Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Ethnopharmacology 90 (2004) 253–259
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Effect of Kuibitang on lipopolysaccharide-induced cytokine production in peripheral blood mononuclear cells of chronic fatigue syndrome patients

Hye-Young Shin [a,b], Nyeon-Hyoung An [b], Yun-Jin Cha [c], Eun-Ju Shin [d], Tae-Yong Shin [e], Seung-Hwa Baek [f], Cheorl-Ho Kim [g], Yeung-Su Lyu [h], Eon-Jeong Lee [h], Hyung-Min Kim [a,*]

[a] *Department of Pharmacology, College of Oriental Medicine, Kyung Hee University, 1 Hoegi-Dong, Dongdaemun-Gu, Seoul 130-701, South Korea*
[b] *College of Pharmacy, VCRC of Wonkwang University, Iksan, Jeonbuk 570-749, South Korea*
[c] *SinnongC Oriental Clinic, Seongdonggu, Seoul, South Korea*
[d] *Department of Health Science, Graduate School of Public Health and Environment, Wonkwang University, Iksan, South Korea*
[e] *College of Phamacy, Woosuk University, Jeonju, Jeonbuk 565-701, South Korea*
[f] *Department of Herbal Resources, Professional Graduate School of Oriental Medicine, Wonkwang University, Iksan 570-749, South Korea*
[g] *Department of Biochemistry and Molecular Biology, College of Oriental Medicine, #707 Sukjang-Dong, Kyung-Ju, Kyungpook 780-714, South Korea*
[h] *College of Oriental Medicine, Wonkwang University, Iksan, Jeonbuk 570-749, South Korea*

Received 9 January 2003; received in revised form 18 June 2003; accepted 7 October 2003

Abstract

Kuibitang (KBT) is clinically used to treat patients suffering from chronic fatigue syndrome (CFS) in South Korea. However, its effect has not been investigated experimentally. Recent reports have shown that CFS patients display an altered cytokine production. We examined the effect of KBT on lipopolysaccharide (LPS)-induced various cytokines production in peripheral blood mononuclear cells (PBMC) of CFS patients and healthy controls. KBT (1 mg/ml) significantly inhibited LPS-induced tumor necrosis factor-α, interleukin-10, and transforming growth factor-β1 production in PBMC of CFS patients. However, LPS-induced interferon-γ production was significantly increased by KBT (0.01 mg/ml). These results provide evidence of a novel activity of the KBT that regulate cytokines production related with CFS.

Keywords: Kuibitang; Lipopolysaccharide; Cytokine; Chronic fatigue

1. Introduction

The chronic fatigue syndrome (CFS) is characterized by unexplained chronic fatigue and pain. Although the pathogenesis of this disease is poorly understood, the Centers for Disease Control has estimated that 500,000 Americans are affected by the disease. The etiology of CFS remains elusive, although some studies have suggested a role of immune dysfunction (Visser et al., 1998; Cannon et al., 1997; Bennett et al., 1997; Buchwald et al., 1997; Levy, 1994). Several reports have shown that CFS patients display an altered functioning of the immune response; i.e., altered cytokine production, low NK-cell function, and differences in the expression of activation markers on lymphocytes (Bearn and Wessely, 1994; Strauss et al., 1993, 1994; Aoki et al., 1993; Caligiuri et al., 1987; Barker et al., 1994; Swanink et al., 1993, 1996; Klimas et al., 1990; Chao et al., 1991; Mawle et al., 1997; Hassan et al., 1998; Tirelli et al., 1994). Others have suggested that an antiviral pathway in CFS patients is upregulated as the consequence of an increased viral reactivation in CFS patients (Visser et al., 1998; Landay et al., 1991); however, until now no infectious agent could be linked to the syndrome.

Cytokines have been suggested to play a role in the pathogenesis and clinical manifestations of CFS (Levy, 1994; Moutschen et al., 1994). CFS is an autoimmune disorder in which activated Th cells and different Th cell cytokine might play an important role. Th cells are divided into three main subset, Th1, Th2, Th3 cells. Th1 cells produce TNF-α, IFN-γ whereas Th2 cells release IL-4 and IL-10 (Romagnani et al., 1997). A characteristic of Th3 cells is their production of the immune-modulating cytokine TGF-β1 (Chen et al., 1994; Fukaura et al., 1996). TGF-β1 inhibits T-cell proliferation and suppresses some Th1 and Th2 cell-mediated autoimmune diseases (Kehrl et al., 1986; Holter et al., 1994; Mosmann and Sad, 1996; Bridoux et al., 1997). A large

* Corresponding author. Tel.: +82-63-850-6805; fax: +82-63-843-3421.
E-mail address: hmkim@khu.ac.kr (H.-M. Kim).

