Effects of aqueous extracts from Lonicera japonica and Tussilago farfara on RAW 264.7 Macrophages

Lee, Eung-Seok[1]·Yang, Su-Young[1]·Park, Yang-Chun[1] *·
Oh, Young-Seon[2]·Lee, Jin-Woo[2]·Lee, Yong-Koo[2]

[1]Div. of Respiratory System, Dept. of Internal Medicine, College of Oriental Medicine, Daejeon University
[2]Hyehwa Hostpital, Daejeon University, Deajeon, Korea

Effects of aqueous extracts from Lonicera japonica and Tussilago farfara on RAW 264.7 Macrophages

Lee, Eung-Seok[1]·Yang, Su-Young[1]·Park, Yang-Chun[1] *·
Oh, Young-Seon[2]·Lee, Jin-Woo[2]·Lee, Yong-Koo[2]
[1]Div. of Respiratory System, Dept. of Internal Medicine, College of Oriental Medicine, Daejeon University
[2]Hyehwa Hostpital, Daejeon University, Deajeon, Korea

Inhalational drug is an attractive modality for local therapy of pulmonary diseases as well as systemic drug delivery. Flower of Lonicera japonica (FLJ) and flower of Tussilag farfara (FTF) are medicinal herbs for respiratory disease in traditional Korean medicine. As a preliminary study for effective inhalable formulation of FLJ and FTF, this study was to provide the toxicity and anti-inflammatory effect on murine macrophages. The dried FLJ and FTF were extracted with distilled water, filtered and freeze-dried. After treatment with FLJ and FTF extract on RAW 264.7 cells, the cell viabilities were measured by MTT assay. FLJ and FTF did not show cytotoxicity on RAW 264.7 cells. LPS stimulated RAW 264.7 cells were treated with 3 and 30 μg/ml of FLJ or FTF. FLJ and FTF did not inhibit TNF-a and IL-6 secretion in both concentration of treatment. We suggest that FLJ and FTF may be useful drugs for respiratory disease. Future work will focus on the physical characteristics for inhalable formulation.

Ⅰ. Introduction

Flower of Lonicera japonica (FLJ) is dried buds of honeysuckle (忍冬), its qi is cold and flavor is sweet. It has known that has effects on heat toxin and sore through its effects of

* 교신저자 : 박양춘, 대전시 동구 용운동 96-3 대전대학교 한의과대학 폐계내과학교실.
투고일 : 2010년 7월 27일, 게재확정일 : 2010년 8월11일.

heat-clearing and detoxicating[1]. FLJ is used for treatment of various respiratory disease, e.g. chronic obstructive pulmonary disease[2,3], asthma[4,5], influenza[6], lung fibrosis[7].

Tussilago farfara, commonly known as coltsfoot (款冬), is a plant in the family Asteraceae. Flower of Tussilago farfara (FTF) is dried buds of coltsfoot, it has been used as a cough suppressant. Its qi is warm and flavor is pungent-sweet[1]. The plant has been used in traditional Korean medicine to treat lung ailments such as asthma as well as various coughs[8,9].

Effectiveness of a drug is determined by the amount reaching to the target in our body. Drugs reaching unintended parts of the body can lead to side effects. Therefore, researches on new drug delivery systems (DDS)[10] which can control the drug distribution and release rate are actively ongoing. However, studies on drug delivery of oriental medicine are almost non-existent.

Therefore, the development of new delivery form is very important to improve the effects and compliance of herbal medicines. So the objective of this study is to assess the cell viabilities and anti-inflammatory effects of FLJ and FTF as preliminary study for new formulation of FLJ and FTF.

Ⅱ. Materials and Methods

1. Materials

FLJ and FTF were purchased from Hyehwa Herb Co. (Daejeon, Korea) and their extracts were prepared in Daejeon University (Daejeon, Korea). RAW264.7 cell lines were purchased from ATCC (Manassas, VA, USA). All media and their components were purchased from Invitrogen (Carlsbad, CA). MTT viability assay kit was obtained from Promega (Fitchburg, WI, USA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2. Preparations of FLJ and FTF extracts

Herbs of FLJ (100 g) and FTF (100 g) were extracted with distilled water (each 1,000 ml) at 100℃ for 3 hours. The extracts were filtered through 0.45 μm filter, freeze dried and kept at -80℃. The dried extaracts were dissolved in PBS and filtered through 0.22 μm filter before use.

