

Sipjeondaebo-Tang, a Traditional Herbal Formula, Inhibits Retinal Neovascularization in a Mouse Model of Oxygen-Induced Retinopathy

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Retinal neovascularization is a common pathology in age-related macular degeneration, retinopathy of prematurity and proliferative diabetic retinopathy. Platelet derived growth factor (PDGF) is a vasoactive factor and has been implicated in proliferative retinopathies. Oxygen-induced retinopathy in the mouse is the standard experimental model of proliferative retinopathies. Sipjeondaebo-tang (SDT) is the most widely used traditional herbal formula in East Asia, also known as Shi-Quan-Da-Bu-Tang in Chinese and Juzen-taiho-to in Japanese. SDT has been known to exert anti-angiogenic activities in several tumor models, but the role of SDT in proliferative retinopathies remains unclear. Thus, the object of the present study is to examine the mechanism of action and efficacy of SDT on retinal neovascularization in oxygen-induced ischemic retinopathy (OIR) mice. Neonatal mice at postnatal day 7 (P7) were exposed to 75% concentration of oxygen for 5 days (P7-P12), and then returned to room air from P12 to P17 to induce retinal neovascularization. SDT were administered once per day for 5 consecutive days (P12-P16) by intraperitoneal injection. Retinal neovascularization was measured at P17. We used a protein array to evaluate the expression levels of angiogenic factors. Inhibitory activity of SDT on PDGF-BB/PDGFR β interaction was evaluated *in vitro*. Retinal neovascularization in the OIR mice was significantly decreased by SDT. SDT decreased the expression levels of PDGF-BB protein and VEGF mRNA. Moreover, SDT dose-dependently inhibited PDGF-BB/PDGFR β interaction ($IC_{50} = 388.82 \pm 7.31 \mu\text{g/ml}$). In conclusion, SDT is a potent inhibitor of retinal neovascularization through inhibiting the pro-angiogenic effect of PDGF-BB.

Keywords: oxygen-induced retinopathy; platelet derived growth factor; retinal neovascularization; Sipjeondaebo-tang; vascular endothelial growth factor

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Introduction

Retinal neovascularization is the major cause of blindness in all ages. Retinopathy of prematurity, proliferative diabetic retinopathy and age-related macular degeneration are caused by abnormal blood vessels growth (Aiello et al. 1994). It is well known that ischemia-mediated overexpression of vascular endothelial growth factor (VEGF) and its receptors plays a central role in development of these diseases (Aiello 1997). The interruption of VEGF signaling is a good pharmacological target for the treatment of retinal neovascularization. VEGF antagonists have an inhibitory effect on retinal neovascularization (Miki et al. 2009). However, a variety of cytokines and growth factors were also released in these ischemic areas, suggesting that other factors may be associated with the angiogenesis process

(Castellon et al. 2002).

Platelet derived growth factor (PDGF) was discovered in 1974 (Ross et al. 1974). PDGF is synthesized and secreted by various cells and has a potent mitogenic and chemotactic activities on several different cell types, including fibroblasts and vascular smooth muscle cells (Vassbotn et al. 1994). The PDGF family consists of five dimeric ligands: PDGF-AA, PDGF-BB, PDGF-AB, PDGF-CC and PDGF-DD (Appelmann et al. 2010). PDGF dimers bind to and activate two membrane receptor tyrosine kinases; PDGF receptor α (PDGFR α) and PDGFR β (Alvarez et al. 2006). PDGF-BB is one of the important regulators of angiogenesis. PDGF-BB is released from endothelial cells, platelets, vascular smooth muscle cells and inflammatory cells at sites of angiogenesis (Heldin and Westermark 1999). PDGF-BB binding to PDGFR β leads to receptor

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dimerization, autophosphorylation and activation of downstream signaling pathways, including the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) (Heldin et al. 1998). Thus, accumulating data have suggested an important role of PDGF-BB in retinal pathogenic angiogenesis.

Sipjeondaebotang (SDT, Shi-Quan-Da-Bu-Tang in Chinese and Juzen-taiho-to in Japanese) is the most widely used traditional herbal formula to treat patients with fatigue, loss of appetite, anemia, atopic dermatitis and rheumatoid arthritis in Korea, Japan and China (Kogure et al. 2005). The formula consists of 12 crude medicinal herbs (Shin et al. 2011). Previous experimental studies demonstrated that SDT possessed some pharmacological activities, such as the stimulation of immune cells, anti-microbial, anti-oxidative and anti-tumor effects (Haranaka et al. 1985; Tagami et al. 2004). It was reported that SDT has an anti-angiogenic effect in malignant glioma (Kamiyama et al. 2005). However, the precise mechanisms of SDT on angiogenesis still are unclear, and to the best of our knowledge there has been no previous report on retinal pathogenic neovascularization of SDT. To address this issue, this study evaluates the effect of SDT on retinal neovascularization in oxygen-induced ischemic retinopathy (OIR) mice.

