

Cataract Surgery Complications

An *In Vitro* Model of Toxic Effects of Ropivacaine and Lidocaine

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Abstract

Background: Intraoperative lidocaine is widely used in controlling discomfort during cataract surgery. However, recent studies have confirmed the toxic effect of lidocaine on ganglion cells. Ropivacaine is an anesthetic recently introduced in clinical practice that couples a long anesthetic effect with a mild vasoconstrictive action.

Objective: The aim of this study was an *in vitro* evaluation of the efficacy of ropivacaine in reducing the degenerative effects usually observed during lidocaine treatment.

Methods: Ropivacaine and lidocaine toxicity has been evaluated in murine fibroblasts 3T6 by measuring percentage of cell death, cell growth inhibition, and DNA degradation. The choice of this cellular line is motivated by the presence of a complete apoptotic system that can be assimilated to the endothelium precursor cells.

Results: We observed that lidocaine 0.25% decreases cell viability and causes DNA degradation in murine fibroblasts 3T6, whereas ropivacaine 0.5% does not cause any cellular or molecular degenerative effect.

Conclusions: Our *in vitro* studies confirm that ropivacaine is less toxic than lidocaine to these cells. Therefore, *in vivo* studies in the anterior chamber could be useful to evaluate the effects of ropivacaine versus lidocaine in intracameral anesthesia in cataract surgery.

Introduction

In the last few years, the frequency of cataract surgery has greatly increased, possibly due to an increase in life expectancy.^[1] The current anesthesia practice does not call for total anesthesia except in pediatrics and handicapped subjects.

Use of local anesthesia (retrobulbar, peribulbar, sub-Tenon's) is limited, even though it is important in eye surgery of anxious subjects and elderly patients who cannot easily control ocular movements. To prevent severe but rare complications, such as globe perforation, optic nerve injury, muscular paresis, diplopia, eyelid ptosis,

and retinal vascular occlusion, safer anesthesia techniques were investigated. Fichman^[2] has introduced topical anesthesia with xylocaine, which has proved to be an effective and safe technique with good postoperative recovery.^[2-4] However, intraoperative discomfort and pain, particularly during phacoemulsification and intraocular lens implantation, have necessitated the association with subconjunctival, retrobulbar, or peribulbar anesthesia.^[5,6] Gills et al.^[7] have reported encouraging results in reducing intraoperative discomfort during cataract surgery through the intracameral injection of 1% unpreserved lidocaine in the presence of topical anesthesia. The same authors have found in their studies that intraocular lidocaine does not damage corneal endothelium (cell count and corneal thickness). These data were confirmed by Kim et al.^[8] in both human endothelium and laboratory animals; however, a transient endothelial cell edema was observed during lidocaine infusion. This was easily evaluated by specular microscopy and electronic scansion microscopy.^[8] Gills et al.^[9] have reported a transient loss of vision in 4 cases in a total of over 15 000 patients. This was essentially due to a dialysis of the posterior capsule with lidocaine interfering with ganglion cell function. Recent *in vitro* and *in vivo* studies have confirmed the toxic effect of lidocaine on ganglion cells.^[10,11] Furthermore, the short duration of the local anesthetic makes administration of a second dose during cataract surgery necessary, thus increasing the risk of toxicity. Ropivacaine, which has recently been introduced in clinical surgery, can represent a valid alternative to lidocaine in intracameral anesthesia. In fact, ropivacaine presents the speculative advantages of a long-duration and bland vasoconstrictive effect.^[12]

In our study, we investigated an *in vitro* evaluation of the efficacy of the local toxicity of ropivacaine versus lidocaine by utilizing murine fibroblasts 3T6 in culture. This cell line was chosen because of its similarities to the endothelium precursors and because apoptosis can be easily induced and controlled.^[13,14] In fact, occurrence of apoptotic phenomena is diagnostic of generalized toxic effect at cellular level.^[15]

Materials and Methods

Cell Cultures

The murine 3T6 fibroblasts are a stable line of derma fibroblasts. These fibroblasts compare well with endothelial cell precursors. Cell cultures were routinely maintained on Dulbecco's modified Eagle's medium (DMEM) and 10% heat-inactivated fetal bovine serum. Cells were maintained at 37°C in a humidified atmosphere of 5% CO₂ and 95% air.