3. Cell viability assay with FLJ and FTF extracts

RAW 264.7 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS). The cells were plated in 96 well plate at the density of 2.5×10^4 per well. After overnight incubation, the media was replaced with 100 μl media containing the original FLJ extract or FTF in the concentrations ranging from 1 - 10,000 μg/ml. The MTT assay was performed after 24 hours of incubation with FLJ extract or FTF. The cell viability was quantified by measuring the UV absorbance at 560 nm with a Tecan microplate reader. The measured absorbance was normalized to the absorbance of non-treated control cells.

4. ELISA of FLJ and FTF extracts

The inhibitory effect of 3 and 30 μg/mL FLJ and FTF on the cytokines TNF-α and IL-6 production from the 1 μg/mL LPS-stimulated RAW 264.7 cells was determined after stimulation and intervention lasting for 24 hours by sandwich ELISA according to related kits instructions provided by manufacturer.

5. Statistical analysis

All data were expressed as mean ± standard deviation (SD). One-way ANOVA was used to determine difference among the groups. A value of $p<0.05$ was considered statistically significant.

Ⅲ. Results and discussion

1. Cell viability of FLJ

RAW 264.7 macrophages were used to assess the effects of FLJ on cell viability. FLJ extract showed cell viabilities from 125.06±6.72% to 79.47±10.23% in all concentration (10 - 10,000 μg/ml) (Fig. 1). Results of present study correspond with the results of earlier studies which reported that FLJ did not showed cytotoxicity[11] and it reversed LPS-induced toxicity in RAW 264.7 cells[12]. It means that FLJ showed the possibility to use by high concentration for further study.

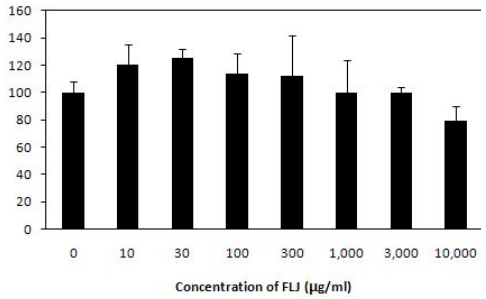

Fig. 1. The cell viability of flower of Lonicera japonica in RAW 264.7 cells. RAW 264.7 cells were treated with various concentration (10 - 10,000 μg/ml). The cell viability was determined by the MTT assay. Each column represents the mean±S.D. from three independent experiments.

2. Cell viability of FTF

RAW 264.7 macrophages were used to assess the effects of FTF on cell viability. FTF extract showed cell viabilities from 128.84±10.95% to 105.1±36.14% in all concentration (10 - 10,000 μg/ml) (Fig. 2). These results are in close agreement with the results of previous studies which reported that FLJ and Tussilagone, isolated from FLJ, have the inhibitory effects on the production of NO and PGE2 in RAW 264.7 cells[13,14]. Our results confirmed that the treatment concentration of FTF for further study.

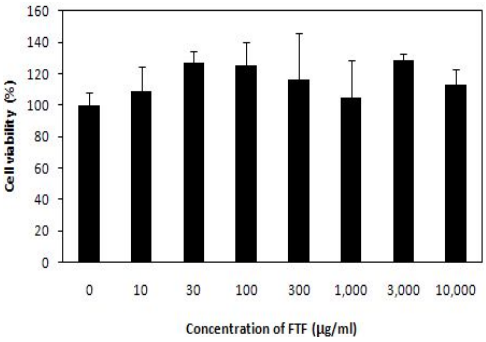

Fig. 2. The cell viability of flower of Tussilago farfara in RAW 264.7 cells. RAW 264.7 cells were treated with various concentration (10 - 10,000 μg/ml). The cell viability was determined by the MTT assay. Each column represents the mean±S.D. from three independent experiments.

3. Effects of FLJ and FTF on TNF-a releasing in RAW 264.7 cells

RAW 264.7 macrophages were used to assess the effects of FLJ and FTF on TNF-a releasing induced by LPS. FLJ and FTF extract did not inhibit TNF-a secretion in both concentration of treatment (Fig. 3).

