Tauroursodeoxycholic Acid Preservation of Photoreceptor Structure and Function in the rd10 Mouse through Postnatal Day 30

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Purpose. Retinitis pigmentosa (RP) is a progressive neurodegenerative disease resulting in blindness for which there is no current treatment. Although the members of the family of RP diseases differ in etiology, their outcomes are the same: apoptosis of rods and then by cones. Recently, the bile acid tauroursodeoxycholic acid (TUDCA) has been shown to have antiapoptotic properties in neurodegenerative diseases, including those of the retina. In this study the authors examined the efficacy of TUDCA on preserving rod and cone function and morphology at postnatal day 30 (P30) in the rd10 mouse, a model of RP.

Methods. Wild-type C57BL/6J and rd10 mice were systemically injected with TUDCA (500 mg/kg) every 3 days from P6 to P30 and were compared with vehicle (0.15 M NaHCO3). At P30, retinal function was measured with electroretinography, and morphologic preservation of the rods and cones was assessed with immunohistochemistry.

Results. Dark-adapted electroretinographic (ERG) responses were twofold greater in rd10 mice treated with TUDCA than with vehicle, likewise light-adapted responses were twofold larger in TUDCA-treated mice than in controls at the brightest ERG flash intensities. TUDCA-treated rd10 retinas had fivefold more photoreceptors than vehicle-treated retinas. TUDCA treatments did not alter retinal function or morphology of wild-type mice when administered to age-matched mice.

Conclusions. TUDCA is efficacious and safe in preserving vision in the rd10 mouse model of RP when treated between P6 and P30. At P30, a developmental stage at which nearly all rods are absent in the rd10 mouse model of RP, TUDCA treatment preserved rod and cone function and greatly preserved overall photoreceptor numbers. (Invest Ophthalmol Vis Sci. 2008;49:2148–2155) DOI:10.1167/iovs.07-1012

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The present study tests the hypothesis that TUDCA preserves rods and cones to P30, the stage at which photoreceptor cell loss peaks. At this stage of degeneration in the rd10 model, most rods have degenerated, and only some cones remain. This stage of degeneration represents the end stage of RP for most patients because it is estimated that only 0.5% of RP patients develop complete blindness (no light perception). Thus, these experiments test the efficacy of TUDCA at a critical stage of degeneration. To test this hypothesis, rd10 mice were treated with TUDCA or vehicle from P6 to P30, and rod and cone function and morphology were assessed by electroretinography, histology, immunohistochemistry, and TUNEL labeling. Although TUDCA and UDCA have been shown to be very well tolerated in animals and humans and have been used to administer a series of increasingly intense light flashes ranging from 3.0 to 2.1 log cd s/m². Dark-adapted ERG recordings were performed using image software (Plus 5.0; ImagePro, Silver Spring, MD). Three sections were counted for each of the four retinal areas in each eye. These values were then averaged, and analysis of variance (ANOVA; SPSS 8.0; SPSS, Inc., Chicago, IL) was performed.

Electroretinographic Methods
Mice were dark adapted overnight, and electroretinography was performed at P30 with a commercial recording system (UTAS 3000; LKC Technologies, Gaithersburg, MD). After anesthesia induction (ketamine, 80 mg/kg; xylazine, 16 mg/kg), the cornea was anesthetized (1% tetracaine), and the pupils were dilated (1% tropicamide, 1% cyclopentolate). Body temperature was maintained at 37°C by placing the mice on a heating pad inside a Faraday cage. The active electrode (1% methylcellulose) and the reference and ground electrodes were placed in the cheek and tail, respectively. A desktop Ganzfeld was used to administer a series of increasingly intense light flashes ranging from –3.0 to 2.1 log cd s/m². Dark-adapted ERG recordings were averaged over 5 to 10 separate flashes per light intensity, with the interstimulus time increasing from 10 to 60 seconds as the flash intensity increased. Animals were then light adapted for 10 minutes using a steady background light (30 cd/m²). Cone-isolating responses were recorded to a seven-step intensity series (−0.82 to 1.88 log cd s/m²) presented at 3 Hz in the presence of the same plastic sections and retina regions described, three independently at the nape of the neck.

