

Original Article

Preventive Effect of *Hwangryunhaedok-tang* on Inflammatory Responses in PHA-stimulated Peripheral Blood Mononuclear Cells from Cerebral Infarction Patients

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Objectives: *Hwangryunhaedok-tang* (HRHDT), a prescription composed of four herbs, has been widely used in Oriental Medicine for the treatment of cerebral infarction. However, the mechanisms by which the herbal formula affects on the production of pro- and anti-inflammatory cytokines in cerebral infarction patients remain unknown yet.

Methods: The levels of pro- and anti-inflammatory cytokines, including tumor necrosis factor (TNF)- α , interleukin (IL)-1b, and IL-6, IL-10, and TGF- β 1 were determined in peripheral blood mononuclear cells (PBMCs) from cerebral infarction patients under our experimental conditions.

Results: The secretory levels of pro- and anti-inflammatory cytokines, including tumor necrosis factor (TNF)- α , interleukin (IL)-1b, and IL-6, and IL-10 were significantly increased in phytohemagglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMCs) from cerebral infarction patients. However, pretreatment with HRHDT significantly inhibited the secretion of pro- and anti-inflammatory in PBMCs. Also, HRHDT induced a significant increase of transforming growth factor (TGF)-b1 in PBMCs.

Conclusions: These data indicate that HRHDT may be beneficial in the suppression of inflammatory processes of cerebral infarct through suppression of TNF- α , IL-1 β , IL-6, and IL-10 and induction of TGF- β 1.

Key Words : *Hwangryunhaedok-tang* (HRHDT), cerebral infarction, pro-inflammatory cytokines, IL-10, TGF- β 1

Introduction

Hwangryunhaedok-tang (HRHDT), a traditional prescription of Oriental Medicine, has been used in treatment of cerebral infarction patients to ameliorate and decrease morbidity and mortality after stroke. HRHDT contains four herbs, *Coptidis Rhizoma*, *Phellodendri Cortex*, *Scutellariae Radix*, and *Gardeniae*

Fructus, which have pharmacological and biological effects, including anti-inflammatory¹⁾ and anti-oxidative²⁻⁴⁾ effects. However, the mechanism of its therapeutic benefits in cerebral infarction patients has not yet been well defined. Cerebral infarct usually causes cerebral ischemic insults with irreversible deterioration of central nervous system (CNS) behaviors. After the onset of cerebral ischemia, the inflammatory process

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triggers acceleration of the early onset and functions as a determinant factor in severity of cerebral damage, morbidity and mortality in neurodegenerative brain diseases^{5,6}. Usually, inflammation occurs in tissues outside the brain with features of redness, swelling, and heat. Under cerebral ischemia, the acute phase of inflammation initiates recruitment of activated inflammatory cells, including macrophages and lymphocytes, into the damaged brain lesions. Macrophages and lymphocytes, circulating immune cells, play an essential role in secreting pro-inflammatory cytokines and activating inflammatory mediators in ischemic status. Other supporting cells, including astrocytes, microglia and endothelia, are also involved in inflammatory processes after cerebral ischemic stroke⁷⁻⁹. Pro-inflammatory cytokines, including tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , and IL-6, are secreted in the ischemic region by activated immune cells, which drive the inflammatory process and accelerate the additional inflammatory processes by inducing inflammatory molecules, such as intercellular adhesion molecules (ICAM), vascular cell adhesion molecules-1 (VCAM-1), and selectin. These inflammatory modulators recruit more circulating leukocytes which infiltrate into ischemic region and lead to further loss of neuronal cells and brain tissue and increase of cerebral infarct size¹⁰⁻¹². Interestingly, blockade of immune reaction and anti-inflammatory agents have been regarded as a potentially therapeutic benefit in cerebral ischemia over the past decade. Therefore, specific inhibition on the role of pro-inflammatory molecules has been focused in treatment of cerebral infarction patients even though molecular mechanisms are not clearly demonstrated in prevention of subsequent neuronal damages during ischemia^{6,13-15}. Anti-inflammatory cytokines, such as IL-10 and transforming growth factor (TGF)- β 1, have been identified to suppress the production of pro-inflammatory cytokine in protection of damaged brain tissues after ischemic stroke. Also, anti-inflammatory cytokines are associated with repairing damaged brain tissues¹⁶⁻¹⁸. To identify the

functional mechanism of HRHDT on cerebral infarct, we herein investigated the regulatory roles of HRHDT on phytohemagglutinin (PHA)-induced inflammatory cytokines in peripheral blood mononuclear cells (PBMCs) from cerebral infarction patients.

