Sub-Tenon Atracurium Injection in Rabbit Eyes; a Histopathologic Study

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Purpose: To evaluate early and late histopathologic changes following posterior sub-Tenon injection of atracurium in the rabbit eye.
Methods: This study was performed on 39 healthy white New Zealand rabbits which received sub-Tenon injection of 0.05-0.08 mg/kg atracurium diluted in 0.5 ml normal saline (N/S) in the left and 0.5 ml N/S in the right eyes. Bilateral enuclation was performed one hour after the injection to evaluate early changes in 19 rabbits and one week later to determine late changes in the remaining 20 animals. After enucleation, the rabbits were euthanized. Enucleated eyes were sent in 10% formalin solution for histopathologic examination. After processing, the specimens were evaluated by light microscopy following staining with hematoxylin and eosin, and trichrome.
Results: Congestion was more common in the control group 1 hour after injection. Liquifaction necrosis was seen in both groups but was significantly increased one week after the injection in the atracurium group.
Conclusion: Congestion is a transient complication related to injection which disappears after one week, but necrosis seems to be an important late complication of atracurium injection.

Key word: Atracurium; Injections, Sub-Tenon; Rabbits

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INTRODUCTION

Local anesthesia entails certain advantages to general anesthesia for ocular surgery including the potential for ambulatory surgery, rapid recovery and lower incidence of significant complications.[1] However, the method of anesthesia may vary based on surgeon's experience, patient's general and ocular conditions and the setting where surgery is being performed.[2]

Local anesthesia in ocular surgery is most commonly performed via retrobulbar or peribulbar routes. Sub-Tenon anesthesia is an alternative to the peribulbar method.[3] Following blunt dissection into the posterior sub-Tenon space, direct injection of local anesthetics is accomplished using a blunt probe. This avoids many complications of peribulbar and retrobulbar injections. Local anesthetics in the posterior sub-Tenon space spread along the extraocular musles and diffuse into the retrobulbar space. This method is relatively painless and provides reliable local anesthesia with a minimum risk of serious compications.[4] The injection can be performed with a 28-gauge needle,[5] a variety of blunt cannulae[6] or a 22-gauge intravenous (IV) catheter.[7]

Non-depolarizing neuromuscular blocking

agents (ND-NMBAs) such as atracurium and verucurium are used as adjuvants for local anesthesia.[8,9] Atracurium has good neuromuscular blocking potential and acts well at physiologic pH and temperature.[10] Atracurium is mostly degraded in the plasma. Some studies have suggested that the addition of low dose atracurium to local anesthetics in peribulbar injections hastens the onset of peribulbar block and provides excellent akinesia without complications.[8] However the injection of atracurium for this purpose has not been thoroughly investigated and its safety is uncertain.

This study was undertaken to determine early and late histopathologic changes following sub-Tenon injection of atracurium in the rabbit eye.

METHODS

This experimental study was performed in accordance with the Association for Research in Vision and Ophthalmology (ARVO) statement for use of animals in ophthalmic and vision research. Supposing 10% adverse effects for atracurium and nil for normal saline (N/S) solution (NaCl 0.9%), α=0.05 and β=0.2; 19 rabbits were required each for evaluation of early and late complications.

Eventually, 39 healthy white New Zealand rabbits of either sex, weighing 2-3 kg and aged 2-3 months were selected for the purpose of the study. The right eyes served as controls and received injection of 0.5 ml N/S, while left eyes underwent an injection of 0.05-0.08 mg/kg atracurium diluted in 0.5 ml N/S. The first group of animals (19 rabbits) underwent bilateral enucleation one hour after the injection to determine early complications and the others (20 rabbits) were enucleated one week later to detect late complications.

All injections were performed under general anesthesia (GA) and aseptic conditions. GA was accomplished using an intramuscular injection of 50 mg/kg ketamine hydrochloride which was repeated if needed. After establishment of GA, a 2 mm incision was made in the conjunctiva 4 mm superotemporal to the limbus using Westcott scissors. Tenon's fascia was dissected and a 22-gauge IV cantheter was inserted into the sub-Tenon space and cautiously moved to the posterior portion of the space to perform the injections. Thereafter, the eyes received gentle digital massage for about 2 minutes.

Both eyes of each rabbit were enucleated under GA one hour and one week after the injections as described above, and all animals were euthanized using an intracardiac injection of pentobarbital sodium under deep anesthesia.[11] All procedures were perfromed by one surgeon (SS).