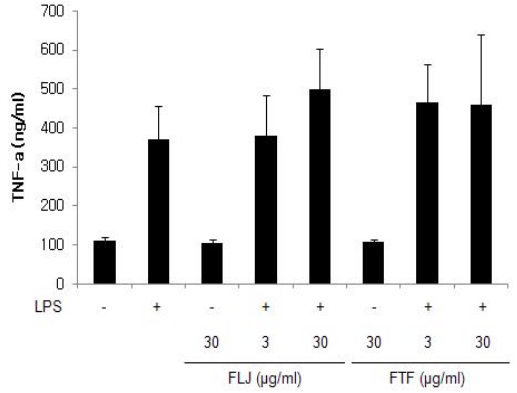

Fig. 3. The inhibitory effects of flower of Lonicera japonica (FLJ) and flower of Tussilago farfara (FTF) on TNF-a secretion in LPS induced RAW 264.7 cells. RAW 264.7 cells were treated with 3 and 30 μg/ml of FLJ or FTF. The productions of TNF-a were measured by ELISA. Each column represents the mean±S.D. from three independent experiments.

4. Effects of FLJ and FTF on IL-6 releasing in RAW 264.7 cells

RAW 264.7 macrophages were used to assess the effects of FLJ and FTF on IL-6 releasing induced by LPS. FLJ and FTF extract did not inhibit IL-6 secretion in both concentration of treatment (Fig. 4).

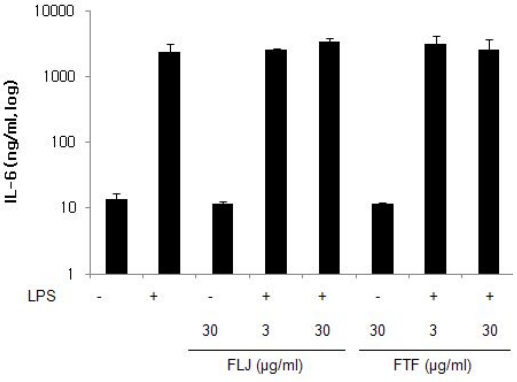

Fig. 4. The inhibitory effects of flower of Lonicera japonica (FLJ) and flower of Tussilago farfara (FTF) on IL-6 secretion in LPS induced RAW 264.7 cells. RAW 264.7 cells were treated with 3 and 30 μg/ml of FLJ or FTF. The productions of IL-6 were measured by ELISA. Each column represents the mean±S.D. from three independent experiments.

In spite of many experimental researches have proven the effectiveness of many herbal medicines on pulmonary disorders including asthma, the herbal medicines are seldom used for these applications. One of the reasons is the way herbal medicines are delivered. Its infusion form has bad flavor and is inconvenient to carry[15]. Generally, oral medicines have difficulty in maintaining consistent drug concentrations since individuals have different levels of drug absorption and metabolism. Also, a large quantity is required in order to reach an effective level of systemic drug concentration[16].

Inhalable systems provide an attractive way of delivering drugs, especially for many respiratory disorders including asthma. The advantages include the abilities to achieve high local (pulmonary) drug concentration and high bioavailability, and to extend the drug absorption time. Therefore, inhalable systems can achieve lung specific activity without increasing systemic side effects[17]. The inhalable systems can improve the challenges in herbal medicine delivery, thereby improving the safety and efficacy of herbal formula, and the patient compliance.

In previous studies, FLJ and fermented FLJ decreased neutrophils, TNF-a and IL-6 level on LPS induced COPD model[2,3]. But in our results FLJ did not inhibit TNF-a and IL-6 on LPS stimulated murine macrophages. We postulate that effects of FLJ on COPD model are due to various mechanism. However, this study has some limitations stemming from its

mere treatment time and dosage. So various attempts will be required to ascertain the effect on cytokines in inflammation. responses. FLJ inhibited expression of IL-8 and ICAM-1 on Th2 related cytokines induced A549 cells[5]. FTF inhibited the tracheal smooth muscle contraction induced by histamine[18], decreased IL-4, IL-5, IL-13, IgE and increased IFN-r in BALF of the OVA induced asthmatic mouse model[19]. The reports demonstrate that FLJ and FTF have possibility as subjects to development of inhalable formulation for drugs for respiratory diseases.

Ⅳ. Conclusion

Flower of Lonicera japonica (FLJ) and flower of Tussilago farfara (FTF) did not show cytotoxicity on RAW 264.7 cells. We suggest that FLJ and FTF may be useful drugs for respiratory disease. Future work will focus on the physical characteristics for inhalable formulation.

Acknowledgements

The authors are highly thankful to professor Yoon Yeo, Purdue University, for thoughtful comment and support for the experiment.

