Baicalein Reduces Inflammatory Process in a Rodent Model of Diabetic Retinopathy

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PURPOSE: This study was designed to elucidate the role of inflammatory process in diabetic retinopathy and to investigate the effect of baicalein treatment on diabetic rat.

METHODS: Retinal microglial cells were identified with CD11b antibody, and retinal Müller cells were identified with glial fibrillary acidic protein (GFAP). The gene expression of interleukin (IL)-18, tumor necrosis factor (TNF)-α, and IL-1β was examined by quantitative real-time PCR. The expression of GFAP and vascular endothelial growth factor (VEGF) was examined by quantitative real-time PCR, immunohistochemistry, and Western blot analysis. Vascular permeability was measured in vivo by bovine serum albumin conjugated with FITC. Baicalein was given by oral administration (150 mg/kg/d) with an animal feeding needle beginning 5 days after streptozotocin (STZ) injection.

RESULTS: By 24 weeks after onset of diabetes, microglial cells were activated and proliferated, and Müller cells upregulated their GFAP and VEGF expression. Pro-inflammatory factors, including IL-18, TNF-α, and IL-1β, were significantly upregulated. Obvious vascular leakage and abnormality were demonstrated, and ganglion cell loss was significant. Baicalein treatment alleviated diabetes-induced microglial activation and pro-inflammatory expression, reduced the GFAP and VEGF expression from Müller cells, and significantly reduced vascular abnormality and ganglion cell loss within the retina.

CONCLUSIONS: Inflammatory process, characterized by microglial activation and Müller cells dysfunction, was implicated in STZ-induced diabetic retinopathy. Baicalein treatment alleviated inflammatory process, and therefore inhibited vascular abnormality and neuron loss in diabetic retinas. (Invest Ophthalmol Vis Sci. 2009;50:2319–2327) DOI:10.1167/iovs.08-2642

Diabetic retinopathy (DR) is the leading cause of adult blindness and the most common complication of diabetes. It affects more than 90% of people with diabetes, ultimately leading to retinal edema, neovascularization, and vision loss in some patients.1,2 Vascular changes, including breakdown of the blood-retinal barrier, thickening of the capillary basement membrane, and reduction in the number of pericytes and increment in the number of acellular capillaries, have been widely documented in DR. Capillary cells are not the only retinal cells that undergo apoptotic death in diabetes. A greater-than-normal frequency of nonvascular cells, mostly ganglion cells, have also been reported to become TUNEL-positive in retinas of humans and animals with diabetes.3

In previous studies, emerging evidence supported the notion that inflammation in the retina, characterized by the activation of microglia and astroglia, is involved in the pathogenesis of DR. DR is a chronic, low-grade inflammatory disease.1–6 Diabetic conditions lead to an elevation of pro-inflammatory cytokine expression within the retina, which activates microglial cells.7 In response to an activating stimulus, quiescent microglia undergo a series of stereotyped morphologic, phenotypic, and functional changes.8 Activated microglia thereby stimulate a cycle of inflammation that recruits leukocytes, causes vascular breakdown, and directly induces glial dysfunction and neuronal cell death through the release of cytotoxic substances.7

The root of Scutellaria baicalensis (Chinese name, Huang-qin) has been used as a folk medicine in China and Japan for the treatment of chronic hepatitis, allergy, thrombotic stroke, and inflammatory disease for centuries.9 Huang-qin is known to contain numerous flavone derivatives,10 and their pharmacological properties have been extensively investigated. Among them, baicalein (5,6,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one) has attracted considerable attention. As the major active component of S. baicalensis, baicalein has also been reported to exhibit potent anti-inflammatory effect. Baicalein was found to inhibit acetic acid-induced increase in vascular permeability in mice and to reduce acute paw edema in the rats induced by compound 48/80.11 It also suppressed development of secondary lesion in adjuvant-induced arthritis in rats.11 Microglial suppressive effect of baicalein is also well documented. In this regard, it has been reported to abolish the LPS-induced expression of inducible nitric oxide synthase (iNOS), tumor necrosis factor (TNF)-α, and interleukin (IL)-1β in murine BV-2 cell and rat primary microglial cells12 and to downregulate the microglial markers CD68, CD80, and CD86 expression in G93A/SOD1 mouse (an animal model of amyotrophic lateral sclerosis).13

Given that inflammatory process plays an important role in the pathogenesis of DR, we speculate that baicalein, with its anti-inflammatory properties, may exert the capacity to block high glucose-induced microglial and astroglial activation, and consequently protect neurons and vascular damage in DR.

