Modulation of diabetic retinopathy pathophysiology by natural medicines through PPAR-γ-related pharmacology

Min K Song, Basil D Roufogalis and Tom H W Huang

Herbal Medicines Research and Education Centre, Faculty of Pharmacy, The University of Sydney, NSW, Australia

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes and remains a major cause of preventable blindness among adults at working age. DR involves an abnormal pathology of major retinal cells, including retinal pigment epithelium, microaneurysms, inter-retinal oedema, haemorrhage, exudates (hard exudates) and intraocular neovascularization. The biochemical mechanisms associated with hyperglycaemic-induced DR are through multifactorial processes. Peroxisome proliferator-activated receptor-γ (PPAR-γ) plays an important role in the pathogenesis of DR by inhibiting diabetes-induced retinal leukostasis and leakage. Despite DR causing eventual blindness, only a few visual or ophthalmic symptoms are observed until visual loss develops. Therefore, early medical interventions and prevention are the current management strategies. Laser photocoagulation therapy is the most common treatment. However, this therapy may cause retinal damage and scarring. Herbal and traditional natural medicines may provide an alternative to prevent or delay the progression of DR. This review provides an analysis of the therapeutic potential of herbal and traditional natural medicines or their active components for the slowdown of progression of DR and their possible mechanism through the PPAR-γ pathway.

Introduction

Diabetic retinopathy (DR) is one of the most common microvascular complications of diabetes and one of the leading causes of blindness worldwide (Fong et al., 2004a). The prevalence of DR increases with the duration of diabetes, and nearly all patients with type I diabetes and more than 60% with type II diabetes have some degree of retinopathy after 20 years (Fong et al., 2003; 2004b; Williams et al., 2004). Therefore, early detection and prevention are the current management strategies (Ciulla et al., 2003). Chronic hyperglycaemia is believed to be the primary pathogenic factor for inducing damage to retinal cells (Knudsen et al., 2002; Hammes, 2005; Yanagi, 2008). However, the mechanisms that lead to DR are not fully understood (West et al., 2006). DR is characterized by microaneurysms, inter-retinal oedema, haemorrhages, exudates (hard exudates) and intraocular pathological neovascularization (Fong et al., 2004a,b). Laser photocoagulation therapy is the most common treatment modality for DR. However, this therapy may damage neural tissue resulting in the deterioration of vision (Bloomgarden, 2007). Therefore, development of new therapeutic strategies for the treatment...
of excessive retinal vasopermeability and angiogenic changes are the basis for further research focus (Garcia et al., 2008).

The peroxisome proliferator-activated receptor-γ (PPAR-γ) is a ligand-inducible transcription factor that belongs to a large superfamily, comprising the nuclear receptors for steroids, thyroid hormones and retinoids (Maioli-Di-Albedi et al., 2008). PPAR-γ plays an important role in adipogenesis, glucose metabolism, angiogenesis and inflammation (Sarafidis and Bakris, 2006). Synthetic PPAR-γ ligands, the thiazolidinediones, including pioglitazone and rosiglitazone, improve insulin resistance in diabetic patients, and have become one of the most popular anti-diabetic drugs in developed countries (Tilg and Moschen, 2008; Tontonoz and Spiegelman, 2008; Zinn et al., 2008). In addition, natural PPAR-γ ligands, such as 15-deoxy-D12,14-prostaglandin J2 (15-dPGJ2) have very potent anti-inflammatory effects that also modulate cellular defence against oxidative stress (Ershov and Bazan, 2000; Straus and Glass, 2001). Moreover, there is accumulating data to show that PPAR-γ activators exert anti-inflammatory, antioxidative and anti-proliferative effects in various cells including normal retinal cells (Yamagishi et al., 2007; Gerry and Pascual, 2008; Giaginis et al., 2008; Sulistio et al., 2008; Yanagi, 2008).

Plants remain an important source of material for maintaining human health with unparalleled diversity and have improved the quality of life for centuries (Li et al., 2005). Moreover, medicinal plants are an abundant source of biologically active molecules that play an important role in past and modern medicine and which act as a ‘stepping stone’ for the discovery of novel pharmacologically active ligands (Barnett et al., 2003). In recent years, numerous plant-derived ligands of PPAR-γ receptors have been identified (Huang et al., 2005). However, very limited research has been undertaken on medicinal plant-derived PPAR-γ ligands in the modulation of DR-related pathophysiology. Thus, the current review is a detailed discussion summarizing the current understanding of the potential of herbal and traditional natural medicines for management and potential reversal of DR-related pathogenesis.

**Diabetic retinopathy**

Diabetes damages all the major cells of the retina, vascular cells (endothelial cells and pericytes; Hammes et al., 1995; Mizutani et al., 1996) and pigment epithelial cells (Decanini et al., 2008). Basement membrane thickening, pericyte dropout and retinal capillary non-perfusion occur prior to the damage, which changes the production pattern of a number of mediators, such as growth factors, vasoactive agents, coagulation factors and adhesion molecules. These result in increased blood flow and capillary diameter, proliferation of the extracellular matrix and thickening of basal membranes, altered cell turnover (apoptosis, proliferation, hypertrophy) and procoagulant/proaggregant patterns, and finally angiogenesis with tissue remodelling. These pathological changes cause increase retinal vasopermeability and breakdown of blood retinal barrier (BRB), resulting in retinal haemorrhages, swelling, exudates and retinal detachment (Gardner et al., 2000; Ciulla et al., 2003; Yam and Kwok, 2007).

**Figure 1**

Pathophysiological cascades implicated in the development of diabetic retinopathy. Schematic representation showing the involvement of PPAR-γ and its receptor system in the progression of diabetic retinopathy (adapted from Yamagishi et al., 2007). VCAM-1, vascular cell adhesion molecule-1.

