

중풍환자에서 양격산화탕에 의한 세포활성 물질 생성의 조절 효과

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Abstract

The Effect of *Yangkyuk-Sanhwa-Tang* on Cytokine Production in the Patients with Cerebral Infarction

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Yangkyuk-Sanhwa-Tang(YST) has been widely used as a formula for the Soyangin cerebral infarction (CI) patients according to Sasang constitutional philosophy. Brain cells produce cytokines and chemokines during the inflammatory process after stroke both in animal models and in patients. Previously, regulation of serum cytokine levels by YST has been observed in individuals at the acute stage of CI disease, but there have not been other scientific investigations on YST. The author investigated the effect of YST on the production of various cytokines using peripheral blood mononuclear cells (PBMCs) from the Soyangin (CI) patients, and Soyangin normal group. The cytokine production was analyzed using enzyme-linked immunosorbent assay (ELISA). The amount of interleukin (IL)-1, IL-1, IL-6, IL-8, and tumor necrosis factor (TNF)- in culture supernatant significantly increased in the LPS-treated cells compared with unstimulated-cells ($P < 0.05$). However, in LPS-stimulated PBMCs, cytokines level in CI patients group was higher than that of normal group. YST (1 mg/ml) significantly inhibited IL-1, IL-1, and IL-8 production in PBMCs stimulated with LPS (about 85% for IL-1, 87% for IL-1, and 53% for IL-8, $P < 0.05$), but did not significantly inhibit IL-6 and TNF- production in the CI patients group. We also show that YST significantly increased LPS-induced IL-1, IL-6, and TNF- production in the normal group. These data suggest that YST has a regulatory effect on the cytokine production, which might explain its beneficial effect in the treatment of CI.

Key words: *Yangkyuk-Sanhwa-Tang*, lipopolysaccharide, cerebral infarction, cytokine

I. Introduction

Sasang Constitution is one of the Oriental medicinal theories, which has not been scientifically proved. Sasang Constitution based on traditional Oriental philosophy consists of four types of people that are

Taeyangin, Taeumin, Soyangin, Soumin. People are grouped in accordance with the four internal organs, which are lung, spleen, liver and kidney. For example, the person with large spleen and small kidney belongs to Soyangin. According to this theory, people who belong to different type of constitution have different prescription respectively to a same disease. *Yangkyuk-Sanhwa-Tang* (YST), a prescription of Traditional Oriental Medicine, has long been used as a specific

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prescription for cerebral infarction (CI) in the type of Soyangin to increase cerebral blood flow and to recover an injured brain cell. Recently, Jeong et al.¹⁾ have reported that the serum level of cytokine was regulated by YST in an acute CI of Soyangin.

Patients with CI due to either thrombi or emboli are frequently suffered from irreversible neurologic deficits that markedly hinder their activity of daily living. Patients may also be suffered from 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 weeks to months.²⁾

Early gene expression of inflammatory cytokines has been reported in the brain following global and focal CI.³⁾ Interleukin (IL)-1 has pleiotropic actions in the central nervous system. During the past decade, a growing corpus of evidence has indicated an important role of this cytokine in the development of brain damage following cerebral ischemia. The expression of IL-1 in the brain is dramatically increased during the early and chronic stage of infarction.⁴⁾ IL-6 is a differentiation factor for B and T lymphocytes, monocytes, neural cells, and hepatocytes.⁵⁻⁷⁾ It has been shown that high levels of IL-6 can occur in the cerebrospinal fluid of neurological patients,⁸⁻¹¹⁾ but the possible etiopathogenetic role of the interleukin in diseases of the central nervous system (CNS) is not clarified. Brain tissue levels of IL-8, a potent neutrophil chemotactic cytokine (chemokine), increased significantly after reperfusion.¹²⁾ The inflammatory cytokine, TNF- α is known to be expressed in brain ischemia. Focal cerebral ischemia in rats produces elevated levels of TNF- α in the ischemic brain region.¹³⁻¹⁴⁾ As described above, various inflammatory cytokines are related to CI, directly or indirectly.

