Subconjunctival Exposure to Carbopol Causes Chronic Histiocytic Inflammatory Response in Rabbits

Lauren A. Dalvin\textsuperscript{1}, Diva R. Salomão\textsuperscript{2}, Raymond Iezzi\textsuperscript{1}, and Andrew J. Barkmeier\textsuperscript{1}

\textsuperscript{1}Department of Ophthalmology, Mayo Clinic, Rochester, MN, USA
\textsuperscript{2}Department of Anatomic/Clinical Pathology, Mayo Clinic, Rochester, MN, USA

Correspondence: Andrew J. Barkmeier, Mayo Clinic, Department of Ophthalmology, 200 First St, SW, Rochester, MN 55905, USA. email: barkmeier.andrew@mayo.edu

Received: 20 July 2017
Accepted: 25 March 2018
Published: 31 May 2018

Keywords: granuloma; conjunctiva; vitreoretinal; carbopol

Citation: Dalvin LA, Salomão DR, Iezzi R, Barkmeier AJ. Subconjunctival exposure to carbopol causes chronic histiocytic inflammatory response in rabbits. Trans Vis Sci Tech. 2018;7(3):9, https://doi.org/10.1167/tvst.7.3.9

Copyright 2018 The Authors

\textbf{Purpose:} We recently reported a clinical association between surgical exposure to the ophthalmic lubricant GenTeal gel and postoperative subconjunctival necrotizing granulomatous inflammation. We used a rabbit model to test our hypothesis that subconjunctival exposure to the carbopol 980 component is responsible for inciting chronic inflammation.

\textbf{Methods:} Of eight adult wild-type Dutch belted rabbits, 16 eyes received subconjunctival injections of the following: hydroxypropyl methylcellulose (HPMC) 0.3% with carbopol 980 (GenTeal gel, \(n = 4\)), HPMC 0.3% (GenTeal tears, \(n = 3\)), 0.1% carbopol 980 in sterile water (\(n = 3\)), HPMC 1.7% (ImproVue, \(n = 3\)), and balanced salt solution (BSS, negative control, \(n = 3\)). Rabbits were examined weekly for clinical evidence of inflammation, and biopsies were taken for final pathology 10 weeks postinjection.

\textbf{Results:} All seven eyes exposed to subconjunctival carbopol 980 (HPMC 0.3% with carbopol 980 or 0.1% carbopol 980) demonstrated conjunctival injection and edema at 4 weeks followed by yellow subconjunctival lesions with scattered pigmentation at 10 weeks. Each of these eyes demonstrated chronic histiocytic inflammation on pathology. No eyes treated with HPMC 0.3%, HPMC 1.7%, or BSS (noncarbopol-containing agents) developed clinical abnormalities or histiocytic inflammation (Fisher’s exact test \(P < 0.0001\)).

\textbf{Conclusions:} Subconjunctival exposure to ophthalmic lubricants containing carbopol 980 stimulates a chronic histiocytic inflammatory response. HPMC-based lubricants lacking carbopol 980 are well tolerated in the subconjunctival space.

\textbf{Translational Relevance:} Ophthalmic lubricants containing carbopol polymers should be avoided during surgical procedures that may result in subconjunctival exposure. Caution should be exercised when considering surgical use of biomedical products containing highly cross linked, high molecular weight acrylic acid polymers

\section*{Introduction}

Ophthalmologists frequently use topical ophthalmic lubricants to maintain corneal clarity during vitreoretinal surgical procedures. GenTeal gel (hydroxypropyl methylcellulose 0.3%, carbopol 980, phoshonic acid, sorbitol, sodium perborate 0.028%; Ciba Vision, Duluth, GA) has recently gained favor due to its ability to maintain corneal clarity longer than another commonly used lubricant that is preserved by benzalkonium chloride (BAK), a corneal epithelial toxin.\textsuperscript{1} However, we recently described an association between intraoperative use of GenTeal gel and postoperative subconjunctival necrotizing granulomatous inflammation in a series of 11 patients who underwent ophthalmic surgical procedures involving large conjunctival incisions.\textsuperscript{2} We suspected that the carbopol 980 component of GenTeal gel was responsible for this reaction. No cases of necrotizing granulomatous inflammation were found among similar operations at our institution that used non-carbopol-containing ophthalmic lubricants.