Histologic Methods
After ERG recordings, deeply anesthetized mice were killed by cervical dislocation. Eyes were immediately enucleated. Fixative was injected into the superior limbus to mark orientation and to aid in the rapid fixation of the retina. All left eyes were immersion fixed in 4% paraformaldehyde for 30 minutes with TUNEL labeling, and all right eyes were fixed overnight in 2.5% glutaraldehyde/2% paraformaldehyde for light microscopy. After fixation, right eyes were dehydrated through a graded alcohol series, infiltrated with propylene oxide, and embedded in resin (Epon 812/Des 756; Electron Microscopy Sciences, Hatfield, PA). Sections (0.5 µm) bisecting the optic disc superiorly to inferiorly were then cut on an ultramicrotome (UltraCut; Leica, Chicago) using a histodiamond knife and collected on glass slides. Slides were stained with 1% aqueous toluidine blue (Sigma, St. Louis, MO). Left eyes were processed through a graded series of alcohols and embedded in paraffin. Sections (5-µm thick) were cut on a rotary microtome, bisecting the optic disc superiorly to inferiorly. Paraffin sections were used for cone opsin immunolabeling and TUNEL stain.

Materials and Methods
Animals
All animal procedures were approved by the Institutional Animal Care and Use Committee at the Atlanta VA Medical Center and conform to the standards of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. To establish a breeding colony, Pde6brd10 mice on a C57BL/6J background (or rd10) were obtained from Jackson Laboratories (Bar Harbor, ME). All mice were housed under controlled lighting conditions on a 14-hour light/10-hour dark cycle (25–200 lux). Each litter was randomly divided at P6 to receive TUDCA treatment (500 mg/kg; Calbiochem, San Diego, CA; n = 17) or vehicle (0.15 M NaHCO₃, 1 mL/kg, pH 7.0; Sigma, St. Louis, MO; n = 14). A breeding colony of WT C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) received the same treatments as the rd10 mice (n = 5 for TUDCA, n = 2 for vehicle treatment) starting at P6. TUDCA and vehicle solutions were made up fresh before every injection and were pH adjusted to 7.4.25 Mice were weighed and injected once every 3 days beginning at P6 and ending at P30, resulting in eight total administrations per animal for the treated and vehicle groups. All injections were made subcutaneously at the nape of the neck.

Histology
Histologic methods were performed on the treated and vehicle groups. All injections were made subcutaneously at the nape of the neck. The mice were then killed at P30 by cervical dislocation. Eyes were immediately enucleated. Fixative was injected into the superior limbus to mark orientation and to aid in the rapid fixation of the retina. All left eyes were immersion fixed in 4% paraformaldehyde for 30 minutes and all right eyes were fixed overnight in 2.5% glutaraldehyde/2% paraformaldehyde for light microscopy. After fixation, right eyes were dehydrated through a graded alcohol series, infiltrated with propylene oxide, and embedded in resin (Epon 812/Des 756; Electron Microscopy Sciences, Hatfield, PA). Sections (0.5 µm) bisecting the optic disc superiorly to inferiorly were then cut on an ultramicrotome (UltraCut; Leica, Chicago) using a histodiamond knife and collected on glass slides. Slides were stained with 1% aqueous toluidine blue (Sigma, St. Louis, MO). Left eyes were processed through a graded series of alcohols and embedded in paraffin. Sections (5-µm thick) were cut on a rotary microtome, bisecting the optic disc superiorly to inferiorly. Paraffin sections were used for cone opsin immunolabeling and TUNEL stain.

