Oral Delivery of the $P2Y_{12}$ Receptor Antagonist Ticagrelor Prevents Loss of Photoreceptors in an $ABCA4^{-/-}$ Mouse Model of Retinal Degeneration

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Purpose. Accumulation of lysosomal waste is linked to neurodegeneration in multiple diseases, and pharmacologic enhancement of lysosomal activity is hypothesized to reduce pathology. An excessive accumulation of lysosomal-associated lipofuscin waste and an elevated lysosomal pH occur in retinal pigment epithelial cells of the $ABCA4^{-/-}$ mouse model of Stargardt's retinal degeneration. As treatment with the $P2Y_{12}$ receptor antagonist ticagrelor was previously shown to lower lysosomal pH and lipofuscin-like autofluorescence in these cells, we asked whether oral delivery of ticagrelor also prevented photoreceptor loss.

Methods. Moderate light exposure was used to accelerate photoreceptor loss in albino $ABCA4^{-/-}$ mice as compared to BALB/c controls. Ticagrelor (0.1%–0.15%) was added to mouse chow for between 1 and 10 months. Photoreceptor function was determined with electroretinograms, while cell survival was determined using optical coherence tomography and histology.

Results. Protection by ticagrelor was demonstrated functionally by using the electroretinogram, as ticagrelor-treated $ABCA4^{-/-}$ mice had increased a- and b-waves compared to untreated mice. Mice receiving ticagrelor treatment had a thicker outer nuclear layer, as measured with both optical coherence tomography and histologic sections. Ticagrelor decreased expression of *LAMP1,* implicating enhanced lysosomal function. No signs of retinal bleeding were observed after prolonged treatment with ticagrelor.

Conclusions. Oral treatment with ticagrelor protected photoreceptors in the $ABCA4^{-/-}$ mouse, which is consistent with enhanced lysosomal function. As mouse ticagrelor exposure levels were clinically relevant, the drug may be of benefit in preventing the loss of photoreceptors in Stargardt's disease and other neurodegenerations associated with lysosomal dysfunction.

Keywords: drug delivery, retinal dystrophy, protection, ABCA4, lysosome

Age-dependent neurodegenerations are frequently associated with excessive accumulation of oxidized lipid waste in lysosome-associated organelles.[1] The accumulation of improperly degraded lipid waste is particularly apparent in the lysosome-associated organelles of retinal pigmented epithelial (RPE) cells in some retinal degenerations.[2–4] As RPE cells provide a glial-like support for the adjacent photoreceptors, this excessive lysosomal storage may impair their ability to protect the neurons.

The $ABCA4^{-/-}$ mouse model of Stargardt's early onset retinal degeneration is characterized by pronounced accumulation of the retinoid N-retinylidene-N-retinylethanolamine (A2E) and of oxidized lipids in lysosome-associated organelles of RPE cells.[5,6] Lysosomal pH is elevated in RPE cells from the $ABCA4^{-/-}$ mice and in ARPE19 cells exposed to A2E.[7] As the degradative enzymes inside the lysosomal lumen are preferentially active under acidic conditions, elevation of lysosomal pH can slow the degradation of lysosomal contents and thus accelerate the accumulation of waste material in the RPE cells.

Reacidifying the lysosomes of RPE cells has been hypothesized to enhance lysosomal degradation and limit the pathology associated with lysosomal dysfunction.[8,9] Several receptor types have been identified that can lower the pH of lysosomes in compromised RPE cells and enhance lysosomal degradation to reduce lipofuscin accumulation. For example, receptors linked to the G_s signaling pathway, such as A_{2A} adenosine receptors, D_5 dopamine receptors, and beta-adrenergic receptors, are particularly effective at lowering lysosomal pH.[7–10] The G_s protein stimulates adenylate cyclase to elevate cytoplasmic cAMP; the ability of protein kinase A (PKA) inhibitors to prevent this acidification in RPE cells implicated PKA in the acidification.[7,11] Even RPE cells from aged $ABCA4^{-/-}$

mice with considerable build-up of lipofuscin material responded to cAMP with lysosomal acidification.[7] Manipulation of cAMP also restored lysosomal pH in fibroblasts from patients with early-onset Alzheimer's disease and mutations in presenilin 1 (PS1).[12] This suggests modulation of cAMP/PKA has broad relevance for improving lysosomal degradation in compromised cells.

