· Original article ·

Effect of Buddleia flavonoids drug-containing plasma on the expression of STAT1 phosphoprotein in lacrimal gland epithelial cells *in vitro*

Fang Wang[1], *Qing-Hua Peng*[2], *Xiao-Lei Yao*[1], *Quan-Long Wu*[2], *Dian Li*[2]

Foundation items: National Natural Science Foundation of China(No. 30772824); Natural Science Foundation of Hunan Province of China (No. 07JJ3049); Doctor-Subject Site Special-Purpose Foundation of High-School of China (No. 200805410004); Scientific Research Foundation of Hunan Provincial Science and Technology Department of China(No. 2009FJ3001); Graduational Innovate Foundation of Hunan Province in 2008 of China

[1]Department of Ophthalmology, Second Affiliated Hospital of Guiyang University of Traditional Chinese Medicine, Guiyang 550003, Guizhou Province, China
[2]Key Disciplines of Chinese Medicine Ophthalmology, the First Affiliated Hospital of Hunan University of Traditional Chinese Medicine, Changsha 410007, Hunan Province, China
Correspondence to: Qing-Hua Peng. Key Disciplines of Chinese Medicine Ophthalmology, the First Affiliated Hospital of Hunan University of Traditional Chinese Medicine, Changsha 410007, Hunan Province, China. pqhz_520@163. com

Received:2009-11-23 Accepted:2010-01-05

Abstract

• AIM: To explore the effect of Buddleia flavonoids drug-containing plasma and androgen receptor(AR) blocker on the expression of STAT1 phosphoprotein.

• METHODS: *In vitro* lacrimal gland epithelial cells were cultivated with H_2O_2 to establish the dry eye apoptosis state. Blank plasma group, Buddleia officinalis plasma total flavonoids interfere with drug-containing group, and the intervention group of testosterone propionate were set. The expressions of STAT1 phosphoprotein of each group were observed by Western blot and AR blocker flutamide was used to explore the intended androgen effect of Buddleia flavonoids.

• RESULTS: After the intervention of drug-containing plasma, the expression of STAT1 Phosphoprotein in Buddleja officinalis drug-containing plasma intervention group (0. 353 ± 0. 494) and testosterone propionate intervention group(0.502 ± 0.036) were enhanced and the differences between the two groups were significant (P < 0. 01). After using the AR blocker in all groups, the expression of STAT1 phosphoprotein in each group (0.268 ± 0. 061, 0. 283 ± 0. 106, 0. 213 ± 0. 071) had no difference.

• CONCLUSION: Buddleja officinalis drug-containing plasma total flavonoids can promote the expression of STAT1 phosphorylation.

• KEYWORDS: dry eye; lacrimal gland epithelial cells; culture; STAT1 phosphoprotein; androgen receptor; flutamide
DOI:10.3969/j. issn. 1672-5123. 2010. 01. 002

Wang F, Peng QH, Yao XL, Wu QL, Li D. Effect of Buddleia flavonoids drug-containing plasma on the expression of STAT1 phosphoprotein in lacrimal gland epithelial cells *in vitro*. *Int J Ophthalmol(Guoji Yanke Zazhi)* 2010;10(1):5-8

INTRODUCTION

Dry eye syndrome is consindered as a result of abnormal quantity and quality of the tear, a group of eye symptoms caused by tear film instability and ocular surface damage, also known as kerato conjunctivitis sicca. It occurrs mostly in more than 60-year-old female, which is a common ocular surface disease. The cause of dry eye is varied, the process of pathophysiology and clinical has no specific "mechanisms". Through observation of the phenomenon that high incidence of dry eye menopausal population. Decline in androgen levels is an important cause of dry eye. Today's drugs have some limitations on dry eye induced by the reduction in the level of androgen. Therefore, it is pressing to find an alternative to androgen therapy drugs in the field of traditional Chinese medicine and get to know its mechanism clearly.

