Tauroursodeoxycholic acid preserves photoreceptor structure and function in the rd10 mouse through post-natal day 30

M. Joe Phillips, Atlanta VA Medical Center; Emory University
Tiffany Austin Walker, Atlanta VA Medical Center
Hee-young Choi, Atlanta VA Medical Center
Amanda E. Faulkner, Atlanta VA Medical Center
Moon K. Kim, Atlanta VA Medical Center
Sheree Sidney, Emory University
Amber Boyd, Emory University
John Nickerson, Emory University
Jeffrey Boatright, Emory University
Machelle Pardue, Emory University

Journal Title: Investigative Ophthalmology & Visual Science
Volume: Volume 49, Number 5
Publisher: Association for Research in Vision and Ophthalmology (ARVO) | 2008-05, Pages 2148-2155
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1167/iovs.07-1012
Permanent URL: http://pid.emory.edu/ark:/25593/fj599

Final published version: http://www.iovs.org/content/49/5/2148.long

Copyright information:
© Association for Research in Vision & Ophthalmology

Accessed April 16, 2019 10:16 PM EDT
Tauroursodeoxycholic Acid Preservation of Photoreceptor Structure and Function in the rd10 Mouse through Postnatal Day 30

M. Joe Phillips,1,2 Tiffany A. Walker,1 Hee-Young Cboi,1,5 Amanda E. Faulkner,1 Moon K. Kim,1 Sheree S. Sidney,2 Amber P. Boyd,2 John M. Nickerson,2 Jeffrey H. Boatright,2 and Macbelle T. Pardue1,2

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) for 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 Ophthalmal Vis Sci. 2008;49: 2148–2155) DOI:10.1167/iovs.07-1012

From the 1Rehabilitation Research and Development Center, Atlanta VA Medical Center and the 2Department of Ophthalmology, Emory School of Medicine, Atlanta, Georgia; and the 3Department of Ophthalmology, Pusan National University, Pusan, Republic of Korea.

Supported by Department of Veterans Affairs, Foundation Fighting Blindness, Research to Prevent Blindness, Knights Templar Education Foundation, and the National Institutes of Health (Grants R01 EY014026, P50 EY06560, R01 EY012514, 5P50 AT000609, R24EY017045, and R01 EY016470). Submitted for publication August 6, 2007; revised October 29 and December 19, 2007; accepted March 14, 2008.

Disclosure: M.J. Phillips, None; T.A. Walker, None; H.-Y. Choi, None; A.E. Faulkner, None; M.K. Kim, None; S.S. Sidney, None; A.P. Boyd, None; J.M. Nickerson, P; J.H. Boatright, P; M.T. Pardue, P

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Machelle T. Pardue, Rehabilitation Research and Development Center, Research Service (151 Oph), Atlanta VA Medical Center, 1670 Clairmont Road, Decatur, GA 30033; mpardue@emory.edu.

R etinitis pigmentosa (RP) is a family of diseases characterized by night blindness and loss of peripheral vision followed by progressive loss of central vision. RP affects approximately 50,000 to 100,000 people in the United States and approximately 1.5 million people worldwide.1 Currently, approximately 200 genes have been identified that cause RP or related retinal diseases (http://www.sph.uth.tmc.edu/ Retnet/), showing the genetic diversity of this group of diseases. However, regardless of the mutation, the final common pathway is programmed photoreceptor cell death, or apoptosis.2

Bear bile has a history of positive effects in ancient Chinese medicine.3,4 but Western medicine has only recently investigated the antiapoptotic effects of bile acids, including tauroursodeoxycholic acid (TUDCA), the taurine conjugate of ursodeoxycholic acid (UDCA). The bulk of the therapeutic effects of TUDCA and UDCA have been shown in the treatment of a wide range of liver and gall bladder diseases.5–7 More recently, TUDCA has been shown to be neuroprotective in animal models of Huntington disease,8,9 Parkinson disease,10 and acute stroke.11,12 TUDCA and UDCA are antiapoptotic agents, and though the exact mechanisms of apoptosis prevention are still under investigation, several key signaling pathways have been implicated. TUDCA modulates cell cycle effector genes, including cyclin D1 and P53.5,13–15 Phosphotidyl-inositol-3-kinase,18–20 mitogen-activated protein kinase, and ERK/Akt21 pathway activation have all been implicated in TUDCA administration. TUDCA and UDCA also stabilize the mitochondrial membrane. They directly inhibit mitochondrial permeability transition, inhibit cytosolic Bax translocation, and suppress mitochondrial release of cytochrome c.22–23 Bile acids block reactive oxygen intermediate production22–24 and may themselves be antioxidants.25 They block caspase activation, including caspase-3,12,22,25,25 and also prevent inactivation of the nuclear enzyme poly(ADP-ribose)polymerase.12,22,25

