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Protective effects of genistein on proinflammatory pathways in human brain microvascular endothelial cells☆

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Abstract

Proinflammatory cerebromicrovascular environment has been implicated in the critical early pathologic events in a variety of neurodegenerative diseases. Recent studies also have demonstrated the potential beneficial effects of soy isoflavones. However, cellular and molecular mechanisms underlying these processes are not fully understood. The present study was designed to examine the hypothesis that soy isoflavone genistein may attenuate cytokine-induced proinflammatory pathways in human brain microvascular endothelial cells. The quantitative real-time reverse transcriptase-polymerase chain reaction and enzyme-linked immunosorbent assay showed that pretreatment of HBMEC with increasing concentrations of genistein significantly and dose-dependently inhibited cytokine-induced up-regulation of mRNA and protein expression of proinflammatory mediators such as tumor necrosis factor-α, interleukin-1β, monocyte chemoattractant protein-1, interleukin-8, and intercellular adhesion molecule-1. In addition, genistein pretreatment significantly reduced cytokine-mediated up-regulation of transmigration of blood leukocytes in a dose-dependent manner. Our results suggest that genistein may attenuate proinflammatory pathways through inhibition of cytokine-induced overexpression of proinflammatory mediators and inflammatory reactions in human brain microvascular endothelial cells.

Keywords: Genistein; Inflammation; Brain microvascular endothelial cells; Blood-brain barrier

1. Introduction

Pro-inflammatory microvascular environment in the brain has been implicated in a variety of neurodegenerative diseases by triggering the cerebrovascular endothelium to initiate several cascades of events leading to perturbations in the functional integrity of brain microvasculature and blood-brain barrier (BBB) leakage [1]. Indeed, alterations of the brain microvasculature and disruption of the BBB are commonly found in patients with neurological disorders such as Alzheimer's disease (AD), Parkinson's disease (PD), and HIV-1 dementia [1–3].

Recent evidence indicates that inflammatory reactions in vascular endothelium are primarily regulated through the production of proinflammatory mediators, such as cytokines, chemokines, and adhesion molecules [4]. In fact, the recruitment of blood leukocytes such as lymphocytes and monocytes/macrophages and their migration throughout the vascular endothelium in the brain are thought to be critical early pathological events in the onset and progression of neurological disorders [5–9]. These processes are promoted by overexpression of proinflammatory mediators and their close interactions in brain microvascular endothelial cells [3,10–14]. In addition, increasing evidence has suggested that treatment with nonsteroidal anti-inflammatory drugs may be associated with a lower risk of developing neurodegenerative diseases including AD and PD [5,9]. These findings provide robust evidence that inflammatory responses in the brain can be critically involved in vascular endothelial dysfunction and may play a significant role in the pathogenesis of neurodegenerative diseases.

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Genistein is one of the major soy isoflavones found in and isolated from the soybean. Compelling body of evidences indicates that soy isoflavones have beneficial health effects including protection against a number of human chronic diseases including cardiovascular diseases, cancer, and osteoporosis [15-17]. In addition, several recent studies have demonstrated the protective effects of genistein on central nervous system (CNS) [18-20]. The cellular and molecular regulatory mechanisms underlying this process, however, are not fully understood. Furthermore, it has not been known whether soy isoflavones can mediate protective effects on the proinflammatory microvascular environments in the brain, which are produced by interactions between blood inflammatory cells and brain endothelial cells in the pathophysiologic process of neurodegenerative diseases. In the present study, we examined whether soy isoflavone genistein can attenuate pro-inflammatory pathways in tumor necrosis factor-α (TNF-α)-stimulated brain microvascular endothelium by inhibiting expression of pro-inflammatory mediators such as cytokines, chemokines, and adhesion molecule in human brain microvascular endothelial cells (HBMEC), as well as by reducing leukocyte transmigration across HBMEC monolayer.

2. Methods and materials

2.1. Cell cultures and reagents

HBMEC were isolated, cultivated, and purified as previously described [21]. These cells were positive for factor VIII-Rag, carbonic anhydrase IV, and Ulex Europeus Agglutinin I; took up fluorescently labeled low-density lipoprotein; and expressed γ-glutamyl transpeptidase, demonstrating their brain endothelial cell characteristics. Contamination of nonendothelial cells such as pericytes and glial cells were less than 1%. HBMEC were cultured in RPMI 1640-based medium with 10% fetal bovine serum, 10% NuSerum, 30 μg/ml of endothelial cell growth supplement, 15 U/ml of heparin, 2 mM L-glutamine, 2 mM sodium pyruvate, nonessential amino acids, vitamins, 100 U/ml of penicillin, and 100 μg/ml of streptomycin. Human monocytic leukemia cells (THP-1) were purchased from ATCC (Manassas, VA, USA) and used to study leukocyte transmigration assay. THP-1 cells were cultured in suspension in RPMI 1640 medium supplemented with 10% fetal bovine serum, 25 mM glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, 50 μM 2-mercaptoethanol, 100 U/ml of penicillin, and 100 μg/ml of streptomycin. Both HBMEC and THP-1 cells were maintained in a humid 5% CO_2 atmosphere at 37°C.

