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Original Article

Comparison between corneal cross-linking, topical antibiotic and combined therapy in experimental bacterial keratitis model

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Abstract

Purpose: This study was conducted to investigate the effects of an experimental bacterial keratitis model on the corneal collagen cross-linking treatment (CXL), and also to compare topical antibiotic treatment with the combined treatment.
Methods: The study involved 40 young adult female Sprague Dawley rats, which had a 2 mm scraped defect of the central corneal epithelium in both eyes. The rats were divided into two equal groups. The first group was inoculated in both eyes with standard *Pseudomonas Aeruginosa* (PA) from a strain suspension prepared from 0.05 ml (Group 1), and the second group was inoculated with standard *Methicillin Resistance Staphylococcus Aureus* (MRSA) strains from a suspension prepared from 0.05 ml (Group 2). Group 1 was divided into four sub-groups: Group 1A was treated by collagen cross-linking (CXL), Group 1C was treated with topical tobramycin drops CXL and also treated by collagen cross-linking (CXL), Group 1D was treated with topical tobramycin drops, and Group 1B was left untreated in order to create a control group. Similarly, Group 2 was also divided into four sub-groups: Group 2A was treated by CXL, Group 2C was treated with topical 5% fortified vancomycin drops CXL and also treated by CXL, Group 2D was treated with topical 5% fortified vancomycin drops, and Group 2B was left untreated in order to create a control group. CXL was performed on the third day following the inoculation and topical drop therapy. Biomicroscopy and microbiologic assessments were performed on the third and seventh days following the inoculation of microorganisms.
Results: In the treatment, which compared baselines in all groups before treatment, the diameter of keratitis infiltrations, corneal clouding, and corneal swab samples were obtained from the reduction in reproduction. The results were statistically significant ($p < 0.01$). Keratitis infiltration groups were conducted on the seventh day for Groups 1C and 1D according to Group 1B, whilst Groups 2A, 2C and 2D were conducted according to Group 2B, which showed a significant statistical reduction ($p < 0.01$). On the seventh day, focal groups were conducted in corneal clouding Group 1D according to Group 1B and in Groups 2A, 2C and 2D according to Group 2B, which revealed a significant statistical reduction ($p < 0.01$). On the seventh day, reproduction in culture was obtained from corneal swab samples in Groups 1C and 1D according to Group 1B; in Groups 1C and 1D according to Group 1A; in Groups 2A, 2C and 2D according to Group 2B; and in Group 2C according to Group 2A, where a significant statistical reduction was observed ($p < 0.01$).
Conclusions: The clinical and microbiological efficacy of the CXL treatment is evaluated in our study. In accordance with the conclusion reached an effective reduction in the density and severity of (infection), occurred as a result of CXL treatment, CXL treatment combined with topical antibiotic treatment and topical antibiotic treatment of *Pseudomonas Aeruginosa* (PA) and *Metisilin Rezistant Staphylococcus Aureus* (MRSA) keratitis infections. From these results, it is shown that topical antibiotics and CXL potentiate each other's effects in the treatment of resistant bacterial keratitis.

Keywords: Cornea, Bacterial Keratitis, Cross-linking

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Introduction

It is currently common practice to treat corneal infections with a broad range of antibiotics but, as the medical profession is faced with the problem of increased bacterial resistance to antibiotic medication, the need to introduce alternative treatments is becoming an issue of growing significance.[1] This is particularly crucial when considering corneal infections, as serious damage to the patient's vision, and even blindness, can ensue, leading to the necessity for more serious treatments, such as chemotherapy or surgery.

One alternative to antibiotic treatment is known as corneal collagen crosslinking (CXL). This treatment works by using UV–A at 365 or 370 nm to activate the photosensitive properties of riboflavin to initiate photochemical reactions which result in covalent bonds or crosslinks in the corneal stroma, which in turn can augment the cornea's biomechanical strength and, thus, potentially arrest the further advancement of keratoconus.[2,3] The idea of using UV rays against microorganisms is certainly not new and is regularly found in research; it is also widely utilised in medical settings such as operating theatres to augment existing sterilisation techniques.[4,5]

The first relevant study in this area was carried out in 1960, when it was revealed that, upon exposure to UV rays, riboflavin would inactivate the RNA of various viruses. Contemporary studies have found that riboflavin also behaves as a photomediator and has the capacity to neutralize not only pathogens in plasma, platelets, and red blood cells[6–8] but also a variety of viruses, bacteria, and parasites.[9]

The treatment of corneal infections using CXL confers an additional benefit, in that resistance of the cornea to enzymatic digestion by microorganisms[10] is increased.