Because of the prominent role of apoptosis in retinal degenerative disease, TUDCA was tested in the Pde6brd10 (rd10) mouse. The rd10 mouse has a missense point mutation in exon 13 of the β-subunit of the rod cGMP phosphodiesterase (β-PDE).25–27 Thus, the rd10 mouse is similar to the popular Pde6brd1 or rd1 mouse, which also shares a mutation in β-PDE gene.28–29 The rate of degeneration in the rd10 mice is similar to that in the rd1 mutant model with a delayed onset; both have rapid rod degeneration followed by cone degeneration.25–27,30

In a previous study, TUDCA treatment of rd10 mice showed significant preservation of photoreceptor function and morphology at postnatal day (P) 18.25 In the rd10 mice, maximal retinal cell loss on a C57BL/6j background occurs at approximately P28.30 Rods degenerate faster than cones in rd10 mice, with rod function decreased by approximately 70% under dark-adapted conditions, whereas cone-isolating, light-adapted conditions show a 50% decline at P30.30 Furthermore, though rod degeneration appears nearly complete by P40 in rd10 mice,30 cones have been identified until 9 months of age.30

Copyright © Association for Research in Vision and Ophthalmology

2148
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 (UDCA has received US Food and Drug Administration approval), these studies have all been performed in adult animals. Thus, in this study, we also sought to determine whether the antipoptotic affects of TUDCA had an effect on early retinal development by treating age-matched C57BL/6J wild-type (WT) mice at the same time points. These studies demonstrate that TUDCA is effective in preserving photoreceptors in the rd10 mice at P30.

**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; 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. 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.

**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 consisted of a silver wire loop that was positioned on the cornea using 1% methylcellulose. Needle electrodes placed in the cheek and tail served as reference and ground, 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 light-adapting background. Light-adapted ERGs were averaged over 25 separate flashes per intensity.

**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 for 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/Deer Tissue; 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 20x 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**

Parafin 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; Alexa-Flour 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 20X 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 indepen-

**ERG a-wave amplitudes were measured from baseline to the first negative wave. The b-wave was measured from the trough of the a-wave to the peak of the first positive wave or, when the a-wave was not present, from baseline to the peak of the first positive wave. Implicit time measurements were made from flash onset to the trough or peak of the a- or b-wave, respectively. Statistical analysis across flash intensity and between treatment groups was conducted using repeated measures ANOVA tests (SPSS, Chicago, IL).**

---

**TUDCA Treatment in rd10 Mice at P30**

---
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 transferase-mediated 2′-deoxyuridine 5′-triphosphate-biotin nick end labeling (TUNEL) using a TUNEL kit (DeadEnd Fluorometric; Promega, Madison, WI) according to the manufacturer’s kit instructions and were counterstained with propidium iodide. Images of TUNEL-stained sections were captured by computer-aided confocal microscopy, and photoreceptor nuclei were counted per field at 20× magnification as described. For each field, ANOVA was used to compare the means between the treatment groups (SPSS 8.0; SPSS).

**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 determine whether TUDCA treatments preserved rod and cone function in rd10 mice, we performed dark- and light-adapted electrophysiology 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 significantly 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 intensities, as assessed by repeated-measures ANOVA (a-wave: F(4, 88) = 2.66, P = 0.038; b-wave: F(4, 96) = 2.55, P = 0.044; n = 16 for TUDCA-treated group; n = 10 for vehicle-treated group).

