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Topical 1% Nalbuphine on corneal sensivity and epitheilization after experimental lamellar keratectomy in rabbits

Nalbufina 1% tópica sobre a sensibilidade e a epitelização corneal após ceratectomia lamelar experimental

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ABSTRACT

The present study was aimed to evaluate the effects of topical 1% nalbuphine on corneal sensitivity and re-epithelialization, after lamellar keratectomy in rabbits. All protocols were approved by the Animal Care Comission of São Paulo State University (Protocol 028793-08) and were conducted in accordance with the Institutional Animal Committee and the Association for Research in Vision and Ophthalmology (ARVO) statement for the use of animals in research. Surgeries were performed on the left eye (Nalbuphine Group) and on the right eye (Control Group). Two groups were formed (n=10) and corneas received either 30µl of 1% nalbuphine (NG) or 30µl of 0,9% saline (CG). Treatments occurred at 7, 11, 15 and 19 hours. After the surgery, the corneas were stained with fluorescein and photographed daily; corneal touch threshold (CTT) was assessed with Cochet-Bonnet aesthesiometer, at 7 and 19 hours, 20 minutes after treatments. Data were statistically compared with repeated measures ANOVA and Bonferroni post-hoc test, and T test (P<0.05). Average ±SD time for corneal re-epithelialization was 7.40±0.47 days (NG) and 8.90±0.31 days (CG) (P=0.11). The results showed that the diameter of the keratectomized area and CTT did not change significantly between both groups (P>0.05); however, a higher area under the curve for both parameters was observed in the NG (2771), in comparison to CG (2164). Topical 1% nalbuphine did not change significantly corneal sensitivity and re-epithelialization, after experimental lamellar keratectomy in rabbits.

Keys words: *rabbit, cornea, re-epithelialization, nalbuphine, pain.*

RESUMO

Avaliaram-se os efeitos da nalbufina 1% sobre o limiar de sensibilidade corneal (LSC) e a epitelização corneal em coelhos submetidos à ceratectomia lamelar unilateral. Os procedimentos foram aprovados pela Comissão de Ética no Uso de Animais da Faculdade de Ciências Agrarias e Veterinárias da Universidade Estadual Paulista (Protocolo nº 028793-08), de acordo com as normas do Institutional Animal Committee and the Association for Research in Vision and Ophthalmology (ARVO). Conceberam-se dois grupos (n=10) e os olhos foram tratados com 30µl de Nalbufina 1% (Olho esquerdo - GN) ou com 30µl de solução salina (Olho direito - GC), às 7, 11, 15 e 19 horas das ceratectomias unilaterais, até sua reepitelização. O limiar de sensibilidade corneal (LSC) foi avaliado 20 minutos após cada tratamento, 48 horas antes e depois da ceratectomia a intervalos regulares de 12 horas (7 e 19 horas) com estesiômetro de Cochet-Bonnet. Após a realização da cirurgia, diariamente, as córneas foram coradas com fluoresceína e registradas em fotos digitais para mensuração em software Image-J. A normalidade dos dados foi avaliada ao teste de Kolmogorov-Smirnov. O limiar de sensibilidade e a área ulcerada foram comparados ao teste de Bonferroni, após ANOVA de medias repetidas (P<0,05). O teste t-Student foi aplicado na avaliação das médias quanto ao tempo de reepitelização. Não houve diferença significativa quanto ao limiar de sensibilidade entre os grupos, em qualquer dos períodos avaliados (P>0,05), todavia, constatou-se maior área sob a curva, relativamente ao LSC, no GN (2771), comparativamente ao GC (2164). O tempo médio±DP de reepitelização no GN foi de 7,40±0,47 dias e de 8,90±0,31 dias no GC, não havendo diferença significativa entre os grupos (P=0,11). Como conclusão, tem-se que o uso tópico de nalbufina 1% não alterou significativamente o limiar de sensibilidade e a reepitelização corneais em coelhos submetidos a ceratectomia lamelar experimental.