Unless stated otherwise, to assess apoptotic and/or necrotic phenomena, 5 × 10⁵ cells were plated in 10 cm Petri dishes and left untreated for 24 hours before experimentation began.

Treatment Protocols

Ropivacaine (Naropine[®]) is a well tolerated, effective, long-duration, local anesthetic. Ropivacaine in low concentrations, unlike lidocaine, reduces cutaneous blood flow and produces bland vasoconstriction.^[12] Ropivacaine and lidocaine were dissolved in isotonic saline buffer (phosphate buffered saline [PBS]) and made up to a final concentration of 10 mmol/L and 5 mmol/L, respectively. To assess toxicity, cells were exposed to ropivacaine or lidocaine for 24 hours.

Measurement of Cell Viability

Cell viability as well as cell growth was measured by trypan blue exclusion. Briefly, 5 × 10⁵ cells were centrifuged for 5 minutes at 100 g. The cell pellet was resuspended in 1 mL PBS. An equal amount of cell suspension and 0.4% trypan blue were mixed. After an incubation time of 3–5 minutes, a drop of cell suspension/trypan blue was applied to a hemacytometer. Unstained (viable) and stained (unviable) cells were counted and the percentage of viability determined.

DNA Degradation

The effect of the treatment was monitored after extraction of DNA according to Hirt^[16] gel electrophoresis and visualization with ethidium bromide.

Statistical Analysis

Quantitative values were presented as mean \pm SD. Significant differences between sets of values for control and test groups were assessed by a one-way ANOVA using a student Newman-Keuls *post hoc* test. A p-value refers to a comparison of a measured parameter in the experimental group with that of the appropriate control. Significance was set at $p < 0.05$.

Results and Discussion

The effects of lidocaine and ropivacaine treatment on cell viability are shown in figure 1a. In particular, cells were treated for 24 hours with lidocaine 5 mmol/L. After 24 hours, the medium was replaced and cell viability measured after 48 hours. Figure 1a shows that during such treatment, lidocaine decreased cell viability to 65%. A partial cellular recovery was observed after 72 hours and 96 hours with a cell viability of 82% and 84%, respectively (figure 1a). By contrast, during the treatment, ropivacaine 10 mmol/L, cell viability did not alter and remained about 95%

(figure 1a). Lidocaine and ropivacaine concentrations were chosen from a dose-response analysis of doses ranging from 1 to 20 mmol/L (unpublished observations, Professor Scarsella).

Cytotoxic effects of lidocaine and ropivacaine were determined by measuring cell proliferation. Figure 1b shows that, compared with the proliferation curve of untreated cells, lidocaine caused a significant inhibition. In particular, after 24 hours, there was almost a 50% decrease in the number of cells due to the toxic effect (figure 1b). By contrast, ropivacaine caused only an initial inhibition of cell proliferation that was reduced after 96 hours of treatment (figure 1b); a time where the differences between ropivacaine and lidocaine were evident. Finally, figure 1c shows that lidocaine caused a DNA degradation represented by an evident smear of heterogeneous fragments after extraction and gel electrophoresis. The production of these fragments is typical in necrotic phenomena (figure 1c, lane d). By contrast, ropivacaine caused only a minor degradation of DNA (figure 1c, lane b).

