In Vivo Ocular Efficacy Profile of Mapracorat, a Novel Selective Glucocorticoid Receptor Agonist, in Rabbit Models of Ocular Disease

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PURPOSE. To compare the efficacy of mapracorat (formerly ZK-245186, and subsequently BOL-303242-X), a novel selective glucocorticoid receptor agonist (SEGRA), with that of dexamethasone (DEX) in rabbit models of ocular disease. The effects of topical BOL-303242-X and DEX on intraocular pressure (IOP) and body weight changes were also evaluated.

METHODS. Dry eye was induced by atropine sulfate administration and was treated with saline, BOL-303242-X (0.1%–1.0%), DEX (0.1%), Restasis 0.05% (Allergan, Inc., Irvine, CA), or Refresh Endura (Allergan, Inc.) three times per day for 7 to 8 days. For paracentesis studies, vehicle, BOL-303242-X (0.1%, 0.5%, and 1.0%), or DEX (0.1%) were repeatedly administered topically 3 hours before paracentesis and continued for 90 minutes afterward. For IOP and body weight measurements, right eyes of rabbits were topically treated with vehicle, BOL-303242-X (1.0% or 0.1%), or DEX (0.1%) four times per day for 6 weeks.

RESULTS. In the dry eye model, BOL-303242-X and DEX were fully efficacious, maintaining tear volume and tear breakup time (TBUT) at baseline levels. Although Restasis improved tear volume compared with vehicle, no changes were observed in TBUT. In the paracentesis study, BOL-303242-X and DEX improved ocular inflammation. BOL-303242-X reduced protein and PGE2 levels. Finally, BOL-303242-X showed no effects on integrated IOP or body weight, whereas DEX significantly increased integrated IOP and prevented the increase of body weight observed in the vehicle-treated animals.

CONCLUSIONS. BOL-303242-X shows full anti-inflammatory efficacy (similar to DEX) in experimental models of dry eye and postoperative inflammation while demonstrating reduced effects in IOP and body weight. These data indicate that mapracorat, a SEGRA, shows efficacy similar to that of traditional steroids while exhibiting an improved side effect profile in IOP and muscle wasting. (Invest Ophtalmol Vis Sci. 2011;52:1422–1430) DOI:10.1167/iovs.10-5598

Glucocorticoids (GCs) are potent and efficacious anti-inflammatory agents that exert their therapeutic effects by binding to the glucocorticoid receptor (GR), leading to the modulation of gene expression by transrepression (i.e., regulating transcriptional regulators such as nuclear factor κB [NF-κB], c-Jun, and c-Fos).1,2 As a consequence of their anti-inflammatory properties, GCs are commonly used in the treatment of ocular inflammation. Ophthalmic formulations of steroids such as dexamethasone (DEX), triamcinolone acetonide, fluorocortolone acetonide, loteprednol etabonate, and prednisolone are commonly used in treating ocular conditions such as conjunctivitis,4–5 uveitis,6,7 allergy,8,9 dry eye,10–12 diabetic macular edema,13–19 and postoperative inflammation in cataract patients.20–22 Indeed, GCs are among the most widely prescribed ophthalmic therapeutics.23

Dry eye has a diverse etiology that includes aging, the environment, and endocrine, immune, and iatrogenic factors,10,24–27 all of which can alter tear film homeostasis, ultimately leading to inflammation. Because of the importance of ocular surface inflammation in the pathophysiology of dry eye, anti-inflammatory therapy, including GCs,10–12 may be of benefit to dry eye patients. Indeed, cyclosporine, an immunosuppressant with purported anti-inflammatory properties, is the first US Food and Drug Administration (FDA)-approved anti-inflammatory therapy for dry eye syndrome (see the DEWS report for a description of currently used treatments).12

In spite of the remarkable anti-inflammatory properties of GCs, chronic use of them often leads to undesired side effects,28 which are believed to be exerted primarily by the ability of the GC-bound GR to bind to specific GC response elements (GREs) in the promoter regions of specific genes, leading to their transactivation.2,29 In ophthalmic applications, repeated or prolonged administration of steroids has been associated with side effects such as increased intraocular pressure (IOP)30–32 and formation of cataracts.2,33 Consequently, an unmet medical need exists for novel nonsteroidal agents that promote the transrepressive activity of the ligand-receptor complex (i.e., anti-inflammatory activity), while minimizing transactivation and, hence, reducing the potential for side effects.

