Evaluation of Anti-TGF-β2 Antibody as a New Postoperative Anti-scarring Agent in Glaucoma Surgery

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PURPOSE. Postoperative subconjunctival wound healing remains the commonest cause of late bleb failure after glaucoma filtration surgery. This study was undertaken to investigate whether the human monoclonal antibody that neutralizes transforming growth factor-β2 (CAT-152; lerdelimumab) could be used as a postoperative agent to prevent scarring after glaucoma surgery and compared it with 5-fluorouracil (5-FU), to benchmark its potential clinical benefit.

METHODS. In a randomized, controlled, masked-observer study, after modified glaucoma surgery, 48 rabbits were randomly allocated to receive a postoperative course of seven subconjunctival injections of CAT-152 (1 mg/mL), 5-FU (50 mg/mL), or no treatment. Bleb characteristics, the presence of subconjunctival drainage, and local reaction to treatment were assessed. Animals were killed on days 10, 21, and 30. Immunohistochemistry, histologic staining and electron microscopy were performed to demonstrate the mechanism of CAT-152-mediated effects on the extracellular matrix.

RESULTS. CAT-152 significantly improved surgical outcome (log rank test, \( P < 0.001 \)) and reduced subconjunctival collagen deposition (\( P < 0.01 \)) compared with 5-FU and control. Median bleb survival was increased in the CAT-152 group (23.5 days) compared with the 5-FU (20 days) and control (16 days) treatment groups. CAT-152 treatment improved bleb morphology (\( P < 0.05 \)) and was well tolerated. 5-FU prolonged the duration of corneal epitheliopathy (\( P < 0.01 \)).

CONCLUSIONS. Postoperative administration of CAT-152 significantly improved surgical outcome, reduced subconjunctival scarring, and minimized the risk of corneal side effects compared with the anti-scarring agent 5-FU. These findings suggest that CAT-152 may offer therapeutic benefit as a postoperative agent to prevent subconjunctival scarring after glaucoma filtration surgery. (Invest Ophthalmol Vis Sci. 2005;44:3394 –3401) DOI:10.1167/iovs.02-0978

The major determinant of the long-term outcome of glaucoma surgery is the wound-healing response. Excessive postoperative scarring at the level of the conjunctiva and sclerostomy sites is associated with poor postoperative pressure control.1-4 The intraoperative use of the antiproliferative agents 5-fluorouracil (5-FU) and mitomycin C (MMC) has increased the success rate of filtration surgery, and this technique has been accepted in clinical practice.5-12 However, despite these intraoperative applications, failure can occur later in the postoperative period. 5-FU has been given subconjunctivally in the postoperative period to treat failing and encysted blebs.13-18 Prevention of a late increase in intraocular pressure may be particularly important in preserving visual function, given the data from the recent Advanced Glaucoma Intervention Study (AGIS) study.19 MMC and 5-FU work by causing widespread cell death and apoptosis and can result in corneal erosions and cystic avascular blebs. They are associated with severe sight-threatening complications.20-24 More physiologically anti-scarring agents are therefore needed for postoperative prevention of bleb failure and increased intraocular pressure.

Of all the growth factors involved in the wound-healing cascade, TGF-β has been shown to be one of the most potent stimulators of scarring in the eye and is involved in the pathogenesis of cataract, proliferative vitreoretinopathy, and conjunctival scarring.25-28 TGF-β2, the most representative of the three mammalian isoforms in the eye, is the most potent growth factor in the aqueous at stimulating conjunctival fibroblast function. Elevated levels of this isoform are found in the aqueous of glaucomatous eyes compared with normal eyes.29-34 These findings suggest that neutralizing the effects of TGF-β2 may reduce conjunctival scarring after glaucoma filtration surgery.