In this study, the regulating effect of YST on inflammatory cytokine production induced by LPS in peripheral blood mononuclear cells (PBMCs) was investigated. An experiment on the regulating effect of

YST on cytokine production in PBMCs was conducted to determine how the regulating mechanism takes place. The author now reports the results that provide the basis for the clinical therapeutic effect on YST in CI patients.

II. Materials and Methods

1. material

1) Reagents

Ficoll-Hypaque, lipopolysaccharide (LPS), avidin-peroxidase, 3-[4,5-Dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), and 2'-azino-bis (3-ethylbenzothiazoline-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). Anti-human IL-1 α , IL-1 β , and TNF- α , biotinylated anti-human IL-1 α , IL-1 β , and TNF- α , and recombinant (r) human IL-1 α , IL-1 β , and TNF- α were purchased from RD systems (Minneapolis, MN, USA). Anti-human IL-6 and IL-8, biotinylated anti-human IL-6 and IL-8, and human IL-6 and IL-8 were purchased from Pharmingen (San Diego, CA, USA).

2) Patient

Patients with CI were examined at ** University. 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%). Blood was obtained from 4 patients (2 males and 2 females, age range 65-70) with CI and 10 healthy adults (5 males and 5 females, age range 60-67) with no medically diagnosable illness as a control group. All samples were collected by centrifugation and quickly frozen and stored in aliquots

at -80°C until assay.

3) Preparation of YST

The ingredients of 44g YST include 8g of *Rehmanniae Radix*, 8g of *Lonicera Japonica*, 4g of *Forsythiae Fructus*, 4g of *Gardeniae Fructus*, 4g of *Menthae Herba*, 4g of *Anemarrhenae Rhizoma*, 4g of *Gypsum Fibrosum*, 4g of *Schizonepetae Herba*, and 4g of *Ledebouriellae Radix*. An extract of YST was prepared by decocting the dried prescription of herbs with boiling distilled water. The plant materials were obtained from the Oriental Medicine Hospital, Wonkwang University and identified by Dr. K.Y. Kim, College of Oriental Medicine, Wonkwang University, and their voucher specimens have been deposited in the Herbarium in the College of Pharmacy, Wonkwang University.

4) PBMCs isolation and culture

PBMCs (CI patients or normal group) 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 2mM L-glutamine, 100U/ml penicillin G, 100g/ml streptomycin, and 10% FBS inactivated for 30min at 56°C . PBMCs were adjusted to a concentration of 2×10^6 cells/ml in 30ml falcon tube, and 100 μl aliquots of cell suspension were placed in a four-well cell culture plate. PBMCs were cultured for 24h 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 .

2. Method

1) MTT assay

The MTT colorimetric assay of cell survival was executed by the method of Trivedi *et al.*¹⁵⁾ with minor modifications. Cell aliquots (2×10^5) were seeded in microplate wells and incubated with 20 μl of a MTT solution (5 mg/ml) for 4h at 37°C under 5% CO_2 and 95% air. Consecutively, 250 μl of DMSO was added to extract the MIT formazan and an automatic microplate

reader read the absorbance of each well at 540nm.

2) ELISA of IL-1 α , IL-1 β , IL-6, IL-8, and TNF- α

Cytokine production was measured by a modified ELISA, as described previously.¹⁶⁾ ELISA for IL-1 α , IL-1 β , IL-6, IL-8 and TNF- α was carried out in duplicate in 96-well, ELISA plates (Nunc) coated with each of 100 μl aliquots of mouse anti-human IL-1 α , IL-1 β , IL-6, IL-8 and TNF- α monoclonal antibodies at 1.0g/ml in PBS at pH 7.4 and were incubated overnight at 4°C . The plates were washed in PBS containing 0.05% Tween-20 (Sigma) and blocked with PBS containing 1% BSA, 5% sucrose and 0.05% NaN_3 for 1h. After additional washes, serum sample or IL-1 α , IL-1 β , IL-6, IL-8, and TNF- α standards were added and incubated at 37°C for 2h. After a 2h incubation at 37°C , the wells were washed and then each of 0.2g/ml of biotinylated anti-human IL-1 α , IL-1 β , IL-6, IL-8, and TNF- α were added and again incubated at 37°C for 2h. After washing the wells, streptavidin-peroxidase was added and plates were incubated for 20min at 37°C . Wells were again washed and ABTS substrate (Sigma) was added. Color development was measured at 450nm using an automated microplate ELISA reader. A standard curve was run on each assay plate using recombinant human IL-1 α , IL-1 β , IL-6, IL-8 and TNF- α (R&D Systems or Pharmingen) in serial dilutions.

