Anesthesia for Intraocular Surgery in Rabbits

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Summary

The purpose of the study was to assess a ketamine/xylazine combination along with retrobulbar block using 4% lignocaine HCl for performing intraocular surgery in rabbits. To do so ten healthy adult New Zealand White rabbits aged 1.5-2 years of either sex weighing 2-3 kgs were selected for unilateral cataract extraction by phacoemulsification . Xylazine HCl was used as preanesthetic at a dose rate of 5 mg/kg, i.m., followed by Ketamine HCl at a dose rate of 35mg/kg, i.m. A retro bulbar block was performed with 4% lignocaine HCL. The anesthetic depth was judged by ear pinching reflex. The time for onset and duration of anesthesia was found to be 7±0.5 minutes and 35.5±1.2 minutes respectively. A central eyeball fixation following retro bulbar block was obtained in all the cases. The mean values recorded before induction of anesthesia and during anesthesia of rectal temperature (38.09^0C±0.2 vs. 37.29^0C±0.2), heart rate in beats per minute (276±1.2 vs.272±1.1), arterial blood pH (7.35±0.02 vs. 7.32±0.02), bicarbonate (16.2±1.2 vs. 20.3±1.8), PaCo2 (26.09±2.3 vs. 27.52±2.3) and PaO2 (84.79±1.9 vs. 80.39±1.1) did not vary significantly (P>0.05). Recovery was smooth and complete in 32.5±2.8 minutes.

Conclusion: the anesthetic regimen provides adequate condition for conducting intraocular surgery in rabbits.

Introduction

The rabbit eye is ideally suited for ophthalmic research for several reasons. The animal is docile, easy to handle and its use is relatively economical in comparison to other mammals like dogs or primates. The large size of the eye is a major advantage for assessment of new technologies as well as ophthalmic surgical procedure (*Arlene, 2008)*. The use of general anesthesia is imperative for conducting intraocular surgery in the rabbit; the optimum ophthalmic requirements for conducting intraocular surgery are central positioning of the globe, relaxation of the extra ocular muscles, and maintenance of intraocular pressure (IOP).

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The combination of xylazine and ketamine has been popular for inducing general anesthesia in rabbits. When considering the suitability of this combination for intraocular surgery it is to be noted that increase in intraocular pressure due to the influence of ketamine (*Bar-Ilan & Pessah, 1986*) may be counteracted by muscle relaxation property of xylazine *(Burke & Potter, 1986*) Though at surgical levels of anesthesia, deviation of the eyeball medially and ventrally in the orbit is observed with various anesthetics, which prevents the optimum exposure of the cornea (*Lumb & Jones, 1996*); hence the globe needs to be rotated back to the central position. The use of stay sutures for this purpose may lead to hemorrhage and tearing of the tissues usually associated with excessive traction. Therefore the anesthetic regimen must be appropriate to maintain the central position of the globe also.

The retrobulbar technique is a useful procedure done with the objective to produce proptosis of the eyeball, turn it and improve exposure of the cornea,

Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

and to provide stability to the eye (*Gelatt & Gelatt*, *2001*) The idea that combined use of a retrobulbar block along with ketamine and xylazine anesthesia to achieve appropriate conditions in rabbits encouraged us to evaluate this anesthetic protocol to perform phacoemulsification in rabbits. The aim was to provide a safe, simple, and effective anesthesia suitable for intraocular surgery in rabbits.

Materials and Methods

The work was carried out with prior permission of Institutional Animal Ethics Committee and abiding by the tenets of Association of Research in Vision and Ophthalmology.

Ten adult white New Zealand rabbits aged 1.5-2 years, of either sex weighing 2-3 kgs were selected for unilateral cataract removal by phacoemulsification. The rabbits were obtained from a registered animal breeding facility (Indian Institute of Chemical Biology, Jadavpur Kolkata, India). The rabbits were housed in individual stainless cages (60 x 50 x 60cm high), received water and a standard pellet diet ad libitum. Bedding material was not used. The humidity ranged between 40 and 60 %. A uniform temperature of 22±2°C was maintained throughout with a 12:12h light: dark cycle.

