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A Comparative Study on the Regulation of Inflammation by Lycium Barbarum Polysaccharide and Exocarpium Citri Grandis

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Abstract

Inhibition of inflammation has important clinical value, and the present study was proposed to compare the effects of *Lycium Barbarum* Polysaccharide (LBP) with those of *Exocarpium Citri Grandis* (ECG) in the regulation of inflammation. In this study, bacterial lipopolysaccharide (LPS) was used to induce the production of inflammatory factors by macrophage cell line MSC-2 cells to construct a cell model of inflammation. LBP and ECG extracts were used to act on this inflammatory cell model, and qPCR was used to detect the changes of inflammatory factors IFN-γ, TNF-α and IL-6. The results showed that LBP down-regulated IFN-γ and IL-6, while TNF-α increased in the inflammatory cell model; the extract of ECG down-regulated all the above three cytokines. In regulating the secretion of inflammatory factors, ECG may be more advantageous, while LBP is recommended to be used with caution for inflammatory diseases in the presence of high TNF-α level.

Keywords: Exocarpium Citri Grandis, inflammation, MSC-2 cells.

1. Introduction

Inflammatory diseases involve complex interactions between pathogens, cells and medications targeted to control inflammatory processes(Nathan, 2002). Infectious inflammation is one of the most common causes of inflammatory diseases, which could possibly result in worldwide epidemic such as COVID-19. The pathogenesis of infectious inflammation is the secretion of large amounts of inflammatory factors by immune cells. Cytokines play an important role in the immune response by regulating cells and transmitting signals. However, immune hyperactivation can lead to cytokine storms, which in turn triggers acute systemic inflammatory effects and secondary organ dysfunction that may ultimately result in death due to multiple organ failure(Fajgenbaum & June, 2020).Current treatment strategies for inflammatory conditions include utilization of antibiotics, adrenal glucocorticoids and monoclonal antibodies (e.g., TNF-a monoclonal antibody, Trastuzumab), but the use of antibiotics may lead to issues like reduced immunity or secondary infection, and monoclonal antibodies are expensive.

Among well-known herbs in traditional Chinese medications, *Lycium barbarum* and *Exocarpium Citri Grandis* (ECG) are often described as "antipyretic" and "anti-inflammatory and analgesic". *Lycium barbarum*, also called goji berry, is commonly used in healthcare products because of its multiple pharmacological efficacies. The main component of goji berry that accounts for its potential anti-inflammatory effects is Lycium Barbarum Polysaccharide (LBP)(Kwok et al., 2019; Tian et al., 2019). Meanwhile, the medicinal effects of ECG were recorded in the "Supplements to Compendim of Materia Medica" written by Zhao Xuemin, an herbalist of the Qing Dynasty, stating that it "treats phlegm very effectively." In the Qing dynasty, Li Yongcui in his work "evidence of the treatment of the Huijin" described the disease "phlegm" as "wet, fluid ..... what phlegm has, if the external wind, summer and dry heat

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invasion ... ...Qi and blood are disturbed, then phlegm is born." This corresponds to mucus and phlegm in inflammatory types such as bacterial pneumonia and Covid(Henig & Kaye, 2017; Ochani et al., 2021). Ancient Chinese medicine practitioners, through their continuous accumulation of experience, justified that the pharmacological efficacy of ECG could be effective in treating inflammation, which provided great inspiration for this study. Meanwhile, in the "COVID Treatment Protocol (Trial Version 6)" published by National Health Commission of the People's Republic of China in February 2020, ECG was included in the recommended prescription for "dampness and toxicity in the lung" used in the general clinical treatment period, suggesting the medicinal value of ECG in the treatment of inflammatory diseases. In this research, whether Lycium Barbarum and ECG can regulate the secretion of inflammatory factors by activated macrophages is examined through experiments.

