D-Penicillamine and Beta-Aminopropionitrile Effects on Experimental Filtering Surgery

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We studied the effect of two compounds, beta-aminopropionitrile (BAPN) and D-penicillamine on the success of experimental glaucoma filtering surgery in cynomolgus monkeys. All animals had laser-induced glaucoma and underwent punch sclerectomy operations. Eyes treated with BAPN or D-penicillamine maintained successful filtration for at least 3 days longer than nondrug-treated controls. This effect, while statistically significant (P < 0.05), was temporary. The lack of more substantial improvement in prolonging filtering patency may have resulted from an inadequate effective drug concentration. Alternatively, these drugs may have limited potency because their action on new collagen synthesis affects only a minor component of the healing process that causes filter failure.


A minority of glaucoma filtering operations ultimately fail. When this surgery fails, it is usually due to the post-operative healing response at the conjunctival/scleral interface. Factors that may influence subsequent bleb closure include excision of Tenon’s capsule and entry of serum into the aqueous humor after breakdown of the blood-aqueous barrier. Corticosteroids and prostaglandin synthetase inhibitors have been advocated to reduce the fibrosis through their anti-inflammatory actions. A recent prospective, randomized trial demonstrated greater success in patients receiving topical steroids after trabeculotomy. A recent prospective, randomized trial demonstrated greater success in patients receiving topical steroids after trabeculotomy.

The antimetabolite 5-fluorouracil, administered by subconjunctival injection, has shown promise in improving the success of filtering surgery in primates and in glaucoma patients. A collaborative clinical trial is now underway to test this drug.

To improve initial success and particularly to approach eyes with past failed filters, investigators have designed new surgical methods. These include the use of the Molteno drainage implant, hydroxyethyl methacrylate capillary strips, silicone tubes, the Krupin-Denver eye valve, and an anterior chamber tube shunt encircling band. While success has been reported in uncontrolled trials, these foreign materials induce inflammatory reactions that may contribute to further filter failure. Others have tried to open failing blebs with either the argon or neodymium-YAG lasers.

This study was designed to determine if two lathyrogenic agents, D-penicillamine and beta-aminopropionitrile fumarate (BAPN), would prolong the patency of a standardized thermal sclerectomy in glaucomatous cynomolgus monkeys. BAPN causes an irreversible inhibition of the enzyme responsible for producing the crosslinks of tropocollagen fibrils. It has been shown to decrease keloid formation and decrease urethral strictures. BAPN decreases the amount of post-traumatic cellular proliferation in the vitreous cavity, and limits the degree of fornix contracture after conjunctival alkali burns in rabbits. The drug may reduce the refractive regression after radial keratotomy in rabbits, but this effect has recently been questioned.

D-penicillamine interferes with de novo collagen synthesis by inhibiting the crosslinking of collagenous fibrils at a later step in the process than BAPN, resulting in an alteration of biochemical solubility and tensile strength of scar tissue. D-penicillamine
also exhibits anti-inflammatory effects. It has been used to treat scleroderma and rheumatoid arthritis.

Materials and Methods

All procedures involving the use of monkeys conformed to the ARVO Resolution on the Use of Animals in Research.

Induction of Glaucoma

Eleven young Macaca fascicularis monkeys of either sex, 2.5-5.0 kg, were regarded as normal if intraocular pressure (IOP) readings with a calibrated Alcon pneumatonometer (Digilab-Biorad Electronics, Cambridge, MA) were normal (between 15-22 mmHg) and stereo photographic disc examination of the individual nerveheads revealed no vascular abnormality or glaucomatous cupping. Under sodium pentobarbital anesthesia, they then underwent bilateral argon laser treatment for 360° of their trabecular meshwork to induce glaucoma, after the method of Kupfer and Gaasterland, as modified by Quigley and Hohman, to obtain a sustained IOP elevation for at least 3 weeks prior to surgery. Monkeys were assigned randomly to treatment groups.