MATERIALS AND METHODS

Materials A total of 120, one-month-old healthy, male, weight 150g-180g, Wistar rats (Supplied by Animal Center of Xiangya Medical College, SPF grade, far inbred) were used to extract the lacrimal glands, then separated and trained the lacrimal gland epithelial cell from the glands. The instruments are mainly ultrasonic cell disruption instrument (the first European science and technology), Water heater thermostat box (blue laboratory instrument factory in Hangzhou, Zhejiang), Grinders (Camry Instrument Factory, Jintan City, Jiangsu Province), DYCZ-24D-based electrophoresis device (Beijing Instrument Factory 61), heating temperature oscillator (blue laboratory instrument factory in Hangzhou, Zhejiang), refrigerated centrifuge (Beckman Inc. USA), E1112X sensitive film (Applygen Technologies Inc). Buddleja officinalis(the qautities are more than 80%. It is mainly linarin and the method is extracting with ethanol and precipitating with water), immune blot test reagents (Boster Biological Technology), Flutamide (Beijing Ding States Biotechnology Co., Ltd.), testosterone propionate (product batch number: 0701102, Hubei Pharmaceutical Group shares

vision Co. , Ltd.), H_2O_2 (Beijing Ding States Biotechnology Co. , Ltd.).

Methods Extract *in vitro* culture primary lacrimal gland epithelial cells, which should also be in good conditon. 2 days after cell fusion, cells were randomly divided into blank plasma intervention group, total flavonoids Buddleja officinalis plasma drug intervention group, androgen intervention group, which were placed in 24-well culture plate, 8 wells in each group. Plasma of healthy male rat was added to blank plasma intervention group, self-prepared Buddleia officinalis drug-containing plasma total flavonoids from male rats were added to total flavonoids Buddleja officinalis plasma drug intervention group (the percentage of the final concentration of 8.95×10^{-2} mol/L), testosterone propionate solution was added to androgen intervention group directly (the final concentration of 1×10^{-6} mol/1). 48 hours after the intervention, H_2O_2 was added respectively whose final concentration was 100mol/L and continued to be fosted for 60 minutes, inducing apoptosis. Aspirated the culture medium containing serum and added serum-free medium after 12 hours holding, drained medium, pre-intervented with drug-containing plasma and Flutamide for 12 hours respectively. Western blot hybridization was used (Western-blot method) for phosphorylated STAT1 protein expression detection.

Statistical Analysis Measurement data between groups was compared with SPSS 13.0 software for analysis of variance, $P < 0.01$ for significant difference.

RESULTS

Expression of STAT1 Phosphoprotein In each group of lacrimal gland epithelial cells, positive band appeared in the relative molecular mass of 84000-90000, representing STAT1 protein (Figure 1). Their average gray ratio of STAT1 phosphoprotein expression ($\bar{x} \pm s$) were 0.169 ± 0.058, 0.353 ± 0.494 and 0.502 ± 0.036 respectively. The blank plasma intervention group had a small amount of STAT1 expression; testosterone propionate intervention group had increased in the expression of STAT1, which had a significant difference compared with the blank plasma intervention group; Buddleja officinalis drug-containing plasma intervention group had increased in the expression of STAT1, which had a significant difference compared with the Blank plasma intervention group; the expression of STAT1 in Buddleja officinalis drug-containing plasma intervention group was lower than that of testosterone propionate intervention group. However, the differences between the two group was significant.

Flutamide Effect The expression of STAT1 protein phosphorylated after the intervention of flutamide had not increased and had no significant difference when compared with blank plasma group(Figure 2).

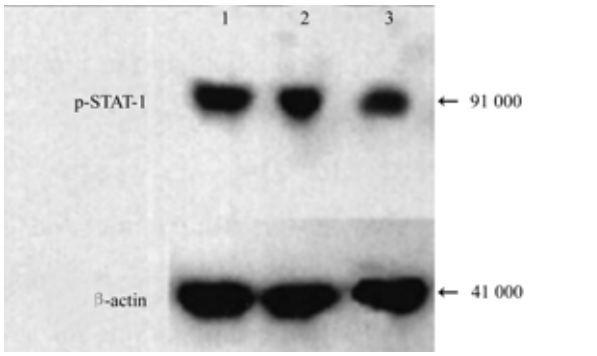

Figure 1 Electrophoresis map of STAT1 phosphoprotein in each group after the intervention of drug-containing plasma