Carbopols are high molecular weight, cross-linked polyacrylic acid polymers that are useful as thickening agents.\textsuperscript{3} However, biocompatibility studies have
suggested that such polymers may stimulate a chronic inflammatory response, and intraoperative exposure to carbopol 934 has been implicated in nonhealing lipoplasty wounds characterized by necrotizing granulomatous inflammation. In order to test the hypothesis that subconjunctival exposure to GenTeal gel and its component carbopol 980 can stimulate a chronic inflammatory response, we designed a rabbit model of ocular surgery to compare the effects of subconjunctival injection of GenTeal gel and carbopol 980 to noncarbopol containing agents.

**Methods**

Of eight adult wild-type Dutch belted rabbits, 16 eyes received a 0.1-mL subconjunctival injection in the superonasal and superotemporal quadrant of each eye on day 0 of the study. For all injections, a skilled veterinary technician administered appropriate anesthesia (35 mg/kg ketamine and 5 mg/kg xylazine intramuscularly). Following induction, an assistant held the upper lid open, and downward rotation of the globe was achieved using a toothed forceps. The appropriate agent was then injected into the subconjunctival space in the superonasal and superotemporal quadrants using a 27-G needle. Research adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and was approved by the Institutional Animal Care and Use Committee.

The following agents were tested: hydroxypropyl methylcellulose (HPMC) 0.3% with carbopol 980 (GenTeal gel; Ciba Vision), HPMC 0.3% (GenTeal tears; Ciba Vision), 0.1% carbopol 980 powder in sterile water, and HPMC 1.7% (Improvue, 1.7% hydroxypropyl methylcellulose, calcium chloride dihydrate, hydrochloric acid, magnesium chloride hexahydrate, potassium chloride, purified water, sodium acetate trihydrate, sodium chloride, sodium citrate dihydrate, and sodium hydroxide; OCULUS Surgical, Port St. Lucie, FL). Carbopol 980 powder was prepared as a 0.1% solution in sterile water due to its poor solubility in more concentrated solutions. Balanced salt solution (BSS) was used as a negative control, and each injection agent had an three eyes from three different rabbits with the exception of HPMC 0.3% with carbopol 980, which had four eyes from four different rabbits as described in the Table.

Following injection, all animals were examined daily by animal housing staff for any signs of distress and weekly by two ophthalmologists to observe for clinically evident inflammation or subconjunctival granulomata. Two rabbits (rabbits 2 and 8) were subjected to survival biopsies of both eyes at week 4 to allow for histologic examination of the effects of subconjunctival HPMC 0.3% with carbopol 980 and carbopol 980 at 1 month. For survival biopsies, anesthesia was administered as for the injection procedure with the addition of buprenorphine (0.01 mg/kg subcutaneous) for analgesia. An assistant held the eyelids open and provided downward rotation of the globe, allowing for an approximately 0.5-cm ellipse snip biopsy of conjunctival and subconjunctival tissue to be taken with Wescott scissors (Fairfield, CT). Tissue was fixed in formalin and processed for paraffin sections that were stained with hematoxylin and eosin (H&E).

At 8 weeks, all eyes were graded by two ophthalmologists on a scale of 0 to 3 based on the observed extent of subconjunctival yellow lesions. A score of 0 indicated no clinically evident abnormalities; 1 corresponded to small yellow subconjunctival lesions covering up to one-third of the injected area without conjunctival pigmentation; 2 represented lesions covering up to two-thirds of the injected area, with subtle conjunctival pigmentation seen only upon close inspection; and 3 indicated yellow subconjunctival lesions underlying the majority of the superior conjunctiva with overlying pigmentation that could be easily recognized as abnormal from a distance.