Materials and Methods

1. Reagents

Ficoll-Hypaque, PHA, and 3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyl-tetrazolium bromide (MTT) were purchased from Sigma (St. Louis, MO, USA). RPMI 1640, ampicillin, streptomycin, and fetal bovine serum (FBS) were bought from Gibco BRL (Grand Island, NY, USA). TNF- α , IL-1 β , IL-6, IL-10, and TGF- β 1 ELISA kits were obtained from R&D system (Minneapolis, MN, USA).

2. Patients

Patients with cerebral infarction were admitted and examined at the Department of Neurology, Wonkwang University Hospital from September 2004 to February 2005. The diagnosis of cerebral infarction was confirmed with computerized tomography (CT), magnetic resonance imaging (MRI), and specific clinical signs, including hemiparesis, hemiplegia, slurred speech, facial palsy, etc. All patients gave informed consent before participating in the research protocols, which was approved by the ethics committee of Wonkwang University Hospital.

3. Preparation of HRHDT

The ingredients of HRHDT were *Coptidis Rhizoma* (16 g), *Phellodendri Cortex* (40 g), *Scutellariae Radix* (40 g), and *Gardeniae Fructus* (40 g). An extract of HRHDT was prepared by decocting the dried prescription of herbs with distilled water (100 g/L). The decoction was filtered, lyophilized, and kept at 4°C. The yield of extraction was about 9.79% (w/w). The HRHDT powder of water extract was

dissolved in sterile saline (100 mg/ml). Final concentrations of 175, 350, and 700 $\mu\text{g/ml}$ HRHDT were used for the experiments. The plant materials were obtained and identified by Professor Byung-Soon Moon from Oriental Medical Hospital, Wonkwang University, Korea.

4. Cell culture

PBMCs from heparinized venous blood of patients with cerebral infarction were isolated by Ficoll-gradient centrifugation, then washed three times in PBS and resuspended in RPMI-1640 medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin G, 100 $\mu\text{g/ml}$ streptomycin, and 10% FBS inactivated for 30 min at 56 °C. PBMCs were adjusted to a concentration of 2×10^6 cells/ml in 30 ml falcon tubes, and 100 ml aliquots of cell-suspension were placed in a four-well cell culture plate. PBMCs were cultured with PHA (10 $\mu\text{g/ml}$) for 24 hr after pretreatment with or without HRHDT (175-700 $\mu\text{g/ml}$) in 95% humidified air containing 5% CO_2 at 37°C; the supernatants were collected by centrifugation and stored at -20 °C.

5. Cell viability test

Cell viability was quantified using 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT). In brief, cells (5×10^5) were cultured in 24-well plates with varying concentrations of HRHDT for 24 h. To determine the cell viability, MTT (0.5mg) was added to 1ml of cell suspension for 4 h. After three washes of cells with phosphate-buffered saline (PBS, pH 7.4), the insoluble formazan product was dissolved in DMSO. Then, the optical density (OD) of each culture well was measured using a Microplate reader (Titertek Multiskan, Flow Laboratories) at 590 nm. The OD in control cells was taken as 100% of viability.