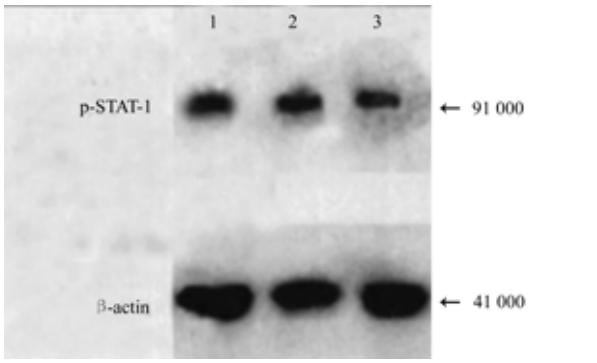

Figure 2 Expression of STAT1 phosphoprotein after the intervention of drug-containing plasma and flutamide

DISCUSSION

The effective part of Buddleia officinalis is flavonoids, which can be isolated into 8 kinds of flavonoids[1]- acacetin, apigenin, luteolin, new glycoside Buddleja officinalis, Menghua glycosides, luteolin-7-0-rutinoside, luteolin-7-0-glucosidase, Qiu ying glycosides. It has been proved that eye is the target organ of hormone and AR has been widely confirmed that in lacrimal gland cornea and other ocular surface tissues of human, rabbit and mouse[2], lacrimal gland epithelial cells are target cells of androgen and AR exists in acinar and on the nucleus of glandular epithelial. Androgen and flavonoids are polyphenolic heterocyclic compounds. Their similarities in chemical structure can be used to combined with AR. Current studies have demonstrated that certain flavonoids have a role to be androgen[3]. They can be used in the treatment of certain diseases caused by reduction in the level of androgen, such as bone loss. The use of radioactive tracer method of marking also showed that the flavonoids are cells and AR is stimulus. Biological effects can be played by the combination of cell and AR[4]. It can be seen that flavonoids contained in Buddleja officinalis can also be combined with AR and have androgen effects, which can be used to treat the diseases caused by reduction in the level of androgen, including dry eye syndrome certainly. Although the coverage that compound traditional Chinese medicine treatment of dry eye are more effective can often be seen. However, at present, there are little relatively clinical and experimental report about using Buddleja as Jun prescription drug to treat this disease. Preliminary studies have proved that flavonoids extracted from Buddleja officinalis had a good therapeutic effect on the dry eye syndrome induced by experimental reduction in the level of androgen. Mainly mechanism is that Buddleja officinalis flavonoids can regulated local lacrimal gland inflammation and apoptosis effectively, it can also improve the lacrimal gland ultrastructure, in order to maintain the basic tear secretion[5,6].

The extraction of Buddleja officinalis flavonoids is a kind of

natural medicine, flavonoids are a kind of phytoestrogen[7]. Clinical application of them has high safety and good prospects. Preliminary studies and literature research support that they are expected to become a new alternative to androgen, the efficacy is certain and can avoid a series of side effects caused by TST. Moreover, the treatment mechanisms is corresponding with pathogenesis of dry eye syndrome caused by the reduction in the level of androgen. It has been shown that eye is the target organ of hormone and androgen, estrogen, progesterone and prolactin receptor have been widely confirmed to be existed in lacrimal gland, meibomian gland, cornea and other ocular surface organization of human, rabbit and mouse[8]. By immunohistochemical localization of the lacrimal gland of androgen receptor (androgen recepter, AR) in SS mouse model, we found that lacrimal gland epithelial cells are the target cells of androgen[9]. AR exists in acinar and on the nucleus of glandular epithelial. Androgen can play a physiological role through the combination of these receptor.