Enucleated eyes were fixed in formaldehyde 10% in phosphate buffer saline (pH= 7.4) for 24 hours, embedded in paraffin and cut into 5 µm sections. Hematoxylin and eosin (H&E) and trichrome staining were performed and histhopathologic examination was performed using light microscopy by one pathologist (HHM) who was masked to the study groups.

Statistical analysis was performed using Chi-square and Fisher's exact tests as well as adjusted residuals to compare histopathologic findings between the groups with significance level set at 0.05.

RESULTS

Based on recoded values of adjusted residuals and with regard to time (early vs. late histopathologic changes) there was no significant difference between the eyes injected with atracurium and N/S. The most common findings were liquefaction necrosis, congestion and hemorrhage (Table 1). In both groups, congestion was seen within 1 hour after injection and liquefaction necrosis was significantly higher one week later.

Table 1 compares early and late complications within the study groups (N/S and atracurium). Differences between early and late complications were stastistically significant in both groups (P=0.023 for N/S group and 0.029 for atracurium group; Fisher's exact test). Based on the values of adjusted residuals, the most significant complication in both groups was tissue congestion (adjusted residual=3.3 for N/S and 2.7 for Atracurium).

Without considering the time of biopsy, there was no statistically significant diffference

between N/S and atracurium groups, such that 11 cases of necrosis were seen in the N/S group vs 10 cases in the atracurium group (P = 0.5). There also was no significant difference between the N/S and atracurium groups in terms of fibrosis (4 vs 10 cases, P = 0.14) (Fig. 1).

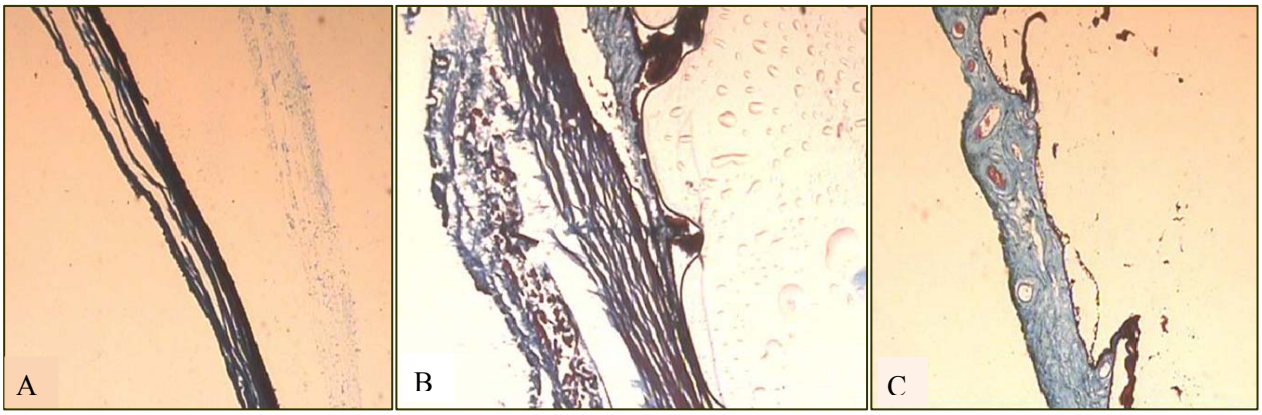

Figure 1 (A) Trichrome staining without visible fibrosis, (B) Trichrome staining showing fibrosis, (C) Trichrome staining (normal).

