Regulatory effects of Seogakjihwang-tang on Cytokines and Growth Factor Production in PBMC from the Patient with Cerebral infarction under Consciousness Disorders

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Seogakjihwang-tang (SJT) was widely used to treat patients suffering from cerebral infarction. But scientific investigation has been carried out very little. The aim of the present study is to investigate the effect of SJT on the production of various cytokines in the patients with cerebral infarction (CI). We investigated interleukin (IL)-4, IL-10 and transforming growth factor (TGF)-1 in the sera of 27 patients with cerebral infarction under consciousness disorders and 10 normal controls using an originally devised sensitive sandwich enzyme-linked immunosorbent assay (ELISA). We found that plasma levels of IL-4 were slightly elevated in patients with cerebral infarction, whereas plasma levels of IL-10 ($P<0.001$) and TGF-1 were reduced. Peripheral blood mononuclear cells (PBMC) obtained from the patient with CI were cultured for 24 h in the presence or absence of lipopolysaccharide (LPS) or phytohaemagglutinin (PHA). The amount of IL-4, IL-10 and TGF-1, in culture supernatant, was significantly increased in the LPS or PHA treated cells compared to unstimulated cells ($P<0.05$), We also show that increased cytokines IL-4, and IL-10 level was significantly inhibited by SJT in a dose-dependent manner. Maximal inhibition rate of IL-4 and IL-10 production by SJT was 45.63.3% and 614.7% for LPS-stimulated cell and 27.31.2% and 83.62% for PHA-stimulated cells, respectively ($P<0.05$). On the other hand, SJT significantly increased the LPS or PHA-induced TGF-1 production ($P<0.05$). These data suggest that SJT has a regulatory effect on the cytokines production, which might explain its beneficial effect in the treatment of CI.

Key words : Seogakjihwang-tang(犀角地黃湯), Cytokines, Lipopolysaccharide, Cerebral infarction, Phytohaemagglutinin

Introduction

People with cerebral infarction (CI) due to either thrombi or emboli frequently suffer irreversible neurologic deficits that markedly hinder their activity of daily living. Patients may suffer disturbances of motor strength and coordination, sensory discrimination, visual function, speech, memory, or other intellectual abilities. Although recovery is often incomplete, partial recovery often occurs in the weeks to months[1]. The injury, such as trauma and ischemia, to the central nervous system initiates inflammatory processes that are implicated in secondary tissue damage. These processes include the synthesis of proinflammatory cytokines, leukocyte extravasation, vasogenic edema, and blood-brain barrier breakdown[39]. Moreover, consciousness disorder results mainly from brain edema[40]. It is known that cytokines are involved in various neuropathologic disease, such as Alzheimer's disease, multiple sclerosis, and acquired immunodeficiency syndrome(AIDS). Also the change of the specific cytokine level was reported in an acute CI patient[37,38]. Early gene expression of inflammatory cytokines has been reported in the brain following global and focal cerebral infarction[2]. Cytokines were classically considered products of inflammatory and immune response, with production by stimulated macrophage and T cells[3-4]. Inflammatory cytokine, interleukin-4 (IL-4) is a pleiotrophic cytokine derived primarily from Th2 lymphocytes and mast cells. Described originally as a B cell growth factor, IL-4 subsequently has been found to influence activities of diverse cell types, including T lymphocytes, monocytes, endothelial cells, and fibroblasts[5-9]. IL-10 is not a typical T-cell-drived cytokine but it is expressed in T-lymphocyte (Th1 and Th2), monocytes and eosinophils[10]. IL-10 has been identified as an anti-inflammatory molecule that can suppress the production of variety of proinflammatory molecules including TNF-, IL-1, and IL-8[11-13]. Administration of IL-10 protects tissue exposed to

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· Received : 2003/03/12 · Revised : 2003/04/18 · Accepted : 2003/05/26

a variety of ischemia-reperfusion regimens[14]. Transforming growth factor (TGF)-1 is expressed as large pro-protein (390-412 amino acid), which includes TGF-1 (25 kDa) at its C-terminus, and TGF-1 latency associated peptide (LAP) at its N-terminus[15]. TGF-1 control many diverse events such as development, differentiation, tissue repair, tumorigenesis, and many immune and endocrine functions[16]. TGF-1 protein is secreted by most mammalian cells and their action is locally mediated through an autocrine/paracrine fashion[17].

Seogakjihwang-tang, a prescription of Traditional Oriental Medicine, has long been used as a specific prescription for CI with consciousness disorder to increase cerebral blood flow and to recover an injured brain cell. But the pharmacological mechanisms of them have not been well defined yet. In the present study, we investigated the various cytokines, IL-4, IL-10 and TGF-1 on the lipopolysaccharide (LPS) or phytohaemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) from the patient with CI. We also investigated the effect of SJT on LPS or PHA-induced cytokines production on the PBMC.

