Minireview

Quercetin and the ocular surface: What we know and where we are going

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Impact statement

The eye represents a small portion of the human body, accounting for one decimal fraction of the anterior body surface. The cornea is an avascular, transparent tissue that acts as a primary barrier against mechanical and infectious damaging agents, protecting the internal structures of the eye. Corneal survival and function are affected by a number of factors including but not limited to injury, trauma, infection, genetics, and environment. Corneal injury, or trauma, often leads to loss of corneal transparency and even blindness. The concept of ''curing'' corneal opacity has been discussed in published form for over 200 years. Currently, full corneal transplant is the only treatment option. There is a strong interest in developing natural therapeutic products that come with minimum side effects. A novel antioxidant flavonoid, quercetin, has been gaining traction as a potential therapeutic to prevent the injured cornea. This review discusses the potential of this antioxidant.

Abstract

Flavonoids are a class of plant and fungus secondary metabolites that serve functional roles in protecting against UV-induced oxidative stress, mediating auxin signaling, and promoting microbial defense. Flavonoids are extremely abundant in nature where their potent antioxidant capacity and very low toxicity makes them highly attractive as potential therapeutic agents. In terms of clinical applications, neither the Food and Drug Administration (FDA) nor the European Food Safety Authority (EFSA) has approved any health claims or drugs related to the use of flavonoids for therapeutic purposes. Quercetin is a common flavonol that has been shown to have potent antioxidant, anti-inflammatory, and anti-fibrotic activities both *in vitro* and *in vivo* in various tissues. Recently, the application of quercetin as a therapeutic has been gaining attention in the ocular surface scientific community in the study of dry eye, keratoconus, inflammation, and neovascularization of the cornea. This review will discuss the latest findings and the use of quercetin for the treatment of dystrophies of the ocular surface.

Keywords: Quercetin, flavonoids, cornea, ocular surface, wound healing, anti-inflammatory, anti-fibrotic

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Introduction

Flavonoids are abundant antioxidants found in nature in a variety of foods, such as green leafy vegetables, tea, berries, apples, and onions.[1]The main structures of flavonoids are defined by a backbone of two fused phenyl rings and an oxygen-containing heterocyclic ring.[2] Variations in the substituents of the rings give rise to the differences in flavonoid classes, as well as chemical reactivity and functionality (Figure 1(a)). Over six major classes of flavonoids have been defined based on substitution patterns of the aromatic rings with over 4000 individual flavonoid compounds identified.[2,5]

Numerous studies have shown protective effects of increased flavonoid uptake in animal models of disease[6,7] and within the human population[8–10] particularly in regards to cardiovascular and Alzheimer's diseases. Despite studies suggesting positive effects of increased dietary intake of flavonoids, the potential health benefits of supplements are questioned by both the Food and Drug Administration (FDA) in the US, as well as the European Food Safety Authority (EFSA) in Europe due to the lack of a solid clinical trial demonstrating therapeutic benefits. Neither one of these organizations have approved any flavonoids as pharmaceutical drugs.

Quercetin is a member of the flavonol subclass that has received considerable attention by the scientific community in recent years (Figure 1(b)). Quercetin, like most flavonoids, is abundant in the human diet. Studies have measured an average daily consumption of quercetin of roughly 16–23 mg/day within human populations.[11,12] Despite the

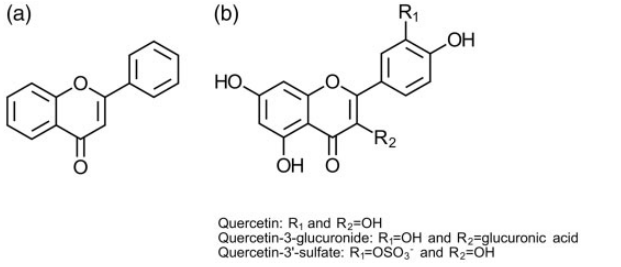

Figure 1 (a) Backbone structure of commonly found flavonoids consisting of fused carbon rings and varying oxygen substituents. (b) Structure of quercetin and common metabolites found in circulation. Addition of a glucose-derived moiety at C-3 position gives rise to the quercetin-3-glucuronide form with a single hydroxyl group making up the aglycone form. Sulfation at the 3′- position represents the quercetin-3′-sulfate metabolite commonly found in plasma. Adapted from Harborne,[2] Rossi *et al.*,[3] and Cao *et al.*[4]

