

# Influence of topical anesthetics on oculocardiac reflex and corneal healing in rabbits

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## Abstract

• **AIM:** To investigate the incidence of oculocardiac reflex (OCR) with two anesthetic regimens and its prevention using topical anesthetics in a rabbit model, and to explore the effect of topical anesthetics on corneal healing.

• **METHODS:** Forty-eight clinically healthy adult New Zealand white rabbits of either sex were divided into two groups (Group A and B) and anesthetized with either ketamine (Group A,  $n=24$ ) or propofol (Group B,  $n=24$ ). The incidence of OCR was recorded in each group with a variety of ocular manipulation with or without the use of topical anesthetics (40g/L lignocaine, 5g/L proparacain, 5g/L bupivacaine). Corneal toxicity and healing following the use of each topical anesthetic was assessed one day after surgery and up to 7 days postoperatively by clinical examination of the eye, histopathology and collagen staining and transmission electron microscopy.

• **RESULTS:** No incidence of OCR was recorded with ocular manipulation under ketamine anesthesia, whereas significant reduction in heart rate ( $P < 0.01$ ) was recorded under propofol anesthesia. Topical anesthetics could successfully prevent the OCR without affecting the corneal healing.

• **CONCLUSION:** Topical anesthetics may be recommended for prevention of OCR without any local adverse effect.

• **KEYWORDS:** oculocardiac reflex; topical anesthetics; corneal healing; ketamine; propofol

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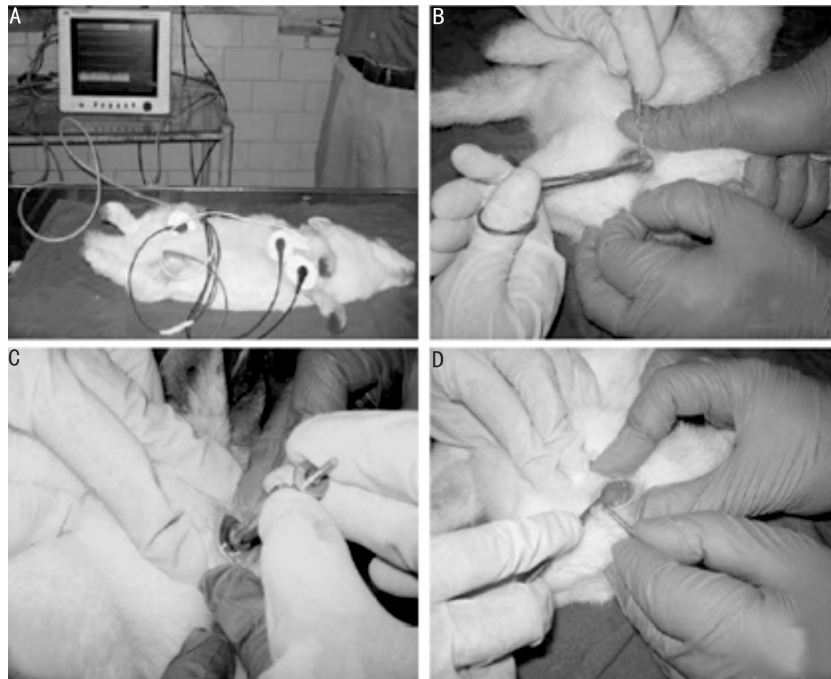
## INTRODUCTION

Oculocardiac reflex (OCR) is a physiological response of the heart to physical stimulation of the eye or the ocular adnexa, characterized by bradycardia or arrhythmia, which sometimes leads to cardiac arrest. It is defined as 10% decreases in heart rate (HR) or occurrence of any arrhythmia induced by traction during the extraocular muscle surgery of recession-resection type or manipulation of extraocular muscles during other eye surgeries. The incidence of OCR has been encountered with strabismus surgery, ranging from 32% to 90%. Transient cardiac arrest could be as frequent as 1 in 2200 corrections of strabismus depending upon the methods used and the criteria of evaluation chosen. The OCR has also been reported in LASIK<sup>[1]</sup>. It has been observed during eye muscle surgery, repair of detached retina, compression of gasserian ganglion, enucleation of eye, by contact lens<sup>[2]</sup>, and repair of nasal fracture under general anesthesia.

