Activation of Sigma 1 Receptor Extends Survival of Cones and Improves Visual Acuity in a Murine Model of Retinitis Pigmentosa

Jing Wang,1,2 Alan Saul,2,3 and Sylvia B. Smith1–3

1Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta University, Augusta, Georgia, United States
2James and Jean Culver Vision Discovery Institute, Augusta University, Augusta, Georgia, United States
3Department of Ophthalmology, Medical College of Georgia, Augusta University, Augusta, Georgia, United States

Correspondence: Sylvia B. Smith, Department of Cellular Biology and Anatomy, Medical College of Georgia, Augusta University, Augusta, Georgia, United States. sbsmith@augusta.edu.

Submitted: June 10, 2019
Accepted: August 21, 2019

Citation: Wang J, Saul A, Smith SB. Activation of Sigma 1 Receptor Extends Survival of Cones and Improves Visual Acuity in a Murine Model of Retinitis Pigmentosa. Invest Ophthalmol Vis Sci. 2019;60:4597–4607. https://doi.org/10.1167/iovs.19-27709

PURPOSE. Retinitis pigmentosa (RP), a retinal photoreceptor degeneration, typically affects rod function and subsequently cones. Activation of sigma 1 receptor (Sig1R) has been shown to preserve cone function through 6 weeks in the rd10 mouse model of RP, when mice were treated systemically with the Sig1R ligand (+)-pentazocine (PTZ). This study determined the extent to which cone function is preserved in rd10 mice when Sig1R is activated.

METHODS. Rd10 mice were administered (+)-PTZ (alternate days beginning at postnatal day [P]14) over a period of 180 days. Mouse visual function and structure were measured in vivo using optokinetic tracking response, scotopic and photopic electroretinography plus photopic assessment using “natural” noise stimuli, and optical coherence tomography (OCT). Immunofluorescent methods were used to detect cones in retinal cryosections.

RESULTS. Visual acuity was maintained in rd10(+)-PTZ-treated mice through P56, whereas rd10 nontreated mice showed marked decline by P28. Cone responses were detected in (+)-PTZ-treated mice through P60, which were more robust when tested with natural noise stimuli; cone responses were minimal in nontreated rd10 mice. OCT revealed significantly thicker retinas in (+)-PTZ-treated rd10 mice through P60 compared to nontreated mice. Cones were detected by immunofluorescence in (+)-PTZ-treated rd10 retinas through P120.

CONCLUSIONS. The extent to which cone rescue could be sustained in (+)-PTZ-treated rd10 mice was evaluated comprehensively, showing that activation of Sig1R is associated with prolonged visual acuity, extended detection of cone function, and detection of cones in retinal histologic sections. The data reflect promising long-term neuroprotection when Sig1R is activated.

Keywords: mouse, retina, pentazocine, retinal degeneration, visual acuity, ERG, OCT

The major cause of untreatable blindness worldwide is retinal degenerative disease, often caused by dysfunction of photoreceptor cells (PRCs).1,2 The retinal degenerative disease retinitis pigmentosa (RP) is caused by numerous mutations in a number of genes and is characterized by progressive loss of PRCs.3 This disease affects ~1:3000 to ~1:5000 humans. Typically in RP, rod PRCs are lost initially, compromising vision in dim light. The more debilitating aspect of this disease is the subsequent loss of cone PRCs, which mediate best vision; cone responses were minimal in nontreated rd10 mice. OCT revealed significantly thicker retinas in (+)-PTZ-treated rd10 mice through P60 compared to nontreated mice. Cones were detected by immunofluorescence in (+)-PTZ-treated rd10 retinas through P120.

Activation of sigma 1 receptor (Sig1R) has been shown to preserve cone function through 6 weeks in the rd10 mouse model of RP, when mice were treated systemically with the Sig1R ligand (+)-pentazocine (PTZ). This study determined the extent to which cone function is preserved in rd10 mice when Sig1R is activated.