broad marketing of quercetin as a beneficial supplement, the FDA and EFSA do not approve any clinical benefits to its consumption. However, quercetin is currently sold as a food supplement by all major pharmacy stores. Studies suggest that quercetin is rapidly cleared following oral consumption, undergoing extensive metabolism, and limiting systemic effects.[13] Scientific studies, however, have been testing the benefits of quercetin with delivery to the target tissue using methods other than oral intake.[14,15] In the treatment of pathologies affecting the ocular surface, the overwhelming delivery method is topical application via eye drops making it easily accessible and non-invasive for human use. This review will discuss the past and present studies that have investigated the bioactivity of quercetin in various ocular surface diseases and dystrophies.

Properties and bioavailability

Quercetin is biosynthesized in plants via the phenylpropanoid pathway. The complete pathway and biosynthesis details were summarized by Winkel-Shirley *et al.*[16,17] in review articles. Quercetin, along with other flavonoids, function as potent antioxidants and serve to scavenge free radicals, bind transition metal ions, and inhibit lipid peroxidation.[18,19] If not controlled, excessive reactive species can lead to protein oxidation and cell damage within the tissue or organ.

Quercetin's oral bioavailability is still debatable with a number of contradictory studies as reviewed by Manach *et al*.[20] The main debate is whether the quercetin aglycone or quercetin glucoside is driving the antioxidant characteristics of quercetin. Hollman *et al*.[21] suggested that the absorption of the glucoside is better that the aglycone, due to the actions of the glucose transporter (SGLT-1). Quercetin glucosides are able to pass through the epithelial cell layer, but they have lower efficiency than the quercetin aglycone.[22] In the same study, Murota and Terao[22] suggested that quercetin absorption depends on the distribution of the sugar groups attached. Interestingly, studies suggest that quercetin aglycone or glucoside is not found in human plasma.[23,24] On a study reported by Day *et al.*,[23] the glucoside and aglycone form of quercetin were not present in human plasma following consumption of onions.[23] However, two metabolites were found: quercetin 3′-sulfate and quercetin-3-glucuronide.[23] Wittig *et al*.[25], in a similar study only with fried onions tested, did not find quercetin aglycone or glucoside in the human plasma. Instead five different quercetin glucuronides were found.[25] It has now been proposed that quercetin, as well as other flavonoids, do not need to be absorbed in order to have an effect. However, the degree to which quercetin can be absorbed following oral intake is still debatable. Overall, the effect of quercetin *in vivo* is still heavily questioned and more studies are necessary in order to identify and fully understand any potential benefits that quercetin might have to offer.

The ocular surface

As the external portion of the eye, the ocular surface serves as a protective and functional barrier for the rest of the eye (Figure 2). Importantly, the cornea also plays a functional role in providing two-thirds of the refractive power of the eye, with the lens and retina providing the remaining one-third of the refractive power within the human eye. Furthermore, the conjunctiva is composed of goblet cells which function to secrete mucins providing lubrication for the anterior segment to reduce infection and barrier breakdown.[28] The cornea is an avascular tissue with immune privilege which must be maintained in order for proper visual function. Ocular surface diseases that affect the structure or function of the cornea or conjunctiva can lead to corneal thinning, inflammation, neovascularization, and scarring. Excess reactive oxygen species (ROS) production due to mitochondrial dysfunction, UV-light, or hypoxia can promote neovascularization and inflammation leading to scarring and visual deficits (Figure 3). A number of pathologies affect the corneal surface and conjunctiva, including dry eye, allergies, microbial infection, keratoconus, chemical burns, Sjögren's syndrome, and many more. Ocular surface diseases of the cornea or conjunctiva may result from prolonged inflammation or corneal defects leading to ocular irritation and reduced visual acuity. The common denominator of these diseases is that they can lead to partial or even complete vision loss, directly affecting quality of life. Unfortunately, to-date, we do not have a non-invasive treatment that can preserve corneal function and transparency. For cases that require surgical intervention, corneal transplantation remains the only solution.