6. Measurement of pro-inflammatory cytokines by ELISA

Secretory level of pro-inflammatory cytokines, including TNF- α , IL-1 β , IL-6, and anti-inflammatory cytokines, including IL-10, and TGF- β 1, in culture supernatant was determined by Quantikine ELISA kit (R&D Systems Inc, Minneapolis, MN, USA). The color generated was determined by measuring the O.D. at 450 nm of spectrophotometric microtiter plate reader (Molecular Devices Corp., Sunnyvale, CA, USA). The minimum detectable concentration of TNF- α , IL-1 β , IL-6, IL-10, and TGF- β 1 was estimated to be 1.6 pg/ml, 1.0 pg/ml, 0.7 pg/ml, 3.9 pg/ml, and 7 pg/ml, respectively. A standard curve was run on each assay plate using recombinant proteins, including TNF- α , IL-1 β , IL-6, IL-10, and TGF- β 1 in serial dilutions.

7. Statistical analysis

The data shown are a summary of the results from at least three experiments and are presented as the mean \pm S.E.M. Statistical evaluation of the results was performed by one-way ANOVA. The results were considered significant at a value of $p < 0.05$.

Results

1. HRHDT significantly inhibited the production of pro-inflammatory cytokines in human PBMCs from cerebral infarction patients

We examined whether HRHDT could regulate pro-inflammatory cytokines in PBMCs from cerebral infarction patients. At first, we confirmed that the concentration of HRHDT used in this study showed no significant effect on viability of PBMCs at 700 $\mu\text{g/ml}$ (Fig. 1). PBMCs from cerebral infarction patients ($n=10$, mean age 56 \pm 12 years; 3 female and 7 males) were treated with HRHDT for 24 hr and cell viability was tested by MTT assay. To evaluate whether

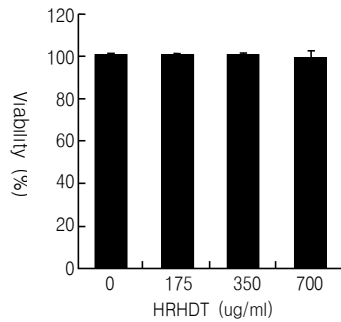


Fig. 1. HRHDT did not affect cell viability of PBMCs from CI patients.

PBMCs were treated with 0.175–0.7 mg/ml HRHDT for 24 h. Cell viability was measured by MTT assay. Results are expressed as means \pm SD of three independent experiments (* p <0.05). (PBMCs: peripheral blood mononuclear cells)

HRHDT suppresses pro-inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6, PBMCs from cerebral infarction patients were pretreated with HRHDT for

1 hr and further maintained with 10 μ g/ml PHA for 24 hr. The culture supernatant was collected to measure the secretion of pro-inflammatory cytokines by ELISA

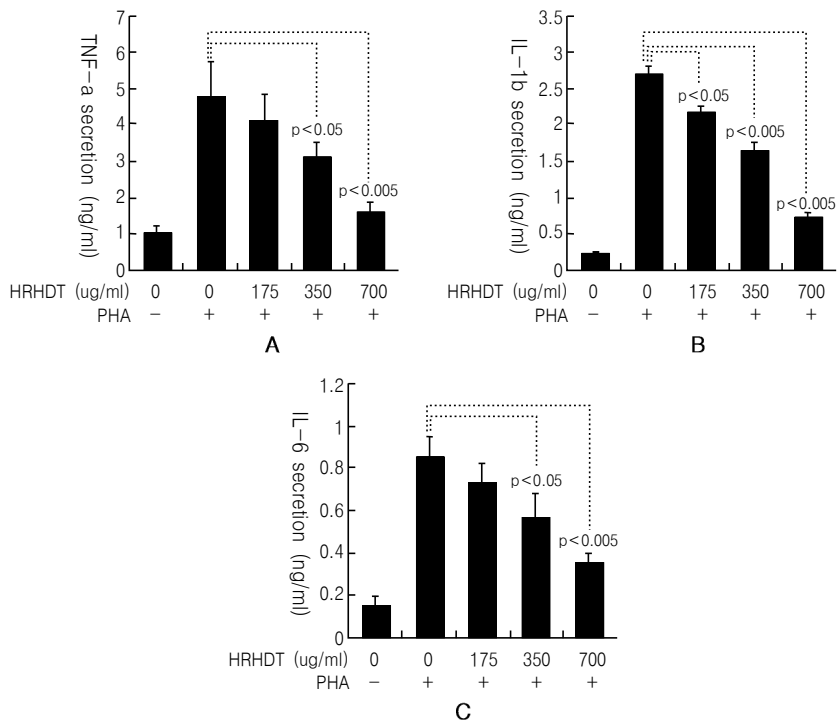


Fig. 2. HRHDT suppressed the secretion of TNF- α , IL-1 β , and IL-6 in PBMCs from CI patients treated with PHA.