In a recent study, we observed dramatic rescue of cone PRCs in the Pde6brd10(rd10) mouse model of RP when mice were treated systemically with (+)-pentazocine (PTZ), a high-affinity Sig1R ligand.16 The rd10 mouse is considered a valuable model of RP and useful for testing rescue approaches.17 The mouse initially loses rods due to a genetic defect in a rod-specific gene (β-subunit of rod phosphodiesterase) and then subsequently cones. The PRC loss is rapid and nearly complete within the first 35 days of life.17 By postnatal day 25 (P25), the outer nuclear layer in which PRC nuclei reside is reduced to two or three rows of PRCs compared to 10 to 12 rows in wild-type (WT) mice. Only a few aberrant cones remain by P45. Owing to the very rapid progression of the retinal phenotype in rd10 mice, our initial study of the effects of activating Sig1R as a neuroprotectant was conducted in mice through P42.16 We performed retinal functional and architectural assessments including evaluation of cone electrophysiological function at P55 and immunohistochemical analysis to detect cones at P42.16 Our electrophysiological assessment of PRC responses to natural stimuli revealed cone function that was significantly preserved in (+)-PTZ-treated mice compared to nontreated mice.
better in (+)-PTZ-treated rd10 mice than in nontreated littermates, and importantly, was similar to that in WT mice. Cones were significantly more abundant in (+)-PTZ-treated rd10 mice than in nontreated rd10 mice. The protective effects of (+)-PTZ treatment are attributable to Sig1R activation as there is no cone preservation in (+)-PTZ-treated rd10/ Sig1R"/ mice. The data showing cone rescue in rd10 mice treated with (+)-PTZ are encouraging, but they reflect only short-term preservation of function and do not address the duration of cone rescue. Thus, we do not know how long cone function can be preserved in this model if Sig1R is activated. We hypothesized that (+)-PTZ treatment can rescue cones beyond P35. In the current study, we evaluated visual function and retinal architecture comprehensively over a 180-day period in (+)-PTZ-treated rd10 mice compared to nontreated mutants. We chose this extended time period anticipating that in this severe retinopathy we would reach the limit of rescue. (+)-PTZ treatment of rd10 mice improved visual acuity through at least P56 and enhanced cone function beyond P60. Indeed, cone function was slightly detectable at P90, though not at P120 or beyond. Cone PRCs continued to be detectable through P90. Thus, activation of Sig1R holds promise as a longer-term neuroprotectant for retinal disease.

**MATERIALS AND METHODS**

**Animals**

Breeding pairs of B6.CXB1-Pde6b"rd10/" (rd10) mice were obtained from the Jackson Laboratories (Bar Harbor, ME, USA). Confirmation of genotype was performed as described.16 The Crb1"Pde6b" mutation responsible for focal retinal disruption in certain mouse strains18 was not detected in any mice used in the study. Mice were fed Teklad Irradiated Rodent Diet 8904 for breeding or Diet 2918 for maintenance (Teklad, Madison, WI, USA). Animals were subjected to a standard 12-hour light/12-hour dark cycle. We adhered to institutional guidelines for humane treatment of animals and to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research. Two groups of mice were evaluated over a period of 180 days: rd10 mice (nontreated) and rd10/+PTZ mice. The rd10/+PTZ mice received an intraperitoneal injection of (+)-PTZ (0.5 mg/kg) (Sigma-Aldrich Corp., St. Louis, MO, USA) on alternate days beginning at P14. The dosage was based on studies showing the numbers of mice analyzed at four time points (P60, P90, P120, and P180) following extended (+)-PTZ treatment.

**Visual Acuity Assessment**

Visual acuity was evaluated in rd10 nontreated and rd10/+PTZ mice ages P21 to P70; a cohort of WT C57Bl/6j mice (Jackson Laboratories) were also included in the assessment for comparison. The visual acuity was measured as described previously.19 Briefly, spatial thresholds for optokinetic tracking of sine-wave gratings were measured using the OptoMotry system (CerebralMechanics, Medicine Hat, Alberta, Canada).20 Mice were placed unrestrained on a pedestal and were presented vertical sine-wave gratings moving at 12'/s or gray of the same mean luminance within the OptoMotry device, which functions as a virtual cylinder. The cylinder hub was continually centered between the mouse’s eyes to establish the spatial frequency of the grating at the mouse’s viewing position as it shifted its position. Gray color was projected while the mouse was moving, but when movement ceased, the gray was replaced with the grating. Grating rotation under these circumstances elicited reflexive tracking, which was scored via live video using a method of limits procedure with a yes/no criterion as recommended by the manufacturer. A measure of spatial resolution was taken as the asymptote of a staircase procedure. The two eyes were tested in an interleaved fashion.

**OptoMotry analysis of vision acuity**

| Mouse Group | n  | Age, Postnatal Days |
|-------------|----|---------------------|
| Wild type   | 4  | 30                  |
| Wild type   | 6  | 60                  |
| Rd10 (no (+)-PTZ) | 9 | 21                  |
| Rd10 (no (+)-PTZ) | 9 | 28                  |
| Rd10 (no (+)-PTZ) | 9 | 35                  |
| Rd10 (no (+)-PTZ) | 9 | 42                  |
| Rd10 (no (+)-PTZ) | 9 | 63                  |
| Rd10 (0.5 mg kg"1 (+)-PTZ) | 9 | 21                  |
| Rd10 (0.5 mg kg"1 (+)-PTZ) | 9 | 28                  |
| Rd10 (0.5 mg kg"1 (+)-PTZ) | 9 | 35                  |
| Rd10 (0.5 mg kg"1 (+)-PTZ) | 9 | 42                  |
| Rd10 (0.5 mg kg"1 (+)-PTZ) | 9 | 49                  |
| Rd10 (0.5 mg kg"1 (+)-PTZ) | 9 | 56                  |
| Rd10 (0.5 mg kg"1 (+)-PTZ) | 9 | 63                  |
| Rd10 (0.5 mg kg"1 (+)-PTZ) | 9 | 70                  |