Given the problems and side effects a corneal transplant may have, scientists have been looking for an alternative for decades.[29,30] Effective ocular drug delivery is dependent on many factors, including drug absorption, bioavailability, and retention on the anterior surface.[31] Lipophilic drugs, in general, are associated with higher corneal epithelial permeability.[32] Solubilization of these compounds into aqueous eye drops has been achieved with complexation to cyclodextrins, which contains a hydrophilic exterior that can interact with the aqueous milieu and internal hydrophobic moieties that stabilize the organic drug.[33] Given the difficulties with bioavailability and degradation associated with oral consumption, topical application of quercetin or other flavonoids may prove more successful in the

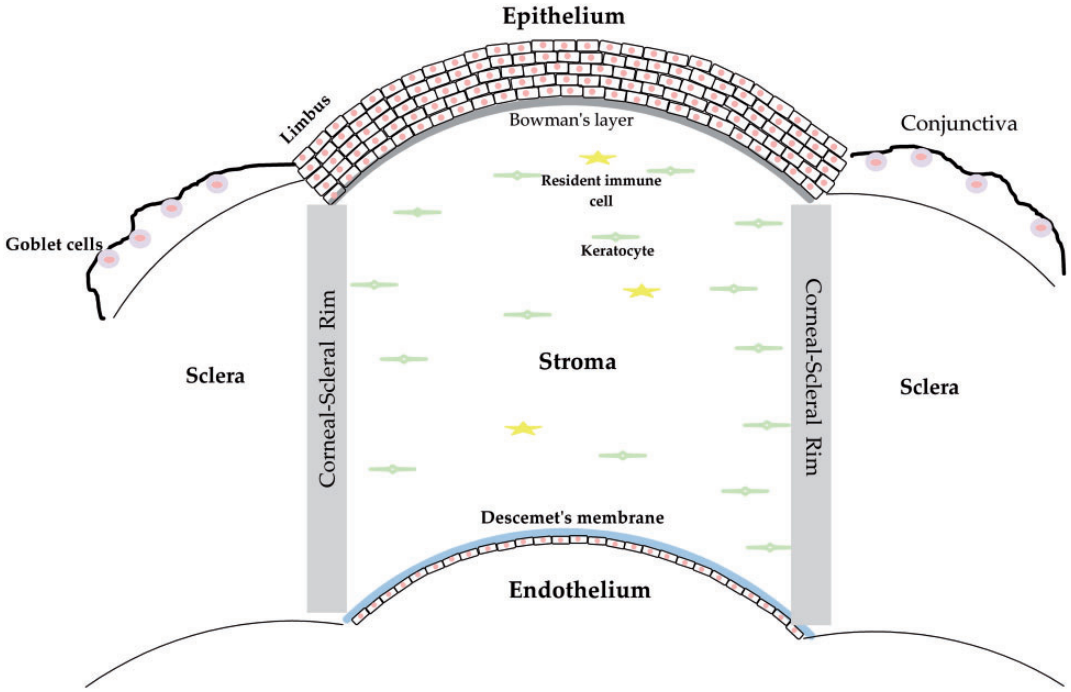

Figure 2 Cartoon representation of the anterior segment of the human eye highlighting the cornea, which is composed of five layers from anterior to posterior: (1) the stratified epithelium that secretes mucin and is continuously regenerated from stem cells found in the limbus, (2) the cell-free collagen matrix, Bowman's layer, (3) the stroma, which makes up 90% of total corneal structure, and is composed of the collagen-secreting keratocyte and resident immune cells, (4) Descemet's membrane, and (5) the single-layer endothelium which regulates fluid flux from the aqueous humor into the cornea. Based on ultrastructure of the cornea in earlier works.[26,27]