Mammalian cells contain seven STAT (SignaL Transducers and Activators of Transcription) coding genes. They are STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B and STAT6 respectively and the amino acid sequence between encoding products have great discreteness. This discreteness makes them to respond to extracellular signaling molecules in lager scope and have a wider distribution of tissue-specific at the same time. The STAT signaling pathway participated by the family members is one of the common pathway of physiological and pathological response of human and it has closely relationship with cell growth, proliferation and differentiation. STAT1 protein contains two domains (SH2 and SH3) in C terminal and it also has conservative tyrosine residues site and the phosphorylation of this site is the basis of STAT1 activation. When the ligand signaling molecule is not exist, STAT1 protein, as transcription factor, located in the cytoplasm and inactive state; after receptor coupled with ligands, STAT1 is activated rapidly and SH2 (Src-homology) domain of its molecular specific binding with the tyrosine residues, whose receptor is phosphorylated. Then, STAT1 is close to the intracellular receptor domain. At this timet conservative tyrosine residues on STAT1 were phosphorylated. The phosphorylated STAT1 can be dissociated from the JAK-receptor complex , form dimmer and then are transferred to the nucleus, relies on its SH3 domain (capable of specifically binding Pro-rich region), which plays a direct role in cis-acting elements regulated by gene transcription and regulates the gene expression, so that the biological effect of a variety of cytokines, growth factors and hormones can be achieved. After the intervention of Buddleja officinalis with total flavonoids plasma and testosterone propionate on lacrimal gland epithelial cells, STAT1 protein transferres from the inactivation state in the cytoplasm to the expression of phosphorylation in activate state in the nucleus. It can be shown that by the time Buddleja officinalis with plasma total flavonoids have effects in increase androgen receptor in lacrimal gland epithelial cells and generated androgen. STAT1 can be activated, phosphorylated it and participate in the cell signal transduction after the effect of Buddleia flavonoids on the lacrimal gland epithelial cells, thus the proposed androgen biological role of Buddleia flavonoids can be achieved completely. However, the intended androgen effect of Buddleja officinalis flavonoids whether have completely relationship with STAT1 pathway's being activated or joint mechanisms with other cell signaling pathway activation, in-depth studies are of great needed.

The principles of androgen receptor blockers is combining the target tissue (or target cells) with the androgen receptor, blocking androgen activity in the form of material and type of material to combine with androgen receptor and inhibiting target tissue's uptaking of androgen and androgenic substances, in order to play the role of anti-androgen. Flutamide has the biological effect mentioned above and can be used to block the androgen receptor in the lacrimal gland epithelial cells to combine with androgenic substances in this research. Experimental results show that after Buddleia flavonoids' intervented with the the lacrimal gland epithelial cells joined with androgen receptor blocker, the expression of STAT1 phosphoprotein and the expression of blank plasma group have no significant difference, which means that Buddleia flavonoids can promote the expression of STAT1 phosphoprotein, activate STAT1 cell signal transduction pathway and promoted androgen effect of Buddleja officinalis to total flavonoids to be achieved through combining with AR. We can see from the above that Buddleja flavonoids drug-containing plasma can promote the expression of STAT1 phosphorylation by a combination with AR, which can also activate STAT1 cell signal transduction pathway and produce the same effect of androgen with testosterone propionate. Whether the activation STAT1 cell signal transduction pathway only has relationship with the combination of Buddleia flavonoids drug-containing plasma and AR, or has combined effects with other biological effects, and whether the intended androgen effect of buddleja officinalis flavonoids have completely relationship with STAT1 pathway's being activated or joint mechanisms with other cell signaling pathway activation, in-depth studies are of great needed.

REFERENCES

1 Li JS, Zhao YY, Li XL. Isolation and identification of Buddleja officinalis flavonoids. *Pharmaceutical Journal* 1996;31(11):849-854

2 Wickham LA, Gao J, Toda I, Rocha EM, Ono M, Sullivan DA. Identification of androgen, estrogen and progesterone receptor mRNAs in the eye. *Acta Ophthalmol Scand* 2000;78(2):146-153

3 Huang XL, Zhou WY, Wang W. Parmacological Research Progress of Epimedium Flavonoids. *Proprietary Chinese medicines* 2005;27(6):719-721

4 Nifli AP, Bosson-Kouame A, Papadopoulou N. Monomeric and oligomeric flavanols are agonists of membrane androgen receptors. *Exp Cell Res* 2005;309(2):329-339

