

Topical Application of a Cyclic GMP Analog Lowers IOP in Normal and Ocular Hypertensive Rabbits

Peter J. Stein* and James W. Clack†

Purpose. To determine whether a new cyclic GMP analog, 2'-O-(4-benzoyl)benzoylguanosine 3',5' cyclic monophosphate (BB-cGMP), significantly reduces intraocular pressure in either normotensive or hypertensive eyes of rabbits.

Methods. Intraocular pressure of normal adult rabbits and/or rabbits with α -chymotrypsin-induced ocular hypertension was measured after topical application of BB-cGMP for up to 72 hours after treatment.

Results. Data indicate that topical application of solutions containing BB-cGMP can significantly reduce intraocular pressure in normotensive eyes and, to a greater extent, hypertensive eyes for periods up to 12 hours for a single dose.

Conclusions. BB-cGMP holds interest both as a clinical agent in the management of intraocular pressure and for in vitro biochemical studies on the regulation of intraocular pressure. Invest Ophthalmol Vis Sci. 1994;35:2765-2768.

Many compounds that change cyclic nucleotide levels in aqueous humor, iris-ciliary body, or scleral-trabecular rings of animal eyes have been shown to reduce intraocular pressure (IOP) in humans and animals. Compounds that have been shown to modulate cAMP concentration and reduce IOP in rabbits include timolol, pirbuterol, vasoactive intestinal peptide, and forskolin.^{1,2,3,4,5} Atrial natriuretic peptides have been shown to activate anterior segment guanylate cyclase and/or elevate cGMP levels; these compounds also reduce IOP.^{6,7,8} Two phosphodiesterase inhibitors, isobutylmethylxanthine and griseolic acid, have been reported to cause small but significant IOP decreases in ocular normotensive rabbits.^{9,10,11} It has recently been reported by two separate groups that subconjunctival and topical application of 8-bromo-cGMP or 8-bromo-cAMP to normotensive rabbit eyes results in reduction of IOP for up to 240 minutes.^{12,13} Topical administration of 8-bromo-cGMP also appears to enhance the hypotensive effect of systemic acetazolamide.

With the exception of timolol, most of the compounds cited above increase anterior segment cyclic nucleotide levels by activating either adenylate or guanylate cyclase. In the case of 8-bromo-cGMP, the cGMP analog is thought to produce IOP reductions either directly or by inhibiting a cGMP phosphodiesterase. Although hypotensive effects of cyclic nucleotide analogs have been demonstrated in rabbits with normal IOP, no definitive studies have been performed to determine the effects of this class of compounds on ocular hypertensive eyes. It was the goal of this work to determine whether a new cGMP photoaffinity analog, 2'-O-(4-benzoyl)benzoylguanosine 3',5' cyclic monophosphate (BB-cGMP; Fig. 1), could effect a reduction in the IOP of either ocular normotensive or hypertensive rabbits. We report here that topical administration of BB-cGMP to the eyes of ocular normotensive rabbits results in significant, prolonged reductions in intraocular pressure in treated eyes. Topical administration of BB-cGMP to eyes of ocular hypertensive rabbits causes much larger and longer-lasting decreases in IOP.

MATERIALS AND METHODS

Chemicals

1,1'-Carbonyldiimidazole and 4-benzoylbenzoic acid were purchased from Aldrich (Milwaukee, WI). Unless

From the *Department of Ophthalmology and Visual Science, Yale University School of Medicine, New Haven, Connecticut, and the †Department of Biology, Indiana University-Purdue University at Indianapolis, Columbus, Indiana. Supported by a contribution from Connecticut Seed Ventures, Avon, Connecticut, and by grant EY07000 from the National Institutes of Health. Submitted for publication September 24, 1993; revised November 29, 1993; accepted December 27, 1993. Proprietary interest category: P. Reprint requests: Dr. James W. Clack, Department of Biology, Indiana University-Purdue University at Indianapolis, 4601 Central Avenue, Columbus, IN 47203.