The planned duration of study was 8 to 12 weeks, with study termination at 8 weeks if granulomata were present and study extension to 12 weeks if no

<table>
<thead>
<tr>
<th>Subconjunctival Injections</th>
<th>Rabbit and Eye Injected</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPMC 0.3% with carbopol 980</td>
<td>Rabbit 1, right eye</td>
</tr>
<tr>
<td>HPMC 0.3%</td>
<td>Rabbit 1, left eye</td>
</tr>
<tr>
<td>0.1% carbopol 980</td>
<td>Rabbit 2, right eye</td>
</tr>
<tr>
<td>HPMC 1.7%</td>
<td>Rabbit 2, left eye</td>
</tr>
<tr>
<td>BSS</td>
<td>Rabbit 3, right eye</td>
</tr>
</tbody>
</table>
Histopathologic abnormalities were found on 8-week tissue harvest from rabbit one. Following grading at week 8, rabbit one was sacrificed, and conjunctival and subconjunctival tissue was harvested from both eyes for histopathologic examination on H&E stained sections. After the discussion of the results of rabbit one with the ophthalmic pathologist, the decision was made to end the study at 10 weeks. At week 10, the remaining rabbits (2–8) were sacrificed, with tissue harvest and H&E sections made at that time. A trained ophthalmic pathologist examined all histology slides. A Fisher's exact test was performed to determine if there was a statistically significant difference between the presence and absence of clinical and pathologic histiocytic inflammation with exposure to carbopol-containing agents compared with noncarbopol-containing agents.

**Results**

Of 8 rabbits, 16 eyes received subconjunctival injections as in the Table. Injections were well tolerated, and no rabbits exhibited signs of discomfort. Immediately following injection, all eyes had two blebs corresponding to the two injection sites. Blebs formed by injection of HPMC 1.7% were localized and significantly elevated consistent with the high viscosity of the solution. Blebs formed by injection of HPMC 0.3% with carbopol 980 or 0.1% carbopol 980 were moderately elevated, and blebs formed by injection of HPMC 0.3% or BSS were more diffuse. At the week 1 exam, all seven eyes injected with HPMC 0.3% with carbopol 980 and 0.1% carbopol 980 had clinically evident conjunctival erythema and edema with mild elevation in the area of injection, which was more severe in 0.1% carbopol-injected eyes; all nine eyes injected with HPMC 0.3%, HPMC 1.7%, or BSS were white and quiet without residual subconjunctival bleb or other clinically evident abnormalities (Figs. 1A–C). By 4 weeks, the conjunctival injection in HPMC 0.3% with carbopol 980 and 0.1% carbopol-injected eyes began to resolve, but mild conjunctival edema remained and there was interval development of subtle underlying yellow lesions (Fig. 1D). Rabbits two and eight underwent biopsy of conjunctival and subconjunctival tissue at that time, and histopathology revealed collections of histiocytes intermixed with eosinophils in the right eye of rabbit two (carbopol 980) and the left eye of rabbit eight (HPMC 0.3% with carbopol 980). The left eye of rabbit 2 (HPMC 2=1.7%) and the right eye of rabbit eight (BSS) demonstrated conjunctival mucosa without abnormalities.

At weeks 8 through 10, all seven eyes injected with HPMC 0.3% with carbopol 980 or 0.1% carbopol 980 had clinically evident subconjunctival yellow lesions, with four having small areas of conjunctival pigmentation (Fig. 2). Grading the extent of subconjunctival lesions at week 8 revealed three grade 1 eyes (all HPMC 0.3% with carbopol 980), three grade 2 eyes (1 HPMC 0.3% with carbopol 980, 2 0.1% carbopol 980), and one grade 3 eye (0.1% carbopol 980). The other nine eyes remained clinically normal and were graded as 0 (Fisher’s exact test, P<0.0001). Rabbit one was sacrificed at week 8, with the remaining rabbits sacrificed at week 10. Tissue was harvested at the time of sacrifice. On histopathology, all seven eyes injected with HPMC 0.3% with carbopol 980 or 0.1% carbopol 980 demonstrated submucosal histiocytes (some pigmented) with chronic inflammation (Fig. 3). One eye injected with BSS (rabbit 5, left eye) exhibited chronic inflammation with intermixed eosinophils but...
no histiocytes (Fig. 4); this is likely unrelated to the injection. The remaining eight eyes showed conjunctival mucosa without abnormalities on histopathologic examination (Fig. 5).