**Cone-mediated, light-adapted ERG waveforms were significantly larger in TUDCA-treated rd10 mice than in vehicle (Fig. 2A). In the representative waveforms shown in Figure 2A, the electroretinogram from the TUDCA-treated animal was clearly larger in amplitude at all intensities, and the b-wave threshold was measurable at −0.8 log cd·s/m² compared with −0.4 log cd·s/m² for the vehicle-treated animal.** When amplitude was plotted across flash intensity, it was seen that there are significant differences in b-wave amplitude between the treatment groups at the brightest flashes (n = 15 TUDCA; n = 10 vehicle; repeated-measures ANOVA F(6, 138) = 6.364; P < 0.001). No significant differences in b-wave amplitudes were measured as a function of flash intensity between the treatment groups. At every light level, the ERG waves in the TUDCA-treated group were significantly larger than in the vehicle control group (Fig. 2B). Comparing TUDCA- and vehicle-treated mice, the electroretinography at P30. The a-wave response 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 < 0.054). TUDCA-treated rd10 retinas contained an average of 353 ± 44 photoreceptor nuclei per region compared with only 68 ± 9 photoreceptor nuclei per 0.5-mm region in the vehicle-treated rd10 retina (mean ± SEM; Figs. 3C, 3D, 3F), a fivefold preservation.

Preservation of Photoreceptor Nuclei: Cone Photoreceptors. To identify the population of cone photoreceptors, paraffin sections were labeled with anticones opsin. Immunolabeled WT retinas showed distinct labeling of cone photoreceptor outer segments (Fig. 4A), as previously reported.35,36 The labeled cone outer segment of WT mice always appeared long and had a distinct tip (Fig. 4A). In TUDCA-treated rd10 retinas, 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. 4C). In TUDCA-treated rd10 mice, the outer segments were not visible, and cone opsin labeling was seen as only small punctuate labeling (Figs. 4D, 4E).

Apoptosis in the rd10 Retina at P30

We have previously shown that rd10 retinas at P18 have numerous TUNEL-positive nuclei, but TUDCA-treated retinas have fewer apoptotic nuclei.25,26 Thus, at P30, we expect to see fewer TUNEL-positive nuclei in vehicle-treated retinas 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 retinas.
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

**FIGURE 4.** Cone opsin labeling in WT and rd10 mice after TUDCA treatments at P30. Each micrograph is presented as a pair, with the DIC image on the right and the cone opsin labeling on the left. (A) Cone opsin immunohistochemistry in a WT retina showing distinct outer segments of cones. (B, C) TUDCA-treated rd10 retinas showing long cone outer segments, similar to WT retina (B) and shorter cone outer segments with more punctuate labeling (C). (D, E) Cone labeling in vehicle-treated rd10 retinas appeared as sparse punctuate labeling. Note that no outer segments are visible in the DIC images of the vehicle-treated mice. These results suggest that TUDCA treatments preserve cone outer segments.

**FIGURE 5.** Quantitative assessment of cones after TUDCA treatment at P30. Cone nuclei in WT (A) and rd10 (B) mice were counted based on heterochromatin pattern. Heterochromatin that formed two or more clumps was counted as a cone (arrows) according to the criteria established by Carter-Dawson and LaVail.35 This contrasts with rod nuclear staining, which appears dark and uniformly stained. (C) Cone counts for each 500-μm retinal region did not show any differences between treatment groups for rd10 or WT mice. However, WT mice had two to three times more cone nuclei per retinal area than rd10 mice (Student’s t-test, P < 0.001). Error bars indicate SEM.
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, $F(4, 20) = 2.020, P = 0.130$; $F(4, 20) = 3.110, P = 0.158$; $F(6, 30) = 0.532, P = 0.779$; 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 50% (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 adulthood9–12 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 expectancy90 and that typical RP patients have a linear rate of retinal function loss,81 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 humans8,2–39 suggests that hydrophilic bile acids may be relevant to the ophthalmic clinic.

**References**

14. Castro RE, Sola S, Ramalho RM, Steer CJ, Rodrigues CM. The bile acid tauroursodeoxycholic acid modulates phosphorylation and translocation of bad via phosphorylidesinositol 3-kinase in glutamate-