ABSTRACT

Objective: To investigate the toxic effects of ropivacaine on corneal endothelium by using the impression cytological method.

Methods: The study was performed between October and December 2004 in Dicle University Hospital, Diyarbakir, Turkey. Twenty-four eyes from 12 rats were used for the research. They were divided into 4 groups, each containing 3 different ropivacaine concentrations and a control. Immediately after enucleation, the corneas were excised and the endothelium was exposed to unpreserved ropivacaine 0.01, 0.1, or 1% and balanced salt solutions (BSS) as a control (6 corneas/group) for 20 minutes. The specimens were obtained by impression cytology method and stained with periodic acid shift. Then, they were examined under light microscope.

Results: Blurring at cell membrane borders, vacuolization at cell cytoplasm, hydropic degeneration and increase in toxic granulation were observed in the 1% ropivacaine group. Cytoplasmic hydropic degeneration was determined in the 0.1% ropivacaine group. Cell structures were normal and almost identical to the control group in the 0.01% ropivacaine group.

Conclusion: In this study, 2 major conclusions were determined. The impression cytology method can be used in examination of corneal endothelium, and exposure of rat corneal endothelium to 0.01% ropivacaine solutions in vitro appears to be non-toxic.

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Topical and intracameral anesthesia has become popular in modern cataract surgery. There are few anesthetic agents used in intracameral and topical anesthesia, and preservative-free lidocaine is used widely in intracameral anesthesia. However, ropivacaine, a new local anesthetic, was used in this study, because, it is also a preservative-free local anesthetic and has a long acting effect that provides longer postoperative analgesia. In several studies, it has been reported that usage of ropivacaine in peribulbar and topical anesthesia has better efficacy and safety in cataract surgery. However, there are no experimental studies related to the toxic effects of ropivacaine on corneal endothelium. Therefore, in this study, it was considered to use ropivacaine for intracameral anesthesia. Electron microscopy is usually used in experimental research to investigate toxic effects of anesthetic agents on the corneal endothelium. However, it is rather an expensive method. The impression cytology method is used in epithelial cell investigations, but not in corneal endothelial cells. In this study, the impression cytology method was used.

Methods. The study was performed between October and December 2004 in Dicle University Hospital, Diyarbakir, Turkey. Mature rats were used in the study. All procedures were performed in compliance with the college’s Ethics committee on experimental research in medicine. Twenty-four eye specimens from 12 rats were used in the study. Rats were sacrificed with 50 mg pentothal. Then, the eyes were enucleated, and corneas were excised 360 degrees from the limbus. The corneal endothelium was carefully protected during this procedure. The cornea specimens were divided into 4 separate groups (6 corneas/group) and exposed to 1%, 0.1%, and 0.01% ropivacaine solutions, and the control group was exposed to balanced salt solution (BSS) for 20 minutes. Then, cytological specimens were obtained from the corneal endothelium with impression paper. Cellulose acetate paper with a 0.20 µm pore diameter was used. Filter papers were cut in the dimensions of 3 x 4 mm, and the rough faces of the paper were slightly pressed to the corneal endothelium for 3-4 seconds by sticking with a smooth stile. The papers were gently removed from the
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Results. In the examination of all specimens, after the results were obtained, the endothelium cell borders of the control group were observed to be definite, and the cell cytoplasm and nucleus were normal (Figure 1). The ropivacaine 1% group had blurred cell borders, cytoplasmic vacuolization, hydropic degeneration and increased toxic granulation (Figure 2). The ropivacaine 0.1% group had hydropic degeneration in the cell cytoplasm (Figure 3). The ropivacaine 0.01% group was determined to be normal and almost identical to the control group (Figure 4).

Discussion. Various methods such as electron microscopy, confocal microscopy, and specular microscopy are used to investigate toxic effects of intracameral applied chemical agents on the corneal endothelium. These methods have perfect visualization quality for corneal endothelium. Impression cytology represents a non- or minimally invasive biopsy of the ocular surface epithelium with no side effects or contraindications. In addition, and mainly during the last decade, its use 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 ropivacaine to the corneal endothelium. Recently, topical anesthesia and intracameral anesthesia have become popular in modern cataract surgery. 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. Kim et al evaluated the in vitro effect of 1% lidocaine on the corneal endothelium. They concluded that 1% lidocaine hydrochloride (HCL) causes a transient endothelial cell edema to the in vitro perfused endothelium of human and rabbit corneas. They highlighted that proper attention should be paid to the type of lidocaine injected intraocularly, namely, concentration, vehicle, preservatives, pH, and

![Figure 1](image1) Specimens from control balanced salt solutions groups were detected to have cell borders with normal cytoplasmic nuclei at light microscopy (x 40, periodic acid shift).

![Figure 2](image2) Cells exposed to 1% ropivacaine had blurred cell borders, cytoplasmic vacuolization, hydropic degeneration and increased toxic granulation (x 200, periodic acid shift).

![Figure 3](image3) Hydropic degeneration at cell cytoplasms were seen at specimens exposed to 0.1% ropivacaine (x 40, periodic acid shift).

![Figure 4](image4) Cell structures from specimens exposed to 0.01% ropivacaine were detected to be normal and almost identical to that of the control group (x 100, periodic acid shift).
osmolarity. Kadonosono et al\textsuperscript{18} evaluated the effects of intracameral anesthesia on the corneal endothelium. They injected unpreserved lidocaine at concentrations of 0.02, 0.2, or 2% and the fellow eye was injected with BSS as a control. They used a bimanual technique for injecting the anesthetic agent. Scanning electron microscopic investigation revealed that intracameral anesthesia with high concentrations of lidocaine risks corneal endothelial damage, but at the low concentration usually used in cataract surgery, did not appear to have an adverse effect. Similarly, Werner et al,\textsuperscript{21} performed an in vitro study of rabbit corneas exposed to lidocaine 1%, lidocaine 5%, and as a control of BSS. They found that, corneas exposed to lidocaine HCL 5% had more marked cell alterations.\textsuperscript{22} Eggeling et al,\textsuperscript{23} examined the potential damaging effect on the corneal endothelium of unpreserved lidocaine at concentrations of 1%, 5%, and 10%, and BSS as a control for 60 minutes. A small subset was exposed to lidocaine HCL 1% only for 30 minutes to simulate clinical conditions. They found that lidocaine 1% used for 30 or 60 minutes did not cause significantly more corneal endothelial damage than did the control group. They concluded that experimental exposure of corneal endothelial cells to higher concentrations of lidocaine resulted in significant cell loss. Lo Martire et al\textsuperscript{23} reported that ropivacaine 1% and lidocaine 2% are safe and effective agents in patients undergoing phacoemulsification using a topical anesthesia. However, ropivacaine provided better operative conditions than lidocaine for the surgeon, and comfortable surgical circumstances for the patient.\textsuperscript{23}

Finally, we showed experimentally that corneas exposed to 0.01% ropivacaine concentration in vitro manifested no serious damage histologically. Also, that the impression cytology method can be used in the investigation of the toxic effect of various intracameral anesthetic agents.

\section*{References}


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