3. Statistical analysis

The experiments shown are presented as the mean \pm SD. Statistical evaluation of the results was performed by independent *t*-test and ANOVA with the Tukey post hoc test. The results were considered significant at a value of $P < 0.05$.

III. Results

1. Cytokine production by LPS in the CI patients and normal group

The amount of IL-1, IL-6, and TNF- α , in CI patients and normal group culture supernatants, was

significantly increased in the LPS treated cells compared with unstimulated cells. The level of cytokine in CI patients group was higher than that of normal group, when LPS (10 ng/ml) stimulated PBMCs (Table 1).

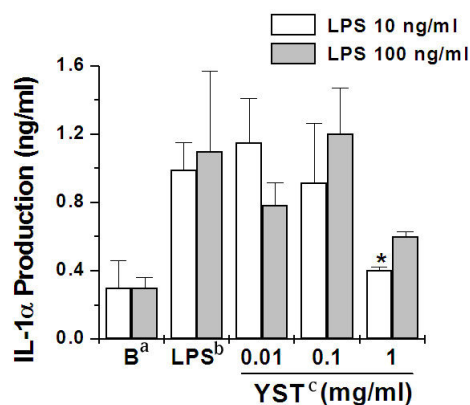
Table 1. Effect of LPS on the Cytokine Production from PBMCs of CI patients Group or Normal Group.

	LPS (10 ng/ml)	IL-1 β (ng/ml)	IL-6 (ng/ml)	TNF- α (ng/ml)
Normal group	-	0.122 \pm 0.01	0.097 \pm 0.01	0.267 \pm 0.01
	+	0.487 \pm 0.10*	0.335 \pm 0.01*	0.346 \pm 0.01
CI group	-	0.612 \pm 0.20	0.375 \pm 0.31	0.755 \pm 0.08
	+	7.885 \pm 2.19*	6.773 \pm 0.65*	4.164 \pm 0.50*

PBMCs (2×10^5) were stimulated with LPS (10ng/ml) for 24h. The cells were separated from the released cytokines by centrifugation at $400 \times g$ for 5min at 4°C. Cytokine levels in culture supernatants were measured using ELISA. Values are the mean \pm SD of duplicate determinations (n=6). * $P < 0.05$, significantly different from the control.

2. Effect of YST on LPS -induced IL-1 α production in the CI patients group

To evaluate the regulatory effect of YST on the IL-1



^aB=unstimulated PBMCs
^bLPS=Treated cells with LPS
^cYST=Treated cells with Yangkyuk-Sanhwa-Tang after LPS stimulation

Fig. 1. Effect of YST on LPS -induced IL-1 α production

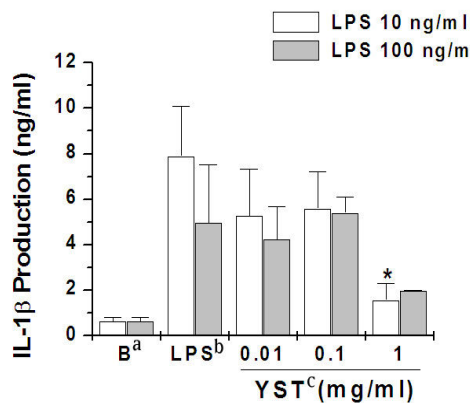
a production, PBMCs were treated with LPS for 24h. The supernatants were analyzed using ELISA for IL-1 α . As shown in Fig. 1, LPS (10 or 100ng/ml) significantly increased cytokine production on the PBMCs (unstimulated cells, 0.303 ± 0.06 ng/ml; LPS 10ng/ml, 0.99 ± 0.17 ng/ml, $P = 0.002$; LPS 100ng/ml, 1.04 ± 0.47 ng/ml, $P = 0.001$). The author also tested the effect of YST on LPS-induced IL-1 α production. IL-1 increased by LPS 10ng/ml was significantly inhibited by YST 1mg/ml. Inhibition rate was $85 \pm 2\%$. Cell cytotoxicity by YST was not observed (Fig. 1).