**Electroretinography (ERG)**

Dark-adapted rd10 nontreated and rd10/+PTZ mice were anesthetized using isoflurane and electrophysiological function was assessed as described.16 Briefly, dark-adapted ERGs were performed using silver-coated nylon fibers joined to flexible wires that were placed on the cornea. The electrical contact was enhanced by placing a drop of hypromellose on the cornea. Optic fibers (1-mm diameter) were positioned in front of the pupil through which highly controllable illumination was delivered to eyes using a 5500° white light-emitting diode. Rod function was assessed using a series of tests with 5-ms flashes of increasing luminance, followed by assessment of cone function using photopic testing with 5-ms flashes above a pedestal. Additionally, a photopic “natural noise” stimulus was presented. This stimulus changes luminance pseudorandomly over time, with the amplitudes of those changes inversely proportional to temporal frequency (as has been described for human subjects), and phase being random. This produces relatively slow, continuous changes in luminance, rather than flashes, and is natural in the sense that real-world visual stimuli.
similarly change slowly. Responses to noise stimuli are also random, but responses are correlated with stimuli to generate kernels that describe how the retina transforms arbitrary stimuli into ERG responses. Mice were evaluated at ages P60, P90, P120, and P180.

Spectral-Domain Optical Coherence Tomography (SD-OCT)

*Rd10* nontreated and *rd10*-PTZ mice were anesthetized with ketamine/xylazine as described.\(^1\) Retinal structure was evaluated in vivo using a Biopitgen Spectral Domain Ophthalmic Imaging System (SDOIS; Biopitgen Envisu R2200, Morrisville, NC, USA) in mice at ages P60, P90, P120, and P180. The imaging protocol included averaged single B-scan and volume intensity scans with images centered on the optic nerve head. Because of the considerable outer retinal disruption in *rd10* retina, it is preferable to use the manual caliper feature to measure retinal layers versus autosegmentation postimaging analysis (a feature of the InVivoVue Diver 2.4 software). We used the manual caliper feature to acquire inner, outer, and total retinal thickness measurements. Inner retina was measured from the superior boundary of inner limiting membrane (ILM) to the lower edge of the inner nuclear layer (INL). Outer retina was measured from the lower edge of the INL to the inferior boundary of the retinal pigment epithelium layer (RPE); if there was separation of photoreceptors from RPE, we obtained outer retinal thickness by adding the RPE layer thickness and the distance from the lower edge of INL to the edge of neuronal retina as described previously.\(^2\)

Intraocular Pressure

Intraocular pressure (IOP) was measured in *rd10* nontreated and *rd10*-PTZ mice at P60, P90, P120, and P180 using a handheld tonometer (Tonolab; Icare Laboratory, Finland) as described.\(^1\) The procedure was conducted under typical laboratory lighting conditions (e.g., 46.50 foot-candles). The tonometer was positioned at the center of the cornea of mice anesthetized by isoflurane inhalation. All IOP measurements were made between 10:00 and 11:00 AM. Three repeated measurements were taken from each animal for quantification.

Immunodetection of Cone Photoreceptor Cells and Retinal Gliosis

Eyes, enucleated from euthanized mice, were flash-frozen in liquid nitrogen, embedded in Tissue-Tek optimal cutting temperature compound (Electron Microscopy Sciences, Hatfield, PA, USA), and cryosections were prepared for immunohistochemistry as previously described.\(^1\) Sections were incubated with fluorescein isothiocyanate–conjugated peanut agglutinin (FITC-PNA, catalog number L7381; Sigma-Aldrich Corp.) or cone arrestin (catalog number AB15282; Millipore, Temecula, CA, USA) to detect cone PRCs. Additional cryosections were incubated with rabbit anti-glial fibrillary acidic protein (GFAP) (catalog number Z0334; Dako Corp., Carpinteria, CA, USA) to detect gliosis. Alexa Fluor 555 and Alexa Fluor 488 anti-rabbit IgG (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) were used as the secondary antibody for cone arrestin and GFAP, respectively. Coverslips were mounted using Fluoroshield with 4',6-diamidino-2-phenylindole (DAPI) (Sigma-Aldrich Corp.). Retinas were examined using a Zeiss (Carl Zeiss, Göttingen, Germany) Axio Imager D2 microscope equipped with a high-resolution camera and processed using Zeiss Zen23pro software.

