Oxidative Stress
Reduction for Dry Eye

Antioxidants’ effects on harmful reactive oxygen species may be helpful in the fight against dry eye.

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What does dry eye have in common with cancer, neurodegenerative disorders, normal aging and heart disease? One answer is oxidative stress. We all know what it’s like to feel stressed out over deadlines or other demands of life. Similarly, our cells experience oxidative stress in response to a variety of molecular disturbances. With dry eye, the pressure might come from exposure to environmental variables such as cigarette smoke, low humidity, sun, wind or pollutants. Certain medications or medical conditions may also contribute to dry eye. Aging itself is associated with decreased tear production and dry eye. All of these variables have the capacity to impact oxidative stress levels on the ocular surface and in so doing contribute to dry eye. Aging itself is associated with decreased tear production and dry eye. All of these variables have the capacity to impact oxidative stress levels on the ocular surface and in doing so contribute to ophthalmic conditions beyond dry eye, including macular degeneration, cataracts, uveitis, keratitis and corneal inflammation.

This month, we take a look at oxidation as a root cause of ocular surface disease, examine how this occurs and consider potential steps toward an antioxidant-based approach to dry-eye treatments.

Oxidative Stress Explained

What exactly is oxidative stress? Oxidative stress occurs when the level of reactive oxygen species produced in cells and tissues exceeds normal levels. ROS are types of free radicals (an atom with one or more unpaired electrons) that play a beneficial role in cell signaling and overall cellular homeostasis. Antioxidants naturally present in tissues usually control ROS levels, but surplus ROS react with nearby proteins, lipids or other cellular components, leading to unpredictable, cumulative and often deleterious effects on normal cell function. Oxidative injury from ROS occurs in the tears and conjunctiva of Sjögren’s syndrome patients, and high levels of ROS and oxidative stress have been identified in the tear film of dry-eye patients and in animal models of dry eye.

A primary source of cellular ROS is mitochondria, the intracellular organelles responsible for oxidation of glucose into H₂O, CO₂ and the chemical energy of adenosine triphosphate; ROS are often a byproduct of this process. Antioxidants such as reduced glutathione or enzymes such as superoxide dismutase provide electrons to convert ROS into less-reactive forms, but the cellular supply of antioxidants can be overwhelmed by too much ROS. As electrons pass along the mitochondrial electron transport chain, a fraction is lost to ROS and subsequent local oxidation events. This theft of electrons by ROS can lead to a host of cellular dysfunctions including membrane disruption. ROS can also inflict damage on DNA, RNA or cell proteins, effects that can ultimately lead to cell apoptosis.

Inflammation

While high ROS levels within the mitochondria lead to oxidative stress and potential organelle damage, ROS outside the mitochondria may be involved in inflammation, a primary mechanism of dry-eye disease. This inflammation can be the result of ROS exiting the mitochondria, or the generation of ROS in other cellular structures. Macrophages and other phagocytes involved in fighting infection use ROS as a weapon against foreign invaders, but control of these...
ROS is not always adequate. In particular, pro-inflammatory cytokines, such as IL-1β, can stimulate ROS to levels that can lead to oxidative tissue injury.

A number of preclinical models have been used to explore the relationship between oxidative stress and inflammation in dry-eye disease. In one study, increased ROS activated the NLRP3 gene, a key player in immune cell recognition of microbial pathogens and stress-related signals. NLRP3 activation in this dry-eye study increased secretion of the pro-inflammatory cytokine IL-1β. The same inflammatory process was confirmed in another dry-eye study using stressed human corneal epithelial cells: Increased ROS activated NLRP3, which in turn stimulated IL-1β and subsequent tissue inflammation. This study also examined 20 dry-eye patients and 15 normal subjects. In the dry-eye subjects, levels of ROS, NLRP3 and IL-1β were elevated in tear samples and conjunctival epithelial cells, indicating that inflammation in dry-eye disease may occur through the ROS–NLRP3–IL-1β signaling pathway. IL-1β levels in the dry-eye patients correlated with ocular surface disease index and Schirmer’s test scores and were elevated compared to control subjects. Previous studies found that NLRP3 is involved in other ocular diseases, including macular degeneration, glaucoma and corneal ulcer, as well as non-ocular diseases. In the future, it’s possible that inhibiting the ROS–NLRP3–IL-1β pathway may turn out to be an effective approach for dry-eye relief.