Mapracorat (also known as ZK-245186, and subsequently as BOL-303242-X) is a novel selective glucocorticoid receptor agonist (SEGRA) under clinical evaluation for the treatment of dermatologic and ocular diseases with an inflammatory component. In numerous preclinical studies related to dermatology, BOL-303242-X has demonstrated anti-inflammatory effects in various in vitro and in vivo models with limited systemic or local side effects.34 In addition, BOL-303242-X exhibits full anti-inflammatory properties compared with traditional ste-
roids in a variety of primary and immortalized ocular cells in vitro.\textsuperscript{35} Equally important, when tested for transactivation-mediated gene regulation in vitro, BOL-303242-X behaves as a partial agonist in activation of the MYOC gene compared with classical GCs, showing significantly reduced ability to elevate myocilin protein levels and gene expression in primary monkey trabecular meshwork cells.\textsuperscript{36} This reduced ability of the BOL-303242-X-ligated GR to transactivate while able to fully transrepress suggests that this agent could provide a beneficial side effect profile compared with traditional GCs for ophthalmologic inflammatory indications.

To date, the promising efficacy and side effect profile for the ophthalmologic use of BOL-303242-X has been studied in vitro and not yet been described in vivo in animal models of ocular inflammation. Therefore, in these studies, we report the anti-inflammatory properties of BOL-303242-X in both the rabbit dry eye and the paracentesis-induced inflammation models.

**Materials and Methods**

**Materials**

BOL-303242-X (ZK 245186; R-1,1,1-trifluoro-4-(5-fluoro-2,3-dihydrobenzofuran-7-yl)-4-methyl-2-([(2-methyl-5-quinolyl)amino]methyl)pentan-2-ol) was provided by Bayer Schering Pharma (Berlin, Germany). DEX sodium phosphate solution (0.1%) and 1.0% atropine sulfate eye drops were obtained from Bausch & Lomb (Tampa, FL). BOL-303242-X was suspended in PBS for the dry eye studies and formulated in PBS containing 10% polyethylene glycol and 1.0% Tween 80 for the paracentesis studies. Restasis (cyclosporine ophthalmic emulsion) 0.05% and Refresh Endura were obtained from Allergan, Inc. (Irvine, CA). Sterile PBS was purchased from Invitrogen (Carlsbad, CA). Sodium fluorescein (1%) was purchased from OcuSoft (Richmond, TX).

**Animals**

For dry eye studies, female New Zealand White rabbits (2.0–2.5 kg; 6–7 rabbits per group) were obtained from Charles River (St. Constant, Canada), and for paracentesis studies male New Zealand White rabbits (1.8–2.0 kg; 4 rabbits per group) were obtained from Morini (Reggio Emilia, Italy). For intraocular pressure studies, 65 female New Zealand–Pigmented (NZP) rabbits (Robinson Services Incorporated, Mocksville, NC; 16–17 rabbits per group) were used. The animals were 15 weeks old at the start of treatment. The choice of animal number was based on previous experience with the models and the ability to detect statistically significant differences with the positive controls included in all the studies.

Animals were housed under standard conditions, with food and water provided ad libitum, in a light-controlled (12-hour light/12-hour dark; lights on at 6 am) room at 21°C ± 3°C and 54% ± 4% humidity. All animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Protocols were reviewed and approved by, and all studies complied with the standards of, the relevant institutional animal care and use committee of the facility in which the work was conducted.

**Dry Eye Model**

To evaluate dry eye disease, tear volume, tear film breakup time (TBUT), and corneal staining were used as end points. Baseline values for tear volume, slit lamp examination of tear film, and corneal integrity were obtained 1 day to 2 days before the induction of dry eye according to an established methodology.\textsuperscript{37} Atropine sulfate 1.0% ophthalmic solution (Bausch & Lomb; 1 drop) was instilled into the lower conjunctival sac of each eye three times a day for the duration of each study. Fifteen minutes after the administration of each dose of atropine sulfate, each eye received 1 drop of the test agent or PBS.