The underlying pathophysiological mechanisms associated with hyperglycaemic-induced DR are through excessive formation of advanced glycation end products (AGEs) and production of excessive oxidative stress (Ciulla et al., 2003; Abu El-Asrar et al., 2009). Moreover, these biochemical mechanisms lead to a cascade of events, such as promotion of apoptosis, inflammation and angiogenesis, which induce damage to diabetic retina, leading to DR (Ciulla et al., 2003; Abu El-Asrar et al., 2009; Figure 1).

**Advanced glycation end products (AGEs) in diabetic retinopathy**

Advanced glycation end products are associated with modification of proteins or lipids that are generated from intermediate glycation products by non-enzymatic reaction of glucose with protein side chains (Schmidt et al., 1994; Goh and Cooper, 2008). These intermediate glycation products undergo further condensation, dehydration or rearrangement, leading to eventual irreversible AGEs formation (Chu and Ali, 2008). AGEs formation occurs normally over time whereas an accelerated rate of AGE formation is accompanied by hyperglycaemia (Munch et al., 1998).

The accumulated AGEs products are detected in the retinal blood vessel wall, responsible for mediating the pathological angiogenesis and hyper-permeability in retina. Several bodies of evidence suggest that the interaction between AGEs and their receptor activates nicotinamide adenine dinucleotide phosphate oxidase and enhances the formation of oxygen radicals, with subsequent activation and translocation of nuclear factor-kappaB (NF-κB), followed by release of pro-inflammatory cytokines and growth factors (Abu El-Asrar et al., 2009). Moreover, AGEs enhance apoptosis in retinal pericytes, corneal endothelial cells, neuronal cells and renal...
mesangial cells through increased oxidative stress or via induced expression of pro-apoptotic cytokines (Kasper et al., 2000; Denis et al., 2002; Kaji et al., 2003). Indeed, AGEs induce apoptosis, angiogenesis, breakdown of BRB, and leukocyte adhesion in the retina. Thus, AGEs are detrimental to the retinal vasculature and contribute to the pathogenesis of DR (Stitt, 2001; Sato et al., 2006).

**Oxidative stress in diabetic retinopathy**

Oxidative stress appears when there is a serious imbalance between generation of reactive oxygen species (ROS) and its clearance by antioxidant defences (Ceriello et al., 2001; Brownlee, 2005). Activation of receptor for advanced glycation end products results in production of oxidative stress (conversely, glycation itself is promoted by oxidative stress), and subsequent activation of NF-κB transcription factor in microvascular endothelial cells, which is considered to be linked to endothelial dysfunction (Moore et al., 2003; Vincent et al., 2007). Retina, a tissue rich in polyunsaturated fatty acid, uses more oxygen and glucose oxidation than any other tissue in the body, and is very susceptible to damage (Schmidt et al., 2003). Diabetic-induced oxidative stress, followed by activation of NF-κB in the retina, are early events in the pathogenesis of DR (Kowluru et al., 1996; 2001; Kowluru, 2005; Obrosova and Julius, 2005). Moreover, oxidative stress has been linked to the accelerated apoptosis of retinal capillary cells and microvascular abnormalities in DR (Allen et al., 2005).

**Nuclear factor-kappaB in diabetic retinopathy**

Nuclear factor-kappaB is a multi-protein complex which can activate many kinds of genes involved in cellular functions. Pathogenic stimuli allow NF-κB to enter the nucleus, and to bind to DNA recognition sites in regulatory regions of target genes (Schreck et al., 1992; Baueerle and Henke, 1994; Boileau et al., 2003). NF-κB is required for maximal transcription of many pro-inflammatory molecules thought to be important in the generation of inflammation, including molecules (intracellular adhesion molecule 1), critical enzymes [inducible nitric oxide synthase, cyclooxygenase-2 (COX-2)] and a number of cytokines (interleukin-1), tumour necrosis factor-α, IL-6) (Siebenlist et al., 1994; Blackwell and Christman, 1997). The activation of NF-κB is considered a key signalling pathway by which high glucose induces apoptosis in endothelial cells (Du et al., 1999). In the retina, NF-κB is localized in sub-retinal membranes and in microvessels (Hammes et al., 1999) and its activation is considered responsible for the accelerated loss of pericytes observed in DR (Romeo et al., 2002).

**Inflammation in diabetic retinopathy**

In recent years inflammation has been linked to vascular leakage in DR, at least in part (Adams, 2002; Joussen et al., 2002). Hyperglycaemia is a contributing risk factor for the development of vascular dysfunction and production of inflammatory markers (Haubner et al., 2007; Tawfik et al., 2009). Indeed, pro-inflammatory cytokines, chemokines and other inflammatory mediators play an important role in the pathogenesis of DR. These lead to persistent low-grade inflammation, the adhesion of leukocytes to the retinal vasculature (leukostasis), breakdown of BRB and neovascularization with subsequent sub-retinal fibrosis or disciform scarring (Carmo et al., 1999; Malchiodi-Albedi et al., 2008). Several inflammatory molecules are involved in the pathogenesis of DR, including tumour necrotic factor (TNF-α), fibronectin, COX-2, intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 and matrix metalloproteinase-9 (MMP-9) (Miyamoto and Ogura, 1999; Yuuki et al., 2001; Joussen et al., 2004).

**Angiogenesis in diabetic retinopathy**

Angiogenesis is defined as the growth of new vessels from pre-existing capillaries which is a complex process comprising endothelial cell proliferation, migration, extracellular proteolysis, tube formation and vessel remodelling (Beck and D’Amore, 1997). In retina, vascular endothelial growth factor (VEGF) is the major angiogenic factor for neovascularization and vascular leakage via the mitogen-activated protein kinase pathway (Miller et al., 1994; Kliffen et al., 1997; De Luca et al., 2008). Additional pro-angiogenic factors, including MMP-9, are also required for the process of ocular neovascularization through either synergistic effects with angiogenic factor or as a stimulant for the secretion of angiogenic factors (Hollborn et al., 2007). In addition, MMP-9 expression acts as a factor in increasing vascular permeability in ocular neovascularization (Hertzlich et al., 2008).