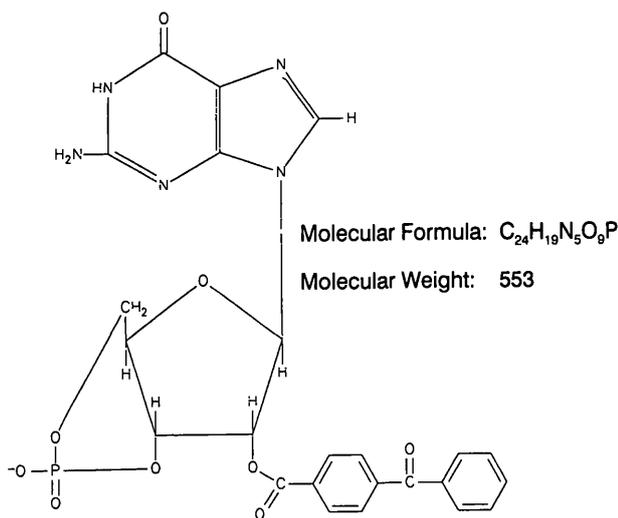


FIGURE 1. Structure, formula, and formula weight of BB-cGMP.

otherwise indicated, all other chemicals were purchased from Sigma (St. Louis, MO).

General Methods

Adult New Zealand albino rabbits (male, 2.5 to 4 kg) were maintained according to the ARVO Resolution on the Use of Animals in Research. Animals were exposed to a constant-temperature isolated environment on a 12-hour light-dark cycle. The rabbits had free access to food and water and were handled by only one of the investigators for all procedures. In experiments on ocular normotensive rabbits, BB-cGMP \ddagger was prepared for topical application by dissolving the Na salt of BB-cGMP in dimethyl sulfoxide at a concentration of 35 mM. This solution was suitably diluted into sterile 0.9% NaCl solution and pH adjusted to between 6.5 and 7.0, if required. In experiments on ocular hypertensive rabbits, BB-cGMP was dissolved directly. The solutions were instilled as a single 50 to 100 μ l aliquot into the lower conjunctival cul-de-sac. In experiments on ocular normotensive rabbits, comparable volumes of vehicle alone were instilled in the contralateral eye.

BB-cGMP Synthesis and Purification

BB-cGMP was synthesized and purified as previously described. BB-cGMP was determined to be a competitive inhibitor of bovine rod outer segment cGMP phosphodiesterase with a $K_i = 0.3 \mu$ M. BB-cGMP was a relatively poor substrate for the PDE, with a hydrolysis V_{max} roughly 1,000-fold less than that for the parent compound, cGMP. The inhibitory effect of BB-cGMP

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on cGMP and cAMP phosphodiesterases of the same class in other tissues ranged from 0.5 to 11 μ M.

Tonometry

After application of 0.5% proparacaine (Alcon Laboratories, Fort Worth, TX), intraocular pressure was measured on unrestrained rabbits with a calibrated pneumotonometer (Aplanatic tonometer, Block Engineering, Cambridge, MA). Normotensive rabbits were accustomed to the procedure by regular tonography for 2 weeks before experiments. In all cases, three separate determinations were made and the results were averaged to generate each reading. IOP was recorded before instillation of BB-cGMP or vehicle and then at regular periods after application for up to 72 hours.

α -Chymotrypsin-Induced Ocular Hypertension

Ocular hypertension was induced in the right eye of 12 male albino rabbits as previously described.^{1,14} Only animals in which ocular pressure developed in the treated eye at least 14 mm Hg higher than in the untreated, contralateral eye were judged to be suitable for this study.

RESULTS

Effect of BB-cGMP in Normotensive Rabbits

Figure 2 shows the results of topical application of BB-cGMP at three different concentrations to one eye of normal white rabbits, normalized to the effect of vehicle alone in the contralateral eye. When 50 μ l of 350 μ M BB-cGMP was instilled in the eye, there was no significant change observed in intraocular pressure. With 50 μ l of both 3.5 mM and 35 mM BB-cGMP concentrations, there was a rapid drop in intraocular pressure that reached its minimum value by 2 hours after instillation. Intraocular pressure began to recover by 4 hours after instillation at 3.5 mM and between 6 and 8 hours at 35 mM. Almost full recovery to the original intraocular pressure level occurred at 10 hours for the higher (3.5 mM and 35 mM) drug concentrations. No significant change in IOP was observed (duration, 4 hours) in either eye of rabbits administered identical volumes of vehicle without BB-cGMP ($n = 4$; data not shown).

