The Long-Term Effects of 5-Fluorouracil and Sodium Butyrate on Human Tenon's Fibroblasts

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The use of subconjunctival 5-fluorouracil (5-FU) in the first weeks after filtration surgery may ensure long-term bleb survival despite a continuing proliferative stimulus such as in eyes with neovascular glaucoma. In addition, long-term side effects may occur, such as increasing bleb thinning. To ascertain the long-term effects of 5-FU and sodium butyrate, an agent with differentiating and antiproliferative properties, we exposed proliferating human Tenon’s capsule fibroblasts to different concentrations of the drugs. The cells were exposed to 5-FU for 1–12 d. The cells were subsequently observed for up to 30 d. Cell proliferation was assessed using cell counting and bromodeoxyuridine uptake, and cell viability was assessed with trypan blue uptake. 5-FU and sodium butyrate inhibited fibroblast proliferation during the treatment period. Higher concentrations of 5-FU (100 and 1000 µg/ml) for as little as 1 d resulted in no significant increase in the number of fibroblasts for at least 29 d after treatment was stopped, despite continued stimulation with serum. When treatment with sodium butyrate was stopped, there was greater recovery of proliferation. At a constant concentration of 1000 µg/ml of 5-FU for 3 or more days, or a concentration of 100 mmol/l sodium butyrate for 12 d, the entire fibroblast population gradually died over the 30 d period. Thus, short-term treatment with 5-FU may result in long-term inhibition of proliferation of fibroblasts. Long-term inhibition depends on the duration of treatment or on the concentration of 5-FU. Short-term treatment may be affecting the ability of the tissues at the bleb site to heal in the long term. Different dosage regimens may have advantages and are discussed.


The use of 5-fluorouracil (5-FU) given by subconjunctival injection after glaucoma filtration surgery has increased the success rate considerably in patients with a high risk of failure.1 However, 5-FU has been associated with complications such as recurrent corneal epithelial erosions and thin conjunctival blebs that may leak, resulting in hypotony and complications such as choroidal effusions and macular edema.2 These thin blebs also may increase the risk of subsequent endophthalmitis.3 The corneal epithelial erosions and thinning of the blebs may continue to worsen even after the subconjunctival injections have been stopped. This has led to an empirical reduction in the number of injections from the initial regimen of two injections twice a day for a week and then once a day for a week to much shorter regimens that achieve similar success rates.4,5

The initial regimen of 5-FU injections was based on the concept of inhibiting fibroblast proliferation over the 2 wk when cellular proliferation is maximal after filtering surgery. The initial cell culture studies that established the concentration of 5-FU required to inhibit the proliferation of fibroblasts by 50% (the inhibitory dose or ID50) were based on continuous exposure to the drug over a short period of 2–3 d.6 However, wound healing is a process that may continue for many months and even years. Although the majority of failures occur in the first few months, surgery may fail many months after surgery.7 In addition, there are eyes in which the stimulus for cellular proliferation may continue unabated, such as those with neovascular glaucoma. Even in these eyes, relatively short-term treatment with 5-FU can have a prolonged effect on the survival of the filtration bleb8 well beyond the treatment period. The longer term effects of up to 30 d of antiproliferative agents on human ocular Tenon’s capsule fibroblasts after exposure to antiproliferatives for periods of up to 2 wk (as per the clinical situation) and the relationship between concentration and time of exposure have not been studied in depth.

5-FU acts as a metabolic blocker via its metabolites by inhibiting thymidylate synthetase and hence DNA
synthesis and by incorporation into RNA. Sodium butyrate is the sodium salt of the naturally occurring four carbon fatty acid that causes certain cancer cells such as neuroblastoma cells to assume the features of a more differentiated cell in tissue culture.\(^9\) It also nonspecifically inhibits cellular proliferation. One of the mechanisms of action may be a direct effect of the expression of various cellular genes by affecting proteins (histones) that bind DNA.\(^{10,11}\) Sodium butyrate has been proposed as a potential agent that inhibits wound healing in the eye.\(^{12,13}\)

The aims of the present study were to:

1. establish the short- and long-term effects on the total cell numbers of proliferating human Tenon's capsule fibroblasts after differing periods of exposure to different concentrations of the antiproliferative agents, 5-FU and sodium butyrate; and
2. establish the short- and long-term effects of exposures to these drugs on fibroblast bromodeoxyuridine uptake and trypan blue exclusion, indices of cellular proliferation and cell viability, respectively.