**Apoptosis in diabetic retinopathy**

Apoptosis is programmed cell death and is characterized by chromatin condensation, fragmentation and formation of apoptotic bodies that can be triggered by various signals (Nagata, 1997; Li et al., 1998; Leal et al., 2009). In healthy organisms apoptosis and cell proliferation are in homeostasis to maintain a physiological balance during normal embryonic development and in the cell turnover throughout life (van den Oever et al., 2010; Zeng et al., 2010). In contrast, retinal microvascular cells are lost selectively via apoptosis before other histopathology is detectable in diabetes (Mizutani et al., 1996; Kern et al., 2000; Kowluru and Odenbach, 2004). Moreover, it has been well established that apoptosis represents a final common pathway of cell loss and hence vision loss (Doonan et al., 2009). Therefore, apoptosis plays an important role in the progression and pathogenesis of DR (Allen et al., 2005; Leal et al., 2009). As oxidative stress is closely linked to apoptosis in diabetes, oxidative stress-induced apoptotic episodes have been demonstrated by retinal abnormalities, potential visual changes and the onset of the first vascular change (Lopes de Faria et al., 2001; Kowluru, 2005). Moreover, apoptotic cell death in retinal regions is a probable stimulus for the increased expression of molecules that enhance the breakdown of the BRB and lead to vascular proliferation (Henkind, 1978; Patz, 1982). Several studies have shown that retinal pigment epithelial (RPE) cells, Glial cells (Zeng et al., 2009) and retinal pericytes (Leal et al., 2009; Zeng et al., 2010) undergo high glucose-induced apoptosis. High glucose causes activation of several proteins involved in apoptotic cell death, including members of the caspase and Bcl-2 family (Allen et al., 2005).
Pathogenesis of retinal pigment epithelium in diabetic retinopathy

The RPE cells form a monolayer between the neuroretina and the choriocapillaris which are the essential component of the outer BRB that maintain physiological and structural balance within the retina (Bok, 1993; Rizzolo, 1997). The main characteristics of RPE cells are the presence of tight junctions at the apical side of their lateral molecules, which limit access of blood components to the retina. Other important functions include regulation of transport of nutrients to the photoreceptors, phagocytosis of damaged or old rod outer segments and production of growth factors (Holtkamp et al., 2001). Moreover, RPE and photoreceptors are particularly susceptible to oxidative stress because of high oxygen consumption by photoreceptors (Beatty et al., 2000). In response to damage caused by the hyperglycaemic condition, RPE cells migrate and proliferate, leading to a breakdown in adhesion between the RPE and the chorioidal capillaries, followed by BRB breakdown compromising blood flow within the RPE layer and leading to eventual retinal oedema (Kennedy et al., 1995). These cascade episodes trigger the serum components and inflammatory cells to enter the vitreous cavity and sub-retinal space, exposing the RPE cells to a variety of cytokines, pro-inflammatory mediators, extracellular matrix proteins and growth factors, causing DR (Kimoto et al., 2004). Several studies have shown that the expression of angiogenic cytokines, growth factors (e.g. VEGF) and metalloproteinases (e.g. MMP-9) are produced by RPE (Grossniklaus et al., 2002). Moreover, the combined effects from chronic sustained inflammation and ROS generation promote the development of RPE damage (Winkler et al., 1999; SanGiovanni and Chew, 2005; de Jong, 2006). There is a strong body of evidence on the prevalence of the variety of anti-angiogenic agents, anti-inflammatory agents, antioxidants and anti-fibrogenesis present in the retinal regions for slowing down the progression of DR (Winkler et al., 1999; Zhang et al., 2006; Kern, 2007; Cheng et al., 2008). Moreover, the discovery of biochemical mechanisms that either protect cells or promote cellular recovery from damage is important (Geiger et al., 2005).

Peroxisome proliferator-activated receptor-γ and diabetic retinopathy

Peroxisome proliferator-activated receptor-γ is heterogeneously expressed in the mammalian eye, prominently present in the retina pigmented epithelium, photoreceptor outer segments, choriocapillaries and retina (Murata et al., 2000; Sarayba et al., 2005; Herzlich et al., 2008). Recent studies have shown that retinal expression of PPAR-γ was suppressed in experimental models of diabetes and in endothelial cells treated with high glucose (Tawfik et al., 2009). Moreover, PPAR-γ ligands are potent inhibitors of corneal angiogenesis and neovascularization (Xin et al., 1999; Muranaka et al., 2006). Administration of 15d-PGJ2 inhibited VEGF-stimulated angiogenesis in rat cornea (Xin et al., 1999). Similarly, choriocapillary neovascularization was markedly reduced by intravitreal injection of troglitazone. Laser photocoagulation-induced lesions in rat and monkey eyes showed significantly less leakage in troglitazone-treated animals (Murata et al., 2000).

Troglitazone demonstrated that intravitreal injections significantly inhibited the percentage of lesions as well as leakage per lesion (Murata et al., 2000). Increase in catalase mRNA has been shown when using known PPAR-γ agonists rosiglitazone and ciglitazone in rat brain microvascular endothelial cells, one of the cell types damaged during inflammatory responses induced by ROS generation (Girunn et al., 2002). In neonatal mice, intravitreous injection of rosiglitazone or troglitazone inhibited development of new retinal vessels. In the same study, thiazolidinediones have been found to inhibit retinal endothelial cell proliferation, migration and tube formation in response to VEGF treatment (Touyz and Schiffrin, 2006). In addition, rosiglitazone inhibits both the retinal leukostasis and retinal leakage observed in experimental diabetic rats which leads to the aggravation of retinal leukostasis and retinal leakage in diabetic mice (Muranaka et al., 2006). Moreover, rosiglitazone has been shown to delay the onset of DR (Shen et al., 2008). These findings suggest that PPAR-γ is involved in the pathogenesis of DR (Figure 1).