Amount of secreted TNF- α (A), IL-1 β (B), and IL-6 (C) from the supernatant of PBMCs for 24 h was measured by ELISA method in the presence and absence of HRHDT (* p <0.05).

assay (Fig. 2). As shown in Fig. 2A, treatment with PHA resulted in a significant increase in secretion of TNF- α (control, 1.026 \pm 0.203 ng/ml; PHA-stimulated, 4.698 \pm 0.962 ng/ml). However, addition of PBMCs with HRHDT significantly suppressed TNF- α production by LPS and PHA in a concentration-dependent manner (350 μ g/ml of HRHDT-pretreated, 3.068 \pm 0.406 ng/ml, p <0.05; 700 μ g/ml of HRHDT-pretreated, 1.602 \pm 0.251 ng/ml, p <0.005).

Also, treatment with PHA resulted in a significant increase in secretion of IL-1 β in PBMCs (control, 0.204 \pm 0.056 ng/ml; PHA-stimulated, 2.684 \pm 0.218 ng/ml). As shown in Fig. 2B, pretreatment with HRHDT dramatically down-regulated IL-1 β secretion by PHA in PBMCs from cerebral infarction patients in a dose-dependent fashion (175 μ g/ml of HRHDT-pretreated, 2.148 \pm 0.211 ng/ml, p <0.05; 350 μ g/ml of HRHDT-pretreated, 1.619 \pm 0.395 ng/ml, p <0.005; 700 μ g/ml of HRHDT-pretreated, 0.731 \pm 0.146 ng/ml, p <0.005). We also measured IL-6 production in PBMCs. Treatment with PHA caused a significant increase of IL-6 production (control, 0.146 \pm 0.056 ng/ml; PHA-stimulated, 0.837 \pm 0.102 ng/ml, respectively) (Fig. 2C). HRHDT markedly suppressed IL-6 production in PBMCs treated with PHA (350 μ g/ml of HRHDT-pretreated, 0.563 \pm 0.120 ng/ml, p <0.05; 700 μ g/ml of HRHDT-pretreated, 0.348 \pm 0.049 ng/ml, p <0.005).

2. HRHDT significantly inhibited the production of IL-10 in human PBMCs of cerebral infarction patients

To evaluate whether HRHDT regulates the production of anti-inflammatory cytokines, PBMCs were pretreated with HRHDT for 1 h and maintained with 10 μ g/ml PHA for 24 h. The culture supernatant was collected to measure the secretion of IL-10 by ELISA assay (Fig. 3). As shown in Fig. 3, treatment with PHA induced a significant increase in secretion of IL-10 (control, 0.08 \pm 0.012 ng/ml; PHA-stimulated, 0.447 \pm 0.071 ng/ml). Addition of PBMCs with HRHDT significantly suppressed IL-10 production by PHA in a concentration-dependent manner (350 μ g/ml of HRHDT-pretreated, 0.304 \pm 0.026 ng/ml, p <0.05; 700 μ g/ml of HRHDT-pretreated, 0.198 \pm 0.008 ng/ml, p <0.005).

3. HRHDT significantly increased the production of TGF- β 1 in human PBMCs of cerebral infarction patients

Also, to assess whether HRHDT produces TGF- β 1, an anti-inflammatory cytokine, PBMCs were treated with HRHDT for 24 h. The culture supernatant was collected to measure the production of TGF- β 1 by ELISA assay (Fig. 4). As shown in Fig. 4, HRHDT markedly up-regulated TGF- β 1 secretion in PBMCs

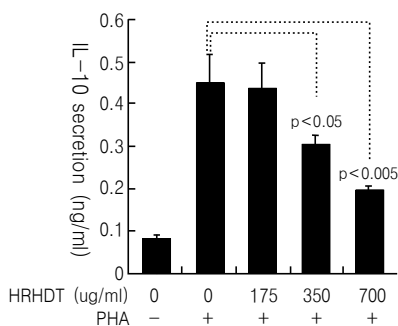


Fig. 3. HRHDT suppressed the secretion of IL-10 in PBMCs from CI patients treated with PHA.