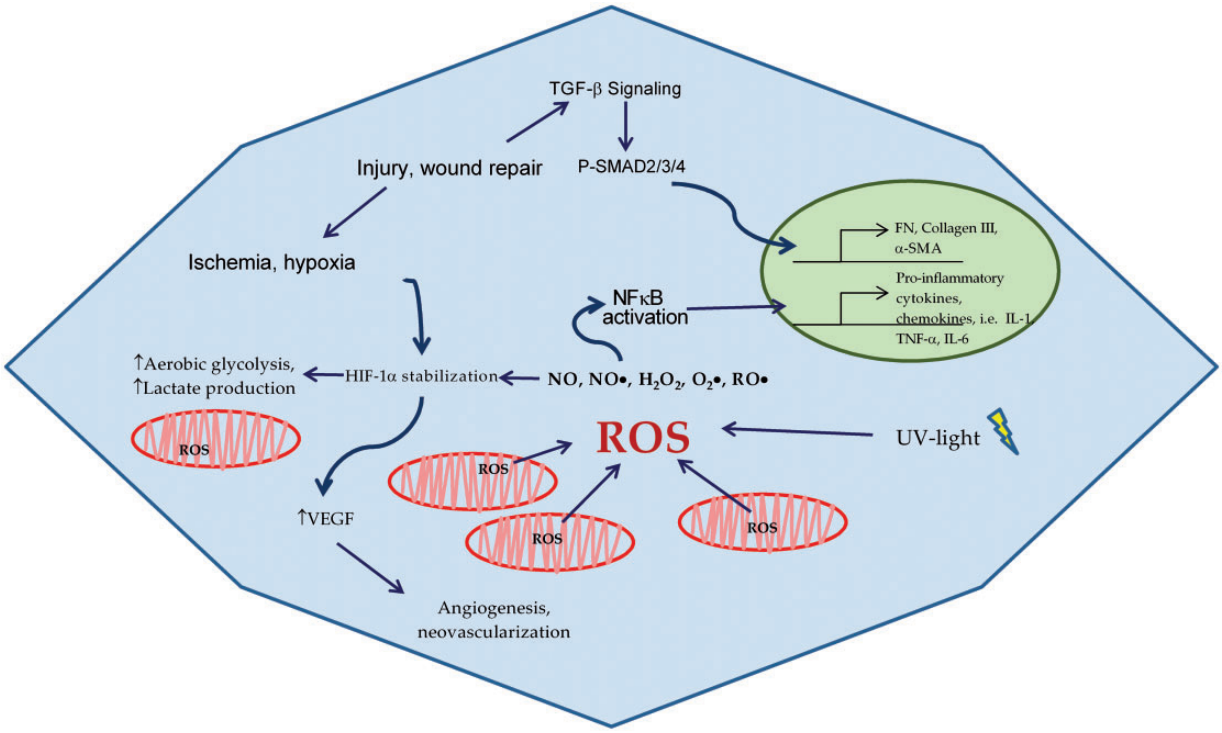

Figure 3 Schematic of reactive-oxygen species (ROS)-mediated effects on cell function and signaling. UV-light, ischemia, hypoxia, and injury can promote ROS production leading to activation of VEGF-induced neovascularization, alterations in cellular metabolism favoring aerobic glycolysis, and canonical TGF-β signaling and myofibroblast differentiation. The ability of the cell to combat oxidative stress determines cell fate and cell differentiation. Within the ocular surface, particularly the avascular cornea, events that lead to neovascularization or inflammation can lead to severe and permanent tissue dysfunction

treatment of conditions that affect the ocular surface. Quercetin and other flavonoids have recently received an enormous amount of interest and several studies on the ocular surface have been performed in order to test their effects. The most significant studies are discussed below.