2. Materials and Methods

Lycium Barbarum Polysaccharide: provided by Shandong Xinnuo Food Company (Zhongning County, Ningxia). Exocarpium Citri Grandis: provided by Guangdong zhanjiang Juhong hall company. Extraction method: Take 5g of dehydrated fruits of Citrus aurantium crushed, material-liquid ratio 1:10, in 80% ethanol solution in 55 ℃ water bath for 4h, to obtain Citrus aurantium extract, the main component is flavonoids. Diluted 160 times with culture medium when used(Hu, 2017). MSC-2 (immortalized MDSCs isolated from the spleens of immunosuppressed BALB/c mice, using a retrovirus encoding the v-myc and v-raf oncogenes) is provided by Francois Ghiringhelli, Department of Medical Oncology, Center GF Leclerc, 2100 Dijon, France. LPS: Sigma, USA.

Modeling procedure: 50,000 MSC-2 cells were inoculated in 6-well plates and cultured for 24 h. LPS solution was added at a final concentration of 0.25 μg/ml. LBP was dissolved in DMEM medium to make 100μg/mL LBP solution and filtered to remove bacteria. The LBP extract was filtered and diluted 160 times with DMEM medium. 10 μL and 20 μL of 100 μg/mL LBP solution and 10 μL and 20 μL of ECG extract were added to 4 wells of the six-well plate, respectively, where LBP solution has been added. The medium was then incubated for 24 hours. Real-time fluorescence quantitative PCR (qPCR) was used to detect cytokine expression. Cells in six-well plates were taken, RNA was extracted by Trizol method, transcribed into cDNA, and the relative amounts of TNF-α, INF-γ, IL-6, and the original mRNA template of the internal reference gene DAPDH were examined respectively.

3. Results

The results showed that the expression of three genes, TNF-α, INF-γ and IL-6, in the LPS control group was higher than that in the blank control group, confirming the success of the inflammation model construction. On this basis, the expression of TNF-α was reduced in the ECG extract groups compared with the LPS control group, but it did not show a significant dose-dependence; while the expression of INF-γ was all reduced to zero, and the expression of IL-6 was reduced, in which the reduction effect was significantly stronger in the high-dose group than in the low-dose group.

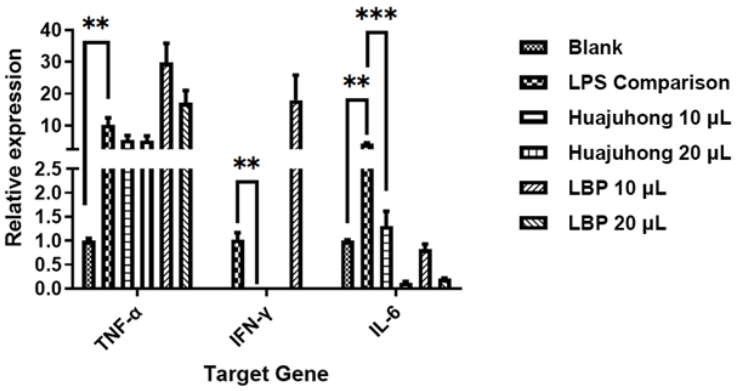

Figure 1. Relative expression of TNF-α, INF-γ and IL-6 in 6 groups. (Huajuhong indicates ECG extracts in this figure)

The expression of TNF-α in the LBP group was higher than that in the LPS control group, and the increase was greater in the low-dose group than in the high-dose group; the expression of INF-γ was higher in the low-dose group than in the control group; the expression of IL-6 was lowered, and the lowering

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effect was significantly stronger in the high-dose group than in the low-dose group, showing some similarity with that of the ECG extract group. Since three samples from each treatment group were tested using qPCR, error bars in the figure were determined using the three results from the according group. Stars between groups indicate the significant differences between data groups, which were determined by p value calculated using t test. P value that is smaller than 0.01 is marked by two stars, and p value smaller than 0.001 is marked by three stars.

4. Discussion

Modern medicine considers inflammation as a spontaneous defense response of the body to inflammatory factors such as bacteria and viruses, a process by which the immune system tries to suppress inflammatory pathogens through multiple pathways, usually manifesting as redness, swelling, heat, pain and dysfunction.