The anesthetic regimen modulates the expression of OCR<sup>[3-5]</sup>. Several preventive strategies<sup>[6,7]</sup> have associated complications<sup>[8]</sup>. Pediatric ophthalmic surgery is usually performed under general anesthesia. We have studied the incidence of OCR under ketamine and propofol anesthesia, commonly used for pediatric ophthalmic surgery<sup>[9,10]</sup>, in a rabbit eye model and its prevention by using topical anesthetic. Topical anesthetics are recognized as excellent corneal analgesic, but their toxic effect on corneal epithelial cells limits its use during corneal epithelial wound healing. Mechanism of the impairment of corneal reepithelialization with topical anesthetics, however, has not been evaluated<sup>[11]</sup>. On the other hand several literatures have reported the safety of using local anesthetics topically on the eye<sup>[12,13]</sup>. We therefore sought to investigate the effect of topical anesthetics on corneal healing.

## MATERIALS AND METHODS

**Materials** The work was carried with prior permission of Institutional Animal Ethics Committee and abiding by the tenets of Association of Research in Vision and Ophthalmology. The study was conducted on forty-eight clinically healthy adult New Zealand white rabbits of either



**Figure 1** Continuous monitoring and recording of ECG in rabbits A: Normal; B: Applying traction on extraocular muscle; C: Creating corneal defects using a 6.5mm trephine; D: Superficial keratectomy

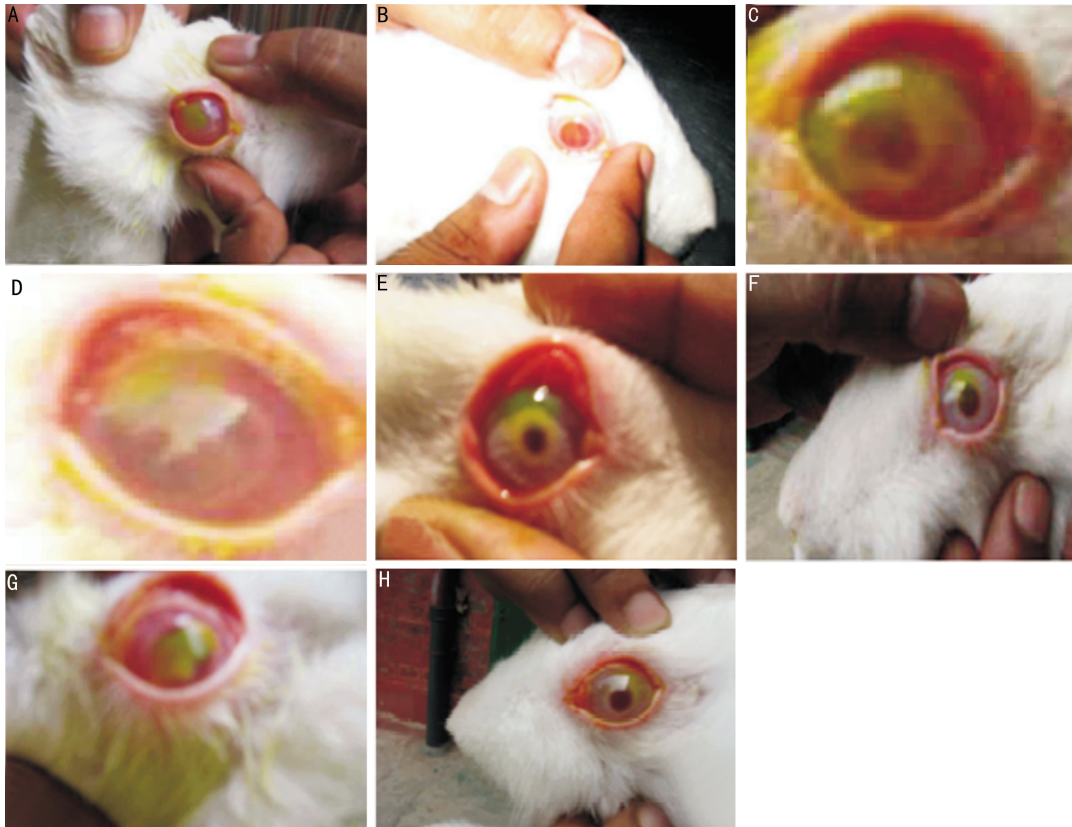
sex between the age group of 1-1.5 years and weighing between 3-3.5kg. Routine clinical evaluation and preoperative ophthalmic examination of both eyes of all the animals was done prior to the experiment.

**Methods** The total number of animals were randomly divided into two groups; Group A and B consisting of twenty-four in each group irrespective of age, weight and sex. In this study, all the 24 animals of Group A, received ketamine hydrochloride (Ketmine 50<sup>®</sup>, Themis Medicare Ltd Gujrat India) at the dose of 35mg/kg as general anesthetic, and animals of Group B were administered propofol (10g/L propofol, 10mL, Cleris Life Sciences L.T.D., Ahmedabad) at the dose of 7mg/kg as general anesthetic.