Effect in Ocular Hypertensive Rabbits

Figure 3 shows the effects of BB-cGMP on chymotrypsin-treated, ocular-hypertensive rabbits. Treatment with vehicle alone resulted in no change in the intraocular pressure of these animals (data not shown). Treatment with 70 μ l of 35 mM BB-cGMP resulted in a drop in intraocular pressure that reached a minimal value at 4 hours. IOP remained depressed until 12 hours after

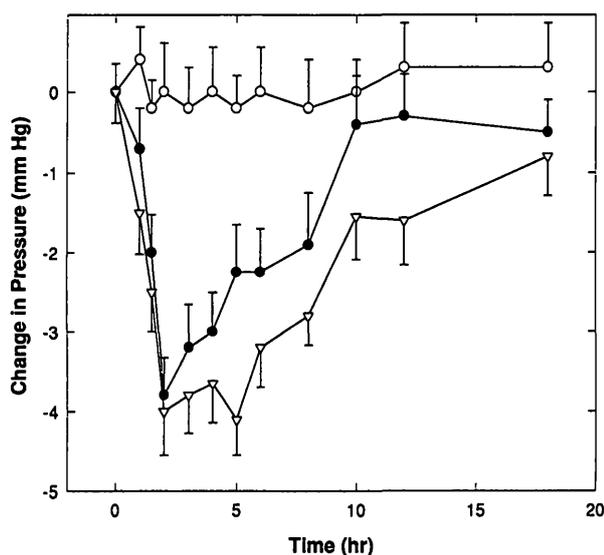


FIGURE 2. Effect of increasing concentrations of BB-cGMP on intraocular pressure of ocular-normotensive rabbits. Intraocular pressures (\pm SEM, $n = 6$) are relative to pressure at time zero and normalized with respect to the contralateral eye. Contralateral eyes received saline solutions (without BB-cGMP) containing DMSO at the same concentrations applied to the BB-cGMP-treated eyes. Open circles, 50 μ l of 0.35 mM BB-cGMP in saline solution containing 10% DMSO applied at $t = 0$. Filled circles, 50 μ l of 3.5 mM BB-cGMP in saline solution containing 10% DMSO administered at $t = 0$. Open triangles, 50 μ l of 35 mM BB-cGMP in saline solution containing 10% DMSO applied at $t = 0$. In this context, normalized means that any difference in the contralateral, sham-treated eye relative to pretreatment IOP was subtracted from that of the drug-treated eye. No significant changes in IOP were observed in the contralateral, sham-treated eye in response to administration of BB-cGMP.

application, when a slow and incomplete recovery began.

Effect of Repeated Dosing

Figure 4 shows the results of an experiment in which BB-cGMP was applied in single doses to the eyes of hypertensive rabbits on three successive days at the same time of day. After each application, there was a large drop in intraocular pressure. Pressure remained minimal between 4 and 12 hours, when a slow and incomplete recovery began. There was a slow, steady decline in the minimal intraocular pressure over successive days. There also appeared to be a steady decline in the value to which IOP "rebounded" just before the next application of BB-cGMP. Dosage in this manner appeared to maintain IOP elevation in the hypertensive eye ≤ 8 mm Hg above the contralateral, normotensive eye at all times.

DISCUSSION

These results show that the cGMP analog BB-cGMP can significantly lower IOP in the eyes of normal rabbits and reduce IOP to a much larger extent in rabbit eyes with elevated IOP. The exact mechanism by which BB-cGMP lowers intraocular pressure remains unknown. However, a large body of evidence suggests that the cyclic nucleotide system may participate in the regulation of aqueous humor dynamics. It has long been thought that cyclic nucleotides, known to serve as second messengers in a variety of signal transduction mechanisms, may play a significant role in the regulation of IOP.^{2,9} Because BB-cGMP is an inhibitor of both cGMP and cAMP phosphodiesterases, it may modulate the cyclic nucleotide system of the ciliary processes of the eye. It is also possible that BB-cGMP acts as a cGMP agonist in a pathway involving cyclic nucleotide-dependent protein kinase(s) rather than as an inhibitor of PDE. Further studies of the biochemical effects of BB-cGMP on ocular tissues are currently in progress.

