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The inhibitory effects of Gami-Phedoc-San on TNF-α, IL-1β and IFN-γ secretion from human monocytes

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SUMMARY

In our study, the several cytokines were determined in phytohaemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) of Adamantiades-Behçets patients. Adamantiades-Behçets disease (ABD) is a systemic inflammatory disorder and might involve immune dysfunction. High levels of TNF-α, IL-1β and IFN-γ indicate the activation of inflammatory reactions and immune system in ABD. Gami-Phedoc-San (GPS) is an Oriental herbal medication, which has been used in Korea for the treatment of ABD. GPS (1 mg/ml) significantly inhibited the secretion of proinflammatory cytokines, TNF-α and IL-1β, compared to absence of GPS (by 50.5±1.9% inhibition for TNF-α and 106.9±16.8% for IL-1β). GPS also inhibited the production of IFN-γ, immunoregulatory Th1 cytokine, by 78.4 ± 2.8%. The inhibitory effects of GPS on cytokine secretion showed dose-dependent manner, and the pre-treatment of 1 mg/ml GPS had better effects than immunosuppressive drug for treatment of ABD, cyclosporin A. Our results suggest that GPS treatment for ABD patients might have pharmacological activity of immune and inflammatory responses through the cytokine modulation.

Key words: Adamantiades-Behçet's Disease; Gami-Phedoc-San; Tumor necrosis factor-α; Interleukin-1β; Interferon-γ

INTRODUCTION

Adamantiades-Behçet's disease (ABD) is a multisystemic disorder with myriad immunologic and pathologic consequences of unknown etiology (Sakane *et al*., 1999; Yazici *et al*., 1999). The disorder was first recognized by B. Adamantiades in 1931 (Adamantiades B, 1931) and described extensively by H. Behçet in 1937 (Behçet H, 1937). ABD is characterized by recurrent oral aphthous ulcers, genital ulcers, uveitis, skin lesions, central nervous system, joint, and gastrointestinal involvement (Kaklamani *et al*., 1998). Patients with ABD are clustered along the ancient Silk Road, expending from Far-East Asian to the Mediterranean basin. The prevalence in Japan, Korea, China, Iran, and Sau di Arabia ranges from 13.5 to 20 cases per 100,000, and ABD is somewhat more common among females in Korea and China, equally common in females and males in Japan (Kastner, 1997; Makae *et al*., 1993; Zouboulis, 1999). Histopathological studies have revealed cellular infiltrations consisting of lymphocytes, plasma cells, macrophages and polymorphonuclear neutrophils in the lesion (Poulter and Lehner, 1989; Emmi *et al*., 1995). As cytokines are involved in the regulation of immune responses and inflammatory reactions, it is important that a regulation of imbalanced cytokines in ABD. Proinflammatory cytokines, Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), have a critical role in inflammatory disease including ABD (Hamzaoui *et al*., 1990). An increase of IL-1β in the serum and from peripheral blood mononuclear cells (PBMC) of patients with active ABD has been described (Katayama *et al*., 1994; Yosipovitch *et al*., 1995). The

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spontaneous production by mononuclear cells and the level in serum of TNF-α are significantly increased in patients with ABD (Mege *et al.*, 1993; Sayinalp *et al.*, 1996). It have been reported that T lymphocytes may be stimulated by unknown causative agents *in vivo* and may spontaneously produce interferon-γ (IFN-γ) (Fujii *et al.*, 1983) and IFN-γ production by PBMC is significantly higher in ABD patients than control (Bacon *et al.*, 1984). High levels of TNF-α, IL-1β and IFN-γ indicate the activation and modulation of inflammatory reactions and immune system in ABD.

Gami-Phedoc-San (GPS) is an Oriental herbal medication that has been used to treat Adamantiades-Behçets disease (ABD), but the mechanism of action of the agent is unclear like other herbal medications.

In our study, the inhibitory effects of GPS on secretion of proinflammatory cytokines, TNF-α and IL-1β, and IFN-γ were investigated in phytohaemagglutinin (PHA)-stimulated PBMC from Adamantiades-Behçets patients. We also investigated whether GPS has same or better effects than cyclosporin A (CsA), an effective immunosuppressive drug for the treatment of ABD.