Peroxisome proliferator-activated receptor-γ and AGEs

Peroxisome proliferator-activated receptor-γ ligands provide a significant prevention of AGEs-induced microvascular complications, including DR (Dolhofer-Bliesener et al., 1996; Watson et al., 2005). Indeed, a body of evidence has shown that PPAR-γ ligands inhibit the formation of AGEs (Rahbar et al., 2000; Sobal et al., 2005). The inhibitory action of PPAR-γ ligands on AGE formation may be ascribed to their antioxidative properties (Yamagishi et al., 2007; Gery and Pascual, 2008; Giaginis et al., 2008; Sulistio et al., 2008). In addition, activation of PPAR-γ by rosiglitazone inhibits AGE-induced inductible NO synthase expression, nitrite release, fibronectin and type IV collagen production (Chang et al., 2004; Yu et al., 2007). Moreover, rosiglitazone has been shown to inhibit extracellular matrix accumulation, fibronectin and type IV collagen in AGE-injected rats, and also inhibits the AGE-induced proliferation and NO production in cardiac fibroblasts (Yu et al., 2007; Li et al., 2008).

Peroxisome proliferator-activated receptor-γ in NF-κB, inflammatory mediators and angiogenesis

Peroxisome proliferator-activated receptor-γ plays a critical role in a variety of biological processes, including inflammation and angiogenesis, mediated through the inhibition of NF-κB (Rosen and Spiegelman, 2001; Lee et al., 2006; Sung et al., 2006; Kim et al., 2007). In streptozotocin-induced DR, rosiglitazone was shown to inhibit both retinal leukostasis and retinal leakage by the inhibition of NF-kB activation, with consequent suppression of ICAM-1 expression (Muranaka et al., 2006). In addition, one recent body of evidence has shown that the suppression of PPAR-γ in diabetic retina is associated with the activation of NF-κB target gene expression (Remels et al., 2009; Tawfik et al., 2009). The activation of PPAR-γ inhibits the pro-inflammatory pathways, including cytokine secretion (Uchimura et al., 2001; Wong et al., 2001) and inducible nitric oxide synthase (iNOS) expression (Petrova et al., 1999; Reilly et al., 2001) in a variety of cell lines. Indeed, PPAR-γ ligands have also been shown to
suppress the expression of iNOS, observed at both mRNA and protein levels (Ricote et al., 1998b). The murine iNOS promoter contains 24 transcriptional factor-binding sites, including NF-xB transcription factor (Chang et al., 2004). Inhibition of ICAM-1 expression and retinal vascular leakage in experimental diabetes has been shown by rosiglitazone, and the increase in the same parameters by depletion of the gene encoding PPAR-γ (Murasaka et al., 2006). PPAR-γ ligands have also been shown to inhibit the expression of VEGF receptors and the subsequent activation of downstream signalling pathway (Xin et al., 1999; Murata et al., 2001). Moreover, rosiglitazone has been shown to inhibit retinal neovascularization in oxygen-induced retinopathy by a mechanism downstream from VEGF-induced angiogenesis (Murata et al., 2001). In addition, it has been suggested that ICAM-1 is involved in VEGF-induced leukocyte–endothelial cell interactions and subsequent BRB breakdown in the diabetic retina (Miyahara et al., 2004). COX-2 has emerged as a key regulator of inflammatory angiogenesis and its expression is induced by VEGF (Wu et al., 2003; Gately and Li, 2004). Moreover, PPAR-γ activation inhibits VEGF-mediated angiogenesis through the modulation of the stimulated COX-2 expression and activity (Scoditti et al., 2009).

**Peroxisome proliferator-activated receptor-γ and apoptosis**

Apoptosis is a complex process, involving a multitude of signalling pathways that regulate the activities of pro- and anti-apoptotic members of Bcl-2 family of proteins which play an important role in various cell types (Fehlberg et al., 2003; Bonne, 2005; Fuenzalida et al., 2007). Moreover, oxidative stress can induce mitochondria dysfunction, followed by cytochrome c release and subsequent activation of caspases, a group of enzymes that execute apoptosis (Danial and Korsmeyer, 2004; Lin and Beal, 2006). A recent study has shown that rosiglitazone protects against oxidative stress-induced apoptosis through up-regulation of anti-apoptotic Bcl-2 family proteins (Ren et al., 2009). Moreover, rosiglitazone and PPAR-γ over-expression protect against apoptosis induced by oxygen and glucose deprivation followed by re-oxygenation and up-regulation of Bcl-2 (Wu et al., 2009). In contrast, down-regulation of NF-xB activation by PPAR-γ ligands protects the cells from destruction via the apoptotic pathways (Grey et al., 1999; Baker et al., 2001). A screen of FDA-approved compounds identified rosiglitazone as a novel anti-apoptotic agent in retinal cells in both in vivo and in vitro (Doonan et al., 2009). In addition, troglitazone has shown cytoprotective activity in apoptotic-induced ARPE-19 cells (Rodrigues et al., 2010). One further study has indicated that 15d-PGJ2 helps RPE cells to maintain mitochondrial integrity by prevention of cytochrome c release and subsequent activation of the apoptosis pathway (Garg and Chang, 2004; Chang and So, 2008).

