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Alpha-lipoic acid restores tear production in an animal model of dry eye

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ABSTRACT

The tear film comprises a major mechanism for protection of the ocular surface against harmful external agents. Disruption of tear production can lead to dry eye syndrome, causing damage ranging from mild discomfort to scarring of the ocular surface with irreversible vision impairment. The production of tears by the lacrimal gland is influenced by neuroendocrine, hormonal, and immunological factors. Reactive oxygen and nitrogen species play an important role in its regulation. We assessed the effects of oxidative stress on antioxidant defenses in the lacrimal gland and ocular surface in ovariectomized rats supplemented with n-3 polyunsaturated fatty acids (n-3 PUFA) and alpha-lipoic acid (ALP). We found that n-3 PUFA did not measurably influence oxidative stress, but ALP had site-specific pro-oxidant and antioxidant effects, and was an important influence on ocular surface dry eye improvement. As an index of oxidative damage to proteins and lipids, we measured levels of carbonyl and malondialdehyde (MDA), respectively. Enzymatic antioxidant defenses were measured as total superoxide dismutase (tSOD) and glutathione peroxidase (GPx), and non-enzymatic defenses were estimated by vitamin C, total glutathione, and indirect oxide nitric levels. PUFA and ALP treatment restored lacrimal production with resulting improvement in the dry eye Schirmer test in all supplemented groups. The results indicated that reactive oxygen species resulting from oxidative stress in the lacrimal gland did not play an important role in dry eye through reactive oxygen species; however, alpha-lipoic acid altered the metabolism of reactive nitrogen species, causing increased activity of lacrimal peroxidase and improved lacrimal production.

1. Introduction

The dry eye syndrome or keratoconjunctivitis sicca (KS) is a multifactorial disease of tears and the ocular surface resulting in symptoms of ocular discomfort, visual disturbance, and tear film instability, with potential damage to the ocular surface. According to the Dry Eye Workshop (DEWS), 2007, dry eye is the main cause of decreased tear production, and is accompanied by increased osmolarity of the tear film and ocular surface inflammation (Barabino et al., 2012). Tear secretion is under neural and hormonal control. It declines with age, and suffers with increased oxidative stress and inflammatory disorders of autoimmune origin, such as Sjögren syndrome (Brignole et al., 2003; Gumus and Cavanagh, 2009; Zoukhri, 2006). The possible involvement of sex hormones and menopause in the pathogenesis of dry eye is controversial, although androgen regulation is well established (Azzarolo et al., 1999; Sullivan et al., 1984). Exposure to atmospheric oxygen damage and ultraviolet (UV) light have been investigated as causes of oxidative stress to the ocular surface, and are thought to be involved in the pathophysiology of dry eye (Demir et al., 2005; Nakamura et al., 2007). Under normal circumstances, reactive species produced by oxidative processes are eliminated by cellular antioxidant systems. Deleterious oxidative processes that occur during chronic inflammatory diseases result in the accumulation of reactive oxygen species (ROS) and reactive nitrogen species that are produced continuously by metabolic processes. The loss of lacrimal gland function associated with oxidative stress and aging is

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accelerated by increased mitochondrial superoxide production and by increased damage to proteins, lipids, and DNA on the ocular surface in the dry eye animal model (Uchino et al., 2012).

In Sjögren syndrome, an autoimmune disorder of exocrine glands including lacrimal and salivary glands, oxidative stress appears to be crucial in the development of dry eye, indicated by an association of ROS generation, lipid peroxidation, and damage to cell membranes in the inflamed ocular surface (Wakamatsu et al., 2013). The expression of nitric oxide (NO) and its byproducts, reactive nitrogen species, which damage the ocular surface, are increased by inflammatory mediators such as interleukin-1B (IL-1B), interleukin-6 (IL-6), interleukin-8 (IL-8), and TNF-α. Reactions between NO and superoxide anions that generate toxic peroxynitrite and lipid peroxides are more pronounced in corneal surfaces irradiated with UVA and UVB, as well as in Sjögren syndrome patients with dry eye (Cejková et al., 2007). Lacrimal gland acinar cells cultured with pro-inflammatory cytokines, such as IL-1B, exhibit increased NO production. NO production is repressed by sex hormones, suggesting that deficiency of these steroids – such as occurs in menopause – leads to cellular damage by oxidative stress with increased reactive nitrogen species, which in turn, may lead to decreased tear production and consequent dry eye (Beauregard and Brandt, 2004). On the other hand, NO and its metabolic byproducts can act as an antioxidant defense in some dry eye models. In postmenopausal women receiving hormone replacement therapy, symptoms of ocular discomfort improved in concert with the hormone-induced increase in lacrimal peroxidase, an antioxidant and antimicrobial enzyme involved in protection of the ocular surface (Marcozzi et al., 2003). In rats, hormonal suppression by ovariectomy results in increased blood levels of hydrogen peroxide and lipid peroxidation, with consequent impairment of enzymatic antioxidant defenses such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione S transferase (GST) (Muthusami et al., 2005). Thus, the relationships between sex hormones and oxidative stress are complex and seemingly paradoxical.