MATERIALS AND METHODS

Animals

After a 16-hour fast, SD female rats weighting 190 to 200 g received a single 60 mg/kg intraperitoneal injection of streptozotocin (STZ; Sigma-Aldrich, St. Louis, MO) in 10 mM sodium citrate buffer (pH 4.5).
Control animals were fasted and received the buffer alone. Animals with blood glucose levels higher than 16.7 mM 5 days after receiving STZ were considered to be diabetic. Body weight, mean systolic blood pressure (by tail cuff plethysmography in conscious rats; equipment from IITC Life Science, Woodland Hills, CA), and blood glucose levels were checked every 4 weeks. All experiments were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All animals were housed in accordance with the guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care.

For the in vivo baicalein (Sigma-Aldrich) treatment, rats were treated by oral administration with an animal feeding needle beginning 5 days after STZ injection. We chose oral administration of baicalein because administration in humans is by the oral route. Baicalein was well absorbed in stomach and small intestine, but relatively less so in colon. Absorption of baicalein itself was negligible, whereas the glucuronides/sulfates of baicalein (namely baicalin) was predominant in the plasma. Previous studies by Hwang et al.17 have applied baicalein orally to ischemia-reperfusion-induced brain injury at the concentrations of 50 mg/kg, and Guo et al.18 have applied baicalein to experimental prostatic hyperplasia intragastrically with the concentrations of 130, 260, and 520 mg/kg. Referring to these previous studies, we chose to give treatment at concentrations of 75, 150, and 300 mg/kg/d. In the present study, there is no significant difference between 75, 150, and 300 mg/kg/d. We chose 150 mg/kg/d as the dosage to present our results in this article.

Tissue Preparation

Animals were euthanized with an overdose of pentobarbital, and their eyes were immediately enucleated and fixed in 4% (w/v) paraformaldehyde (PFA) in PBS for 1 hour. The anterior segments were removed and the posterior segments were further fixed in the same fixative for an additional period of 5 hours. The tissue samples were transferred to 20% sucrose buffer overnight at 4 °C for cryoprotection and then embedded in OCT compound. Frozen sections were cut 8 μm thick through the optic nerve head and ora serrata with a cryostat, and the sections were kept in a −80 °C freezer until use.

Flatmounted retinas were prepared by bisecting the eyes at the ciliary body into anterior and posterior sections. The lens and vitreous were removed, and the retina was subsequently separated from the underlying retinal pigment epithelium and choroid. Flatmounted retinas were kept at 4 °C until use.

Vessel Leakage Study

For leakage study, rats were deeply anesthetized using a ketamine-xylazine mixture, and were perfused through the left ventricle using a FITC-dextran stock solution, 10 mg/mL (Sigma-Aldrich), at a 0.05 mL/g body weight concentration. Animals were killed 1 minute after perfusion. The eyes were enucleated and fixed in 4% PFA overnight. The corneas and lens were then removed, and the peripheral retinas were dissected and flatmounted on microscope slides for examination under confocal laser scanning microscope (510 META; Carl Zeiss, Oberkochen, Germany).

Immunohistochemical Study

For immunohistochemical study, the frozen sections were fixed with chilled fresh acetone for 10 minutes. Sections were incubated with mouse anti-VEGF monoclonal (1:50; Santa Cruz Biotechnology, Santa Cruz, CA) or goat anti-VEGF polyclonal (1:50; SC-9065; Santa Cruz Biotechnology) antibodies. The secondary anti-rabbit or anti-mouse antibodies were labeled with HRP. Control sections were treated in the same way, but with omission of primary antibodies. The sections were developed with DAB.

Microglial cells were identified with CD11b. The immunohistochemical study in flatmounted retinas was performed as described previously. Briefly, the flatmounted retinas were first fixed in absolute ethanol for 10 minutes at 4 °C and rehydrated in 0.01 M PBS for 20 minutes, made permeable by incubating in 1% Triton X-100 containing 1% bovine serum albumin for 1 hour, and incubated in primary antibodies to CD11b (1:50, MCA275R; Serotec Ltd., Oxford, UK) at 4 °C overnight. Finally the retinas were incubated with corresponding HRP labeled secondary antibody (1:200, STAR77; Serotec Ltd.) for 3 h at 37°C, and developed with DAB. Flatmounted retinas were examined by confocal microscope (Carl Zeiss) and counted by a masked observer. Positively stained microglial cells in the inner and middle retinal layer were counted in a standard area of retina using a graticule at 40 × objective magnifications. At least 10 nonadjacent areas per eye were counted for each stain.