Palavras-chave: *coelho, córnea, reepitelização, nalbufina, dor.*

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INTRODUCTION

Corneal ulcerations are very painful affections that may arise by trauma, infection, neurogenic conditions, autoimmune diseases, chemical burns, among others causes (SZUCS et al., 2000). The high density noniceptive network makes the cornea one the most sensitive tissues in the body (STILES et al., 2003).

Topical instillation of non-steroidal anti-inflammatory drugs (NSAIDs) is an option to control the discomfort that arises from corneal abrasions. However, NSAIDs used with this purpose may delay corneal wound healing due to epithelial toxicity and the enhancement of lytic enzymes (REVIGLIO et al., 2003; WOLF et al., 2009).

Data suggest that topically applied opioids, notably morphine may provide analgesia through peripheral receptors via the neuroimmune pathway since opioid receptors have been indentified in peripheral nerve terminals in dogs (STILES et al., 2003).

Mild to moderate analgesia can be achieved with nalbuphine, a ê agonist, μ antagonist opioid (HOSKIN & HANKS, 1991). The agent has been largely used pre-operatory and during the post-operatory period (MINAI & KHAN, 2003)

The instillation of naltrexone, an antagonist primarily at μ and ê opioid receptors, accelerates corneal re-epithelialization in rabbits, humans, and in diabetic rats by inhibiting the action of opioid growth factor (ZAGON et al., 1998). One may suppose that the mixed action of nalbuphine may accelerate corneal epithelialization by blocking mu and opioid growth factor receptors, and producing analgesia via kappa receptors.

The results found in the current literature about local delivery of nalbuphine are controversial. Topical nalbuphine did not promote corneal analgesia in healthy horses and dogs, a well as in dogs with experimental corneal ulcers (CLARK et al., 2009; WOTMAN & UTTER, 2010). However, AQUINO et al. (2005) observed that the agent was able to decrease corneal sensation after topical administration in healthy dogs.

Studies assessing topical administration of nalbuphine have not been carried out in rabbits, animals largely used in ophthalmic research. The present study aimed to investigate the analgesic effects of topical administration of 1% nalbuphine until completion of corneal re-epithelialization in keratectomized rabbits and the effects of this treatment on corneal wound healing time.

MATERIAL AND METHODS

Twenty adult male White New Zeland rabbits with an average weight of 3.0±0.04kg were used. All animals were subjected to ophthalmic examination based on Shirmer tear test, applanation tonometry, slit-lamp biomicroscopy, indirect binocular ophthalmoscopy and fluorescein test. Once selected, they were individually housed in a ventilated environment and fed dry pellets twice a day. Water was available *ad libitum*.

After induction of anesthesia with an intramuscular injection of 15mg kg^{-1} of ketamine (Cetamin S+®, Cristália, São Carlos, Brasil) combined with 0.5mg kg^{-1} of midazolam (Dormire®, Cristália, São Carlos, Brasil), the rabbits were maintained under inhalant anesthesia with isoflurane diluted in 100% oxygen delivered through a facial mask (BORKOWSKY & KARAS, 1999). One drop of proxymetacaine (Anestalcon®, Alcon, São Paulo, Brasil) was instilled and a central unilateral ulcer was created with a trephine of 6mm of diameter and 200μm of depth (Steel Inox, São Paulo, Brasil). After trephination, the corneal button was removed with an angled crescent knife (Unique Edge, Mohnton, PA, USA) under magnification (20X) (MC-3101, DF Vasconcellos, São Paulo, Brasil).

A preservative-free 1% nalbuphine solution was industrially manufactured (736/08, Cristália, Campinas, Brasil). Hydrochloric acid and sodium hydroxide were added to adjust the pH to 6.0 to avoid precipitation of the salt.