Genistein (4′,5,7-trihydroxyisoflavone; Fig. 1) was purchased from Sigma (St. Louis, MO, USA) and dissolved in dimethyl sulfoxide (DMSO). Levels of DMSO in the experimental medium were less than 0.1% and did not affect endothelial cells. Control cells were not pretreated with genistein but the same amount of DMSO as in genistein-treated cells was added to control cultures.

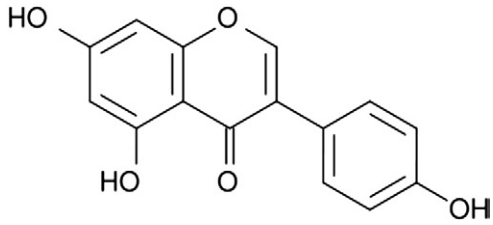

Fig. 1. Chemical structure of genistein (4′,5′,7-Trihydroxyisoflavone, $C_{15}H_{10}O_5$).

2.2. Real-time reverse transcriptase-polymerase chain reaction

Total RNA was isolated and purified using RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the protocol of the manufacturer. One microgram of total RNA was reverse-transcribed at 25°C for 15 min, 42°C for 45 min, and 99°C for 5 min in 20 μl of 5 mM $MgCl_2$; 10 mM Tris–HCl, pH 9.0; 50 mM KCl; 0.1% Triton X-100; 1 mM dNTP; 1 U/μl of recombinant RNasin ribonuclease inhibitor; 15 U/μg of AMV reverse transcriptase; and 0.5 μg of random hexamers. For quantitative polymerase chain reaction (PCR), amplifications of individual genes were performed on ABI 7300 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using TaqMan Universal PCR Master Mix, gene-specific TaqMan PCR probes and primers, and a standard thermal cycler protocol (50°C for 2 min before the first cycle, 95°C for 15 s, and 60°C for 1 min, repeated 45 times). For specific probes and primers of PCR amplifications, TaqMan Gene Expression Assay Reagents for human TNF-α, interleukin-1β (IL-1β), monocyte chemoattractant protein-1 (MCP-1), interleukin-8 (IL-8), intercellular adhesion molecule-1 (ICAM-1), VCAM-1, E-selectin, and β-actin were obtained from Applied Biosystems. The threshold cycle (C_T), which indicates the fractional cycle number at which the amount of amplified target gene reaches a fixed threshold, from each well was determined using by the Applied Biosystems Sequence Detection Software v1.2.3. Relative quantification, which represents the change in gene expression from real-time quantitative polymerase chain reactions between experimental groups, was calculated by the comparative C_T method, as described earlier [22,23]. The data were analyzed using equation $2^{-\Delta\Delta CT}$, where $\Delta\Delta C_T$= [C_T of target gene−C_T of housekeeping gene]$_{\text{treated group}}$−[C_T of target gene−C_T of housekeeping gene]$_{\text{untreated control group}}$. Evaluation of $2^{-\Delta\Delta CT}$ indicates the fold change in gene expression, normalized to a housekeeping gene (β-actin) and relative to the control group.

2.3. Enzyme-linked immunosorbent assay and cell-based enzyme-linked immunosorbent assay

The protein expression levels of MCP-1 and IL-8 in cell culture supernatants as well as TNF-α and IL-1β in cell lysates were determined by enzyme-linked immunosorbent assay (ELISA) using Human Immunoassay kits (R and D Systems, Minneapolis, MN, USA) according to the protocol

of the manufacturer. Cell surface expression levels of adhesion molecule ICAM-1 were quantified by cell-based ELISA (CELISA) using ELISA Development kits (R and D Systems) according to the manufacturer's procedure with modifications [24,25]. Briefly, HBMEC monolayers were incubated with antihuman ICAM-1 monoclonal antibody (2.5 μg/ml) for 1 h at 37°C. The cells were then incubated with biotinylated goat antimouse IgG antibody (1:1,000 dilution) for 1 h at 37°C. After washing the wells thoroughly, the working dilution of streptavidin-horseradish peroxidase (HRP) were added to each well and incubated for 20 min at room temperature. The cells were incubated with HRP substrate solution for 20 min at room temperature with subsequent addition of stop solution. After color development, absorbance from each well was measured by a microtiter plate reader at 450–570 nm.

