OPEM
www.opem.org

Oriental Pharmacy and Experimental Medicine 2006 **6(1)**, 39-44

Effects of the water of yellow soil, Ji-Jang-Soo on cell viability and cytokines production in immune cells

Hyun-Ja Jeong[1,3], Gab-Soo Hwang[2], No-Il Myung[1], Joon-Ho Lee[1], Ju-Young Lee[1], Jae-Young Um[3], Hyung-Min Kim[3] and Seung-Heon Hong[1,*]

[1]College of Pharmacy, VCRC of Wonkwang University, Iksan, Jeonbuk, 570-749, South Korea; [2]School of Civil and Environmental Engineering, Kunsan National University, 68 Miryong-dong, Kunsan, Jeonbuk, 573-701, South Korea; [3]College of Oriental Medicine, Kyung Hee University, 1 Hoegi-Dong, Dongdaemun-Gu, Seoul, 130-701, South Korea

SUMMARY

Ji-Jang-Soo (JJS) is known to have a detoxification effect. However, it is still unclear how JJS has these effects in experimental models. In this study, we investigated the effect of JJS on the viability of cells and production of cytokines in human T-cell line, MOLT-4 cells, and human mast cell line, HMC-1 cells. The MOLT-4 cells were cultured for 24 h in the presence or absence of JJS. As the result, JJS (1/100 dilution) significantly increased the cell viability about 78% ($P < 0.05$) and also increased the interleukin (IL)-2, and interferon (IFN)-γ production compared with media control at 24 h. But had no effect on IL-4 production. Hypoxia mimic compound, desferroxamine (DFX) decreased the immune cell viability. Cell viability decreased by DFX was increased by JJS. In conclusion, these data indicate that JJS may have an immune-enhancing effect.

Key words: Ji-Jang-Soo; Cell viability; Cytokines; Immune-enhancing effect

INTRODUCTION

Yellow soil, which covers about 10% of the surface of the earth, includes a large quantity of $CaCo_3$. Yellow soil has a feature that it is not broken easily and has stickiness because of the chemistry of $CaCo_3$. Also it is turned into clay with the addition of water. Clay consists of SiO_2, Al_2O_3, Fe, Mg, Na, and K etc. As yellow soil that is fulfilled these elements ratio and various enzymes radiates the ultrared ray, it is called alive life. About two hundred million of microorganisms live at one spoon, which causes wide variety of a circular function. The four component of Yellow soil are catalase, dephenol oxydase, protease and saccharase. These enzymes play a role of elimination of the toxin, decomposition, fertilizer, and action of purification.

Ji-Jang-Soo (JJS) indicates the yellow water of yellow soil. After digging a 60 cm hole of the sunny ground or the deep mountain ridge, and mix it with suitable ratio of water and give several stirs. After adequate time pass, pale yellow water float, this water is called JJS. A property of JJS is cold and sweet. Although JJS has been used for treatment of various diseases in Korea, its outstanding effect on various other diseases has not been investigated experimentally.

T-cells play a crucial role in immune functions as they act both as effectors (cytotoxic T-cells, Tc cells) and regulators (helper and suppressor T-cells, Th

*Correspondence: Seung-Heon Hong, College of Pharmacy, VCRC of Wonkwang University, Iksan, Jeonbuk, 570-749, South Korea. Tel: +82-63-850-6805; Fax: +82-63-843-3421; E-mail: jooklim@wonkwang.ac.kr

and Ts cells). Tc cells can kill virus-infected cells and cells that undergo malignant transformation. Their activation depends on antigen challenge and signals sent from activated Th cells. Th cells mediate the link between antigen-presenting and the triggering of other cellular (natural and lymphokine-activated killer cells, macrophages, granulocytes) and humoral (B cell-produced antibodies) components of the immune response (Riddell *et al.*, 2002). Especially, Th cells are known to have two different subsets, Th1 and Th2. They are distinguished by cytokines they secret (Mosmann and Coffman, 1989; Prete *et al.*, 1991). Th1 lymphocytes produce interleukin (IL)-2, interferon (IFN)-γ and tumor necrosis factor (TNF), which promote cell-mediated immunity. Th2 lymphocytes produce IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13, which promote humoral antibody-mediated immune response (Carter and Dutton, 1996; Stephens *et al.*, 2002). As described above, various cytokines such as IFN-γ, IL-2, IL-4, IL-12 and TNF-α are related to immune reaction, directly or indirectly.