PBMCs (2×10^5) were treated with various concentration of YST for 1 h and then stimulated with LPS (10ng/ml or 100ng/ml) for 24h. Cytokine concentrations were measured from cell supernatants using ELISA method. Values are the mean \pm SD of duplicate determinations (n=6). * $P < 0.05$; significantly different from the LPS-stimulated cells.

3. Effect of YST on LPS-induced IL-1 β production in the CI patients group

To assess the regulatory effect of YST on IL-1 β production, PBMCs were treated with LPS for 24h. The supernatants were analyzed using ELISA for IL-1 β . As shown in Fig. 2, LPS (10 or 100ng/ml) significantly increased cytokine production on the PBMCs (unstimulated cells, 0.61 ± 0.2 ng/ml; LPS 10ng/ml, 7.88 ± 2.19 ng/ml, $P = 0.00$; LPS 100ng/ml, 4.96 ± 2.55 ng/ml, $P = 0.01$). The author also tested the effect of YST on LPS-induced IL-1 β production. IL-1 β increased by LPS 10ng/ml was significantly inhibited by YST 1mg/ml. Inhibition rate was $87 \pm 8\%$ (Fig. 2).

PBMCs (2×10^5) were treated with various concentration of YST for 1h and then stimulated with LPS (10ng/ml or 100ng/ml) for 24h. Cytokine concentrations were measured from cell supernatants using ELISA method. Values are the mean \pm SD of duplicate determinations (n=6). * $P < 0.05$; significantly different from the LPS-stimulated cells.

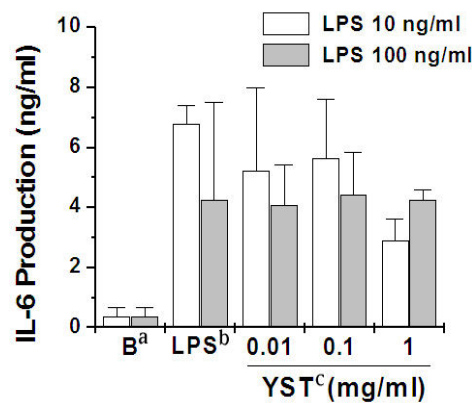


^aB=unstimulated PBMCs

^bLPS=Treated cells with LPS

^cYST=Treated cells with Yangkyuk-Sanhwa-Tang after LPS stimulation

Fig. 2. Effect of YST on LPS -induced IL-1 β production



^aB=unstimulated PBMCs

^bLPS=Treated cells with LPS

^cYST=Treated cells with Yangkyuk-Sanhwa-Tang after LPS stimulation

Fig. 3. Effect of YST on LPS -induced IL-6 production

4. Effect of YST on the IL-6 production in the CI patients group

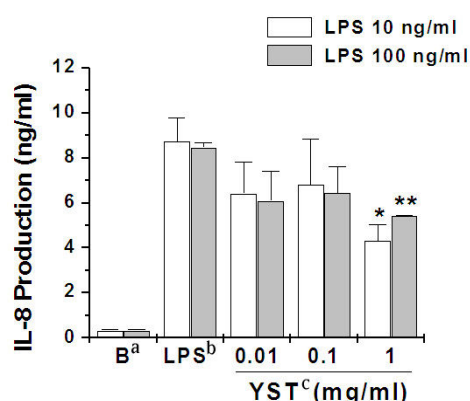
To evaluate the regulatory effect of YST on the IL-6 production, PBMCs were treated with LPS for 24h. The supernatants were analyzed using ELISA for IL-6. As shown in Fig. 3, LPS (10ng/ml) significantly increased cytokine production on the PBMCs (unstimulated cells, $0.37 \pm 0.31 \text{ ng/ml}$; LPS 10ng/ml, $6.77 \pm 0.65 \text{ ng/ml}$, $P=0.001$), but did not significant at LPS 100ng/ml ($4.25 \pm 3.27 \text{ ng/ml}$, $P=0.099$). The author also tested the effect of YST on LPS-induced IL-6 production. IL-6 increased by LPS 10ng/ml was inhibited by YST, but there was no significant difference. YST had no effect on the LPS 100ng/ml-induced IL-6 production (LPS 10ng/ml, YST 1mg/ml, $P=1$; LPS 100ng/ml, YST 1mg/ml, $P=0.569$) (Fig. 3).