After induction of corneal ulceration, the animals were picked at random and divided into two groups of 10 individuals. Twelve hours after the surgeries, 50μl of 1% nalbuphine were instilled on the left eye of the rabbits of the nalbuphine group (NG) every day at 7, 11, 15 and 19 hours until the corneal re-epithelialization. The animals on the control group (CG) received 0.9% NaCl using the same regimen described for the NG. Both groups were also topically treated with 0.3% tobramycin (Tobrex, Alcon®, São Paulo, SP) at the same time points before mentioned, observing a 10 minutes interval after instillations of nalbuphine or saline solution.

Central corneal touch threshold (CCT) was assessed by a blinded examiner using a Cochet-Bonett® esthesiometer (Lunaeu, France). The rabbits were conditioned with the procedure for 24 hours; on the next day, baseline values were recorded 10 minutes after treatment with either 1% nalbuphine or 0.9% NaCl, at 7, 11, 15, and 19 hours. On the day of surgery, CCT

were measured 12 hours after keratectomies, 20 minutes after treatments. On the following days, CCT were assessed twice a day, always at 7 and 19 hours. Nylon filament length was converted to grams per millimeters squared (g mm^{-2}) in accordance with instructions provided by the manufacturer.

After the end of the keratectomy procedures and at 14 hours on the consecutive days, fluorescein was instilled on the corneas for evaluation with slit lamp (SL-15, Kowa Optimed Inc., Japan) and cobalt filter by the same blinded examiner. Images were taken with a digital camera (Cybershot 7.1® Sony do Brasil, São Paulo, SP, Brasil) at a fixed distance of 10cm and the dimensions of the wound areas were measured using an image analysis software (Image J, rsbweb.nih.gov/ij/). The percentage of the wound area was calculated as described previously (YANG et al., 2010).

Statistical analysis was performed using SigmaStat 3.0® software (Systat Software Inc, San Jose, USA). Data were normally distributed by Komogorov-Smirnov test (P>0.01), therefore repeated measures ANOVA followed by Bonferroni multicomparison test was used to check for differences in the percentage of wound area and corneal touch threshold between each time point. Two tailed unpaired Student t-test were used to assess if there were differences in the corneal wound healing rate between groups. In each test, P<0.05 was considered significant. All results will be expressed as mean and standard deviation (±SD). Area under the curve was calculated after analysis of the percentage of wound area and CTT.

RESULTS

Before induction of corneal ulceration, corneal touch threshold (CCT) (g mm^{-2}) did not change significantly after 1% nalbuphine instillation at any time point (P=0.91).

Twelve hours after keratectomy, no significant differences in average CTT (P>0.05) and baseline (P>0.05) values between NG and CG were found. From 24 hours after the surgery and until corneal re-epithelialization, average CTT values increased in NG when compared to those of CG, however such difference was not significant, at any time point (P>0.05) (Figure 1). A larger area under the curve was observed in NG (2771), in comparison to CG (2164) (Figure 1).

Corneas of NG re-ephitelialized in a faster rate (7.40±0.47 days) than corneas of CG (8.90±0.31 days), but such difference was not significant (P=0.11) (Figure 2). The percentage of wound was not significant between right and left eyes in both groups at my time point (P>0.05).

DISCUSSION

Control of corneal pain may be achieved by local delivery of non steroidal anti-inflammatory drugs (AINES). However, AINES such agents may be deleterious to the corneal epithelium and increase the expression of lytic enzymes, such as matrix metallopoteinases (REVIGLIO et al., 2003). Such statements motivated the present study with topical nalbuphine.