CAT-152 (lerdelimumab) is a novel human monoclonal antibody that was isolated and developed in vitro by the technique of antibody phage display. It displays high affinity and specificity for the active form of TGF-β2 and has been designed for therapeutic use. We have demonstrated in vitro that CAT-152 inhibits TGF-β2-induced human Tenon’s fibroblast migration and proliferation. Furthermore, in our in vivo model of aggressive conjunctival scarring we have shown that subconjunctival administration of CAT-152 at the time of glaucoma surgery and in the immediate postoperative period successfully improves surgical outcome, reduces subconjunctival fibrosis, and is safe and well tolerated.26 In the first human trial of CAT-152 in patients undergoing trabeculectomy, good tolerance and safety were reported with a treatment regimen of both intra- and postoperative injection.35 A multicenter phase II study is under way.

However to date, we have made no assessment of isolated postoperative application of CAT-152. Using the same model of glaucoma filtration surgery, the present study was designed to determine whether postoperative application alone of CAT-152 can improve bleb survival and to compare the effectiveness of CAT-152 with the currently used postoperative anti-scarring agent 5-FU.

METHODS

Forty-eight New Zealand White rabbits aged between 12 and 14 weeks and weighing 1.5 to 2.2 kg were used in this prospective randomized,
controlled, masked-observer study, which was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Glaucoma filtration surgery was performed on the left eyes of rabbits under general anesthesia (intramuscular muscular ketamine [50 mg/kg] and xylazine [10 mg/kg]) using a technique previously described by Cordeiro et al. A partial-thickness 60° silk corneal traction suture (Ethicon, Edinburgh, UK) was placed at 12 o’clock, to gain exposure to the superior conjunctiva. A fornix-based conjunctival flap was raised, and blunt dissection of the subconjunctival space was performed to a distance of 15 mm behind the limbus. An MVR blade (Vistech, Warwickshire, UK) was used to fashion a partial-thickness scleral tunnel, starting 4 mm behind the limbus and continuing until the blade was just visible in the anterior cornea stroma. A 22-gauge/25-mm intravenous cannula (Venflon; BD Biosciences, Helsingborg, Sweden) was then passed through the scleral tunnel until the cannula needle was visible in the clear cornea. The cannula needle entered the anterior chamber, the cannula was advanced to the mid-pupillary area, and the needle was withdrawn. Finally, the cannula was trimmed and beveled at its scleral end so that it protruded 1 mm from the insertion point, and a 10-0 nylon suture was used to fix the tube to the scleral surface. The conjunctival incision was closed with two interrupted sutures and a central mattress-type 10-0 nylon suture on a needle (B/V 100-4 needle, Ethicon) to give a water-tight closure. One drop of atropine sulfate 1% and betamethasone sodium phosphate was instilled at the end of surgery. No other adjunctive treatment was given at the time of surgery. The animals were randomly allocated to receive a postoperative course of seven subconjunctival injections (100 μL) of CAT-152 (1 mg/mL), 5-FU (50 mg/mL), or no treatment. The subconjunctival injections were given on days 2, 3, 4, 7, 9, 11, and 14 after surgery (day 0) under topical anesthesia (proxymetacaine hydrochloride 0.5% eye drops, 1 drop per eye), using a 30-gauge needle (Myjector 100u; Terumo, Tokyo, Japan). CAT-152 was injected 5 mm behind the limbus at the nasal margin of the superior rectus muscle. 5-FU was administered 180° from the site of surgery. The rationale for the injection site selection was in keeping with the method used in the original studies of subconjunctival 5-FU in glaucoma filtration surgery and widespread clinical practice. Injections were given by an independent clinician, because the different injection sites for each treatment precluded masking.

Clinical Evaluation of Postoperative CAT-152

Baseline observations were performed before glaucoma filtration surgery. Measurement of intraocular pressure in both eyes was made with a handheld tonometer (Tonopen; Mentor, Norwell, MA) after topical instillation of 0.5% proxymetacaine HCl eye drops, 1 drop per eye, using a 30-gauge needle (Myjector 100u; Terumo, Tokyo, Japan). CAT-152 was injected 5 mm behind the limbus at the nasal margin of the superior rectus muscle. 5-FU was administered 180° from the site of surgery. The rationale for the injection site selection was in keeping with the method used in the original studies of subconjunctival 5-FU in glaucoma filtration surgery and widespread clinical practice. Injections were given by an independent clinician, because the different injection sites for each treatment precluded masking.

