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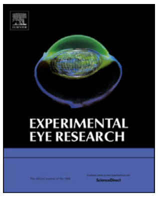

Research article

Short-term effects of extremely low-frequency pulsed electromagnetic field and pulsed low-level laser therapy on rabbit model of corneal alkali burn

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ABSTRACT

This study was conducted to investigate the effect of combining extremely low frequency–pulsed electromagnetic field (ELF-PEMF) and low-level laser therapy (LLLT) on alkali-burned rabbit corneas. Fifty alkali-burned corneas of 50 rabbits were categorized into five groups: ELF-PEMF therapy with 2 mT intensity (ELF 2) for 2 h daily; LLLT for 30 min twice daily; combined ELF-PEMF and LLLT (ELF + LLLT); medical therapy (MT); and control (i.e., no treatment). Clinical examination and digital photography of the corneas were performed on days 0, 2, 7, and 14. After euthanizing the rabbits, the affected eyes were evaluated by histopathology. The clinical and histopathologic results were compared between the groups. On days 7 and 14, no significant difference in the corneal defect area was evident between the ELF, LLLT, ELF + LLLT, and MT groups. Excluding the controls, none of the study groups demonstrated a significant corneal neovascularization in both routine histopathology and immunohistochemistry for CD31. Keratocyte loss was significantly higher in the MT group than in the ELF, LLLT, and ELF + LLLT groups. Moderate to severe stromal inflammation in the LLLT group was comparable with that in the MT group and was significantly lower than that in the other groups. In conclusion, combining LLLT and ELF was not superior to ELF alone or LLLT alone in healing corneal alkali burns. However, given the lower intensity of corneal inflammation and the lower rate of keratocytes loss with LLLT, this treatment may be superior to other proposed treatment modalities for healing alkali-burned corneas.

1. Introduction

Persistent corneal epithelial defects from alkali burns may cause corneal ulceration and perforation that are unresponsive to conventional medical treatment and corneal transplantation (McCulley, 1987; Singh et al., 2013; Pfister et al., 1981; Eslani et al., 2014). Because multiple therapeutic modalities such as medical and surgical treatments have not been completely successful for preserving corneal epithelial integrity (Singh et al., 2013; Pfister et al., 1981; Eslani et al., 2014), magnetic therapy was attempted in our previous study as a new treatment strategy with favorable but short-term results, which were comparable to those of conventional medical methods (Rezaei Kanavi et al., 2012). Furthermore, other experimental studies have reported successful corneal wound healing by using this method (Basu et al., 1989; Sta Iglesia and Vanable, 1998; Reid et al., 2005; Ghaffarieh et al., 2012). However, as demonstrated in our previous study (Rezaei Kanavi et al., 2012), the implementation of extremely low frequency–pulsed electromagnetic field (ELF-PEMF) of 2 mT (mT) field intensity was effective but insufficient in healing experimental corneal alkali burns and exerted no significant influence on reducing inflammatory cell infiltrates. Therefore, seeking a new ideal treatment strategy for corneal chemical burns is necessary.

Low-level laser therapy (LLLT) applied by using a specified set of laser wavelengths exerts positive effects on wound healing (Bjordal et al., 2008; Mao et al., 2012; Houreld, 2014; Peplow et al., 2010). The use of LLLT has recently been extended beyond wound healing, and its potential benefits have been reported for retinal disorders

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such as retinitis pigmentosa and age-related macular degeneration (Ivandic and Ivandic, 2014, 2008). The exact mechanism of action of LLLT is unclear, although improved cellular hemostasis as the result of a stimulatory effect on the mitochondria and increased cellular adenosine triphosphate has been proposed (Ivandic and Ivandic, 2014, 2008; Bjordal et al., 2006; Tafur and Mills, 2008; Karu, 2008; Hu et al., 2007; Houreld et al., 2012; Masha et al., 2013). This form of phototherapy promotes tissue repair and regulates the biological behavior of cells by increasing cellular migration, proliferation, and viability; collagen production; nitric oxide and growth factors levels; and gene regulation (Mao et al., 2012; Masha et al., 2013; Esmaeelinejad et al., 2014; Ayuk et al., 2012; Sekhejane et al., 2011; Houreld and Abrahamse, 2007; Hawkins and Abrahamse, 2006; Houreld et al., 2010; Hakki and Bozkurt, 2012). It downregulates apoptosis and inhibits inflammation by decreasing the levels of proinflammatory cytokines and matrix metalloproteinases (MMPs) (Mao et al., 2012; Sekhejane et al., 2011; Gavish et al., 2006; Frigo et al., 2010).

Endogenous electric field in normal corneas is significantly increased after corneal wounding. Cellular galvanotropism in form of cellular polarity, directional migration, and outgrowth may be induced by enhancement of the endogenous electric field (Sta Iglesia and Vanable, 1998; Reid et al., 2005). Pulsed electromagnetic field, through interacting with cell membranes and modulating the rate of ion binding and/or transport (Rezaei Kanavi et al., 2012; Basu et al., 1989), and low level laser through improvement of cellular hemostasis (Mao et al., 2012) may alter the biophysiological cascade of cellular responses to injuries and boost intrinsic healing mechanisms. To the best of our knowledge, there is no study concerning the effects of LLLT on alkali-burned corneas. Hence, this study investigated whether adding LLLT to ELF-PEMF is advantageous for healing of animal corneas with alkali burns over LLLT alone, ELF-PEMF alone, or medical treatment. Because of the effectiveness of the ELF-PEMF with field intensity of 2 mT on the healing of corneal defects from alkali burns (Rezaei Kanavi et al., 2012) and the effectiveness of LLLT with wavelength range of 790–940 nm and fluency of 2.5–10 J/cm^2 on the healing of animal skin wounds (Santos et al., 2010), the field intensity of 2 mT for ELF-PEMF and pulsed infrared laser with 810 nm wavelength and energy density of 0.5 J/cm^2 were used in this experiment. According to current available international standards, exposing the eyes to a pulsed low-level laser with this energy density does not present an optical hazard (Eadie et al., 2009).