Although these previous studies provide proof of concept that targeting cAMP could lower lysosomal pH and enhance degradation of lysosomal waste, treatment of patients with chronic diseases of accumulation will require a drug that is tolerated in the aged population over an extended period. We propose that $P2Y_{12}$ receptor antagonists are well suited to target lysosomal acidification in RPE cells. The $P2Y_{12}$ receptor is coupled to G_i proteins that inhibit adenylate cyclase, and thus, blocking of the $P2Y_{12}$ receptor elevates cytoplasmic cAMP.[13] Inhibition of the $P2Y_{12}$ receptor was recently shown to lower lysosomal pH in RPE cells and reduce autofluorescence in cultured RPE cells fed photoreceptor outer segments while treated with the lysosomotropic agent chloroquine.[14] In this initial study, the addition of the $P2Y_{12}$ receptor antagonist ticagrelor to mouse chow also lowered the pH of lysosomes in RPE cells and reduced autofluorescence.[14] Although this suggested that ticagrelor given orally could target lysosomes of RPE cells in ABCA4$^{-/-}$ mice, the effects on vision were not determined.

The current study extends these findings to test the ability of ticagrelor to protect retinal function and photoreceptor survival in ABCA4$^{-/-}$ mice. As photoreceptors in even albino ABCA4$^{-/-}$ mice are surprisingly hardy, moderate exposure to light was used to augment the loss of photoreceptors in this model. Protection was evaluated functionally by measuring the a- and b-waves of the ERG and structurally using both ocular coherence tomography (OCT) to determine outer nuclear thickness and histology to monitor loss of photoreceptor nuclei in the outer nuclear layer (ONL). The effect of ticagrelor on RPE lysosomes was determined through expression of marker *LAMP1*. Together, these findings suggest ticagrelor can protect photoreceptors and retinal function in the ABCA4$^{-/-}$ mouse model of Stargardt's disease by reacidification of lysosomes in RPE cells.

Methods

Animal Handling

All animal procedures were carried out in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Albino ABCA4$^{-/-}$ mice were received from Roxana Radu (University of California at Los Angeles, CA). Mice were exposed to light from 7:00 AM and 7:00 PM with free access to water.

Drug Delivery Strategies

Mice were pretreated with ticagrelor by using a custom mouse diet made by MP Biomedicals (Santa Ana, CA, USA). This custom diet contained Purina Lab Meal 5001 with ticagrelor provided by AstraZeneca (Gothenburg, Sweden). Two separate chow preparations were made, namely, one with 0.1% and one with 0.15% ticagrelor. Untreated food pellets or those containing ticagrelor were added at 100 to 200 g every week and the remainder weighed to determine total food consumption. No adverse side effects were observed in mice treated with ticagrelor for up to 10 months.

Mice were processed in age-matched pairs (ticagrelor-treated versus untreated ABCA4$^{-/-}$ mice). In total, 37 mice were used, including 18 treated with ticagrelor and 19 untreated. Mice were treated in five batches, including batch A: 6 to 7 months old at time of light damage, 0.15% ticagrelor for 4 weeks beforehand, used for ERG and histology; batch B: 3 to 4 months old, 0.15% ticagrelor for 4 weeks, used for ERG and histology; batch C: 7 to 9 months old, 0.15% ticagrelor for 5 weeks used for ERG and histology; batch D: 14 to 20 months old, 4 months 0.1% ticagrelor and 6 months 0.15% ticagrelor used for ERG, PCR, and histology; and batch E: 17 months old, 0.1% ticagrelor for 8 months used for OCT and fundus images.

Light Damage

Although the loss of photoreceptors is well documented in patients with mutations in the ABCA4 gene in recessive Stargardt's disease,[15] little photoreceptor loss occurs in ABCA4$^{-/-}$ mice.[16] A minor loss of photoreceptors has been documented in albino ABCA4$^{-/-}$ mice,[17] but the loss was negligible in our hands. To determine whether ticagrelor actually protected vision, we exposed mice to a moderate level of light to trigger retinal pathology. Mice were dark adapted for >1 hour and then exposed to 10,000 lux cool light for 6 hours. This protocol led to greater photoreceptor loss in the albino ABCA4$^{-/-}$ mice than the wild type BALB/c albino mice (see Results), as found by others.[18] After this 6-hour exposure to light, mice were returned to cages with ticagrelor-treated or untreated chow for the indicated time (up to 14 days) and exposed to standard light regimes.