**Peroxisome proliferator-activated receptor-γ and RPE cells**

A number of studies have shown that RPE might be the prime target for oxidative stress and PPAR-γ ligands modulate cellular defence against the oxidative stress (Chang et al., 2008). 15d-PGJ2 protects RPE cells from oxidative stress by elevating GSH and enhancing mitogen-activated protein kinase activation through the PPAR-γ independent pathway (Qin et al., 2006). In addition, 15d-PGJ2, independent of its PPARγ activity, protects RPE cells from oxidative injury by raising intracellular GSH levels and extending hydrogen peroxide-induced activation of JNK and p38, suggesting the possible application of the agents in preventing ocular diseases from oxidative stress (Garg and Chang, 2003; Qin et al., 2006). It has also been shown that PPAR-γ ligands inhibit VEGF-induced choroidal angiogenesis, without apparent toxicity to the adjacent retina, in a laser-induced model of choroidal neovascularization (Murata et al., 2000). A recent study has shown that PPAR-γ inhibits the expression of MMP-9 in response to 15d-PGJ2, and synthetic PPAR-γ ligands by antagonizing the activities of NF-xB to decrease vascular permeability or neovascularization (Ricote et al., 1998a,b; Herzlich et al., 2008). Moreover, rosiglitazone inhibits the endothelial effects of VEGF (Murata et al., 2000). Interestingly, synthetic PPARγ agonists, AG195037 and rosiglitazone, have been shown to be less effective in the protection of RPE cells against hydrogen peroxide-induced injury. Hence, the cytoprotective effect of this agent has not been conclusively demonstrated (Qin et al., 2006; Chang et al., 2008). There is growing interest in natural products with combined anti-glycation and antioxidant properties as they may have reduced toxicity. Indeed, a number of plant-derived flavonoids with antioxidant activity (quercetin, rutin and kaempferol) have been reported to inhibit glycation (Ahmed, 2005).

**Herbal and traditional medicines and diabetic retinopathy**

In recent years, there has been growing attention to alternative therapies and the therapeutic use of plant-origin natural products (Li et al., 2005). More than 400 traditional medicinal plants and the effective constituents derived from over 800 herbal compounds are reported to possess therapeutic potential or efficacy for the treatment of diabetes (Bailey and Day, 1989; Al-Rowais, 2002; Dey et al., 2002). Herbal and traditional medicines have been globally used for diabetic complications for centuries. Moreover, it may provide an alternative to prevent or delay the progression of DR (Head, 1999). Despite modern pharmacotherapeutics and advancement in an ever changing world of biotechnology, a lack of understanding still exists on the bioactivity of these herbal medicines and related active ligands towards the modulation of DR pathogenesis (Bailey and Day, 1989). This has prompted research to understand the mechanism of action of these compounds and seek new products for better management of diabetes and its complications (Ceylan-Isik et al., 2005). This section summarizes the current research on various active components of herbal and traditional medicines capable of modulating DR pathogenesis (Table 1).

**Traditional Chinese medicines in diabetic retinopathy**

Traditional Chinese medicines (TCMs) have been used for more than 2000 years in China and other Asian countries with the fundamental aim of targeting not only the treatment of diabetes but also the prevention of its complications (Covington, 2001; Li et al., 2004). The main concept on
<table>
<thead>
<tr>
<th>Herbal medicines</th>
<th>Active component</th>
<th>Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gogi berry (Lycium barbarum L.)</td>
<td>Lycium barbarum polysaccharides (LBP)</td>
<td>Beneficial effect for maintaining macular pigment density in age-related macular degeneration by increase fasting plasma zeaxanthin levels in human clinical trial. Effective in glaucoma and modulating immune system in retinal ganglion cells in a rat hypertension model. LBP antagonizes glutamate excitotoxicity in rat cortical neurons and increase Bcl-2 level in lens epithelial cells of the whole lens exposed to hydrogen peroxide.</td>
<td>(Cheng et al., 2005) (Chiu et al., 2009) (Ho et al., 2009) (Wang et al., 2002)</td>
</tr>
<tr>
<td>Danshen dripping pills</td>
<td>Salviae miltiorrhiae</td>
<td>Improve visual acuity, control micro-haemorrhage. Improve ischaemia of the retina and the visual field in early diabetic retinopathy; reduce the number of microaneurysms.</td>
<td>(Qi et al., 2007) (Deng et al., 2005)</td>
</tr>
<tr>
<td>Ansidus tanguticus</td>
<td>Anisodamine</td>
<td>Increase neural tissue oxygen tension in retinal hypoxia. Increase blood flow and oxygen delivery to the retina-choroid and iris-ciliary body of the eye, and lipid peroxidation in the retinal cells.</td>
<td>(Linsenmeier et al., 1989; Zhang et al., 1990)</td>
</tr>
<tr>
<td>Stephania tetrandra S. Moore (STMS) (Han Fang Ji)</td>
<td>Tetrandrine</td>
<td>Direct effect on retinal capillary of posterior ocular region and suppression of neovascularization of the retinal capillary in diabetic rats.</td>
<td>(Liang et al., 2002)</td>
</tr>
<tr>
<td>Radix Purerariae</td>
<td>Not known</td>
<td>The ethanol extract of Radix Purerariae inhibits glycosylation of rat lens protein. Discovery of a new key chemical constituent proven to be useful in DR by inhibiting aldose reductase.</td>
<td>(Hirakura et al., 1989) (Duan et al., 2000)</td>
</tr>
<tr>
<td>Gymnema sylvestre</td>
<td>A polyl, conduritol A</td>
<td>Beneficial effect on cataract by inhibiting lens aldose reductase.</td>
<td>(Miyatake et al., 1994)</td>
</tr>
<tr>
<td>Astragalus radix</td>
<td>Astragalosides</td>
<td>Potentially useful for the prevention of clinical diabetic complications such as diabetic retinopathy by inhibiting AGE product.</td>
<td>(Motomura et al., 2009)</td>
</tr>
<tr>
<td>Hachimi-Jio-gan (Trhmannia Eight Formula)</td>
<td>Not known</td>
<td>Controls the balance of sodium, potassium and calcium ions for maintenance of lens transparency. This finding may lead the possibility of prophylactic use in eye diseases such as diabetic cataract.</td>
<td>(Kamei et al., 1987)</td>
</tr>
<tr>
<td>Trigonella foenum-graecum (Fenugreek)</td>
<td>Saponins, Flavanoids, 4-hydroxyisoleucine</td>
<td>Beneficial effect for abnormal accumulation of sorbitol and fructose in the diabetic lens. Restore disintegration of the inner nuclear layer cells with reduction in rough endoplasmic reticulum and swelling of mitochondria in the diabetic retina. Cataract-suppressing effect by inhibiting lens aldose reductase.</td>
<td>(Miyatake et al., 1994) (Preet et al., 2006)</td>
</tr>
<tr>
<td>Vaccinium myrtillus (billberry)</td>
<td>Anthocyanidins</td>
<td>In a clinical study, 12 adult diabetic patients were treated with 600 mg of anthocyanosides daily for 2 months, followed by significant decrease in the biosynthesis of connective tissue to protect against diabetic retinopathy. In 31 patients suffering from various types of retinopathy, treatment with V. myrtillus extract was associated with a reduced tendency towards retinal haemorrhage.</td>
<td>(Boniface and Robert, 1996) (Perossini et al., 1988)</td>
</tr>
<tr>
<td>Resveratrol (trans-3, 5, 4-trihydroxystilbene)</td>
<td>Not known</td>
<td>Protects high glucose-induced ARPE-19 cells by reducing low-grade inflammation mediators, interleukin-6 and interleukin-8, COX-2, VEGF and connexion 43 down-regulations.</td>
<td>(Losso et al., 2010) (Srivastava et al., 2009)</td>
</tr>
<tr>
<td>Saptamrita lauha</td>
<td>Flavanoid-like compounds but not specified</td>
<td>Control retinal haemorrhage absorption and its recurrence in retinopathic eyes.</td>
<td>(Sharma et al., 1992)</td>
</tr>
</tbody>
</table>
mechanisms of DR in TCMs are *yin* vacuity with dryness and heat leading to accumulation of phlegm and blood stasis, and causing not nourishing the eyes and static blood obstructing the network vessels of the eyes (Flaws et al., 2002). Many clinical studies have shown the benefit of TCMs in reducing symptoms, improving visual acuity and visual field and improving the outcome under ophthalmoscopy (Ma et al., 2004; Song et al., 2006). Moreover, a growing body of evidence has shown the benefit of combining TCMs with Western treatment strategies (Donnelly et al., 2006). Indeed, a combination of laser therapy with TCMs has been explored for DR in China (Gong, 2007). Traditionally, ‘blood-vitalizing herbs’ are used to treat microvascular-related DR (Han and Xu, 1988).