Total Photoreceptor Cell Counts
Plastic sections were analyzed using light microscopy (DMRB; Leica, Bannockburn, IL) to determine photoreceptor cell counts. Four retinal regions in the vertical meridian (0.5 mm in width) were photographed at 20× magnification. Locations in reference to the optic nerve head were 2.0 to 1.5 mm superior, 1.0 to 0.5 mm superior, 0.5 to 1.0 mm inferior, and 1.5 to 2.0 mm inferior. Photoreceptor nuclei counts were performed using image software (Plus 5.0; ImagePro, Silver Spring, MD). Three sections were counted for each of the four retinal areas in each eye. These values were then averaged, and analysis of variance (ANOVA; SPSS 8.0; SPSS, Inc., Chicago, IL) was performed.

Cone Photoreceptor Labeling
Paraffin sections were deparaffinized with xylene, followed by a graded series of alcohol rinses. After an initial blocking step with 5% goat serum (Chemicon, Temecula, CA) made with blocking buffer (Superblock; Pierce, Rockford, IL), sections were incubated in antiopsin green/red and blue (1:500; Chemicon) for 48 hours at room temperature. The primary antibody was visualized by labeling with goat anti-rabbit IgG secondary antibody (1:500; AlexaFluor 488; Abcam, Cambridge, MA) for 1 hour, after optimization with a titrated series for both the primary and the secondary antibody. Each slide contained a negative control by eliminating primary antibody from one section per slide. Sections were then coverslipped with an aqueous mounting medium (Gel/Mount; Biomeda, Foster City, CA). Digital micrographs were captured of images at 20× magnification using a confocal microscope. All micrographs were taken from sections stained the same day with the same camera settings.

Cone Photoreceptor Nuclei Counts
Rod and cone photoreceptor nuclei have differently shaped heterochromatin such that rods have a dense central clump and cones have irregularly shaped heterochromatin that can appear as one to three clumps in tissue sections. Thus, cone photoreceptor nuclei were quantified by counting all photoreceptor nuclei with two or more clumps of heterochromatin in toluidine blue-stained plastic sections (see Fig. 5 for examples of cone vs. rod photoreceptor nuclei). Using the same plastic sections and retina regions described, three independently at the nape of the neck.
The same doses. of RP and those testing TUDCA in age-matched WT mice with
dent observers counted cone nuclei at 20×. Different treatment
groups and strains were compared using ANOVA (SPSS 8.0; SPSS).

**TUNEL Labeling**

Paraffin sections were stained by terminal deoxynucleotidyl trans-
ferase-mediated 2′ deoxyuridine 5′ triphosphate-biotin nick end label-
ing (TUNEL) using a TUNEL kit (DeadEnd Fluorometric; Promega,
Madison, WI) according to the manufacturer’s kit instructions and
were counterstained with propidium iodide.25 Images of TUNEL-
stained sections were captured by computer-aided confocal micros-
copy, and photoreceptor nuclei were counted per field at 20× magni-

**RESULTS**

Results are broken into two broad sets of experiments, those
dealing with the efficacy of TUDCA in the rd10 mouse model of
RP and those testing TUDCA in age-matched WT mice with
the same doses.

**Efficacy of TUDCA in the rd10 Mouse**

**TUDCA Preserves Retinal Function at P30.** To deter-
mine whether TUDCA treatments preserved rod and cone
function in rd10 mice, we performed dark- and light-adapted
electroretinography at P30. We found that TUDCA treatment
significantly preserved both rod and cone function in rd10
mice. Comparing TUDCA- and vehicle-treated mice, dark-
adapted ERGs from TUDCA-treated animals showed signifi-
cantly larger waveforms (Fig. 1A). At the highest flash intensity,
the a-wave amplitude was approximately five times larger in
TUDCA-treated mice than in the control treatment groups,
whereas the b-wave amplitude was twice as large as were the
other treatment groups (Fig. 1). Furthermore, mice treated
with TUDCA showed significant preservation of mean a- and
b-wave amplitudes (Fig. 1B) over a range of ERG flash intensi-
ties, as assessed by repeated-measures ANOVA (a-wave: F(4,
88) = 2.66, P = 0.038; b-wave: F(4, 96) = 2.55, P = 0). We conclude that TUDCA is efficacious in
preventing the loss of ERG signals in response to a range of scotopic flashes.