Methods

Preparation of SDT

A standardized powder of SDT was kindly provided by Dr. Hyeun-Kyoo Shin (Korea Institute of Oriental Medicine, Daejeon, Korea). SDT was prepared as a lyophilized powder of a hot water extract obtained from ten herbs in the following ratio: Panax ginseng (3.75 g), Cinnamomum cassia (3.75 g), Cnidium officinale (3.75 g), Rehmannia glutinosa (3.75 g), Poria cocos (3.75 g), Glycyrrhiza uralensis (3.75 g), Astragalus membranaceus (3.75 g), Angelica gigas (3.75 g), Paeonia lactiflora (3.75 g), Atractylodes japonica (3.75 g), Zingiber officinale (3.75 g) and Zizyphus jujuba (3.75 g). The HPLC fingerprint and contents of ten major compounds of SDT is described in the previous report (Shin et al. 2011).

In vitro ligand blocking assay

Purified recombinant human PDGFR β -Fc chimera (R&D systems, MN, USA) in a concentration of 1 μ g/ml in 100 μ l phosphate buffered saline (PBS)/well were coated on the surface of 96-well ELISA plates overnight at 4°C. The wells were washed three times, and the nonspecific binding was blocked with PBS containing 3% bovine serum albumin for 1 hour at room temperature. Blocking solution was removed, and then 1 μ g of horseradish peroxidase (HRP)-conjugated PDGF-BB (R&D systems, MN, USA) was added with or without SDT in increasing concentrations (0-1,000 μ g/ml). HRP-labeled PDGF-BB was prepared using an HRP Labeling kit (Dojindo Laboratories, Kumamoto, Japan). After incubation for 1 hour at 37°C, free PDGF-BB was removed by three washes. Peroxidase activity was quantified using tetramethylbenzidine. Nonspecific binding was determined in the presence of an excess of unlabeled PDGF-BB. Blocking of the binding of PDGF-BB to PDGFR β was expressed as the percentage of optical density. We calculated the 50% inhibition concentration (IC₅₀) of ligand binding.

Animals and experimental design

Ischemic retinopathy was induced in C57BL/6 mouse pups. Experiment was performed on a total of 40 mouse pups of both sexes. Neonatal mice at postnatal day 7 (P7) with their nursing mothers were exposed to 75 \pm 2 % concentration of oxygen for five days (P7-P11) and then were returned to room air from P12 to P17. At P12, mice were randomly assigned to three groups: (group 1, *n* = 10) vehicle-treated OIR mice, (group 2, *n* = 10) OIR mice treated with SDT (50 mg/kg/day) and (group 3, *n* = 10) OIR mice treated with SDT (100 mg/kg/day). Normal control mice (group 4, *n* = 10) were maintained in room air from P0 to P17. In human, the daily dose of SDT is about 45-g dried herb (Shin et al. 2011), which is equivalent to 10.53 g of the SDT extract (yield = 23.4%). Considering a mean body weight of an adult of 60-80 kg, this dose for human is equal to 131-175 mg/kg of SDT extract. We chose two doses of SDT (50 and 100 mg/kg) based on the minimum human equivalent dosage of raw herbs. SDT dissolved in saline and injected intraperitoneally once a day for five days (P12-P16). The other groups were given the same amount of vehicle for five days. All experiments were approved by the Korean Institute of Oriental Medicine Institutional Animal Care and Use Committee.

Fluorescein isothiocyanate-dextran microscopy

At P17, mice were anesthetized with zolazepam (Zoletil, Virbac, Carros, France) and then circulated through the left ventricle with 0.1 ml of sterile PBS containing 5 mg of fluorescein isothiocyanate (FITC)-dextran (Sigma, MO, USA). The eyes were enucleated and fixed in 4% paraformaldehyde for 3 hours. The retinas were dissected away and incised radially. The retinal flat mounts generated, and images were captured using an Olympus BX51 microscope and DP72 camera (Olympus, Tokyo, Japan). Quantification of the central non-perfused area of the retina was measured by ImageJ software (NIH, MD, USA).