Postoperative Anti-TGF-β2 in Glaucoma Surgery

Bleb survival was taken as the primary efficacy end point in the analysis of CAT-152 in rabbit filtration surgery. Bleb failure was defined as the appearance of a flat, vascularized, and scarred bleb in the presence of a deep anterior chamber. Kaplan-Meier and log rank statistics were used to compare treatment groups in bleb and intraocular pressure failure (defined as the return of the intraocular pressure in the surgical eye to baseline level). Bleb area and height, anterior chamber depth and activity, and conjunctival vascularity per quadrant were all analyzed with a repeated-measures procedure and the generalized linear model (SPSS; SPSS Inc., Chicago, IL). This allowed comparison of

FIGURE 1. The effect of CAT-152 (n = 8), 5-FU (n = 8), or no treatment (n = 7) on (A) bleb survival, (B) bleb area, and (C) bleb height. CAT-152 significantly prolonged bleb survival compared with 5-FU and the untreated control group, as shown in the Kaplan-Meier survival curve (P = 0.0009 log rank test). CAT-152 treated eyes had significantly larger blebs (area and height, P < 0.05).
treatment groups over the whole study period, using between-subject tests. Duration corneal epithelopathy and avascularity were analyzed using the Kruskal-Wallis test and the Dunn test. Finally, intraocular pressure was analyzed with the multivariate analysis of variance with Bonferroni’s modification, to compare differences between treatments and the effects of time and treatment. The level of significance applied to the statistical analysis was \( P < 0.05 \).

The experiment was performed in two phases. In the first phase, 24 rabbits underwent the experimental protocol and were killed on day 30. Observational analysis of the effect of CAT-152 on glaucoma surgery and histologic analysis of the ocular tissue were performed on these animals. In the second phase, the experiment was repeated using an additional 24 rabbits which were killed on days 10 (\( n = 12 \)) and 21 (\( n = 12 \)) to provide histologic information at these earlier time points.

**Analysis of Rabbit Tissues**

**Histology.** On days 10, 21, and 30 animals were killed with a lethal intravenous injection of phenobarbitone, and the tissues were processed for histology. Both eyes were enucleated. The upper lid was left intact, attached to the globe to preserve the architecture of the superior fornix and conjunctival tissues around the drainage site. All the eyes were fixed in 10% buffered formal saline for 24 hours, stored in 70% alcohol and fixed in paraffin wax. Sequential 5-µm sections of the operative wound site were prepared, and histologic staining was performed to demonstrate cellularity and extracellular matrix deposition including: hematoxylin and eosin (for total cellularity); picrosirius red and Gamori’s trichrome (for scar formation, collagen density, and orientation); oxidation aldehyde fuchsin (elastic fibers), α-smooth muscle actin immunohistochemistry (myofibroblast phenotype identification), and proliferating cell nuclear antigen (PCNA) immunohistochemistry (recent cell division). These parameters were graded by two masked observers, using a modified scoring system (based on that described by Shah et al.) which incorporated visual reference standards for each grade on a scale of 0 to 4: 0, same as control eye; 1, 1%-25% of control; 2, 26%-50% of control; 3, 51%-75% of control; 4, more than 75% of control. All surgical eyes were compared with contralateral, nonsurgical eyes. Mean scores of histologic parameters for each treatment group per time point were calculated, and this semiquantitative data were analyzed by analysis of variance (SPSS).

**Electron Microscopy.** We also compared the electron micrograph characteristics of two rabbit eyes treated with postoperative CAT-152 and two untreated eyes. The surgical technique and postoperative injection procedure used in the main study were repeated. Animals were killed on day 10, and the operative wound site was processed for both scanning and transmission electron microscopy. The day 10 time point was selected to provide maximum information on the comparative bleb appearances between treated and untreated animals before the onset of bleb failure.

**Results**

**Experimental Details**

Of the 24 rabbits observed for the duration of the 30-day experimental period, 23 completed the experimental protocol. One animal was observed to be showing signs of developing severe intraocular infection 3 days after surgery. The rabbit was killed and an independent observer (to preserve masking) identified that the animal was in the control group. Observational analysis was therefore performed on eight animals that received CAT-152, eight animals that received 5-FU, and seven untreated control animals.