The rabbits were screened for pre-existing ocular disorders and clinical assessment was performed to ensure adequate health status. Topical medication initiated prior to surgery included prednisolone (Prednisolone Acetate ophthalmic suspension®, Allergan India Pvt. Ltd., Bangalore, India), flubiprofen (FNB, eye drops® Microlabs Ltd., Bangalore, India), and chloramphenicol eye drops (Bromycetin®, Martin and Brown Pharmaceuticals, Hissan, India). Mydriasis was achieved by 10 % phenylephrine (Decomic®, Klas Sehen Pvt. Ltd., Kolkata, India), given topically BID from 3 days prior to surgery, and were given TID on the day before surgery and applied every 30 min up to initiation of surgery. Preoperative IOP of the eyes to be operated upon for each rabbit (mean value of three reliable averaged readings) were recorded following instillation of topical anesthetic, lignocaine hydrochloride 4% (Xylocaine Topical®; Astra Zeneca Pharma India Ltd., Bangalore, India) by applanation tonometry (Tonopen, Reichert). The rabbits scheduled for unilateral cataract extraction were placed in lateral recumbency. Arterial blood was drawn from the central auricular artery and kept in ice for blood gas analysis. Anesthesia was induced with a combination of xylazine HCl (Xylaxin®; Indian Immunologicals Ltd., Gottapadu, India) at a dose of 5 mg / Kg, i.m., followed by ketamine HCl (Ketmin50®; Themis Medicare Ltd., Mumbai, India) at a dose of 35mg /Kg, i.m. The anesthetic depth was judged by ear pinching reflex. Absence of ear pinch response were considered as the surgical stage of anesthesia. For endotracheal intubation the head and neck was held in atlantooccipital extension to displace the epiglottis to provide a straight passage for the endotracheal tube. Local anesthetic 2% lignocaine HCl was sprayed into the larynx, the mouth was opened and a neonatal endotracheal tube of internal diameter 2.5mm (Portex® Tracheal Tube, Smiths Medical ASD, Mexico) was advanced into the larynx by mild rotation placed by listening for breath sounds through the tube.

A retrobulbar nerve block was performed by inserting a 2.5 cm, 20-gauge needle at the lateral canthus of the eye. The needle was passed between the eyeball and the orbit and directed towards the opposite mandible until the medial orbital wall could be felt and 0.3 mL of 4 % Lignocaine HCl (Xylocaine®; AstraZeneca Pharma India Ltd) was deposited extraconaly immediately behind the globe. The eye position and IOP were recorded immediately after achieving general anesthesia and retro bulbar block. The time of onset, depth and duration of anesthesia and recovery from anesthesia were recorded. Monitoring during anesthesia was done by recording rectal, temperature, respiratory rate, heart rate and blood gas analysis., the first three being measured throughout the procedure (BPL Excello Cardiac monitor).Oxygen was supplied to all the rabbits during surgery at a rate of 400ml/min and was continued for up to 5 minutes after completion of surgery.

Phacoemulsification was performed and an intraocular lens was placed in all rabbits. The corneal incision was closed with two 10-0 nylon simple interrupted sutures. The time of recovery from anesthesia was recorded as the period of between return of the ear pinch reflex to the animal being able to walk. Any signs of distress during recovery were recorded. Postoperative medication comprised of analgesics flubiprofen (FNB, eye drops® Microlabs Ltd., Bangalore, India), antibiotics (ciplox eye drop) and anti-inflammatory drugs prednisolone (Prednisolone Acetate ophthalmic suspension®, Allergan India Pvt. Ltd., Bangalore, India).

Figure 3. Testing for positive intubation using cotton wisk.

Figure 1. Recording IOP in anesthetized rabbit using applanation tonometer.

Figure 4. Collection of arterial blood from central auricular artery of rabbit for blood gas analysis.

Figure 2. Performing blind endo tracheal intubation in rabbit.

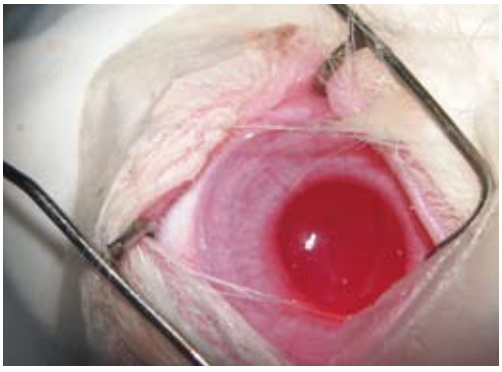

Figure 5. Ventromedial deviation of globe at surgical stage of anesthesia.