Amount of secreted IL-10 from the supernatant of PBMCs for 24 h was measured by ELISA method in the presence and absence of HRHDT (* p <0.05).

of cerebral infarction patients in a dose-dependent fashion (control, 30.75 ± 8.26 ng/ml; 350 μ g/ml of HRHDT-pretreated, 0.062 ± 0.009 ng/ml, $p < 0.005$; 700 μ g/ml of HRHDT-pretreated, 0.106 ± 0.008 ng/ml, $p < 0.005$).

Discussion

In this study, we demonstrated that HRHDT suppressed the production of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6 in PBMCs treated with PHA (Fig. 2). The secretion of IL-10, an anti-inflammatory cytokine, was increased by PHA, which was significantly decreased by pretreatment with HRHDT in PBMC of cerebral infarction patients (Fig. 3). HRHDT also induced the production of TGF- β 1, an anti-inflammatory cytokine (Fig. 4). Thus, we suggest that the one of the beneficial roles of HRHDT in treatment of cerebral infarction patients is associated with its regulatory effects on the inflammatory process.

Each herbal extract of HRHDT has been used as oriental traditional medicine and reported their pharmacological effects as follow. Berberine, an isoquinoline derivative alkaloid isolated from *Coptidis Rhizoma*, has many pharmacological and biological effects, including anti-inflammatory, antioxidant, antihypertensive, and anti-diabetic effects^{19,20}. *Phellodendri Cortex* has

been demonstrated to reduce glucose level, prevent the development of diabetic nephropathy in streptozotocin-induced diabetic rats, and inhibit gene expression and production of iNOS and TNF- α in LPS-stimulated microglia^{21,22}. Oroxylin A isolated from *Scutellariae Radix* induces G2/M phase cell-cycle arrest via inhibiting Cdk7-mediated expression of Cdc2/p34 in human gastric carcinoma BGC-823 cells²³. *Gardeniae Fructus* has been reported to inhibit matrix metalloproteinases activity in HT1080²⁴, LPS- and PGE₂-induced aqueous flare elevation in pigmented rabbits²⁵.

Under cerebral ischemia state, inflammatory cells infiltrate into the ischemic brain region and trigger to activate inherent brain cells, including astrocytes, microglia and endothelia. Inflammatory responses which affect these cells with substances, including pre-inflammatory cytokines, vasoactive substances and adhesion molecules, play an important role in the pathogenesis of cerebral lesions following cerebral ischemia^{13,26}.

The most important pro-inflammatory cytokines in post-ischemic inflammation include TNF- α , IL-1 β , and IL-6. TNF- α and IL-1 β play the major role to initiate the inflammatory response mediated by the induction of inflammatory metabolites and increased expression of adhesion molecules on the surface of endothelial cells. TNF- α and IL-1 β secreted from

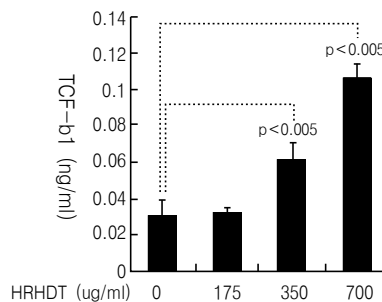


Fig. 4. HRHDT increased the secretion of TGF- β 1 in PBMCs from CI patients.