0378-8741/$ – see front matter © 2003 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2003.10.006

number of immune abnormalities, including cytokine production in CFS, have been reported (Klimas et al., 1990; Buchwald and Komaroff, 1991; Gupta and Vayuvegula, 1991; Gupta, 1992; Jone, 1991; Lloyed et al., 1989; Straus et al., 1989). Recent insights into autoimmune type disease have suggested a pivotal role for the pro-inflammatory cytokine tumor necrosis factor (TNF)-α. TNF-α has been shown to have a variety of in vivo effects including pain, inflammation and fatigue (Krakauer et al., 1999). Interferon (IFN) has been used therapeutically to treat a number of diseases, and the therapy is associated with a number of adverse effects, including fatigue (Dusheiko, 1997). IL-10 is a cytokine that is produced by a variety of cell types, including Th2 type T cells (Howard et al., 1992). IL-10 inhibits cytokines produced by Th1 type T cells, including IL-2 and IFN-γ. Furthermore, IL-10 inhibits monokine production by activated macrophages, including IL-6 and TNF-α (Fiorentino et al., 1991). Transforming growth factor (TGF)-β1 is expressed as a large pro-protein (390–412 amino acids), which includes TGF-β1 as its C-terminus, and TGF-β1 latency associated peptide (LAP) at its N-terminus, TGF-β1 remains in covalent association with LAP due to limited intracellular proteolytic cleavage (Lopez et al., 1992). It appears that during both in vivo and in vitro mycobacterial infections at least some TGF-β1 produced by mononuclear cells is detected in its active form (Aung et al., 2000; Hirsch et al., 1994). Most recently it was shown that TGF-β1 is one of the key negative regulators of immune homeostasis and its absence leads to activation of a self-targeted immune response (Gorelik and Flavell, 2000).

Recently Kuibitang (KBT) has been successfully used for regulation of the immune response in South Korea (Lim et al., 1999). KBT consists of 13 different herbs. This prescription was also composed on the basis of the theory of Oriental medicine to maximize its efficacy. Some main components they are known to have are as follows; saponins, glycosides, and polysaccharides etc (Matsuda et al., 1999; Ye et al., 2001).

Based upon the clinical presentation of CFS, we hypothesized that cytokines may play a role in the pathogenesis of the disease. In the present study, we investigated the effect of KBT on lipopolysaccharide (LPS)-induced cytokines production in PBMC of eight CFS patients and five healthy controls. Our data demonstrated that KBT treatment inhibited production of TNF-α, IL-10, TGF-β1 and increased production of IFN-γ in PBMC of CFS patients.

2. Materials and methods

2.1. Reagents

Ficoll-Hypaque, LPS, bovine serum albumin (BSA), abidin-peroxidase and 2-azino-bis(3-ethylbenzithiazoline-6-sulfonic acid) tablets substrate (ABTS) 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). IL-6, IL-10, TGF-β1 and recombinant human IL-6, IL-10, TGF-β1 were purchased from Pharmingen (San Diego, CA, USA). Anti-human TNF-α, IFN-γ and recombinant human TNF-α, IFN-γ were purchased from R & D Systems Inc. (Minneapolis, MN, USA).

2.2. Preparation of KBT

The plant materials were obtained from the Wonkwang Oriental Medicine Hospital (Chonju, Chonbuk) and authenticated by Professor Y.S. Lyu, College of Oriental Medicine, Wonkwang University. A voucher specimen (number 02-03-21) was deposited at the Herbarium of the College of Pharmacy, Wonkwang University. An extract of KBT was prepared by decocting the dried prescription of herbs with boiling distilled water. The duration of decoction was about 3 h. The decoction was filtered, lyophilized and kept at 4 °C. The yield of extraction was about 14% (w/w). The KBT water extract powder was dissolved in sterile saline (50 mg/ml). The ingredients of KBT include 4 g of Angelicae Gigantis Radix, 4 g of Longanae Arilus, 4 g of Zizyphi Spinosi Semen, 4 g of Polygalae Radix, 4 g of Ginseng Radix Alba, 4 g of Astragali Radix, 4 g of Atractylodis Rhizoma Alba, 4 g of Poria, 4 g of Prunellae Spica, 2 g of Saussureae Radix, 1.2 g of Araliae Cordatae Radix, five piece of Zingiberis Rhizoma, two piece of Zizyphi Fructus. These ingredients correspond to parts of the following plants: *Angelica gigas* NAKAI (Umbelliferae), *Euphoria longan* (LOUR.) STEUD. (Sapindaceae), *Ziziyphus spinosa* HU. (Rhamnaceae), *Polygala tenuifolia* WILLD. (Polygalaceae), *Panax ginseng* C.A. MEY. (Araliaceae), *Astragalus membranaceus* BUNGE (Leguminosae), *Atractylodes macrocephala* KOIDZ. (Compositae), *Poria cocos* (SCHW.) WOLF (Polyporaceae), *Prunella vulgaris* var. *lilacina* NAKAI (Labiatae), *Aucklandia lappa* DECNE. (Compositae), *Glycyrrhiza uralensis* FISCH. (Leguminosae),*Zingiber officinale* ROSC. (Zingiberaceae), *Zizyphus jujuba* MILL. var. *inermis* REHDER(Rhamnaceae), respectively.

2.3. Patients

Patients between 20 and 50 years of age fulfilled the Centers for Disease Control-criteria as defined by Fukuda et al. (1994). Patients suffering from somatic and psychiatric disorders and patients using beta-blockers, psychotropic drugs, or diuretics were excluded from the study. Furthermore patients with an alcohol intake of more than four units of alcohol per day and a body mass index of greater than 45 were excluded from the study.