PBMCs (2×10^5) were treated with various concentration of YST for 1h and then stimulated with LPS (10 or 100ng/ml) for 24h. Cytokine concentrations were measured from cell supernatants using ELISA method. Values are the mean \pm SD of duplicate determinations ($n=6$).

5. Effect of YST on LPS-induced IL-8 production in the CI patients group

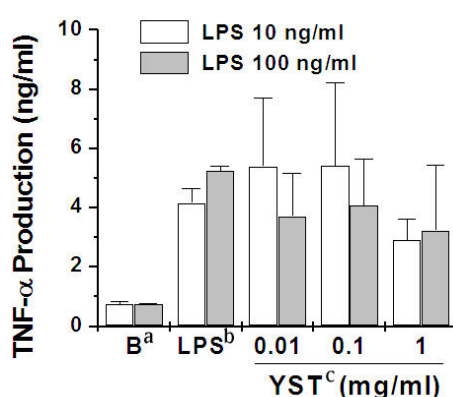
To assess the regulatory effect of YST on IL-8 production, PBMCs were treated with LPS for 24h. The supernatants were analyzed using ELISA for IL-8. LPS (10 or 100ng/ml) significantly increased cytokine production on the PBMCs (unstimulated cells, $0.33 \pm 0.03 \text{ ng/ml}$; LPS 10ng/ml, $8.73 \pm 1.07 \text{ ng/ml}$, $P=0.00$; LPS 100ng/ml, $8.46 \pm 0.2 \text{ ng/ml}$, $P=0.00$). The author tested the effect of YST on LPS-induced IL-8 production. IL-8 increased by LPS was significantly inhibited by YST 1mg/ml ($P<0.05$). Inhibition rate of LPS 10ng/ml and 100ng/ml was about 53% and 37.2%, respectively (Fig. 4).

PBMCs (2×10^5) were treated with various concentration of YST for 1 h and then stimulated with LPS (10 or 100ng/ml) for 24h. Cytokine concentrations were measured from cell supernatants using ELISA method. Values are the mean \pm SD of duplicate determinations ($n=6$). $*P<0.05$: significantly different from the LPS 10ng/ml-stimulated cells. $**P<0.05$: significantly different from the LPS 100ng/ml-stimulated cell.



^aB=unstimulated PBMCs
^bLPS=Treated cells with LPS
^cYST=Treated cells with Yangkyuk-Sanhwa-Tang after LPS stimulation

Fig. 4. Effect of YST on LPS -induced IL-8 production



^aB=unstimulated PBMCs
^bLPS=Treated cells with LPS
^cYST=Treated cells with Yangkyuk-Sanhwa-Tang after LPS stimulation

Fig. 5. Effect of YST on LPS -induced TNF-α production

6. Effect of YST on LPS induced TNF-α production in the CI patients group

To evaluate the regulatory effect of YST on the TNF-α production, PBMCs were treated with LPS for 24h. The supernatants were analyzed using ELISA for TNF-α. LPS (10 or 100ng/ml) significantly increased cytokine production on the PBMCs (unstimulated cells, 0.75±0.08ng/ml; LPS 10ng/ml, 4.16±0.5ng/ml, *P*=0.001; LPS 100ng/ml, 5.24±0.15ng/ml, *P*=0.00). The author tested the effect of YST on LPS-induced TNF-α production. TNF-α increased by LPS was inhibited by YST 1mg/ml, but there was no significant difference (LPS 10ng/ml, YST 1mg/ml, *P*=0.72) (Fig. 5).