Materials and Methods

Cell Culture

Fibroblasts were grown from human Tenon's capsule biopsies taken from a 17-yr-old donor eye. The explants were placed in 25 cm\(^2\) tissue culture flasks in Dulbecco's modified Eagle's medium (DMEM; Gibco, Paisley, UK) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin (Gibco) and grown at 37°C in 5% humidified CO\(_2\). All cells used were between 3rd and 6th passage. The cells then were passaged 1:3 with trypsin/EDTA. This procedure was repeated using the same cells treated with sodium butyrate (Sigma, Poole, UK) at concentrations of 0, 1, 11, 110, 1100, and 11,000 \(\mu\)g/ml (equivalent to 0, 0.01, 0.1, 1, 10, and 100 mmol/l solutions) for 1, 3, 6, and 12 d.

Cell Proliferation

Fibroblasts were seeded onto eight 96-well plates at a density of 5000 cells per cm\(^2\) and allowed to settle for one day. Each plate was divided into 12 groups of four wells which were treated with different concentrations of 5-FU in DMEM/10% FBS; 0, 1 and 100 \(\mu\)g per ml for different periods of 1, 3, 6 and 12 days. At days 0, 1, 3, 6, 12, 18, 24 and 30 one plate was incubated with 1:1000 bromodeoxyuridine (BrDU) for four hours. The plate was then washed gently with HBSS, fixed in 70% methanol, and air dried and frozen at \(-20^\circ\)C. The rest of the plates were treated with the different concentrations of 5-FU for the varying time periods, washed with HBSS and refed with DMEM/10% FBS after the treatment period was over. The cells were fed every three days.

At the end of 30 d, the cells in all of the fixed plates were rehydrated with phosphate buffered saline (PBS) and incubated with 3% hydrogen peroxide. They then were washed and incubated in 5% goat serum, and mouse antibrromodeoxyuridine antibody (Amer-sham, UK) was added for 1 hr at 37°C. Biotinylated rabbit anti-mouse IgG antibody (Dakopatts, High Wycombe, UK) then was added, diluted in 1% goat serum/PBS. Peroxidase conjugated avidin (Dako-patts) then was added and 3-amino-9-ethylcarbazole with \(\text{H}_2\text{O}_2\) was used to produce a red color reaction. The cells then were stained with hematoxylin and granul lent pipetted into the wells. The wells were viewed and photographed with a phase contrast microscope. Five fields of 0.75 \(\times\) 0.55 mm per well were used. The total number of cells per field and the total number of cells positive for BrDU were counted, with an average calculated for each well. Five fields from each of the other three wells that received the same treatment regimen also were counted using five fields. This was repeated for the other three wells. Differences in cell number were assessed for statistical significance using analysis of variance and Bonferroni's multiple testing criteria. Significance was defined as \(P < 0.05\).
Cell Viability

The same procedure, using 96-well plates, was conducted as above with the same concentrations of drugs and treatment periods. At days 0, 1, 3, 6, 12, 18, 24, and 30, one plate was taken and each well in turn was filled with a concentration of 0.25% trypan blue, DMEM. After one minute of incubation, the number of trypan blue positive cells were counted in each of five fields and an average was calculated. The well then was washed gently with HBSS and fixed with methanol. The numbers of cells in each of five fields were counted. The percentage of trypan blue positive cells was calculated by dividing the average number of trypan blue positive cells by the average total number of cells after fixation.

Results

Cell Numbers

5-FU and sodium butyrate inhibited the increase in cell numbers that occurred when preconfluent human Tenon's fibroblasts were stimulated with serum (Figs. 1 and 2). This inhibition was statistically significant after 1 d of treatment only with the highest concentration of 5-FU (1000 µg/ml), but was statistically significant only after 3 or more days of treatment with lower doses of 5-FU and with sodium butyrate. With an increasing period of treatment, the relative inhibition of cell division became more apparent as the control cells continued to increase. At a concentration of 1000 µg/ml of 5-FU, a fall in cell number with a gradual loss of the cell population was seen after removal of the drug and refeeding with serum and media, even after only 3 d treatment. At the 100 µg/ml concentration of 5-FU, gradual cell loss occurred after 12 d of treatment, even after cessation of treatment. With sodium butyrate, cell loss also occurred at the 11,000 µg/ml (100 mmol/l) concentration. Most of the cell loss with the sodium butyrate occurred during treatment, unlike the cell loss at the 100 µg/ml concentration of 5-FU, where a lot of the cell loss occurred after cessation of treatment. During treatment, at doses of 5-FU and sodium butyrate that caused growth arrest but not death, the cells assumed a spread out appearance with a much larger surface area, in contrast to their normal, more spindle shaped appearance.