Amount of secreted TGF- β 1 from the supernatants of PBMCs treated with HRHDT at individual concentration for 24 h was measured by ELISA method ($p < 0.05$).

brain cells share a high homology in structure and function. TNF- α and IL-1 β are also responsible for the accumulation of inflammatory cells in the peripheral nucleus of cerebral infarct and induce a second inflammatory response mediated by IL-6. IL-6 is playing a central role in acute inflammatory processes exhibiting pro-inflammatory activities in many different brain pathologies including cerebral ischemia and excitotoxic brain damage.²⁷⁻³⁰⁾ In addition, to ameliorate brain damages followed by stroke and ischemic attack, there have been reported various therapeutic trials, including anti-TNF- α antibody and TNF- α soluble receptor type 1^{31,32)}, IL-1 β receptor antagonist³³⁾, and recombinant IL-1ra^{17,34)}. Accumulated evidence has proved that the suppressive approaches of specific inflammatory mediators are believed to be one of best solutions to prevent leading to secondary ischemia and damage of brain cells under cerebral ischemia.

IL-10, a Th2-type response and an anti-inflammatory cytokine, modulates immune processes by inhibiting Th1-type response, which is associated with the function of antigen presenting cells. Especially, it reduces ischemic infarct size and the production of other pro-inflammatory mediators in the brain after stroke. Through deactivation of macrophage-like cells and astrocytes, IL-10 plays a role of inhibiting secondary inflammatory processes by them within the brain. However, cerebral infarction patients have been reported to have a significantly lower IL-10 serum level than people without.^{16,35)} In our results, HRHDT might prevent inflammatory signaling transduction in early stage by inhibiting the production of IL-10, which is a defensive response to inflammatory stimuli.

TGF- β 1, a Th3-type response and an anti-inflammatory cytokine, regulates immune processes through the suppression of Th1-type response. TGF- β 1 is implicated in tissue repair, differentiation, and various immune functions. After ischemic stroke, TGF- β 1 in the brain functions on tissue repairing and angiogenesis. Wang et al. reported that it protects the cerebral

hemispheres from damage induced by stroke³⁶⁻³⁸⁾.

In conclusion, our data demonstrate that HRHDT functions to suppress the production of pro-inflammatory cytokines, including TNF- α , IL-1 β , and IL-6 in PBMCs from cerebral infarction patients. Simultaneously, HRHDT induces a significant increase of anti-inflammatory cytokines, TGF- β 1. Also, HRHDT prevents the production of anti-inflammatory cytokines, IL-10 in response to inflammatory stimuli, PHA. Taken together, HRHDT has many functions on normalization of homeostatic balance between anti- and pro-inflammatory processes mediated by mediator, including cytokines.

Furthermore, works are undergoing to identify the crucial components of herbal constituents of HRHDT and the precise signaling pathway of HRHDT on inhibitory mechanism *in vitro* and *in vivo* animal model of brain ischemia.

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References

1. Baek SI, Bae JH, Kim JY, Sim SH, Kang KH, Lee SY, et al. Anti-allergic inflammatory effects of Hwangyounhaedog-tang on PMA plus A23187-stimulated RBL-2H3 cells. *Korean j Oriental Physiology & Pathology*. 2008; 22(3):600-6.
2. Moon JY. Effects of *Hwangryunhaedok-tang* on DNA Damage, Antioxidant Enzymes Expression and Acetylcholinesterase Activity. *Korea j herbology*. 2007; 22(1):7-12.
3. Ahn SH, Kim JW, Kang CH, Whang WW. The effects of Hwangryunhaedoktang and herbs on Mouse neuroblastoma 2a cells damaged by hypoxia-reoxygenation. *J Oriental Neuropsychiatry*. 2006; 17(2):37-59.