Tear volume was evaluated by the Schirmer test. Schirmer strips (Eagle Vision, Memphis, TN) were carefully placed in the posterior (i.e., temporal) lower fornix for 60 seconds, and the wetted area was read in millimeters as an index of tear volume. TBUT was determined after instillation of 5 μL of 2% sodium fluorescein in sterile PBS onto the lower eyelid. Fluorescein was distributed on the cornea by manual blinking of the lids. Under slit lamp (SL-D7; Topcon, Paramus, NJ) with cobalt blue filter (Boston Slit lamp filter kit 7503; Winchester Optical, Elmira, NY) to enhance the fluorescein patterns, the time from opening of the eyes until the appearance of the first black spot or streak on the cornea was recorded. The procedure was conducted three times consecutively, and the average of three readings was used. Corneal epithelial-staining was evaluated using the National Eye Institute grading system.\textsuperscript{38} The corneal surface was divided into five areas, 0 to 3 staining severity for each area was designated, and the grades were added, providing a total severity of 0 to 15. All measurements were conducted in conscious animals in the following order: TBUT, corneal staining, and Schirmer test.

**Paracentesis Model**

Rabbits were randomly assigned to five groups (four animals per group). Each rabbit was placed in a restraining device, and the test articles were instilled (50 μL) into the conjunctival sac of each eye 180, 120, 90, and 30 minutes before paracentesis and 15, 30, and 90 minutes after the intervention. To perform the paracentesis, animals were anesthetized by intravenous injection of 5 mg/kg agent (Zoletil [Virbac Laboratories, Carros, France], 2.5 mg/kg ketamine HCl, and 2.5 mg/kg zolazepam HCl) and 1 drop of local anesthetic (Novocain; Novartis, Basel, Switzerland) administered to the eye. Anterior chamber paracentesis was performed with a 26-gauge needle attached to a tuberculin syringe; the needle was introduced into the anterior chamber close to the limbus, with care taken not to damage the tissues, and 100 μL aqueous humor was removed and discarded. Two hours after the first paracentesis, a second paracentesis was performed to collect the aqueous humor for biochemical evaluation. The animals were then euthanatized with 0.4 mL euthanatizing agent (Tanax; Intervet International/Schering-Plough Animal Health, Merck & Co., Inc., New York, NY). The pupillary diameter of each eye was measured with a Castroviejo caliper 180 minutes and 5 minutes before the first paracentesis and 5 minutes before the rabbits were killed (approximately 2 hours after the second paracentesis to collect aqueous humor for further evaluation). Aqueous humor samples were collected and split into four aliquots, which were stored at −80°C until analysis. Then both eyes were enucleated and the iris-ciliary body was carefully excised, placed in polypropylene tubes, and stored at −80°C until analysis. The clinical evaluation of both eyes was performed with a slit lamp (4179-T; Bausch & Lomb; Flóra, Firenze, Italy) at 180 minutes and 5 minutes before the paracentesis and 115 minutes after the paracentesis. Clinical signs were graded according to the following scheme: 0 = normal, 1 = discrete dilatation of iris and conjunctival vessels; 2 = moderate dilatation of iris and conjunctival vessels; 3 = intense iridal hyperemia with flare in the anterior chamber; 4 = intense iridal hyperemia with flare in the anterior chamber and presence of fibrous exudates.

Aqueous humor PGE\textsubscript{2} levels were measured with a standard PGE\textsubscript{2} immunoassay kit (R&D Systems; Minneapolis, MN), and aqueous humor protein concentrations were measured (Protein Quantification Kit; Fluka, Milan, Italy). In both cases, samples were treated after the assay procedure described in the kit. Polymorphonuclear leukocytes (PMNs) were quantified in aqueous humor using a hemocytometer (Fisher Scientific, Pittsburgh, PA) and a microscope (Polyvar 2; Reichert-Jung, Depew, NY). The PMN count was expressed as number of cells per microliter of aqueous humor. The activity of myeloperoxidase (MPO) was measured as previously described.\textsuperscript{39}

**Intraocular Pressure Measurements**

Animals were randomly distributed into four treatment groups. Their right eyes were treated with one of the following preparations for 6 weeks: BOL-303242-X vehicle, BOL-303242-X 0.1% or 1.0%, or DEX in BOL-303242-X vehicle 0.1%. One drop was instilled every 6 hours into
each lower conjunctival sac by gently pulling the lower lid away from the eye, placing the drop in the pocket, and closing the eyelid. Before administration, the preparations were stirred so that they formed a homogeneous suspension. Left eyes were not treated.