Mast cells are derived from $CD34^+$ hematopoietic progenitor cells, such as those present in adult bone marrow, mature mast cells typically do not circulate in the blood but complete their differentiation in vascularized tissues. The ability of mast cells to substantially enhance the recruitment of neutrophils in the context of bacterial infection has been well documented. Mast cells play a role of immune response (Galli *et al.*, 1991).

Hypoxia is an essential developmental and physiological stimulus that plays a key role in the pathophysiology of heart disease, cancer, neuron death, cerebrovascular disease and chronic lung disease, which presented the most common causes of mortality in western cultures (Semenza, 2001).

To investigate the effect of JJS on cytokines production in the present study, we analyzed the production of IL-2, IL-4, and IFN-γ on the JJS treated MOLT-4 cells. We also examined the effect of JJS on the viability of desferroxamine (DFX)-treated human mast cell line, HMC-1.

MATERIALS AND METHODS

Reagents

Avidin-peroxidase, DFX, and 2'-AZINO-bis (3-ethylbenzithiazoline-6-sulfonic acid) tablets substrate were purchased from Sigma (St. Louis, MO, USA). RPMI 1640, ampicillin, streptomycin and fetal bovine serum (FBS) were purchased from Gibco BRL (Grand Island, NY, USA). Anti-human IL-2, 4, and IFN-γ, biotinylated anti-human IL-2, 4, and IFN-γ, and recombinant (r) human IL-2, 4, and IFN-γ were purchased from R & D Systems (Minneapolis, MN, USA).

MOLT-4 cell culture

The T cell line, MOLT-4 was used in this study. The cells were maintained in RPMI-1640 medium (Gibco BRL, USA) with 10% FBS (JRH BIOSCIENCE, USA) at 37°C under 5% CO_2 in the air.

HMC-1 cell culture

The cells were maintained in IMDM medium (Gibco BRL, USA) with 10% fetal bovine serum (JRH BIOSCIENCE, USA) at 37°C under 5% CO_2 in air. HMC-1 cells suspensions (3×10^5 cells) were stimulated with DFX for 24 h.

Preparation of JJS

We prepare an earthenware pot for JJS and then put yellow soil into an earthenware pot. The earthenware spot full with water and then stir it enough in order to make it mixed well, and wait for about 1 - 2 h. Supernatant (JJS) was used at this study.

MTT assay

Cell aliquots (3×10^5) were seeded in microplate wells and incubated with 20 μl of a MTT solution (5 mg/ml) for 4 h at 37°C under 5% CO_2 and 95% air. Consecutively, 250 μl of DMSO were added to extract the MTT formazan and an automatic microplate reader read the absorbance of each well at 540 nm.

Enzyme-linked immunosorbent assay (ELISA) of cytokines

Sandwich ELISA for IL-2, IL-4, and IFN-γ was carried out in duplicate in 96-well ELISA plates (Nunc, Denmark) coated with each of 100 μl aliquots of anti-human IL-2, IL-4, and IFN-γ monoclonal antibodies at 1.0 μg/ml in PBS at pH 7.4 and was incubated overnight at 4°C. The plates were washed in PBS containing 0.05% Tween-20 (Sigma, St. Louis, MO, USA) and blocked with PBS containing 1% BSA, 5% sucrose and 0.05% NaN_3 for 1 h. After additional washes, sample or IL-2, IL-4, and IFN-γ standards were added and incubated at 37°C for 2 h. After 2 h incubation at 37°C, the wells were washed and then each of 0.2 μg/ml of biotinylated anti-human IL-2, IL-4, and IFN-γ were added and again incubated at 37°C for 2 h. After washing the wells, avidin-peroxidase was added and plates were incubated for 20 min at 37°C. Wells were again washed and ABTS substrate was added. Color development was measured at 405 nm using an automated microplate ELISA reader. A standard curve was run on each assay plate using recombinant IL-2, IL-4, and IFN-γ in serial dilutions.