MATERIALS AND Methods

1. Reagents

Ficoll-Hypaque, LPS, avidin-peroxidase and 2'-AZINO-bis (3-ethylbenzithiazoline-6-sulfonic acid) tablets substrate (ABTS) and PHA 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-4, 10 and TGF-1, biotinylated anti-human IL-4, 10 and TGF-1, and recombinant (r) human IL-4, 10 and TGF-1 were purchased from Pharmingen (USA).

2. Patients

Twenty-seven patients with CI were examined at the Department of Neurology Wonkwang University school of medicine from March, 2002 to September, 2002. The diagnosis of CI was confirmed with computerized tomography (CT) and magnetic resonance imaging and clinical signs (hemiparesis, hemiplegia, slurred speech, facial palsy etc). Signs and symptoms at cerebral infarction included: vertigo (100%), gait disturbance (60%), headache (85%), slurred speech (60%), weakness (20%), drowsiness (60%) and sensory disturbances (40%). For cytokines assay blood was obtained from ten patients (14 males and 13 females, mean age 63.5, range 50 70) with CI and ten healthy adults (5 males and 5 females, mean age 62.5, range 41 68) with no medically diagnosable illness as a control group. Informed consent was obtained from all subjects before performing these studies. All samples were collected in a sterile glass tube and allowed to clot spontaneously for 15min. Serum was then collected by centrifugation and quickly frozen and stored in aliquots at -80C until assay.

3. Preparation of SJT

The ingredients of SJT are Rhizoma Rehmanniae(12g), Radix Paeoniae(8g), Cortex Moutan Radicis(4g), and Cornu Bubalus bubalis(4g). An extract of SJT was prepared by decocting the dried prescription of herbs with boiling distilled water. The plant materials were obtained from Oriental Medicine Hospital ,Wonkwang University and identified by professor Gang-Gyeong Seong, College of Oriental Medicine, Wonkwang University, and their voucher specimens have been deposited at the Herbarium at the College of Pharmacy, Wonkwang University.

4. PBMC isolation and culture

PBMC (2 patiens with CI under consciousness disorders) from heparinized venous blood were isolated by Ficoll-gradient centrifugation, washed three times in phosphate-buffered saline (PBS) solution and resuspended in RPMI 1640 medium (GIBCO) supplemented with 2 mM L-glutamin, 100 U/ml penicillin G, 100 g/ml streptomycin, and 10% FBS inactivated for 30 min at 56C. PBMC were adjusted to a concentration of 2 10^6 cells/ml in 30 ml falcon tube, and 100 l aliquots of cell suspension were placed in a four-well cell culture plate. PBMC were cultured for 24 h in 95% humidified air containing 5% CO_2 (37C), in the presence or the absence of LPS or PHA, and the supernatants were collected by centrifugation and stored at -20C. For TGF-1 assay, cell supernatants were adjusted to pH 3 with 1 N HCl and then the acidified samples were incubated at 4C for 60 min. After incubation, the samples were neutralized by treating with 1 N NaOH and stored at -70C.

5. ELISA of IL-4, 10 and TGF-1

Sandwich ELISA for IL-4, 10 and TGF-1 was carried out in duplicate in 96-well ELISA plates (Nunc, Denmark) coated with each of 100 l aliquots of anti-human IL-4, 10 and TGF-1 monoclonal antibodies at 1.0 g/ml in PBS at pH 7.4 and was incubated overnight at 4C. The plates were washed in PBS containing 0.05% Teeen-20 (Sigma, St. Lousis, 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-4, 10 and TGF-1 standards were added and incubated at 37C for 2 h. After 2 h incubation at 37C, the wells were washed and then each of 0.2 g/ml of biotinylated anti-human IL-4, 10

and TGF-1 were added and again incubated at 37C for 2 h. After washing the wells, avidin-peroxidase was added and plates were incubated for 20 min at 37C. Wells were again washed and ABTS substrate (Sigma) 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-4, 10 and TGF-1 in serial dilutions.

6. MTT assay

The MTT colorimetric assay of cell survival was executed by the method of Trivedi et al.,[18] with minor modifications. Cell aliquots (3 10^5) were seeded in microplate wells and incubated with 20 l of a MTT solution (5 mg/ml) for 4 h at 37C under 5% CO_2 and 95% air. Consecutively, 250 l of DMSO was added to extract the MTT formazan and the absorbance of each well at 540 nm was read by an automatic microplate reader.