**RESULTS**

Activation of Sig1R Enhances Visual Acuity in *rd10* Mice

Prior to conducting the electrophysiological and retinal architectural analyses in mice over the 180-day time period,
we measured visual acuity in rd10 mice that had or had not received (±)-PTZ treatment and compared the findings with WT mice. The optokinetic tracking response performed using the OptoMotry system in the left and right eye of each animal measured the reflex response for clockwise and counterclockwise movement of gratings drifting at 12°/s. Data were recorded as the asymptotic convergence of a staircase procedure that estimates spatial resolution, or acuity, in units of cycles per degree (c/d). WT mice had an average visual acuity of 0.4 c/d (Fig. 1A). The visual acuity of rd10 nontreated mice was ~0.30 c/d at P21 and ~0.35 c/d at P28, but decreased rapidly such that by P35 it was reduced to 0.1 c/d. Beyond P35, the visual acuity of rd10 nontreated mice remained minimal (note responses at P42 and P63, Fig. 1B). In contrast, the rd10 mice treated with (±)-PTZ sustained visual acuity through P56 (median visual acuity was ~0.32 c/d at P35, ~0.3 c/d at P42, ~0.25 c/d at P49, ~0.2 c/d at P56) (Fig. 1C). By P63 visual acuity was reduced to ~0.1 c/d. The data indicate that (±)-PTZ treatment postpones the precipitous decline in visual acuity observed in nontreated rd10 mice. The averaged values for the visual acuity data are presented for comparisons between rd10 nontreated and rd10-PTZ mice at P21, P35, P42, and P65 (Fig. 1D).

Activation of Sig1R Extends Scotopic and Photopic ERG Responses in rd10 Mice

Given that visual acuity was extended significantly in rd10 mice administered (±)-PTZ through ~60 days compared to nontreated rd10 mice (Fig. 1), we were interested in determining the extent to which electrical impulses, indicative of retinal cellular activity, could be detected in rd10 mice versus (±)-PTZ-treated rd10 mice. Mice, administered (±)-PTZ on alternate days beginning at P14, were subjected to comprehensive ERG analysis at P60, P90, P120, and P180 to assess rod (scotopic) and cone (photopic) function (Figs. 2–5). Responses were compared with age-matched rd10 nontreated mice. Representative scotopic tracings are shown for rd10 mice, and responses were minimal (Figs. 2A–D). There was significant rod function detected at higher luminous intensities in rd10 (A–D) and rd10-PTZ (E–H) are plotted against luminous intensity (I–L, at P60, P90, P120, and P180, respectively). *Significantly different from nontreated animals: P < 0.05.

The data indicate that (±)-PTZ function is discernible in the (±)-PTZ-treated rd10 mice through P60,
which is significantly longer than our original observation of improved scotopic response through P35.16

Regarding cone responses, the b-wave amplitude measured in the photopic ERG response was nearly the same in rd10+PTZ mice compared to nontreated rd10 mice (Figs. 3A–L). There was improvement in cone response at the highest contrast (Fig. 4A) in rd10+PTZ mice at P60, although not in rd10+PTZ mice at P90, P120, and P180 (Figs. 4B–D). Stronger rescue of cone function was observed in rd10+PTZ mice at P60 and P90 when they were tested with a stimulus that changed more slowly in time (Figs. 5A, 5B, 5E). This natural stimulus (‘green noise’) detected responses in rd10+PTZ mice that were significantly greater than in nontreated rd10 mice at P60 (Fig. 5A) and P90 (Fig. 5B). This improved response was no longer observed in (+)-PTZ-treated rd10 mice at P120 or P180. The quantification of the green noise amplitude in rd10+PTZ versus rd10 nontreated mice is shown in Figure 5E.

**Activation of Sig1R Sustains Retinal Architecture Measured In Vivo by SD-OCT in rd10 Mice**

We visualized the structure of retinas in situ using SD-OCT. Representative images are shown (Figs. 6A–D). At P60, the rd10 mouse retina was ~90 to 100 μm thick (Fig. 6A). There is often significant separation of the neural retina from the RPE in rd10 mice by this age as the inner and outer segments are markedly diminished. In (+)-PTZ-treated rd10 mice the retinas at P60 were significantly thicker (~110 μm) than in nontreated (Figs. 6A, 6E), and the neural retina–RPE separation was attenuated. The outer retina (Fig. 6F) and inner retina (Fig. 6G) of (+)-PTZ-treated rd10 mice were significantly thicker than in nontreated mice at P60 as well. Interestingly, while the differences between (+)-PTZ-treated and nontreated rd10 mice were only slightly significant or nonsignificant at P90 and P120 (Figs. 6E–G), by P120 the differences were highly significant for total retinal thickness (Fig. 6E) as well as inner retinal thickness (Fig. 6G).

In addition to the aforementioned analyses (Figs. 1–6), we also assessed the IOP of the mice as part of a comprehensive ophthalmologic evaluation. Altered IOP has not been reported in rd10 mice, and our data indicate that pressure was within normal limits for all mice examined (Supplementary Fig. S1).