2.4. Leukocyte transmigration assay

HBMEC were pretreated with indicated concentrations of genistein for 1 h, followed by exposure to 10 ng/ml of TNF-α for 16 h, and culture supernatants were obtained for cell migration assay. Transmigration of blood leukocytes, THP-1 (1.0×10^6 cells/ml), was determined using QCM Chemotaxis 5μm 96-Well Cell Migration Assay kit (Chemicon International) according to the protocol of the manufacturer. It provides a quick and efficient system for quantitative determination of monocyte/macrophage migration. The migratory cells on the bottom of the insert membrane were dissociated from the membrane when incubated with cell detachment buffer. These cells were subsequently lysed and detected by the CyQuant GR dye (Molecular Probes). This green fluorescent dye exhibits strong fluorescence enhancement when bound to cellular nucleic acids. The fluorescence was measured using a fluorescence multiwell plate reader with excitation and emission wavelengths of 480 and 520 nm.

2.5. Statistical analysis

Statistical analysis of data was completed using SigmaStat 3.5 (Systat Software, Point Richmond, CA, USA). One-way analysis of variance was used to compare mean responses among the treatments. For each end point, the treatment means were compared using Bonferroni least significant difference procedure. Differences among the means were considered significant at $P<.05$.

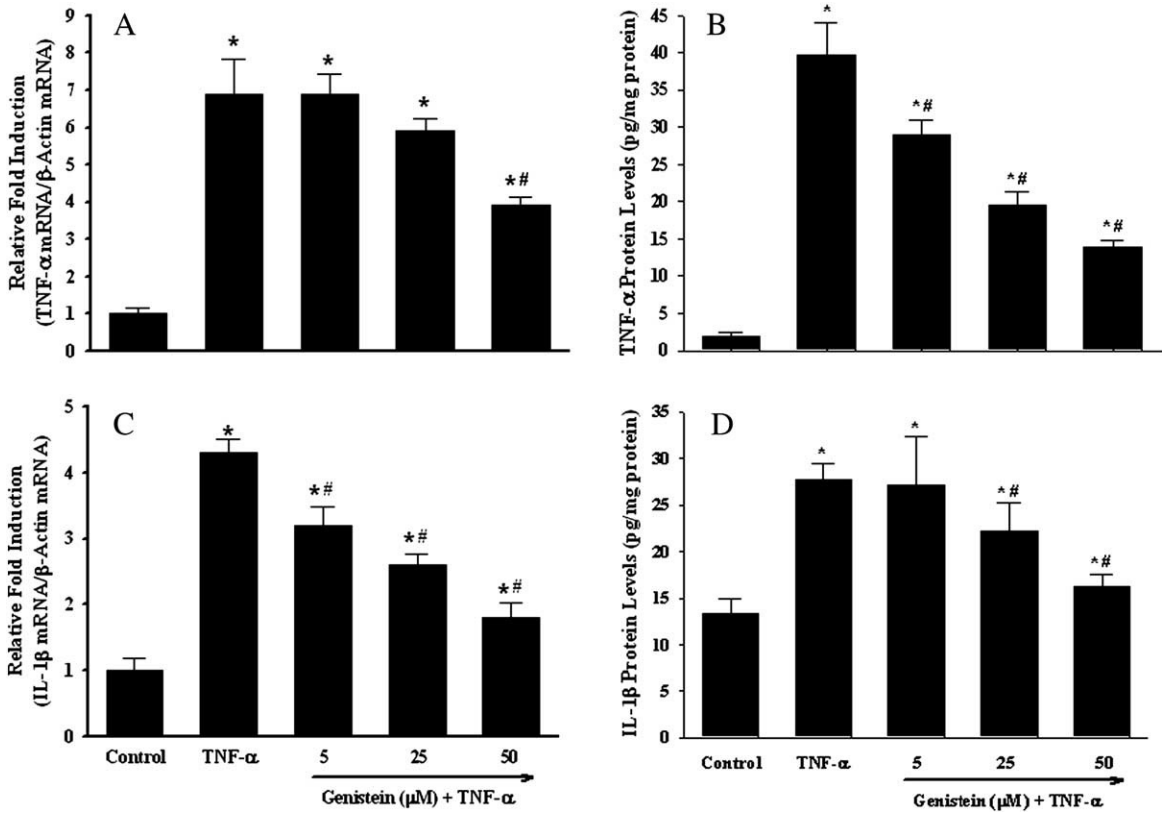

Fig. 2. Genistein attenuates the mRNA and protein expression of proinflammatory cytokines in human brain microvascular endothelial cells stimulated with TNF-α. HBMEC were pretreated with indicated concentrations of genistein for 1 h and then exposed to 10 ng/ml of TNF-α for either 4 (gene expression) or 16 h (protein expression). The mRNA levels of TNF-α (A) and IL-1β (C) were determined by quantitative real-time RT-PCR. Data are means±S.E.M. of 4 determinations. The protein expression levels of TNF-α (B) and IL-1β (D) were measured by ELISA. Data are means±S.D. of 4 determinations. *Statistically significant as compared to control cultures ($P<.05$); #Levels in the groups treated with genistein plus TNF-α are significantly different as compared to those in cultures treated with TNF-α alone ($P<.05$).