Extracts used in TCMs provide therapeutic effects that are dependent on its related chemical compounds (Shi et al., 2008). Despite the growing expenditure of TCMs for DR management, there is still no conclusive evidence to support its efficacy and issues of safety (Yeh et al., 2003; Liu et al., 2004; West et al., 2006). Thus, the study of active compounds from the extract and its possible applicable mechanisms are required for better understanding the effect of TCMs in DR (Su et al., 2007).

One human supplementation trial showed that the *Lycium (L.) barbarum* (Goji berry) intake increases the fasting plasma zeaxathin levels, beneficial for maintaining macular pigment density in age-related macular degeneration (Cheng et al., 2005). *L. barbarum* polysaccharides, most abundantly present in Goji berry, induce an activation of microglia (brain macrophages) in retina (Chiu et al., 2009). Indeed, Goji berry has been shown to be effective in glaucoma and in modulating immune response in retinal ganglion cells in a rat hypertension model (Chan et al., 2007; Chiu et al., 2009). Moreover, LPB has been shown to antagonize glutamate excitotoxicity in rat cortical neurons (Ho et al., 2009). In addition, *L. barbarum* polysaccharides has been shown to increase anti-apoptotic protein Bcl-2 level in lens epithelial cells of the whole lens incubated in culture medium and exposed to hydrogen peroxide (Wang et al., 2002).

Clinical study of DR treated with compound Danshen dripping pills (*Salviae miltiorrhiae*, Radix notoginseng and borneol) showed improvement in visual acuity, and control of micro- and macrohemorrhage and microaneurysm of fundus. Moreover, the pills were effective in visual function recovery of DR (Qi et al., 2007). Another study on the compound Danshen dripping pill was shown to improve ischaemia of the retina and the visual field in early DR, and to reduce the number of microaneurysms (Deng et al., 2005).

Anisosadamine is an alkaloid, originally isolated from *Anisodus tanguticus* which was found to increase neural tissue oxygen tension of the retina (Linsenmeier et al., 1989). Moreover, anisosadamine has been shown to increase blood flow and oxygen delivery to the retina-choroid and iris-ciliary body of the eye, and to decrease lipid peroxidation in the retinal cells (Zhang et al., 1990).

The ethanol extract of *Radix (R.) Pureratae* has shown to inhibit glycosylation of rat lens protein (Duan et al., 2000). Moreover, a new key chemical constituent 7-(6-O-malonyl-β-D-glucopyranosyl)-3-(4-hydroxyphenyl)-4H-1benzopyran-4-one was isolated from *R. Pureratae*. This new aldose reductase-inhibitory compound proved to be useful in DR (Hirakura et al., 1989). The active components astragalo-sides from crude extract of *Astragalis (A.) Radix* has also been shown to be potentially useful for the prevention of clinical diabetic complications such as DR by inhibiting AGEs production (Motomura et al., 2009).

A study of therapeutic efficacy of the root extracts of *Stephania tetrandra* S. Moore for delaying the progression of retinopathy was carried out in the streptozotocin-induced diabetic rat model. It was considered to work through the activation by its active component, tetrandrine, of suppression of neovascularization of the retinal capillary (Liang et al., 2002).

Traditional Chinese formula Hachimi-Jio-gan (Trhmannia Eight Formula) controls the balance of sodium, potassium and calcium ions for maintenance of lens transparency. This finding leads to the possibility of prophylactic use of Hachimi-Jio-gan in eye diseases such as diabetic cataract (Kamei et al., 1987). However, further study is warranted for human application.

**Ayurvedic herbal medicines and diabetic retinopathy**

Ayurveda has been widely used in India and some European countries, and recognition of its modality is increasing worldwide (Saxena and Vikram, 2004). More than 800 plants are used as traditional remedies for diabetes and its complications in the ancient Indian Ayurvedic and Unani system of medicine (Alarcon-Aguilara et al., 1998; Saxena and Vikram, 2004). However, only a few medicinal herbs have been evaluated by scientific investigations (Saxena and Vikram, 2004).