Each eye was titrated with laser therapy (the number of burns varied from 115 to 44 (47-57) Joules of energy) to obtain a sustained IOP rise. Since the individual monkey eye's response to intraocular surgery is unique, both eyes of each animal were treated and each eye included separately in our data analysis. When the animals manifested clinical glaucoma or glaucomatous cupping. Under sodium pentobarbital anesthesia, they then underwent bilateral argon laser treatment for 360° of their trabecular meshwork to induce glaucoma, after the method of Kupfer and Gaasterland, as modified by Quigley and Hohman, to obtain a sustained IOP elevation for at least 3 weeks prior to surgery. Monkeys were assigned randomly to treatment groups.

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Surgical Procedure

All monkeys were operated on by one of two surgeons (LJBM or RPM). Each monkey was first sedated with 0.4 cc (100 mg/ml) of ketamine hydrochloride (Bristol Laboratories), then anesthetized with 0.75 cc (65 mg/ml) of intravenous sodium pentobarbital (Harvey Laboratories).

After sterile preparation, a pediatric Baraquer speculum was placed in the eye. A lateral canthotomy was performed and a #4-0 silk stay suture was passed under the superior rectus muscle. A limbus-based conjunctival flap was made superiorly for about 120 degrees. Tenon's capsule was removed in the area of the conjunctival flap. The conjunctival flap was dissected anteriorly using a spring-action-iris scissors and a Beaver (Rudolph Beaver Inc., Waltham, MA) blade (minblade #6400).

The anterior chamber was entered with a knife (Weck 30 degree) for 2 mm. The Kelly punch was used to make three 1/2-3/4 mm semicircular bites from the posterior lip of the incision. A standard-sized 2 mm diameter hole was made by measurement with a millimeter ruler. A peripheral iridectomy was made with one cut of the DeWecker scissors and remaining prolapsed iris was reposited. Any bleeding iris vessels were tamponaded with a cotton tip applicator through the cornea or were cauterized. No eye had greater than a 3% hyphema the first day after surgery.

The conjunctival flap was tightly closed with a running locked suture of #8-0 collagen.

Post-Surgical Treatment

Immediately after surgery, monkeys received either D-penicillamine, BAPN or saline. Subconjunctival injections were given in the inferior quadrant 180° away from the sclerectomy site using a 25 gauge needle on a tuberculin syringe. Monkeys were sedated with 0.4 cc (100 mg/ml) of ketamine hydrochloride for ocular examination and drug injections. All injections or ointment applications were given after gonioscopic examination and after IOP was measured with the Alcon pneumotonometer.

The eyes of animals treated with D-penicillamine received 0.5 cc of a 10% solution (pH = 6.9-7.0) of D-penicillamine (Merck, Sharp & Dohme, West Point, PA) subconjunctivally each day for 7 days. All D-penicillamine solutions were made up fresh weekly with distilled water and kept under refrigeration. Control animals for the D-penicillamine group received subconjunctival injections of 0.5 cc of distilled water for 7 days. The injections could not be masked due to the smell of the D-penicillamine solution. D-penicillamine was given subconjunctivally only, due to the negative results found with intraperitoneal injections in rabbits in a similar study. That preliminary study also showed positive results with subconjunctival injections of D-penicillamine in rabbits.

BAPN was given both as an ointment and intraocularly in the same manner shown to limit the degree of posttraumatic vitreous proliferation in rabbits. Immediately after surgery the monkeys received an injection of 1 g of aqueous BAPN (Hoffmann LaRoche, Nutley, NJ) into their quadriceps femoris and then 100 mg/kg/day was given intramuscularly daily until filter failure. A ribbon of 33% BAPN ointment (Hoffmann LaRoche) was given in the inferior fornix immediately after surgery in each eye and once daily thereafter until filter failure. All BAPN solutions were made up fresh daily with BSS® solution before injection. Control animals for the BAPN group received 1 ml of BSS® solution intramuscularly daily and this group of animals received...
their injections in a masked fashion until filter failure. Each monkey eye received atropine 1% drops and bacitracin ointment to the lower fornix once only, immediately after either D-penicillamine or BAPN drug therapy.

