The Effect of Total Glucosides of Peony on Lipopolysaccharide Induced Monocyte Immune Inflammation

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Abstract: This study aimed to explore the effect of total glucosides of peony (TGP) on the lipopolysaccharide induced secretion of IL-1, IL-6 and TNF-α in monocytes. Human peripheral blood was used to isolate and culture monocytes, with 25μg/mL Porphyromonas gingivalis lipopolysaccharide (Pg LPS) as a stimulating factor, then we observed the inhibit influence of 1, 5, 10, 20, 50μg/mL concentrations of TGP on the secretion of IL-1, IL-6, and TNF-α. Enzyme-linked immunosorbent assay (ELISA) was used on the concentration determination of IL-1, IL-6, and TNF-α. This study discovered that TGP had a significant inhibitory effect on Pg LPS induced monocyte secretion of IL-1 and IL-6, and the inhibitory effect on IL-1 was enhanced with the increase of TGP concentration. However, when the TGP reached a certain concentration, the inhibitory effect on IL-6 weakens. No effect was observed on the inhibitory effect of TNF-α. The results of the experiments showed that TGP have a significant inhibitory effect on the secretion of IL-1 and IL-6 by monocytes induced by Pg LPS within a certain concentration range, which might be one of its mechanisms in treating periodontitis.

Keywords: Total Glucosides of Paeony; Porphyromonas Gingivalis Lipopolysaccharide; Periodontitis; Traditional Chinese Medicine; Therapy

1. Introduction

Chronic periodontitis is a multifactorial infectious diseases with plaque as the initiating factor. From the perspective of inflammatory cytokines, it is found that in the gingival crevicular fluid of diabetes patients with periodontitis, the levels of inflammatory factors such as IL-1, IL-6 and TNF-α are significantly increased, and may activate signaling pathways such as NLRP3, thereby exacerbating periodontal tissue damage [1]. Traditional Chinese medicine has used in China for a long time to treat various diseases. Utilizing the anti-inflammatory and immunosuppressive properties of traditional Chinese medicine to intervene in periodontitis has been one of the research hotspots in recent years.

Total glucosides of peony (TGP) is an effective ingredient extracted from the roots of Chinese traditional medicine, Paeonia lactiflora. Pharmacological analysis shows that TGP mainly contain components such as paeoniflorin, hydroxypaeoniflorin, paeoniflorin, paeoniflorin, and benzoyl paeoniflorin. The pharmacological research results on TGP have found that it has pharmacological effects such as inhibiting inflammatory response, promoting cell proliferation, antioxidant stress damage, and inhibiting macrophage infiltration [2]. Therefore, using TGP as a medication to treat periodontitis has high feasibility.

This study isolated and cultured monocytes, and induced them with Porphyromonas gingivalis lipopolysaccharide (Pg LPS) to construct an immune environment in the pathological state of periodontitis. Subsequently, TGP intervention was used to explore the effect of TGP on the local immune environment of periodontitis.

2. Materials and Methods

2.1 Reagents and Instruments

TGP Capsules (National Medical Approval Letter H20055058, Ningbo Lihua Pharmaceutical), Dexamethasone Injection (National Medical Approval Letter H12020516, Tianjin Jinyao Pharmaceutical), Pg LPS (Invivogen, USA), RPMI-1640 Culture Medium (Gibco, USA), Fetal Bovine Serum (Ausbian, Australia), and Human Lymphocyte Isolation Medium (Tianjin Haoyang, China).

Human IL-1, IL-6, and TNF-α ELISA kits were all provided by Shanghai Enzyme linked Biotechnology.

Multifunctional enzyme-linked immunosorbent assay (Tristar² S LB 942, Berthold, Germany), and washing machine (EL406, BioTek, USA).

2.2 Isolation and Culture of Human Blood Monocytes

Take 100ml of fresh anticoagulant human whole blood and dilute it with Hanks solution in a 1:1 volume ratio. Perform gradient density centrifugation using human lymphocyte separation solution according to the instructions, and suspend in RPMI 1640 culture medium containing 10% fetal bovine serum and dual antibodies (100U/mL each for penicillin and streptomycin).

Use a cell counting plate to adjust the cell density to 2×10^6/mL. Inoculate the above cell suspension into a culture bottle and culture for 3 hours. After confirming cell adhesion through microscopic examination, replace the culture medium and continue to culture for 24 hours. After changing the medium, obtain monocytes for experimentation. Trypan blue staining confirms cell viability>95%.

