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Original article

The Effect of MS14 on Production of Pro-inflammatory Cytokines by Macrophages

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Abstract

Objective(s)
Using herbal medicines as a complementary treatment method is increasing in wide variety of diseases. MS14-an herbal-marine preparation-is reported to have anti-inflammatory and immunomodulatory activities; however, the mechanism underlying its therapeutic effect is not known. Macrophages play an important role in host defense mechanisms and carry out their role by producing various mediators including proinflammatory cytokines (TNFα, IL-1β). In this study the effects of orally administered MS14 on TNFα and IL-1β production of BALB/c mice peritoneal macrophages were evaluated.

Materials and Methods
MS14 at 100 mg/kg was orally administered for 5 days to BALB/c mice in MS14 group. Sterile normal saline was administered to mice in control group. Peritoneal macrophage were isolated from control and MS14 groups and cultured, then the supernatants were collected and the cytokines IL-1β and TNFα were measured by ELISA test.

Results
Significant decrease in TNFα and IL-1β production of macrophages both at the presence and absence of stimulators was observed. TNFα levels were 64.7±4.6 and 51.1±4.2 pg/ml in drug and control groups respectively ($P< 0.05$) and 298.7±31.3 and 177.0±26.5 pg/ml in stimulated (PMA+fMLP) cultures of drug and control groups respectively ($P< 0.007$). The IL-1β levels was 130.1±2.8 pg/ml in control and 65.1±5.6 in MS14 group ($P< 0.000$).

Conclusion
It could be concluded that MS14 is able to cause a decline in some inflammatory responses of immune system, which could be considered as at least one of its immunomodulatory mechanisms.

Keywords: BALB/c mice, Interleukin-1beta (IL-1β), Macrophages, MS14, Tumor necrosis factor-alpha (TNFα)

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Introduction

Macrophages play an important role in host defense mechanisms, when activated; inhibit the growth of a wide variety of tumor cells and microorganisms (1). They produce some mediators such as NO and inflammatory cytokines; the most important amongst are tumor necrosis factor-alpha (TNFα) and Interleukin-1beta (IL-1β). TNF-α is produced by activated macrophages, fibroblasts, and many different types of cells and has also been recognized and well characterized as one of important defense molecules of body with potent proinflammatory effects (2). The cytokine IL-1β is produced during injury, inflammation, immunological challenge or infection, and contributes to the inflammatory response that may have an important effect on CNS diseases, such as multiple sclerosis and Alzheimer's disease (3). The biological activities make IL-1β the most closely related to TNFα, although the structure and receptors for IL-1β and TNFα are clearly distinct and belong to different families of molecules (3).

For many years, investigators have been looking for a drug which can regulate the immune system. Immunomodulators are very important in clinical medicine, since they can manipulate immune responses (4). Herbs have recently become attractive as health-beneficial foods and as a source material for the development of drugs (1, 5). Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, with relatively little knowledge regarding their modes of action (1).

MS14 is a natural (herbal-marine) product, contains 90% *Penaeus latisculatus* (king prawn), 5% *Apium graveolens* (Umbelliferae) and 5% *Hypericum perforatum* L. (St. John's Wort). This preparation is produced for using in treatment of multiple sclerosis patient symptoms (6) as well as ameliorating experimental allergic encephalomyelitis (7). It had no toxicity even in very high doses, and has been reported to possess anti-inflammatory effects (8). In the current study, we evaluated the effect of orally administered MS14 (100 mg/kg dose) on IL-1β and TNFα production of mice peritoneal macrophages.

Materials and Methods

Animals and MS14

Pathogen free 6–8 weeks old female BALB/c mice were obtained from Animal Lab of Shahed University. Mice were housed under normal laboratory conditions, with free access to standard rodent food and water.

MS14 powder was produced by Pharmacology Department of Shahed University (7). The MS14 powder (100 mg/kg) was dissolved in sterile normal saline and administered orally (the volume was 100 microliter) using feeding tube for 5 days to drug group mice. The mice in control group were administered the same volume of normal saline orally the same as drug group.