2.4. Recruitment of patients and controls

Patients suffered from severe fatigue for more than 6 months resulting in a reduction of their daily activity by

more than 50%. Patients were included if more than four of the following symptoms were present with a duration of at least 6 months: impaired memory or concentration, sore throat, tender cervical or axillary lymph nodes, muscle pain, multi-joint pain, new headaches, unrefreshing sleep, and post-exertion malaise. Patients with CFS were recruited through the Wonkwang Oriental Medicine Hospital (Jeonju, Jeonbuk). By the inspection of the available medical records, it was established whether the prospective patients met the above-defined criteria. This study involved eight CFS patients and five healthy controls. Informed consent was obtained from all the patients.

2.5. PBMC isolation and culture

PBMC (CFS patient with conscious disorder) from heparinized venous blood were isolated by Ficoll-gradient centrifugation, washed three times in phosphated-buffered saline (PBS) solution and resuspended in RPMI 1640 medium (GIBCO) supplemented with 2 mM L-glutamin, 100 U/ml penicillin G, 100 μg/ml streptomycin, and 10% FBS inactivated for 30 min at 56 °C. PBMC were cultured for 24 h in 95% humidified air containing 5% CO_2 (37 °C), in the presence or the absence of LPS, and the supernatants were collected by centrifugation and stored at −20 °C. For TGF-β1 assay, cell supernatants were adjusted to pH 3.0 with 1N HCl and then the acidified samples were incubated at 4 °C for 60 min. After incubation, the samples were neutralized by treating with 1N NaOH and stored at −70 °C.

2.6. MTT assay

Cell viability was determined by the MTT assay. Briefly, 500 μl of PBMC suspension (2.5×10^4 cells) was cultured in four-well plates for 24 h after treatment by each concentration of purple bamboo salt. 20 μl of MTT solution (5 mg/ml) was added and the cells were incubated at 37 °C for an additional 4 h. After washing the supernatant out, the insoluble formazan product was dissolved in DMSO. Then, optical density of 96-well culture plates was measured using an enzyme-linked immunosorbent assay (ELISA) reader at 540 nm. The optical density of formazan formed in untreated control cells was taken as 100% of viability.

2.7. Cytokine assays

Cytokines were determined by modification of an ELISA as described previously (Scuderi et al., 1986). For the measurement of TNF-α and IFN-γ, monoclonal anti-human TNF-α, IFN-γ (1 μg/ml) were used as a capture antibody; biotinylated anti-human TNF-α, IFN-γ (0.1 μg/ml) were used as a detecting antibody; whereas human recombinant TNF-α, IFN-γ served as a standard. For the detection of IL-10 and TGF-β1 the plates were coated with purified rat anti-human IL-10, TGF-β1 monoclonal antibody (1 μg/ml) and biotinylated rat anti-human IL-10, TGF-β1 monoclonal antibody were used in a concentration of 0.1 μg/ml. Recombinant human IL-10 and TGF-β1 that were diluted in PBS containing 0.05% tween 20 were used as a standard. The standard curves ranged from 1000 to 10 pg/ml.

$$\text{Inhibition}(\%) = \frac{(a-b)\times 100}{a}$$

where '*a*' is cytokine secretion without KBT and '*b* is cytokine secretion with KBT.

2.8. HPLC analysis

The chromatographic system consisted of a pump (Shimaduz LC-10AT HPLC pump), a UV detector (Shimaduz SPD-10A) and an autosampler (Shimaduz SIL-10AD autosampler). A Cosmosil 5C18-AR-IIcolumm (250 mm, Nacalai Tesgue Inc., Japan) was used. Acetonitrile-H2O-Acetic acid (100:900:10) was used as the mobile phase. Detection of the peaks at 254 nm and the sensitivity was set of 0.50 AUFS. The injection volume was 20 μl and flow rate was 1.0 ml/min. Standard solution was prepared by dissolving in distilled water (10 mg/100 ml). The solution was filtered through 0.45 μm membrane filter and applied to HPLC.

2.9. Statistical analysis

The experiments shown are a summary of the data from at least three experiments and are presented as the mean ± S.E.M. Statistical analysis was performed using the Mann–Whitney *U*-nonparametric test for matched pairs. Results with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Inhibitory effect of KBT on LPS-induced TNF-α production

We examined the effect of KBT on LPS-induced TNF-α production from PBMC of CFS patients and healthy controls. PBMC were stimulated with 10 ng/ml LPS in the absence or presence of various concentrations of KBT, and the supernatants were harvested after 24 h. Culture supernatants were assayed for TNF-α levels by ELISA method. As shown in Table 1, addition of 1 mg/ml of KBT significantly inhibited TNF-α production by 46.08 ± 0.59% ($P < 0.05$). PBMC cytotoxicity was not observed at the same condition by KBT (Fig. 1).