4. Kim HY, Shin SH, Lee I. Neuroprotective Effect of *Hwangryunhaedok-tang* Against Glucose-deprivation Induced Cytotoxicity in PC-12 Cells. Korean j Oriental Physiology & Pathology. 2008; 22(6):1462-9.
5. Bémeur C, Ste-Marie L, Desjardins P, Vachon L, Butterworth RF, Hazell AS, et al. Dehydroascorbic acid normalizes several markers of oxidative stress and inflammation in acute hyperglycemic focal cerebral ischemia in the rat. Neurochem Int. 2005; 46:399-407.
6. Lee JC, Cho GS, Kim HJ, Lim JH, Oh YK, Nam W, et al. Accelerated cerebral ischemic injury by activated macrophages/microglia after lipopolysaccharide microinjection into rat corpus callosum. Glia. 2005; 50:168-81.
7. Kim Y, So HS, Youn MJ, Kim HJ, Woo WH, Shin SH, et al. Anti-inflammatory effect of Sasim extracts in PHA-stimulated THP-1 and peripheral blood mononuclear cells from CI patients. J Ethnopharmacol. 2007; 112(1):32-9.
8. Barone FC, Schmidt DB, Hillebrand LM, Price WJ, White RF, Feuerstein GZ, et al. Reperfusion increases neutrophils and leukotriene B4 receptor binding in rat focal ischemia. Stroke. 1992; 23:1337-47.
9. Kochanek PM, Hallenbeck JM. Polymorphonuclear leukocytes and monocytes/macrophages in the pathogenesis of cerebral ischemia and stroke. Stroke. 1992; 23:1367-79.
10. Wang PY, Kao CH, Mui MY, Wang SJ. Leukocyte infiltration in acute hemispheric ischemic stroke. Stroke. 1993; 24:236-40.
11. Garcia JH, Liu KF, Yoshida Y, Chen S, Lian J. Brain microvessels: factors altering their patency after the occlusion of a middle cerebral artery (Wistar rat). Am J Pathol. 1994; 145: 728-40.
12. Lucas SM, Rothwell NJ, Gibson RM. The role of inflammation in CNS injury and disease. Br J Pharmacol. 2006; 1:232-40.
13. Kim Y, So HS, Youn MJ, Kim ES, Song MS, Chai KY, et al. Anti-inflammatory effect of So-Pung-Tang, a Korean traditional prescription for cerebral infarction patients. J Ethnopharmacol. 2007; 114(3):425-31.
14. Castillo J, Leira R. Predictors of deteriorating cerebral infarct: role of inflammatory mechanisms. Would its early treatment be useful? Cerebrovasc Dis. 2007; 1:40-8.
15. Nilupul PM, Ma HK, Arakawa S, Howells DW, Markus R, Rowe CC, et al. Inflammation following stroke. J Clin Neurosci. 2006; 13:1-8.
16. Yamasaki Y, Matsuo Y, Zagorski J, Matsuura N, Onodera H, Itoyama Y, et al. New therapeutic possibility of blocking cytokine-induced neutrophil chemoattractant on transient ischemic brain damage in rats. Brain Res. 1997; 759:103-11.
17. Kariko K, Weissman D, Welsh FA. Inhibition of toll-like receptor and cytokine signaling--a unifying theme in ischemic tolerance. J Cereb Blood Flow Metab. 2004; 24:1288-304.
18. Nakamura K, Ueno T, Yamamoto H, Iguro Y, Yamada K, Sakata R. Relationship between cerebral injury and inflammatory responses in patients undergoing cardiac surgery with cardiopulmonary bypass. Cytokine. 2005; 29:95-104.
19. Jung HA, Yoon NY, Bae HJ, Min BS, Choi JS. Inhibitory activities of the alkaloids from *Coptidis Rhizoma* against aldose reductase. Arch Pharm Res. 2008; 31(11):1405-12.
20. Youn MJ, So HS, Cho HJ, Kim HJ, Kim Y, Lee JH, et al. Berberine, a natural product, combined with cisplatin enhanced apoptosis through a mitochondria/caspase-mediated pathway in HeLa cells. Biol Pharm Bull. 2008; 31(5): 789-95.
21. Kim HJ, Kong MK, Kim YC. Beneficial effects of *Phellodendri Cortex* extract on hyperglycemia and diabetic nephropathy in streptozotocin-induced diabetic rats. BMB Rep. 2008; 41(10):710-5.
22. Park YK, Chung YS, Kim YS, Kwon OY, Joh