MATERIALS AND METHODS

Reagents

Phytohaemagglutinin (PHA), cyclosporin A (CsA), Ficoll-Hypaque (Histopaque-1077), avidin-peroxidase, ABTS substrate and other reagents were obtained from Sigma (St. Louis, MO, USA). Cell culture medium, RPMI 1640 was purchased from Gibco BRL (Grand Island, NY USA) and fetal bovine serum (FBS) was from Life Science (Grand Island, NY USA). Anti-human TNF-α/IL-1β/IFN-γ Abs, biotinylated anti-human TNF-α/IL-1β/IFN-γ Abs, and recombinant human TNF-α/IL-1β/IFN-γ were purchased from R&D Systems (Minneapolis, MN USA).

Preparation of GPS extracts

Extract of GPS was prepared by decocting the dried prescription of herbs with boiling distilled water. The extraction decocted for approximately 3 h was filtered, lyophilized, and kept at 4°C. The GPS water extract powder was dissolved in phosphate-buffered saline (PBS) and was filtered with 0.2 μm syringe filter. GPS include 3.75 g of *Ostericum koreanum* (MAX) radix, *Aralia cordata* radix, *Peucedanum decursivum* (MIQ) radix, *Bupleurum chinense* radix, *Cnidium officinale* rhizoma, *Poncirus trifoliata* (L) fructus, *Platycodon grandiflorum* (JACQ) redix, *Panax ginseng* radix, *Ledebouriella divaricata* (TURCZ) radix, *Schiwonepeta tenuifolia var. japonica* herba, *Atractylodes japonica* rhizoma, *Atractylodes macrocephala* rhizoma, *Paeonia lactiflora* radix, *Angelica gigas* radix, *Rehmannia glutinosa* radix, 1.87 g of *Mentha arvensis var. piperascens* herba, and *Glycyrrhiza uralensis* radix, respectively.

Isolation and culture of PBMC

Peripheral blood was drawn in EDTA-treated tubes from 2 patients with ABD. To minimize the effect *in vitro* on the activation status of the cells, PBMC were immediately purified by rapid Ficoll-Hypaque (Histopaque-1077) centrifugation at room temperature. PBMC were washed twice with PBS and once more with RPMI 1640 medium. PBMC were grown in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10% heat-inactivated FBS at 37°C in 5% CO_2 and 95% humidity. Stabilized cells (3×10^6 cells/ml) were seeded in 24 well culture plates and treated GPS and CsA for 30 min prior to stimulation with PHA.

Determination of cytokine levels

PBMC (3×10^6 cells/ml) were cultured and stimulated with PHA in the absence or presence GPS and CsA. After 24 h of incubation, cell-free supernatants were collected and stored at -70°C until assayed for cytokines. The amount of TNF-α, IL-1β and IFN-γ produced by PBMC was measured by modified enzyme-linked immunosorbent assay (ELISA) as described previously (Kim and Lee, 1999).

Statistical analysis

The results were expressed as mean ± SEM for the number of experiments. In single mean comparisons, data were analyzed by Students *t*-test. All analyses were performed using the software SPSS 9.0 for windows, and considered statistically significant for P values <0.05.

RESULTS

Clinical features of Subjects

We obtained peripheral blood from two patients, 30-year-old woman and 50-year-old man with 5-year history and 10-year history of ABD, respectively. Two patients presented with clinical signs and symptoms of active ABD. They have been at least 3 major symptoms such as oral (Bang *et al.*, 1995) or ocular (Nussenblatt, 1997), genital ulcer (Sakane *et al.*, 1999) and skin lesion (Sakane and Takeno, 2000).

Effects of GPS on cytokine secretion

The amount of cytokines released into supernatants after stimulation with PHA was determined by ELISA. PHA strongly induced cytokine secretion in PBMC from patients with ABD. We examined the inhibitory effects of GPS on TNF-α, IL-1β, and IFN-γ secretion. Pre-treatment of GPS inhibited the TNF-α secretion by 3.26 ± 0.10 ng / ml (for 0.1 mg / ml GPS) and 2.53 ± 0.04 ng / ml (for 1.0 mg / ml GPS) compared to value of 3.60 ± 0.08 ng / ml (absence of GPS) (Fig. 1). Also, GPS blocked IL-1β secretion in PHA-stimulated PBMC (Fig. 2). IL-1β secretion decreased in pre-treatment GPS 7.53 ± 0.21 ng / ml (for 0.1 mg / ml GPS) and 5.46 ± 0.41 ng / ml (for 1.0 mg / ml GPS) compared to value of no treatment of GPS (8.07 ± 0.07 ng / ml). Secretion of proinflammatory cytokines, TNF-α and IL-1β, by GPS was blocked in dose-dependent manner. We tested an inhibitory effect of GPS on IFN-γ secretion from PHA-stimulated PBMC (Fig. 3). Pre-treatment of GPS inhibited the IFN-γ secretion by 0.491 ± 0.043 ng / ml (for 0.1 mg / ml of GPS) and 0.265 ± 0.031 ng / ml (for 1.0 mg / ml of GPS) compared to value of