Intracameral injection of lidocaine 1% in addition to topical anesthesia was an important

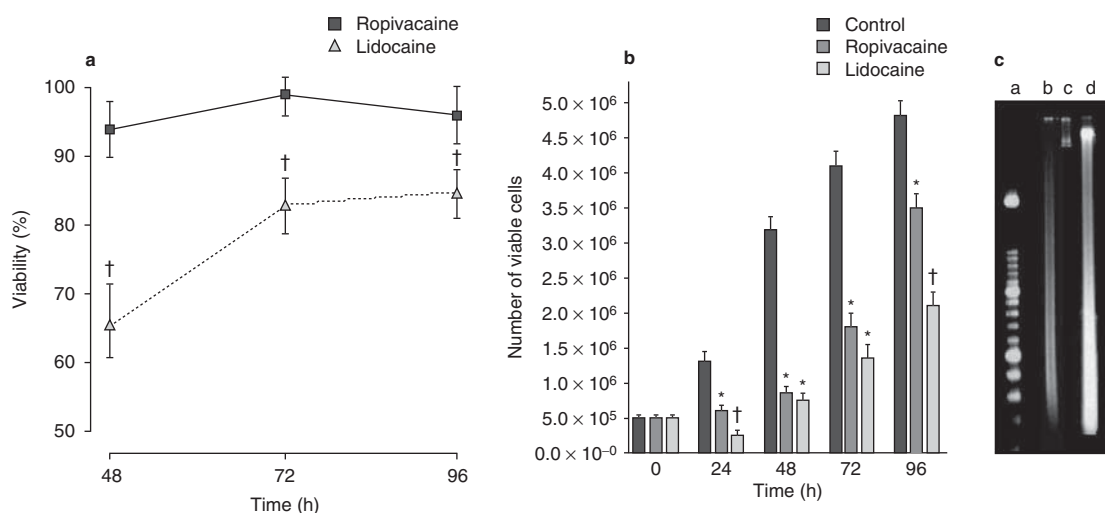


Fig. 1. Cytotoxic effects of lidocaine and ropivacaine. (a) Percentage cell viability after 48, 72, and 96 hours. (b) Number of viable cells after 0, 24, 48, 72, and 96 hours. (c) Gel electrophoresis (lane a shows the DNA molecular weight marker; lane b shows ropivacaine-treated cells; lane c shows control untreated cells; and lane d shows lidocaine-treated cells). * $p < 0.05$ vs control, † $p < 0.05$ vs ropivacaine.

advancement in ocular anesthesia during cataract surgery.^[7,9] However, safer and newer drugs are being investigated in ocular anesthesia to improve surgical techniques. In particular, ropivacaine may represent a safer alternative to lidocaine, thanks to its long-term efficacy and modest vasoconstrictive action, which is usually an advantageous side effect in anterior segment surgery. By contrast, lidocaine causes vasodilation and has a short anesthetic effect (about 10 minutes). Therefore, supplemental injections of anesthetic in anterior chamber surgery to obtain the desired anesthesia are often reported, thereby increasing the risk of toxicity and impairing surgical procedure. The duration of the anesthetic effect is related to the extent of protein binding, which in ropivacaine is 94%, while in lidocaine it is about 64%. In addition, in ropivacaine, there is a marked difference in effect between sensitive and motor blocks that enables surgeons to use very low doses of the drug to inhibit only sensitive function. Therefore, the long duration and the vasoconstrictive action typical of ropivacaine are a definite advantage in anterior chamber surgery. Our results indicate that, in addition to the duration and vasoconstriction effects reported in this study, ropivacaine has the advantage that it does not cause any cellular damage.

In particular, some authors have observed that perfusion of lidocaine 1% in human and murine endothelial corneal cells causes a transient cellular edema.^[8,17] In our experimental model, comparable with endothelial cells, ropivacaine versus lidocaine does not present degenerative effects even at a concentration as high as 10 mmol/L.