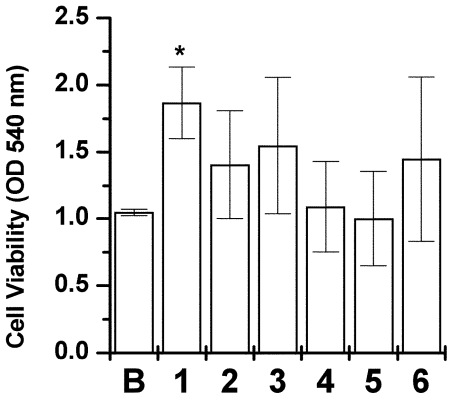

Fig. 1. Effect of JJS on the cell viability. MOLT-4 cells (3×10^5) were treated with various concentrations of JJS or DW for 24 h. Cells were then collected and assessed for viability using MTT. Values are the mean ± SEM of duplicate determinations from three separate experiments ($P < 0.05$). B, untreated; 1, JJS 1/100 dilution; 2, JJS 1/50 dilution; 3, JJS 1/25 dilution; 4, DW 1/100 dilution; 5, DW 1/50 dilution; 6, DW 1/25 dilution.

Statistical analysis

The experiments shown are a summary of the data from at least-three experiments and are presented, as the mean ± SEM. Statistical significance of the data was determined using the ANOVA with Tukey post hoc test; a value of $P < 0.05$ was accepted as statistically significant.

RESULTS

Effect of JJS on the viability of MOLT-4 cells

T-cells play a crucial role in immune functions as they act both as effectors and regulators (Riddell *et al.*, 2002). To assess the effect of JJS on the viability of T cell, we performed MTT assay. As a result, JJS increased the viability of MOLT-4 about 78% (unstimulated cells, 1.05 ± 0.02; JJS 1/100 dilution, 1.87 ± 0.27, $P < 0.05$). Distilled water (DW) did not affect the cell viability.

Effect of JJS on the production of IL-2, IL-4 and IFN-γ on MOLT-4

To assess the effects of JJS on the production of various cytokines, MOLT-4 cells were treated with various concentrations of JJS for 24 h. The levels of IL-2, IL-4 and IFN-γ were analyzed by ELISA method. As shown in Fig. 2, JJS increased the IL-2 production compared with media control (0.16 ± 0.04 ng/ml) in a dose-dependent manner (0.24 ± 0.05 ng/ml at 1/25 dilution). JJS also significantly increased IFN-γ production (0.43 ± 0.06 ng/ml for IFN-γ) compared with media control and DW (0.11 ± 0.07 ng/ml for media control and 0.31 ± 0.13 ng/ml for DW, $P < 0.05$). But JJS had no effect the IL-4 production. IFN-γ production showed quite a complicated manner in dependency on JJS concentrations. Their productions tended to be increased at low concentrations of JJS, but they decreased at high concentrations.

