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Action of Ropivacaine as a Surface Anaesthetic on the Cornea of Rabbits

N. Chandrasekhar, T. Jayasree, M. Vinay, Rohit Dixit, V.S. Manohar and N. Jagan Rao
Department of Pharmacology, Mamata Medical College, Rotary Nagar, Khammam Andhra Pradesh, India

Abstract: The aim of the present study was to investigate the role of Ropivacaine as a surface anesthetic on the cornea of rabbits. Twenty Albino rabbits (weighing 2.5 to 3.0 kg of either sex) were randomly allocated to 2 treatment groups-Group I and II. The upper and lower eye lashes of all the rabbits were carefully clipped off. The conjunctival sac of right eye was held open to form a pocket. Into these pockets, animals in Group I were delivered 1 drop (containing 535.71 μg of Ropivacaine) of the standard solution of Ropivacaine and those in Group II were delivered 2 drops (containing 1071.42 μg of Ropivacaine) of the same solution. The left eye served as the control. The corneal reflex was elicited by touching the cornea from the side using a cotton wisp. The time between the disappearance and reappearance of corneal reflex (duration of action) was registered. Statistical analysis revealed highly significant differences between group I and group II ($p<0.001$). The mean onset of action for group I was 10 min and for group II was 5 min. The mean duration of action for group I was 29±4.595 and for group II was 48±5.869. Ropivacaine can be used as surface anaesthetic as Proparcaine and Tetracaine for removal of foreign bodies and other clinical conditions in ophthalmology.

Key words: Ropivacaine, corneal reflex, surface anesthesia, epiduralanaesthesia, conjunctival sac

INTRODUCTION

Surface anesthesia or anesthesia of mucous membranes of the nose, mouth, throat, tracheobronchial tree, esophagus and genitourinary tract can be produced by direct application of aqueous solutions of salts of many local anesthetics or by suspension of the poorly soluble local anesthetics. Tetracaine (2%), lidocaine (2 to 10%) and cocaine (1 to 4%) are typically used. The local anesthetics are absorbed rapidly into the circulation following topical application to mucous membranes or denuded skin (Catterall and Mackie, 2006).

Cardiac toxicity of bupivacaine stimulated interest in developing a less toxic and long-lasting local anesthetic. One result of the search was the development of the amino ethylamide Ropivacaine (Catterall and Mackie, 2006). It is a newer bupivacaine congener which is superior to bupivacaine for epidural anesthesia because of its decreased potency for motor block. It has been recently approved for use in adults. It is less toxic to the central nervous system and heart and interferes less with motor function than bupivacaine (Lonnqvist *et al*., 2000). The S-enantiomer was chosen because it has a lower toxicity than the R-isomer (Catterall and Mackie, 2006). It blocks Aβ and C fibres (involved in pain transmission) more completely than Aβ fibres which control motor function. Though equi-effective concentrations of Ropivacaine are higher than those of Bupivacaine, a greater degree of separation between sensory and motor block has been obtained with epidural Ropivacaine (Tripathi, 2008).

Ropivacaine is the S-enantiomer of 1-propyl-2', 6'-pipecoloxylidide (Catterall and Mackie, 2006). It is a new, long-acting local anaesthetic of the amide type. It contains a single chiral centre and is used as the pure S-(-) enantiomer. *In vivo* racemization does not occur after systemic administration of the drug. Its physiochemical properties includes-wt. 274.4 (base), pK 8.1 and log D (pH 7.4 n-octanol vs. buffer) 2.15. Intravenous Ropivacaine shows linear pharmacokinetics and the drug is almost completely metabolized, with less than 1% of the dose excreted unchanged (Arlander *et al*., 2003).

Several studies have been done to reveal the scope of Ropivacaine for use in various diseases and conditions. Infiltration of split skin grafts donor site with Ropivacaine improves postoperative pain during 48 h. This is a safe and efficient method to improve comfort in addition to a standardized occlusive dressing (Trost *et al*., 2005). Dural surface area influences the spread of epidural anesthesia with Ropivacaine and posterior fat volume influences the duration of epidural anesthesia in healthy patients within a narrow age range. Epidural venous plexus velocity might also influence the duration of epidural anesthesia with Ropivacaine (Higuchi *et al*., 2004). Topical anesthesia with Ropivacaine is safe and effective in pterygium surgery

Corresponding Author: Dr. N. Chandrasekhar, Department of Pharmacology, Mamata Medical College, Rotary Nagar, Khammam Andhra Pradesh, India Tel: (0)9959421956

(Caccavale *et al*., 2010). Ropivacaine proved to be effective for pain relief after hernia repair in ilioinguinal blocks accompanying general anesthesia (Wulf *et al*., 1999).