Rabbit body weights were monitored at baseline and once a week. Intraocular pressure was measured using a classic pneumotonometer (Solan Model 30; Medtronic, Minneapolis, MN). Measurements were conducted three times per week, and they were taken within 2 hours of the administration of the first drop for that day. Three IOP readings were taken at each time point, and a median value was used for analysis.

**Statistical Analysis**

All data are expressed as mean ± SEM unless indicated otherwise. For IOP measurements, integrated changes from baseline were determined by calculating the areas under the curve (AUCs) using the trapezoidal rule across the time course. Statistical analysis was conducted using either one-way ANOVA followed by the Dunnett’s or Tukey-Kramer test (IOP studies) or two-way ANOVA with repeated measures followed by Tukey-Kramer test on raw data (tear volume and corneal staining) or data elevated to the power 0.4 depending on whether the criteria of normality and variance homogeneity were satisfied (specific data transformations are discussed in the figure legends). Statistical analysis was conducted with statistical software (JMP; SAS Institute, Cary, NC). $P < 0.05$ was predetermined as the criterion of statistical significance.

**RESULTS**

**Dry Eye Model**

Repeated topical administration of atropine followed by PBS (vehicle) significantly reduced tear volume (3–5 mm) compared with baseline (~12 mm) at all time points studied (Fig. 1, left, solid circles). Both BOL-303242-X (0.5% and 1.0%) and DEX (0.1%) significantly inhibited atropine-induced tear volume reductions starting on day 1 and day 3, respectively, and lasting throughout the study period (Fig. 1). Both agents maintained tear volume at baseline levels, with no statistically significant differences from baseline at any time point. These data indicate that the treatments completely prevented atropine-mediated effects in tear volume.

When evaluating the tear integrity of atropine-treated eyes as measured by TBUT, trends similar to those observed in tear volume were identified. PBS-treated eyes showed a statistically significant and immediate reduction in TBUT that started at day 1 (reduction of TBUT to ~4.4 seconds) and lasted until the end of the study (TBUT of ~3.5 seconds) when compared with baseline TBUT (~36 seconds; Fig. 1, middle). In contrast, BOL-303242-X (0.5% and 1.0%) or DEX (0.1%) maintained TBUT close to baseline values at all time points (Fig. 1). As with tear volume, these data indicate that the treatments prevented the effects of atropine treatment on tear stability. In this study, corneal staining was not a useful efficacy end point because corneal staining was not altered by atropine treatment (Fig. 1).

To further evaluate the dose-dependent effect of BOL-303242-X, lower doses (0.1% and 0.3%) were also tested in the dry eye model. As with the higher concentrations, BOL-303242-X at 0.1% and 0.3% maintained baseline tear volume at all time points with no clear dose-dependent effects (Fig. 1). Dose-dependent effects were observed for BOL-303242-X on TBUT. The 0.1% dose of BOL-303242-X was not completely effective at blocking reductions in TBUT because no statistically significant differences were observed between 0.1% BOL-303242-X–treated and the vehicle-treated groups. However, treatment with 0.1% BOL-303242-X avoided the decline in TBUT observed in the PBS-treated group by day 7 (i.e., TBUT returned to baseline levels because no differences were observed when comparisons were conducted vs. baseline readouts). With the 0.3% dose group, BOL-303242-X prevented atropine-induced reductions in TBUT at the 3- and 7-day time points in a statistically significant manner compared with the atropine/PBS-treated groups at the same time points (Fig. 2, middle). As in the previous experiment no changes were observed in corneal staining (Fig. 2). Overall, these studies demonstrated that doses as low as 0.1% to 0.3% BOL-303242-X were effective at preventing atropine-induced changes in tear volume in rabbits with borderline effects in TBUT.