A polyl, conduritol A from *Gymnema sylvestre* may be responsible for the cataract-suppressing effect by inhibiting lens aldose reductase (Miyatake et al., 1994).

The seeds of *Trigonella foenum graecum*, commonly known as fenugreek, have been known for hypoglycaemic, hypoholesterolemic and hyperinsulinemic effects in diabetic patients and experimental animals (Khosla et al., 1995; Puri et al., 2002). It has been shown that fenugreek has a beneficial effect on abnormal accumulation of sorbitol and fructose in the diabetic lens. Moreover, disintegration of the inner nuclear layer cells with reduction in rough endoplasmic reticulum and swelling of mitochondria in the diabetic retina have been restored by fenugreek. Saponins, flavonoids and 4-hydroxyisoleucine are potential plausible effectors in reversing the histopathological and biochemical abnormalities observed in DR and warrants further investigation (Preet et al., 2006). Clinical study has shown that an indigenous drug, *Saptamrita lauha* may have a role in control of retinal haemorrhage absorption and its recurrence in retinopathic eyes (Sharma et al., 1992).

**Western herbal medicines and diabetic retinopathy**

Western herbal medicine is the most widely used form of herbal medicine although Ayurvedic and Chinese herbal medicines are becoming better known (Wohlmuth et al., 2002).

The fruits of *Vaccinium (V.) myrtillus* (bilberry), containing 25% anthocyanidins, have been widely used in Europe to prevent and treat DR (Murray, 1997; Camire, 2000). In a clinical study, 12 adult diabetic patients were treated with 600 mg of anthocyanosides daily for 2 months. Following
Diabetic retinopathy and natural medicines

Table 2
Modern research on herbal and traditional medicines affecting PPAR-γ activation and its possible mechanisms in diabetic retinopathy

<table>
<thead>
<tr>
<th>PPAR-γ activity in herbal medicine</th>
<th>Active component</th>
<th>Functions in diabetic retinopathy</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Astragalus membranaceus</em> and <em>Pueraria thomsonii</em></td>
<td>Biochanin A</td>
<td>Inhibitory effect on protein glycosylation in diabetic retinopathy</td>
<td>(Asgary <em>et al.</em>, 2002)</td>
</tr>
<tr>
<td><em>Swietenia mahagony</em></td>
<td>Not known</td>
<td>Antioxidant activity of <em>Swietenia mahagony</em> seed may provide a new avenue against diabetic retinopathy</td>
<td>(Rodrigues <em>et al.</em>, 2010)</td>
</tr>
<tr>
<td><em>Korean red ginseng</em></td>
<td>Ginsenoside 20(S)</td>
<td>Prevention of high glucose-induced apoptosis through decreased in Bcl-2 expression and increased Bax expression</td>
<td>(Park, 2008)</td>
</tr>
<tr>
<td></td>
<td>Ginsenoside Rb2</td>
<td>Protective effect against oxidative stress in the eye of diabetic rats which can be effective in the prevention of diabetic microvasculopathy</td>
<td>(Cho <em>et al.</em>, 2006)</td>
</tr>
<tr>
<td></td>
<td>Ginsenoside Re</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tetrandrine</td>
<td></td>
<td>(Motomura <em>et al.</em>, 2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Liang <em>et al.</em>, 2002)</td>
</tr>
<tr>
<td><em>Turmeric</em></td>
<td>Curcumin</td>
<td>Inhibition of VEGF level in diabetes-induced retina</td>
<td>(Zhou <em>et al.</em>, 2007)</td>
</tr>
<tr>
<td></td>
<td>Curcuminoids</td>
<td>Antioxidation property by inhibition of Ca2+ entry and protein kinase activity</td>
<td>(Kowluu and Kanwar, 2007; Mrudula <em>et al.</em>, 2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(Balasubramanyam <em>et al.</em>, 2003)</td>
</tr>
</tbody>
</table>

One study has shown the beneficial outcome of the treatment of DR with laser photocoagulation therapy when combined with *Ginkgo (G.) dipyridolum* injection than the laser therapy alone in human clinical trials (Wei *et al.*, 2005). In addition, the therapeutic efficiency of the *G. biloba* extract was estimated for early DR, associated with diabetic dyschromatopsia in a double-blind trial. An improvement tendency was evidenced by *G. biloba* treatment without retinal ischemia (Lanthony and Cosson, 1988).

**Herbal and traditional medicines that modulate diabetic retinopathy through PPAR-γ activation**

Over the last two decades, herbal plants or their active components have been major sources of bioactive agents and continue to represent a potential ‘goldmine’ for novel ligands to be discovered (Egan, 2002; Shen *et al.*, 2003). However, the mechanisms of action for most of the herbal medicines associated with modulating DR have not been fully elucidated. Nevertheless, the experience obtained from their traditional use over many years should not be ignored (Elvin-Lewis, 2001). Despite numerous plant-derived PPAR-γ activators having been identified from plants in recent years (Huang *et al.*, 2009), there are no conclusive studies on DR and its mechanism of modulation through the PPAR-γ activation. The next section of this review summarizes the current studies on herbal or traditional medicine associated with PPAR-γ activation and the possible mechanisms relevant to the management of DR (Table 2).

Biochanin A is a common isoflavone from *Astragalus membranaceus* and *Pueraria thomsonii* which has been shown to induce adipocyte differentiation activation *in vitro* through or at least in part, PPAR-γ as measured by reporter-gene activity. This finding has suggested the potential value of Biochanin A as an anti-diabetic agent (Shen *et al.*, 2006). Moreover, biochanin A has shown beneficial effect in DR by inhibition of protein glycosylation (Asgary *et al.*, 2002). *Swietenia mahagony* is native to the West Indies, and is traditionally used for the treatment of hypertension and malaria as a folk medicine in Indonesia (Nagalakshmi *et al.*, 2001). This medicinal herb has shown anti-diabetic activity with PPAR-γ transcriptional regulatory function as one of its *in vivo* mechanisms (Li *et al.*, 2005). Moreover, antioxidant activity of *Swietenia mahagony* seed may provide a new approach to diabetic-related complications (Rodrigues *et al.*, 2010).