3. Results

TNF-α has been widely recognized as the critical mediator of inflammatory events in the central nervous system. Indeed, TNF-α is a potent activator of nuclear factor κB and AP-1 and inducer of a variety of pro-inflammatory mediators, and thus, it can significantly contribute to the alterations of the BBB and leukocyte infiltration into the brain [26]. In addition, it has been demonstrated that overexpression of TNF-α precedes the infiltration of inflammatory cells in the injured regions of the brain [27]. Furthermore, TNF-α can exert direct injury effects to vascular endothelial cells. For example, treatment with TNF-α can induce endothelial cell oxidative stress, decrease cellular glutathione and ATP levels, increase intracellular calcium, activate matrix metalloproteinases, as well as induce endothelial cell apoptosis [28–31]. Therefore, in the present study, HBMEC were stimulated with TNF-α as an in vitro experimental model to examine the ability of soy isoflavone genistein to ameliorate cerebrovascular inflammation.

3.1. Genistein attenuates overexpression of proinflammatory mediators in cytokine-stimulated human brain microvascular endothelial cells

It is generally accepted that pro-inflammatory cytokines, such as TNF-α and IL-1β, are well-known inducers of inflammatory reactions and can stimulate expression of cytokines, chemokines, and adhesion molecules. Therefore, the effects of genistein on the mRNA and protein expression of TNF-α and IL-1β were investigated in cytokine-stimulated human brain microvascular endothelial cells. Real-time reverse transcriptase-PCR (RT-PCR) and ELISA showed that treatment with 10 ng/ml of TNF-α markedly and significantly up-regulated the mRNA and protein expression of TNF-α and IL-1β in HBMEC (Fig. 2). Pretreatment with genistein, however, significantly attenuated cytokine-mediated up-regulation of TNF-α and IL-1β mRNA expression (Fig. 2A and C) in a dose-dependent manner. In addition, a significant and dose-dependent inhibition of overexpression of TNF-α and IL-1β proteins (Fig. 2B and D) was observed in cytokine-stimulated HBMEC pretreated with genistein.