Anti-inflammatory properties

Numerous studies have shown that ROS levels regulate many cellular functions including activation and apoptosis of leukocytes,[34–36] as well as expression of pro-inflammatory factors, such as vascular cell adhesion molecules (VCAMs), interleukins (IL), and vascular endothelial growth factor (VEGF), by epithelial and endothelial cells[37–40] (Figure 3). Antioxidants have been reported to have inherent anti-inflammatory properties through the direct inhibition of ROS-promoted activation of NFκB signaling, which functions as a transcription factor promoting the expression of pro-inflammatory cytokines and chemokines.[41,42] A number of studies have highlighted the anti-inflammatory properties of quercetin primarily via downregulation of NFκB both *in vitro*[43,44] and *in vivo*.[45,46] Various studies have identified potent anti-angiogenic properties of quercetin.[47,48] A study by Ljubimov *et al.*[49] found that inhibition of protein kinase CK2 (casein kinase 2) by quercetin (50 μM) resulted in reduced retinal neovascularization in a mouse model of oxygen-induced retinopathy with the direct regulation of expression of pro-angiogenic factors. Interestingly, quercetin's role as an inhibitor of CK2 with rather broad specificity[50] may give rise to the observed anti-angiogenic properties. CK2 has also been shown to play a role in proliferative and tumorigenic properties[51–53] suggesting that the anti-cancer properties correlated with quercetin[54,55] may also relate to its modulatory effects on CK2 activity.[56]

A study from Abengózar-Vela *et al*.[57] showed the efficacy of quercetin as a therapeutic for corneal inflammation *in vitro*. The authors tested the anti-inflammatory and antioxidant effects of quercetin on human conjunctival and corneal epithelial cell lines. Results showed significant down regulation of IL-6 and IP-10 secretion, following quercetin stimulation, in a dose-dependent manner in both cell lines.[57] Ultraviolet-B induced significant up-regulation of ROS, *in vitro*, and was significantly down-regulated by quercetin confirming its antioxidant efficacy.[57]

Reduced tear production, infection, injury, or allergy can compromise the immune privilege of the cornea and lead to ocular irritation, scarring, and reduced visual acuity. Quercetin has been found to have immunoregulatory properties when applied topically on the ocular surface in dry eye mouse models.[58] In the most recent *in vivo* study, Oh *et al*.[59] examined the effects of quercetin using an experimental dry eye mouse model; 0.5% quercetin eye drops resulted in significant tear volume increase and restoration of smooth corneal surfaces without detaching the corneal epithelium. Quercetin also increased goblet cell density[59] suggesting that quercetin treatment may increase tear film production by directly modulating cell number.

Within the cornea, prolonged and uncontrolled pro-inflammatory processes are often associated with neovascularization within the cornea. Unlike the *in vivo* studies, *in vitro* studies investigating the effects of quercetin on the ocular surface did not appear until recently. One of the first studies was published by Donnini *et al*.[60]. In that article, the authors investigated the effects of quercetin and its main circulating conjugates (quercetin-3′-sulfate: Q3′S, and quercetin 3-glucuronide: Q3G) on cultured bovine endothelial cells.[60] The authors correlated their findings with *in vivo* studies on the rabbit cornea following VEGF-induced angiogenesis. The results showed that Q3G and quercetin itself had no effect on quiescent endothelium, *in vitro*, while they inhibited endothelial function and angiogenesis *in vivo*.[60] On the other hand, Q3′S significantly increased the growth of quiescent endothelial cells *in vitro* and had no effects on angiogenesis *in vivo*. The authors concluded that the ratio between Q3′S and Q3G is critical for the inhibition or activation of angiogenesis.[60]

Haynes *et al*.[61] used computerized image analysis to evaluate quantitatively the ability of topically applied small molecules to reduce corneal neovascularization in a rat cornea.[61] A large number of molecules were tested including quercetin, esculetin, prednisolone acetate, ketorolac, and others. The authors reported no significant effects on corneal neovascularization following treatment with 1% quercetin. While a small range of concentrations were tested during this study, it is important to take into account these observations when designing a quercetin-based ocular treatment.[61]