**FIGURE 1.** Dark-adapted retinal function in rd10 mice after TUDCA treatment at P30. ERG recordings show-
ing rod-dominated retinal function in rd10 mice treated with TUDCA or vehicle. (A) Representative ERG
waves to a series of flash intensities from a TUDCA- or vehicle-treated mouse. At every light level, the
TUDCA-treated mice had a larger response than the vehicle-treated rd10 mouse. The a-wave is much larger in the
TUDCA-treated animals. (B) Plot of average ERG amplitude (±SEM) for a- and b-waves from the two treat-
groups. TUDCA (n = 16) and vehicle (n = 10). TUDCA-treated animals had significantly greater a- and
b-wave amplitudes than the vehicle
group (n = 10) (F(6,138) = 6.364, P < 0.001). The a-wave amplitude is not statistically significantly different between the groups
(repeated-measures ANOVA; F(6, 120) = 2.075, P = 0.061). We conclude that TUDCA is efficacious in preserving cone-mediated electrical responses in the retina.
significant differences were detected between the treatment groups for the small light-adapted a-wave (repeated-measures ANOVA F(6, 120) = 2.075; P = 0.061).

Preservation of Photoreceptor Nuclei: Rod and Cone Photoreceptors. To determine whether TUDCA treatments preserved photoreceptors, we examined photoreceptor structure and numbers in rd10 mice. TUDCA treatments significantly preserved photoreceptor numbers and the inner and outer segments in the rd10 mice (Fig. 3C) compared with vehicle-treated controls (Fig. 3D). The number of photoreceptor nuclei in a 0.5-mm field at four locations from inferior to superior in TUDCA-treated rd10 mice at P30 showed significant preservation of photoreceptors (Fig. 3F; ANOVA F(3, 87) = 3.013; P < 0.043). TUDCA-treated retinae have significantly more photoreceptors across all areas sampled than vehicle-treated mice (ANOVA F(3, 87) = 3.013, P = 0.034). We conclude that TUDCA treatment is efficacious in preventing or slowing photoreceptor degeneration up to P30 in the rd10 mouse model of RP.

Preservation of Photoreceptor Nuclei: Cone Photoreceptors. To identify the population of cone photoreceptors, paraffin sections were labeled with anticone opsin. Immunolabeled WT retinae showed distinct labeling of cone photoreceptor outer segments (Fig. 4A), as previously reported.37,38 The labeled cone outer segment of WT mice always appeared long and had a distinct tip (Fig. 4A). In TUDCA-treated rd10 retinae, distinct cone labeling was still visible (Figs. 4B, 4C). Labeled segments correlated with the extent of preservation. Retinas with the most preservation of photoreceptors and longer outer segments had the most cone labeling (Fig. 4B). In rd10 mice with less preservation from the TUDCA treatments, the cone outer segments were smaller and the cone opsin labeling appeared as small round structures or punctuate in the region of the outer segments (Fig. 4C). In vehicle-treated rd10 mice, the outer segments were not visible, and cone opsin labeling was seen as only small punctuate labeling (Figs. 4D, 4E). Although some variability in cone opsin labeling was present in all treatment groups, TUDCA-treated retinae more frequently presented with a continuous line of outer segments and longer cone outer segments compared with vehicle-treated retinae (Figs. 4B, 4C vs. 4D, 4E).