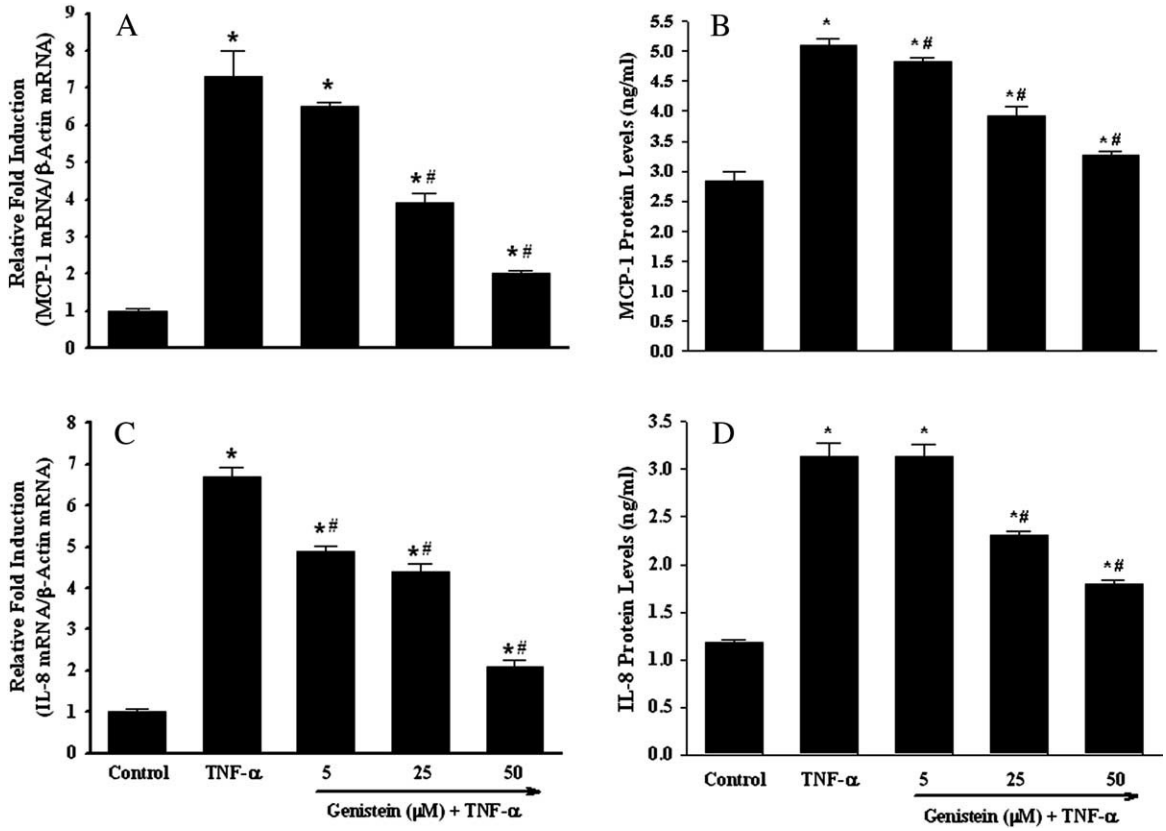

Fig. 3. Genistein attenuates the mRNA and protein expression of chemokines in human brain microvascular endothelial cells stimulated with TNF-α. HBMEC were pretreated with indicated concentrations of genistein for 1 h and then exposed to 10 ng/ml of TNF-α for either 4 (gene expression) or 16 h (protein expression). The mRNA levels of MCP-1 (A) and IL-8 (C) were determined by quantitative real-time RT-PCR. Data are means±SEM of 4 determinations. The protein expression levels of MCP-1 (B) and IL-8 (D) were measured by ELISA. Data are means±S.D. of 6 determinations. *Statistically significant as compared to control cultures ($P<.05$); #Levels in the groups treated with genistein plus TNF-α are significantly different as compared to those in cultures treated with TNF-α alone ($P<.05$).

In addition to proinflammatory cytokines, enhanced expression of chemokines, such as MCP-1 and IL-8, and adhesion molecules, such as ICAM-1, VCAM-1 and E-selectin, in the vascular endothelium facilitates recruiting and adhering inflammatory cells into the vessel wall and subsequently stimulates transendothelial migration. Consistent with the data shown in Fig. 2, exposure of HBMEC to TNF-α dramatically and significantly increased the mRNA and protein expression levels of chemokines MCP-1 and IL-8 (Fig. 3) and adhesion molecules ICAM-1 (Fig. 4), VCAM-1, and E-selectin (data not shown). In contrast, pretreatment with genistein significantly and dose-dependently attenuated up-regulation of MCP-1, IL-8, and ICAM-1 expression in cytokine-stimulated HBMEC (Figs. 3 and 4). On the other hand, genistein did not affect the cytokine-mediated induction of VCAM-1 and E-selectin expression in HBMEC (data not shown).

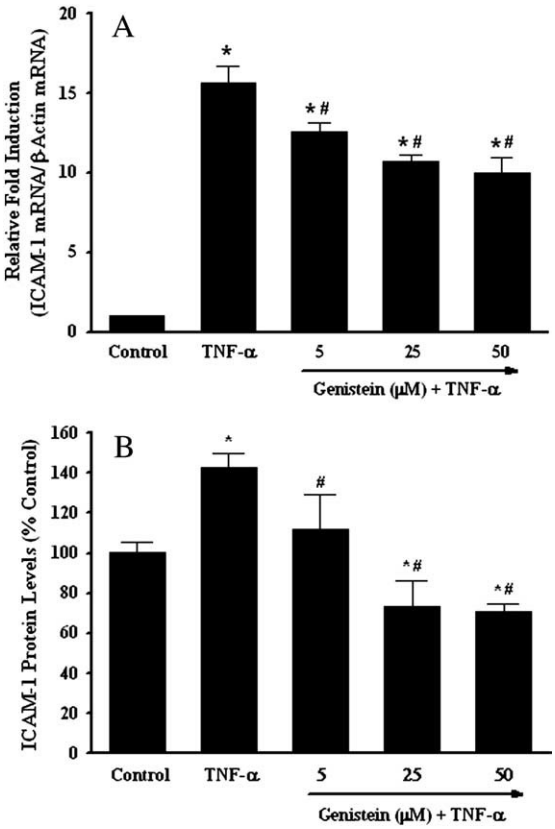

Fig. 4. Genistein attenuates the mRNA and protein expression of adhesion molecule in human brain microvascular endothelial cells stimulated with TNF-α. HBMEC were pretreated with indicated concentrations of genistein for 1 h and then exposed to 10 ng/ml of TNF-α for either 4 (gene expression) or 16 h (protein expression). The mRNA levels of ICAM-1 (A) were determined by quantitative real-time RT-PCR. Data are means±S.E.M. of four determinations. The cell surface expression levels of ICAM-1 protein were measured by CELISA. Data are means±S.D. of four determinations. *Statistically significant as compared to control cultures ($P<.05$); #Levels in the groups treated with genistein plus TNF-α are significantly different as compared to those in cultures treated with TNF-α alone ($P<.05$).