Lectin staining

The flat mounted retinas were incubated with PBS containing 5% Triton X-100 and 1% bovine serum albumin for 3 hours at 37°C. The retinas were then washed 3 times with PBS and labeled with tetramethylrhodamine-conjugated isolectin B4 from *Bandeiraea simplicifolia* (1:50 Sigma, MO, USA) diluted in PBS. The retinal vessels were viewed with an Olympus BX51 microscope (Olympus, Tokyo, Japan). ImageJ software (NIH, MD, USA) was used to measure the neovascular area of the retina.

Angiogenesis-related protein array

The expression levels of 55 angiogenesis-related proteins in SDT-treated ischemic retinas were evaluated using an antibody array analysis (Mouse Angiogenesis Array Kit, R&D Systems, MN, USA). Blocking, hybridization, washing conditions and chemiluminescent detection steps were performed according to the instructions supplied by the manufacturer. Arrays were captured and quantified using LAS-3000 image analyzer (Fujifilm, Tokyo, Japan). Proteins detectable by the mouse angiogenesis array kit included: a disintegrin and metalloproteinase with thrombospondin motifs 1 (ADAMTS-1), amphiregulin, angiogenin, angiopoietin-1, angiopoietin-3, coagulation factor III/tissue factor (TF), chemokine (C-X-C motif) ligand 16 (CXCL16), cysteine-rich protein 61 (Cyr61/CCN1), insulin-like growth factor binding protein-10 (IGFBP-10), delta like ligand 4 (Dll4), dipeptidyl peptidase-4 (DPPIV), epidermal growth factor

(EGF), endoglin/cluster of differentiation (CD) 105, endostatin/collagen XVIII, endothelin-1, acidic fibroblast growth factor (FGFa/FGF-1), basic fibroblast growth factor (FGFb/FGF-2), FGF-7, fractalkine/chemokine (C-X3-C motif) ligand 1 (CX3CL1), granulocyte-macrophage colony-stimulating factor (GM-CSF), heparin-binding EGF-like growth factor (HB-EGF), hepatocyte growth factor (HGF), IGFBP-1, IGFBP-2, IGFBP-3, interleukin-1 α (IL-1 α), IL-1 β , IL-10, interferon gamma-induced protein (IP-10)/CXCL10, keratinocyte-derived chemokines (KC)/CXCL1/growth-related oncogene α (GRO α), leptin, monocyte chemoattractant protein-1 (MCP-1)/chemokine (C-C motif) ligand 2 (CCL2), macrophage inflammatory protein 1 α (MIP-1 α)/CCL3, matrix metalloproteinase-3 (MMP-3), MMP-8 (pro form), MMP-9 (pro and active form), nephroblastoma overexpressed (NOV)/IGFBP-9, osteopontin, platelet-derived endothelial cell growth factor (PD-ECGF), PDGF-AA, PDGF-AB/PDGF-BB, pentraxin-3, platelet factor 4/CXCL4, placenta growth factor (PlGF), prolactin, proliferin, stromal cell-derived factor 1 (SDF-1), serpin E1, serpin F1, trombospondin-2, tissue inhibitor of metalloproteinase 1 (TIMP-1), TIMP-4, VEGF and VEGF-B. The list and spot location of 55 antibodies can be found at the manufacture web page (<http://www.rndsystems.com/index.aspx>).

Real-time PCR analysis

Total retinal RNA was extracted using a TRIzol reagent (Invitrogen, CA, USA), and cDNA was synthesized with 0.5 μ g of total RNA using a PrimeScript 1st Strand cDNA Synthesis kit (TaKaRa, CA, USA). Quantitative real-time PCR was performed with specific primers for VEGF, PDGF-B and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) using an iQ5 Continuous

Fluorescence Detector System (Bio-Rad, CA, USA). The primers were: for VEGF, 5'-TTACTGCTGTACCTCCACC-3' and 5'-ACAGGACGGCTTGAAGATG-3'; for PDGF-B, 5'-GCAGGGTGA GCAAGGTTGTAATG-3' and 5'-TGAAGGAAGCAGAAGGAA CGG-3'; for GAPDH, 5'-AACGACCCCTTCATTGAC-3' and 5'-TCCACGACATACTCAGCAC-3'. All real-time PCR experiments were performed in triplicate. Values of VEGF and PDGF-B mRNA expressions were normalized to GAPDH gene expression using iQ5 optical system software (Bio-Rad, CA, USA).