**Effects on Filtration Surgery**

CAT-152 significantly improved glaucoma filtration surgery outcome in this animal model of aggressive postsurgical scarring. CAT-152 significantly prolonged bleb survival compared with the 5-FU group and the untreated control group, as shown in the Kaplan-Meier survival curve in Figure 1A (log rank test; \( P = 0.0009 \)). The rate of bleb failure and percentage survival in each treatment group is shown in Table 1. All the blebs in the control and 5-FU groups had failed by day 22; however, 62.5% of the CAT-152 treatment group had functioning blebs. By day 30 all but one of the operations had failed; the only animal with

**TABLE 1. Incidence of Bleb Failure and the Percentage of Bleb Survival in Rabbits Undergoing Glaucoma Filtration Surgery: Effect of CAT-152 and 5-FU Treatment Compared to Control**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0</th>
<th>14</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
<th>28</th>
<th>29</th>
<th>30</th>
<th>Survival 30</th>
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<tr>
<td>CAT-152</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1/8</td>
</tr>
<tr>
<td>% Survival</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>87.5</td>
<td>75</td>
<td>62.5</td>
<td>50</td>
<td>37.5</td>
<td>25</td>
<td>12.5</td>
<td>12.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>5-FU</td>
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<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
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<td>0</td>
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<td>0</td>
<td>0</td>
<td>0/8</td>
</tr>
<tr>
<td>% Survival</td>
<td>100</td>
<td>100</td>
<td>87.5</td>
<td>62.5</td>
<td>37.5</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0/7</td>
</tr>
<tr>
<td>No treatment</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>1</td>
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<td>1</td>
<td>0</td>
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<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>% Survival</td>
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<td>57.1</td>
<td>42.9</td>
<td>28.6</td>
<td>14.3</td>
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</table>

**FIGURE 2. Bleb morphology at day 21 after glaucoma filtration surgery.** One representative animal is shown per group. Animals were treated with CAT-152 (A) or 5-FU (B) or received no treatment (C). Treatment with CAT-152 was associated with elevated, diffuse, fleshy looking blebs compared with the flat, scarred blebs in the 5-FU and control groups. Black arrows: bleb border; white arrow: cannula.
a functioning bleb had received CAT-152. The median (range) survival rates were 23.5 (20–30), 20 (16–22), and 16 (14–21) days in the CAT-152, 5-FU, and control groups, respectively. The presence of a well formed bleb is an important indicator of effective filtration. Subconjunctival scarring causes contraction and flattening of the bleb. Figure 2 shows the typical appearances of the filtration blebs on day 21. Treatment with CAT-152 was associated with elevated, diffuse, fleshy looking blebs compared with the flat, scarred blebs in the other groups. Analysis of both bleb area and bleb height using the repeated measures of the generalized linear model, revealed significant differences in both these variables after treatment with CAT-152 compared with the 5-FU or no-treatment regimens (P < 0.005 area, P < 0.001 height; Figs. 1B, 1C).

Analysis of mean intraocular pressure in the surgical eyes showed no significant differences between treatment groups over the entire study period (P > 0.05). Corneal epitheliopathy is a recognized adverse effect associated with the clinical use of 5-FU and is associated with both ocular discomfort and the risk of infection. Given this, a grading system was included in the methods to assess the severity of this variable. In this experiment only mild punctuate staining of the cornea (superficial punctuate keratitis, grade 1) was detected in all treatment groups, and this was a transient finding. The duration of the staining was significantly longer in the 5-FU-treated group (P < 0.01). The duration of staining in the CAT-152 group was similar to that observed in the no-treatment control group (Table 2).

One of the features of existing cytotoxic anti-scarring regimens is their production of nonperfused, avascular areas in locally treated tissues. These areas of avascularity are associated with thin-walled, cystic blebs and the attendant risks of leakage and infection. In all the rabbit eyes, a small region of avascularity was noted in the nasal side of the bleb (<3 mm), within the first 7 days. This was transient finding. The duration of the avascular segment tended to be longer in the 5-FU-treated animals, but did not reach statistical significance (P = 0.159). The duration of avascularity in the CAT-152 group was similar to that observed in the no-treatment control group (Table 2).