PBMCs (2×10⁵) were treated with various concentration of YST for 1 h and then stimulated with LPS (10 or 100ng/ml) for 24h. Cytokine concentrations were measured from cell supernatants using ELISA

Table 2. Effect of YST on the LPS-Induced cytokine Production from PBMCs of Normal Group.

YST (mg/ml)	IL-1β (ng/ml)	IL-6 (ng/ml)	TNF-α (ng/ml)
-	0.487 ± 0.10	0.335 ± 0.01	0.346 ± 0.001
0.01	0.764 ± 0.10	0.419 ± 0.09*	0.406 ± 0.12
0.1	1.080 ± 0.30*	0.400 ± 0.01	0.519 ± 0.10*
1	-	0.415 ± 0.06	0.625 ± 0.10

method. Values are the mean ± SD of duplicate determinations (n=6).

7. Effect of YST on LPS induced cytokine production in the normal group

The effect of YST on LPS induced IL-1β, IL-6, and TNF-α production in PBMCs was evaluated. These cytokines are an important inflammatory cytokine. LPS induce inflammatory cytokine production by PBMCs. As shown in Table 2, YST increased the LPS-induced IL-1β, IL-6, and TNF-α production.

PBMCs (2×10⁵) were treated with various concentration of YST for 1h and then stimulated with LPS (10ng/ml) for 24h. The cells were separated from the released cytokines by centrifugation at 400Xg for 5min at 4°C. Cytokine levels in culture supernatants were measured using ELISA. Values are the mean±SD of duplicate determinations (n=6). **P*<0.05, significantly different from the control.

IV. Discussion

In this study, the regulatory effect of YST on LPS-induced cytokine production in PBMCs was

investigated. YST (1mg/ml) significantly inhibited IL-1 α , IL-1 β , and IL-8 production in PBMCs stimulated with LPS, but did not significantly inhibit IL-6 and TNF- α production in the CI patients group. The author also shows that YST significantly increased LPS-induced IL-1 β , IL-6, and TNF- α production in the normal group.

Endotoxin LPS can initiate a variety of cell activation pathways. These unique macromolecules have been extensively studied in order to elucidate and define relevant pathophysiological parameters of endotoxin shock, profound life-threatening consequence of bacterial sepsis.¹⁷⁾ These bacterial products have generated intense interest due to their pluripotential immunostimulatory activity as manifested by the activation of host cells, B lymphocyte and macrophage to differentiate functionally.¹⁸⁾ Cell activated by LPS produce cytokines including interferons, IL-1, IL-6, IL-8, TNF- α , platelet activating factor, and procoagulant tissue factor. These cytokines induced by LPS involved in inflammation.¹⁹⁾

IL-1 is an endogenous pyrogen, the activating factor of lymphocyte products. It is made in many cells, especially macrophage cells, where it is made most. IL-1 activates T-cell and B-cell, and causes the inflammatory response. According to Touzani et al.'s recent report,²⁰⁾ IL-1 has an important role in the development of brain damage following cerebral ischemia. The expression of IL-1 in the brain is dramatically increased during the early and chronic stages of infarction. The most direct evidence that IL-1 contributes significantly to ischemic injury is that²¹⁾ central administration of IL-1 exacerbates brain damage, and²²⁾ injection or over-expression of IL-1 receptor antagonist, and blockade of IL-1 converting enzyme activity dramatically reduce infarction and improve behavioral deficit. The mechanisms underlying IL-1 actions in stroke have not been clearly elucidated, and it seems likely that its effects are mediated through stimulation and inhibition of a wide range of

pathophysiological processes. The chemokine IL-8 is produced by stimulated monocytes, endothelium and other tissue cells. It is a potent chemoattractant for polymorphonuclear leukocyte (PMNLs) and also stimulates degranulation and the respiratory burst of these cells.²³⁾ IL-8 may therefore contribute to the accumulation of PMNLs in ischemic brain injury, a process that is considered to exacerbate tissue damage by vessel plugging and the release of oxygen radicals, proteinases and proinflammatory cytokines.²⁴⁻²⁵⁾ Accordingly, neutralizing IL-8 reduced brain edema and infarct size in a reperfusion model in rabbits²³⁾. Grau et al., reported that IL-8 levels increased in acute cerebral ischemia.²⁷⁾ Recent studies had shown that IL-8 is increased in cerebrospinal fluid (CSF) early after stroke.²⁸⁾ In this study, the author showed that YST effectively inhibited the production of IL-1 and IL-8 cytokines in LPS-stimulated PBMC cells. These results suggest that YST might have a beneficial effect in the treatment of CI.