Metabolic regulator

Various studies have shown that quercetin is a regulator of systemic metabolism in physiological and pathological conditions.[62–64] Notably, quercetin has been shown to exhibit anti-diabetic properties when ingested orally in streptozotocin (STZ)-induced Type 1 diabetic mouse model,[65] as reviewed in Mukhopadhyay *et al*.[66] *In vivo* studies investigating the effects of quercetin on the ocular surface date back to the late 1980s. However, not many studies have been reported since that time with regard to quercetin's metabolic activity on the ocular surface. Lee *et al*.[67] reported the distribution of ketone reductase activity in the rabbit cornea and its influence on ocular metabolism. The authors tested several inhibitors on ocular ketone reductase activity: quercetin, barbital, pyrazole, and dicoumarol.[67] Quercetin was effective in inhibiting the ketone reductase activity in all the ocular tissues, being more so in the lens and the iris-ciliary than in the conjunctiva and corneal epithelium.[67]

Stoddard *et al*.[68] investigated the bioavailability and efficacy of antioxidants in human corneal limbal epithelial cells (HCLE). Quercetin was the most potent at quenching ROS. On the other hand, quercetin was more slowly taken up by the cells than other compounds tested in the study.[68] Even though this is an *in vitro* study, the availability of a topical ophthalmic formulation *in vivo* is relatively short due to frequent tear production and clearance (0.08–0.4 μL/min) depending on age.[69] Therefore, any treatment needs to be taken up by corneal cells quickly if corneal diseases or dystrophies are to be treated topically and non-invasively.

A corneal thinning disease termed Keratoconus has been associated with increased susceptibility to ROS-induced apoptosis,[70–73] altered cellular metabolism favoring aerobic glycolysis,[74] and mitochondrial dysfunction[75–77] within the corneal stroma. The specific metabolic effects of quercetin were investigated by our group in 2015 showing that quercetin upregulated the endogenous antioxidant pathway, the pentose phosphate pathway, in human keratoconus corneal cells.[78] Our results showed that quercetin reduced lactate production by human keratoconus corneal stromal cells to levels similar to that of healthy controls favoring more favorable ATP production via the citric acid cycle.[78,79] These studies suggest that quercetin may prove useful in targeting oxidative stress within the cornea in the context of Keratoconus by modulating energy production.

Anti-fibrotic

Scarring of the cornea is one of the most devastating outcomes following infection or trauma and is a major cause of blindness worldwide.[80] The mechanisms of corneal wound healing are known to involve cytokine- and growth factor-mediated interactions within the epithelium and stroma in a cascade of events that result in increased matrix deposition and wound closure.[81,82] Amongst other corneal diseases and dystrophies, corneal scarring occurs in a number of Keratoconus patients and is a leading cause of corneal transplantation in the US.[83] In 2015, our lab investigated the anti-fibrotic effects of quercetin on human keratoconus corneal cells and concluded that it exhibited therapeutic benefit *in vitro*.[78,79] Results showed that quercetin down-regulated key fibrotic markers, Collagen III and α-smooth muscle actin, by human keratoconus corneal stromal cells.[79] We also showed that quercetin downregulated transforming growth factor-β2 (TGF-β2) in the presence and absence of excess lactate.[79] It is well established that TGF-β signaling is one of the key players in corneal fibrosis, and various models have been proposed on how to inhibit its activity.[81,84] Quercetin may be a successful therapeutic option for inhibiting corneal scarring by controlling TGF-β expression. Further studies are warranted to verify the anti-fibrotic effects of quercetin *in vivo*.

Gupta et al.[85] examined the ability of inhibitors of arachidonic acid metabolism to influence the rate of epithelial closure in organ cultured rat corneas following 3-mm diameter central epithelial debridement. One of the inhibitors tested was quercetin at 100 μM concentration and was compared to indomethacin (1 μM), esculetin (100 μM), and flurbiprofen (1 μM).[85] The authors observed a delayed epithelial wound healing rate following treatments with esculetin, as well as quercetin.[85] While no further concentrations were tested, the role of these inhibitors is still to be investigated. This is the only organ culture study reported so far looking at the effects of quercetin on the anterior part of the eye.[85]