Conclusions

In the light of our results, neither drug caused apoptosis; however, lidocaine did give rise to evident necrosis. Therefore, lidocaine may have relevant cytotoxic effects for the endothelium in cataract surgery. Our data are in agreement with the cytotoxic effects observed by Grosskreutz et al.^[10] both *in vitro* and *in vivo*. It is therefore reasonable to conclude that ropivacaine is safer than lidocaine because, at very low con-

centrations, it can assure long duration of action coupled with mild but useful vasoconstriction, without causing any degenerative effects on endothelial cells.

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References

1. Ashwin PT, Shah S, Wolffsohn JS. Advances in cataract surgery. *Clin Exp Optom* 2009; 92: 333-42
2. Fichman RA. Use of topical anesthesia alone in cataract surgery. *J Cataract Refract Surg* 1996; 22: 612-4
3. Husted RF, Hamilton RC. Pharmacology. In: Gills JP, Husted RF, Sanders DR, editors. *Ophthalmic anesthesia*. Thorofare (NJ): Slack, Inc., 1993: 69-102
4. Borazan M, Karalezli A, Oto S, et al. Comparison of a bupivacaine 0.5% and lidocaine 2% mixture with levobupivacaine 0.75% and ropivacaine 1% in peribulbar anaesthesia for cataract surgery with phacoemulsification. *Acta Ophthalmol Scand* 2007; 85: 844-7
5. Patel BC, Burns TA, Crandall A, et al. A comparison of topical and retrobulbar anesthesia for cataract surgery. *Ophthalmology* 1996; 103: 1196-203
6. Leaming DV. Practice styles and preferences of ASCRS members: 2003 survey. *J Cataract Refract Surg* 2004; 30: 892-900
7. Gills JP, Cherchio M, Raanam MG. Unpreserved lidocaine to control discomfort during IOL surgery under topical anesthesia. *J Cataract Refr Surg* 1997; 23: 545-50
8. Kim T, Holley G, Lee J, et al. The effects of intraocular lidocaine on the corneal endothelium. *Ophthalmology* 1998; 105: 125-30
9. Gills JP, Jonsons DE, Cherchio M. Anesthesia intraoculare. In: Davis DB, Mandel MR, editors. *Oftalmologia clinica del Nord America*. Rome: Anestesia Oftalmologia, Verduci Editore, 1998; 10 (3): 65-71
10. Grosskreutz CL, Katowitz WR, Freeman EE, et al. Lidocaine toxicity to rat retinal ganglion cells. *Curr Eye Res* 1999; 18: 363-7
11. Borazan M, Karalezli A, Akova YA, et al. Comparative clinical trial of topical anaesthetic agents for cataract surgery with phacoemulsification: lidocaine 2% drops, levobupivacaine 0.75% drops, and ropivacaine 1% drops. *Eye (Lond)* 2008; 22: 425-9
12. McClure JH. Ropivacaine. *Br J Anaesth* 1996 Feb; 76 (2): 300-7
13. Bonincontro A, Iacoangeli A, Melucci-Vigo G, et al. Apoptosis dependent decrease of the intramembrane ion traffic in cultured mouse fibroblasts shown by conductivity dispersion. *Biosci Rep* 1997; 17: 547-56

14. Pescosolido N, Del Bianco G, De Feo G, et al. Induced acute ocular hypertension: mode of retinal cell degeneration. *Acta Ophthalmol Scand* 1998; 227 (Suppl.): 20-1
15. Borazan M, Karalezli A, Oto S, et al. Induction of apoptosis of rabbit corneal endothelial cells by preservative-free lidocaine hydrochloride 2%, ropivacaine 1%, or levobupivacaine 0.75%. *J Cataract Refract Surg* 2009; 35: 753-8
16. Hirt B. Selective extraction of polyoma DNA from infected mouse cell cultures. *J Mol Biol* 1967; 14; 26: 365-9
17. Shah AR, Diwan RP, Vasavada AR, et al. Corneal endothelial safety of intracameral preservative-free 1% xylocaine. *Indian J Ophthalmol* 2004; 52: 133-8

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