In a separate experiment and to validate the model, we evaluated the activity of a marketed dry eye product in this

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**Figure 1.** BOL-303242-X ophthalmic suspension (0.5% and 1.0%) is as effective as DEX ophthalmic solution (0.1%) in maintaining tear volume and TBUT in rabbits treated with repeated topical administration of atropine sulfate (1%). In this study repeated administration of atropine did not alter the corneal surface. Data are presented as mean ± SEM. *P < 0.05*, statistically significant differences compared with baseline of the same group. Two-way analysis of variance with repeated measures followed by the Tukey-Kramer test on raw data (tear volume and corneal staining) or data elevated to the power 0.4 (TBUT). $n = 14$ eyes for the vehicle-treated group; $n = 12$ eyes for the rest of the groups.
rabbit model, Restasis, compared with its vehicle, Refresh Endura. Although Refresh Endura failed to maintain tear volume at baseline levels, Restasis administered three times a day maintained tear volume (11.2–12.6 mm) close to baseline values (13.2 mm) 8 days after atropine treatment (Fig. 3). However, both Restasis and Refresh Endura failed to maintain tear film integrity as measured by TBUT, though TBUT in Restasis-treated eyes was significantly longer than that in Refresh Endura–treated eyes on days 3, 5, and 8 (Fig. 3). As in the other studies, corneal staining was not a useful end point in this investigation because atropine treatment failed to modify corneal staining in the vehicle group (though some apparent Restasis treatment effects were observed because of a higher baseline in the Restasis group; Fig. 3).

**Paracentesis Model**

BOL-303242-X (0.5% and 1.0%) and DEX (0.1%) significantly reduced clinical signs of inflammation compared with the control group, whereas at 0.1% BOL-303242-X was inactive in this model (Fig. 4, top). Furthermore, BOL-303242-X (0.5% and 1.0%) reduced aqueous humor protein levels in a statistically significant manner, whereas DEX and 0.1% BOL-303242-X were not effective (Fig. 4, bottom). BOL-303242-X at all doses reduced aqueous humor PGE2 concentrations, whereas DEX was ineffective (Fig. 5). BOL-303242-X (0.1%, 0.5%, and 1.0%) and DEX also reduced leukocyte count in the anterior chamber and MPO activity in a statistically significant manner compared with the control group (Fig. 6). No statistically significant differences in pupil diameter were observed across treatment groups (data not shown). Overall, these data indicate that BOL-303242-X is at least as efficacious as DEX in the paracentesis model and even better in some parameters (such as PGE2).

**Intraocular Pressure Measurements**

DEX treatment (0.1%) increased IOP by 1.2 mm Hg (geometric mean of the mean change from baseline) with 95% confidence limits of 0.05 to 2.66 mm Hg, whereas in vehicle-treated animals the geometric mean was 0.22 mm Hg with 95% confidence limits ranging from 1.57 to 1.54 mm Hg. In contrast, BOL-303242-X at 0.1% showed an increase in IOP of 0.15 mm Hg (geometric mean of the mean change from baseline).
line) with 95% confidence limits of 0.82 to 1.28 mm Hg. At the 1.0% dose, the geometric mean of the mean IOP change from baseline was 0.64 mm Hg with 95% confidence limits of 0.03 to 1.29 mm Hg. Although these changes in the context of this experiment may not be clinically significant, the data clearly indicate that DEX-treated eyes show a greater propensity to higher IOP than those treated with vehicle or BOL-303242-X, even at a BOL-303242-X concentration 10 times higher than DEX. Analysis of the cumulative effects using the area under the IOP curve reinforced this notion, and the results are presented in Figure 7. DEX treatment significantly increased integrated IOP in the DEX-treated eyes (Fig. 7, left, black bars) compared with vehicle-treated eyes (Fig. 7, left, white bars). This effect was also observed in the untreated, fellow eye, as demonstrated by the statistically significant increase in integrated IOP for the fellow eye compared with 0.1% BOL-303242-X–treated animals (Fig. 7, middle, solid vs. light gray bars).