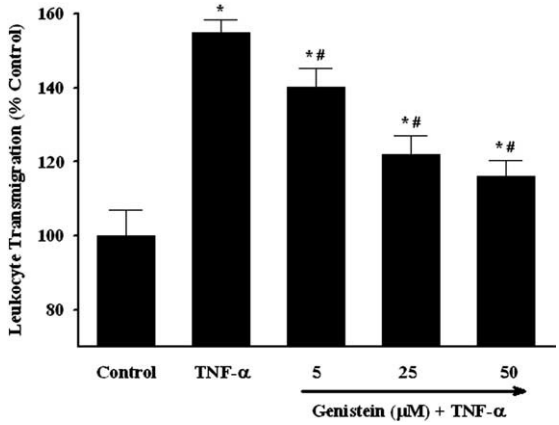

Fig. 5. Genistein inhibits TNF-α-stimulated transendothelial migration of leukocytes. HBMEC were pretreated with indicated concentrations of genistein for 1 h, and then exposed to 10 ng/ml of TNF-α for 16 h. Transmigration of THP-1 cells was quantified as described in methods and materials. *Statistically significant as compared to control cultures ($P<.05$). #Levels in the groups treated with genistein plus TNF-α are significantly different as compared to those in cultures treated with TNF-α alone ($P<.05$).

3.2. Genistein inhibits cytokine-induced up-regulation of transmigration of blood leukocytes

To determine whether genistein can attenuate cytokine-induced inflammatory reactions in human brain microvascular endothelial cells, leukocyte transmigration assays were performed. As shown in Fig. 5, exposure of HBMEC to TNF-α resulted in a marked and significant increase in transmigration of THP-1 human monocytic leukemia cells. In contrast, pretreatment with genistein significantly and dose-dependently inhibited cytokine-induced leukocyte transmigration (Fig. 5).

These results indicate that genistein can mitigate proinflammatory cerebrovascular environment via down-regulation of cytokine-mediated overexpression of various inflammatory mediators and reduction of transmigration of blood leukocytes.

4. Discussion

The vascular endothelium consists of a monolayer of endothelial cells and lines the internal surface of the blood vessels. Brain microvascular endothelial cells are responsible in large part for the structure and function of the BBB, a highly selective barrier between blood and brain which regulates the entry of intravascular substances into the brain tissue [1]. Emerging evidence has indicated that overexpression of proinflammatory mediators contributes to the brain microvascular endothelial cell dysfunction and BBB disruption leading to the development of neurodegenerative diseases. For example, amyloid β, the major constituent of amyloid plaque in the brains of AD patients, can lead to an inflammatory cascade via secretion of interferon-γ and IL-1β, as well as increase the ability of monocytes/macrophages

to transmigrate across the brain microvascular endothelial cell monolayer [10,11]. Additionally, a recent postmortem study showing reduced *p*-glycoprotein function in PD patients strongly supports BBB dysfunction as a causative mechanism of PD [32]. The proinflammatory pathways in the brain microvasculature and their interactions with surrounding brain cells such as astrocytes and microglia, however, have not yet been fully understood.

A number of epidemiological, clinical, and experimental studies have suggested that the consumption of nutritious diet is important for maintaining human health and decreasing the risk for chronic diseases. In particular, soybeans and foods containing soy isoflavones are believed to provide immense health benefits that may help prevent and/or treat a variety of human chronic diseases including neurological disorders [15–20]. For example, genistein, the primary soybean isoflavone, has shown to exert neuroprotective activities in mouse model of familial amyotrophic lateral sclerosis and stroke [18,33]. It was also found that pretreatment with genistein protects cortical neurons from apoptotic cell death induced by tertiary butylhydroperoxide or thapsigargin [19,34]. In addition, Wang et al. [20] recently reported that genistein may protect dopaminergic neurons by inhibiting the production of proinflammatory mediators in lipopolysaccharide-stimulated microglia. These previous in vivo and in vitro studies provide compelling evidence indicating the potential neuroprotective role of genistein. The precise mechanism for the protective effect of genistein on CNS injury, however, remains to be further investigated. In the present study, we first examined whether proinflammatory cytokine TNF-α can stimulate an inflammatory response in HBMEC. A significant increase in the mRNA and protein expression of cytokines (TNF-α and IL-1β) (Fig. 2), chemokines (MCP-1 and IL-8) (Fig. 3), and adhesion molecule (ICAM-1) (Fig. 4) was observed in TNF-α-treated HBMEC. Additionally, TNF-α markedly stimulated the transmigration of blood leukocytes across HBMEC monolayer (Fig. 5). These data indicated that the cytokine employed in this study produces the proinflammatory cerebrovascular environment that can be used as an in vitro experimental model to examine the ability of genistein to ameliorate cerebrovascular inflammation.