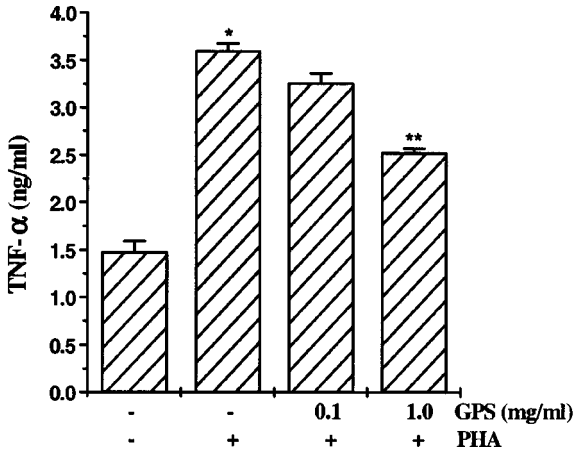

Fig. 1. TNF-α secretion from PBMC stimulated by PHA (25 μg / ml) in the absence or presence of GPS (0.1 and 1.0 mg / ml) for 30 min prior to stimulation. TNF-α levels are presented as mean ± SEM. **P*<0.05 compared to saline value and ***P*<0.05 PHA-treated alone value (Student *t*-test).

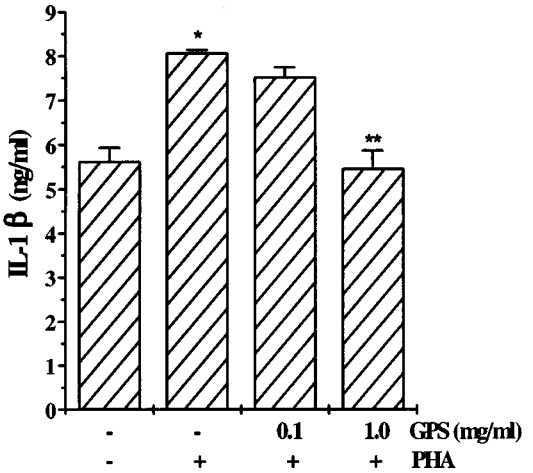

Fig. 2. IL-1β secretion from PBMC of ABD patients was measured. PBMC were cultured with or without GPS (0.1 and 1.0 mg / ml) for 30 min and then stimulated with PHA for 24 h. Each datum represents the mean ± SEM. **P*<0.05 compared to saline value and ***P*<0.05 PHA-treated alone value (Student *t*-test).

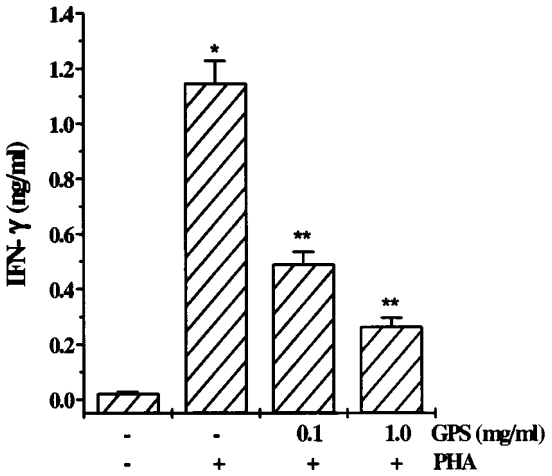

Fig. 3. IFN-γ secretion from PBMC stimulated by PHA (25 μg / ml) in the absence or presence of GPS (0.1 and 1.0 mg / ml) for 30 min prior to stimulation. TNF-α levels are presented as mean ± SEM. **P*<0.05 compared to saline value and ***P*<0.05 PHA-treated alone value (Student *t*-test).

Table 1. Inhibitory effects of GPS on cytokine secretion

Cytokine	Inhibition (%)	
	GPS	CsA
TNF-α	50.5 ± 1.9	30.9 ± 10.1
IL-1β	106.9 ± 16.8	89.1 ± 5.9
IFN-γ	78.4 ± 2.8	59.4 ± 4.1

PBMC (6×10^5 cells / ml) were stimulated with PHA (25 μg / ml) for 24 h in the presence of GPS (1 mg / ml) or CsA (5 μg / ml). The amount of TNF-α, IL-1β and IFN-γ secreted by PBMC was measured by ELISA. Inhibition rate was calculated from PHA-treated alone value.

absence of GPS, 1.149 ± 0.079 ng / ml.