Post-Surgical Examinations

All monkey eyes were monitored daily with slit lamp examination, gonioscopy and pneumatonometry. When IOP exceeded 25 mmHg on at least 3 consecutive days the operation was defined as having failed at the first day. Usually when the IOP first exceeded 25 mmHg it remained elevated or elevated further on successive readings.

The mean IOP of the group of D-penicillamine-treated (six eyes) monkeys was compared to the mean IOP of the group of BAPN-treated (eight eyes) monkeys and each of these was compared to the mean IOP of the control monkeys (seven eyes) by a student t-test for each day after surgery.

Results

Results are reported on 21 of 22 eyes of 11 monkeys. One eye of one monkey was used in another experiment evaluating a third collagen cross-linking inhibitor. The mean IOP of the D-penicillamine-treated monkey eyes prior to surgery (six eyes) was 44 mmHg, SD ± 6. The average IOP of the BAPN-treated monkey eyes prior to surgery (eight eyes) was 37 mmHg, SD ± 6; the average IOP of the control monkey eyes prior to surgery (seven eyes) was 39 mmHg, SD ± 11. Neither average IOP of the two treated groups of monkey eyes was significantly different from the average IOP of the control eyes prior to surgery (P = 0.47 for D-penicillamine vs controls, and P = 0.41 for BAPN vs controls). For the first 4 days after surgery, all three groups had mean IOP below 10 mmHg. On the fifth post-operative day, the control group began a rapid rise back to elevated IOP levels. By the seventh day, the mean control group IOP was 25 mmHg, SD ± 8 mmHg.

D-penicillamine-treated eyes had significantly lower mean IOP than control eyes on days 6 through 9 (P < 0.05, Fig. 1). BAPN-treated eyes had lower than control mean IOP on days 3 through 8 (P < 0.05, Fig. 2). Thus, the IOP lowering effect of surgery ceased by the seventh day in controls and was prolonged for an additional 48–72 hr by drug treatment.

D-penicillamine-treated eyes developed a grey filmy membrane, visible gonioscopically at the internal opening, by day 9. Similarly, all BAPN-treated eyes began to show an internal obstruction by day 8. As the filtering operations continued to fail by days

Fig. 1. Mean IOP of D-penicillamine-treated eyes (n = 6) compared to control eyes (n = 7) after glaucoma filtering surgery. From elevated IOP levels prior to surgery, both groups fell below 10 mmHg. The control group returned to high IOP faster as their operations failed more quickly. The area between the perforated lines shows the period during which D-penicillamine-treated eyes maintained significantly lower IOP than controls due to better filter function (P < 0.05). However, the treated group also failed to maintain low IOP, and had failed before 2 weeks elapsed. Only one standard deviation bar is included on one side of each curve for clarity.

Fig. 2. Mean IOP of BAPN-treated eyes (n = 8) compared to control eyes (n = 7). As in Figure 1, both groups were hypotonous for 4 days, but the control eyes rose to a mean of >25 mmHg by the seventh day. Mean IOP in BAPN-treated eyes was significantly lower than control from day 3 to 8 (dotted lines, bracket, P < 0.05), but rose to equal that of the untreated group before the end of 2 weeks. Only one standard deviation bar is included on one side of each curve for clarity.
10 and 9, respectively, the greyish membrane became more obvious. The exact nature of this membrane-like tissue will be reported separately. Filter closure was not due to incarceration of the iris, ciliary body or lens in the internal opening. Control eyes began to have an internal membrane by day 5, several days earlier than either of the two treatment groups.

As IOP rose to preoperative levels, no further filtering site could be discerned gonioscopically. The white tissue covering the site was not pigmented or vascular.

The following paragraphs discuss possible surgical complications or drug-induced side effects observed during the course of filtration failure.

A diffuse bleb with microcystic changes was looked for by slit lamp examination as a sign of adequate filtration. Control and treated eyes had diffuse conjunctival blebs immediately after surgery. Microcystic changes of the blebs were evident during the first post-operative week. Bleb reaction in the control and treated eyes was similar. When filtration failure occurred the blebs flattened, yet the conjunctiva appeared thicker than normal for several more days.