7. Statistical analysis

The experiments shown are presented as the mean SD. Statistical evaluation of the results was performed by Mann-Whitney's u test. The results were considered significant at a value of $P<0.05$.

RESULTS

1. IL-4 levels in plasma

As can be seen in Fig. 1, CI group showed IL-4 levels comparable to that of normal group. The average IL-4 plasma level in CI group was slightly higher than that in normal group (108.251.2 pg/ml vs 101.717.2 pg/ml). But there were no significant differences between CI group and normal group.

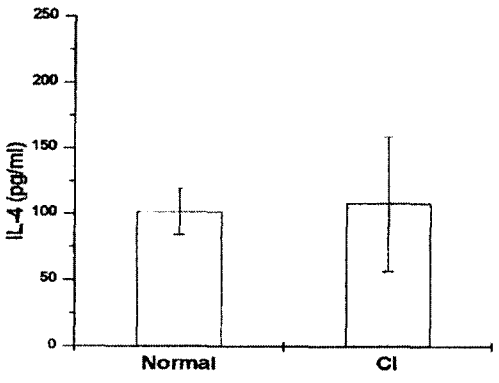

Fig. 1. Serum IL-4 levels were not significantly elevated in patients with CI

2. IL-10 levels in plasma.

Lower levels of plasma IL-10 than normal group (374.7132 pg/ml) were measured in the CI group (119.451 pg/ml) (Fig. 2). There were significant differences between CI group and normal group by Mann-Whitney U-test at $P<0.001$.

3. TFG-1 levels in plasma.

As can be seen in Fig. 3, CI group showed TFG-1 levels comparable to that of normal group. The average TFG-1 plasma level in CI group was lower than that in normal group (47.812 pg/ml vs 16521 pg/ml). But there were no significant differences between CI group and normal group.

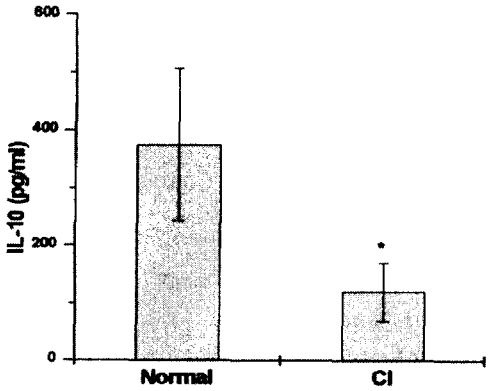

Fig. 2. Serum IL-10 levels were decreased significantly in patients with CI.

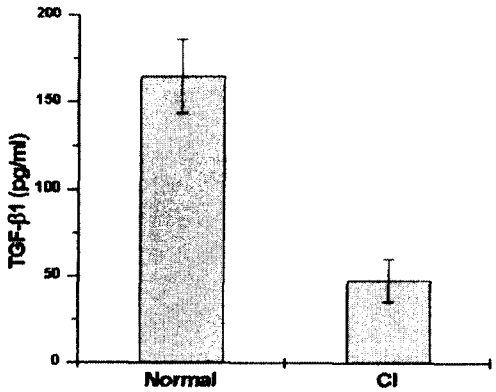

Fig. 3. Serum TGF-1 levels were not significantly decreased in patients with CI.

4. Inhibitory effect of SJT on LPS or PHA-induced IL-4 production

To evaluate the regulatory effect of SJT by LPS, we tested the effect of SJT on LPS-induced cytokines production. PBMC cells were pretreated for 1 h with various concentration of SJT, followed by 24 h of incubation with LPS (10 ng/ml). The supernatants analysed by ELISA method for IL-4. As shown in

Fig. 4A, LPS significantly increased cytokine production on the PBMC cells (unstimulated cells, 0.080.001 ng/ml; LPS stimulated cells, 0.2390.017 ng/ml, P=0.019). Increased the levels of IL-4 also significantly inhibited by SJT in a dose dependent manner on LPS-induced cytokine production. Maximal inhibition rate was 45.63.32% at 1 mg/ml SJT. Experiments were also conducted to determine whether SJT altered cytokine production patterns in mitogen-activated PBMC. Human PBMC were incubated with varying concentrations of SJT for 1 h and then stimulated with 10 g/ml PHA in the presence of the SJT for 24 h. IL-4 was up regulated by PHA at this time. Treatment of cells with SJT resulted in a significant and dose-dependent inhibition of IL-4 secretion by approximately 28% at 1 mg/ml SJT (P=0.021, Fig4. B). Cell cytotoxicity by SJT was not observed.