Western Blot Assays

After the animals were killed as described previously, the eyes were enucleated and bisected, and the retinas were peeled from the eyecups and immediately homogenized with 0.5 mL ice-cold lysis buffer. The insoluble material was removed by centrifugation at 12,000g for 20 minutes. Final protein concentrations were determined using a protein assay kit (BCA; Pierce Biotechnology, Rockford, IL) according to the manufacturer’s specifications. Western blot analysis was then performed. Antibodies specific for GFAP and VEGF were used for immunodetection. Blots were visualized using an enhanced chemiluminescent technique (SC-2048; Santa Cruz Biotechnology). As a control for equal loading of proteins, a β-actin antibody (1:2000, A2228; Sigma-Aldrich) was used. For quantitative evaluation of the Western blot studies, the films were scanned and the optical densities were quantified with analysis software (Quantity One 1-D; Bio-Rad, Hercules, CA). The Western blot experiments were repeated four times from separate samples.

Quantitative Real-Time PCR

After the animals were killed as described previously, the eyes were enucleated and bisected, and the retinas were peeled from the eyecups and immediately homogenized in a RNA isolation agent (TRIzol; Invitrogen Corp.). Real-time PCR was performed in 96-well plates using standard protocols with a fluorescent detection dye (SYBR Green; Invitrogen Corp.) in a real-time detection system (iCycler; Bio-Rad). All PCR reactions were at a final volume of 30 μL comprised of fluorescent dye/PCR mix, 600 μM forward and reverse primers, and 1 ng of cDNA. We used the following PCR cycle parameters: polymerase activation for 15 minutes at 95°C, 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 1 minute. Fluorescence was measured at 72°C. The quantity of mRNA was calculated by normalizing the C_T (threshold cycle) of the target gene to the C_T of the β-actin housekeeping gene in the same sample, according to the following formula: The average β-actin C_T (each multiplex PCR was performed in triplicate) was subtracted from the average target gene C_T; the result represents the ΔC_T. This ΔC_T is specific and can be compared with the ΔC_T of a calibration sample. The subtraction of control ΔC_T from the ΔC_T of the target gene is referred as ΔΔC_T. The relative quantification of expression of a target gene (in comparison with control) was determined by using 2−ΔΔC_T.
### Morphologic Alterations by Baicalein

Amelioration of Diabetes-Induced Retinal

<table>
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<th>Treatment Group (n = 8)</th>
<th>Weight (g)</th>
<th>Blood Pressure (mm Hg)</th>
<th>Blood Glucose (mmol/L)</th>
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Data are expressed as the mean ± SE. *Significantly different from age-matched control rats (P < 0.05).

### Statistical Analysis

Data are summarized as the mean ± SD. Statistical comparisons were made by a single-factor ANOVA followed by the least-significant difference post hoc test for multiple comparisons. A P value less than 0.05 was considered significant.

### Results

#### Animals

The body weight, blood pressure, and blood glucose levels for diabetic rats with or without baicalein treatment and the age-matched control rats are shown in Table 1. By 4 weeks after onset of diabetes, diabetic rats treated with or without baicalein weighted significantly less than the control rats. The age-matched control rats had a 46% gain in weight from 4 to 24 weeks after onset of diabetes, whereas the diabetic rats had no weight gain, and the baicalein-treated diabetic rats had a 2% gain in weight during this period. The blood pressure levels had no significant difference among control rats and diabetic rats with or without baicalein treatment at all ages examined. The blood glucose levels differed significantly between control rats and diabetic rats with or without baicalein treatment at all ages studied.

#### Amelioration of Diabetes-Induced Retinal Morphologic Alterations by Baicalein

In the age-matched control retinas, the photoreceptor inner and outer segments as well as the photoreceptor nuclei were well aligned (Fig. 1A). By 24 weeks after onset of diabetes, the outer and inner segments of photoreceptor cells showed moderate disorganization, and large number of pyknotic nuclei appeared in the inner nuclear layer (INL) (Fig. 1B). After treatment with baicalein, the disorganized inner and outer segment as well as the pyknotic nuclei in the INL disappeared (Fig. 1C). By 24 weeks after onset of diabetes, the thickness of retinas of diabetic rats was less than that in age-matched control and the baicalein-treated rats.