2. Material and methods

2.1. Animal models, preparation, and grouping

Fifty New Zealand albino female rabbits (Razi Institute for Vaccine and Serum Research, Karaj, Iran) that weighed approximately 2 kg were used. All experimental procedures were conducted in adherence to the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in ophthalmic research and were approved by the ethics committee of the Ophthalmic Research Center at the Shahid Beheshti University of Medical Sciences (Tehran, Iran). Before starting the experiments, all rabbits were examined for the presence of any ocular or systemic disease.

On day 0, the rabbits were anesthetized with an intramuscular injection of 10% ketamine HCL (Alfamine; 30 mg/kg; Alfasan, Woerden, Holland) and xylazine (Rompun; 3 mg/kg; Bayer, Leverkusen, Germany) (Vennen and Mitchell, 2009). After instilling one drop of topical 0.5% tetracaine eye drops (Anestocaine, Sina Darou Laboratories, Tehran, Iran) in the right eye, a 5-mm diameter round filter paper that had been soaked in 2.5 N sodium hydroxide was adhered to the center of the cornea for 30 s to produce a well circumscribed central corneal burn. The ocular surface was then thoroughly irrigated with normal saline solution (0.9% NaCl) for 3 min. The rabbits were then randomized into five groups of 10 animals (10 eyes): (1) the ELF group, ELF-PEMF of 2 mT field intensity and frequency of 25 Hz for 2 h daily for 14 days; (2) the LLLT group, pulsed LLLT of 810 nm wavelength and energy density of 0.5 J/cm^2 for 30 min twice daily for 14 days; (3) the combined group, a combination of ELF-PEMF of 2 mT field intensity and frequency of 25 Hz for 2 h daily and LLLT of 810 nm wavelength and energy density of 0.5 J/cm^2 for 30 min twice daily for 14 days; (4) the medical therapy (MT) group (Rezaei Kanavi et al., 2012; Crispin, 2005; Ralph, 2004), chloramphenicol eye drops 0.5% (Chlobiotic, Sina Darou Laboratories) four times daily, betamethasone 0.1% eye drops (Betasonit, Sina Darou Laboratories) four times daily, sodium citrate 10% eye drops (Aurocitrate; Aurolab, Madurai, India) every 6 h, ascorbic acid 10% solution prepared from an ascorbic acid ampule containing 500 mg in 5 mL, (Vitamin C, Daru Pakhsh Pharmaceutical Company, Tehran, Iran) four times daily, and atropine 1% eye drops (Sina Darou Laboratories) once daily for 14 days; and (5) the control group, no treatment.

2.2. Treatment by ELF-PEMF

Treatment by ELF-PEMF has been described previously (Rezaei Kanavi et al., 2012). In brief, to produce ELF-PEMF, a plastic ocular shield was attached to a ring-shaped solenoid electromagnet (Helmholtz coil) of 5-cm diameter with 250 wire loops.

The eye could be observed through a 2-cm circular opening in the center of the shield during the magnetic therapy. Without any anesthesia, the head of the animal was restrained and the shield was placed on the right eye and stabilized in place by fixing an elastic–cotton strip behind the head. The pulse amplitude of the direct generator unit was altered to achieve a field intensity of 2 mT.

2.3. Low-level laser therapy

Low-level laser therapy was applied to the injured cornea by using an infrared probe assembled on the lateral branch of a headset. The headset was stabilized on the rabbit's head by fixing an elastic-cotton strip behind the head and below the chin. The procedure was performed without anesthesia. Infrared diode laser with wavelength of 810 nm, maximum output power of 100 mW, and energy density of 0.5 J/cm^2 was used in our study.

2.4. Combined ELF-PEMF and LLLT treatment

After assembling an infrared probe and a ring-shaped Helmholtz coil on the lateral branch of the headset, the headset was stabilized on the rabbit's head and the injured cornea was exposed to a combination of electromagnetic and phototherapies (Fig. 1). The field intensity of 2 mT and the wavelength of 810 nm with energy density of 0.5 J/cm^2 were set through an electrical generator unit.

2.5. Clinical analysis

By using an ophthalmic operating microscope (OMS-300; Topcon, Tokyo, Japan), the eyes were examined on days 0, 2, 7, and 14 for the presence of a corneal defect, perforation, vascularization, limbal ischemia, or symblepharon formation. To measure a corneal defect, one drop tetracaine 0.5% eye drop (Sina Darou Laboratories) was instilled. A fluorescein dye strip (Negah Fluorescein Paper; Toosnegah Co., Mashhad, Iran) was then placed in the inferior fornix. The fluorescein-stained corneal epithelial defect with an