**Activation of Sig1R Prolongs the Immunodetection of Cones in rd10 Mice**

Following functional visual assessments, animals were euthanized and eyes were processed to determine whether cones were retained in (+)-PTZ-treated rd10 retinas. In earlier studies, cone PRCs were detected in (+)-PTZ-treated rd10 retinas through P42. In the present study, we were interested in determining whether cones persisted over a longer treatment regimen. We...
detected cone cells using FITC-PNA. We observed some labeling in rd10 nontreated retinas at P60 as well as in rd10−PTZ mice at P60 (Fig. 7A, arrowheads). FITC-PNA labeling in the rd10 nontreated mice was minimal at P90, P120, and P180 but was discernible in rd10−PTZ retinas at P90 and P120 (Figs. 7B–D). The data suggest that some cones are retained in the (+)-PTZ-treated rd10 retinas, at least through P120. Thus, activation of Sig1R via the treatment with (+)-PTZ contributes to cone rescue beyond P60. These data were confirmed using a second antibody to detect cones (cone arrestin) as shown (Supplementary Fig. S2).

In healthy retinas, GFAP is present primarily in retinal astrocytes, which reside in the retinal ganglion cell layer; its expression is minimal in the remainder of the retina. Under stress, however, GFAP is detected at much higher levels in retina, particularly in the radially oriented Müller cells. This was reported in rd10 mice and attenuated with (+)-PTZ treatment through P42. Here, we investigated whether long-term (+)-PTZ treatment alters gliosis in rd10 retinas; however, we did not observe quantitative differences in GFAP immunofluorescence at ages P60 and beyond (Supplementary Fig. S3). Our data suggest that gliosis is considerable as the severe rd10 retinopathy progresses regardless of (+)-PTZ treatment.

**DISCUSSION**

Blindness exacts an extremely burdensome personal, emotional, and financial toll and represents an urgent unmet health care need demanding novel therapeutic strategies. Sig1R represents a novel target for delaying neuronal loss in retinal disease. While earlier reports indicated impressive rescue of cone PRCs in the rd10 mouse model of RP, the extent to which this rescue could be sustained was not explored, but is clinically significant. In the current study we evaluated, over an extended time period, the effects of activating Sig1R in rd10 mice by assessing visual acuity, retinal electrophysiological function, retinal architecture, and immunodetection of cones. The most profound observations in mice treated with the Sig1R ligand were prolonged visual acuity, extended detection of cone function using a natural noise stimulus in electrophysiological analysis, and detection of cones in retinal histologic sections.

Regarding visual acuity, we observed a dramatic improvement in visual acuity in rd10−PTZ mice compared with nontreated animals. The nontreated mice had an average acuity of ~0.35 c/d at P28 and by age P35 the average acuity was less than 0.1 c/d, which is markedly decreased compared to the normal (WT) value of ~0.4 c/d. In contrast, the (+)-PTZ-treated rd10 mice had visual acuity >0.5 c/d at P35 and ~0.5 c/d at P42 and continued to demonstrate a robust, albeit decreased, response through P56. Only when tested at P65 and beyond did rd10−PTZ mouse responses compare similarly to the nontreated rd10 mice. Other investigations of therapeutic intervention strategies in rd10 mice have also evaluated visual acuity, although generally for a limited time period. For example, in a study evaluating the benefit of rasagiline, an anti-Parkinsonism compound that inhibits monoamine oxidase B, the rd10-treated mice showed improvement in visual acuity. However, the study was terminated at P30, hence it is unknown whether the benefits of this compound extended beyond the period of greatest PRC death in the mutant model. Similarly, the benefit of exercise (active running wheel) on visual acuity has been evaluated in rd10 mice. One of these studies evaluated optokinetic tracking in exercised mice at P35 and P42 and found improved responses at both ages compared to nonexercised mice, although the spatial frequency threshold at P42 was only ~0.175 c/d in the exercised group. The other exercise-related study spanned a longer time frame and examined exercised and standard conditioned mice at 1 year. By that advanced age, the visual acuity in the exercised mice was quite low (~0.1 c/d), although it was greater than in those reared under standard conditions.

An intriguing study, conducted through P60, demonstrated promising effects on visual acuity in rd10 mice treated with myriocin, an inhibitor of serine-palmitoyl transferase, the rate-limiting factor in de novo ceramide synthesis. The assessment of visual acuity is an important measure of the efficacy of treatment strategies, and it appears that activation of Sig1R...
affords robust improvement compared to nontreatment and to several other strategies as well. In addition to extended visual acuity observed in rd10+PTZ mice, we also observed extended electrophysiological function in this cohort of mice compared to nontreated animals. There was discernible PRC function in rd10+PTZ mice at P60, which was not observed in rd10 nontreated mice at this age. There was no rod function observed at P90 or beyond regardless of (+)-PTZ treatment. Regarding cone function, the (+)-PTZ-treated mice had more robust responses at P60 (when measured using the standard photopic ERG), which could be appreciated even more when cone function was interrogated using a pseudorandom luminance noise test that has power at low temporal frequencies. Indeed, there were detectable responses at P60 and P90. This is quite encouraging for treatment of retinal disease. As with the assessment of visual acuity mentioned above, electrophysiological function assessments have frequently been performed during the acute phase of rod loss through ~P42 in rd10 mice.25,26 The studies limiting ceramide synthesis using myriocin were quite promising because they preserved cone function for a longer time period.28 Indeed, those studies and our own are noteworthy because in neither case is the treatment strategy directly targeting the genetic mutation; rather these studies are addressing disruption of a biological function. In the case of the myriocin studies, rod function was likely preserved because the compound inhibited ceramide, which can be toxic to retinal cells.29