Recent studies have highlighted the protective effects of dietary isoflavones on the inflammatory responses in vascular endothelium. For instance, soy isoflavones genistein and daidzein significantly decreased MCP-1 secretion induced by TNF-α in human umbilical vein endothelial cells (HUVEC) [35]. It was also found that genistein inhibits monocyte adhesion to cytokine-stimulated human aortic endothelial cells and HUVEC through activation of anti-inflammatory PPAR-γ [36]. However, there are no reports on soy isoflavones-mediated effects on human brain microvascular endothelial cells. Therefore, we determined whether genistein treatment has anti-inflammatory effects in TNF-α-stimulated HBMEC. As shown in Figs. 2–5, pretreatment of HBMEC with genistein significantly and dose-dependently attenuated cytokine-stimulated up-regulation of proinflammatory mediators and blood leukocyte transmigration. These findings suggest the potential protective role of genistein in proinflammatory pathways in human microvascular endothelium in the brain. Further studies, however, needed to elucidate the exact mechanisms of the genistein's protective effects on proinflammatory cerebrovascular environment.

In conclusion, the present study provides the first evidence that genistein exerts its anti-inflammatory action against human brain microvascular endothelial injury through down-regulation of cytokine-mediated overexpression of various pro-inflammatory mediators, such as TNF-α, IL-1β, MCP-1, IL-8, and ICAM-1, and reduction of transmigration of blood leukocytes. These data may contribute to a better understanding of cellular and molecular mechanisms of action for soy isoflavones-mediated protection against cerebrovascular injury and subsequent progression of neurodegenerative diseases.

References

[1] Rubin LL, Staddon JM. The cell biology of the blood-brain barrier. Annu Rev Neurosci 1999;22:11–28.
[2] Staddon JM, Herrenknecht K, Schulze C, Smales C, Rubin LL. Signal transduction at the blood-brain barrier. Biochem Soc Trans 1995;23:475–9.
[3] Toborek M, Lee YW, Flora G, Pu H, Andras IE, Wylegala E, Hennig B, Nath A. Mechanisms of the blood-brain barrier disruption in HIV-1 infection. Cell Mol Neurobiol 2005;25:181–99.
[4] Ross R. Atherosclerosis is an inflammatory disease. Am Heart J 1999;138:S419–20.
[5] Perry VH, Bell MD, Brown HC, Matyszak MK. Inflammation in the nervous system. Curr Opin Neurobiol 1995;5:636–41.
[6] Fiala M, Zhang L, Gan X, et al. Amyloid-β induces chemokine secretion and monocyte migration across a human blood-brain barrier model. Mol Med 1998;4:480–9.
[7] Neuroinflammation Working Group. Inflammation and Alzheimer's disease. Neurobiol Aging 2000;21:383–421.
[8] McGeer PL, Yasojima K, McGeer EG. Inflammation in Parkinson's disease. Adv Neurol 2001;86:83–9.
[9] McGeer PL, McGeer EG. Inflammation and the degenerative diseases of aging. Ann N Y Acad Sci 2004;1035:104–16.
[10] Suo Z, Tan J, Placzek A, Crawford F, Fang C, Mullan M. Alzheimer's β-amyloid peptides induce inflammatory cascade in human vascular cells: the role of cytokines and CD40. Brain Res 1998;807:110–7.
[11] de la Torre JC, Stefano GB. Evidence that Alzheimer's disease is a microvascular disorder: the role of constitutive nitric oxide. Brain Res Rev 2000;34:119–36.
[12] Lee YW, Hennig B, Fiala M, Kim KS, Toborek M. Cocaine activates redox-regulated transcription factors and induces TNF-α expression in human brain endothelial cells. Brain Res 2001;920:125–33.
[13] Lee YW, Hennig B, Yao J, Toborek M. Methamphetamine induces AP-1 and NF-κB binding and transactivation in human brain endothelial cells. J Neurosci Res 2001;66:583–91.
[14] Toborek M, Lee YW, Pu H, Malecki A, Flora G, Garrido R, et al. HIV-Tat protein induces oxidative and inflammatory pathways in brain endothelium. J Neurochem 2003;84:169–79.
[15] Messina M, Perskey V, Setchell KD, Barnes S. Soy intake and cancer risk: a review of the in vitro and in vivo data. Nutr Cancer 1994;21:113–31.