One study has evaluated the effect of three traditional anti-diabetic herbs on the progression of diabetes. The results showed that mulberry leaf, Korean red ginseng and banana leaf extracts significantly increased insulin sensitivity and...
improved hyperglycaemia through the PPAR-γ pathway (Park et al., 2005). In particular, ginsenoside 20(S), one of the ginsenoside metabolites, was found to increase PPAR-γ transactivation activity and expression of glucose transporter 4 (GLUT 4) in 3T3-L1 adipocytes. Moreover, ginsenoside Rb2, an active component from Korean red ginseng, prevents high glucose-induced apoptosis through an increase in Bcl-2 expression and decrease in Bax expression in ARPE-19 cells (Park, 2008). Another active component, ginsenoside Re also shows a protective effect against oxidative stress in the eye of diabetic rats, which is effective in the prevention of diabetic microvasculopathy (Cho et al., 2006). Dan-shao-hua-xian, a TCM preparation, has been shown in a recent article to play a role in treating hepatic fibrosis by up-regulation of the gene expression and generation of PPAR-γ, and also by suppressing TNF-α production through down-regulation of the NF-kB signalling pathway (Wang and Cheng, 2008). Moreover, Stephania tetrandra S. Moore, one of the ingredients in Dan-shao-hua-xian, has been shown to suppress neovascularization of retinopathy in diabetes through the activation of a major chemical component, tetrandrine (Li et al, 2002). In addition, one of the other ingredient of Dan-shao-hua-xian, A. Radix, and its active components astragalosides, have been shown to be beneficial for the prevention of DR by inhibiting AGEs formation (Motomura et al., 2009). Curcumin, the phytochemical component in turmeric has been shown to interrupt the platelet-derived growth factor and epidermal growth factor signalling pathway by stimulation of PPAR-γ gene expression in rat activated hepatic stellate cell (Zhou et al., 2007). Moreover, a recent review has highlighted the importance of the anti-inflammatory property of curcumin component from turmeric by suppression of TNF-α expression and release, which is potentially mediated by the up-regulation of PPAR-γ (Jacob et al., 2007). The anti-inflammatory property of curcumin has also been shown to inhibit VEGF level in streptozotocin-induced diabetic rat retina which gives a potential role in DR (Kowluru and Kanwar, 2007; Mrudula et al., 2007). Curcuminoids in turmeric have shown antioxidation properties by inhibition of calcium entry and protein kinase activity, which may have therapeutic implications in DR (Balasubramanyam et al., 2003).

Terpenoids are one of many bioactive phytochemicals in medicinal plants, which include carotenoids, isoprenoids and isoprenols (He et al., 1997). Studies have shown that terpenoids are significant in cancer and cardiovascular diseases (Takahashi et al., 2002). On the other hand, typical isoprenols from herbs and fruits, including farnesol and geranylgeraniol, activate PPAR-γ in 3T3-L1 adipocytes and HepG2 hepatocytes. In addition, these herbal derivatives up-regulate the expression of some lipid metabolic target genes of PPAR-γ, including an adipocyte-specific gene, aP2 (He et al., 1997; Takahashi et al., 2002). Moreover, terpenoids such as ginkgolides from G. biloba improve blood flow by dilating blood vessels and reducing the stickiness of platelets for eye disorders, including DR (Braquet et al., 1985; Baudouin et al., 1999).

**Conclusion**

Diabetic retinopathy remains one of the major risk factors and a leading cause of preventable blindness worldwide. The increasing impotence of understanding the specific molecular and biochemical changes in DR leads to the requirement for development of novel therapeutic interventions. Although it is an important cause of blindness, initially DR presents few visual or ophthalmic symptoms until complete visual loss occurs (Fong et al., 2004a,b). Current treatments of DR rarely improve visual function and are limited to surgical options in an advanced stage, with excessive side effects and significant financial burden. Hence, emerging treatments, possibly in combination with standard therapy, may provide superior efficacy and safety profile for the treatment or prevention of DR. Moreover, the new strategies move to a paradigm in treating the early stages of DR. The recent advancements in the knowledge of the pathogenic alterations driving ocular damage and vision loss in DR strongly focus on PPAR-γ as a valuable target to control high glucose-induced inflammation and apoptosis. PPAR-γ functions as a transcription factor and thereby controls cellular processes at the level of gene expression, through modulation by its nuclear receptor activity of selective downstream gene expression (Huang et al., 2005). The complexity of PPAR-γ activation not only provides beneficial effects but also introduces risks from undesirable side effects, such as cardiovascular complications with long-term application (Roehr, 2010). PPAR-γ is an attractive and relatively unexploited target for herbal-derived medicines in DR. This review confirms that natural and traditional herbal medicines have potential as alternative or combination therapy for DR, and emphasizes their possible mechanisms through PPAR-γ activity. Despite the long history of herbal and natural traditional medicines for the management of DR, there is still no conclusive evidence on their effectiveness and safety profiles. Therefore, future studies are warranted for extensive investigation to gather proof of efficacy in various preclinical and clinical settings.

**Acknowledgements**

This work was supported by a grant from the National Institute of Complementary Medicine (NICM) to B. D. R.

**Conflict of interest**

The authors declare no conflict of interest.

**References**


Diabetic retinopathy and natural medicines


Li DD, Chen JH, Chen Q, Li GW, Chen J, Yue JM et al. (2005). Swietenia mahagoni extract shows agonistic activity to PPAR(gamma) and gives ameliorative effects on diabetic db/db mice. Acta Pharmacol Sin 26: 220–222.


Diabetic retinopathy and natural medicines


Diabetic retinopathy and natural medicines

British Journal of Pharmacology (2012) 165 4–19