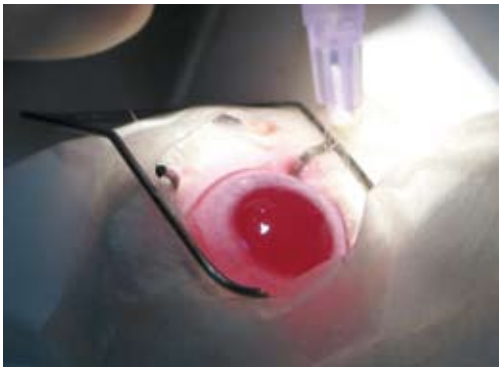

Figure 6. A retro bulbar nerve block being performed by inserting a 2.5 cm, 20-gauge needle.

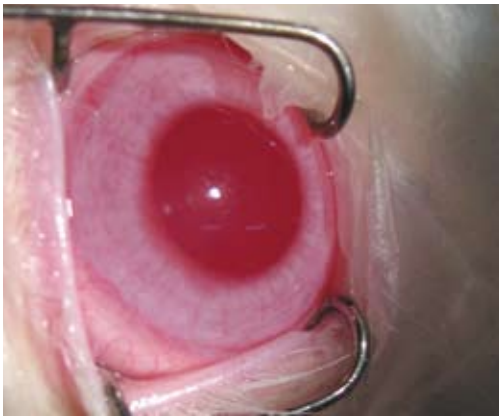

Figure 7. Excellent central fixation of globe achieved after successful retrobulbar block.

Statistical analysis

Statistical analyses were performed using Students t-test. Values of $P < 0.05$ were considered statistically significant.

Results

Endotracheal intubation was performed successfully in all the rabbits. The exposure of the eyeball in all rabbits was adequate; the desired central eye position was obtained in all rabbits. The mean pre-anesthetic (30.8±1.5) and post-anesthetic (31.4 ±1.7) IOP did not differ significantly (P>0.05) (Table 1). The time for onset of anesthesia was 7±0.5 minutes.

Table 1. Mean IOP before and after induction of anesthesia.

Pre-anesthetic IOP (mmHg)	Post-anesthetic IOP (mmHg)
30.8±1.5	31.4 ±1.7

The data represented as mean±SEM. N=10. *P>0.05.*

The anesthesia achieved with a combination of xylazine and ketamine was of adequate depth to carry out the operation and was maintained for an average period of 35.5±1.2 minutes until completion of surgery. No major intraoperative complication was encountered, following the phacoemulsification and placement of intraocular lens; corneal sutures could be successfully placed. The mean values of various parameters recorded before induction of anesthesia and during maintenance of anesthesia i.e. rectal temperature (38.09^0C±0.2 vs. 37.29^0C±0.2), heart rate in beats per minute (276±1.2 vs.272±1.1), arterial blood pH (7.35±0.02 vs. 7.32±0.02), bicarbonate (16.2±1.2 vs. 20.3±1.8) in mmol/L, PaCo2 (26.09±2.3 vs. 27.52±2.3) and PaO2 (84.79±1.9 vs. 80.39±1.1) in mm of Hg did not vary significantly (P>0.05), though a significant (P<0.05) change in respiratory rate (100.0± 0.3vs. 69.0±1.1) in breaths/min, was recorded (Table 2). No major changes in rectal temperature, heart rate, and respiratory rate were recorded intraoperatively in the continuous monitor. No immediate postoperative complication was observed. The anesthetic recovery was smooth in all the rabbits and complete recovery period was 32.5±2.8 min; absence of blepharospasm and retention of normal blinking reflex were recorded immediately after recovery.

Discussion

Rabbits are extensively used in eye research for their large eyes, easy handling and economical maintenance compared to dogs or primates. Rabbits have proved to be very useful especially for evaluation

Table 2. Values of rectal temperature, respiratory rate, heart rate, pH, bi-carbonate, PaCo2, PaO2 immediately before induction of anesthesia and during anesthesia.

	Temperature in^{0}C	Respiratory rate in breaths / min	Heart rate in Beats/ min	Arterial blood pH	Bicarbonate In mmol/L	PaCo2 In mm of Hg	PaO2 In mm of Hg
Before induction of anesthesia	38.09±0.23	100.00± 0.27	276±1.24	7.35±0.018	16.2±1.19	26.09±2.3	84.79±1.9
During anesthesia	37.29±0.25	69*±1.08	272±1.09	7.32±0.019	20.3±1.77	27.52±2.3	80.39±1.1

The data represented as mean± SEM. N=10. *P>0.05 vs.* pre-anesthetic value.

and adaptation of new ophthalmic technologies and surgical procedures (*Arlene, 2008*)

General anesthesia is imperative for all intraocular surgical procedures in rabbits. Primary objectives of anesthesia for ocular surgery are to maintain normal intraocular pressure, cardiopulmonary function and complete immobilization of the eye (*Lumb and Jones, 2001)*