Ticagrelor Measurement

Ticagrelor plasma concentrations were determined based on a method previously described.[19] In brief, blood collected from the tail-vein was placed on ice and plasma prepared within 30 minutes of blood sampling by centrifugation at 1500*g* for 10 minutes at approximately 4°C. The plasma was transferred into tubes stored at or below −20°C within 1 hour of sample collection. Plasma concentration of ticagrelor was determined by a protein precipitation and liquid chromatographic-mass spectrometric method. Chromatographic separation was performed using an ACQUITY UPLC I-class system (Waters Corporation, Milford, MA, USA). Analytical column ACQUITY UPLC HSS T3, 2.1 × 30 mm was used with a 1.8-μm particle size. The mass detector was a Waters Quattro Premier XE triple quadrupole mass spectrometer (Waters Corporation, Milford, MA, USA) using electrospray ionization. The lower limit of quantification was 0.05 μM. Plasma was obtained from mice in groups A and D; no significant difference in plasma levels of ticagrelor was detected between groups.

ERG Recordings

Mice were dark-adapted >2 hours and anesthetized with 1.5% isoflurane at a flow rate of 1.0 L/min. Pupils were topically dilated with 1.0% tropicamide (Mydriacyl; Alcon, New York, NY, USA) and mice were placed on a heated platform, with artificial tears used to keep the eye hydrated (Systane, Alcon, TX, USA). Scotopic responses were stimulated at light intensity increments of 0.01 cd·s/m^2 (50 repetitions averaged), 0.1 cd·s/m^2 (30 repetitions), or 1.0 cd·s/m^2 (25 repetitions) by using the TOUCH/TOUCH protocol with the Diagnosys Celeris ERG system (Diagnosys LLC, Lowell, MA, USA). ERGs were recorded from both eyes simultaneously by placing the electrode/stimulators in contact with each cornea. The peak of the ERG a-waves (first negative ERG component) and b-waves (first positive ERG component) were quantified automatically by using the Espion software supplied.

Investigative Ophthalmology & Visual Science

Optical Coherence Tomography

Mice were anesthetized by intraperitoneal injection of ketamine/xylazine (80/8 mg/kg) and pupils were dilated with 1% tropicamide. Mice were placed on a positioning stage, and the corneas were kept moist with application of artificial tears. OCT imaging was acquired with the Bioptigen spectral-domain OCT device Envisue R4310 (Leica Microsystems Inc., Buffalo Grove, IL, USA) and analyzed with the associated software (InVivoVue 2.4; Bioptigen, Inc., Durham, NC, USA). The thickness of the retinal ONL was manually measured 400 μm away from the optic nerve head in nasal and temporal retinal quadrants by using the software's ruler tool.

Histology

Mice were perfused intracardially, and dissected eyes were postfixed with 4% paraformaldehyde. Retinal sections (12 μm) were obtained through the optic nerve on the inferior/superior axis and stained with 4′,6-diamidino-2-phenylindole. Slides were mounted in Slow Fade Gold Antifade Mountant (ThermoFisher Scientific, Waltham, MA, USA) and imaged using a Nikon Eclipse 600 microscope (Nikon USA, Melville, NY, USA). Nuclei rows were counted at 0.25, 0.5, 0.75, and 1 mm from the optic nerve along the superior to inferior axis.

Fundus Photography

Fundus images were acquired using the Brightfield settings on the Micron III fundus camera following the manufacturer's instructions (Phoenix Research Laboratories, Inc., Pleasanton, CA, USA). Mice were anesthetized with a mixture delivering ketamine/xylazine 80/8 (mg/kg). The pupils were dilated with topical application of 1% tropicamide eye drops.