Proposed mechanism of action

Quercetin has been found to have pleiotropic bioactivity regulating inflammation, angiogenesis, cellular metabolism, and extracellular matrix deposition. As a potent antioxidant, quercetin has the ability to directly modulate ROS levels produced during normal cellular metabolism, as well as during pathological conditions, such as hypoxia, injury, or mitochondrial dysfunction. ROS regulate a number of signaling processes, in particular activation of NFκB, which as a transcription factor activates pro-inflammatory genes, including cytokines and chemokines that promote recruitment of inflammatory cells (Figure 3). Activation of pro-inflammatory genes within the corneal stroma or conjunctiva can lead to tissue damage due to prolonged inflammation and neovascularization. Evidence suggests that quercetin reduces ROS-induced activation of NFκB thereby reducing inflammation. Targeting oxidative stress is important in a number of tissues and may also be a novel target in the treatment of oxidative-stress-related corneal dystrophies, such as Keratoconus. Many studies have also shown that quercetin inhibits TGF-β signaling by regulating canonical SMAD2/3 phosphorylation suggesting that quercetin may serve as a potent anti-scarring agent. Further studies are required to determine if quercetin exhibits anti-fibrotic characteristics in preventing corneal scarring *in vivo*.

Future directions

Corneal defects are a leading cause of blindness worldwide, second only to cataracts.[80] For decades, we and others have worked towards the goal of keeping the cornea and ocular surface transparent in order to ensure quality vision. To achieve this, several strategies have been employed ranging from eye drops to full corneal transplants.[86–89] The data indicate that these strategies have succeeded in some occasions and failed in others. One of the most common observed failures has been the inability to ''rescue'' a chronic corneal scar or injury without the need of a corneal transplant. A study reporting global data from 2012 to 2013 showed a significant shortage of corneal tissue worldwide with only 1 out of 70 individuals in need of a corneal transplant were able to receive the surgery.[90] However, we should acknowledge that successes have also been observed with corneal transplantation,[91] and despite the long-term side effects, many people have regained their vision. Clearly, the global need to restore vision due to corneal blindness remains a driving force for alternative drug development.

Given the accelerated interest in flavonoids and recently in quercetin as a potent anti-inflammatory[45,58] and anti-fibrotic[79,92] agent, it is critical to accelerate our efforts towards more translational and clinical studies aimed at the development of non-invasive therapeutics for ocular surface diseases. A recent search of the ClinicalTrials.gov database (accessed on 19 May 2016) revealed that approximately 44 clinical trials are currently investigating the effects of quercetin or quercetin-included supplements on various diseases. Surprisingly, none of these studies are related to ocular pathologies. It is therefore clear that we need to increase our efforts towards the development of novel therapeutics for ocular surface diseases.

One of the main reasons quercetin as well as other flavonoids are attractive to pharmaceutical companies is that they come with a very low cost tag and extremely high safety profile.[93,94] It is also possible that these natural compounds can be used in conjunction with other current chemotherapeutic drugs in order to enhance their effectiveness. These kinds of studies are almost non-existent and require in-depth pharmacokinetics and pharmacodynamics analyses.

The study of quercetin is complex because of the scarcity of data in both *in vitro* and *in vivo* studies, on short- and long-term effectiveness, as well as bioavailability. There is, however, great promise based on the studies reported that this flavonoid can help with the treatment of at least some ocular surface diseases. As we look forward to the future, there are several factors that need to be considered when designing and executing these studies: (1) the lack of mechanism by which quercetin exerts its beneficial effects, (2) uncertainty about its bioavailability in a pharmacological form, and (3) the complexity of the numerous ocular surface diseases within the human population.

Conclusions

Quercetin and flavonoids in general are considered safe compounds, since unwanted toxic effects in humans are not frequently encountered. In fact, they can be administered in humans at high concentrations without any major threats. Unfortunately, only few flavonoids have made it to the clinical setting in terms of disease treatment and/or prevention. Quercetin is a very promising flavonoid with significant *in vitro* and *in vivo* beneficial effects for the ocular surface. Clearly, there is a great need for more effective medical therapy, and this warrants continued investigation both at the cellular and clinical level.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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