Ropivacaine has efficacy similar to lidocaine, with slightly longer onset and duration of the motor blockade. In addition, Ropivacaine (0.75%) induces lower intraocular pressure and less pain on injection than does lidocaine (2%) when used in peribulbar anesthesia for cataract surgery (Olmez *et al*., 2004). Compared with lidocaine, intravenous regional anesthesia with Ropivacaine appears to be comparable but has longer-lasting residual anesthesia (Chan *et al*., 1999). Topical Ropivacaine performed at least as well as topical lidocaine in efficacy and safety in cataract surgery. It provided sufficient and long-lasting analgesia without the need for supplemental intracameral anesthesia in most cases (Martini *et al*., 2002).

The 0.5% Ropivacaine with 1:200,000 epinephrines is equivalent to 0.5% bupivacaine with 1:200,000 epinephrines in pharmacologic action. The duration of pulpal anesthesia is less for Ropivacaine without epinephrine. Ropivacaine with epinephrine has the potential to replace bupivacaine with epinephrine in clinical dental practice because of the decreased potential for cardiac and central nervous system toxicity (Kennedy *et al*., 2001).

However, the action of Ropivacaine as a surface anesthetic has not been studied so far. The aim of the present study was to investigate the role of Ropivacaine as a surface anesthetic on the cornea of rabbits.

MATERIALS AND METHODS

The study was performed between 5/2/2010 to 24/6/2010 for a period of four months at Mamata Medical College (MMC), Khammam Andhra Pradesh (AP). Twenty albino rabbits (weighing 2.5 to 3.0 kg of either sex) were obtained from the Research Animal House of MMC and used in the study. The animals were housed at a temperature of 24±2°C and relative humidity of 30-70%. All animals had free access to water and standard pelleted laboratory animal diet. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee of MMC, Khammam, AP and were in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Calculation of doses from standard solution of ropivacaine: Commercially available standard solutions of Ropivacaine containing 0.75% Ropivacaine in 1 mL of solution (7.5 mg mL^{-1}) were used to prepare the test solutions.

One milliter of solution contains 7.5 mg or 7500 μg of Ropivacaine.

One milliter of solution contains 14 drops.

Thus, 1 drop contains 7500/14 = 535.71 μg of Ropivacaine.

Two drops contains 535.71×2 = 1071.42 μg of Ropivacaine.

Delivering the test solutions to experimental groups: The animals were placed in rabbit holding cages and were randomly allocated to 2 treatment groups-Group I and Group II. The upper and lower eyelashes of all the rabbits were carefully clipped off to avoid the corneal reflex initiated by accidental touching of the eyelashes. The conjunctival sac of right eye was held open to form a pocket. Into these pockets, animals in group I were delivered 1 drop (containing 535.71 μg of Ropivacaine) of the standard solution of Ropivacaine and those in Group II were delivered 2 drops (containing 1071.42 μg of Ropivacaine) of the same solution. The left eye served as the control.

The corneal reflex was elicited by touching the cornea from the side using a cotton wisp. The test was started 5 min after the application of the drug in both groups and repeated every 5 min until the corneal reflex was lost (blinking lost) followed by reappearance of the corneal reflex (blinking reappears). The time between the disappearance and reappearance of corneal reflex (duration of action) was registered.

The results obtained were recorded and tabulated. All data was expressed in terms of a Mean±Standard deviation. To compare the intergroup differences for onset of action of the anesthetic and the duration of action of the anesthetic, a student "t" test was done using the software "Primer for Biostatistics" Version 5.0. All probability values less than 0.05 ($p<0.05$) were considered significant and those less than 0.001 ($p<0.001$) were considered highly significant.

RESULTS

The time of disappearance of corneal reflex and the time between the disappearance and reappearance of corneal reflex were recorded in 20 albino rabbits after the administration of two doses of a standard solution Ropivacaine. At baseline (0 min), all the rabbits in both group I and II (n = 20) showed the presence of a normal corneal reflex. Five min after the application of the test solution, all rabbits in-group I still retained a normal corneal reflex while that in-group II lost their corneal reflex (Table 2). By 10 min, group I rabbits also had lost their corneal reflex (Table 1). In the group I rabbits, 1 rabbit regained corneal reflex after 30 min (duration of action =

Table 1: Corneal reflex of group I rabbits (n = 10), Dose: 1 drop (535.71 μg)