Statistical analysis

The data were expressed as the mean \pm s.e. The statistical significance was determined by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test or by unpaired Student's *t*-test. Differences with $P < 0.01$ were considered statistically significant.

Results

SDT inhibits retinal neovascularization in OIR mice

The pups treated with SDT in OIR mice resulted in a dose-dependent inhibition of pathological changes occurred in ischemic retinopathy. SDT prevented pathogenic neovascularization and promoted revascularization of the central retina compared with ischemic retinas in OIR group at P17 (Figs. 1 and 2). At P17, the avascular area in OIR mice average 22.9% of total retinal area. Intraperitoneal administration both dose of SDT did not have a significant effect on avascular area, but 100 mg/kg of SDT slightly decreased

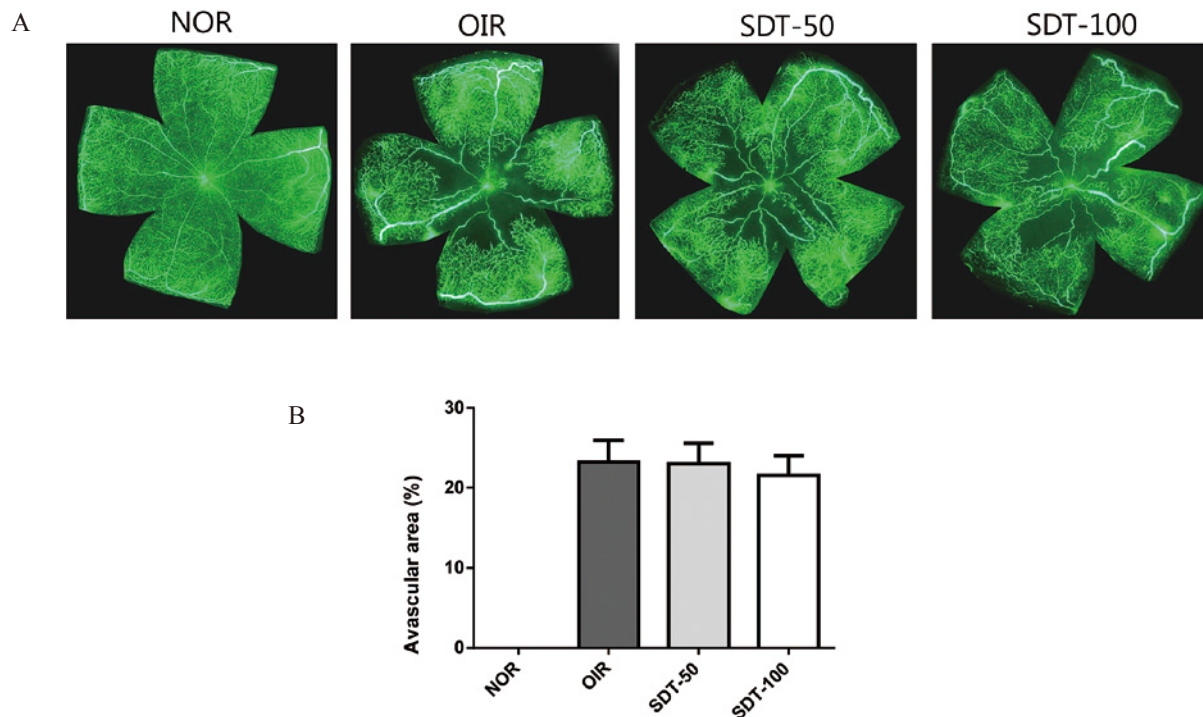


Fig. 1. Effect of SDT on central retinal vascular obliteration in OIR mice.

(A) FITC-dextran perfusion of the retinal blood vessels was observed under a fluorescent microscope. NOR, normal mice; OIR, vehicle-treated OIR mice; SDT-50, OIR mice treated with SDT (50 mg/kg); and SDT-100, OIR mice treated with SDT (100 mg/kg). (B) Quantification results are expressed as the percentage of central avascular area to total retinal area. The values in the bar graph represent mean \pm s.e. ($n = 10$).