Cones nuclei were quantified by counting in toluidine blue plastic-embedded sections. All photoreceptor nuclei with two or more clumps of heterochromatin were classified as cones.39 Figure 5A (arrows) shows cone nuclei in a WT retina, whereas Figure 5B (arrows) shows cone nuclei identified in an rd10 TUDCA-treated retina. Figure 5C shows that average cone nuclei counts across the retinal areas from TUDCA- and vehicle-treated rd10 retinae were similar, 2.92 ± 0.27 versus 4.08 ± 0.49 cones/500 μm (TUDCA, n = 14; vehicle, n = 11; ANOVA F(3, 69) = 1.25, P = 0.298). In addition, no differences in cone counts in WT retinae were found between treatment groups (Student’s t-test, P = 0.93). WT mice had 10.5 ± 1.1 nuclei/500 μm (significantly more cone nuclei than rd10 mice), as expected (Student’s t-test, P < 0.001).

Apoptosis in the rd10 Retina at P30

We have previously shown that rd10 retinas at P18 have numerous TUNEL-positive nuclei, but TUDCA-treated retinae have few apoptotic nuclei.25,26 Thus, at P30, we expect to see fewer TUNEL-positive nuclei in vehicle-treated retinae because of the prior massive loss of photoreceptors before this stage. In contrast, because of the delay in degeneration produced by TUDCA treatments, we expect to observe some apoptotic nuclei in TUDCA-treated retinae.

Figure 3. Retinal morphology after TUDCA treatment at P30. Retinal micrographs from P30 WT C57BL/6 mice treated with TUDCA (A) or vehicle (B). All retinal layers are intact, and the photoreceptor layers are normal. Qualitatively, (A) and (B) show no changes between treatment groups in any of the layers of the retina of WT mice. This finding suggests that TUDCA treatment at the given dose and for the indicated duration is safe. Retinal micrographs from rd10 mice treated with TUDCA (C) or vehicle (D). The photoreceptor layer has been reduced to approximately 1 row of nuclei in the vehicle-treated mouse (D), whereas the TUDCA-treated retina retains three to four rows of nuclei (C). (G) and (D) show clear differences in the thicknesses of the ONL, outer segments (OS), and inner segments (IS), with TUDCA treatment demonstrating an efficacious delay of retinal degeneration. (E, F) Plots of the total number of photoreceptors at each retinal location from WT and rd10 mice with reference to the optic nerve. Inset: schematic diagram of the retina and optic nerve. Each shaded square indicates a sampling region, as indicated on the graphs. (E) Photoreceptor cell counts in the WT mice from the two treatment groups show no differences across the retina. (n = 5 TUDCA, n = 2 vehicle. ANOVA F(3, 12) = 0.397, P = 0.757) (F). Photoreceptor cell counts from rd10 mice treated with TUDCA (n = 17) or vehicle (n = 14). The TUDCA-treated mice have significantly more photoreceptors across all areas sampled than vehicle-treated mice (ANOVA F(3, 87) = 3.013, P = 0.034). We conclude that TUDCA treatment is efficacious in preventing or slowing photoreceptor degeneration up to P30 in the rd10 mouse model of RP.
Our results show that TUDCA-treated rd10 retinas and vehicle-treated retinas had similar numbers of apoptotic nuclei. The average number of TUNEL-positive nuclei in TUDCA-treated retinas per microscope field was 6.8 ± 1.1 (mean ± SEM; n = 10) compared with 11.2 ± 2.9 (n = 3) in vehicle-treated retinas. These differences were not significant (Student’s t-test, P = 0.29).

TUDCA Studies in WT Mice

**TUDCA Treatment Does Not Affect Normal Retinal Function.** To test whether TUDCA has adverse affects on normal retinal function when given early in development, we recorded electroreginograms from TUDCA- and vehicle-treated mice. Figure 6A shows the typical dark-adapted ERG waveform to a series of flash intensities. Waveforms from TUDCA-treated retinas were larger than those from vehicle-treated retinas. The mean dark-adapted ERG amplitudes for the a- and b-waves were nearly identical for the TUDCA- and vehicle-treated mice (Figs. 6B, 6C; repeated-measures ANOVA, F(4, 20) = 2.020, P = 0.130, and F(4, 20) = 3.110, P = 0.138, respectively; n = 5 for TUDCA-treated and n = 2 for vehicle-treated). Light-adapted b-wave amplitudes were also similar between treatment groups (Fig. 6D; repeated-measures ANOVA, F(6, 30) = 0.532, P = 0.779).

