

Effects of total glucosides of peony on expression of VEGF mRNA in skin tissue and peripheral blood of psoriasis mice.

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Abstracts

To analyze total glucosides of paeony's (TGP) influence on expression of VEGF mRNA in skin tissue and peripheral blood of Psoriasis mice and to explore the possible mechanism of TGP's treatment of Psoriasis. 60 mice were divided into six groups: Model group, treatment group, blank group and low-dose, middle-dose and high-dose TGP group, the mice in every group were applied 5% imiquimod cream on their backs except those in blank group. All the mice in each group were executed on the 8th day except those in treatment group, which were offered with middle-dose TGP treatment, continuously applied 5% imiquimod cream and executed until the skin lesion has vanished entirely. VEGF expression levels among different groups were surveyed by quantitative real-time PCR. On the 15th day of experiment, the skin lesion of mice in treatment group fades, and the tissue pathology doesn't show any abnormality. The VEGF mRNA expression amounts of mice skin tissue and peripheral blood from model group are all higher than those from blank group and the VEGF mRNA expression amounts of mice skin tissue from blank group, treatment group, high-dose, and middle-dose TGP group are all lower than those from model group, with a statistical significant ($P < 0.05$). Therefore, TGP can produce a therapeutic effect on psoriatic lesions by inhibiting expression of VEGF mRNA in the psoriasis model.

Keywords: TGP, VEGF, Quantitative real-time PCR.

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Introduction

Psoriasis is a kind of common, chronic and inflammatory skin disease [1], it affects 1-3% of the general people [2,3]. The pathology of psoriasis is featured by abnormal epidermal dysplasia, infiltration of a large number of inflammatory cells in the dermis and neovascularization [4,5]. Among the three histopathologic changes, the abnormality of the dermal papillary vasculature appears first, indicating that neovascularization perhaps be one of the key characteristic of psoriasis pathogenesis [6]. VEGF was identified as a strongest and most specific direct angiogenic diathesis that was intensively up-regulated in psoriatic skin lesions [7]. Therefore, VEGF occupies a significant position in the occurrence of psoriasis [8-11]. Extraction of Radix Paeoniae Alba monomer is mainly a group of glycoside substances, including paeoniflorin, hydroxy paeoniflorin, paeonin, albiflorin, benzoylpaeoniflorin, collectively referred to as the total glucosides of paeony (total glucosides of peony, TGP). It has been reported that TGP has effects on skin tissue. However, the mechanism was still unknown.

Materials and Methods

Animal experiments and drug treatment

Sixty adult female BALB/c mice, weighing 18-20 g (offered by animal experiment center of Southern Medical University) were employed in study. The mice were randomly divided into six groups: Treatment group, model group, blank group and different concentration of TGP groups: low-dose, middle-dose and high-dose TGP group, with 10 mice in each. A mice model of psoriasis was produced by applying 5% imiquimod cream on it's naked backs 50 mg each, once a day. Except those in blank group, the other mice of five groups establishes psoriasis animal models.

TGP dissolves in normal saline according to the required dose and doses by gavage, once a day, 0.4 ml each. The mice from low-dose, middle-dose and high-dose when they were modeled, were given 50 mg/kg•d, 100 mg/kg•d, 200 mg/kg•d TGP respectively, while those from model group and blank group were given normal saline by gavage with the same quantity, and executed on the 8th day. On the 9th after the modeling of the treatment group, the mice were continuously applied 5% imiquimod cream on their backs, offered with 100

mg/kg•d TGP and executed until the skin lesion had been vanished entirely.

Statistical analysis

All outcomes were expressed as “median (P25~P75)”. Measurement data were analyzed using a rank-sum test and SPSS17.0 software analysis. A result of $p < 0.05$ was supposed to be statistically significant.

Results

The changing of skin tissues in mice

The model group shows: 1 day after application of imiquimod (IMQ), red spots appear on the skin from model group; 2-3 days, scales appear; 4-5 days, the most severe condition appears; 6 days skins lesion turns better; 7-8 days the skin deteriorates again and thickens constantly. Pathology slice shows: corneum thickening and parakeratosis appear in the skin; stratum granulosum attenuates; stratum spinosum thickens, and epidermal ridge lengthens etc., in the skin. These histologic changes are similar to those of human psoriasis patients.

