby contribute to VSMC proliferation. PD098059, an antagonist for MAPK and ERK kinase (MEK), can inhibit the activation of ERK1/2 and enhance the content of MKP-1. Blank plasma slightly inhibited ERK1/2 activation and ameliorated the reduction of PDGF-induced MKP-1 content. These effects

of blank plasma may be the result of partial deprivation of calcium from culture medium by EDTA-Na_2 in the plasma. Atorvastatin-plasma significantly inhibited the activation of PDGF-induced ERK and enhanced the content of MKP-1, which suggests that atorvastatin may inhibit the transcription, synthesis of factors promoting cell proliferation through the inhibition of ERK activation, thereby inhibiting VSMC proliferation. TPNS-plasma inhibited the activation of PDGF-induced ERK and promoted the MKP-1 the contents like PD098059 and atorvastatin. It is possible that TPNS can inhibit the proliferation of VSMCs through the same mechanisms. However, whether atorvastatin or TPNS can inhibit PDGF-induced VSMC proliferation through inhibiting ERK upstream kinase activation, or they directly inhibit ERK activation, or they inhibit the activation of ERK through activating MKP-1 remains a question that needs to be further studied in the future.

Conclusions

The plasma pharmacology method is a feasible way of evaluating the activity of TCM. TPNS could inhibit VSMC proliferation via inhibiting the activation of ERK signalling pathway.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This study was supported by a grant from the National Natural Science Foundation of China (No. 30572301).

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