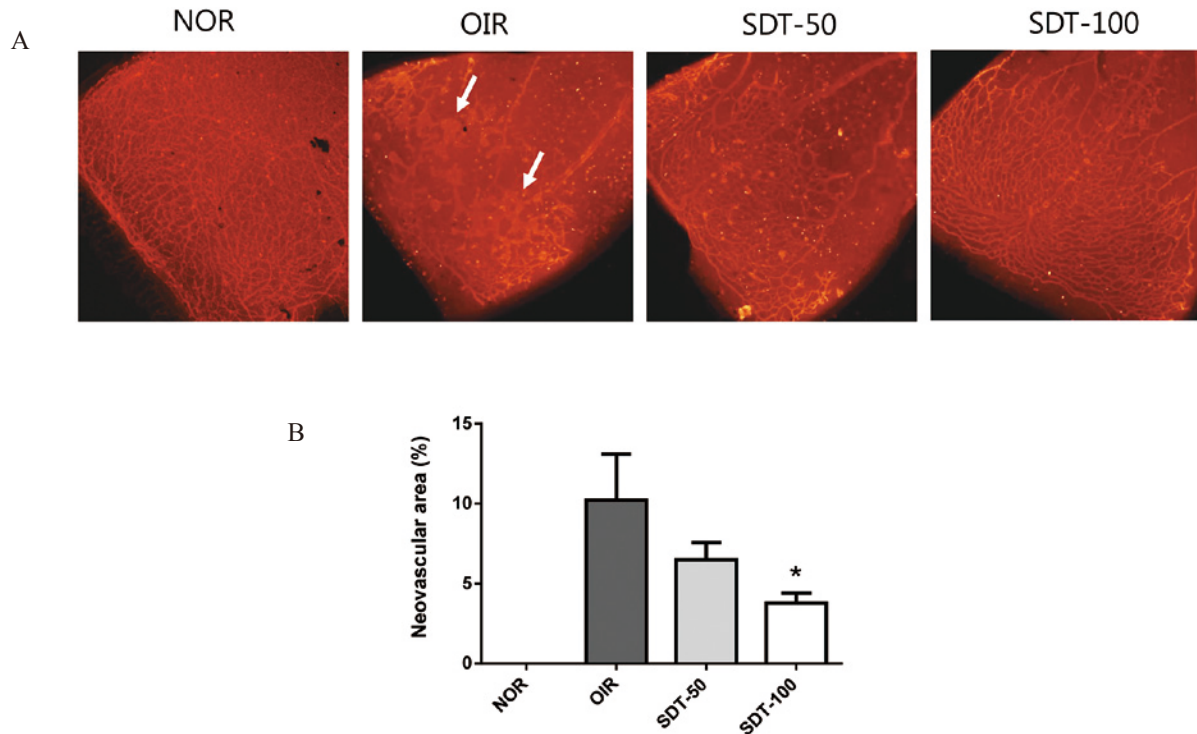


Fig. 2. Effect of SDT on retinal neovascularization in OIR mice.

(A) Isolectin B4-stained retinal flat mounts. Arrows represent neovascular tufts. NOR, normal mice; OIR, vehicle-treated OIR mice; SDT-50, OIR mice treated with SDT (50 mg/kg); and SDT-100, OIR mice treated with SDT (100 mg/kg). (B) Quantification of retinal surface covered by neovascular tufts. The values in the bar graph represent mean \pm s.e. ($n = 10$; * $p < 0.01$ vs. vehicle-treated OIR mice).

retinal avascular area as compared with ischemic retinas in OIR group (Fig. 1). Intraperitoneal administration of 50 mg/kg and 100 mg/kg of SDT prevented retinal neovascularization by 36.5% and 63.1%, respectively, compared to vehicle (Fig. 2). Thus, SDT helps to maintain a relatively normal vascular structure in proliferative retinopathy.

SDT regulates the expression of angiogenesis-associated factors in OIR mice

As shown in Fig. 3, SDT decreased dose-dependently and significantly ($p < 0.01$) at the highest dose the expression of pro-angiogenic factors (heparin-binding epidermal growth factor-like growth factor, leptin, and PDGF-AB/BB) as compared with vehicle-treated OIR mice. By contrast, anti-angiogenic factors such as pigment epithelium-derived factor and thrombospondin-2 were significantly downregulated by SDT ($p < 0.01$). This suggests that SDT exerts anti-angiogenic properties through inhibiting the expressions of heparin-binding epidermal growth factor-like growth factor, leptin, and PDGF-AB/BB. Upregulation of pigment epithelium-derived factor and thrombospondin-2 may be a protective mechanism against angiogenesis. Consistent with previous report (Giddabasappa et al. 2012), VEGF protein was not detected in the angiogenesis protein array in either vehicle- or SDT-treated OIR retinas, possibly due to low sensitivity of this antibody on the array. Since VEGF is a key player in angiogenesis, we further investi-

gated gene expression changes at the mRNA level.