Ali et al.²⁹⁾ have reported that in the brain, the expression of the pleiotropic cytokine IL-6 is enhanced in various chronic or acute CNS disorders and focal cerebral ischemia in rats early up-regulated the expression of IL-6 mRNA. Additionally, the striatal injection of N-methyl-D-aspartate in rats, a paradigm of excitotoxic injury activated the expression of IL-6 mRNA. TNF- α is pleiotropic cytokine and appears to be involved in blood-brain barrier, inflammatory, thrombogenic, and vascular changes associated with brain injury. TNF- α levels in brain tissue, cerebrospinal fluid, and plasma have been found to be elevated in several CNS disorders, including Alzheimer's disease, multiple sclerosis, Parkinson's disease, meningococcal meningitis, and HIV infection.³⁰⁾ Recent studies have indicated that blocking of TNF- α reduced brain injury and attenuated ICAM-1 expression during transient cerebral ischemia.³¹⁾ In this study, the author showed that YST inhibited the production of IL-6 and TNF- α cytokines in LPS-stimulated PBMC cells, but there was

no significant difference. On the other hand, YST increased the LPS-induced IL-6 and TNF- α production in the Soyangin normal group. Recently, Jeong et al. reported that YST reduced the IL-6 and TNF- α levels, and improved outcome in focal stroke patients.¹⁾ Therefore, these results suggest that YST might have a beneficial effect in the treatment of CI. Further study was necessary to clarify mechanism of the IL-6 and TNF- α regulation by YST.

The Sasang Constitutional Medicine was established by Je-Ma Lee of Korea in 1894. In Sasang Constitutional Medicine, the symptoms of diseases in each constitutional type are classified according to exterior and interior diseases. Sasang Constitutional Medicine forms the basis of treatment by harmonizing the interrelationship between the internal organs, thereby recovering the body's homeostatis and enabling the body to prevent disease. In other words, if the human body maintains a balanced and harmonious states, its immune system will be working at an optimal level and the body's ability to prevent or fight off most diseases will be strengthened. Therefore, we can enhance the body's ability to prevent disease and to recuperate from disease damage by promoting beneficial immune responses and repressing the harmful ones.

The Sasang Constitutional medical approach to disease is distinctive in that it focuses on endogenous etiological factors rather than exogenous ones. Even in diseases caused by exogenous etiological factors it focuses on natural treatment through self-recovery of body's immune function by remedying endogenous etiological factors.³²⁾

Therefore, YST is thought to be applicable to the treatment of the acute CI. Nevertheless, the precise mechanism of YST should be established in the future.

V. Conclusion

The author has obtained the following results

through the study of YST's effect on the production of various cytokines using peripheral blood mononuclear cells (PBMCs) from the Soyangin (CI) patients, and Soyangin normal group.

1. The amount of interleukin (IL)-1 α , IL-1 β , IL-6, IL-8, and tumor necrosis factor (TNF)- α in culture supernatant significantly increased in the LPS-treated cells compared with unstimulated-cells ($P < 0.05$).

2. In LPS-stimulated PBMCs, cytokines level in CI patients group was higher than that of normal group.

3. YST (1mg/ml) significantly inhibited IL-1 α , IL-1 β , and IL-8 production in PBMCs stimulated with LPS (about 85% for IL-1 α , 87% for IL-1 β , and 53% for IL-8, $P < 0.05$), but did not significantly inhibit IL-6 and TNF- α production in the CI patients group.

4. YST significantly increased LPS-induced IL-1 β , IL-6, and TNF- α production in the normal group.

These results suggest that YST has a regulatory effect on the cytokine production, which might explain its beneficial effect in the treatment of CI.

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