3.2. Inhibitory effect of KBT on LPS-induced IL-10 production

Next, we examined the effect of KBT on LPS-induced IL-10 production from PBMC of CFS patients and healthy controls. PBMC were stimulated with 10 ng/ml LPS in the

Table 1
Inhibitory effect of KBT on LPS-induced TNF-α production from PBMC of healthy control and CFS patients

Treatment	TNF-α production (ng/ml)		Inhibition (%)
	Control	Patients	Patients
Saline	0.51 ± 0.08	0.62 ± 0.24	–
LPS	2.24 ± 0.52	2.04 ± 0.56	–
KBT (mg/ml)			
0.01	1.87 ± 0.35	1.92 ± 0.59	5.88 ± 0.74
0.1	1.76 ± 0.50*	1.86 ± 0.41	8.82 ± 0.41
1	1.76 ± 0.48*	1.10 ± 0.73*	46.08 ± 0.59*

PBMC (2×10^5) from healthy control and CFS patients were stimulated with 10 ng/ml LPS in the presence of KBT. The supernatants were harvested after 24 h of culture. IL-10 secreted into the medium are presented as the mean ± S.E.M. of six independent experiments.

* $P < 0.05$: significantly different from LPS.

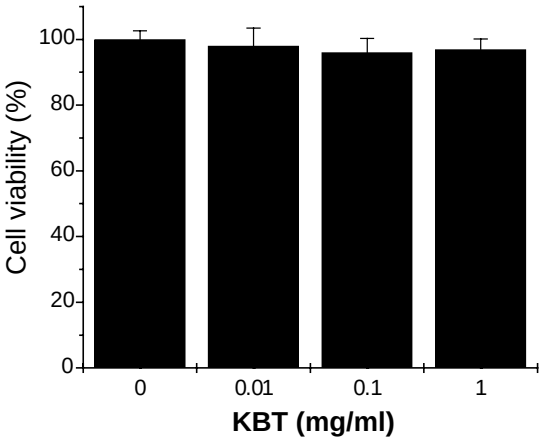

Fig. 1. Effect of KBT on the cell viability in PBMC. Cell viability was evaluated by MTT assay 24 h after KBT treatment (0.01–1 mg/ml) in PBMC. The percentage of viable cells was over 95% in each condition. Data represent the mean ± S.E.M. of six independent experiments.

absence or presence of various concentrations of KBT, and the supernatants were harvested after 24 h. As shown in Table 2, KBT (1 mg/ml) significantly inhibited IL-10 production by 50.00 ± 0.27% ($P < 0.05$).

Table 2
Inhibitory effect of KBT on LPS-induced IL-10 production from PBMC of healthy control and CFS patients

Treatment	IL-10 production (ng/ml)		Inhibition (%)
	Control	Patients	Patients
Saline	0.29 ± 0.77	0.47 ± 0.14	–
LPS	1.56 ± 0.52	2.08 ± 0.56	–
KBT (mg/ml)			
0.01	0.98 ± 0.69*	1.67 ± 0.75	19.71 ± 0.75
0.1	0.94 ± 0.56*	1.44 ± 0.71	30.77 ± 0.71
1	0.67 ± 0.45*	1.04 ± 0.26*	50.00 ± 0.27*

PBMC (2×10^5) from healthy control and CFS patients were stimulated with 10 ng/ml LPS in the presence of KBT. The supernatants were harvested after 24 h of culture. IL-10 secreted into the medium are presented as the mean ± S.E.M. of six independent experiments.

* $P < 0.05$: significantly different from LPS.

Table 3
Inhibitory effect of KBT on LPS-induced TGF-β1 production from PBMC of control and CFS patients

Treatment	TGF-β1 production (ng/ml)		Inhibition (%)
	Control	Patients	Patients
Saline	0.09 ± 0.03	0.08 ± 0.02	–
LPS	2.06 ± 1.27	2.11 ± 0.79	–
KBT (mg/ml)			
0.01	–	1.45 ± 0.23	31.28 ± 0.23
0.1	1.837 ± 0.28	1.16 ± 0.75	45.02 ± 0.75
1	1.61 ± 0.27	1.04 ± 0.59*	50.71 ± 0.59*

PBMC (2×10^5) from healthy control and CFS patients were stimulated with 10 ng/ml LPS in the presence of KBT. The supernatants were harvested after 24 h of culture. TGF-β1 secreted into the medium are presented as the mean ± S.E.M. of six independent experiments.

* $P < 0.05$: significantly different from LPS.

3.3. Inhibitory effect of KBT on LPS-induced TGF-β1 production

LPS-induced TGF-β1 production was measured from PBMC of CFS patients and healthy controls after KBT treatment. PBMC were stimulated with 10 ng/ml LPS in the absence or presence of various concentrations KBT, and the supernatants were harvested after 24 h. All supernatants were measured as described in Section 2. As shown in Table 3, KBT (1 mg/ml) significantly inhibited TGF-β1 production by 50.71 ± 0.59% ($P < 0.05$).

3.4. Inhibitory effect of KBT on LPS-induced IFN-γ production

We finally examined the inhibitory effect of KBT on LPS-induced IFN-γ production from PBMC of CFS patients and healthy controls. PBMC were stimulated with 10 ng/ml LPS in the absence or presence of various concentrations KBT, and the supernatants were harvested after 24 h. As shown in Table 4, KBT (0.01 mg/ml) significantly increased IFN-γ production ($P < 0.05$).