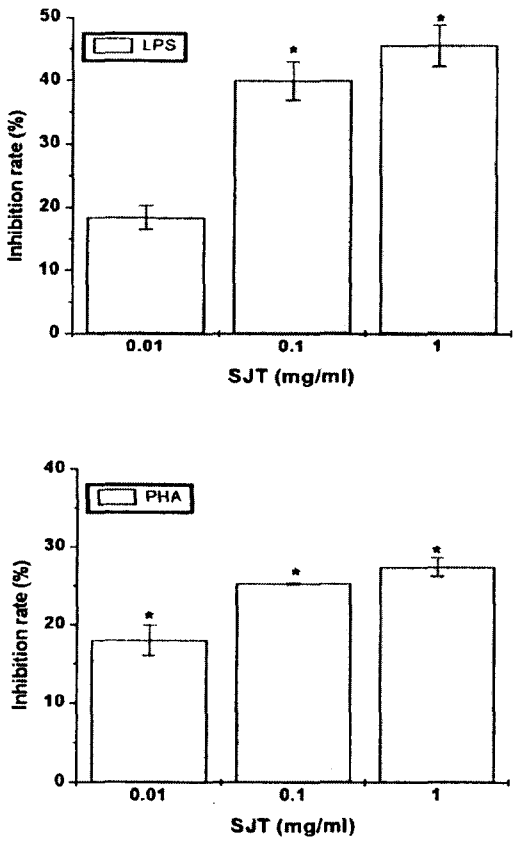

Fig. 4. Inhibitory effect of SJT on LPS or PHA-induced IL-4 produciton. PBMC cells (2 10^5) were treated with various concentration of SJT for 1 h and then stimulated with LPS (10 ng/ml) or PHA (10 g/ml) for 24 h. Cytokine concentrations were measured from cell supernatants using ELISA method. Values are the mean SEM of duplicate determinations from three separate experiments. *P<0.05: significantly different from the LPS or PHA-stimulated cells.

5. Inhibitory effect of SJT on LPS or PHA-induced IL-10 production

To evaluate the regulatory effect of SJT by LPS or PHA, we tested the effect of SJT on LPS or PHA-induced cytokines production. PBMC cells were pretreated for 1 h with various concentration of SJT, followed by 24 h of incubation with LPS (10 ng/ml) or PHA (10 g/ml). The supernatants analysed by ELISA method for IL-10. As shown in Fig. 5, LPS or PHA significantly increased cytokine production on the PBMC cells (unstimulated cells, 0.0960.011 ng/ml; LPS stimulated cells, 3.7471.45 ng/ml and PHA stimulated cells, 7.122.68 ng/ml, P=0.026 and P=0.04, respectively). Increased the levels of IL-10 significantly inhibited by SJT in a dose dependent manner on LPS or PHA-induced cytokine production. Maximal inhibition rate was 614.7% and 83.62%, respectively, at SJT 1 mg/ml (P<0.05)

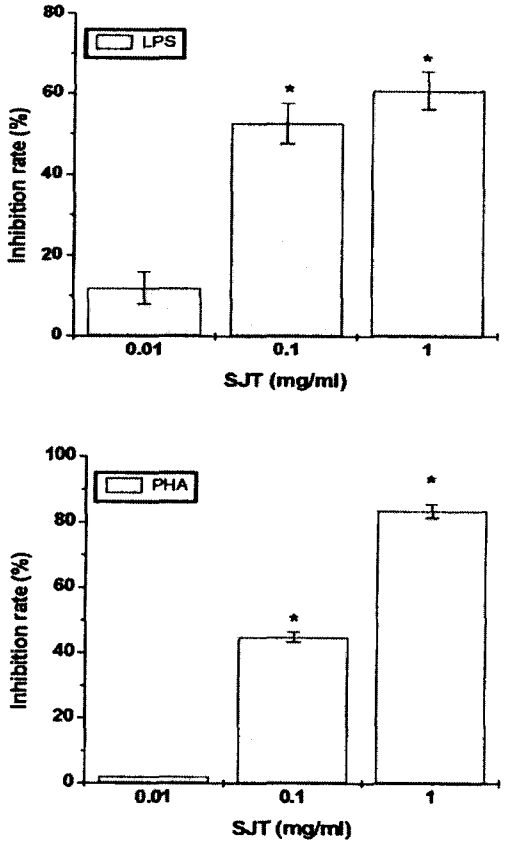

Fig. 5. Inhibitory effect of SJT on LPS or PHA-induced IL-10 produciton. PBMC cells (2 10^5) were treated with various concentration of SJT for 1 h and then stimulated with LPS (10 ng/ml) or PHA (10 g/ml) for 24 h. Cytokine concentrations were measured from cell supernatants using ELISA method. Values are the mean SEM of duplicate determinations from three separate experiments. *P<0.05: significantly different from the LPS or PHA-stimulated cells.