After induction of anesthesia, ECG was recorded using continuous cardiac monitor (Excello+REC Multiparamonitor BPL Limited India) (Figure 1A) immediately in both the groups. In Group B, ECG recorded 3 minutes after induction of anesthesia was considered basal heart rate for this group. All ocular manipulations were performed in surgical stage of anesthesia. The depth of anesthesia was assessed by head shaking in response to ear pinching and pedal reflex. At the surgical stage of anesthesia the entire study for OCR was studied in two phases. In initial phase all procedures to incite OCR were done without the use of topical anesthesia. A gap of five minutes was allowed after each type of ocular manipulation i.e. traction on extraocular muscle, application of digital pressure on various sites on the eye ball and superficial keratectomy. This was to register the effect of each type manipulation on the heart rate. A gap of five

minutes was allowed to abolish the effect of the previous manipulation. Surgical manipulations were done by applying traction on medial rectus, superior rectus; lateral rectus (Figure 1B). A conjunctival forceps was used to hold the extraocular muscle which was easily identified through the thin conjunctiva in the rabbit eye. Each muscle was held and traction was applied. Any change in heart rate was registered, OCR was incited by applying digital pressure on the eyeball, and the index finger was used to apply pressure on the eye ball on all superior, inferior medial and lateral positions. Uniform corneal defects were created using a 6.5mm trephine (Figure 1C), followed by mechanical superficial keratectomy using No.11 blade(Figure 1D). ECG was recorded continuously during the ocular manipulations. A 10% reduction in heart rate was considered positive for oculocardiac reflex.

In the second phase still maintaining the surgical level of anesthesia, the rabbits of each group were divided into four subgroups, with six animals in each subgroup. The animals were given rest for few minutes following surgical manipulation of the first phase till the heart rate came back to normal baseline as observed by continuous cardiac monitoring. Similar surgical manipulation by the same surgeon was repeated in each subgroup after using 2 drops either of the topical anesthetics instilled on the cornea, lignocaine 40g/L (Xylocaine<sup>®</sup> 40g/L topical, Astra Zeneca LTD., Bangalore), bupivacaine 5g/L (Sensorcaine<sup>®</sup> 5g/L 20mL, Astra Zeneca LTD., Bangalore), proparacaine 5g/L (Carecain<sup>®</sup> 5g/L, Ajanta Pharma India LTD Bangalore)



**Figure 2** Corneal healing as detected by fluorescein dye test A: Day 1 untreated; B: Day 4 untreated; C: Day 1 with lignocaine; D: Day 4 with lignocaine; E: Day 1 with proparacaine; F: Day 4 with proparacaine; G: Day 1 with bupivacain; H: Day 4 with bupivacain

while in the fourth subgroup no topical anesthetic was administered and used as control.

Extent of corneal injury (wound), on the treated eyes of all 48 animals, was examined on 1<sup>st</sup> postoperative day. After one day of experiment, six rabbits from each subgroup (subgroups of different topical anesthesia and control) were randomly selected and euthanized with overdose of thiopental sodium (Thiosol<sup>®</sup> 500mg, Neon Laboratories, Mumbai) and corneal tissues were collected in glutaraldehyde for transmission electron microscopy (TEM) (Techni g-2 Biotwin Fet Holland, Philips).

Fluorescein dye test was performed on 6 rabbits from each subgroup following surgery for recording the course of clinical healing. Postoperative intraocular changes were monitored by slit lamp biomicroscopy and ophthalmoscopy. After 7 days the animals were euthanized by the same procedure, corneal tissues were collected in 40g/L formalin for histopathology and the corneal tissue sections were stained with hematoxylin eosin and sirius red following standard procedure.

Postoperative medication comprised of analgesic Inj meloxicam (Melonex<sup>®</sup>, Intas Pharmaceuticals, Ahmedabad, India) after recovery from anesthesia and topical instillation of eye drops Ciprofloxacin (Ciplox<sup>®</sup>, Cipla India) and Flurbiprofen (Flur<sup>®</sup>, Allergan India), twice a day till euthanasia. Postoperative treatment comprised of topical