At 5-FU concentrations of 0.01, 0.1, and 1 µg/ml, the cell numbers began to gradually increase after treatment was stopped. However, the cells treated for 12 d with 1 µg/ml did not show an increase in cell number, even 18 d after treatment was stopped (day 30 of the experiment). At a concentration of 10 µg/ml, treatment periods of 3 d or more resulted in no

![Graphs showing cell numbers after exposure to 5-FU for 1, 3, 6, and 12 days, respectively, at different concentrations.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933168/)
Butyrate treatment for 1 day

Butyrate treatment for 3 days

Butyrate treatment for 6 days

Butyrate treatment for 12 days

Fig. 2. Cell numbers after exposure to sodium butyrate for 1, 3, 6, and 12 days, respectively, at different concentrations.

increase in cell number up to day 30. In contrast, with sodium butyrate treatment, the cell numbers began to increase after treatment was stopped at all dosing regimens except for the treatment for 12 d with 11,000 μg/ml (100 mmol/l) butyrate, during which most of the cells died. Many of the cells treated with fluorouracil and butyrate at mid range doses showed an increase in cell size. However, although cell contact did occur at lower densities, there were many areas where the nonproliferating cells were not in contact where BrDU uptake was not occurring.

The ID_{50} doses are shown in Tables 1 and 2. A 50% inhibition of the cell counts relative to the control was not reached until day 3 for the 5-FU and sodium butyrate treatment. However, after 1 d of treatment with 5-FU, the ID_{50} level was reached from day 3 onward. This reflected the gradual increase in the control cell counts and the continued inhibition of the increase in cell counts with the 5-FU treated cells. In comparison, the cells treated for 1 d with sodium butyrate achieved only a 50% inhibition at days 12 and 18 as the cells recovered and increased in number relative to the control cells. This trend also was seen in the ID_{50} levels for 3, 6, and 12 d of treatment. There was only a small change in the ID_{50} levels after treatment with 5-FU was stopped, whereas the ID_{50} levels rose when treatment was stopped with the sodium butyrate.

The dose of sodium butyrate required to inhibit the increase in cell numbers to the same degree as that done by 5-FU during treatment was about 100 times greater on a weight for weight basis (1 μg/ml 5-FU was about equivalent to 110 μg/ml sodium butyrate).

Table 1. ID_{50} concentrations for 5-FU-treated cells (μg/ml)

<table>
<thead>
<tr>
<th>Day</th>
<th>1-day Rx</th>
<th>3-day Rx</th>
<th>6-day Rx</th>
<th>12-day Rx</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>Not reached</td>
<td>Not reached</td>
<td>Not reached</td>
<td>Not reached</td>
</tr>
<tr>
<td>1</td>
<td>Not reached</td>
<td>Not reached</td>
<td>Not reached</td>
<td>Not reached</td>
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<tr>
<td>3</td>
<td>420</td>
<td>20.4</td>
<td>8.48</td>
<td>32.1</td>
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<td>6</td>
<td>822</td>
<td>7.56</td>
<td>0.76</td>
<td>0.66</td>
</tr>
<tr>
<td>12</td>
<td>8.45</td>
<td>2.17</td>
<td>0.81</td>
<td>0.28</td>
</tr>
<tr>
<td>18</td>
<td>12.3</td>
<td>5.08</td>
<td>1.25</td>
<td>0.45</td>
</tr>
<tr>
<td>24</td>
<td>6.71</td>
<td>1.45</td>
<td>0.62</td>
<td>0.40</td>
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<tr>
<td>30</td>
<td>11.3</td>
<td>2.15</td>
<td>0.68</td>
<td>0.39</td>
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</table>
Table 2. ID₅₀ concentrations for sodium butyrate-treated cells (μg/ml)