Table 4
Effect of KBT on LPS-induced IFN-γ production from PBMC of control and CFS patients

Treatment	IFN-γ production (ng/ml)	
	Control	Patients
Saline	0.36 ± 0.08	0.40 ± 0.01
LPS	1.30 ± 0.29	0.62 ± 0.10
KBT (mg/ml)		
0.01	1.74 ± 0.06*	1.07 ± 0.02*
0.1	1.33 ± 0.21	0.75 ± 0.04
1	1.39 ± 0.25*	0.62 ± 0.03

PBMC (2×10^5) from healthy control and CFS patients were stimulated with 10 ng/ml LPS in the presence of KBT. The supernatants were harvested after 24 h of culture. IFN-γ secreted into the medium are presented as the mean ± S.E.M. of six independent experiments.

* $P < 0.05$: significantly different from LPS.

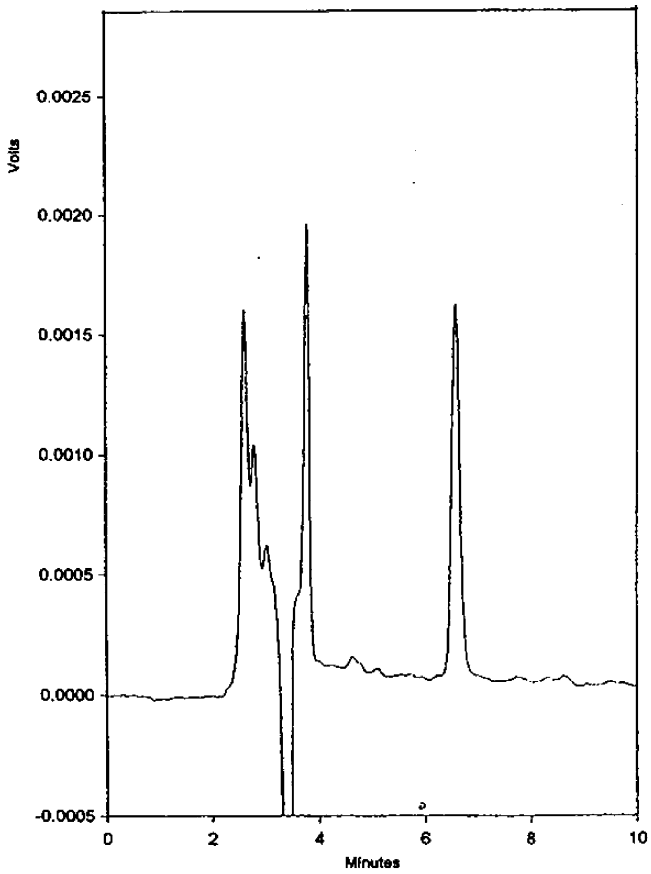

Fig. 2. HPLC chromatogram of the KBT. Standard solution of KBT was prepared by dissolving in distilled water (10 mg/100 ml). The injection volume was 20 μl and the detection was made at 254 nm.

3.5. Characterization of the principal components of KBT

The KBT was analyzed by HPLC. Chromatogram of the KBT is shown in Fig. 2. Peaks of the principal components have not yet been identified in this study (Fig. 2).

4. Discussion

It has been suggested that abnormal production of cytokines may play a role in the pathogenesis and clinical manifestations of CFS (Buchwald and Komaroff, 1991). A number of investigators have reported levels of certain cytokines in serum and in culture supernatants. TNF-α is a pro-inflammatory molecule that appears to play a role in the pathogenesis of AIDS and multiple sclerosis, both associated with chronic fatigue (Matsuyama et al., 1991; Brosnan et al., 1988). TNF provokes slow-wave sleep when placed in the lateral ventricles of experimental animals (Shoham et al., 1987). Chao et al. reported increased TNF-α production by PBMC of CFS in response to LPS (1991). In the present study, we observed a significant inhibition of LPS-induced TNF-α production in PBMC of CFS patients by KBT. In CFS patients, spontaneously produced IL-10 by both adherent monocytes and non-adherent lymphocytes and by phytoagglutinin-activated non-adherent monocytes were decreased (Gupta et al., 1997). Fatigue is a condition which is also associated with multiple sclerosis. Multiple sclerosis patients show decreased IL-10 levels, in particular, during active disease (van Boxel-Dezaire et al., 1999). LPS-induced cytokine secretion in whole blood cultures showed a significant increase in IL-10 as compared with controls (Visser et al., 2001). TGF-β1 has been implicated in the pathogenesis of a number of diseases including infection with intracellular pathogens (Border and Ruoslahti, 1992; Koyanagi et al., 2000; Barral et al., 1993; Reed, 1999). Serum bioactive TGF-β1 levels were higher in patients with CFS and Chronic idiopathic thrombocytopenic purpura (ITP) than in control subjects (Chao et al., 1991). Chronic ITP patients with active disease had a reduced PBMC production of the TGF-β1 (Andersson et al., 2002). In the present study, LPS-induced TGF-β1 production was increased in PBMC cultures derived from patients with CFS and controls. However, KBT inhibited production of TGF-β1 in LPS induced PBMC of CFS patients. These results indicate that KBT can regulate TGF-β1 production in PBMC treated with LPS.