Table 1 Histopathologic findings according to different biopsy times

Complications	Biopsy time: No (%) [Adjusted residual]		
	1 hr after injection	1 wk after injection	Total
N/S group			
Acute inflammatory infiltrate	1 (5.3) [0.0]	0 (0) [-1.0]	1 (2.6)
Liquefaction necrosis	4 (21.1) [-1.0]	7 (35.0) [1.0]	11 (28.2)
Congestion	8 (42.1) [3.3]	0 (0) [-3.3]	8 (20.5)
Hemorrhage	2 (10.5) [0.1]	2 (10.0) [1.0]	4 (10.3)
Fibrosis	1 (5.3) [-1.0]	3 (15.0) [1.0]	4 (10.3)
Normal	3 (15.8) [-1.7]	8 (40.0) [1.7]	11 (28.2)
Total	19 (100)	20 (100)	39 (100)
Atracurium group			
Acute inflammatory infiltrate	1 (5.3) [1.0]	0 (0) [-1.0]	1 (2.6)
Chronic inflammatory infiltrate	1 (5.3) [1.0]	0 (0) [-1]	1 (2.6)
Liquefaction necrosis	2 (10.5) [-2.1]	8 (40.0) [2.1]	10 (25.6)
Congestion	6 (31.6) [2.7]	0 (0) [-2.7]	6 (15.4)
Hemorrhage	1 (5.3) [1.0]	0 (0) [-1.0]	1 (2.6)
Fibrosis	5 (26.3) [-1.0]	5 (25.0) [-1.0]	10 (25.6)
Normal	3 (15.8) [-1.4]	7 (35.0) [1.4]	10 (25.6)
Total	19 (100)	20 (100)	39 (100)

DISCUSSION

Direct injection of local anesthetics into the posterior sub-Tenon space avoids many complications related to peribulbar and retrobulbar injections; however, the use of an adjuvant to accelerate the onset of akinesia could further improve its efficacy. Atracurium is used as an adjuvant to local anesthetics in order to provide better akinesia and accelerate the onset of anesthesia. Atracurium has been used in regional blocks in humans, such as Bier's wrist block[12] and intravenous regional anesthesia.[13]

The addition of atracurium to local anesthetics does not affect analgesia, but because of its effect on motor nerves, it induces akinesia in extraocular muscles and orbicularis occuli therefore optimizing the setting for ophthalmic surgeries. In the study by McGlone et al,[12] application of 2 mg atracurium in regional blocks

facilitated surgery for reduction of a wrist fracture. Elhakim et al[13] reported that the addition of 2 mg atracurium to lidocaine for intravenous regional anesthesia provided musle relaxation and facilitated reduction of fractures. In a study by Kurt et al[14] adding atracurium to IV regional block in the arm hastened the onset of block but did not affect akinesia. Kucukyavuz et al[8] showed that the addition of atracurium to local anesthetics for peribulbar blocks hastens the onset of akinesia and provides better conditions without adverse events.

Application of atracurium in regional eye blocks requires special considerations. First of all, it should not effect intraocular pressure (IOP). Atracurium and pancuronium are known not to increase IOP during general anesthesia.[15,16] Secondly, it should be safe for ocular tissues. Since the half life of atracurium is about 20 minutes, its immediate side effects can be detected one hour after injection. We used a 0.05-0.08 mg/kg dose of atracurium and diluted it with N/S, because larger doses may lead to apnea and death.

The current study demonstrated that fibrosis was comparable in the study groups. Normal sclera is composed of compact collagen which stains with trichrome. Therefore, severe staining is indicative of fibrosis (Fig. 2B). Cases of fibrosis observed 1 hour post-injection were accepted as normal sclera or pre-existing fibrosis due to causes other than injection. Congestion was encountered more frequently in the N/S group than the atracurium group and was of a transient nature. Although the overall rate of necrosis was similar in the study groups, the trend toward greater necrosis one week after atracurium injection suggests that this agent may induce necrosis. All cases of necrosis were of the liquefaction type and were seen near scleral tissue (probably from Tenon's or conjunctival tissue). Since normal saline is tissue-compatible and no histopathologic side effects are expected following its injection, the necrosis observed one hour post-injection can be due to volume trauma or be injection-related.

Amann et al[17] reported that atracurium and cisatracurium inhibit proliferation of human cell lines in vitro, but mivacurium did not. This effect was alleviated by glutathione and N-acetylcysteine, as well as by carboxyl esterase, indicating that the inhibition may be caused by reactive acrylate metabolites. In other studies, cisatracurium induced apoptosis in human endothelial cells of the umblical vein[18] and prevented the survival and axonal growth of neonatal and adult rat peripheral neurons in vitro.[19] Another study reported that long-term use of atracurium could be associated with decreased myosin filaments and muscle thickness.[20] Atracurium may affect endothelial cells and progressively decrease neovascularization at the site of injection leading to poor healing process and necrosis.

In conclusion, the use of atracurium as an adjuvant to sub-Tenon anesthesia seems to be of questionable safety according to this animal model due to the risk of induced necrosis. Additional studies are needed to validate our findings and to evaluate long-term adverse effects associated with injection of atracurium into the sub-Tenon space.

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