Additional measurements were made of the number of cells in the ganglion cell layer (GCL) of diabetic and control rats. Retinas of control and baicalein-treated rats had a GCL in which the cells were densely packed; except for occasional blood vessels, there was little space intervening between cells. In contrast, diabetic retinas without baicalein treatment demonstrated a loss of cells in the GCL, with some areas devoid of any cells.

To determine whether there is a significant difference in the number of cells in the GCL of diabetic versus control rats, the cells were counted in sections by a masked observer. The cells in the ganglion cell layer were counted in a standard length of retina (1.2 mm) centered on the optic nerve head using a graticule at 40× objective magnification. As shown in Figure 1D, control retinas typically had from 27 to 35 cells in cross-sections of this layer. By 24 weeks after onset of diabetes, there were significantly fewer (40%) cells in the GCL in diabetic retinas compared with that in control retinas (18.00 ± 2.92 vs. 30.00 ± 3.39). After treatment with baicalein, the number of cells in the GCL was 48% more than that in untreated retinas (26.8 ± 1.93 vs. 18.00 ± 2.92), and there did not appear to be a significant difference when compared with age-matched control retinas (26.8 ± 1.93 vs. 30.00 ± 3.39).

#### Prevention of Diabetes-Induced Vascular Changes by Baicalein

Increases in retinal vascular permeability in animal models of diabetes have been reported by multiple investigators.19,20 In the present study, retinal vascular permeability was measured by extravasation of BSA-FITC. Diabetic rats without baicalein treatment (Fig. 2B) had significant lesions in the retinal capillary network as well as large vessels compared with that in age-matched controls (Fig. 2A) and baicalein-treated (Fig. 2C) rats. The diabetic retina demonstrated focal dilation of retinal capillaries, large-vessel tortuosity or beading, and small-like bulges in the vessel wall. Obvious leakage of the fluorescein from capillaries and larger vessels was also noted in the flat-mounted retinas of diabetic rats without baicalein treatment.

#### Reduction of Microglial Reactivity in the Retina of Diabetic Mouse by Baicalein

Microglias were visualized in flatmounted retinas from control and diabetic rats with or without baicalein treatment. Two subpopulations of CD11b-labeled microglial cells were identified in age-matched control retina. One subpopulation had
long, slender processes, localized at the perivascular region in the neural fiber layer and GCL (Fig. 3A). The other, also with long and slender processes, was mostly found in the retinal parenchyma, did not run along the retinal vessels, and was more localized in the inner plexiform layer (Fig. 3D).

In the diabetic retinas, the microglial cells in the inner retinal layer were characteristically ameboid, with few stout and dark-stained processes localized at the vascular walls (Fig. 3B). The microglial cells in the middle retinal layer were characterized with much shorter and wider processes (Fig. 3E). The number of microglial cells both in the inner and middle retinal layer was significantly higher than those in the control retinas (Fig. 3G). The morphologic and number changes show that microglial cells in the diabetic retinas were activated and proliferated. In diabetic retinas with baicalein treatment, the microglial cells in the inner retinal layer were ramified with dark-stained processes (Fig. 3C), and the cell number was significantly fewer than that in the untreated diabetic retinas, but it was still higher than that in the control retinas (Fig. 3G). The microglial cells with baicalein treatment in the middle retinal layer showed more slender processes (Fig. 3F), and the cell number was significantly fewer than that in the untreated diabetic retinas, but there was no significant difference when compared with the control retinas (Fig. 3G). The morphologic and number changes show that baicalein treatment ameliorated high glucose-induced microglial activation and proliferation.

To determine whether activated microglial cells in the inner retinal layer were responsible for the cell loss in the GCL, we double-labeled TNF-α/H9251 and CD11b on diabetic retinas. Our results demonstrated that TNF-α expression was most evident in the inner retinal layer (Fig. 3H). CD11b labeled microglial cells was most evident in the inner and middle retinal layers (Fig. 3I). Colocalization of TNF-α/H9251 and CD11b showed that TNF-α was mainly expressed by microglial cells in the inner retinal layer; microglial cells in the middle retinal layer did not express TNF-α (Fig. 3J).