Rabbits used in this study were only 15 weeks old and were expected to gain weight throughout the experimental period. Indeed, body weights of vehicle-treated animals increased in a statistically significant manner when compared with baseline body weights (Fig. 7, right panel). Repeated topical administration of BOL-303242-X had no statistically significant effects on body weights at either of the doses tested, and, as expected, body weights also increased from baseline in a manner similar to that of the vehicle-treated animals (Fig. 7, right, gray triangles). Body weight in BOL-303242-X–treated animals was significantly greater than that observed at baseline by days 15 and 22 for the 0.1% and 1.0% doses, respectively. In contrast, DEX (0.1%) prevented the increase in body weight observed in the vehicle- and BOL-303242-X–treated animals (Fig. 7, right). In fact, body weights in DEX-treated animals were not significantly greater than those of vehicle-treated animals (Fig. 7, right). In summary, topical treatment...
with BOL-303242-X had no significant effect on body weight gain or IOP elevation, whereas DEX treatment increased IOP and blunted the normal increase in body weight observed in the vehicle- and BOL-303242-X–treated animals.

**Discussion**

BOL-303242-X (also known as ZK-245186) represents the prototype of a new class of drugs termed SEGRA. In in vitro studies, BOL-303242-X exhibits full anti-inflammatory properties when compared with traditional steroids in a variety of primary and immortalized ocular cells. In these studies, we have demonstrated that BOL-303242-X is as efficacious as DEX in a model of dry eye induced by topical atropine administration in rabbits. In addition, our data clearly show that the compound is as active as the steroid in paracentesis-induced inflammation in rabbits. In both models, we were able to demonstrate that the anti-inflammatory efficacy of BOL-303242-X was comparable to that of DEX, a potent GC commonly used to treat ocular inflammation, which corroborates the findings of our in vitro studies by demonstrating the full efficacy of SEGRA compared with traditional steroids. During evaluation of transactivation-mediated gene regulation in vitro, BOL-303242-X behaved as a partial agonist in the activation of the MYOC gene compared with classical GCs, showing significantly reduced ability to elevate myocilin protein levels and gene expression in primary monkey trabecular meshwork cells. This reduced ability of the BOL-303242-X–liganded GR to transactivate while able to fully transrepress suggests that this agent could potentially provide a beneficial side effect profile compared with traditional GCs for ophthalmologic inflammatory indications. Our data indicate that BOL-303242-X has a reduced overall impact on IOP compared with DEX after chronic treatment in vivo; this is particularly apparent when a cumulative parameter such as integrated IOP is evaluated to assess the overall impact of the drugs on IOP. It must be noted, however, that the actual day-to-day changes in IOP are small and probably not clinically significant in this model. Furthermore, although DEX abolished the increase in body weight observed during the experimental period, the body weight time course for the animals treated with BOL-303242-X resembled that observed in the vehicle-treated animals, suggesting a reduced ability of SEGRA to induce muscle wasting, a common side effect of GC treatment.

Both SEGRAs and GCs exert their pharmacologic actions by binding to the GR. On GC binding, translocation to the nucleus, and dimerization, activated GR regulates the transcription of many genes. This regulation occurs primarily by transactivation through the binding of GRE and by transrepression through either direct binding to negative GREs or, more importantly, interference with transcription factors such as NF-kB and cJun-Fos. The liganded GR interacts with a number of associated proteins (coactivators and corepressors) that exhibit DNA remodeling activities, RNA polymerase II, and other components of the basal transcription machinery. Binding of diverse ligands to GR results in distinct interactions with amino acids facing the binding pocket. These interactions, in turn, lead to conformational changes of the receptor surface that are specific to the interacting ligand. Recent structural data have confirmed this notion by showing subtle changes in helix 12 positioning in crystals liganded with steroids compared with a nonsteroidal selective GR agonist such as GSK866. These changes in the shape of receptor surface coactivator binding motifs would alter the binding of coactivators. Similarly, there is crystallographical evidence to indicate that GR antagonists such as RU486 induce such drastic conformational changes to the receptor that coactivator motifs in the receptor surface are essentially hidden, leading to markedly limited transcriptional activity of GR. These changes in surface conformation and altered binding motifs for specific cofactors seem responsible for differences in transcriptional activity of the liganded receptors. Binding of SEGRAs such as BOL-303242-X to the GR is thought to induce distinct conformational changes in the receptor surface that may favor interactions with cofactors that preferentially induce transrepression while limiting transactivating interactions. Specific cofactor interaction profiles for BOL-303242-X–liganded GR have not yet been explored; however, other investigators have demonstrated modified coactivator profiles for SEGRAs such as AL-43847 and LGD-5552. As does BOL-303242-X, these compounds show full anti-inflammatory activity but reduced transactivation properties and transactivation-mediated effects, and, hence, improved side effect profiles. Our data after chronic administration of BOL-303242-X in the rabbit indicate a reduced tendency for elevated IOP and an inability to reduce body weight. In these studies, DEX elevated IOP and markedly reduced body weight. The reduction in the tendency for BOL-303242-X to induce IOP elevation could be related to its partial agonist profile to induce the myocilin gene compared with DEX.