It is the case that 90% of the thickness of the cornea consists of the stroma, which is made up of collagen fibrils caught up within an environment of proteoglycans, proteins, glycoproteins and keratocytes located amid the collagen lamellae.

Some microorganisms, including bacteria and fungi, emit enzymes that have the capacity to digest collagen, in turn leading to melting and perforation of the cornea. Notwithstanding microbiological remedy, deterioration of collagen and proteolysis as a result of enzymatic action may well still be apparent in the form of progressive ulceration. Use of the CXL treatment in porcine testing has had very positive results against enzymatic degeneration by collagenase, trypsin and pepsin. It can therefore be supposed that CXL is not only able to neutralise the microorganisms causing the infection, but also confer the benefit of corneal rigidity, thereby lessening vulnerability of the stroma to proteolysis and the advancement of corneal melt.[10–12]

Material and methods

Preparations of the strains

The standard strains PA ATCC 9027 and MRSA ATCC 33591 were utilized in this study. It was necessary for the strains to be kept at a temperature of −86 °C until required for testing purposes, at which point they were revived with agar containing 5% sheep blood; was necessary for them to be passaged twice with blood agar. The suspensions of the strains are dense and they were prepared in sterile physiological serum. The actual concentration of bacteria required was 1.0 × 108 CFU/ml in each 50 μl suspension to be introduced into the rat eyes; this was achieved by means of dilution using physiological saline solution spectrophotometrically.

Animals

The study included 40 young adult female Sprague Dawley rats weighing between 230 and 250 kg. All rats were treated in accordance with the Association for Research in Vision and Ophthalmology statement for the use of animal in ophthalmic research, and the protocol was approved by the Institutional Ethics Committee. The rats were anesthetized with 50 mg/kg of intraperitoneal ketamine hydrochloride and 7 mg/kg of xylazine before all interventions. Corneal anesthesia was attained using 0.5% topical proparacaine hydrochloride. The central corneal epithelium was marked with a 2 mm disposable dermatological skin punch (Acu-Punch, Acuderm, Ft. Lauderdale, FL). The marked corneal epithelium was scraped using a number 11 scalpel (Fig. 1a). The rats were divided into two equal groups. The first group was inoculated into both eyes with standard *Pseudomonas Aeruginosa* (PA) from a strain suspension prepared from 0.05 ml (Group 1), and the second group was inoculated with standard *Methicillin Resistance Staphylococcus Aureus* (MRSA) strains from a suspension prepared from 0.05 ml (Group 2) (Fig. 1b). Three days later, bio microscopic examination revealed keratitis in both eyes of all rats. All rats were swallowed from the cornea of both eyes and microbiologically appeared to be infected.

Group 1 was divided into four sub-groups: Group 1A was treated by collagen cross-linking (CXL), Group 1C was treated with topical tobramycin drops (CXL) and also treated by collagen cross-linking (CXL), Group 1D was treated with topical tobramycin drops, and Group 1B was left untreated in order to create a control group. Similarly, Group 2 was also divided into four sub-groups: Group 2A was treated by CXL, Group 2C was treated with topical 5% fortified vancomycin drops (CXL) and also treated by (CXL), Group 2D was treated with topical 5% fortified vancomycin drops, and Group 2B was left untreated in order to create a control group (Table 1).

Treatment

The CXL procedure was performed three days after the inoculation of *Pseudomonas Aeruginosa* (PA) and *Methicillin Resistance Staphylococcus Aureus* (MRSA), conducted under sterile conditions in an operating room. Topical drop therapy was undertaken within those groups which were exposed to CXL treatment after CXL (on the third day), and no CXL treatment (only topical antibiotics treatment the third day after introduction of the microorganism suspension). The rats were anesthetized with intramuscular 50 mg/kg of ketamine hydrochloride and 7 mg/kg of xylazine before all interventions and corneal anesthesia was attained using 0.5% topical proparacaine hydrochloride. Using a number 11 scalpel, a 2 mm central fragment of the corneal epithelium was carefully detached. As a photosensitizer, a riboflavin 0.1% solution (10 mg of riboflavin-5-phosphate in 10 mL of dextran–T-500 20% solution) was applied every five minutes for 30 min before

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