		Experimental group I (Right eye)									
Time (min)	Control (Left eye)	1	2	3	4	5	6	7	8	9	10
0	+	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+
10	+	–	–	–	–	–	–	–	–	–	–
15	+	–	–	–	–	–	–	–	–	–	–
20	+	–	–	–	–	–	–	–	–	–	–
25	+	–	–	–	–	–	–	–	–	–	–
30	+	–	–	–	–	–	–	–	–	–	+
35	+	–	–	–	–	–	–	–	+	+	+
40	+	+	+	+	+	+	–	–	+	+	+
45	+	+	+	+	+	+	+	+	+	+	+
50	+	+	+	+	+	+	+	+	+	+	+
55	+	+	+	+	+	+	+	+	+	+	+
60	+	+	+	+	+	+	+	+	+	+	+

+: Corneal reflex present; –: Corneal reflex absent

Table 2: Corneal reflex of group II rabbits (n = 10), Dose: 2 drops (1071.42 μg)

		Experimental group II (Right eye)									
Time (min)	Control (Left eye)	1	2	3	4	5	6	7	8	9	10
0	+	+	+	+	+	+	+	+	+	+	+
5	+	–	–	–	–	–	–	–	–	–	–
10	+	–	–	–	–	–	–	–	–	–	–
15	+	–	–	–	–	–	–	–	–	–	–
20	+	–	–	–	–	–	–	–	–	–	–
25	+	–	–	–	–	–	–	–	–	–	–
30	+	–	–	–	–	–	–	–	–	–	–
35	+	–	–	–	–	–	–	–	–	–	–
40	+	–	–	–	–	–	–	–	–	–	–
45	+	+	+	–	–	–	–	+	–	–	–
50	+	+	+	+	+	+	–	+	–	–	–
55	+	+	+	+	+	+	+	+	–	–	–
60	+	+	+	+	+	+	+	+	+	+	+

+: Corneal reflex present; –: Corneal reflex absent

25 min), 2 rabbits regained their corneal reflex after 35 min (duration of action = 30 min), 5 rabbits regained their corneal reflex after 40 min (duration of action = 35 min) and 2 rabbits regained their corneal reflex after 45 min (duration of action = 40 min) (Table 1). In the group II rabbits, 2 rabbits regained their corneal reflex after 45 min (duration of action = 45 min), 3 rabbits regained their corneal reflex after 50 min (duration of action = 50 min), 2 rabbits regained their corneal reflex after 55 min (duration of action = 55 min) and 3 rabbits regained their corneal reflex after 60 min (duration of action = 60 min) (Table 2). For the control sites (left eye), the corneal reflex was positive throughout the period of the experiment.

Statistical analysis revealed highly significant differences between group I and II ($p<0.001$). The mean onset of action for group I was 10 min and for group II was 5 min (Table 3). The mean duration of action for group I was 29±4.595 and for II was 48±5.869 (Table 4).

Table 3: Mean onset of action of two different doses of ropivacaine

Group	n	Mean	SD	SEM	p-value
I	10	10	0	0	0.000*
II	10	5	0	0	<0.001

n: Sample size, SD: Standard deviation, SEM: Standard error mean, *Statistically significant

Table 4: Mean duration of action of two different doses of ropivacaine

Group	n	Mean	SD	SEM	p-value
I	10	29	4.595	1.453	0.000*
II	10	48	5.869	1.856	<0.001

n: Sample size, SD: Standard deviation, SEM: Standard error mean, *Statistically significant

DISCUSSION

Topical anesthetic agents used clinically in ophthalmology include Cocaine, Proparcaine and Tetracaine drops and Lidocaine gel. Proparcaine and Tetracaine are used for removal of foreign bodies, for tonometry and for superficial corneal surgeries with no side effects (Catterall and Mackie, 2006). Ropivacaine is a local anaesthetic agent mainly used for both epidural and regional anesthesia. Side effects with topical Ropivacaine have not been studied extensively but one study with impression cytology presents a non-invasive or minimally invasive biopsy of the ocular surface epithelium with no side effects (Soker *et al*., 2007). Ropivacaine has also been used safely and effectively in pterygium surgery. The Long-lasting anesthesia with this agent permitted performing the surgical procedures with autograft conjunctival graft and fibrin glue to attach the flap with low pain perceived by the patients, low surgical invasivity and short duration of surgery (Caccavale *et al*., 2010).

Recently, topical anesthesia and intracameral anesthesia have become popular in modern cataract surgery. During the last decade, the use of Ropivacaine as a research tool has experienced an enormous growth and has greatly contributed to the understanding of ocular surface pathology, including investigation of the toxic effects of anterior camera applied chemicals, for instance, application of Ropivacaine to the corneal endothelium. However, some authors advise caution with the use of intracameral anesthetic agents because of possible toxic effects in intraocular structure, especially the corneal endothelium. Many experimental studies were performed to investigate endothelial toxicity. One percent lidocaine hydrochloride (HCL) causes a transient endothelial cell edema to the in vitro perfused endothelium of human and rabbit corneas (Martini *et al*., 2002).