Effects of SDT on expression of VEGF and PDGF-B mRNAs in OIR mice

The effect of SDT on retinal VEGF and PDGF-B mRNA expression was examined by real-time PCR. In OIR mice, the relative expression level of VEGF mRNA was increased, compared with control mice. Decreased VEGF mRNA expression was observed in the SDT-treated OIR mice (Fig. 4A). Similarly, low levels of PDGF-B mRNA were detected in the SDT-treated OIR mice compared with the ischemic retinas in OIR group (Fig. 4B).

Blocking activity of SDT on PDGF-BB/PDGFR β interaction

To determine whether SDT blocks the binding of PDGF-BB to PDGFR β in vitro, we developed a ligand-binding assay for PDGFR β . In this assay, SDT dose-dependently inhibited the binding of PDGF-BB to PDGFR β with IC₅₀ values of 388.82 ± 7.31 μ g/ml (Table 1).

Discussion

The OIR model using neonatal mice has been widely used to study hypoxia-induced retinal neovascularization (Kociok et al. 2006). When the hyperoxia-exposed mice are returned to normoxia (P12-P17), hypoxic state by vasoobliteration of the central part of retina strongly stimulates

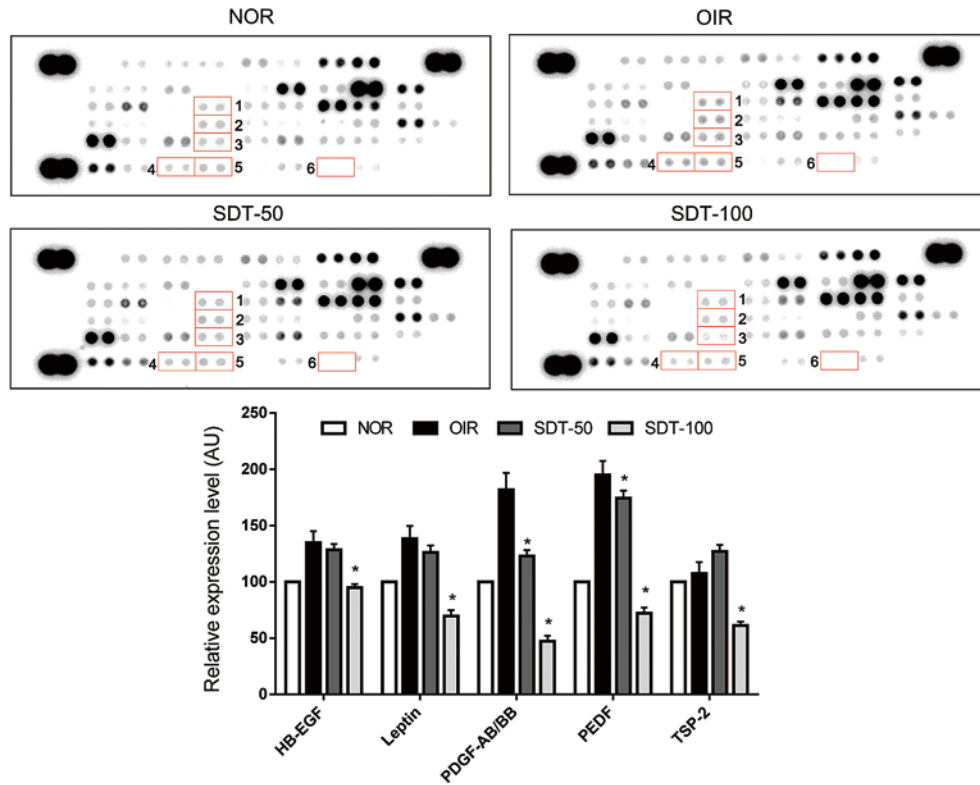


Fig. 3. Expression levels of 55 angiogenesis-related proteins in OIR mice.

Levels of pro- and anti-angiogenic factors were analyzed in retinas using protein arrays and quantified by LAS-3000 image analyzer. Positive controls are located in three corners of the arrays, and negative control is located in lower right corner of the arrays. Modulated proteins in retina treated with SDT are highlighted with red squares and indicated by numbers. NOR, normal mice; OIR, vehicle-treated OIR mice; SDT-50, OIR mice treated with SDT (50 mg/kg); and SDT-100, OIR mice treated with SDT (100 mg/kg). Square 1: HB-EGF, heparin-binding epidermal growth factor-like growth factor; Square 2: leptin; Square 3: PDGF, platelet derived growth factor; Square 4: PEDF, pigment epithelium-derived factor; Square 5: TSP, thrombospondin-2; Square 6: VEGF, vascular endothelial growth factor. The values in the bar graph represent mean \pm S.E. ($n = 4$; $*p < 0.01$ vs. vehicle-treated OIR mice).