The microglial cells with baicalein treatment in the middle retinal layer showed more slender processes (Fig. 3F), and the cell number was significantly fewer than that in the untreated diabetic retinas, but there was no significant difference when compared with the control retinas (Fig. 3G). The morphologic and number changes show that baicalein treatment ameliorated high glucose-induced microglial activation and proliferation.
To further demonstrate the relationship between activated microglial cells and vascular abnormality, we double-labeled CD11b and anti-Factor VIII related Ag, which labeled vascular endothelial cells, on diabetic retinas. Our results demonstrated that CD11b labeled microglial cells were most evident in the inner and middle retinal layers (Fig. 3K). The anti-Factor VIII-related Ag expression was evident in the inner and middle retinal layers, which corresponded to the vascular distribution in the retina (Fig. 3L). Colocalization of CD11b and anti–Factor VIII-related Ag showed that some of the activated microglial cells surrounded the abnormal vascular in the inner and even in the middle retinal layer (Fig. 3M).

**Reduction of GFAP and VEGF Immunoreactivity in the Retina of Diabetic Mouse by Baicalein**

The expression of GFAP and VEGF was determined by quantitative real-time PCR, immunohistochemistry, and Western blot analysis. In the age-matched control retinas, the GFAP-positive staining was mainly located in the neural fiber layer, where the stellate astrocyte is located; the Müller cells were not labeled (Fig. 4A). By 24 weeks after onset of diabetes, a marked increase in the number of GFAP-positive processes of Müller cells was observed throughout the whole neural retinal layer (Fig. 4B). After treatment with baicalein, the GFAP-positive labeling was much weaker than that in untreated retinas (Fig. 4C). A representative gel image of GFAP protein expression is shown in Figure 4D. The GFAP mRNA expression was detected by real-time PCR analysis using forward primer (GFAP): TTGGAAGGATGGTTGT GGATTC. The results show that the age-matched control retina consistently expressed modest quantities of GFAP. By 24 weeks after onset of diabetes, the GFAP expression was significantly upregulated. After treatment with baicalein, the GFAP expression was significantly downregulated when compared with that in untreated retinas, and...
there did not appear to be a significant difference when compared with age-matched control retinas (Fig. 4E and F).

In the age-matched control retina, no clear VEGF-positive staining was observed (Fig. 5A). By 24 weeks after onset of diabetes, clear vertical thread-like positive-VEGF staining was observed (Fig. 5B). Colocalization study demonstrated that VEGF was expressed in Müller cells (data not shown). After treatment with baicalein, the VEGF-positive labeling was much weaker than that in untreated retinas (Fig. 5C). A representative gel image of VEGF protein expression is shown in Figure 5D. The VEGF mRNA expression was detected by real-time PCR analysis using forward primer (VEGF): CTGTACCTCCA CCATGCCAAGT and reverse primer (VEGF): CTGCGCTGGTA- GACGTCCAT. The results show that age-matched control retinas expressed very low levels of VEGF protein. By 24 weeks after onset of diabetes, the gene expression of IL-18, TNF-α, and IL-1β was significantly upregulated. After treatment with baicalein, the gene expression of IL-18, TNF-α, and IL-1β was significantly downregulated when compared with those in untreated retinas, but there did not appear to be a significant difference when compared with age-matched control retinas (Fig. 6).

**DISCUSSION**

Our studies demonstrated that inflammatory process, characterized by microglial activation and Müller cell dysfunction, is implicated in STZ-induced DR. Anti-inflammatory drug baicalein treatment ameliorated inflammatory process, therefore inhibited vascular abnormality and neuron loss in diabetic retinas.