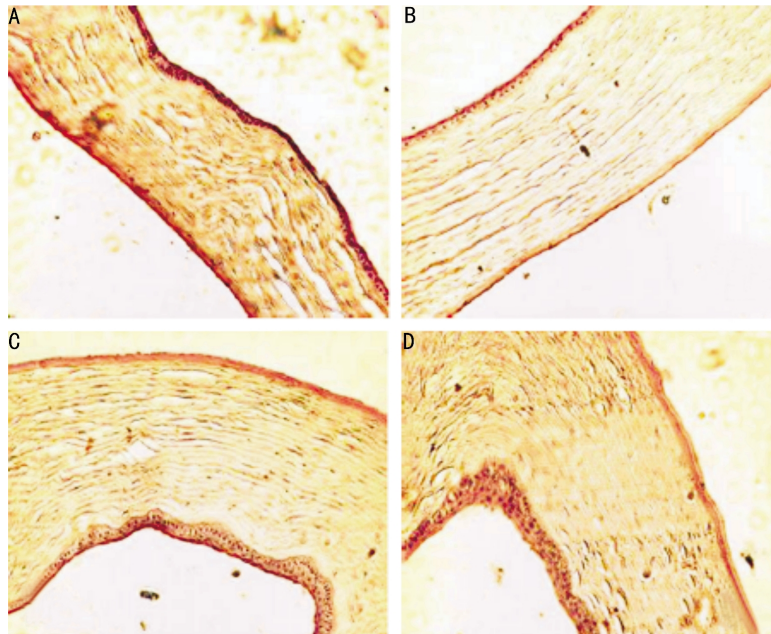
instillation of ciplox eye drops and flurbiprofen eye drops. A temporary tarsorrhaphy was done in all rabbits for 24 hours.

## RESULTS

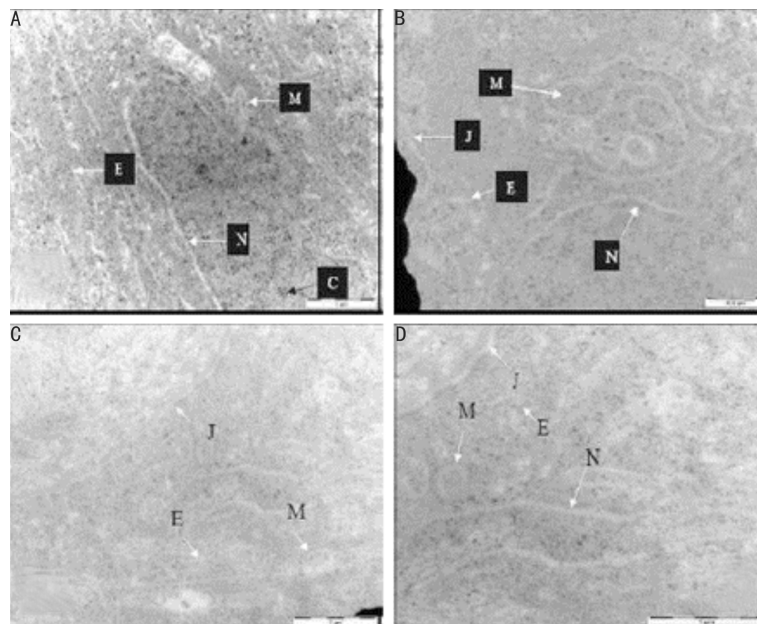
All rabbits attained surgical stage of anesthesia as assessed by response to ear pinching and pedal reflex. The mean basal heart rate immediately after ketamine anesthesia ( $275 \pm 9/\text{min}$ ) did not vary significantly ( $P < 0.01$ ) after ocular manipulation ( $270 \pm 10/\text{min}$ ). Use of either of the topical anesthetics proparacaine, lignocaine, bupivacaine before ocular manipulations in this group of animal, did not significantly change the heart rate ( $270 \pm 7$ ,  $270 \pm 6$ ,  $264 \pm 6/\text{min}$ ) recorded with ocular manipulation under ketamine alone.

A significant reduction in heart rate was recorded after ocular manipulation ( $245 \pm 11/\text{min}$ ,  $P < 0.01$ ) under propofol anesthesia as compared to the basal heart rate ( $278 \pm 8/\text{min}$ ) recorded after propofol anesthesia. Use of topical anesthetics proparacaine, lignocaine, bupivacaine prior to ocular manipulation in this group of animal significantly ( $P < 0.01$ ) prevented the decreased heart rate recorded after ocular manipulation under propofol anesthesia alone ( $vs$   $270 \pm 5$ ,  $277 \pm 4$ ,  $276 \pm 6/\text{min}$ ).