6. Effect of SJT on the TGF-1 production

To evaluate the regulatory effect of SJT by LPS or PHA, we tested the effect of SJT on LPS or PHA-induced cytokines production. PBMC cells were pretreated for 1 h with various concentration of SJT, followed by 24 h of incubation with LPS (10 ng/ml) or PHA (10 g/ml). The supernatants analysed by ELISA method for TGF-1. All supernatants were acid activated

as described in Materials and methods. SJT increased TGF-1 production by itself. The levels of TGF-1 in culture supernatants were significantly increased in response to LPS or PHA as compared to unsitmulated culture supernatant ($P<0.05$ or $P<0.01$, respectively for LPS or PHA). The amount of TGF-1 (2.73-fold for LPS and 2.6-fold for PHA, respectively) was significantly higher in the SJT (1 mg/ml) plus LPS or PHA treated cells than LPS or PHA treated cells ($P<0.05$, Fig. 6).

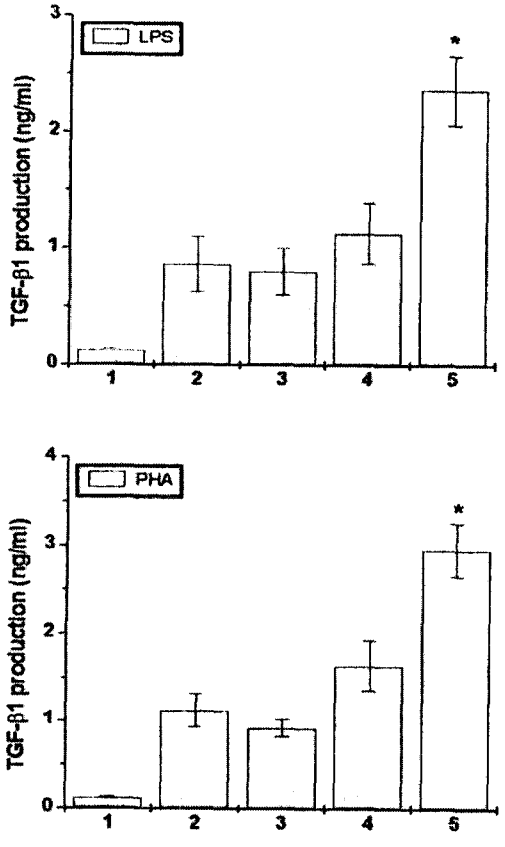

Fig. 6. Effect of SJT on TGF-1 production. PBMC cells (2 10^5) were treated with various concentration of SJT for 1 h and then stimulated with LPS (10 ng/ml) or PHA (10 g/ml) for 24 h. Cytokine concentrations were measured from cell supernatants using ELISA method. Values are the mean SEM of duplicate determinations from three separate experiments. 1, unstimulated cells; 2. LPS or PHA; 3, LPS (PHA) + SJT (0.01 mg/ml); 4, LPS (PHA) + SJT (0.1 mg/ml); 5, LPS (PHA) + SJT (1 mg/ml). *P(0.05: significantly different from the LPS or PHA-stimulated cells.