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<tr>
<th>Day</th>
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<th>3-day Rx</th>
<th>6-day Rx</th>
<th>12-day Rx</th>
</tr>
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<tbody>
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<td>Not reached</td>
<td>Not reached</td>
<td>Not reached</td>
</tr>
<tr>
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<td>Not reached</td>
<td>Not reached</td>
<td>Not reached</td>
<td>Not reached</td>
</tr>
<tr>
<td>3</td>
<td>1489 (13.5 mM)</td>
<td>105.2 (0.96 mM)</td>
<td>30 (0.27 mM)</td>
<td>29.7 (0.27 mM)</td>
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<tr>
<td>6</td>
<td>Not reached</td>
<td>2038 (18.5 mM)</td>
<td>57.6 (0.52 mM)</td>
<td>100.1 (0.91 mM)</td>
</tr>
<tr>
<td>12</td>
<td>1222 (11.1 mM)</td>
<td>105.2 (0.96 mM)</td>
<td>30 (0.27 mM)</td>
<td>29.7 (0.27 mM)</td>
</tr>
<tr>
<td>18</td>
<td>4816 (43.7 mM)</td>
<td>2778 (25.2 mM)</td>
<td>92.7 (0.84 mM)</td>
<td>73.1 (0.66 mM)</td>
</tr>
<tr>
<td>24</td>
<td>Not reached</td>
<td>3063 (27.8 mM)</td>
<td>140 (1.27 mM)</td>
<td>139.9 (1.27 mM)</td>
</tr>
<tr>
<td>30</td>
<td>Not reached</td>
<td>10,845 (98.5 mM)</td>
<td>267 (2.43 mM)</td>
<td>156.8 (1.42 mM)</td>
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</tbody>
</table>

**Cell Proliferation**

For the control cells, BrDU incorporation as a percentage of the total number of cells gradually fell as the cells became confluent (Fig. 3 and 4). The fall in BrDU incorporation relative to the control cells was apparent by day 1 for 5-FU and butyrate-treated cells. Regarding the cells treated with 1 d of 5-FU, they continued to take up BrDU when treatment was stopped and the uptake gradually decreased as the cells became more confluent. However, with longer treatment periods of 3, 6, and 12 d, the cells did not substantially increase their uptake of BrDU when treatment was stopped. For all treatments with 5-FU, the percentage of cells positive for BrDU after release from treatment never rose significantly above the level of BrDU uptake during treatment.

In comparison, the sodium butyrate-treated cells increased their uptake of BrDU to above the treatment levels when released from the butyrate media.

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**Fig. 3.** Percentage of cells positive for BrDU after 1, 3, 6, or 12 days of exposure to three concentrations of 5-FU.
This marked increase in uptake occurred within 2 d or less of when the sodium butyrate treatment was stopped and was sustained until the cells began to approach confluence.

Cell Viability

At doses of 1 μg/ml 5-FU and 110 μg/ml (1 mmol/l) sodium butyrate, the percentage of trypan blue positive cells remained relatively constant, but rose slightly as the period of treatment increased (Figs. 5 and 6). At doses of 100 μg/ml of 5-FU and 1,000 μg/ml (100 mmol/l) of sodium butyrate, there was a conspicuous increase in the number of cells that were trypan blue positive during the period of treatment. This was apparent after about 3 d of treatment with 5-FU and sodium butyrate. With sodium butyrate treatment, the percentage of trypan blue positive cells rose and then gradually fell, compared to the 5-FU treated cells, where the percentage of trypan blue positive cells continued to increase even after treatment had been stopped.

Discussion

There are limitations in applying cell culture findings to the situation in the eye after glaucoma filtration surgery. The cells are monolayers growing on plastic as opposed to cells in a tissue, and the majority of the cells are actively proliferating under the influence of serum that may make them more susceptible to the effects of antiproliferative agents. Nonetheless, many of our aims in fibroblast inhibition after filtration surgery are based on the cell culture studies that established the concentrations required to inhibit fibroblast proliferation by 50%. Clinically, the aim has been to achieve these concentrations at the filtering site after filtration surgery. As such, our findings have implications for the clinical situation.

The most interesting finding was the prolonged effect on proliferation of short-term exposure to 5-FU, up to and including the 30 d limit of the experiments. Treatment for only 1 d at concentrations of 100 or 1000 μg/ml caused marked inhibition of proliferation for at least 30 d. Treatment at a 5-FU concentration...
Fig. 5. Percentage of cells with trypan blue uptake after 1, 3, 6, or 12 days of exposure to three concentrations of 5-FU.

of 1 μg/ml for 12 d had a similar effect in "arresting growth" in the cells for at least 30 d. Of interest is that depriving normal cells of pyrimidine nucleotides can result in simple growth arrest, in which a quiescent state can be maintained for prolonged periods without cell death.

