

## The Effect of Two Kinds of Chinese Medicine Monomer on the Expression of Surface Proteins in Antigen Presenting Cells

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### Abstract

**Gastrodin (GAS) is one of the rare traditional Chinese medicinal materials in our country and it has been widely used in the treatment of cardiovascular and cerebrovascular diseases. Baicalein is a traditional Chinese herbal medicine, the active ingredient of Scutellaria baicalensis Georgi has a wide range of pharmacological effects. In recent years Fan Jinghui et al found that baicalein has a significant anti-tumor effect. In this study, we used LPS as a positive control, different concentrations of gastrodin and baicalein respectively stimulated DC2.4 cell line and RAW264.7 cell line. After 48 hours, flow cytometry was used to detect the expression of CD80, CD86, MHCI, MHCII expression changes.**

### Keywords

**Gastrodin, Baicalein, Antigen presenting cell.**

### 1. Introduction

Gastrodin has a protective effect on the nervous system and cardiovascular system[1, 2]. It is a safe and effective traditional Chinese medicine monomer. Scutellaria baicalensis Georgi is one of the traditional Chinese medicine traditional Chinese medicine, mainly in Hebei, Shanxi and other places. Scutellaria bitter, cold, with heat and dampness, detoxification, miscarriage and other effects[3-5]. Baicalin as a commonly used traditional Chinese medicine has been received in the "China Pharmacopoeia", used for wet temperature, damp, stuffy chest vomiting, damp heat diarrhea, jaundice, abdominal fullness, lung heat cough, high fever, polydipsia, vomit blood, the treatment of carbuncle sore and fetal irritability and other symptoms[6, 7]. In modern clinic, it is mainly used for clearing away heat and toxic, water and damp pathogen, virus, mycoplasma and Chlamydia infection[8].

In vitro experiments of Yang et al. Showed that the tumor cells treated with baicalein could induce immune response and induce the death of tumor cells The anti-tumor immune response is mainly mediated by cellular immunity, and cell immune response priming requires APC present antigen to T cells, the proliferation of naive T cells activated to exert its antitumor activity, therefore, the study of APC cells in innate immunity is the basis of anti tumor immunity.

### 2. Treatment of drugs and DC2.4 and RAW264.7 cells

(1)LPS and gastrodin were dissolved in sterile double distilled water, and baicalein was dissolved with methanol and adjusted to 1 mg / ml. 1mg / ml of LPS was diluted to 6µg / ml with serum-free medium, and 1mg / ml of baicalein was diluted with serum-free medium into 10, 20, 40 and 80µg / ml by dilution method. 1 mg/ml of gastrodin was diluted with serum-free medium to 30, 60, 120 and 240 µg/ml.

(2)DC2.4 and RAW264.7 cells were cultured in a cell incubator containing 5% CO<sub>2</sub> in 37°C. When the cells were grown to logarithmic growth phase, DC2.4 and RAW264.7 cell suspension were prepared. The cell concentration was adjusted to 2×10<sup>6</sup>/ml, DC2.4 and RAW264.7 cells were plated in 96-well plates, each hole is 100µl.

(3)When the cells were in the logarithmic growth phase, we abandoned the old culture medium and then added the culture medium containing 10% fetal bovine serum, and added 20ul different concentrations of gastrodin and baicalein to each hole, 20 $\mu$ l of 6 $\mu$ g/ml LPS as a positive control, 20  $\mu$ l of medium containing 10% fetal bovine serum was added as a negative control, and the final volume of each solution was 120 $\mu$ l.

(4)The above-mentioned 96-well cell culture plates were labeled and placed in a 37 $^{\circ}$ C cell incubator containing 5% CO<sub>2</sub>.

### 3. Flow cytometry

(1)Cell culture 24,36 and 48 hours respectively in the incubator, the cells with PE anti-mouse H-2Kb (MHC-I), PE anti-mouse I-A/I-E (MHC-II), PE anti-mouse CD80, PE anti-mouse CD86 staining, stained at 4 $^{\circ}$ C for 30min.

(2)Washed once with PBS, discard the supernatant, add 1ml PBS to resuspend the cells and collected in 1.5ml centrifuge tube, gently spread with a pipetting.

(3)The sequence of four concentration gradients was tested in each antibody negative control, positive control, and drug treatment group using Guava Soft flow cytometry.