Our results showed that LPS-induced IFN-γ production was slightly increased comparing to unstimulated PBMC and that LPS-induced IFN-γ production was increased by KBT. IFN-γ is an immunoregulatory molecule, enhancing both NK cell cytotoxicity and causing inhibition of suppressor T lymphocyte activity (Targan and Stebbing, 1982; Knop et al., 1982). Two groups have found impaired IFN-γ production on mitogenic stimulation of PBMC from CFS patients (Visser et al., 1998; Klimas et al., 1990). In contrast, Morte et al. (1988), observed normal interferon production, and Altmann et al. (1988), and Rasmussen et al. (1991), observed increased production in CFS (1988, 1991). Further research in this area will shed more light on the usefulness of IFN-γ in the diagnosis and treatment of CFS patients.

We can reasonably conclude that KBT treatment profoundly affect the LPS-induced TNF-α, IL-10, TGF-β1, and IFN-γ production in PBMC of CFS patients. However, the effect was not directly proportional to concentration. We assume that may be because KBT is a crude extract or was slightly absorbed by the cells, but further detailed study is needed. In addition, the immune-profiling of CFS patients will provide valuable aid for the individuals to guarantee homogeneity of the PBMC.

Acknowledgements

This work was supported by Korea Research Foundation Grant (KRF-2001-042-F00118).

References

Altmann, C., Larratt, K., Golubjatnikov, R., Kirmani, N., Rytel, M., 1988. Immunologic markers in the chronic fatigue syndrome. Clinical Research 36, 845A.

Andersson, P.O., Olsson, A., Wadenvik, H., 2002. Reduced transforming growth factor-beta1 production by mononuclear cells from patients with active chronic idiopathic thrombocytopenic purpura. British Journal of Hematology 116, 862–867.

Aoki, T., Miyakoshi, H., Usuda, Y., Herberman, R.B., 1993. Low NK syndrome and its relationship to chronic fatigue syndrome. Clinical Immunology and Immunopathology 3, 253–265.

Aung, H., Toossi, Z., McKenna, S.M., Gogate, P., Sierra, J., Sada, E., Rich, E.A., 2000. Expression of transforming growth factor-beta but not tumor necrosis factor-alpha, interferon-gamma, and interleukin-4 in granulomatous lung lesions in tuberculosis. Tubercle and Lung Disease 80, 61–67.

Barker, E., Fujimura, S.F., Fadem, M.B., Landay, A.L., Levy, J.A., 1994. Immunologic abnormalities associated with chronic fatigue syndrome. Clinical Infectious Diseases Supplemnt 1, S136–S141.

Barral, A., Barral-Netto, M., Yong, E.C., Brownell, C.E., Twardzik, D.R., Reed, S.G., 1993. Transforming growth factor beta as a virulence mechanism for *Leishmania braziliensis*. Proceedings of the National Academy Sciences USA 90, 3442–3446.

Bearn, J., Wessely, S., 1994. Neurobiological aspects of the chronic fatigue syndrome. European Journal of Clinical Investigation 2, 79–90.

Bennett, A.L., Chao, C.C., Hu, S., Buchwald, D., Fagioli, L.R., Schur, P.H., Peterson, P.K., Komaroff, A.L., 1997. Elevation of bioactive transforming growth factor-beta in serum from patients with chronic fatigue syndrome. Journal of Clinical Immunology 2, 160–166.

Border, W.A., Ruoslahti, E., 1992. Transforming growth factor-beta in disease: the dark side of tissue repair. Journal of Clinical Investigation 90, 1–7.

Brosnan, C.F., Selmaj, K., Raine, C.S., 1988. Hypothesis: a role for tumor necrosis factor in immune-mediated demyelination and its relevance to multiple sclerosis. Journal of Neuroimmunology 18, 87–94.

Buchwald, D., Komaroff, A.L., 1991. Review of laboratory findings for patients with chronic fatigue syndrome. Reviews Infectious Disease 13, S12–S18.

Buchwald, D., Wener, M.H., Pearlman, T., Kith, P., 1997. Markers of inflammation and immune activation in chronic fatigue and chronic fatigue syndrome. Journal of Rheumatology 2, 372–376.

Caligiuri, M., Murray, C., Buchwald, D., Levine, H., Cheney, P., Peterson, D., Komaroff, A.L., Ritz, J., 1987. Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome. Journal of Immunology 139, 3306–3313.

Cannon, J.G., Angel, J.B., Abad, L.W., Vannier, E., Mileno, M.D., Fagioli, L., Wolff, S.M., Komaroff, A.L., 1997. Interleukin-1 beta, interleukin-1 receptor antagonist, and soluble interleukin-1 receptor type II secretion in chronic fatigue syndrome. Journal of Clinical Immunology 3, 253–261.

Chao, C.C., Janoff, E.N., Hu, S.X., Thomas, K., Gallagher, M., Tsang, M., Peterson, P.K., 1991. Altered cytokine release in peripheral blood mononuclear cell cultures from patients with the chronic fatigue syndrome. Cytokine 3, 292–298.

Chen, Y., Kuchroo, V.K., Inobe, J., Hafler, D.A., Weiner, H.L., 1994. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science 265, 1237–1240.