In the field of Sig1R biology, the mechanism of neuronal protection may involve attenuating oxidative stress, perhaps by modulation of NRF2.11,12 Other studies have targeted oxidative stress in the rd10 mouse, especially the Campochiaro laboratory.30 They administered the antioxidant compound N-acetylcysteine via the drinking water to rd10 mice and observed improved photopic responses through P50.30 There are other modalities of neuronal protection in the visual system ascribed to activation of Sig1R. For example, Sig1R is able to differentially modulate the extracellular signal-regulated protein kinase (ERK1/2) in a cell type–specific manner.31–33 This is relevant to retinal neuronal protection because of the

**Figure 5.** Kernels derived from natural noise stimulation in Pde6b<sup>rd10</sup> (rd10) mice administered (+)-PTZ versus rd10 nontreated mice assessed over 180 days. The rd10 nontreated and (+)-PTZ-treated rd10 mice were tested with photopic “natural” noise stimuli, which is a slowly varying luminance time series with amplitude inversely proportional to temporal frequency. Kernels were computed from responses to natural noise stimuli. This assessment reflects how the retina transforms luminance modulations that include relatively slower changes than can be seen with the brief flashes. (A–D) Averaged kernels from natural noise stimulation for (+)-PTZ-injected rd10 versus rd10 nontreated mice at P60, P90, P120, and P180, respectively. The rd10+PTZ mice were treated every other day with (+)-PTZ beginning at P14. (E) Kernel (RMS) amplitude plotted versus mouse age (P60, P90, P120, P180 days) for rd10 and rd10+PTZ groups. *Significantly different from nontreated animals: P < 0.05.
The regulatory role played by ERK1/2 in fundamental cellular processes including survival, proliferation, and differentiation. There is evidence also that Sig1R regulates mitochondrial function, including restoring mitochondrial membrane potential and cytochrome c oxidase activity in retinal cells.\(^{34}\) That study showed that depriving cells of oxygen and glucose deprivation impacted mitochondrial function negatively, but overexpression of Sig1R improved mitochondrial function.\(^{35,36}\) There is considerable evidence that activation of Sig1R modulates retinal cell survival and attenuates apoptosis by modulating L-type voltage-gated calcium channels.\(^{35,36}\) Finally, studies show that activation of Sig1R in models of cone and rod degeneration may mediate neuroprotection by influencing autophagy.\(^{57}\)

The (+)-PTZ-treated rd10 mice used in the present study demonstrated improved retinal architecture when examined by OCT over the extended time period. Moreover, the separation of outer retina from RPE, frequently observed in this mutant mouse, was diminished when mice were treated for the extended time period. Additionally, the immunohistochemical studies detected many more cones in the (+)-PTZ-treated animals than in nontreated animals through P90 and P120.

Taken collectively, our data indicate that activation of Sig1R is beneficial in attenuating cone death in this mutant mouse model. It is encouraging for the field of neuroprotection that benefits were observed at time points significantly exceeding the known loss of rods and cones. There are, however, limitations to the study, one of which is the extremely rapid loss of rods and cone cells in rd10 mice.\(^{17}\) While not as rapid a degeneration perhaps as in the rd1 mouse,\(^{38}\) the phenotype of the rd10 mouse has been described as a “catastrophic” retinal dystrophy. Thus while it serves the critical purpose of identifying promising targets, it may not be as informative about long-term benefits. This may not be because a particular treatment strategy is inherently flawed, but simply because the
model system has limitations. It is extremely important that promising interventions take advantage of models that are more similar to human disease. A new mouse model exhibiting the P23H mutation of opsin has gained attention because it reflects the most common form of autosomal dominant RP. What is noteworthy about the model is that heterozygous mice (P23H/+) have photoreceptor degeneration that spans several months, mimicking more closely the human condition and allowing an extended period of time to assess therapeutic efficacy. Indeed, in this comprehensive study from Sakami et al., data are provided for the retinal function and architecture in human patients afflicted with RP, allowing comparison to the murine model. Given that we have had such promising findings in attenuating retinal disease when Sig1R is activated in short-term studies as well as in longer-term studies (described here), it will be critically important to validate the benefits of Sig1R activation on retinal structure and function (especially long-term benefits) in a highly clinically relevant model, such as the P23H mouse.