### 4. Statistical analysis.

All data are presented as the mean  $\pm$  standard deviation. Statistical analysis was performed using the Student's t-test and P<0.05 was considered to indicate a statistically significant difference

## 5. Results

### 5.1 GAS enhanced APC activation in vitro.

To determine the effect of GAS on APCs in vitro, DC line DC2.4 and macrophage line RAW264.7 were treated with different concentrations of GAS (30 ug/mL, 60 ug/mL, 120 ug/mL, 240 ug/mL) and LPS (positive control), and the expression levels of CD80, CD86, MHCI and MHCII in APCs were detected. Results showed that the expression levels of CD80 and MHCI were increased in DC2.4 line at the concentration of 60 ug/mL and 120 ug/mL of GAS compared to negative control; the expression levels of CD80, CD86, MHCII and MHCI were all increased in DC2.4 line at the concentration of 240 ug/mL of GAS compared to negative control. The expression levels of CD80, CD86, MHCI were up-regulated in RAW264.7 cell line at the concentration of 30 ug/mL, 60 ug/mL, 120 ug/mL and 240 ug/mL of GAS compared to negative control, while the expression levels of MHCII was not regulated at any concentration of GAS in RAW264.7 cell line compared to negative control (Fig.1). The average expression level of four surface proteins of DC2.4 cells stimulated by gastrodin was shown in Table 1 and the average expression level of four surface proteins of RAW264.7 cells stimulated by gastrodin was shown in Table 2

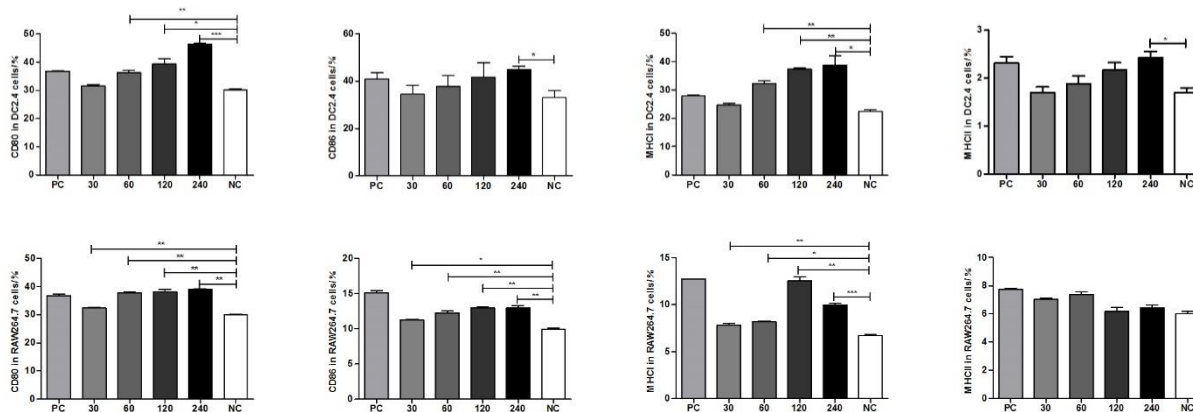


Fig 1. Effects of GAS on APC activation

Table 1. The average expression level of four surface proteins of DC2.4 cells stimulated by gastrodin

protein	PC	30	60	120	240	NC
CD80	36.76	31.53	36.27	39.27	46.44	30.11
CD86	41.08	34.52	37.87	41.77	44.93	33.13
MHCI	27.79	24.71	32.34	37.35	38.76	22.37
MHCII	2.31	1.71	1.89	2.17	2.43	1.71

Table 2. The average expression level of four surface proteins of RAW264.7 cells stimulated by gastrodin

protein	PC	30	60	120	240	NC
CD80	36.82	32.49	37.87	38.27	38.89	29.92
CD86	15.14	11.24	12.26	12.96	12.97	9.95
MHCI	12.75	7.80	8.18	12.54	9.97	6.73
MHCII	7.73	7.01	7.37	6.17	6.44	6.00

**5.2 Baicalein enhanced APC activation in vitro.**

the expression levels of CD80, CD86, MHC I and MHC II were increased in DC2.4 line and RAW264.7 line at the concentration of 80 ug/mL of GAS compared to negative control (Fig.2). The average expression level of four surface proteins of DC2.4 cells stimulated by baicalein was shown in Table 3. The average expression level of four surface proteins of RAW264.7 cells stimulated by baicalein was shown in Table 4

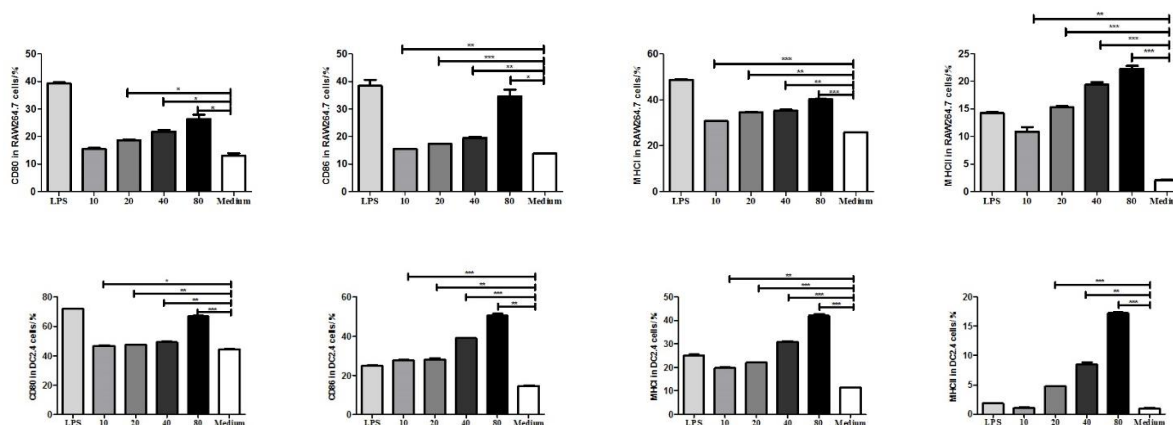


Fig 2. Effects of baicalein on APC activation

Table 3. The average expression level of four surface proteins of DC2.4 cells stimulated by baicalein