Dusheiko, G., 1997. Side effects of alpha interferon in chronic hepatitis C. Hepatology 26, 112S–121S.

Fiorentino, D.F., Zlotnik, A., Mosmann, T.R., Howard, M., O'Garra, A., 1991. IL-10 inhibits cytokine production by activated macrophages. Journal of Immunology 147, 3815–3822.

Fukaura, H., Kent, S.C., Pietrusewicz, M.J., Khoury, S.J., Weiner, H.L., Hafler, D.A., 1996. Induction of circulating myelin basic protein and proteolipid protein-specific transforming growth factor-beta1-secreting Th3 T cells by oral administration of myelin in multiple sclerosis patients. Journal of Clinical Investigation 98, 70–77.

Fukuda, K., Straus, S.E., Hickie, I., Sharpe, M.C., Dobbins, J.G., Komaroff, A., 1994. The chronic fatigue syndrome: a comprehensive approach to its definition and study, International Chronic Fatigue Syndrome Study Group. Annals International Medicine 121, 953–959.

Gorelik, L., Flavell, R.A., 2000. Abrogation of TGFbeta signaling in T cells leads to spontaneous T cell differentiation and autoimmune disease. Immunity 12, 171–181.

Gupta, S., Aggarwal, S., See, D., Starr, A., 1997. Cytokine production by adherent and non-adherent mononuclear cells in chronic fatigue syndrome. Journal of Psychiatric Research 31, 149–156.

Gupta, S., 1992. Recent developments in immunological aspects of chronic fatigue syndrome. In: The Clinical and Scientific Basis of Myalgic Encephalomyelitis Chronic Fatigue Syndrome. New York: The Nightingale Research Foundation. pp. 545–550.

Gupta, S., Vayuvegula, B., 1991. A comprehensive immunological analysis in chronic fatigue syndrome. Scandinavian Journal of Immunology 33, 319–327.

Hassan, I.S., Bannister, B.A., Akbar, A., Weir, W., Bofill, M., 1998. A study of the immunology of the chronic fatigue syndrome: correlation of immunologic parameters to health dysfunction. Clinical Immunology and Immunopathology 87, 60–67.

Hirsch, C.S., Yoneda, T., Averill, L., Ellner, J.J., Toossi, Z., 1994. Enhancement of intracellular growth of Mycobacterium tuberculosis in human monocytes by transforming growth factor-beta 1. Journal of Infectious Diseases 170, 1229–1237.

Howard, M., O'Garra, A., Ishida, H., Malefyt, R., de Vries, J., 1992. Biological properties of interleukin 10. Journal of Clinical Immunology 12, 239–347.

Jone, J.F., 1991. Serologic and immunologic responses in chronic fatigue syndrome with emphasis on the Epstein-Barr virus. Reviews Infectious Disease 1, S26–S31.

Klimas, N.G., Salvato, F.R., Morgan, R., Fletcher, M.A., 1990. Immunologic abnormalities in chronic fatigue syndrome. Journal of Clinical Microbiology 28, 1403–1410.

Knop, J., Stremer, R., Nauman, C., deMaeyer, E., Macher, M., 1982. Interferon inhibits the suppressor T cell response of delayed hypersensitivity. Nature 296, 757–759.

Koyanagi, M., Egashira, K., Kubo-Inoue, M., Usui, M., Kitamoto, S., Tomita, H., Shimokawa, H., Takeshita, A., 2000. Role of transforming growth factor-beta1 in cardiovascular inflammatory changes induced by chronic inhibition of nitric oxide synthesis. Hypertension 35, 86–90.

Krakauer, T., Vilcek, J., Oppenheim, J.J., 1999. Proinflammatory cytokines: TNF and IL-1 families, chemokines, TGF-β, and others. In: Paul, W.E. (Ed). Fundamental Immunology. 4th ed. Lippincott-Raven, New York, pp. 775–811.

Landay, A.L., Jessop, C., Lennette, E.T., Levy, J.A., 1991. Chronic fatigue syndrome: clinical condition associated with immune activation. Lancet 338, 707–712.

Levy, J.A., 1994. Viral studies of chronic fatigue syndrome. Clinical Infectious Diseases 18, S117–S120.

Lim, D.W., Kim, D.H., You, D.Y., 1999. Effect of Kamiguibitang on immunologic control function. The Journal of Oriental Gynecology 12, 253–280.

Lloyed, A.R., Wakefield, D., Dwyer, J., Boughton, C., 1989. Immunological abnormalities in chronic fatigue syndrome. Medical Journal of Australia 151, 122–124.

Lopez, A.R., Cook, J., Deininger, P.L., Derynck, R., 1992. Dominant negative mutants of transforming growth factor-beta 1 inhibit the secretion of different transforming growth factor-beta isoforms. Molecular and Cellular Biology 12, 1674–1679.

Matsuda, H., Murakami, T., Ikebata, A., Yamahara, J., Yoshikawa, M., 1999. Bioactive saponins and glycosides. XIV. Structure elucidation and immunological adjuvant activity of novel protojujubogenin type triterpene bisdesmosides, protojujubosides A, B, and B1, from the seeds of Zizyphus jujuba var. spinosa (Zizyphi Spinosi Semen). Chemical and Pharmaceutical Bulletin 47, 1744–1748.