BOL-303242-X was completely ineffective in reducing body weight in rabbits, whereas DEX completely blunted the inflammatory indication. Our data indicate that BOL-303242-X exhibits a better side effect profile than DEX in IOP and maintenance of body weight in rabbits. The effects of vehicle, BOL-303242-X (0.1% or 1.0%), or DEX (0.1%) are shown on integrated IOP in treated (left) and fellow (center) eyes and on body weight (right). Data are mean ± SEM. The integrated IOP was analyzed using a one-way ANOVA followed by Tukey-Kramer test on raw data for the treated eye or the square of the data for the fellow eye. Changes in body weights were analyzed using a two-way ANOVA with repeated measures followed by Tukey-Kramer test on data elevated to 0.6 power. *P < 0.05, statistical significance with respect to vehicle-treated control at the same time point. †P < 0.05, statistical significance with respect to BOL-303242-X 0.1%-treated animals. n = 16 animals for vehicle, 1.0% BOL-303242-X–treated, and DEX groups; n = 17 animals for the 0.1% BOL-303242-X–treated group.
crease in body weight observed in vehicle- and BOL-303242-X–treated animals. It is well documented that GCs induce muscle wasting in rats.49 This, in turn, is likely responsible for the reduced body weight observed in DEX-treated animals. The sarcopenic effects of GCs seem mediated by transactivation of the myostatin gene. The first line of evidence pointing to myostatin as the mediator of GC-induced muscle wasting comes from the identification of multiple GREs in the promoter region of the myostatin gene, and the demonstration, using reporter assays, that DEX treatment increases promoter-mediated transcriptional activity, which is blocked by cotreatment with RU486.50 In addition, these in vitro data were recapitulated in the in vivo studies conducted in rats, which demonstrated an association between DEX-induced muscle loss and increased intramuscle mRNA levels for myostatin, effects that were blocked (gene activation) or markedly reduced (muscle atrophy) by coadministration of RU486.51 More recent studies have clearly identified that the myostatin gene is required for observing the sarcopenic effects of GCs because GC-induced sarcopenia is not observed in myostatin knockout mice.59 Our data with BOL-303242-X are intriguing in the sense that though the compound exhibits anti-inflammatory activity similar to that of DEX in dry eye and postoperative inflammation models, it is distinct because BOL-303242-X–treated rabbits exhibit the same body weight increase observed in vehicle-treated animals, whereas DEX blunts the expected body weight increase. These data, therefore, support the notion that BOL-303242-X–liganded GR is not active in inducing transactivation of the myostatin gene, in contrast to GR activated by traditional GCs such as DEX.49–51 However, further studies are needed to evaluate this hypothesis.

Dry eye syndrome is a multifactorial disease caused by any disorder to the lacrimal functional unit (lacrimal glands, ocular surface, lids, sensory and motor nerves) leading to tear film instability with potential damage to the ocular surface. It is accompanied by increased osmolarity and inflammation.12 Restasis, a putative immunosuppressant (0.05% cyclosporine formulation), is the only anti-inflammatory–type FDA-approved prescription drug for the treatment of the more severe form of dry eye disease. In two pooled independent phase 3 clinical trials, Restasis has been shown to significantly increase tear volume at baseline levels, increased TBUT, and reduced corneal staining compared with its vehicle. As was the case with Restasis in the dry eye model, BOL-303242-X improved tear volume and TBUT and the observed effects were equivalent to those elicited by the reference compound, DEX. In the present studies, these agents were unable to markedly improve corneal staining; however, the atropine-induced dry eye model reported here appears relatively ineffective at inducing corneal staining.