A consideration regarding administration of (+)-PTZ is that its elimination half-life is ~3.6 hours (range, 1.5–10 hours); ~60% of the total dosage is eliminated within 24 hours. In our administration scheme (injecting every other day), we provide a booster at a time when the available compound will have reached a very low level. Whether administration of (+)-PTZ in a sustained-release route would be more efficacious than our present dosing regimen is not known, but could be explored in future studies. Future studies are also necessary to establish whether cone rescue observed as a consequence of (+)-PTZ treatment is through a direct effect on PRCs or whether the effect is mediated by actions on supportive cells, such as the Müller glial cell or the RPE.

Acknowledgments

The authors thank Jing Zhao and Xuezhi (Rachel) Cui for their assistance with mouse injections for a portion of this study, the members of the Augusta University EM/Histology Core and Imaging Core, and the Culver Vision Discovery Institute and Augusta University for financial support in acquiring the Opto-Motry device for visual acuity studies. Supported by the National Institutes of Health/National Eye Institute (RO1-EY028103) and Foundation Fighting Blindness (TA-NMF0617-0721-AUG).
Disclosure: J. Wang, None; A. Saul, None; S.B. Smith, None

References

1. Dowling JE. Restoring vision to the blind: introduction. Trans Vis Sci Technol. 2014;3(7):2.
2. Cuenca N, Fernández-Sánchez L, Campello L, et al. Cellular responses following retinal injuries and therapeutic approaches for neurodegenerative diseases. Prog Retin Eye Res. 2014;43:17–75.
3. Dias MF, Joo K, Kemp JA, et al. Molecular genetics and emerging therapies for retinitis pigmentosa: basic research and clinical perspectives. Prog Retin Eye Res. 2018;63:107–131.
4. Smith SB, Wang J, Cui X, Mysona BA, Zhao J, Bollinger KE. Sigma 1 receptor: a novel therapeutic target in retinal disease. Prog Retin Eye Res. 2018;67:130–149.
5. Yang H, Fu Y, Liu X, et al. Role of the sigma-1 receptor chaperone in rod and cone photoreceptor degenerations in a mouse model of retinitis pigmentosa. Mol Neurodegener. 2017;12:68.
6. Schmidt HR, Zheng S, Gurpiner E, Koehl A, Manglik A, Kruse AC. Crystal structure of the human σ1 receptor. Nature. 2016;532:527–530.
7. Su TP, Su TC, Nakamura Y, Tsai SY. The Sigma-1 receptor as a pluripotent modulator in living systems. Trends Pharmacol Sci. 2016;37:262–278.
8. Nguyen L, Lucke-Wold BP, Mookerjee SA, et al. Role of sigma-1 receptors in neurodegenerative diseases. J Pharmacol Sci. 2015;127:17–29.
9. Jiang G, Mysona B, Dun Y, et al. Expression, subcellular localization, and regulation of sigma receptor in retinal Müller cells. Invest Ophthalmol Vis Sci. 2008;49:5776–5802.
10. Mavlyutov TA, Epstein M, Guo LW. Subcellular localization of the sigma-1 receptor in retinal neurons - an electron microscopy study. Sci Rep. 2015;5:10689.
11. Wang J, Zhao J, Cui X, et al. The molecular chaperone sigma 1 receptor mediates rescue of retinal cone photoreceptor cells via modulation of NRF2. Free Radic Biol Med. 2019;134:604–616.
12. Wang J, Shumamugam A, Markand S, Zorrilla E, Ganapathy V, Smith SB. Sigma 1 receptor regulates the oxidative stress response in primary retinal Müller glial cells via NRF2 signaling and system xc(-), the Na(+)-dependent glutamate-cystine exchanger. Free Radic Biol Med. 2015;86:25–36.
13. Bucolo C, Draghi F, Lin RR, Reddy VN. Sigma receptor ligands protect human retinal cells against oxidative stress. Neuroreport. 2006;17:287–291.
14. Yu DY, Cringle SJ. Retinal degeneration and local oxygen metabolism. Exp Eye Res. 2005;80:745–751.
15. Campochiaro PA, Mir TA. The mechanism of cone cell death in retinitis pigmentosa. Prog Retin Eye Res. 2018;62:24–37.
16. Wang J, Saul A, Roon P, Smith SB. Activation of the molecular chaperone, sigma 1 receptor, preserves cone function in a murine model of inherited retinal degeneration. Proc Natl Acad Sci U S A. 2016;113:E3764–E3772.
17. Gargioli C, Terzibasi E, Mazzoni F, Strettoi E. Retinal organization in the retinal degeneration 10 (rd10) mutant mouse: a morphological and ERG study. J Comp Neurol. 2007;500:222–238.
18. Chang B, Hurd R, Wang J, Nishina P. Survey of common eye diseases in laboratory mouse strains. Invest Ophthalmol Vis Sci. 2013;54:4974–4981.