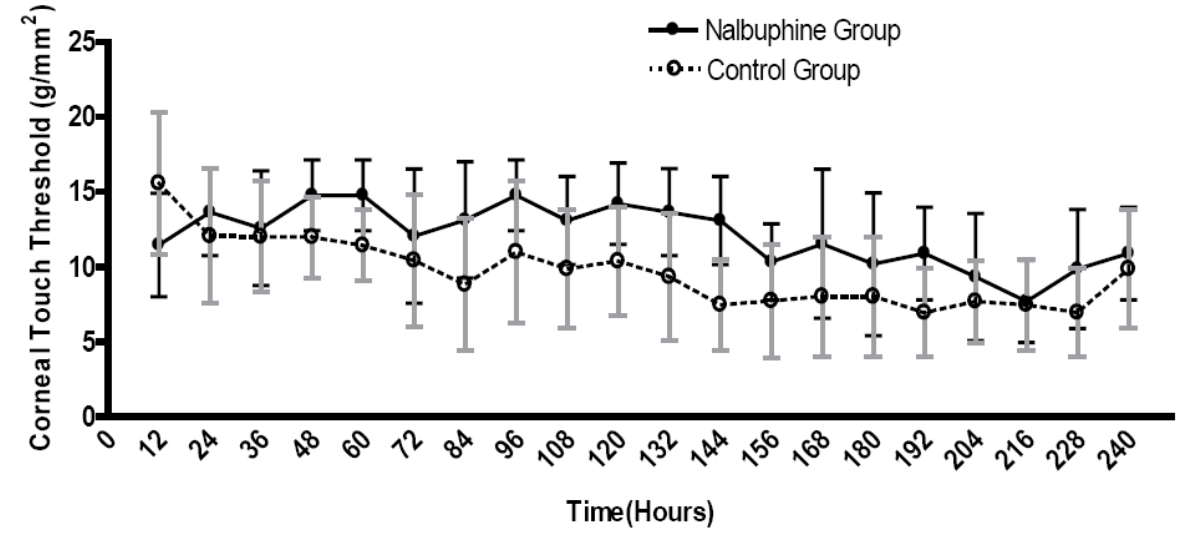

Figure 1 - Mean±SEM* corneal touch threshold values (g mm^{-2}) of control and nalbuphine-treated groups after keratectomy. *Bonferroni test (P>0.05)

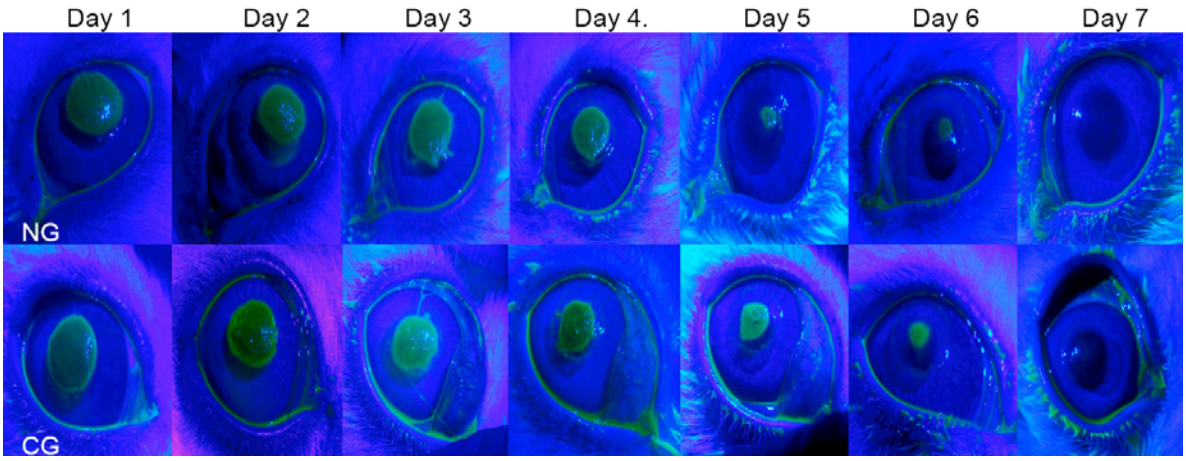

Figure 2 - Representative figure showing evolution of corneal wound healing, from day 1 to day 7 in white New Zealand rabbits of control (CG) and nalbuphine-treated group (NG).