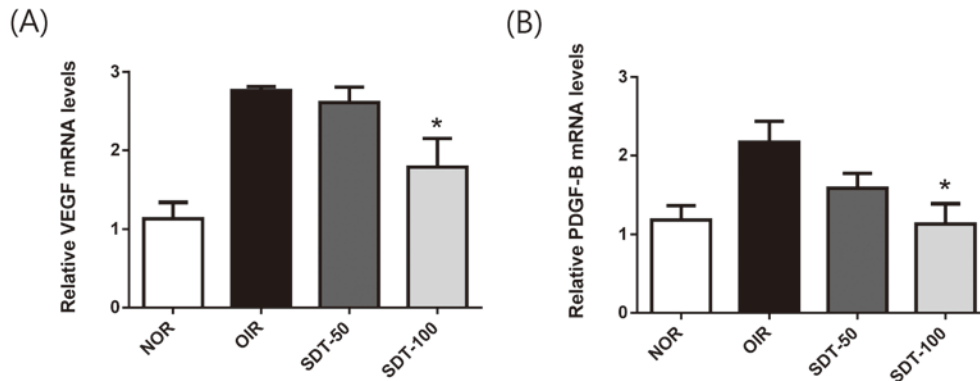


Fig. 4. Real-time PCR analysis of VEGF and PDGF-B mRNA levels in OIR mice.

When compared with normal controls, the relative expression levels of VEGF (A) and PDGF-B (B) mRNAs were markedly increased in retinas of OIR mice, and dramatically decreased after SDT treatment. NOR, normal mice; OIR, vehicle-treated OIR mice; SDT-50, OIR mice treated with SDT (50 mg/kg); and SDT-100, OIR mice treated with SDT (100 mg/kg). Data show mean \pm S.E. ($n = 4$; $*p < 0.01$ vs. vehicle-treated OIR mice).

the release of pro-angiogenic factors and induces neovascularization on the surface of the retina (Kociok et al. 2006). In present study, SDT, a well-known Korean traditional medicine, could prevent retinal neovascularization in OIR

mice. We also showed that SDT treatment dramatically inhibited the expression of PDGF-BB and VEGF in OIR mice. Moreover, our in vitro ligand-binding assay showed that SDT inhibited the binding PDGF-BB to its receptor,

Table 1. Inhibitory effect of SDT on PDGF-BB/PDGFR β interaction.

Sample	Concentration ($\mu\text{g/ml}$)	Inhibition (%)	IC ₅₀ ($\mu\text{g/ml}$)
SDT	50	28.90 \pm 2.39	388.82 \pm 7.31
	100	36.95 \pm 8.26	
	500	55.80 \pm 11.28	

Inhibitory activity was expressed as mean \pm s.e. of quadruplicate samples. The value of 50% inhibition concentration (IC₅₀) was calculated from the dose inhibition curve.

PDGFR β . These results indicate that SDT has anti-angiogenic effects on retinal neovascularization.

Although SDT has been usually administered orally in humans, we used intraperitoneal injection as the route of drug administration. The oral route is the most convenient route of administration. However, the neonatal mouse has historically used the intraperitoneal route of administration for dosing (McClain et al. 2001). There is no method of minimally invasive oral drug administration that is accurate and reliable for delivering drug compounds to neonatal mice (Butchbach et al. 2007).