Effect of JJS on the viability of DFX-stimulated mast cells

To assess the effect of JJS on the viability of DFX-

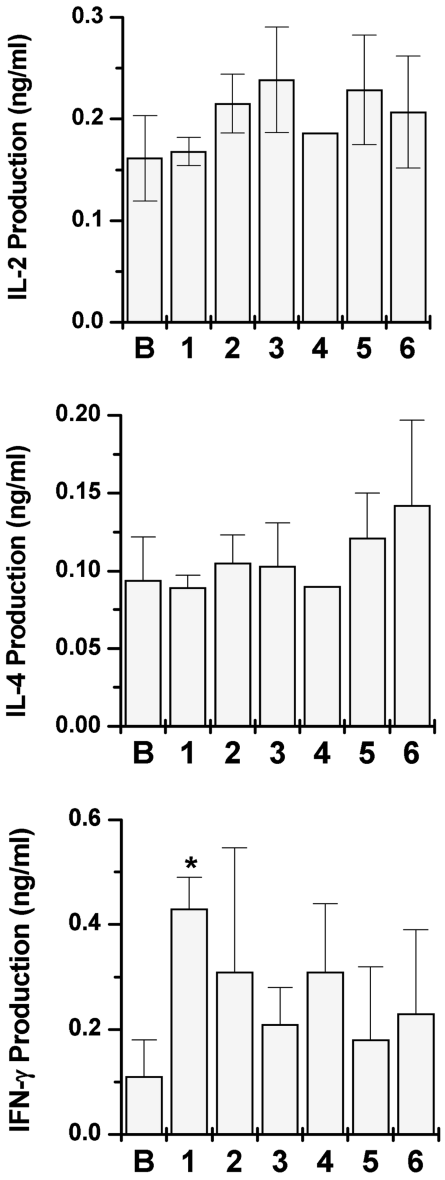

Fig. 2. Effect of JJS on IL-2, IL-4 and IFN-γ production in the MOLT-4 cells. Culture supernatant was collected from none or JJS treated MOLT-4 cells, which were cultured for 24 h. Cytokines levels in culture supernatant were measured using ELISA. ($^{*}P < 0.05$). B, untreated; 1, JJS 1/100 dilution; 2, JJS 1/50 dilution; 3, JJS 1/25 dilution; 4, DW 1/100 dilution; 5, DW 1/50 dilution; 6, DW 1/25 dilution.

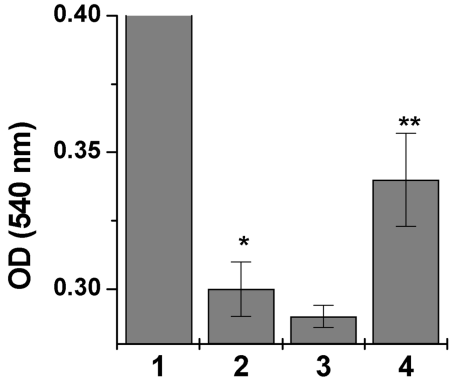

Fig. 3. Effect of JJS on the cell viability. HMC-1 cells (3×10^5) were treated with JJS or DW for 1 h and then stimulated with DFX for 24 h. Cells were then collected and assessed for viability using MTT. Values are the mean ± SD of duplicate determinations. 1, unstimulated cells; 2, DFX; 3, DFX + DW; 4, DFX + JJS. $^{*}P < 0.05$: significantly different from the unstimulated cells, $^{**}P < 0.05$: significantly different from the DFX-treated cells.

treated mast cell, we performed MTT assay. As shown in Fig. 3, the levels of cell viability in DFX-treated mast cells decreased. But JJS significantly increased the viability compared with DFX-treated mast cells. Distilled water had no effect on the viability of DFX-treated mast cells.

DISCUSSION

JJS has long been used for arthritis, muscle pain, back pain and autonomic ataxia. JJS have a good effect for skin care because it dissolves impurities and have autopurification. JJS hasten blood and promote metabolism. JJS remove the toxin of the interior body and relieve a pain. JJS also eliminate the inflammation and suppress the growth of cancerous cells. In the present study, we showed that JJS increased the viability of T cells and mast cells. JJS also induced the production of IL-2 and IFN-γ.