Reference

1. Kim SH, Lee KH, Lee YJ, An DK, Lee SI, Seo YB, et al. Herbalogy. 3rd ed. Seoul, Younglimsa, pp198-9, 482-3, 1995.
2. Lee DJ. Effects of Lonicera japonica on the murine model of chronic obstructive pulmonary disease. Seoul, Kyunghee Univ., 2009.
3. Kim YD. Effects of lactobacillus fermented flos lonicerae on the murine model of chronic obstructive pulmonary disease. Seoul, Kyunghee Univ., 2009.
4. Jung HJ, Park SK, Jung SK, Rhee HK. The inhibitory effects of Lonicerae Flos and Paeoniae Radix on IL-6, IL-16, GM-CSF mRNA level by BEAS-2B human epithelial cells. J Korean Oriental Med, 24(3):145-54, 2003.
5. Chung KJ, Jung HJ, Jung SK, Rhee HK. Lonicerae Flos contributes to the chemotaxis of eosinophils and secretion of cytokines in A549 human epithelial cells. Korean J Oriental Int Med, 26(1):129-42, 2005.
6. Suhr SS, Jung SK. Antiviral effects of fermented Lonicerae Flos on A type influenza virus. Korean J Oriental Int Med, 30(3):465-80, 2009.
7. Lee JS, Jung HJ, Jung SK, Rhee HK. The effects of Lonicerae Flos on bleomycin-induced lung fibrosis. Korean J Oriental Int Med, 25(1):71-80, 2004.
8. Namba T. The encyclopedia of Wakan-Yaku (Vol. 2). Tokyo, Hoikusha, p525, 1980.
9. Cho JS, Kim HY, Ryu JH, Jeong YS, Lee YS, Jin CB. Neuro-protective and antioxidant effects of the ethyl acetate fraction prepared from Tussilag farfara L. Biol Pharm Bull, 28:455-60, 2005.
10. Hillery AM, Lloyd AW, Swarbrick J. Drug delivery and targeting; for pharmacists and pharmaceutical scientists. London, TaylorandFrancis, p2, 2001.
11. Lee DE, Lee JR, Kim YW, Kwon YK, Byun SH, Shin SW et al. Inhibition of

lipopolysaccharide-inducible nitric oxide synthase, TNF-a, IL-1b and COX-2 expression by flower and whole plant of Lonicera japonica. Korean J Oriental Physiol & Pathol, 19(2):481-9, 2005.
12. Yun YG, Kim GM, Lee SJ, Ryu SH, Jang SI. Inhibitory effect of aqueous extract from Lonicera japonica flower on LPS-induced inflammatory mediators in RAW 264.7 macrophages. Korea J Herbology, 22(3):117-25, 2007.
13. Lee HJ, Kim JS, Jina C, Ryu JH. Inhibitory activity of medicinal herbs on nitric oxide synthesis in activated macrophages. Natural Product Sciences. 11(1):16-21, 2005.
14. Hwangbo C, Lee HS, Park J, Choe J, Lee JH. The anti-inflammatory effect of tussilagone, from Tussilago farfara, is mediated by the induction of heme oxygenase-1 in murine macrophages. Int Immunopharmacol. 9(13-14):1578-84, 2009.
15. Choi HJ, Bang NY, Song BW, Kim NJ, Rhyu BH. Survey on the preference for the dosage forms of oriental herbal medicine. Kyunghee Medicine, 20(1): 46-57, 2004.
16. Derendorf H, Nave R, Drollmann A, Cerasoli F, Wurst W. Relevance of pharmacokinetics and pharmacodynamics of inhaled corticosteroids to asthma. Eur Respir J, 28(5):1042-50, 2006.
17. Tayab ZR, Hochhaus G. Pharmacokinetic/ pharmacodynamic evaluation of inhalation drugs: application to targeted pulmonary delivery systems.Expert Opin Drug Deliv. 2005;2(3):519-32.
18. Han JH, Kang SY, Yu KS, Jin SS, Ha KH, Lee KJ. Farfarea Flos inhitits histamine-induced contractile reponses of airway smooth muscle. Korean J Oriental Int Med, 17(1):210-7, 1996.
19. Kwak SK, Choi SM, Park YC. Effect of Farfarae Flos extract drug on immune cells and cytokines in BALF of OVA-induced asthmatic mice. Korean J Oriental Pathol & Physiol, 19(3):716-21.