Discussion

In our prior case series, we described 11 eyes of 11 patients with subconjunctival necrotizing granulomatous inflammation following vitreoretinal surgery with large conjunctival incisions.\(^2\) Clinically, these granulomata had the appearance of yellow subconjunctival nodules. The suspected cause of inflammation, after careful investigation of all instruments and materials used during surgery, was HPMC 0.3% with carbopol 980. No granulomata were observed in patients subjected to similar surgical procedures when agents lacking carbopol 980 were used to maintain corneal clarity. Furthermore, granulomata were only identified in patients exposed to topical HPMC 0.3% with carbopol 980 during procedures including large conjunctival incisions that may have introduced this topically applied gel into the subconjunctival space.\(^2\) Herein, we discuss the results of a rabbit study evaluating the effects of subconjunctival exposure to carbopol 980.

We injected 16 eyes of eight rabbits with HPMC 0.3% with carbopol 980 ($n = 4$), HPMC 0.3% ($n = 3$),...
0.1% carbopol 980 powder in sterile water (n = 3), HPMC 1.7% (n = 3), and BSS (n = 3). Subconjunctival injections in rabbits simulated subconjunctival exposure encountered during surgery with large subconjunctival cutdowns without subjecting rabbits to invasive surgical procedures that could be associated with significant postoperative discomfort. Furthermore, avoidance of large conjunctival incisions in this rabbit model removes the variable of surgical manipulation.

Eyes were observed for evidence of conjunctival and subconjunctival inflammatory reactions similar to those seen in patients from our prior series.2 Blebs from HPMC 1.7%, HPMC 0.3%, and BSS had completely resolved without residual clinical abnormalities within 1 week, while areas injected with HPMC 0.3% with carbopol 980 and 0.1% carbopol 980 had residual minor elevation with new development of conjunctival erythema and chemosis. After 8 to 10 weeks, all eyes exposed to subconjunctival carbopol 980 (HPMC 0.3% with carbopol 980 and 0.1% carbopol 980) had developed clinically evident yellow subconjunctival lesions that corresponded to chronic histiocytic inflammation on pathology. No eyes exposed to noncarbopol-containing ophthalmic lubricants developed histiocytic inflammation or clinical abnormalities. Importantly, HPMC 1.7%, a more viscous alternative for maintenance of corneal clarity lacking carbopol 980, was not associated with clinically evident acute or chronic inflammation or histopathologically evident histiocytic inflammation.

We hypothesized that the cross-linked polymer structure and high molecular weight of carbopol 980 may stimulate a foreign body reaction and/or prevent adequate clearance from the subconjunctival space. While histologically apparent granulomata did not develop in any eyes, the histiocytic inflammation and yellow lesions found on exam support our hypothesis that the carbopol 980 component of GenTeal gel is capable of stimulating a chronic inflammatory response with histiocytic infiltration if introduced into the subconjunctival space. It remains possible that histopathologically evident granulomata may have developed in the rabbits following a longer exposure time. Of note, all biopsies in our prior clinical series were performed more than 3 months postoperatively.2

We recognize that our sample size is small in this largely qualitative study, and that additional subjects would have allowed for a more robust statistical analysis. However, given the consistency of clinical and histopathologic findings, we felt that these results were sufficiently convincing such that it was not necessary to subject additional rabbits to further investigation.

Given these results, we conclude that subconjunctival exposure to agents containing carbopols can stimulate a chronic inflammatory response characterized by histiocytic infiltration. We recommend against the use of ophthalmic lubricants containing these polymers during surgical procedures that involve larger conjunctival incisions. Furthermore, we encourage careful investigation of any biomedical gel or product containing a high molecular weight, cross-linked, polyacrylic acid polymer that may be exposed to tissue during surgical procedures. Further investigation is necessary to determine whether or not careful irrigation prior to closure of conjunctival incisions can effectively prevent granulomatous inflammation. Noncarbopol-containing ophthalmic lubricants, such as HPMC 1.7%, do not appear to stimulate histiocytic inflammation and can be safely substituted for topical use during ocular surgery.

Acknowledgments

Supported, by grants from Research to Prevent Blindness, Inc. and a grant from the VitreoRetinal Surgery Foundation. The Mayo Clinic CTSA through grant number UL1 TR000135 made this publication possible from the National Center for Advancing Translational Sciences (NCATS), a component of the National Institutes of Health (NIH).

Disclosure: L.A. Dalvin, None; D.R. Salomao, None; R. Iezzi, None; A.J. Barkmeier, None

References