Studies in animal models predicted that VEGF plays a major role and this has been validated in clinical trials. VEGF antagonists provide major benefits in patients with neovascular age-related macular degeneration and other types of ocular neovascularization (Campochiaro 2013). However, growing evidence suggests that PDGF-BB has been implicated in ischemic retinopathies such as proliferative diabetic retinopathy and choroidal neovascularization. Normal vessels showed very low to undetectable levels of PDGF-BB, but the high levels of PDGF-BB and its receptors are produced in vascular cells following injury (Raines 2004). Increased expression of PDGF-BB in the retina causes plays an important role in the pathogenesis of proliferative diabetic retinopathy and retinal detachment (Butchbach et al. 2007). Inhibition of PDGFR signaling by antibodies was also shown to enhance therapeutic effect of anti-VEGF treatment in multiple mouse models of ocular neovascularization (Kociok et al. 2006). In addition, the PDGF-BB/PDGFR signal pathway has an important role in supporting vascular integrity and stability (Zehetner et al. 2014). Pericytes are recruited by PDGF-BB secreted from endothelial cells during the process of neovascularization (Benjamin et al. 1998). In pericyte-stripped vessels induced by the inhibition of PDGF-BB, the endothelium becomes more susceptible to VEGF blockade, resulting in regression of the neovascularization (Zehetner et al. 2014). Thus, it has been proposed that PDGF-B is a second validated target against retinal neovascularization (Campochiaro 2013). In our present study, SDT blocked PDGF-BB/PDGFR β interaction *in vitro* and prevented retinal neovascularization through down-regulation of both PDGF-BB and VEGF *in vivo*. This is first report demonstrating that SDT is a potent inhibitor of PDGF-BB.

SDT is known to have multifunctional properties in

clinical applications. SDT has an excellent safety record (Shin et al. 2011). The most important finding in our experiment is the anti-angiogenic effect of SDT in ischemic retinopathy. There are very limited reports of anti-angiogenic studies using crude extracts or oral administration of herbal medicines. Recent reports showed that herbal medicine can prevent angiogenesis during tumor progression (Yance and Sagar 2006; Ruma et al. 2014). Moreover, some botanical extracts and phytochemicals have anti-angiogenic activity on retinal neovascularization (Cao et al. 2010; Hua et al. 2011; Tanaka et al. 2012; Kumar Gupta et al. 2013). In many traditional herbal medicines, a mixture of medicinal herbs has been known to possess additive and synergistic effects of phytochemicals present in the different herbs (Lee et al. 2006). Interestingly, in multi-herbal formulas, the therapeutic activity, dosages and toxicology of single herb are altered by addition of other herbs. SDT stimulates the intestinal immune functions, but each single herb in SDT has no such activity (Kiyohara et al. 2004). SDT contains 10 major compounds (5-hydroxymethyl-2-furaldehyde, albiflorin, paeoniflorin, liquiritin, cinnamic acid, ginsenoside Rb1, ginsenoside Rc, glycyrrhizin, formonoetin and decursin). The paeoniflorin (1.108%) and glycyrrhizin (0.847%) contents of SDT are higher than those of the other compounds (Shin et al. 2011). Paeoniflorin prevented apoptosis induced by oxidative stress in human retinal pigment epithelial cells (Wankun et al. 2011) and decreased the production of VEGF in rat synovium with adjuvant arthritis (Zheng et al. 2007). The expressions of PDGF-BB and PDGFR α mRNAs were inhibited by paeoniflorin (0.1-10 $\mu\text{g/ml}$) in oxidized low-density lipoprotein-treated vascular smooth muscle cells (Ji et al. 2006). The IC₅₀ of SDT for inhibiting the binding of PDGF-BB to PDGFR β is 388.82 $\mu\text{g/ml}$. The concentration of paeoniflorin in 388.82 $\mu\text{g/ml}$ of SDT is about 4.3 $\mu\text{g/ml}$. The peak plasma concentration of paeoniflorin after oral administration of paeoniae radix extracts in rats was 8 $\mu\text{g/ml}$ (Hsiu et al. 2003). These findings suggest that the effective plasma concentration of paeoniflorin is able to reach by administration of 100 mg/kg of SDT. Glycyrrhizin suppressed angiogenesis during tumorigenesis in mice (Kim et al. 2013). Glycyrrhizin blocked the advanced glycation end products-induced VEGF production in rat retinal ganglion cells (Lee et al. 2012). Moreover, glycyrrhizin is known as a selective inhibitor of high-mobility group box-1, a potent pro-angiogenic factor and

attenuated retinal neovascularization in OIR mice (Lee et al. 2013). Therefore, it is suggested that the inhibition of retinal neovascularization by SDT may be due to synergic effects of paeoniflorin and glycyrrhizin. However, the detailed mechanism of combination effects of SDT on retinal neovascularization remains obscure.

In conclusion, SDT blocked the binding of PDGF-BB to PDGFR β in vitro, and 100 mg/kg/day of SDT exerted an anti-angiogenic effect in the mouse model of ischemic retinopathy. The daily dose of SDT in human is about 175.5 mg/kg/day. Therefore, SDT would be clinically useful as an herbal medicine for human ischemic retinopathy.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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