Local reaction to treatment was assessed by the degree of anterior chamber inflammation and conjunctival vascularity. No significant difference was found between treatment groups for either of these indicators of the inflammatory response (vascularity, superior P = 0.402, temporal P = 0.434, nasal P = 0.668; anterior chamber inflammation P = 0.430).

The depth of the anterior chamber was assessed as an indirect indicator of drainage. On day 1 after surgery the anterior chamber was flat in most of the animals. Over the next 7 days the anterior chamber gradually deepened. No significant difference was found between the treatment groups in the time taken for the anterior chamber to deepen (P = 0.302).

### Histologic Effects

CAT-152 treatment reduced scarring at a microscopic level. The greatest histologic difference between treatment groups was seen on day 10 (Fig. 3). At this time point, total scar formation, as judged by the staining characteristics of picrosirius red, was significantly reduced by CAT-152 treatment (P = 0.01, Figs. 3D–F, Fig. 5). In addition, CAT-152 significantly reduced the population of cells expressing α-smooth muscle actin, indicating less fibroblast differentiation into the myofibroblast phenotype (P = 0.01; Figs. 4, 5). No other significant differences were found between treatment groups in total cellularity, elastic fiber deposition, or proliferating cell nuclear antigen (PCNA) at day 10. By day 30 subconjunctival scarring at the wound site consisted characteristically of densely packed collagen and fibroblasts. In contrast, the only surviving CAT-152-treated bleb showed much looser architecture with visible conjunctival bleb formation (Fig. 6).

We compared electron microscopic (EM) characteristics of CAT-152–treated and untreated control animals. Morphologically, scanning EM demonstrated looser subconjunctival bleb architecture with CAT-152 treatment (Figs. 7A, 7C, 7E). At a cellular level, transmission EM showed a corresponding reduction in inflammatory cell infiltrate and collagen fibril formation within the blebs of CAT-152–treated animals (Fig. 7, compare E and F).

### TABLE 2. Duration of Low-Grade Corneal Epitheliopathy or Avascularity after Treatment with CAT-152 or 5-FU Compared with the No-Treatment Control Animals

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>CAT-152</th>
<th>5-FU</th>
<th>NT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corneal Epitheliopathy</td>
<td>1.63 ± 0.53</td>
<td>10.71 ± 1.71*</td>
<td>1.57 ± 0.48</td>
</tr>
<tr>
<td>Avascularity</td>
<td>9.13 ± 1.33</td>
<td>16.88 ± 5.2</td>
<td>10.57 ± 2.5</td>
</tr>
</tbody>
</table>

Data are the mean ± SE.

*P < 0.01 comparing CAT-152 or 5-FU treatment groups to the no-treatment control group using Kruskal-Wallis test and Dunn’s test. NT, no-treatment group.

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**FIGURE 3.** Histologic characteristics of filtration blebs on day 10. (A–C) Hematoxylin and eosin– and (D–F) picrosirius red–stained sections are shown of one representative animal per group: CAT-152–treated (A, D), 5-FU–treated (B, E), and the control group (C, F). CAT-152 treatment reduced subconjunctival scarring at the microscopic level. The picrosirius red staining demonstrates a reduction in the density of subconjunctival collagen deposition (green and yellow) in CAT-152–treated animals. Bar, 500 μm.
neutralizing the effects of TGF-
out some of the side effects associated with antiproliferative
use of CAT-152 appears more efficacious than 5-FU and with-
animal model of aggressive scarring. In addition, postoperative
by subconjunctival administration of a novel antibody (CAT-
in certain eyes.37 However, even short exposure to MMC
target of CAT-152 over MMC and 5-FU lies in its more physio-
tissue architecture in the absence of side effects.

The anti-metabolites 5-FU and MMC are currently the back-
bon of anti-scarring treatments. The intraoperative regimen
of mitomycin C has gained favor due to the convenience of a
single treatment and the delivery of lower intraocular pressure
in certain eyes.37 However, even short exposure to MMC
results in local irreversible tissue destruction.12,20 The advan-
tage of CAT-152 over MMC and 5-FU lies in its more physio-
logical method of action, potentially providing long-term titrat-
able intraocular pressure control, while maintaining normal
tissue architecture in the absence of side effects.