Comparison with GPS and CsA on cytokine modulation effects

To confirm inhibitory effects of GS-Tang on cytokine secretion, we compared to effects of an effective immunosuppressive drug, CsA (Table 1). This has been introduced for the treatment of uveitis in patients with ABD for about 15 years and is still the most effective drug and widely used (Sakane and Takeno, 2000). The inhibitory effects of GPS on the secretion of proinflammatory cytokines (TNF-α and IL-1β) and an immunoregulatory cytokine (IFN-γ) were better than those of immunosuppressive drug, CsA.

DISCUSSION

GPS is an Oriental medication, which has been successfully used for the treatment of ABD, but its mechanism of action still remains unknown. Our study suggested that GPS inhibited the secretion of proinflammatory cytokines, TNF-α and IL-1β, and IFN-γ in PHA-stimulated PBMC from ABD patients. This result showed GPS had effective pharmacological activity and ability of regulation of immune and inflammatory responses by cytokine modulation in ABD.

The etiology and pathogenesis of ABD has yet to be fully elucidated. Environmental factors including infectious agents, immune abnormalities, and genetic predisposition have been implicated, and in particular there are considerable data implicating abnormalities of the immune system and inflammation thought to be T helper cell type 1 (Th1)-mediated (Arbesfeld and Kurban, 1988). The abundance of the various cytokines and chemokines possibly involved in ABD and recent studies have been focused on the correlation of various cytokines with ABD and its severity. Proinflammatory cytokines, such as TNF-α and IL-1β, are part of a larger network of inflammatory regulation and play an important role in inflammatory diseases including ABD (Mege *et al*., 1993; Yosipovitch *et al*., 1995). An increase in IL-1β in the serum and from PBMC of patients with active BD has been described, however another study did not find such IL-1β elevations (Hamzaoui *et al*., 1990; Sayinalp *et al*., 1996). IL-6 is also an important mediator promoting inflammation and IL-6 concentrations in cultured PBMC of patients with active BD were significantly high compared with patients with inactive BD and healthy controls (Yamakawa *et al*., 1996). Interleukin-8 (IL-8), a major chemokine known to attract and activate leukocytes (Chertov *et al*., 1996; Taub *et al*., 1996), has recently been under focused investigation because of its possible participation in the evolution of ABD. Increased IL-8 levels have been found in ABD patients serum and IL-8 is a more reliable marker for disease (al-Dalaan *et al*., 1995; Katsantonis *et al*., 2000; Ozoran *et al*., 1995; Zouboulis *et al*., 2000).

TNF-α seems to be a target in the therapy of inflammatory and immune diseases including Crohns disease and rheumatoid arthritis (RA) (Bondeson and Maini, 2001). Hassard *et al*. suggested that anti-TNF-α Ab might be an effective new therapy for gastrointestinal ABD (Hassard *et al*., 2001). An effective immunomodulatory and anti-inflammatory drug, thalidomide, has long been used successfully in several immune dysregulatory disorders including Behçets syndrome (Calabrese and Fleischer, 2000). The inhibitory effect of thalidomide on TNF-α production from human monocytes was reported (Sampaio *et al*., 1991). CsA as well as thalidomide significantly inhibited IFN-γ (Th1 cytokine) production in PHA-stimulated human PBMC cultures (McHuge *et al*., 1995). Although CsA is still one of the most effective drugs for the treatment in BD (Bang, 1997; Masuda *et al*., 1989), several problems have emerged in clinical use, such as reduced efficacy and neurotoxicity (Kotake *et al*., 1999; Ozyazgan *et al*., 1992). But Oriental herbal medication, GPS seems

to have no side effects in human treatment of ABD. IFN-γ is an immunoregulatory cytokine that influences both the growth and differentiation of immunologically active cells. IFN-γ production by PBMC is significantly higher in ABD patients than control (Bacon *et al*., 1984; Fujii *et al*., 1983). In our study, we confirmed that GPS inhibited the secretion of proinflammatory cytokines (TNF-α and IL-1β) and Th1 cytokine (IFN-β) in PHA-stimulated PBMC from ABD patients. Although our study cant be investigated with healthy control and the number of patients is too small, the outcome supports evidence that GPS plays a pivotal role in cytokine modulation. The further studies must be investigated whether GPS can regulate the imbalanced Th1/Th2-cell derived cytokines, chemokine, and other biological marker in active ABD patients.

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