Preparation of peritoneal macrophages and cell cultures

After 5 days, the mice were anesthetized by diethyl ether, and then peritoneal macrophages were isolated from mice at sterile condition with peritoneal lavage using 10 ml cold normal saline. The collected cells were washed with RPMI 1640 (Sigma, USA) and counted. 4×10^5 cell/well were cultured on a flat-bottom 96-well culture plate and then incubated for 2 hr at 37 ^oC in a humidified 5% CO_2 incubator. After removing the nonadherent cells; the monolayered macrophages were remained in RPMI 1640 supplemented with 10% FBS (Sigma, EU) for 24 hr. The stimulators which have been added to half of samples included phorbol myristate acetate (PMA) (Merk, Germany) at final concentration of 25 ng/ml, N-formyl-met-Leu-phe (fMLP) (Sigma, EU) at final concentration of 50 μg/ml and lipopolysaccharide (LPS) (Sigma, USA) at final concentration of 10 μg/ml.

Immunoassay of TNFα and IL-1β

Supernatant of macrophage culture were collected after 12 hr for TNFα and after 20 hr for IL-1β. The amount of murine IL-1β and TNFα was measured by enzyme-linked immunosorbent assay (ELISA) according to the instructions of the manufacturer (Biosource, Switzerland). Briefly, a 96-well flat-bottomed microtiter plate was pre-coated overnight with an anti-mouse cytokine monoclonal antibody, followed by blocking and several washings and then the standards and samples (collected

supernatants from macrophage cultures) were added. After washings, the anti-mouse cytokine monoclonal antibody was added to each well and incubated for 2 hr. Substrate solution was then added, followed by the addition of stop solution, and the absorbance was read using a microtiter plate reader (ICNFlow, model: MK11) at wavelength at 450 nm.

Statistical analysis

Results were expressed as the mean±standard error. Statistical differences were assessed by the Student unpaired T-test, and $P< 0.05$ was considered statistically significant.

Results

MS14 at 100 mg/kg diminishes TNFα production of peritoneal macrophages

As shown in Figure 1, mouse peritoneal macrophages of MS14 administered group produced lower amounts of TNFα than control group (51.1±4.2 and 64.7±4.6 pg/ml respectively) which was statistically significant ($P< 0.05$). The same results were obtained with stimulated macrophages (PMA+fMLP) i.e. lower amounts of TNF-α were produced by peritoneal macrophages of MS14 group compared to the control group (298.7±31.3 and 177.0±26.5 pg/ml respectively) ($P< 0.007$). PMA-fMLP stimulates the TNFα production of macrophages about 6 times more than non-stimulated macrophages in control group and about 2.7 times in MS14 group.

MS14 at 100 mg/kg diminishes IL-1β production of peritoneal macrophages

IL-1β production of mice peritoneal macrophages in MS14 group (65.1±5.6, pg/ml), was reduced in comparison with the control group (130.1±2.8, pg/ml) ($P< 0.000$). As shown in Figure 2, the same results were obtained with stimulated macrophages (PMA+LPS) i.e. lower amounts of IL-1β were produced by peritoneal macrophages of MS14 group compared to the control group (47.8±4. and 142.0±4.07 pg/ml respectively, $P< 0.000$). The effect of stimulator on IL-1β production was minor but statistically significant i.e. about 9% increases in control group was observed. The amount of IL-1β production in stimulated macrophages of MS14 group was about 73% of non-stimulated macrophages.

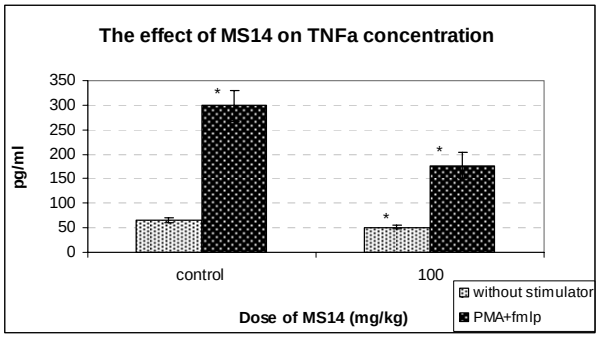

Figure 1. The effect of oral administration of MS14 (100 mg/kg) on TNF-α production of mouse peritoneal macrophages. The macrophages were cultured (4×10^5 cells in each well), and stimulators were added to half of the cultures and the TNFα concentration was assayed after 12 hr. All data are represented as means±SEM.