protein	LPS	10	20	40	80	Medium
CD80	39.25	15.54	18.69	21.91	26.36	13.14
CD86	38.37	15.47	17.43	19.56	34.68	13.87
MHCI	48.77	30.65	34.62	35.34	40.22	25.89
MHCII	14.27	10.93	15.31	19.41	22.26	2.11

Table 4. The average expression level of four surface proteins of RAW264.7 cells stimulated by baicalein

protein	LPS	10	20	40	80	Medium
CD80	71.94	46.95	47.41	49.41	67.05	44.56
CD86	25.08	27.61	28.27	39.01	50.45	14.79
MHCI	25.10	19.87	22.09	30.79	42.03	11.39
MHCII	1.86	1.06	4.77	8.51	17.20	0.97

## 6. Conclusion

The immune process can be divided into three steps: (1) APC processing and antigen presentation.(2) the activation of tumor antigen specific or non-specific T cells. (3) Antigen specific cytotoxic T cells (cytotoxic lymphocyte, CTL) migrate to the tumor site and exert specific anti-tumor effects As the most basic immune response cells[8-10], APC plays an important role in immune recognition, immune response and immune regulation, immune response and immune regulation in APC cells by the expression of surface protein important: CD80, CD8, MHCI, MHCII and IL-12 secretion is an important anti-tumor effect, TNF- etc play a role[11-13]. Therefore, the study of APC cells in innate immunity is the basis of anti-tumor immunity. So, in this experiment we study the traditional Chinese medicine monomer could enhance the immune activity of APC cells, the results showed that gastrodin and baicalein provided primary cell surface protein expression was enhanced in different degree, lay a theoretical foundation for the study of anti tumor

## References

- [1] Matias, M, et al. *Gastrodia elata* and epilepsy: Rationale and therapeutic potential. *Phytomedicine*, 2016. 23(12): p. 1511-1526.
- [2] Shen, C.Y, et al. Anti-ageing active ingredients from herbs and nutraceuticals used in traditional Chinese medicine: pharmacological mechanisms and implications for drug discovery. *Br J Pharmacol*, 2016.
- [3] Xiao, M.M, et al. Gastrodin protects against chronic inflammatory pain by inhibiting spinal synaptic potentiation. *Sci Rep*, 2016. 6: p. 37251.
- [4] Wang, X, et al. Gastrodin ameliorates memory deficits in 3,3'-iminodipropionitrile-induced rats: possible involvement of dopaminergic system. *Neurochem Res*, 2014. 39(8): p. 1458-66
- [5] Cai, Z, et al. Preparation and evaluation of sustained-release solid dispersions co-loading gastrodin with borneol as an oral brain-targeting enhancer. *Acta Pharm Sin B*, 2014. 4(1): p. 86-93.
- [6] Gong, W.Y, et al. Exploring the chemopreventive properties and perspectives of baicalin and its aglycone baicalein in solid tumors. *Eur J Med Chem*, 2017. 126: p. 844-852.
- [7] Gaire, B.P., S.K. Moon, and H. Kim. *Scutellaria baicalensis* in stroke management: nature's blessing in traditional Eastern medicine. *Chin J Integr Med*, 2014. 20(9): p. 712-20.
- [8] Lin, J.J, et al. A meta-analysis of trials using the intention to treat principle for glutamine supplementation in critically ill patients with burn. *Burns*, 2013. 39(4): p. 565-70.
- [9] Hrycek, E, et al. [Accelerated arteriosclerosis in systemic lupus erythematosus patients and the role of some adipocytokines in this process]. *Wiad Lek*, 2016. 69(6): p. 778-785.
- [10] Xu, L, et al. Click hybridization of immune cells and polyamidoamine dendrimers. *Adv Healthc Mater*, 2014. 3(9): p. 1430-8.
- [11] Merves, J, et al. Eosinophilic esophagitis. *Ann Allergy Asthma Immunol*, 2014. 112(5): p. 397-403.
- [12] Shen, W.J, S. Zhang, and H.S. Wong, An effective and efficient peptide binding prediction approach for a broad set of HLA-DR molecules based on ordered weighted averaging of binding pocket profiles. *Proteome Sci*, 2013. 11(Suppl 1): p. S15.
- [13] Zhang, X, et al. Plasmacytoid dendritic cells engagement by influenza vaccine as a surrogate strategy for driving T-helper type 1 responses in human neonatal settings. *J Infect Dis*, 2014. 210(3): p. 424-34.