Microglia belongs to the mononuclear phagocyte system, and forms the resident macrophages in the brain and retina. Resting microglial cells promote neuronal cell viability and maintain homeostasis in the normal CNS. However, reactive microglial cells were implicated in the progression of many retinal diseases, including retinal degeneration, glaucoma, and DR. Consistent with previous studies, our work demonstrates that microglial cells in the inner and middle retinal layers were markedly activated in diabetic retinas. Furthermore, localization studies demonstrate that some of the activated microglial cells surround the abnormal vascular in the inner and even in the middle retinal layer. Krady et al. have hypothesized that activated microglia stimulate a cycle of inflammation that recruits leukocytes, causing vascular break-

**FIGURE 4.** Expression of glial fibrillary acidic protein (GFAP) in the age-matched control and diabetic retinas with or without baicalein treatment. (A) Retina from the age-matched control rat. (B) Retina from the diabetic rat (24 weeks after onset of diabetes) exhibited strong GFAP-positive staining of Müller cells. (C) With baicalein treatment (treated by oral administration with 150 mg/kg/d beginning 5 days after STZ injection) for 24 weeks, the GFAP-positive labeling was significantly decreased. Western blot analysis (D, E) and real-time PCR analysis (F) demonstrated that GFAP expression was significantly upregulated in the diabetic retinas, and baicalein treatment markedly reduced high glucose-induced GFAP expression.
Mueller cell dysfunction was involved in STZ-induced diabetic expression was demonstrated in Mueller cells, indicating that high levels of GFAP. In the present study, increased GFAP contributed to the enhanced death of neurons in these diseases. proinflammatory and/or cytotoxic factors might be major contributors to the inflammatory response within the retina, can propagate the inflammatory response within the retina, and baicalein treatment significantly reduced high glucose-induced VEGF expression.

Vision loss and blindness from diabetic retinopathy are usually the result of vascular leakage or ischemia. Vascular leakage involves hemorrhage or the formation of hard exudates. Ischemia from vascular damage and disruption in local perfusion result in angiogenesis and neovascularization. The new blood vessels formed are fragile and prone to hemorrhage, which can impair vision, ultimately causing blindness. VEGF is a major regulator of blood vessel formation and function. It controls several processes in endothelial cells, such as proliferation, survival, and migration. Retinal VEGF expression is correlated with diabetic blood-retinal barrier breakdown and ischemia related neovascularization in animals and humans. In the present study, VEGF expression in Muller cells was significantly upregulated in diabetic retina, indicating that VEGF overexpression plays a crucial role in retinal vascular abnormality in STZ-induced diabetes.

Baicalein has been shown to exert anti-inflammatory effects. In the present study, baicalein treatment ameliorated high glucose-induced microglial activation and their secretion of inflammatory and/or cytotoxic factor expression; baicalein treatment also inhibited high glucose-induced Muller cell dysfunction and VEGF overexpression. Limitations of the in vivo studies prevented the present study from providing a direct answer to the question of whether the inhibitory effect on activated microglial and Muller cells contributed to the rescue of retina, or the inhibitory effect on activated microglial and Muller cells were the result of the rescued retina. Nevertheless, several groups have confirmed baicalein’s microglial inhibitory
effect in the in vitro and in vivo systems. In vitro studies from Ignacio et al.\textsuperscript{13} demonstrated that treatment of G93A/SOD1 mouse (an animal model of amyotrophic lateral sclerosis) with baicalein downregulated the microglial markers CD68, CD80, and CD86 expression, all of which were upregulated in untreated mutant animals. Furthermore, studies by Suk et al.\textsuperscript{42} suggest that baicalein could be a key template for the development of therapeutic agents to selectively modulate inflammatory responses and cellular apoptosis in central nervous system. Although we cannot exclude the possibility that baicalein may execute protective activity through other mechanisms, from the evidence we mentioned above and our own studies, we suggest that baicalein treatment attenuated the inflammatory process in diabetic retinas, and subsequently inhibited high glucose–induced vascular abnormality and neuronal cell loss in diabetic retinas.

Aside from its antimicroglial and anti-Müller effect, oral baicalein dramatically reduced sorbitol levels in RBC without affecting blood glucose levels. Its mechanism is presumably through inhibition of aldose reductase.\textsuperscript{43} Antioxidant effect of baicalein is also well documented. It has been reported to scavenge reactive oxygen species, including superoxide, hydrogen peroxide, and hydroxyl radicals.\textsuperscript{44} Antioxidants and aldose reductase inhibitors have been found to have potent anti-inflammatory actions in diabetic retinopathy.\textsuperscript{45,46} From previous studies, baicalein or baicalin treatment has few side effects, which facilitates its application for human DR.

In conclusion, our study demonstrated that inflammatory process plays an important role in vascular abnormality and neuron cell loss in DR. Drugs designed to counter the inflammatory process should, therefore, have numerous benefits in disease such as DR.

References


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