Rabbit models exhibit an exaggerated healing response
compared with human tissue. Demonstration of efficacy in
such models is therefore likely to be reproduced in the clinical
setting. The surgical model of glaucoma surgery used in this
study localizes scarring to the level of the conjunctiva. This is
achieved by maintaining a permanent fistula to drain aqueous
into the subconjunctival wound site. Experience has shown
that intraocular pressure is not a reliable indicator of filtration
in this model of glaucoma surgery. Furthermore, in this study
no significant differences were detected in IOP between
groups. This can be explained by the fact that basal preoper-
ative intraocular pressure in this model of glaucoma filtration
surgery is within the normal range (this is a model of subcon-
junctival scarring, not of glaucoma). Given this, bleb failure
rather than intraocular pressure has always been defined as the
primary outcome variable representing failure of surgery in this
model.

We found that isolated postoperative 5-FU had the same
bleb survival end point as the control in this experiment. This
model exhibits an extremely aggressive scarring response that
may explain this finding. However, conversely, all the treat-
ments that have improved bleb survival in this model have
worked in a subsequent clinical setting.12,26 Only a limited
number of studies have looked at efficacy of 5-FU as an isolated
postoperative agent in animal experiments. Doyle et al.38 com-
pared the effect of five postoperative injections of 5-FU with
single intraoperative 5-FU, and combined intra- and postope-
rate 5-FU in rabbit filtration surgery. Control animals received
intraoperative distilled water only. No significant difference
was shown in bleb survival between postoperative 5-FU treat-
ment and control in this study. However, what can be seen in
the survival curve from Doyle et al. and our data is that post-
operative injections of 5-FU appear to shift the survival curve
to the right of the control at the early time points, without
affecting the final end point.

The landmark Fluorouracil Filtering Surgery Study6 repre-
ents the only definitive report in which postoperative injec-
tions of 5-FU were shown to improve surgical outcome. In this
study, 21 subconjunctival injections were administered: two
injections per day on days 1 to 7 after surgery and one injection
per day on days 7 to 14 after surgery. If this number of
injections had been used, we may well have shown efficacy of
postoperative 5-FU in this model. When designing the protocol
we chose a postoperative regimen that more closely reflected
current clinical subconjunctival 5-FU use.

Anterior chamber depth was included in the observations as
an indirect indicator of the drainage of fluid through the tube
into the subconjunctival space. The rabbit anterior segment is
very crowded with a very large lens and small anterior cham-
ber. Therefore, in practice, this measurement was fairly diffi-
cult to grade. In most of the animals, the anterior chamber was
flat on day 1 and gradually deepened over the next 7 days. No
significant difference was found between the treatments in the
time taken for the anterior chamber to deepen. Overall, this
observation may not be as true a representation of anterior
fluid dynamics as had been originally anticipated.

Ideally all subconjunctival injections would have been given
at the same site. In all the preclinical and clinical studies of
CAT-152 in glaucoma filtration surgery, the drug has been

Figure 5. A histologic grading system was used to quantify the effect
of treatment with CAT-152 or 5-FU compared with the control on
collagen deposition and myofibroblast transformation on day 10. CAT-
152 significantly reduced staining for picrosirius red and α-smooth
muscle actin.

Figure 4. Fibroblast differentiation to the myofibroblast phenotype is characterized by the expression
and assembly of α-smooth muscle actin into stress fibers. Immunohistochemical staining of the filtration
blebs on day 10, using a diaminobenzidine (DAB) detection system, demonstrates a reduction in the
number of α-smooth muscle actin expressing cells after CAT-152 treatment (A) compared with 5-FU (B)
and the control (C). Bar, 500 μm.
Figure 6. By day 30, only one bleb (CAT-152 treated) was still functioning. In failed blebs, subconjunctival scarring consisted of dense collagen fibers and fibroblasts (B). In contrast, the surviving bleb showed much looser architecture and visible evidence of bleb formation (A). Bar, 100 μm.