5 Yao XL, Peng QH, Wu QL, Chen M. Effect of Dense flower extract on the dry eye rabbit castration resulted in apoptosis of the lacrimal gland. *Chin Med J Ophthalmol* 2007;17(3):139-144

6 Yao XL, Peng QH, Wu QL, Chen M. Preventive effect of Dense flower extract on dry eye castrated male rabbits. *Zhonghua Yanke Zazhi* 2008;44(11):1011-1019
7 Yao XL, Peng QH. Dry eye caused by phytoestrogens and hormone imbalance. *Zhonghua Yanke Zazhi* 2008; 44 (11) :1011-1019
8 Li QK. Traditional Chinese Medicine Ophthalmology. Beijing: People's Health Publishing House 2004:261
9 Ono M, Rocha FJ, SuLLivan DA. ImmunocytochemicaL Location and hormonaL controL of androgen receptors in LacrimaL tissues of the femaLe MRL/Mp-Lpr/Lpr mouse modeL of Sjogren's syndrome. *Exp Eye Res* 1995; 61(6):659-666

密蒙花总黄酮含药血浆干预干眼症细胞凋亡模型 STAT1 磷酸化蛋白表达

王 方[1],彭清华[2],姚小磊[1],吴权龙[2],李 点[2]

基金项目:中国国家自然科学基金资助项目(No. 30772824);中国湖南省自然科学基金资助项目(No. 07JJ3049);中国教育部高等学校博士学科点专项基金资助(No. 200805410004);中国湖南省科技厅科研基金资助项目(No. 2009FJ3001);中国 2008 湖南省研究生创新基金资助项目

(作者单位:[1] 550003 中国贵州省贵阳市,贵阳中医学院第二附属医院眼科;[2] 410007 中国湖南省长沙市,湖南中医药大学第一附属医院中医眼科学重点学科)
作者简介:王方,女,医学博士,毕业于湖南中医药大学,主要从事眼表疾病的研究。
通讯作者:彭清华,男,教授,主任医师,博士,博士生导师,1999 年获教育部全国高等院校青年教师奖,2000 年获湖南省人民政府优秀博士学位论文奖,2001 年获上海颜德馨中医药人才奖,2005 年获湖南省青年科技奖,2003 年度卫生部有突出贡献中青年专家,第二届全国百名杰出青年中医,2008 年入选湖南省 121 人才工程第一层次,2002 年享受国务院政府特殊津贴,兼中国中西医结合学会眼科专业委员会副主任委员、世界中医药学会联合会眼科分会常务理事、中华中医药学会眼科分会常务委员、湖南省中西医结合眼科专业委员会主任委员,主要从事中西医结合防治眼底病、青光眼和眼表疾病的研究. pqhz_520@163. com

摘要

目的:探讨密蒙花总黄酮含药血浆和雄激素受体阻滞剂对泪腺上皮细胞中 STAT1 的磷酸化蛋白表达的影响。

方法:体外分离及培养泪腺上皮细胞。以 H_2O_2 诱导大鼠泪腺上皮细胞凋亡,建立雄激素水平下降所致干眼症的细胞凋亡状态。设立空白血浆组、密蒙花总黄酮含药血浆干预组、丙酸睾酮干预组,分别观察各组 STAT1 的磷酸化蛋白表达情况;并应用雄激素受体阻滞剂氟他胺,考察密蒙花总黄酮的拟雄激素效应。

结果:免疫印迹结果表明,含药血浆干预后,密蒙花总黄酮含药血浆干预组中(0.353±0.494)与丙酸睾酮干预组中 STAT1 的磷酸化蛋白(0.502±0.036)的表达增强,两组间的差异有统计学意义($P<0.01$)。各组加入雄激素受体阻滞剂后,各组间(分别是 0.268±0.061,0.283±0.106,0.213±0.071)的 STAT1 的磷酸化蛋白表达间无差异。

结论:密蒙花总黄酮含药血浆可促进 STAT1 的磷酸化表达,并激活 STAT1 细胞信号传导通路,而产生与丙酸睾酮相同的雄激素效应。

关键词:干眼症;泪腺上皮细胞;培养;STAT1 的磷酸化蛋白;雄激素受体;氟他胺