TNF-α, the predominant inflammatory factor, induces increase of multiple inflammatory factors expression and leads to ischemia and thrombosis(Idriss & Naismith, 2000). Inappropriate or excessive activation of TNF-α signaling is associated with chronic inflammation and may eventually lead to the development of pathological complications, such as autoimmune diseases(Jang et al., 2021). IFN-γ induces phagocytosis of blood cells by macrophages, leading to hemocytopenia in patients with inflammation. IL-6 is also is a pro-inflammatory cytokine that increases antibody production and induces acute phase reactants(Fajgenbaum & June, 2020).

How to reduce the expression of inflammatory factors is a key issue in clinical treatment. In severe pneumonia, TNF-α and IFN-γ acting together can lead to inflammatory cell death, requiring the use of antibodies to inhibit the secretion of inflammatory factors(Karki et al., 2021). Trastuzumab is effective in reducing the effects of IL-6(Tanaka, Narazaki, & Kishimoto, 2014), but it has the problem of high price. Therefore, drugs that can effectively reduce these major inflammatory factors are urgently needed to be discovered.

Present studies showed that 70% ethanol-extracted ECG components exhibited great anti-cough, anti-phlegm and anti-inflammatory effects when applied to ammonia-induced cough model in mice, phenol red excretion in mice and xylene-guided ear swelling model in mice(Jiang et al., 2014). In addition, in mice models of air pollution particle-induced pulmonary inflammation, ECG flavonoids significantly inhibited PM2.5 -stimulated overproduction of TNF-α, IL-1β, IL-6, and IL-18 and increased the numbers of white blood cell, neutrophils, lymphocytes, and monocytes in bronchoalveolar lavage fluid of the model mice(Zhu et al., 2019). The anti-inflammatory effects of ECG flavonoids were also studied using LPS-induced RAW264.7 cell model(Hu, 2017). The MSC-2 cells used in this study are characterized by a high degree of differentiation and some inflammatory properties(Apolloni et al., 2000). Studies using this cell type can provide in-depth validation of the anti-inflammatory potency of ethanolic extracts of ECG and provide experience for future studies.

In this study, the effects of LBP and ECG extracts on the regulation of TNF-α, IFN-γ and IL-6 were investigated. The results showed that ECG extract had a significant reduction effect on IL-6 and IFN-γ, but the reduction of TNF-α was relatively limited and did not meet the needs of clinical treatment well. LBP treatment group showed small increase in TNF-α, and the reduction of IFN-γ only existed in groups with high LBP concentration (20 μL). These variations of amount changes among different cytosines could be caused by the difference in structure of LBP and ECG extracts, since LBP is a type of polysaccharide while ECG extracts are mainly composed of flavonoids. Whereas their ways of acting might be similar because of the shared effect of reducing IL-6. Exact mechanisms of their regulatory effects still need further research.

Therefore, the combination of ECG and TNF-α monoclonal antibodies may have better anti-inflammatory effects and has application potential. In addition, some traditional Chinese medicine formulas also suggest the possibility of combined use of ECG and other herbal medicines. For example, in the "Pulmonary Disease System Pill" recorded in the "Beijing Selected Traditional Chinese Medicine Formulas", the combination of ECG, shiso, licorice and poria was used together. The licorice extract can reduce the expression of TNF-α(Yang et al., 2015), and platycoside E in poria can reduce the expression of TNF-α and IL-1β(Ji et al., 2020). The good combination of these herbs can reduce the expression of TNF-α, IFN-γ, IL-6 and other cytokines, which can well compensate for the deficiency of ECG in reducing TNF-α.

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5. Conclusion

Both LBP and ECG embodied some anti-inflammatory effects, and the ECG extract showed stronger effects in reducing TNF-α and IFN-γ. The experimental results confirm that the active ingredients of these two herbs can inhibit the secretion of inflammatory factors to a certain extent, but the different inhibitory effects on different types of inflammatory factors also indicate that a single type of herbal extract is not effective in treating inflammatory diseases. This suggests that future research could investigate the medical potential of different species of plant extracts used together.

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