Both control and treated eyes were evaluated under the slit lamp for evidence of wound leaks. The presence of the collagen inhibitors did not seem to affect wound healing adversely. Flat chambers did not persist in any eye beyond day 2.

A deliberate corneal examination was undertaken in both control and treated eyes to search for signs of corneal toxicity. Several eyes showed minor corneal epithelial defects the first day after surgery, but these healed uneventfully within 24 hr. There were no corneal dellen or stromal opacities.

There were no other significant post-operative complications. There was a mild anterior chamber reaction after surgery in almost all eyes, but this too cleared uneventfully. No hyphemas were evident 1 day following surgery. No eyes developed lenticular opacities through day 16 after surgery. The retinas appeared normal ophthalmoscopically throughout the study.

No signs of systemic lathyrism were seen with either agent. No loss of hair or skeletal deformity appeared. No monkey died suddenly (aortic aneurysm) or developed any joint injury or hernia. Autopsies to look for vessel wall weakness, avulsed tendons, or slipped epiphyseal plates were not done.

Discussion

We used a primate model for testing agents to assist human glaucoma surgery because non-primate animals have a dramatically different anterior segment anatomy and quantitatively different inflammatory response to surgical insult. The rabbit, for example, provides little space between peripheral iris and cornea, making it difficult to enter the anterior chamber without inadvertently entering the vitreous cavity.

Even in monkeys, we found that the healing response is so vigorous that all control glaucoma procedures began to fail promptly at 1 week after surgery. Post-operative inflammation causes an hypotony that also lasts 1–2 weeks. Therefore, it can be difficult to distinguish eyes with functioning blebs from those that are simply hypotonous without filtration. We added the induction of elevated IOP by laser angle treatment to our model system to provide greater sensitivity in determining when the filters fail. Simple inspection of bleb or internal opening is clearly not adequate to define the point of failure. Of course, when a dramatic success with long-term filtration occurs, as with the dose of 5-fluorouracil used by Gressel et al, the addition of artificial glaucoma is perhaps unnecessary. But for determination of a wider range of drug effects, it seems prudent to take the extra step.

Two inhibitors of collagen crosslinking, D-penicillamine and BAPN, produced a small but detectable prolongation of experimental filtering surgery patency. D-penicillamine was given for only 7 days, and its effects ceased 2 days after therapy was terminated. Possibly, had drug been continued, the apparent benefit would have been longer. However, BAPN was given continuously, and its significant effects ceased 8 days after surgery.

We delivered both agents at their maximum solubilities, but only once per day. Perhaps in human eyes, where more frequent drop delivery is feasible, the effect would be greater.

We have no data on penetration of the agents through the subconjunctival space nor tissue levels of drug after injection of aqueous solutions.

It may be that agents acting only on the formation of collagen provide too limited a protection against the closure of a filtration opening. Preliminary histologic examination of some eyes in this study suggests that other extracellular components, such as glycosaminoglycans, may play a primary role in the healing response that ruins filtering operations. To affect this component, we might use other agents.

Combinations of drugs will likely represent the most effective means to assure filtering success. Use of the antiproliferative agent 5-fluorouracil has shown that suppression of cell proliferation (or possibly migration) provides an important adjuvant to the anti-inflammatory effects of corticosteroids. Future efforts should be directed to determining the most beneficial chemotherapeutic regimen, with agents that act on each component of the healing response.

The potential aid of new agents must be weighed against their possible toxicity. The antiproliferative
drugs 5-fluorouracil\textsuperscript{1} and daunorubicin\textsuperscript{33} can cause corneal epithelial erosion and unwanted failure of the conjunctival incision to heal. We detected no ocular or systemic toxicity from D-penicillamine or BAPN. However, more frequent dosing might elicit detrimental effects.

Key words: beta-aminopropionitrile, collagen synthesis, D-penicillamine, filtering surgery, sclerectomy

Acknowledgments

The authors wish to thank Earl M. Addicks and Gregory Dunkelberger, for lab help; Merck, Sharp & Dohme for providing D-penicillamine powder; and Hoffmann LaRoche for providing BAPN ointment and powder.

References