The activation of tumor antigen-specific Th and Tc cells or non-specific macrophages and natural killer (NK) cells using immunotherapeutic approaches may lead to the subsequent destruction of tumor tissue (Dredge *et al.*, 2002). Previous reports have demonstrated that the induction of Th1-promoting

cytokine, using specific adjuvants, can enhance anti-tumor immunity and can reduce or even prevent tumor growth (Dredge *et al.*, 2002). The immune response can be broadly categorized into a cellular or humoral mediated response. The production of IL-2, IFN-γ, TNF-α and IL-12 lead to a Th1-type cellular response, while production of IL-4 and IL-6 lead to Th2-type humoral immunity (Parronchi *et al.*, 1991; Romagnani, 1991; Zurwski and Vries, 1994). Many cancer vaccines, particularly in combination with immune adjuvants, elicit strong cellular immune responses leading to the production of Th1 type cytokines such as IL-2, IFN-γ, TNF-α and IL-12 (Dalgleish, 2000). First of all, IL-2 cytokine (also known as T-cell growth factor) has multiple immunoregulatory functions and biological properties. IL-2, together with other factors and in conjunction with antigens, mitogens, or anti-immunoglobulin antibodies, controls B cell proliferation and differentiation into antibody-producing plasma cells (Jelinek and Lipsky, 1987). NK and lymphokine-activated killer cells, monocytes and macrophages all have the ability to respond to IL-2 with increased activity or proliferation (Kuziel and Greene, 1991; Minami *et al.*, 1992). IFN-γ is also an important cytokine in the host defense against infection by viral and microbial pathogens (Samuel, 2001). IFN-γ induces a variety of physiologically significant responses that contribute to immunity. IL-12, which is primarily produced by activated macrophages, stimulates T cells and NK cells. It induces IFN-γ and plays a role in promoting Th1 cell responses. Others reported IFN-γ produced by Th1 cells inhibits the development of Th2 cells as well as humoral responses, whereas the production of IL-4 by Th2 cells inhibits development and activation of Th1 cells (Peleman *et al.*, 1989; Fitch *et al.*, 1993). Previously, we reported that levels of Th2 cytokine were higher than that of Th1 cytokine in various diseases including cerebral infarction (CI), allergy and asthma (Kim *et al.*, 2000; Jeong *et al.*, 2002). In the present study, we found JJS increased the production of Th1 cytokines. These results suggest that JJS might have a beneficial effect in the treatment of various diseases (CI, asthma and cancer) through the immune-enhancement.

Mast cells are strategically stationed at sites such as lung, skin, gastrointestinal and urogenital tract. Mast cells have been implicated in the expression of a wide variety of immune responses, including immediate hypersensitivity, host responses to parasites and neoplasms, angiogenesis, tissue remodeling, and immunologically non-specific inflammatory, and fibrotic conditions (Galli *et al.*, 1991). Mast cells express complex- and partially overlapping-roles in acquired and innate immunity.

Cells undergo numerous changes in gene transcription, enzyme activities, and mitochondrial function in response to hypoxia (Li and Jackson, 2002). On exposure to severe hypoxia beyond cellular adaptive capability, immune cells are irreversibly injured and die in necrosis or apoptosis (Malhotra *et al.*, 2001). Iron chelator, DFX induced the hypoxia probably by replacing or removing the central iron of the putative heme oxygen sensor. In this study, we showed that hypoxia induced by DFX induced the apoptosis of immune cells. JJS inhibited the DFX-induced immune cell apoptosis. JJS contained SiO_2, Al_2O_3, Fe, Mg, Na, and K etc. Therefore, we supposed that JJS inhibited the hypoxia-induced apoptosis by maximization of oxygen utilization.

In conclusion, our results demonstrate that JJS increases the Th1 cytokines production. And this encourages us to suggest that JJS has an immune-enhancement effect. Further study would be necessary to clarify the role of the cytokines increased by JJS.

ACKNOWLEDGEMENTS

This work was supported by the Association of Purple Bamboo Salt.