DISCUSSION

Consciousness disorders are caused by many medical conditions, such as intracranial lesion, endocrine disease, cardiovascular disease, drug intoxication and psychiatric factor. Among them, consciousness disorders in cerebrovascular disease results mainly from brain edema[40]. Brain edema induces the increase of the intracranial pressure and decreases cerebral blood flow, therefore causes metabolic disorder of brain tissue and insufficiency of brain function[40]. The injury, such as trauma and ischemia, to the central nervous system initiates inflammatory processes that are implicated in secondary tissue damage. These processes include the synthesis of proinflammatory cytokines, leukocyte extravasation, vasogenic edema, and blood-brain barrier breakdown[39]. It is known that cytokines are involved in various neuropathologic disease, such as Alzheimer's disease, multiple sclerosis, and acquired immunodeficiency syndrome(AIDS). Also the change of the specific cytokine level was reported in an acute CI patient[37,38]. Early gene expression of inflammatory cytokines has been reported in the brain following global and focal cerebral infarction[2]. Th cells are thought to be important in the development of various diseases. While Th cells of the Th1 type predominantly produce IL-2 and IFN-, and are involved in cell-mediated immune responses, Th cells of Th2 type produce large quantities of IL-4 and IL-6, which promote the development of inflammation[19-21]. An alteration of cytokine levels could shift the Th1/Th2 balance toward Th2 dominance, the results being augmented eosinophil recruitment and inflammation[22]. Other researchers also reported that Th2 cytokine level was higher than Th1 cytokine levels in various diseases including cerebral infarction, allergy and asthma[23-24]. Proinflammatory cytokines, IL-4 has been called the prototypic immunoregulatory cytokine and involved in hemostatic and immunological imbalance leading to enlargement of various tissue damages. IL-4 has also an important role in regulating antibody production, hematopoiesis and inflammation, and the development of effecter T-cell responses[25]. Previously, we reported that levels of IL-4 elevated in the patients with cerebral infarction during the acute stage[23]. Yuldahansotang (has been developed as a formula to prevent and treat CI) inhibited secretion of IL-4 from human astrocytes stimulated with LPS[26]. In this study, we reported that plasma IL-4 levels in the CI group were higher than in normal group. We also reported that SJT effectively inhibited the production of IL-4 cytokines in LPS or PHA-stimulated PBMC cells. These results suggest that SJT might have a beneficial effect in the treatment of CI. Anti-inflammatory cytokine, IL-10 significantly inhibited the production of other proinflammatory mediators, such as reactive oxygen intermediates, reactive nitrogen intermediates and prostanglandins in monocyte/macrophages[27]. Stroke patients had significantly lowered IL-10 serum levels. Lower levels of IL-10 indicate that anti-inflammatory response is down-regulated in acute stroke patients[28]. Pelidou et al reported that number of myelin basic protein-reactive IL-10 secreting PBMC were elevated in a proportion of the patients with stroke and hemorrhage[29]. IL-10 also reduces rat brain

injury following focal stroke[30]. In the present study, we reported that plasma IL-10 levels in the CI group were lower than in normal group. We also demonstrated that LPS or PHA increased IL-10 production from PBMC of CI patients. From this, we suggest that anti-inflammatory cytokine IL-10 could play a pivotal role in ischemic stroke as well as cerebral infarction. We also reported that IL-10 was inhibited by SJT. Therefore, we can speculate that SJT may contribute to regulate the IL-10 production. The presence of an inflammatory response in the pathophysiology of acute brain ischemia is relatively well established, but less is known about the anti-inflammatory mechanisms. Therefore, further study is necessary to clarify the role of the IL-10 decreased by SJT.

Excess activity of the macrophage deactivating, anti-inflammatory, and pro-fibrotic cytokines, TGF-1, has been implicated in the pathogenesis of a number of diseases including cerebral infarction[31]. TGF-1 increases bad phosphorylation and protects neurons against damage[32]. Krupinski et al., also reported that TGF-1 might be involved in angiogenesis after ischemic stroke in humans[33]. Recent studies indicate that Glial cell line-derived neurotrophic factor (GDNF), a member of the TGF-superfamily, can protect the cerebral hemispheres from damage induced by middle cerebral arterial ligation[34]. In addition, Border et al. reported that TGF-1 is an important regulatory cytokine involved in tissues repair whose sustained production in many tissues underlies the development of fibrosis[35]. Nakao also reported that TGF-1 has been implicated in tissue repairing[36]. In this study, plasma TGF-1 levels in the CI group were lower than in normal group. We also showed that TGF-1 was increased by treatment of SJT. Therefore, we can speculate that SJT-induced TGF-1 expression may contribute to repairing processes, enduring cerebral infarction.

SJT is a prescription which was recorded for the first time in Cheongeumnyobang[千金要方] and treated blood stasis of Upper Burner[41] and has been used on disease with disorder of consciousness in oriental medicine. SJT is composed of Rhizoma Rehmanniae, Radix Paeoniae, Cortex Moutan Radicis, and Cornu Bubalus bubalis[41]. SJT has effects of heat-clearing therapy(淸熱), removing toxic material therapy(解毒), Cooling blood therapy(凉血) and removing blood stasis therapy(散瘀)[42]. Those effects are as good as anti-inflammatory, improvement of blood flow and immunity in western medicine[43]. Overall our results suggest that SJT is a regulator of cytokine production in PBMC cells, which might explain its beneficial effect in the treatment of CI. The exact mechanism and effect of SJT at a molecular level should be established in the future.

CONCLUSIONS

The author has obtained the following results through the study of SJT effect on the regulation of various cytokines and growth factor in peripheral blood mononuclear cells from the patient with cerebral infarction under consciousness disorders. IL-10 and TGF-1 levels were decreased in patients with CI, whereas IL-4 levels were slightly higher than in control. SJT had an effect on LPS or PHA-stimulated PBMC culture supernatant from the patient with CI. Production of IL-4 and IL-10 was significantly decreased by treatment of SJT. But TGF-1 production was significantly increased by treatment of SJT.