BB-cGMP, besides being a potent inhibitor of cyclic nucleotide PDE molecules, is also a photoaffinity analog of cGMP (owing to its UV light-induced cova-

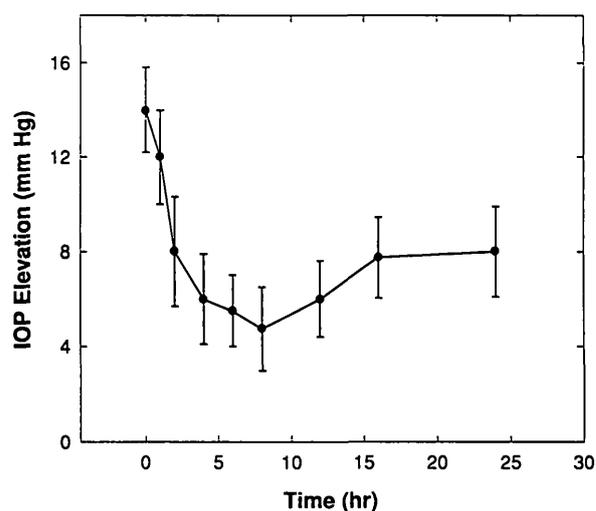


FIGURE 3. Effect of BB-cGMP on IOP in ocular hypertensive rabbits. Ocular hypertension was induced in rabbits by injection of chymotrypsin. Eight weeks after injection, when eye pressures had stabilized, the effects of BB-cGMP were observed on the intraocular pressure of hypertensive eyes. At time zero, 70 μ l of BB-cGMP (35 mM) suspended in a commercial buffered saline solution (Hypotears, Isolab, Norton, OH) containing 1% DMSO was instilled in the hypertensive eye. The closed circles represent the mean \pm SEM ($n = 4$) pressure elevation above the contralateral (normotensive) eye.

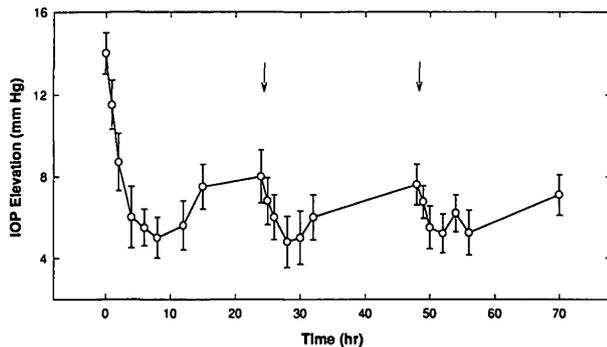


FIGURE 4. Effect of repeated doses of BB-cGMP on hyper-tensive eyes. Rabbits with ocular hypertension were treated on three successive days (denoted as time = 0 and the two downward arrows) with a single dose of $70 \mu\text{l}$ of 35 mM BB-cGMP. The open circles show the mean intraocular pressure elevation (\pm SEM, $n = 4$) in the hypertensive eyes normalized to the contralateral control eyes.

lent reactivity). This gives rise to the possibility of identifying the site of (IOP-lowering) action of BB-cGMP by use of photoaffinity labeling. Use of this compound and similar compounds to probe the biochemical nature of cyclic GMP analogs in lowering IOP should provide important insights into the regulation of IOP.

In addition, the finding that cyclic GMP analogs, such as 8-bromo-cGMP,¹³ griseolic acid,¹⁰ and BB-cGMP, are active in lowering IOP (and possibly controlling IOP elevation in certain types of glaucoma) may open the way for a new class of anti-glaucoma agents. These agents could be used either alone or in conjunction with other classes of anti-glaucoma drugs.

Key Words

cyclic GMP, intraocular pressure, phosphodiesterase inhibitor, glaucoma

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