Ketamine HCl and xylazine HCl were selected as a combination to study as it is effective, easy to administer and inexpensive (*Lipman et al., 1990)*. Ketamine HCl is a cyclohexanone compound, which provides a state of dissociative anaesthesia with analgesia and deep sedation. When it is combined with xylazine, good muscle relaxation can be achieved, and analgesia is prolonged (*White & Holmes, 1976; Hall & Clarke, 1983*). In our previous study, we reported ketamine HCl, xylazine HCl along with retrobulbar block to be an excellent anesthetic regimen for performing intraocular surgery in the dog *(Hazra et al., 2008).*

In contrast to the report of unpredictable results with this combination (*Peeters et al.,1998),* in the present study we achieved surgical anesthesia in all the rabbits (*Lipman et al., 1990)*. There was no significant change in the intraocular pressure with this anesthetic regimen. Ketamine HCl is reported to increase IOP in rabbits (*Schutten & Van Horn, 1977*). In the present study, the effects of ketamine HCl on IOP might be counterbalanced by xylazine HCl, which is reported to reduce intraocular pressure *(Burke & Potter, 1986)*

Central fixation of the eyeball was successfully achieved in all the cases following retrobulbar block with 4% lignocaine HCl. The retrobulbar technique is a useful procedure to produce a central eye and improve the exposure of the cornea and to provide stability to the eye (*Gelatt & Gelatt, 2001*). In spite of possible hazards associated with retrobulbar block e.g. retrobulbar hemorrhage, undesirable proptosis of the eyeball, anterior displacement of the vitreous humor and accidental penetration of the meningeal sheath surrounding the optic nerve *(Lumb & Jones, 1996)*, we did not encounter any complication while performing the retrobulbar block. The preanesthetic values of rectal temperature, heart rate, blood pH and bicarbonate PaO2, PaCo2 did not change significantly (P>0.05) during anesthesia whereas a significant (P<0.05) change in respiratory rate, was recorded. There was marked respiratory depression and metabolic alkalosis. Metabolic alkalosis has been previously reported with xylazine alone and a xylazine/ketamine combination (*Peeters et al., 1998; Nuh Kilic 2004*)

As respiratory depression is normally responsible for decrease in PaO2 and increase in PaCo2, en-

dotracheal intubations had been was performed in all the rabbits. Oxygen supplementation was done throughout the procedure based on recommendations of previous workers (*Nuh Kilic, 2004)*. Intubation in the rabbit is difficult; the diameter of the larynx being less than that of the trachea limits the size of the endotracheal tube that can be passed. Despite this, enodotracheal intubation can be accomplished by direct visualization or by blind passage of the tube *(Lumb & Jones, 1996)*

Rabbits are used by practitioners, scientists and surgeons associated with biological experimentation and experimental surgery. There is need for a safe anesthetic protocol, which would be easy to perform in absence of sophisticated anesthetic machines. Among the various injectable drug combinations used to produce successful anesthesia in the rabbit, fentanyl /fluniasone, ketamine /acepromazine, ketamine / diazepam are good options but have a prolonged duration (*Mero et al.,* 1987) or cause hypoxia *(Mero et al*., 1999).In a study, combination of ketamine / medatomidine and ketamine/xylazine anesthetic regimen in rabbits, both the techniques produced effective anesthesia, but ketamine /medatomidine was reported to have a prolonged duration of action (*Nuh Kilic , 2004*) In our study the depth and duration of anesthesia and analgesic effect with a combination of xylazine HCl and ketamine HCl was appropriate for surgery in all the rabbits and recovery was smooth, supplementation of oxygen aimed to prevent hypoxaemia. A retro bulbar block, in addition to maintaining the central eye position, also produces pupillary dilatation and reduction in intraocular pressure, which are prerequisites of intraocular surgery and makes this anesthetic protocol appropriate for eye surgery in rabbits. To our understanding this is the first report of an improvised anesthetic technique for performing intraocular surgery in rabbits.

Conclusion

The combination of ketamine HCl and xylazine HCl along with retrobulbar block with Lignocaine HCL is a suitable anesthetic regimen for performing intraocular surgery in the rabbit.

Acknowledgements

The author acknowledges the financial support of Council of Scientific and Industrial Research as part of research project for conducting the study and Dept of Veterinary Surgery & Radiology, West Bengal University of Animal and Fishery Sciences, Kolkata, India , for providing the necessary infrastructure and facilities.

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