**Benefits and Limitations**

Most of us have heard about the purportedly miraculous qualities of antioxidants in food or nutritional supplements that allegedly can keep us healthy and help keep various diseases at bay. Antioxidant-rich foods (such as blueberries) are highly recommended by health experts, and sales of antioxidant supplements (such as vitamin C, vitamin E and Coenzyme Q) have skyrocketed. Although the evidence for a role of oxidative damage in conditions from diabetes, cancer or heart disease is undeniable, efforts to use antioxidants as therapeutics have been hit-or-miss: Antioxidant supplements have shown beneficial effects in some trials, while other studies have found little or no benefit. This is true both for overall health and for eye health specifically.

In one preclinical study, nutritional polyunsaturated fatty acid supplementation produced statistically significant changes in serum fatty acids and a dose-related inhibition of rabbit corneal infiltrates and corneal angiogenesis. This process involved modulation of eicosanoid precursors, changes in corneal neovascularization and in alkali-induced inflammation. A subsequent study, however, was unable to reproduce the effect of nutritional supplementation with the same PUFAs, gamma-linolenic acid, eicosapentaenoic acid or a combination of the fatty acids used in the prior study. The latter study is in line with more recent publications showing little or no effect of fish oils in reducing ocular inflammation.

For macular degeneration, a combination of antioxidant vitamins C and E, plus beta-carotene and zinc, afforded a statistically significant protection in disease onset in the Age-Related Eye Disease Study. A second AREDS study showed that lutein plus zeaxanthin—which are two carotenoids—can substitute for beta-carot-
tene, which has been associated with an increased risk of some types of cancer. While the effects are modest, the AREDS studies represent the most prominent examples of the benefits of antioxidants in the eye. Other studies have suggested that selenium or lactoferrin supplementation may similarly protect the corneal epithelium from oxidative stress. Antioxidants have also been explored as potential therapy in many other conditions associated with ROS. For example, supplements of vitamin E, vitamin C and Coenzyme Q have yielded some relief from the ocular complications associated with diabetes, although overall effects are mixed.

It’s important to note that there are also a few other studies that have suggested an increased risk of disease associated with the use of antioxidant supplements. Two studies showed an increase in cancer risk for people who were heavy smokers or were exposed to asbestos and were taking beta-carotene or a beta-carotene/vitamin A combination. It’s possible that the mixed results of antioxidant supplementation in reducing oxidative stress and disease may be due to the limited ability of natural antioxidants to reach a cell’s mitochondria and accumulate there, due to the relatively poor bioavailability, pharmacokinetics or stability of these antioxidant supplements. For some degenerative diseases, for example, very large doses were necessary to show a significant treatment benefit. And although antioxidant supplements have been beneficial for certain eye conditions, it’s also true that, except for the retina, enzymatic antioxidant activity in the eye is limited, with few protections against reactive oxygen species.

Targeted Antioxidants

Because of the limitations of natural antioxidant supplements in reducing ROS damage, mitochondrial-targeted antioxidants have been developed that are capable of accumulating in mitochondria. These therapies have shown beneficial effects for ocular and non-ocular diseases in some animal and clinical studies, although other studies did not confirm these results.

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Mice lacking the enzyme superoxide dismutase displayed degenerative loss of retinal pigment epithelial cells; this defect was corrected in these superoxide dismutase knockout mice by directed RPE expression of superoxide dismutase, which is capable of reducing mitochondrial and extracellular ROS generation. Using this same mouse model, another study showed that superoxide dismutase knockout resulted in abnormalities in lacrimal gland tissue, tear quantity and stability and the ocular surface. This murine model may be useful for future dry-eye studies.