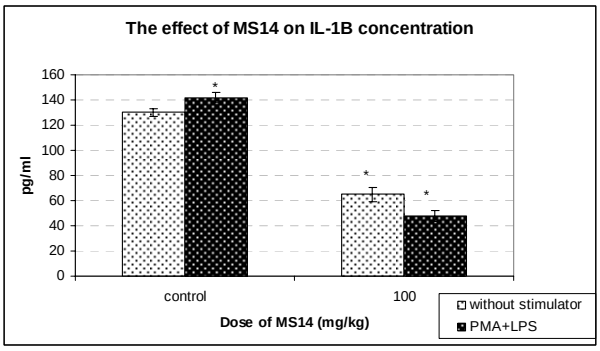

Figure 2. The effect of oral administration of MS14 (100 mg/kg) on IL-1β production of mouse peritoneal macrophages. The macrophages were cultured (4×10^5 cells in each well) stimulators were added to half of the cultures and the IL-1β concentration was assayed after 20 hr. All data are represented as means±SEM.

Discussion

Macrophages are a major cell population in the innate immune system. They play an important role in mounting inflammatory responses, by secreting a number of cytokines and chemokines (9). Activated macrophage produce potent pro-inflammatory cytokines such as tumor necrosis factor alpha (TNFα), and interleukin 1 (IL-1), these inflammatory cytokines are beneficial to the host defense, although they can also trigger pathological conditions when expressed in excess (10).

Unregulated levels of cytokines has been implicated as a potential pathogenic factor in the development of conditions associated with several chronic inflammatory diseases, including

type-2 diabetes, cardiovascular disease, inflammatory bowel disease, rheumatoid arthritis, major depression, and even normal aging (11). TNFα directly plays a fundamental role in pathology of diseases that cause inflammation; e.g. destruction and the co-morbidities associated with RA, and even TNF-alpha blockade is utilized as a treatment for RA (12). Also it has been shown in many researches that IL-1β is very important in SLE (13), MS (14), etc. Interestingly, IL-1β and TNFα cooperate in pathologic conditions most of the time. Thus regulation of these cytokines could be considered as an approach for treatment and inhibition of many autoimmune diseases.

Herbal medicine could be studied as a suitable candidate for this purpose. It assumed that some plant compounds are able to modulate these cytokines (15). MS14 was provided in Pharmacology Department of Shahed University, for use in treatment of MS patients. It has no undesired side effects on clinical symptoms of MS patients as claimed by Naseri and colleagues (6) and no significant toxic effects in rat when administered at very high doses (8). The study of Tafreshi and colleagues showed that MS14 ameliorated experimental allergic encephalomyelitis (7). As we have shown in another study, MS14 at 100 mg/kg dose and 5 days administration had not adverse effects on body weight and spleen index of BALB/c mice; and the histopathology study of liver, lung, bone marrow, lymph node, and spleen were normal; although lymphoid organs were more active than control group (16). In this study the effects of orally administered MS14 on TNFα and IL-1β production of BALB/c mice peritoneal macrophages were evaluated.

The results of our study shows that MS14 at 100 mg/kg and 5 days consecutive administration, significantly decreases TNFα production of macrophages at the presence or absence of stimulators, and similar results were obtained for IL-1β i.e. macrophages in MS14 group produced significantly lower amount of (less than half of) IL-1β compared to the control group. It means that MS14 by reducing the production of the most important pro-inflammatory cytokines has a potent anti-inflammatory effect and exerts its effect both on activated and resting or non-activated macrophages at the experiment's condition. The IL-1β production of stimulated macrophages in MS14 group was surprisingly lower than non-stimulated cells which may need further studies.

However, the exact mechanism responsible for the effects of MS14 remains unclear. There are some studies considering the immunoregulatory effect of some MS14 components alone. For example Kimberly and colleagues have shown that *Hypericum perforatum* inhibit prostaglandin E2 production (17) and Mencherini and colleagues have found that *Hypericum perforatum* inhibits iNOS synthase expression (18); both could be considered as anti-inflammatory effects. However, in experimental model of Candida sepsis, MS14 not only did not suppressed normal immune response, but by increasing the number of macrophages helped protection of the mice against sepsis (19). MS14 in another study caused significant increase in IL-5, IL-10 as well (20). Altogether it seems that MS14 does not exert a suppressing effect on immune system but it selectively downregulates some responses (mostly pro-inflammatory responses) and simultaneously activates some others (mostly anti-inflammatory responses) which in general may modulate the response to desired condition. Regarding these data MS14 could be considered as a good candidate for further studies on its potential effect in treatment of inflammatory diseases in which TNFα and IL-1 β have important pathogenic role.

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