2.3 Monocyte Grouping and Drug Intervention

Treat the above-mentioned adherent cells with cell brushes, suspend them in RPMI 1640 culture medium, and inoculate them into a 24 well culture plate. Each well contains 1mL of cells, with 4 wells in each group. After continuing to cultivate for 24 hours and adhering to the wall, discard the original culture medium, wash twice, and then add medication.

TGP are dissolved in a 1% sodium carboxymethyl cellulose solution before use. The grouping of this study is as follows: blank control group (without any medication), LPS group, LPS+TGP group (final concentrations of TGP are: 1, 5, 10, 20, 50μg/mL, respectively), LPS+dexamethasone group (final concentration of dexamethasone is 2μg/mL), the final concentration of LPS in all groups was 25μg/mL.

2.4 ELISA Determination of Cytokine Concentrations

After culturing each group of cells with drugs for 48 hours, extract the supernatant of the culture medium, and use ELISA method to determine the concentration of IL-1, IL-6, and TNF-α in each group according to the process and requirements of the reagent kit manual.

2.5 Statistical Processing

After collecting and organizing ELISA data, statistical analysis was performed by Microsoft Excel, and t-tests were used to compare whether there were significant differences in cytokine concentrations among each groups.

3. Results

3.1 Effects of The Secretion of IL-1

From the experimental results, TGP have a significant inhibitory effect on Pg LPS induced monocyte secretion of IL-1. When the concentration of TGP reaches 5μg/mL, there was a significant difference between the LPS group and the other groups, and the inhibitory effect was enhanced with the increase of TGP concentration (Table 1 and Figure 1).

Table 1. Inhibition of TGP on LPS Induced IL-1 Secretion in Monocytes

Groups	IL-1(ng/mL)	Inhibitory rate(%)
Control	1.21±0.18	-
LPS	2.25±0.05	0
LPS+1μg/mL TGP	1.95±0.14	13.33
LPS+5μg/mL TGP	1.43±0.08*	36.44
LPS+10μg/mL TGP	1.32±0.09*	41.33
LPS+20μg/mL TGP	1.11±0.07*	50.67
LPS+50μg/mL TGP	0.95±0.08*	57.78
LPS+2μg/mL dexamethasone	0.67±0.15*	70.22

*Compared with the LPS group, $P<0.05$

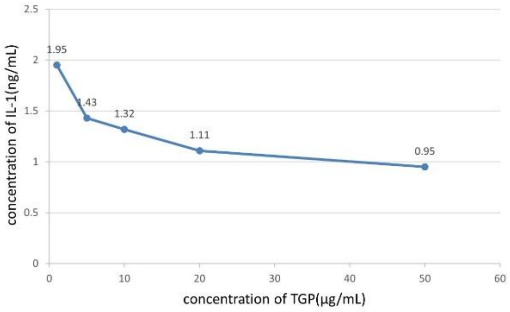

Figure 1. Dose-Response Curve of TGP on LPS Induced Inhibition of IL-1 Secretion in Monocytes

3.2 Effects of The Secretion of IL-6

The TGP also had a significant inhibitory effect on Pg LPS induced IL-6 secretion in

monocytes, but the inhibitory effect weakens when the TGP reach a certain concentration (Table 2 and Figure 2).

Table 2. Inhibition of TGP on LPS Induced IL-6 Secretion in Monocytes

Groups	IL-6(ng/mL)	Inhibitory rate(%)
Control	1.31±0.27	-
LPS	1.95±0.13	0
LPS+1μg/mL TGP	1.77±0.11	9.23
LPS+5μg/mL TGP	1.54±0.14*	21.03
LPS+10μg/mL TGP	1.23±0.09*	36.92
LPS+20μg/mL TGP	1.16±0.06*	40.51
LPS+50μg/mL TGP	1.87±0.08	4.10
LPS+2μg/mL dexamethasone	0.91±0.16	53.33

*Compared with the LPS group, $P<0.05$

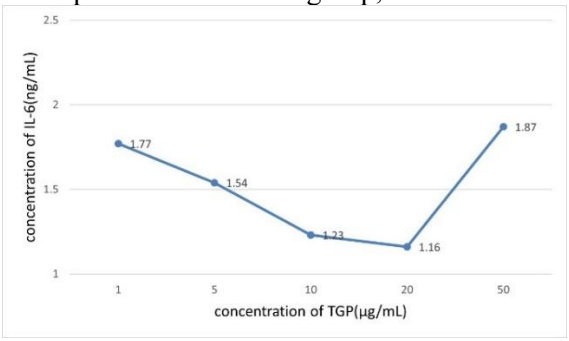

Figure 2. Dose-Response Curve of Total Paeoniflorin on LPS Induced Inhibition of IL-6 Secretion in Monocytes

3.3 Effects of The Secretion Of TNF-A

There was no significant inhibitory effect on TNF-α secretion, and no significant differences in concentration between the TGP groups compared to the LPS group were observed (Table 3 and Figure 3).