The long chain omega-3 polyunsaturated fatty acids (n-3 PUFA) are mostly found in cold water marine fish. Dietary supplementation with n-3 PUFA generates the byproducts docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which compete with omega-6 polyunsaturated fatty acids (n-6 PUFA), such as arachidonic acid, from animal fat in the formation of membrane phospholipids. N-3 PUFA play important roles in cardiovascular, neurological, and physiological regulatory processes, and, as precursors of anti-inflammatory mediators, are involved in the inflammatory response (Calder, 2001). Macsai (2008) showed a decrease in the plasma and red blood cells membrane n-6 PUFA/n-3 PUFA ratio and improvements in clinical tests for tear film dysfunction in patients with dietary supplementation of n-3 PUFA. Results from dry eye animal models showed that a deficiency of n-3 PUFA in the diet does not correlate with the severity of dry eye (Viau et al., 2011), whereas n-3 PUFA dietary supplementation contributes to n-3 PUFA incorporation in the lacrimal gland, altering lipid homeostasis and partially interfering with the course of dry eye, without altering lacrimal production of prostaglandins (Viau et al., 2009). Wojtowicz et al. (2010) showed an increase in average tear production, as measured by the Schirmer test, and improvement in dry eye symptoms in patients receiving oral supplementation of fish oil containing EPA and DHA.

Alpha-lipoic acid (ALP) is an endogenously synthesized cofactor for mitochondrial enzyme complexes. ALP has both antioxidant and pro-oxidant activity, depending on the biological system and levels (Çakatay, 2006). The biological effects of ALP include sequestration of ROS, interaction and regeneration of other antioxidants such as vitamin C and vitamin E, redox regulation and conformation of thiol groups of proteins, and effects on gene expression and apoptosis (Demir et al., 2005). As an antioxidant, ALP decreases lipid peroxidation and nitric oxide production and increases activity of the enzymes CuZnSOD, GPx, and catalase in brain and retina of rats after induced stress (Akpinar et al., 2008). Improvements in neurodegenerative disorders cognitive decline have been seen following treatment with ALP alone or ALP in combination with other antioxidants and anti-inflammatory drugs (Luil, 2008). A protective effect of ALP against mitochondrial oxidative damage by lipid peroxidation has been found, especially in old compared to young rats (Palaniappan and Dai, 2007). The role of ALP in hormonal regulation and nitric oxide metabolism is unknown, and the use of ALP for the treatment of lacrimal deficiency has not been described.

Dry eye is prevalent among postmenopausal women, and the availability of a dietary supplement for treating dry eye is a pressing need for these women. This study aimed to evaluate the role of dietary supplementation with DHA, EPA, and ALP in an animal model of postmenopausal dry eye. We evaluated the antioxidant enzymatic and non-enzymatic responses and oxidative damage in lacrimal glands and ocular surface tissues and their participation in the redox balance of these tissues in hormonally suppressed female rats.

2. Materials and methods

2.1. Animals

This study employed 50 three-month-old Wistar female rats (*Rattus norvegicus*). Rats were housed in polypropylene cages with five animals per cage. All animal studies followed the rules from the EU Directive for animal experiments 2010/63/EU and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (DHEW Publication No. (NIH) 85-23, revised in 1996, Office of Science and Health Reports, Division of Research Resources/NIH, Bethesda, MD, USA) and were approved by the Ethics Committee of the Universidade Federal do Rio Grande do Sul. The animal house was kept on a 12 h light/dark cycle at a temperature of 24 ± 1 °C. Animals were fed standard lab chow and drinking water ad libitum.

The rats were divided into five groups of ten animals each. Four groups were subjected to bilateral ovariectomy and one group was sham operated, but without removal of the ovaries (SHAM group). Ovariectomy was performed during diestrus (determined by vaginal smear). Animals in SHAM group were-assessed and euthanized during diestrus (Freeman, 1994). The surgical procedure was performed under general anesthesia, administered as an i.p. injection of a combination of xylazine (10 mg/kg) and ketamine (60 mg/kg). Immediately after surgery, while still under anesthesia, the rats received a combination of antibiotics and anti-inflammatory drugs (Pencivet PPU Plus®, Intervet/Schering-Plough Animal Health, 0.1 ml/100 g, i.m.; containing (per 100 ml): procaine benzylpenicillin G, 10.000.000 IU, benzathine benzylpenicillin G, 10.000.000 IU dihydrostreptomycin, 10.5 mg, piroxicam 1.0 mg). After surgery, the animals were maintained under a heat lamp until recovered from anesthesia. Animals received analgesia with acetaminophen (Paracetamol®, MSD) at a dose of 200 mg/kg, diluted in the drinking water for 3 days. After the period of study of 16 weeks, the animals were euthanized under general anesthesia by i.p. injection of combination of xylazine (10 mg/kg) and ketamine (60 mg/kg) for removal of the main lacrimal gland and eye tissues and withdrawal of blood for hormonal analysis. Tissues were separated and identified as the lacrimal gland, cornea, and conjunctiva of the eye.