The longer term effects of 5-FU after short-term exposures have been studied in animal cells. Using an MTT color absorbance assay, Senderoff and colleagues exposed Chinese hamster ovary cells to 5-FU for 3 d and then washed the cells and fed them with media and serum to assess recovery. Recovery began to occur 72 h after cessation of treatment. However, although no actual drug concentration was given, the concentrations used appear to be between 0.1 and 1 μg/ml. Likewise, at these levels in the present study, we found recovery after 3 d treatment. Yamamoto and colleagues examined the effects of 5-FU and mitomycin-C on rabbit subconjunctival fibroblasts using three concentrations (0.33, 1, and 3.3 μg/ml) of 5-FU for treatment periods of 2, 4, 7, and 15 d. The fibroblasts then were counted at day 15. Exposure of the cells to the highest concentration of 3.3 μg/ml for a 2 d period resulted in only an 8% increase in cell number at day 15. However, higher concentrations of 5-FU were not used, so no gradual cell death was observed over the course of 30 d, as observed in our study.

Are the higher concentrations of 5-FU used in these experiments relevant to the clinical situation? Earlier pharmacokinetic experiments in the rabbit, albeit given in the upper fornix, have established that 5 mg of subconjunctival 5-FU results in a concentration of 5-FU in the conjunctiva and sclera 180° opposite the injection of between 0.3 and 0.8 μg/ml for most of the day. This range encompasses the ID₅₀ concentrations for ocular fibroblasts after 2-10 d of continuous exposure to 5-FU in tissue culture established in several studies. However, the conjunctival cells also were exposed to concentrations from 50,000 μg/ml at the time of the injection to 2374 μg/ml at 1 hr and 88.3 μg/ml at 3 hr at the site of the injection. Even
180° from the injection, which in clinical practice is usually the actual bleb site, the levels were 65.3 μg/ml at 1 hr and 10.1 μg/ml at 3 hr. If twice daily 5-FU injections were given, the conjunctival cells clearly would have been exposed to these high concentrations for much longer periods of the day.

Our data suggest that proliferating Tenon’s capsule fibroblasts are sensitive to concentration or duration of 5-FU treatment. Either a high concentration for a short period or a lower concentration (closer to the theoretical ID_{50} concentration) for a longer period can result in “growth arrest” of the fibroblasts. This suggests that the original regimen of 5 mg twice a day for the first week, followed by 5 mg once a day for the second week, was too high a dose if the target was to achieve only 50% inhibition of fibroblast proliferation. However, we do not know whether a 50% inhibition of fibroblast proliferation is the appropriate inhibition to aim for in a clinical context. Lowering the dose or duration of treatment with 5-FU should achieve similar inhibition of fibroblast proliferation compared to higher concentrations, with less long-term fibroblast toxicity. This conclusion already has been applied in practice based on clinical experience with 5-FU, with lower doses being used. However, this is complicated by the fact that many of the injections in these studies were given closer to the filtering bleb, which may have resulted in higher local concentrations.

At the other extreme, even higher concentrations of 5-FU or other antiproliferative therapies for a much shorter period may have the same effect as the lower concentrations for a longer period. There is evidence to support the concept that short exposures to the appropriate dose of an antiproliferative agent may have a prolonged effect on fibroblast proliferation. Beta radiation delivered to the filtering site immediately after filtering surgery in children with congenital glaucoma has considerably increased the success rate in these patients, where there is a very high risk of failure. Cell culture experiments from our laboratory show that a two-and-a-half minute exposure to similar...
doses of beta irradiation inhibits the proliferation of human Tenon’s capsule fibroblasts for at least 2 wk.21 Very short exposures of 5 min to mitomycin-C at the filtering site have been reported to have profound effects on the appearance and long-term survival of filtering blebs, similar to the effects seen with 5-FU.22-24 In addition, if the injections of 5-FU were given into the bleb site, the concentration achieved would be much higher than that achieved by an injection at 180° away, and very few injections should be needed to produce significant inhibition of fibroblast proliferation at the filtering site. Loftfield and Ball2 gave an average of only 1.9 injections directly into the bleb site, at the time of surgery and after. Despite this small number of injections, the 5-FU treated group had a lower intraocular pressure (11.9 mmHg vs 14 mmHg) and a significantly increased number of complications relating to the bleb site, including wound leaks (9/23 vs 3/20) and maculopathy related to hypotony (4/23 vs 0/20).