The results suggest that the regulation of cytokines and growth factor production in PBMC is closely involved in the therapeuthic effect in an cerebral infarction patient under consciousness disorders by SJT.

Acknowledgements

This work is supported by the Wonkwang University Research Fund in the year 2002.

REFERENCES

1. Kawamata T, Dietrich WD, Schallert T, Gotts JE, Cocke RR, Benowitz LI, Finklestein SP. Intracisternal basic fibroblast growth factor enhances functional recovery and up-regulates the expression of a molecular marker of neuronal sprouting following focal cerebral infarction. Proc Natl Acad Sci USA 94: 8179-8184, 1997.
2. Wiessner C, Gehrmann J, Lindholm D, Topper R, Kreutzberg GW, Hossmann KA. Expression of transforming growth factor-beta 1 and interleukin-1 beta mRNA in rat brain following transient forebrain ischemia. Acta Neuropathol (Berl) 86(5):439-46, 1993.
3. Nathan CF, Prendergast TJ, Wiebe ME, Stanley ER, Platzer E, Remold HG, Welte K, Rubin BY, Murray HW. Activation of human macrophages. Comparison of other cytokines with interferon-gamma. J Exp Med 160(2):600-5, 1984.
4. Baadsgaard O, Fisher GJ, Voorhees JJ, Cooper KD. Interactions of epidermal cells and T cells in inflammatory skin diseases. J Am Acad Dermatol 23(6 Pt 2):1312-6; discussion 1316-7, 1990.
5. Paul WE. Interleukin-4: a prototypic immunoregulatory lymphokine. Blood 77(9):1859-70, 1991.
6. Mitchell LC, Davis LS, Lipsky PE. Promotion of human T lymphocyte proliferation by IL-4. J Immunol 142(5):1548-57, 1989.

7. te Velde AA, Huijbens RJ, Heije K, de Vries JE, Figdor CG. Interleukin-4 (IL-4) inhibits secretion of IL-1 beta, tumor necrosis factor alpha, and IL-6 by human monocytes. Blood 76(7):1392-7, 1990.
8. Howells G, Pham P, Taylor D, Foxwell B, Feldmann M. Interleukin 4 induces interleukin 6 production by endothelial cells: synergy with interferon-gamma. Eur J Immunol 21(1):97-101, 1991.
9. Sempowski GD, Beckmann MP, Derdak S, Phipps RP. Subsets of murine lung fibroblasts express membrane-bound and soluble IL-4 receptors. Role of IL-4 in enhancing fibroblast proliferation and collagen synthesis. J Immunol 152(7):3606-14, 1994.
10. Lamkhioued B, Aldebert D, Gounni AS, Delaporte E, Goldman M, Capron A, Capron M. Synthesis of cytokines by eosinophils and their regulation. Int Arch Allergy Immunol 107(1-3):122-3, 1995.
11. de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10(IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of *IL-10* produced by monocytes. J Exp Med 174(5):1209-20, 1991.
12. Geng Y, Gulbins E, Altman A, Lotz M. Monocyte deactivation by interleukin 10 via inhibition of tyrosine kinase activity and the Ras signaling pathway. Proc Natl Acad Sci U S A 91(18):8602-6, 1994.
13. Howard M, O'Garra A. Biological properties of interleukin 10. Immunol Today 13(6):198-200, 1992.
14. Le Moine O, Louis H, Demols A, Desalle F, Demoor F, Quertinmont E, Goldman M, Deviere J. Cold liver ischemia-reperfusion injury critically depends on liver T cells and is improved by donor pretreatment with interleukin 10 in mice. Hepatology. 31(6):1266-74, 2000.
15. Aung H, Sherman J, Tary-Lehman M, Toossi Z. Analysis of transforming growth factor-beta 1 (TGF-beta1) expression in human monocytes infected with Mycobacterium avium at a single cell level by ELISPOT assay. J Immunol Methods 259(1-2):25-32, 2002.
16. Lijnen PJ, Petrov VV, Fagard RH. Induction of cardiac fibrosis by transforming growth factor-beta(1). Mol Genet Metab 71(1-2):418-35, 2000.
17. Saltis J, Agrotis A, Bobik A. Regulation and interactions of transforming growth factor-beta with cardiovascular cells: implications for development and disease. Clin Exp Pharmacol Physiol 23(3):193-200, 1996.
18. Ben Trivedi A, Kitabatake N, Doi E. Toxicity of dimethyl sulfoxide as a solvent in bioassay system with HeLa cells evaluated colorimetrically with 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl-tetrazolium bromide. Agric Biol Chem 54:2961-2966, 1990.
19. Romagnani S. Human TH1 and TH2 subsets: doubt no more. Immunol Today 12(8): 256-7, 1997.
20. Parronchi P, Macchia D, Piccinni MP, Biswas P, Simonelli C, Maggi E, Ricci M, Ansari AA, Romagnani S. 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(10):4538-42, 1991.
21. Zurawski G, de Vries JE. Interleukin 13, an interleukin 4-like cytokine that acts on monocytes and B cells, but not on T cells. Immunol Today 15(1):19-26, 1994.
22. Alenius H, Laouini D, Woodward A, Mizoguchi E, Bhan AK, Castigli E, Oettgen HC, Geha RS. Mast cells regulate IFN-gamma expression in the skin and circulating IgE levels in allergen-induced skin inflammation. J Allergy Clin Immunol 109(1):106-13, 2002.
23. 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. 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(3):191-6, 2000.
24. Jeong HJ, Kim BS, Kim KS, Kim HM. Regulatory effect of cytokine production in asthma patients by SOOJI CHIM (Koryo Hand Acupuncture Therapy). Immunopharmacol Immunotoxicol 24(2):265-74, 2002.
25. Brown MA, Hural J. Functions of IL-4 and control of its expression. Crit Rev Immunol 17(1):1-32, 1997.
26. Choi JS, Jung SW, Ju JC, Lee SW, Kim KY, Kim HM. Cytokine production regulation in human astrocytes by a herbal combination (Yuldahansotang). Immunopharmacol Immunotoxicol 24(1):55-67, 2002.
27. Gazzinelli RT, Oswald IP, James SL, Sher A. IL-10 inhibits parasite killing and nitrogen oxide production by IFN-gamma -activated macrophages. J Immunol 148(6): 1792-6, 1992.
28. Perini F, Morra M, Alecci M, Galloni E, Marchi M, Toso V. Temporal profile of serum anti-inflammatory and pro-inflammatory interleukins in acute ischemic stroke patients. Neurol Sci 22(4):289-96, 2001.
29. Pelidou SH, Kostulas N, Matusevicius D, Kivisakk P, Kostulas V, Link H. High levels of IL-10 secreting cells are present in blood in cerebrovascular diseases. Eur J Neurol 6(4):437-42, 1999.
30. Spera PA, Ellison JA, Feuerstein GZ, Barone FC. IL-10 reduces rat brain injury following focal stroke. Neurosci Lett 251(3):189-92, 1998.
31. Krupinski J, Kumar P, Kumar S, Kaluza J. Increased expression of TGF-beta 1 in brain tissue after ischemic stroke in humans. Stroke 27(5):852-7, 1996.