MitoQ’s mitochondrial-targeted drug MitoQ (ubiquinone, which is identical to the antioxidant moiety in Coenzyme Q10) has been tested both in animals and in humans. In rodent studies MitoQ protected cells from pathological mitochondrial oxidative changes associated with effects such as cardiac damage, hypertension, liver damage, kidney damage and processes related to Parkinson’s disease. In human studies, MitoQ had mixed but promising results. In subjects with hepatitis C it significantly reduced liver enzyme levels, suggesting a reduction in liver inflammation, although viral levels were not significantly reduced. MitoQ didn’t slow the progression of Parkinson’s disease in an Australian/New Zealand study, possibly because it may be too late to rescue remaining dopamine neurons once the clinical signs of Parkinson’s are present.

Compounds with known antioxidant activity are chemically diverse. For example, another approach is the use of Szeto-Schiller peptides, short sequences of alternating aromatic and basic amino acids that are selectively taken up by mitochondria and are capable of reducing ROS at nanomolar concentrations. These peptides have shown promise in treating several conditions associated with inflammation or oxidative stress, including cardiac ischemia/reperfusion injury, insulin resistance and Parkinson’s. This approach may also be relevant as an ocular therapeutic, although no studies have been published to date.

Other antioxidant therapeutics targeting mitochondria include plastoquinone derivatives such as SKQ1 (Mitotech, Luxembourg). This compound is an approved treatment for dry eye in Russia. At the cellular level, SKQ1 reduces cell damage caused by excessive ROS by modulating the mitochondrial membrane electrical potential, the driving force for the electron transfer chain, ATP production and ROS formation. An important feature of SKQ1 is that its oxidation chemistry is such that it is recycled in the mitochondria, allowing it to serve as a renewable antioxidant. In addition to its antioxidant proper-
ties, studies in cell cultures of human conjunctival epithelial cells showed that SKQ1 reduced the production of prostaglandin E2, a pro-inflammatory signaling molecule that has been implicated in dry eye. Other SKQ1 studies of human endothelial cells indicated that mitochondria ROS are involved in regulation of the immune response.23

In mouse models of dry eye, SKQ1 reduced corneal staining and appeared to have a rapid onset and long duration of action. Other studies of the compound showed SKQ1 or related plastoquinones had beneficial therapeutic effects in animal models of retinopathy, glaucoma, macular degeneration, and UV damage to the lens.24,25 Systemic benefits in ischemia-related diseases have also been documented.25

In Russian clinical trials, SKQ1-induced reductions in dry-eye signs and symptoms were significantly greater than those seen with an artificial tear control.26 SKQ1 improved corneal cell function, increased tear-film stability and reduced dryness, burning, grittiness and blurred vision. In a subsequent U.S. clinical trial, SKQ1 reduced corneal and conjunctival staining, improved ocular discomfort scores and was generally superior to placebo control treatment.27 Of note, this study demonstrated SKQ1 improvements in both signs and symptoms of dry eye evoked through the use of the controlled adverse environment, a model that is designed to exacerbate dry-eye inigstigators, including oxidative stress effects. In both clinical trials, the compound exhibited a good safety profile and was well-tolerated by subjects.

Results with the compound SKQ1 confirm the importance of the mitochondria as a target for reducing oxidative stress in the body, and also support the notion that ROS are important contributors to dry-eye disease.

Looking Ahead

Novel treatments for dry eye hold promise in the not-to-distant future; treatments that hone in on the initial damaging events of intracellular oxidation could halt dry eye signs and symptoms in their tracks. Treating dry eye from the inside out may very well de-stress the cells that are under the onslaught of oxidative stress, allowing both the patient and the ophthalmologist some much needed respite from this disease. REVIEW

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