It is possible that some of the 5-FU-associated bleb and corneal complications may be a result of gradual fibroblast and corneal epithelial cell death after treatment with high doses of 5-FU, or even a result of acute cell toxicity when exposed to extremely high doses of 5-FU or agents such as mitomycin-C. Exposure to 5-FU concentrations of 1000 μg/ml for a day or more, or 100 μg/ml for 12 d, causes gradual loss of viability and death of the fibroblast population in tissue culture. This may explain the gradual increase in thinning seen in some filtering blebs even after 5-FU has been stopped for some time. Progressive worsening of corneal complications after treatment has been stopped also may be due to gradual cell death over time. Loss of cell viability and cell death also occurred with sodium butyrate, but this was mainly during the treatment period, after which the percentage of nonviable cells fell as the cell population “recovered.”

Our cell culture studies suggest that the fibroblasts can be “growth arrested” for at least 30 d without causing gradual cell death by maintaining 5-FU concentrations of 1 μg/ml for 12 d, 10 μg/ml for three or more d, or 100 μg/ml for 3 d. Unfortunately, steady tissue concentrations of 5-FU cannot be maintained with twice or once daily subconjunctival injections. A very high concentration must be given (25,000–50,000 μg/ml) to ensure a concentration of 0.1–1.0 μg/ml for most of the day, thus exposing the cells to very high levels of 5-FU for part of the day. The peak concentration of 5-FU may be as important as the final baseline levels, as has been previously suggested.22 Also, an injection of less than 5 mg may provide a baseline concentration of less than the theoretical ID90 for most of the day, but produce the same inhibition of proliferation with less cell death.

More frequent injections of a much lower concentration of 5-FU also would achieve a more consistent tissue level, but this is not practical. Several slow release systems under development seem to have the potential to maintain much more consistent levels of antiproliferative agents.26 Two wk periods of exposure to 5-FU may not be required if levels higher than 0.1–1.0 μg/ml are maintained in the tissues. Finally, it is interesting to speculate whether very short-term exposure of a few minutes to a very high dose of 5-FU, similar to that with mitomycin-C at the time of surgery, would produce long-term effects on the proliferation of Tenon’s capsule fibroblasts. 5-fluorouridine, which is taken up and metabolized by cells much more rapidly than 5-FU, may be a better drug for short-term high dose exposure. The advantages of this type of delivery include convenience for the patient and reduced corneal complications if treatment is restricted to the inner surface of Tenon’s capsule and the sclera. However, by the very nature of high-dose short-term exposure, the margin between inhibition of proliferation and frank toxicity is probably small.

Long-term inhibition of fibroblast proliferation may be occurring at the bleb site. This is particularly important because the local conjunctival and scleral fibroblast population is a relatively limited resource. Renewal can occur only by local proliferation or migration from surrounding areas, not by repopulation from the blood, as was previously thought. The distance over which migration can occur may be limited, and if the local fibroblast proliferation is impaired for a prolonged period, healing of the conjunctiva over the bleb site will also be impaired for a prolonged period. This may explain some of the cases of poor healing in leaking blebs after previous 5-FU treatment. We may be permanently impairing the ability of the tissues to heal in some cases.

Although the majority of filtration failures occur in the first few months, the wound healing process may go on for months and even years. Long-term inhibition of proliferation may be a disadvantage in eyes that have excessively thin leaking blebs. However, it may be an advantage in many cases and may account for the success of filtering surgery in 5-FU-treated eyes that have a continued stimulus to local cell proliferation, such as eyes with neovascular glaucoma. Drugs such as sodium butyrate, in which the inhibition of proliferation was much more “reversible,” may be advantageous in wound healing situations where the healing stimulus is maximal only during a short period that can be covered by treatment. This group may or may not include patients at low risk for surgical failure undergoing filtration surgery. However, we do not fully understand the complex mechanisms and time course of healing after filtration surgery in differ-
ent patients. Further research is required to identify different categories of patients.

**Key words:** 5-fluorouracil, sodium butyrate, proliferation, long-term effects, human Tenon’s fibroblasts

References