Uniform gradual reduction in the diameter of created corneal defect was observed after topical fluorescein dye test in all the groups (Figure 2 A-H). Any intraocular changes i.e. uveitis, hypema, lens opacity or retinal changes were not observed in any animal during the period of study. Lack of



**Figure 3 Histopathology of rabbit treated with topical anesthetics** A: Proparacain; B: Lignocain; C: Bupivacain; D: Control



**Figure 4 Ultrastructure of rabbit cornea treated with topical anesthetics** A: Normal; B: Bupivacaine; C: Lignocain; D: Proparacain. (N: Nucleus; M: Mitochondria; E: Endoplasmic reticulum; J: Cellular junction)

anterior epithelial cells and uniform arrangement of collagen in the stromal layer was seen in sections of cornea subjected to superficial keratectomy (Figure 3A-D). Histological sections stained either with hematoxylin eosin or sirius red did not reveal any difference in corneal healing and collagen deposition between the control and topical anesthetics group (Figure 4A-D).

Ultrastructural study of the portion of the intact cornea treated with topical anesthetic showed no intracellular changes and was similar to section of the untreated cornea. The cellular junctions appeared normal. The nuclear membrane integrity was preserved and the chromatin appeared uniformly distributed. No dilatations of the

endoplasmic reticulum and intact mitochondrial outer and inner membrane were observed.

#### DISCUSSION

Oculocardiac reflex (OCR) is mainly encountered during strabismus surgery, with incidence of OCR ranging from 32% to 90%. It is also observed during LASIK<sup>[1]</sup>, eye muscle surgery, repair of detached retina, compression of gasserian ganglion, enucleation of eye, by contact lens<sup>[2]</sup>, and repair of nasal fracture under general anesthesia. As the expression of OCR is influenced by the anesthetic regimen, we have investigated the incidence of oculocardiac reflex with ketamine and propofol anesthesia by a variety of ocular stimulus in rabbit. We have also used topical anesthetics for

prevention of OCR. No preanesthetics i.e. atropine sulphate or other anesthetic combination was used to avoid any masking effect of preanesthetics on the incidence OCR with the two anesthetics under study.

Rabbit is a preferred model for assessment of new technologies as well as ophthalmic surgical procedures for its large size of the eye<sup>[14]</sup>, docile nature and lower cost compared to dog or primate. Rabbit has been used as a model for studies on oculocardiac, oculo-respiratory reflex. In the present study ocular manipulations under ketamine anesthesia did not produce any significant change in the heart rate. However, a significant reduction in heart rate was recorded after ocular manipulation under propofol anesthesia. Ten percent decrease in heart rate or occurrence of any arrhythmia induced by extraocular muscle manipulation is considered as OCR in the present study. In case of propofol anesthesia the basal heart rate was recorded 3 minutes after induction to eliminate the transient increase in heart rate<sup>[15]</sup> which occurs after propofol anesthesia and to make the basal heart rates of both anesthetics comparable. Similar studies report lower incidence of OCR with ketamine anesthesia in comparison to other anesthetic combinations<sup>[16]</sup>. Low incidence of OCR under ketamine anesthesia could be attributed to its profound analgesic property as we hypothesize and also due to inhibition of the parasympathetic reflex. On the contrary propofol has a potency to increase bradycardia by central sympatholytic effect and vagal stimulation. Hahnenkamp *et al*<sup>[17]</sup> explored the incidence of OCR using sevoflurane, halothane, propofol and ketamine as the main anesthetics and found the least incidence with ketamine and the highest with propofol.

Proparacaine, lignocaine, bupivacaine significantly prevented the decrease in heart rate resulting from various ocular manipulations under propofol anesthesia in the present study. Effect of topical anesthetics for prevention of OCR has been studied mainly in strabismus surgery<sup>[18,19]</sup>. Use of topical anesthetics on the cornea for the prevention of OCR and its influence on corneal healing has not been studied so far. Instillation of topical anesthetics directly on the medial rectus muscle significantly reduced the incidence of the OCR in pediatric and adult patients undergoing strabismus surgery, this involves dissection of tissues to expose the rectus muscles. Topical application of bupivacaine, procaine, and benzocaine, lidocaine, did not show any toxic effect on cornea and corneal reepithelization. But tetracaine and proparacaine are reported to have toxic effects on stromal keratocytes related not only to drug concentrations but also to time exposure. This finding raises concern that widespread use of topical anesthetic might affect the stromal wound healing. Contrary to such concern effect of single topical application of lignocaine, bupivacain and proparacain

did not influence the corneal healing following superficial keratectomy in our study. The healing was comparable to the untreated control group as evident from clinical observation, histopathological study, and collagen staining and ultrastructural study of cornea.

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