**TUDCA Treatment Does Not Affect Normal Retinal Morphology.** Retinal morphology appeared normal in WT mice from all treatment groups (Figs. 3A, 3B, 3E). The average number of photoreceptor nuclei was similar, with 795.4 ± 27.4 and 754.2 ± 46.9 nuclei/region in TUDCA-treated and vehicle-treated retinas, respectively. The number of nuclei...
across retinal areas was not significant between treatment groups in the WT mice (repeated-measures ANOVA, $F(3, 12) = 0.397, P = 0.757$; Fig. 3E).

**TUDCA Treatment Alters Body Weight in rd10 and WT Mice.** Animal weights were also compared across time with TUDCA versus vehicle treatment. TUDCA-treated WT mice were found to have significantly lower body weights than vehicle-injected mice (Fig. 7A; repeated-measures ANOVA, $F(5, 25) = 4.276, P = 0.006$). As treatments progressed, the TUDCA-treated mice gained less weight than did vehicle-treated mice. The largest differences were not reached until P24. TUDCA-treated rd10 mice also showed a significant decrease in body weight over the treatment period (Fig. 7B; repeated-measures ANOVA, $F(7, 245) = 10.973, P < 0.001$). In rd10 mice, the effect of TUDCA on body weight was apparent by P9, much sooner than in the WT mice.

**FIGURE 6.** Studies in WT mice. Retinal function measurements were recorded from WT C57BL/6J mice treated with TUDCA or vehicle from P6 to P30. (A) Representative dark-adapted ERG waveforms for WT TUDCA- and vehicle-treated mice. Dark-adapted a-wave (B), dark-adapted b-wave (C), or light-adapted b-wave (D) amplitudes from WT mice treated with TUDCA ($n = 5$) or vehicle ($n = 2$) show no differences in amplitude or timing across different flash intensities (repeated-measures ANOVA, $F(4, 20) = 2.020, P = 0.130; F(4, 20) = 3.110, P = 0.138; F(6, 30) = 0.532, P = 0.779$; respectively). We concluded that TUDCA treatment from P6 to P30 has no deleterious effect on retinal function, as measured with the ERG.

**FIGURE 7.** Body weights in WT and rd10 mice after TUDCA treatments from P6 to P30. (A) WT mice treated with TUDCA ($n = 5$) showed a reduction in body weight compared with vehicle ($n = 2$; repeated-measures ANOVA, $F(5, 25) = 4.276, P = 0.006$). (B) TUDCA-treatment of rd10 mice ($n = 24$) resulted in significantly lower body weight than vehicle ($n = 13$) from P15 to P27 (repeated-measures ANOVA, $F(7, 245) = 10.973, P < 0.001$). We conclude that TUDCA treatments suppressed body weight in the WT and rd10 mice. Symbols represent mean ± SD.


**DISCUSSION**

The present study demonstrates that TUDCA treatment, through the advanced degenerative time point of P30 in the rd10 mouse, is remarkably effective in sustaining photoreceptor cells and their function. We have previously shown successful preservation of rod function and structure through P18 in this model.25 Though slightly delayed compared with that in rd1 mice, retinal degeneration in rd10 mice is rapid and aggressive. In untreated rd10 mice, the outer nuclear layer (ONL) is reduced to a single nuclear layer by P30.25–27 Functionally, the dark-adapted a-wave is largely undetectable (only 3% of WT response), and the b-wave is greatly diminished (14% of WT response).25–27 At this stage, rods are almost completely absent and cones are degenerating. This study shows that TUDCA treatments starting at P6 sustain retinal function and morphology through this critical stage from 30% (dark-adapted a-wave) to 45% (light-adapted and dark-adapted b-waves and photoreceptor numbers) of WT responses. These results show significant preservation of the retina by a systemic agent at this stage of degeneration.