REFERENCES

Carter LL, Dutton RW. (1996) Type 1 and type 2: a fundamental dichotomy for all T-cell subsets. *Curr.*

Opin. Immunol. **8**, 336-342.
Dalgleish AG. (2000) Cancer vaccines. *Br. J. Cancer* **82**, 1619-1624.
Del Prete GF, De Carli M, Mastromauro C, Biagiotti R, Macchia D, Falagiani P, Ricci M, Romagnani S. (1991) Purified protein derivative of Mycobacterium tuberculosis and excretory-secretory antigen(s) of Toxocara canis expand in vitro human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. *J. Clin. Invest.* **88**, 346-350.
Dredge K, Marriott JB, Todryk SM, Dalgleish AG. (2002) Adjuvants and the promotion of Th1-type cytokines in tumour immunotherapy. *Cancer Immunol. Immunother.* **51**, 521-531.
Dredge K, Marriott JB, Todryk SM, Muller GW, Chen R, Stirling DI, Dalgleish AG. (2002) Protective antitumor immunity induced by a costimulatory thalidomide analog in conjunction with whole tumor cell vaccination is mediated by increased Th1-type immunity. *Immunol.* **168**, 4914-4919.
Fitch FW, Mckisic MD, Lancki DW, Gajewski TF. (1993) Differential regulation of murine T lymphocyte subsets. *Annu. Rev. Immunol.* **11**, 29-48.
Galli SJ, Gordon JR, Wershil BK. (1991) Cytokine production by mast cells and basophils. *Curr. Opin. Immunol.* **3**, 865-872.
Jelinek DF, Lipsky PE. (1987) Regulation of human B lymphocyte activation, proliferation, and differentiation. *Adv. Immunol.* **40**, 1-59.
Jeong HJ, Kim BS, Kim KS, Kim HM. (2002) Regulatory effect of cytokine production in asthma patients by SOOJI CHIM (Koryo Hand Acupuncture Therapy). *Immunopharmacol. Immunotoxicol.* **24**, 265-274.
Kim HM, Shin HY, Jeong HJ, An HJ, Kim NS, Chae HJ, Kim HR, Song HJ, Kim KY, Baek SH, Cho KH, Moon BS, Lee YM. (2000) Reduced IL-2 but elevated IL-4, IL-6, and IgE serum levels in patients with cerebral infarction during the acute stage. *J. Mol. Neurosci.* **14**, 191-196.
Kuziel WA, Greene WC. (1991) Interleukin-2. In A. Thomsen (Ed), The cytokine handbook, Academic Press, London, pp. 83-102.
Li C, Jackson RM. (2002) Reactive species mechanisms of cellular hypoxia-reoxygenation injury. *Am. J. Physiol.* **282**, C227-C241.
Malhotra R, Lin Z, Vincenz C, Brosius FC. (2001) Hypoxia induces apoptosis via two independent pathways in Jurkat cells: differential regulation by glucose. *Am. J. Physiol.* **281**, 1596-1603.
Minami Y, Kono T, Yamada K, Taniguchi T. (1992) The interleukin-2 receptors: insights into a complex signalling mechanism. *Biochim. Biophys. Acta* **1114**, 163-177.
Mosmann TR, Coffman RL. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* **7**, 145-173.
Parronchi P, Macchia D, Piccinni MP, Biswas P, Simonelli C, Maggi E, Ricci M, Ansari AA, Romagnani S. (1991) Allergen- and bacterial antigen-specific T-cell clones established from atopic donors show a different profile of cytokine production. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 4538-4542.
Peleman R, Wu J, Fargeas C, Delespesse G. (1989) Recombinant interleukin 4 suppresses the production of interferon gamma by human mononuclear cells. *J. Exp. Med.* **170**, 1751-1756.
Riddell SR, Murata M, Bryant S, Warren EH. (2002) T-cell therapy of leukemia. *Cancer Control* **9**, 114-122.
Romagnani S. (1991) Human TH1 and TH2 subsets: doubt no more. *Immunol. Today* **12**, 256-257.
Samuel CE. (2001) Antiviral actions of interferons. *Clin. Microbiol. Rev.* **14**, 778-809.
Semenza GL. (2001) Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology. *Trends Mol. Med.* **7**, 345-350.
Stephens R, Eisenbarth SC, Chaplin DD. (2002) T helper type 1 cells in asthma: friend or foe? *Curr. Opin. Allergy Clin. Immunol.* **2**, 31-37.
Zurawski G, de Vries JE. (1994) Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. *Immunol. Today* **15**, 19-26.