The effects of anti-inflammatory agents in the atropine-induced dry eye model, as manifested in the maintenance of tear volume at baseline levels, are an interesting phenomenon. How atropine-induced reductions in tear volume can be ameliorated by anti-inflammatory treatment is difficult to understand at first glance. Taken at face value, these data indicate that the anticholinergic induction of dry eye may encompass an inflammatory syndrome in the lacrimal glands of the treated animals. There are preliminary indications that this may indeed be the case because other studies in the atropine-induced mouse model have demonstrated that cyclosporine reversed the effect of atropine on tear volume.53 recapitulating our observations in the present study. Moreover, in a similar rabbit model, nonsteroidal anti-inflammatory drugs such as nimesulide (0.1%) and ketorolac (0.5%) have been shown to improve tear volume, TBUT, and corneal staining.54 These data reinforce the notion that atropine-induced changes in tear volume in this model may be associated with an inflammatory component, which, after treatment with anti-inflammatory drugs, provides the window observed in the model with these agents.

The anti-inflammatory effect of BOL-303242-X compared with classical GC has been shown in a number of ocular cells and a monocytic cell line, THP-1.55 The anti-inflammatory action of BOL-303242-X was comparable to that of DEX or triamcinolone acetonide in inhibiting key inflammatory cytokines such as IL-1β, IL-6, IL-8, monocyte chemotactic protein-1 (MCP-1), and TNF-α in all the cell types studied and intracellular adhesion molecule-1 (ICAM-1) in human retinal endothelial cells. Furthermore, BOL-303242-X inhibited the phosphorylation of the transcriptional factors NF-κB, p38, and JNK.55 In the present study, we also evaluated the anti-inflammatory properties of BOL-303242-X compared with DEX in a model of postoperative inflammation, the paracentesis-induced inflammation model. The rabbit paracentesis model is a relevant system that mimics ocular trauma induced during surgery in the anterior segment with breakdown of the blood-aqueous barrier. In this model, animals respond to GC treatment by showing inhibition of clinical signs indicating an inflammatory response and biomarkers of inflammation such as PGE_2. The anti-inflammatory activity of DEX in experimental postoperative inflammation and downregulation of PGE_2 has been previously demonstrated in postsurgical (vitrectomy or lensectomy) rabbit inflammation models.55 Therefore, this animal model represents a good platform for screening anti-inflammatory agents, particularly those that modulate GR activity. Pretreatment with BOL-303242-X improved clinical anti-inflammatory outcomes in the paracentesis model. In addition, more objective measures of inflammation, which include flare, levels of PGE_2, and infiltration of inflammatory cells in aqueous humor, were also favorably affected by treatment with the compound. More important, the data indicate that BOL-303242-X is as effective as DEX in improving inflammatory readouts in this model, demonstrating that the compound shows full efficacy when looking at its anti-inflammatory properties. However, DEX was ineffective in reducing levels of PGE_2. The discrepancy between these data and findings by Scheib et al.57 may be due to the duration of our study and the time points at which the samples were assayed. The differences observed between BOL-303242-X or DEX may be due to differences in ligand-receptor interaction and morphologic changes that may result in the inhibition of specific inflammatory mediators; however, it is also possible that there are differences in the PK properties of BOL-303242-X and DEX that could result in different concentrations attained in the target tissue.

In summary, our data demonstrate that BOL-303242-X is an effective anti-inflammatory agent when tested in experimental atropine-induced dry eye and paracentesis-induced inflammation models in rabbits. The data also indicate that BOL-303242-X is at least as efficacious as DEX, a traditional GC commonly used to treat ocular inflammation, in a model of dry eye and in paracentesis-induced inflammation in rabbits. Furthermore, BOL-303242-X has a reduced overall impact in IOP when compared with DEX after chronic treatment. In addition, though DEX abolished the increase in body weight observed during the experimental period, the body weight time course for the animals treated with BOL-303242-X resembled that observed in the vehicle-treated
animals. These data suggest a reduced ability of SEGRA to induce muscle wasting, a common side effect of GC treatment, highlighting that BOL-303242-X represents a valid and potentially safer alternative than traditional steroids and warranting further clinical evaluation of the compound for the treatment of ocular inflammation, particularly for chronic conditions.

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