32. Zhu Y, Yang GY, Ahlemeyer B, Pang L, Che XM, Culmsee C, Klumpp S, Krieglstein J. Transforming growth factor-beta 1 increases bad phosphorylation and protects neurons against damage. J Neurosci 22(10):3898-909, 2002.
33. Krupinski J, Issa R, Bujny T, Slevin M, Kumar P, Kumar S, Kaluza J. A putative role for platelet-derived growth factor in angiogenesis and neuroprotection after ischemic stroke in humans. Stroke 28(3):564-73, 1997.
34. Wang Y, Chang CF, Morales M, Chiang YH, Hoffer J. Protective effects of glial cell line-derived neurotrophic factor in ischemic brain injury. Ann N Y Acad Sci 962:423-37, 2002.
35. Matsunaga Y, Kawasaki H, Terada T. Stromal mast cells and nerve fibers in various chronic liver diseases: relevance to hepatic fibrosis. Am J Gastroenterol 94:1923-1932, 1999.
36. Border WA, Ruoslahti E. Transforming growth factor-beta in disease: the dark side of tissue repair. J Clin Invest 90:1-7, 1992.
37. Brosnan CF, Selmaj FK and Raine DS. Hypothesis: a role for tumor necrosis factor in immune-related demyelination and its relevance to multiple sclerosis. J. Neuroimmunol 18:87, 1988.
38. Kim HM, Shin HY, Jeong HJ, An HJ, Kim NS, Chae HJ, Kim HR, Song HJ, Kim KY, Baek SH, Cho KH, Moon BS and Lee YM. 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, 2000.
39. Knoblach Susan M. Interleukin-10 improves outcome and alters proinflammatory cytokine expression after experimental traumatic brain injury. Experimental Neurology 153(1): 143-151, 1998.
40. Kenneth W. Lindsay, Ian Bone : Clinical neurology, Seoul, Korea medicine Publishers Ltd 127-144, 393-402, 1997.