Figure 7. Electron microscopy (EM) was performed on CAT-152–treated animals (A, C, E) and untreated control animals (B, D, F). Morphologically, scanning EM (A–D) demonstrated looser subconjunctival bleb architecture with CAT-152 treatment. At a cellular level, transmission EM showed the associated reduction in inflammatory cell infiltrate and collagen fiber deposition in the CAT-152–treated animals. Bars: (A, B) 1 mm; (C, D) 100 μm; (E, F) 10 μm.
administered by subconjunctival injection in the superior nasal quadrant adjacent to the drainage bleb. The same injection site was therefore selected in this study. Clinically, the site of subconjunctival 5-FU administration varies, depending on clinical preference. Some clinicians favor injections adjacent to the bleb and some 90° and others 180° from the operation site. The main concern with injecting 5-FU adjacent to the bleb relates to the possibility of intraocular penetration, because the pH of 5-FU is 9. We decided to base our study protocol on the pioneering studies of 5-FU in glaucoma surgery performed by the Fluorouracil Filtering Surgery Study group. In this study 5-FU was injected 180° from the site of surgery. 4 Given the different injection sites for the CAT-152 and 5-FU groups a universal injection site for a control vehicle was not possible and therefore the no-treatment control group was introduced.

Aqueous flow bathes the wound and provides a unique and changeable environment that influences postoperative healing. Of all the growth factors in the aqueous TGF-β is the most potent stimulator of human Tenon’s fibroblast activity. 46 Latent TGF-β2 is produced by tissues within the eye (ciliary body and trabecular meshwork) before activation by plasmin and thrombospondin released from blood components. 42,43 Aqueous humor in glaucomatous eyes contains increased level of TGF-β2. 44 After glaucoma surgery, elevated levels of activated TGF-β2 at the wound site are therefore likely to be related to aqueous concentration, the flow of aqueous, and breakdown of the blood aqueous barrier. In addition TGF-β2 also displays the ability to autoinduce its own production thereby initiating a perpetuating cascade of activation. 45,46 In a mouse model of conjunctival scarring, peak levels of TGF-β2 have been shown at the wound site at day 7. 47 Without treatment, the rabbit model fails by day 14. We propose that isolated postoperative administration of CAT-152, between days 2 and 14 in this model, can still neutralize subconjunctival TGF-β2 levels at the wound site below a threshold required to mediate downstream effects on the extracellular matrix.

Histologic analysis of the rabbit tissues showed that CAT-152 significantly reduced subconjunctival collagen deposition compared with both the 5-FU and control groups. CAT-152 also significantly reduced the population of cells expressing α-smooth muscle actin, indicating an inhibition of fibroblast differentiation into the myofibroblast phenotype. Myofibroblasts are specialized fibroblasts that play an important role in wound healing. They are present transiently during tissue repair and are thought to generate the contractile force that is integral to normal wound closure. Excessive or abnormal contraction of granulation tissue leads to pathologic scarring. Fibroblast differentiation into the myofibroblast phenotype, characterized by the expression and assembly of α-smooth muscle actin into stress fibers, is modulated by cytokines. 44 TGF-β has been shown to be a direct inducer of the myofibroblast phenotype and is capable of upregulating α-smooth muscle actin, both in vivo and in vitro. 45,46 We have shown in vitro that CAT-152 significantly inhibits TGF-β2-stimulated collagen contraction. 46 These observations suggest that the beneficial effects of CAT-152 in glaucoma surgery may be mediated by a reduction in TGF-β2-induced collagen production and contraction.

Repetitive injections of subconjunctival 5-FU in clinical practice are known to cause corneal epitheliopathy. Similar findings were associated with the use of 5-FU in this study. The more serious complications of avascular bleb formation, bleb-related infection and chronic hypotony reflect the cytotoxic mechanism of action of antiproliferative agents. CAT-152 treatment provides a more physiological alternative. We present evidence that postoperative TGF-β2 inhibition with this novel monoclonal antibody can prevent failure of experimental glaucoma surgery by inhibition of subconjunctival scarring. This represents a potentially useful development in the prevention of late surgical failure and may provide us with a safer therapy to maintain maximal IOP control in the longer term.

References