19. Naveen S, Zhao J, Wang J, et al. Hyperhomocysteinemia-induced death of retinal ganglion cells: the role of Müller glial cells and NRF2. Redox Biol. 2019;24:101199.
20. Prusky GT, Alam NM, Beckman S, Douglas RM. Rapid quantification of adult and developing mouse spatial vision using a virtual optomotor system. Invest Ophthalmol Vis Sci. 2004;45:4611–4616.
21. Saul AB, Still AE. Multifocal electroretinography in the presence of temporal and spatial correlations and eye movements. Vision. 2017;1:3.
22. Mezú-Ndubuisi OJ, Taylor LK, Schoephoerster JA. Simultaneous confocal angiography and spectral domain optical coherence tomography correlate retinal thickness changes to vascular abnormalities in an in vivo mouse model of retinopathy of prematurity. J Ophthalmol. 2017;2017:9620876.
23. Saszik SM, Robson JG, Frishman LJ. The scotopic threshold response of the dark-adapted electroretinogram of the mouse. J Physiol. 2002;543:899–916.
24. Reichenbach A, Bringmann A. New functions of Müller cells. Glia. 2013;61:651–678.
25. García-Delgado AB, Valdés-Sánchez L, Calado SM, Díaz-Corralles FJ, Bhattacharya SS. Rasagiline delays retinal degeneration in a mouse model of retinitis pigmentosa via modulation of Bax/Bcl-2 expression. CNS Neurosci Ther. 2018;24:448–455.
26. Hanif AM, Lawson EC, Prunty M, et al. Neuroprotective effects of voluntary exercise in an inherited retinal degeneration mouse model. Invest Ophthalmol Vis Sci. 2015;56:6839–6846.
27. Barone I, Novelli E, Strettoi E. Long-term preservation of cone photoreceptors and visual acuity in rd10 mutant mice exposed to continuous environmental enrichment. Mol Vis. 2014;20:1545–1556.
28. Piano I, Novelli E, Gasco P, Ghiodini R, Strettoi E, Gargioli C. Cone survival and preservation of visual acuity in an animal model of retinal degeneration. Eur J Neurosci. 2013;37:1853–1862.
29. Sreekumar PG, Ding Y, Ryan SJ, Kannan R, Hinton DR. Regulation of thioredoxin by ceramide in retinal pigment epithelial cells. Exp Eye Res. 2009;88:410–417.
30. Lee SY, Usui S, Zafar AB, et al. N-Acetylcysteine promotes long-term survival of cones in a model of retinitis pigmentosa. J Cell Physiol. 2011;226:1843–1849.
31. Zhao J, Mysona BA, Wang J, Gonsalvez GB, Smith SB, Bollinger KE. Sigma 1 receptor regulates ERK activation and promotes survival of optic nerve head astrocytes. PLoS One. 2017;12:e0184421.
32. Zhao J, Mysona BA, Qureshi A, et al. (−)-Pentazocine reduces NMDA-induced murine retinal ganglion cell death through a σR1-dependent mechanism. Invest Ophthalmol Vis Sci. 2016;57:453–461.
33. Mueller BH II, Park Y, Ma HY, et al. Sigma-1 receptor stimulation protects retinal ganglion cells from ischemia-like insult through the activation of extracellular-signal-regulated kinases 1/2. Exp Eye Res. 2014;128:156–169.
34. Ellis DZ, Li L, Park Y, He S, Mueller B, Yorio T. Sigma-1 receptor regulates mitochondrial function in glucose- and oxygen-deprived retinal ganglion cells. Invest Ophthalmol Vis Sci. 2017;58:2755–2764.
35. Tchchedre TK, Huang RQ, Dibas A, Krishnamoorthy RR, Dillon GH, Yorio T. Sigma-1 receptor regulation of voltage-gated calcium channels involves a direct interaction. Invest Ophthalmol Vis Sci. 2008;49:4993–5002.
36. Mueller BH II, Park Y, Daudt DR III, et al. Sigma-1 receptor stimulation attenuates calcium influx through activated L-type voltage gated calcium channels in purified retinal ganglion cells. Exp Eye Res. 2013;107:21–31.
37. Wang H, Fu Y, Liu X, et al. Role of the sigma-1 receptor chaperone in rod and cone photoreceptor degenerations in a...
38. Kalloniatis M, Nivison-Smith L, Chua J, Acosta ML, Fletcher EL. Using the rd1 mouse to understand functional and anatomical retinal remodelling and treatment implications in retinitis pigmentosa: a review. *Exp Eye Res*. 2016;150:106–121.

39. Sakami S, Maeda T, Bereta G, et al. Probing mechanisms of photoreceptor degeneration in a new mouse model of the common form of autosomal dominant retinitis pigmentosa due to P23H opsin mutations. *J Biol Chem*. 2011;286:10551–10567.

40. Ritschel WA, Hoffmann KA, Willig JL, Frederick KA, Wetzelsberger N. The effect of age on the pharmacokinetics of pentazocine. *Methods Find Exp Clin Pharmacol*. 1986;8:497–503.