Matsuyama, T., Kobayashi, N., Yamamoto, N., 1991. Cytokines and HIV infection: is AIDS a tumor necrosis factor disease? AIDS 5, 1405–1417.

Mawle, A.C., Nisenbaum, R., Dobbins, J.G., Gary Jr., H.E., Stewart, J.A., Reyes, M., Steele, L., Schmid, D.S., Reeves, W.C., 1997. Immune

responses associated with chronic fatigue syndrome: a case-control study. Journal of Infectious Diseases 175, 136–141.

Morte, S., Castilla, A., Civeira, M.P., Serrano, M., Prieto, J., 1988. Gamma-interferon and chronic fatigue syndrome. Lancet 2, 623–624.

Moutschen, M., Triffaux, J.M., Demonty, J., Legros, J.J., Lefebvre, P.J., 1994. Pathogenic tracks in fatigue syndromes. Acta Clinica Belgica 49, 274–289.

Rasmussen, A.K., Nolesen, H., Andersen, V., Barington, T., Bendtzen, K., Hansen, M.B., Nielsen, L., Pedersen, B.K., Wiik, A., 1991. Chronic fatigue syndrome-a controlled cross sectional study. Journal of Rheumatology 21, 1527–1531.

Reed, S.G., 1999. TGF-beta in infections and infectious diseases. Microbes and Infection 1, 1313–1325.

Scuderi, P., Sterling, K.E., Lam, K.S., Finley, P.R., Ryan, K.J., Ray, C.G., Petersen, E., Slymen, D.J., Salmon, S.E., 1986. Raised serum levels of tumour necrosis factor in parasitic infections. Lancet 2, 1364–1365.

Shoham, S., Davenne, D., Cady, A.B., Dinarello, C.A., Krueger, J.M., 1987. Recombinant tumor necrosis factor and interleukin 1 enhance slow-wave sleep. American Journal of Physiology 253, R142–R149.

Straus, S.E., Dale, J.K., Peter, J.B., Dinnarello, C.A., 1989. Circulating lymphokine levels in the chronic fatigue syndrome. Journal of Infectious Diseases 160, 1085–1086.

Strauss, S.E., Komaroff, A.L., Wedner, H.J., 1994. Chronic fatigue syndrome. Point and counterpoint. Journal of Infectious Diseases 170, 1–6.

Strauss, S.E., Fritz, S., Dale, J.K., Gould, B., Strober, W., 1993. Lympocyte phenotype and function in the chronic fatigue syndrome. Journal of Clinical Immunology 13, 30–40.

Swanink, C.M.A., Vercoulen, J.H.M.M., Bleijenberg, G., Fennis, J.F.M., Galema, J.M.D., van der Meer, J.W., 1993. Chronic fatigue syndrome: a clinical and laboratory study with a well matched control group. Journal of International Medicine 237, 499–506.

Swanink, C.M.A., Vercoulen, J.H.M.M., Galama, J.M.D., Roos, M.T., Meyaard, L., van der Ven-Jongekrijg, J., de Nijs, R., Bleijenberg, G., Fennis, J.F., Miedema, F., van der Meer, J.W., 1996. Lymphocyte subsets, apoptosis, and cytokines in patients with chronic fatigue syndrome. Journal of Infectious Diseases 173, 460–463.

Targan, S., Stebbing, N., 1982. In vitro interactions of purified cloned human interferons on NK cells: enhanced activation. Journal of Immunology 129, 934–935.

Tirelli, U., Marotta, G., Improta, S., Pinto, A., 1994. Immunological abnormalities in patients with chronic fatigue syndrome. Scandinavian Journal of Immunology 40, 601–608.

van Boxel-Dezaire, A.H., Hoff, S.C., van Oosten, B.W., Verweij, C.L., Drager, A.M., Ader, H.J., van Houwelingen, J.C., Barkhof, F., Polman, C.H., Nagelkerken, L., 1999. Decreased interleukin-10 and increased interleukin-12p40 mRNA are associated with disease activity and characterize different disease stages in multiple sclerosis. Annals of Neurology 45, 695–703.

Visser, J., Blauw, B., Hinloopen, B., Brommer, E., de Kloet, E.R., Kluft, C., Nagelkerken, L., 1998. CD4 T lymphocytes from patients with chronic fatigue syndrome have decreased interferon-gamma production and increased sensitivity to dexamethasone. Journal of Infectious Diseases 2, 451–454.

Visser, J., Graffelman, W., Blauw, B., Haspels, I., Lentjes, E., de Kloet, E.R., Nagelkerken, L., 2001. LPS-induced IL-10 production in whole blood cultures from chronic fatigue syndrome patients is increased but supersensitive to inhibition by dexamethasone. Journal of Neuroimmunology 119, 343–349.

Ye, Y.N., Liu, E.S., Li, Y., So, H.L., Cho, C.C., Sheng, H.P., Lee, S.S., Cho, C.H., 2001. Protective effect of polysaccharides-enriched fraction from Angelica sinensis on hepatic injury. Life Science 69, 637–646.