- TH. Inhibition of gene expression and production of iNOS and TNF- α in LPS-stimulated microglia by methanol extract of *Phellodendri cortex*. *Int Immunopharmacol.* 2007; 7(7):955-62.
23. Yang Y, Hu Y, Gu HY, Lu N, Liu W, Qi Q, et al. Oroxylin A induces G2/M phase cell-cycle arrest via inhibiting Cdk7-mediated expression of Cdc2/p34 in human gastric carcinoma BGC-823 cells. *J Pharm Pharmacol.* 2008; 60(11):1459-63.
 24. Yang JG, Shen YH, Hong Y, Jin FH, Zhao SH, Wang MC, et al. Stir-baked *Fructus gardeniae* (L.) extracts inhibit matrix metalloproteinases and alter cell morphology. *J Ethnopharmacol.* 2008; 117(2):285-9.
 25. Nagaki Y, Hayasaka S, Zhang XY, Hayasaka Y, Nakamura N, Terasawa K. Effects of topical instillation of traditional herbal medicines, herbal extracts, and their components on prostaglandin E2-induced aqueous flare elevation in pigmented rabbits. *Jpn J Ophthalmol.* 2003; 47(3):249-53.
 26. Zheng Z, Yenari MA. Post-ischemic inflammation: molecular mechanisms and therapeutic implications. *Neurol Res.* 2004; 26:884-92.
 27. Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA. Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. *J Clin Invest.* 1985; 76:2003-11.
 28. Dihne M, Block F. Focal ischemia induces transient expression of IL-6 in the substantia nigra pars reticulata. *Brain Res.* 2001; 889:165-73.
 29. Park YG, Kang SK, Kim WJ, Lee YC, Kim CH. Effects of TGF- β , TNF- α , IL- β and IL-6 alone or in combination, and tyrosine kinase inhibitor on cyclooxygenase expression, prostaglandin E2 production and bone resorption in mouse calvarial bone cells. *Int J Biochem Cell Biol.* 2004; 36:2270-80.
 30. Hosomi N, Ban CR, Naya T, Takahashi T, Guo P, Song XY, et al. Tumor necrosis factor- α neutralization reduced cerebral edema through inhibition of matrix metalloproteinase production after transient focal cerebral ischemia. *J Cereb Blood Flow Metab.* 2005; 25:959-67.
 31. Dawson DA, Martin D, Hallenbeck JM. Inhibition of tumor necrosis factor- α reduces focal cerebral ischemic injury in the spontaneously hypertensive rat. *Neurosci Lett.* 1996; 218:41-4.
 32. Loddick SA, Rothwell NJ. Neuroprotective effects of human recombinant interleukin-1 receptor antagonist in focal cerebral ischaemia in the rat. *J Cereb Blood Flow Metab.* 1996; 16:932-40.
 33. Relton JK, Martin D, Thompson RC, Russell DA. Peripheral administration of Interleukin-1 Receptor antagonist inhibits brain damage after focal cerebral ischemia in the rat. *Exp Neurol.* 1996; 138:206-13.
 34. Stroemer RP, Rothwell NJ. Cortical protection by localized striatal injection of IL-1ra following cerebral ischemia in the rat. *J Cereb Blood Flow Metab.* 1997; 17:597-604.
 35. Frenkel D, Huang Z, Maron R, Koldzic DN, Moskowitz MA, Weiner HL. Neuroprotection by IL-10-producing MOG CD4+ T cells following ischemic stroke. *J Neurol Sci.* 2005; 233(1-2): 125-32.
 36. Buisson A, Lesne S, Docagne F, Ali C, Nicole O, MacKenzie ET, et al. Transforming growth factor- β and ischemic brain injury. *Cell Mol Neurobiol.* 2003; 23(4-5):539-50.
 37. Krupinski J, Kumar P, Kumar S, Kaluza J. Increased expression of TGF- β 1 in brain tissue after ischemic stroke in humans. *Stroke.* 1996; 27(5):852-7.
 38. Zhu Y, Yang GY, Ahlemeyer B, Pang L, Che XM, Culmsee C, et al. Transforming growth factor- β 1 increases bad phosphorylation and protects neurons against damage. *J Neurosci.* 2002; 22(10):3898-909.