In this study, topical instillation of 1% nalbuphine did not alter corneal touch threshold (CTT) in intact corneas. Similar results had been found for healthy corneas of rats, rabbits, dogs, and horses treated with topical morphine and nalbuphine (STILES et al., 2003; WENK et al., 2003; RIBEIRO et al., 2010; WOTMAN & UTTER, 2010). Our findings are in agreement with those of STILES et al. (2003) and WENK et al. (2003) which stated that P substance, calcitonin gene-related peptide, and prostaglandins synthesis are necessary for opioids to promote corneal analgesia. Another explanation to the inefficacy of nalbuphine 1% used in healthy corneas may be related to low concentration of the agent. One study showed that the same concentration of this drug promoted corneal analgesia in healthy corneas of dogs, 30 minutes after instillation (AQUINO et al., 2005), while in another, analgesic effects was not seen in intact and keratectomized corneas of dogs (CLARK et al., 2009). Such discrepancies may be attributed to previous conditioning of the dogs and the examiners with the procedures (AQUINO et al., 2005; CLARK et al., 2009; WOTMAN & UTTER, 2010).

Even without statistical significance, 12 hours following keratectomies, rabbits treated with nalbuphine showed higher CTT scores, in comparison with controls. The results observed in the present study suggest that the kappa agonist fraction of nalbuphine may promote some analgesic effect. The presence of SP and opioid receptors µ and ä in the corneas of dogs and rats is reported in the literature (STILES et al., 2003: SELBACH et al., 2005). RIBEIRO et al. (2010) observed corneal analgesia 11 hours after lamellar keratectomy in rabbits treated with topical morphine, pointing out that opioid receptors may be synthesized in inflamed corneal tissue of this species. One may consider that, in rabbits, the contingent of ê receptors (site of action of nalbuphine) may be lower than the µ receptors, justifying the action of morphine (pure opioid agonist) in corneas of this species (RIBEIRO et al., 2010).

In dogs, local delivery of 1% nalbuphine did not alter CTT after keratectomy (CLARK et al., 2009). Such results differ from the ones seen in the present study. It has been shown that 1% nalbuphine solution is unstable in temperature and pH considerated physiologic (SPATOLA et al., 2009). In addition, factors other than pH that may affect bioavailability of agents delivered locally include corneal penetrability, corneal stromal metabolism, and corneal stromal protein binding (WARD, 1996). Particle size may be the most important formulation in determining the bioavailability of the active molecule suspension (ROBERTS & NELSON, 2007).

Corneal analgesia was not observed in human beings with corneal ulcers treated with 10µg of topical fentanil (ZÖLLNER et al., 2008). The authors concluded that a failure in achieving analgesia may be related to the low concentration used, uncompatibility of the agent with the adjuvant, decreasing corneal penetration or rapid penetration to the anterior chamber due to high liposolubility of the fentanil (ZÖLLNER et al., 2008).

In the present study, topical 1% nalbuphine did not decrease corneal epithelialization rate. Similar results were reported by CLARK et al. (2009), in dogs with experimental keratitis treated with the same drug. In vitro, toxicity to stromal cells of dogs placed in 1% nalbuphine was seen in acute manner; lower toxicity was produced in the corneal epithelium in the same experiment (SPATOLA et al., 2009).

Opioid growth factor (OGF) and its receptors are present in the mammalian cornea (ZAGON et al., 1998; ROBERTSON & ANDREW, 2003). The instillation of naltrexone, an unspecific opioid receptor antagonist, accelerates corneal re-epithelialization in rabbits, humans, and in diabetic rats by inhibiting the action of opioid growth factor (ZAGON et al., 1998). However, local morphine, a pure opioid agonist was not deleterious to corneal epithelialization in rabbits (RIBEIRO et al., 2010). Even without statistical significance corneas of rabbits treated with 1% nalbuphine re-epithelized, on average, 2 days earlier than the ones treated with saline. This may have occurred due to the antagonist effects of nalbuphine over OGF receptors and μ receptors.

CONCLUSION

In conclusion, it is reasonable to admit that topically applied 1% nalbuphine did not promote corneal analgesia, nor accelerated corneal epithelialization rate in rabbits after experimental lamellar keratectomy.

BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

Protocol Approval (protocolo nº 028793-08).

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