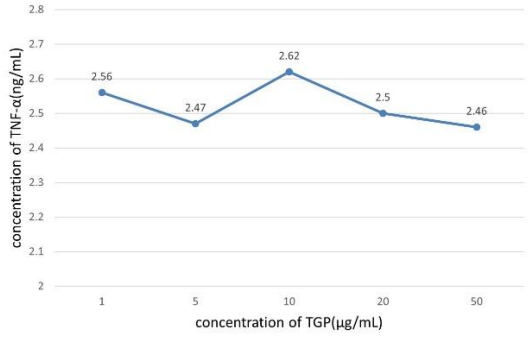

Figure 3. Dose-Response Curve of Total Paeoniflorin on LPS Induced Inhibition of TNF-A Secretion in Monocytes

Table 3. Inhibition of TGP on LPS Induced TNF-A Secretion in Monocytes

Groups	TNF-α(ng/mL)
Control	1.45±0.34
LPS	2.67±0.23
LPS+1μg/mL TGP	2.56±0.18
LPS+5μg/mL TGP	2.47±0.22
LPS+10μg/mL TGP	2.62±0.24
LPS+20μg/mL TGP	2.50±0.37
LPS+50μg/mL TGP	2.46±0.35
LPS+2μg/mL dexamethasone	0.63±0.22*

*Compared with the LPS group, $P<0.05$

4. Discussion

The immune inflammatory response induced by periodontal pathogenic bacteria LPS such as Porphyromonas gingivalis is an important cause of periodontal tissue inflammation and alveolar bone resorption[3], and it is also an important pathological change in the onset of periodontitis. Therefore, if the immune inflammatory response induced by periodontal pathogenic bacteria LPS can be suppressed, it provides an important approach for the treatment of periodontitis.

Using natural drugs and their extracts for topical or systemic treatment of periodontitis is one of the hotspots in periodontal disease research, emodin, gallnut water extract, astragaloside, and etc were reported [4]. And a systematic review showed that adjunctive natural products better reduced the periodontal pocket depth when compared to scaling and root planing alone or scaling and root planing plus placebo in a follow-up of 3-6 months [5]. From the results of this study, we found that TGP had a significant inhibitory effect on Pg LPS induced monocyte secretion of IL-1 and IL-6, with the inhibitory effect on IL-6 limited to a certain concentration range. From this, it can be seen that using TGP for the treatment of periodontitis is highly feasible. TGP can exert therapeutic effects by inhibiting the immune inflammatory response induced by periodontal pathogenic bacteria LPS.

In previous studies, TGP were mostly used as adjunctive therapy for autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), psoriasis, and etc[6]. Their efficacy has been confirmed through basic and clinical medical studies. However, there are few studies on the using of TGP in the treatment of periodontal diseases.

Some scholars had studied the therapeutic mechanism of TGP on patients with both periodontitis and coronary heart disease. By measuring the CD3+, CD4+, CD8+, and CD4+/CD8+subsets of peripheral blood T lymphocytes before and 3 months after treatment with TGP, it was found that TGP had a bidirectional regulatory effect on the immune function of patients with both periodontitis and coronary heart disease, and periodontal indicators such as PD, AL, BI, and PLI had also been significantly improved, reducing the degree of local inflammation in the periodontium. This study indicated that TGP might be an effective medication for periodontitis[7].

Based on the results of our study, it can be seen that TGP has high feasibility in the treatment of chronic periodontitis, and its mechanism of action may mainly be to regulate the systemic and local immune inflammatory response of patients, then periodontal tissue damage can be suppressed. The conclusion of this study provides some clues for the use of TGP in the treatment of periodontitis, and it is feasible to use TGP for local or systemic treatment of periodontitis. In particular, localized drug delivery system combined with scaling and root planing therapy for periodontitis is a potential tool since it increases drug efficacy and minimizes negative effects by managing drug release[8]. However, further in-depth research is suggested on its mechanism of action, and clinical research conclusions also needed to provide evidence-based medicine support.

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