[16] Fournier DB, Erdman Jr JW, Gordon GB. Soy, its components, and cancer prevention: a review of the in vitro, animal, and human data. Cancer Epidemiol Biomark Prev 1998;7:1055–65.
[17] Barnes S. Soy isoflavones—phystoestrogens and what else? J Nutr 2004;134:1225S–8S.
[18] Trieu VN, Uckun FM. Genistein is neuroprotective in murine models of familial amyotrophic lateral sclerosis and stroke. Biochem Biophys Res Commun 1999;258:685–8.
[19] Sonee M, Sum T, Wang C, Mukherjee SK. The soy isoflavone, genistein, protects human cortical neuronal cells from oxidative stress. Neurotoxicology 2004;25:885–91.
[20] Wang X, Chen S, Ma G, Ye M, Lu G. Genistein protects dopaminergic neurons by inhibiting microglial activation. Neuroreport 2005;16: 267–70.
[21] Stins MF, Gilles F, Kim KS. Selective expression of adhesion molecules on human brain microvascular endothelial cells. J Neuroimmunol 1997; 76:81–90.
[22] Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{-DDCT} method. Methods 2001;25:402–8.
[23] Deng X, Li H, Tang YW. Cytokine expression in respiratory syncytial virus-infected mice as measured by quantitative reverse-transcriptase PCR. J Virol Methods 2003;107:141–6.
[24] Lee YW, Eum SY, Chen KC, Hennig B, Toborek M. Gene expression profile in interleukin-4-stimulated human vascular endothelial cells. Mol Med 2004;10:19–27.
[25] Lee YW, Eum SY, Nath A, Toborek M. Estrogen-mediated protection against HIV Tat protein-induced inflammatory pathways in human vascular endothelial cells. Cardiovasc Res 2004;63:139–48.
[26] Duchini A, Govindarajan S, Santucci M, Zampi G, Hofman FM. Effects of tumor necrosis factor-alpha and interleukin-6 on fluid-phase permeability and ammonia diffusion in CNS-derived endothelial cells. J Investig Med 1996;44:474–82.
[27] Feuerstein GZ, Liu T, Barone FC. Cytokines, inflammation, and brain injury: role of tumor necrosis factor-alpha. Cerebrovasc Brain Metab Rev 1994;6:341–60.
[28] Hanemaaijer R, Koolwijk P, le Clercq L, de Vree WJ, van Hinsbergh VW. Regulation of matrix metalloproteinase expression in human vein and microvascular endothelial cells. Effects of tumour necrosis factor alpha, interleukin 1 and phorbol ester. Biochem J 1993;296: 803–9.
[29] Koga S, Morris S, Ogawa S, et al. TNF modulates endothelial properties by decreasing cAMP. Am J Pathol 1995;268:C1104–13.
[30] Toborek M, Barger SW, Mattson MP, McClain CJ, Hennig B. Role of glutathione redox cycle in TNF-α mediated endothelial cell dysfunction. Atherosclerosis 1995;117:179–88.
[31] Rahman I, MacNee W. Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches. Free Radic Biol Med 2000;28:1405–20.
[32] Kortekaas R, Fnders KL, van Oostrom JCH, Vaalburg W, Bart J, Willemsen ATM, Hendrikse NH. Blood-brain barrier dysfunction in parkinsonian midbrain in vivo. Ann Neurol 2005;57:176–9.
[33] Trieu VN, Dong Y, Zheng Y, Uckun FM. In vivo antioxidant activity of genistein in a murine model of singlet oxygen-induced cerebral stroke. Radiat Res 1999;152:508–16.
[34] Linford NJ, Dorsa DM. 17β-Estradiol and the phytoestrogen attenuate neuronal apoptosis induced by the endoplasmic reticulum calcium–ATPase inhibitor thapsigargin. Steroids 2002;67:1029–40.
[35] Gottstein N, Ewins BA, Eccleston C, Hubbard GP, Kavanagh IC, Minihane AM, Weinberg PD, Rimbach G. Effect of genistein and daidzein on platelet aggregation and monocyte and endothelial function. Br J Nutr 2003;89:607–15.
[36] Chacko BK, Chandler RT, Mundhekar A, et al. Revealing anti-inflammatory mechanisms of soy isoflavones by flow: modulation of leukocyte-endothelial cell interactions. Am J Physiol Heart Circ Physiol 2005;289:H908–15.