After 7 days 100 mg/kg•d total glycosides of paeony treatment by gavage, the skin lesion of mice in treatment group fades entirely. HE staining: epidermis and dermis of skin tissue are approximately normal, without corneum thickening, hyperkeratosis, and parakeratosis, etc.

Effect of TGP inhibition in expression of VEGF mRNA in skin tissue of psoriasis mice

The VEGF mRNA expression amounts in treatment group and different concentration of TGP groups are all lower than those of the model group, with obvious discrepancy ($P < 0.05$) while compared with blank group, there is no statistically significant difference; the VEGF mRNA expression amounts in low-dose TGP group are higher than those of the blank group, with a statistically significant ($P = 0.01$) while compared with the model group, there is no statistically significant ($P = 0.32$) (Table 1).

Effect of TGP inhibition in expression of VEGF mRNA in Peripheral blood of psoriasis mice

The VEGF mRNA expression amounts in treatment group and different concentration of TGP groups are compared respectively with those of the model group and blank group with no statistically significant difference (Table 2).

Table 1. VEGF mRNA expression level in skin tissue.

Group	Skin Tissue	Values of P	
Low-dose TGP group	1.57 (0.48~3.07)	0.32*	0.01#
Middle-dose TGP group	0.36 (0.21~1.32)	0.02*	0.21#
High-dose TGP group	0.23 (0.03~1.43)	0.00*	0.55#

Treatment group	0.21 (0.12~0.91)	0.01*	0.42#
Blank group	0.15 (0.38~0.57)	0.00*	-
Model group	5.77 (0.86~9.63)	-	0.00#

Note: *Compared with the model group ;#Compared with the blank group

The correlation analysis between different concentrations of TGP and the expression amount of VEGF mRNA

There is a negative correlation between the concentration of TGP and the expression amount of VEGF mRNA in skin tissue, (Kendall's tau_b: $r = -0.312$ $p = 0.036$; Spearman's rho: $r = -0.383$ $p = 0.040$).

Table 2. The expression of VEGF mRNA levels in peripheral blood.

Group	Peripheral blood	Values of P	
Low-dose TGP group	3.51 (0.76~4.09)	0.45*	0.14#
Middle-dose TGP group	1.58 (0.45~8.81)	0.36*	0.35#
High-dose TGP group	1.73 (0.91~8.89)	0.85*	0.07#
Treatment group	0.45 (0.24~11.87)	0.34*	0.97#
Blank group	0.48 (0.22~0.75)	0.00*	
Model group	11.69 (1.19~59.59)		0.00#

Note: *Compared with the model group; #Compared with the blank group

Discussion

TGP is a kind of immunomodulator whose main components include paeoniflorin, hydroxy-paeoniflorin, paeonin, albinorin, benzoylpaeoniflorin and so on, while paeoniflorin takes 90% of amount of total glycosides [12]. It is widely used in the treatment of autoimmune disorder such as lichen planus, eczema, SLE and so on. Recent study shows TGP treatment of psoriasis has obtained preferable efficacy [13], but there is no clear mechanism. The past research shows: In psoriasis patients, pathologic changes such as neovascularization occur due to the over expression of VEGF [14,15], and the over expression of VEGF also promotes the psoriasis pathologic changes on the epidermis of mice which are similar to those of psoriasis people. This study found that TGP has therapeutic effect on psoriasis and it can refrain the VEGF mRNA in skin tissue from expression. However, in skin tissue, low-dose TGP cannot inhibit the expression of VEGF mRNA and there is a negative correlation between the concentration of TGP and the expression amount of VEGF mRNA in skin tissue.

The signal transduction pathway of p38 MAPK plays a significant part in pathological process of psoriasis [16], and the activity of P38 in skin lesion area of psoriasis patients is significantly higher than those in non-lesion area of normal people. It will adjust the secretion of many kinds of protein kinases and transcription factors in downstream including VEGF and over activation of P38 can cause the increase of VEGF expression. Previous studies found that TGP can refrain

p38 MAPK from activation [17], thus TGP playing an anti-inflammatory role.

Conclusion

Inferred from the above results, TGP's treatment mechanism on psoriasis can be related to its refraining VEGF mRNA from expression by the method of p38 MAPK and only when the concentration of TGP reaches to a certain degree, can TGP refrain VEGF mRNA in skin tissue from expression.

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