A pan-retinal preservation of retinal function and photoreceptor nuclei was found in the TUDCA-treated rd10 mice at P30. Rod function and rod photoreceptors were significantly greater in TUDCA-treated rd10 mice (Figs. 1–3). In addition, the number of total photoreceptor nuclei was fivefold greater in TUDCA-treated mice than in vehicle-treated mice (Fig. 5).

Interestingly, TUDCA treatments appeared to preserve cone outer segment morphology compared with vehicle treatment (Fig. 4), which correlates well with the increased amplitudes in the light-adapted TUDCA-treated retinas (Fig. 2). However, no differences were found in the number of cone nuclei between the treatment groups (Fig. 5). These data may suggest that TUDCA causes an overall increase in preservation of the photoreceptors. Given that cone degeneration occurs secondarily in this model, the significant preservation of rods from TUDCA treatments may sustain the cones. A later time point may be needed to exclusively evaluate the effect of TUDCA treatments on cone preservation. However, because humans rely most on cone vision, the finding of greater cone function and healthier cone outer segments after TUDCA treatments suggests that this drug might be useful for preserving cone vision in people.

The similarity in TUNEL labeling between TUDCA- and vehicle-treated rd10 retinas may suggest that TUDCA is delaying the death of photoreceptors in the rd10 model. In our analysis of rd10 retinas at P18, vehicle-treated retinas showed a vastly greater number of TUNEL-positive nuclei compared with TUDCA-treated retinas.25 At P30, as might be expected, the vehicle-treated rd10 retinas do not have many nuclei remaining to undergo apoptosis. Conversely, the TUDCA-treated retinas have many remaining nuclei, some of which are apoptotic. WT mice showed no significant differences between the treatment groups.

Similar to other studies that showed TUDCA to be well tolerated in adult animals,8–12 we found no evidence at the functional or morphologic level that TUDCA itself had any significant adverse effects on the retina in WT mice when given from P6 to P30. However, TUDCA treatments did produce a decrease in body weight in WT and rd10 mice (Fig. 7). The mean body weight of TUDCA-treated WT mice dropped 22%, whereas weights in TUDCA-treated rd10 mice decreased 23%. These values are slightly below the 25% loss of body weight that is considered an end point criterion by our Institutional Animal Care and Use Committee. Other studies have not reported a reduction in body weight after TUDCA treatment, perhaps because other animal models were treated during adulthood8–12 and not early postnatal development, as in this study. Future studies will determine whether a different vehicle would have less effect on body weight. Nevertheless, the lack of toxic effects on the retina complement other studies that showed the safety of UDCA treatment in human patients with liver disease,32,39 of TUDCA treatment in rodent models of Huntington disease,8,9 and of TUDCA treatment in rodent models of stroke.11,12 If TUDCA continues to show effects on body weight in young animals, drug delivery approaches could be pursued to administer TUDCA exclusively to the retina.

Comparing the preservation of the rd10 retina between P18 and P30, TUDCA appears to delay degeneration in rd10 mice by approximately 12 days, or approximately 35%, over the course of the degeneration period. Because it has been reported that different species have surprisingly similar rates of degeneration based on maximal life expectancy30 and that typical RP patients have a linear rate of retinal function loss,41 a prediction of TUDCA ability to preserve vision in RP patients can be made. TUDCA might preserve retinal function for approximately 18 years in a patient whose photoreceptor loss began in his or her 20s and ended in his or her 70s. Coupling this possibility of efficacy with the demonstrated lack of toxicity of hydrophilic bile acids in several animal models8–12 and humans8,9,39 suggests that hydrophilic bile acids may be relevant to the ophthalmic clinic.

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