Ropivacaine 1% and lidocaine 2% are safe and effective agents inpatients undergoing phacoemulsification using a topical anesthesia. However,

Ropivacaine provided better operative conditions than lidocaine for the surgeon and comfortable surgical circumstances for the patient (Nicholson *et al*., 1999). Topical anesthesia with ropivacaine was safe, feasible and more effective than lidocaine in cataract surgery (Borazan *et al*., 2008). One of the studies showed experimentally that, corneas exposed to 0.01% Ropivacaine concentration in vitro manifested no serious damage histologically. The impression cytology method can be used in the investigation of the toxic effect of various intracameral anesthetic agents (Soker *et al*., 2007). The efficacy of 1% ropivacaine for topical anesthesia in dentistry was comparable with 20% benzocaine gel and eutectic mixture of local anesthetics 2.5% lidocaine and 2.5% prilocaine (Franz-Montan *et al*., 2007). The intraperitoneal instillation of ropivacaine was effective in reducing postoperative pain and in shortening the recovery course after laparoscopic colectomy. The additional instillation of ropivacaine at the end of the surgery proved even more effective (Baek *et al*., 2010). In another study, they concluded that administration of intraperitoneal ropivacaine reduced pain during the post-operative period after laparoscopic appendectomy (Kang and Kim, 2010).

Ropivacaine is a pure S-enantiomer that is less lipid-soluble and less cardiotoxic than bupivacaine but more cardiotoxic than lidocaine. Ropivacaine is the newest long-acting, enantiomerically pure (S-enantiomer) amide local anaesthetic, designed by modification of an existing one. Chemically, it is very similar to bupivacaine and mepivacaine. All of these three anesthetics come from the family of molecules known as pipecolyl xylidines which combine the piperidine ring of cocaine with xylidine from lidocaine. Substitution of methyl, butyl and propyl groups on the piperidine ring give rise to mepivacaine, bupivacaine and Ropivacaine, respectively. The high level of potency and lipid solubility of Ropivacaine suggests a CNS toxicity profile similar to that of bupivacaine. Studies on anaesthetized rats showed that the cumulative doses of levobupivacaine and Ropivacaine that produced seizures were similar and were larger than those of bupivacaine. The predicted cardiac toxicity profile of Ropivacaine has been extensively studied and animal studies confirm an arrhythmogenicity of Ropivacaine that is intermediate between that of mepivacaine and bupivacaine. The cumulative doses of levobupivacaine that produced dysrhythmias and asystole were smaller than the corresponding doses of Ropivacaine, but they were larger than those of bupivacaine. Ropivacaine-induced cardiac arrest was more susceptible to treatment than that induced by bupivacaine or levobupivacaine (Lonnqvist *et al*., 2000; Soker *et al*., 2007; Tripathi, 2008; Marron-Pena and Rivera-Flores, 2008).

Another study on rats concluded that Ropivacaine, even at equipotent dose, is less toxic than bupivacaine. In rabbits and pigs, an indication was found that Ropivacaine is less cardiodepressive and arrhythmogenic than bupivacaine. Ropivacaine, according to animal data, is less neurotoxic and cardiotoxic than bupivacaine. Based on available clinical data, Ropivacaine appears to be as active and well tolerated as bupivacaine, when equianalgesic doses are compared and to block nerve fibres involved in pain transmission (A delta and C fibres) to a greater degree than those controlling motor function (A beta fibres). The greater degree of separation between motor and sensory blockade seen with Ropivacaine relative to bupivacaine at lower concentrations (approximately 5 mg kg^{-1}) will be advantageous in certain applications (Nicholson *et al*., 1999).

This study was done to see whether Ropivacaine produces surface anesthesia or not. This study shows that Ropivacaine is a potent surface anaesthetic of fast onset with intermediate duration of action with dose of one drop i.e., 187.5 μg the onset of action (loss of corneal reflex is 10 min) and duration of action (reappearance of corneal reflex is 29 min) as shown in Table 4. With 2 drops i.e., 375 μg of Ropivacaine dose the onset of action (loss of corneal reflex is 5 min) and duration of action (reappearance of corneal reflex is 48 min) as shown in Table 4.

CONCLUSION

Like Proparcaine and Tetracaine, Ropivacaine can also be used as surface anaesthetic for removal of foreign bodies and other clinical conditions in ophthalmology. Different studies show safety and potency of the drug as topical anaesthetic with minimal cardiac and neurotoxicities. However, further studies should be done with different doses of Ropivacaine and further comparative studies with lidocaine and bupivacaine should be done to elicit the potency of Ropivacaine as surface anaesthetic.

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