Quantification of LAMP1 mRNA

Total RNA was isolated from fresh mouse RPE/choroid using Trizol and the RNeasy mini kit (Qiagen Inc., City, State, Country). RNA yield was determined by a Nanodrop 2000 spectrophotometer; 100 ng of total RNA was converted into cDNA using the High Capacity RNA-to-cDNA kit (catalog number 4387406; Applied Biosystems, Germantown, MD, USA). The quantitative PCR was performed using the Power SYBR Green detector (Life Technologies Inc.) on the 7300 RealTime PCR system (Applied Biosystems Corp., Foster City, CA, USA), starting with 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. The final primer concentration in each well was 0.5 μM for forward and 0.5 μM for reverse primer with 0.5-μL cDNA. The relative expression of *LAMP1* was normalized internally to housekeeping gene *GAPDH* and analyzed using the delta-delta CT approach, with results expressed as fold change in gene expression. Primer pairs for mouse *LAMP1* are forward, CAGCACTCTTTGAGGTGAAAAAC, and reverse, AC GATCTGAGAACCATTCGCA (104 base pairs); and for mouse *GAPDH* are forward, TCACCACCATGGAGAAGGC, and reverse, GCTAAGCAGTTGGTGGTGCA (169 base pairs).

Data Analysis

All data are given as mean ± standard error of the mean (SEM). Analysis was performed using SigmaStat (Systat Software Inc., San Jose, CA, USA) or Prism (Graphpad Software Inc., San Diego, CA, USA). Differences between treatments were analyzed using a one-way analysis of variance (ANOVA) with indicated posthoc tests as appropriate.

Results

Ticagrelor Protects Photoreceptor Function, as Determined With the ERG

Albino ABCA4$^{-/-}$ mice fed ticagrelor showed a significant protection of photoreceptor function compared to mice receiving standard chow, as determined from the ERG readings (Fig. 1). The ERG response obtained from untreated mice exposed to light was noticeably smaller than that from mice treated with ticagrelor (Fig. 1A, 1B). The negative-going a-wave, considered an early measure of photoreceptor activity, was significantly greater in the ticagrelor-treated mice at light intensities of 0.1 and 1.0 cd·s/m^2 (Fig. 1C). Protection of the b-wave by ticagrelor was even more substantial (Fig. 1D); as the b-wave is considered to represent the amplification of the photoreceptor responses, the greater amplitude of both a- and b- waves together in mice treated with ticagrelor is consistent with greater photoreceptor activity.

The difference in amplitude of the a- and b- waves between ticagrelor-treated and untreated mice was observed 1, 7, and 14 days after light exposure (Fig. 1E, 1F), implying a permanent protection. The increased ERG response was also observed across multiple batches of ticagrelor-treated mice (see Methods).

Ticagrelor Protects Against Retinal Thinning as Detected by Using OCT

As the ERG recordings implied that ticagrelor provided a permanent protection of photoreceptor function, additional complementary approaches were used to determine whether ticagrelor treatment protected photoreceptors from loss. OCT is used clinically to identify structural changes in retinal tissue corresponding to events such as neural death. Treatment with ticagrelor for an extended period did not affect the ONL thickness before exposure to light (Fig. 2A, 2B). Although both sets of mice showed some change in retinal thickness 3 weeks after exposure to the light protocol, mice treated with ticagrelor had increased ONL thickness (Fig. 2C). When the effects of light exposure were compared within the same retinal section, the protection found in eyes receiving ticagrelor was even greater (Fig. 2D).

Ticagrelor Treatment Prevents the Death of Photoreceptors, as Determined From Counts of the Photoreceptor Nuclei in Retinal Sections

The number of nuclei rows present in the ONL was determined to provide a further assessment of photoreceptor survival in retinal sections from ticagrelor-treated and untreated ABCA4$^{-/-}$ mice exposed to light (Fig. 3A, 3B). The loss of photoreceptor nuclei was significantly less in mice receiving ticagrelor than in untreated mice (Fig. 3C). Spider graphs of the number of nuclei rows as a function of the distance from the optic nerve suggested the protection by ticagrelor was found throughout the retina (Fig. 3D).

Lysosomal Implication and Additional Controls

Several controls were performed to confirm the neuroprotective effects of ticagrelor. First, the effect of ticagrelor treatment on expression of *LAMP1* mRNA in the RPE of ABCA4$^{-/-}$ mice was tested. Our previous findings indicate that oral treatment with ticagrelor lowers the lysosomal pH of RPE cells[14] and proposed that lowering lysosomal pH of RPE cells protects the health of the outer retina.[9] The expression of lysosomal genes such as *LAMP1* are often inversely related to lysosomal activity,

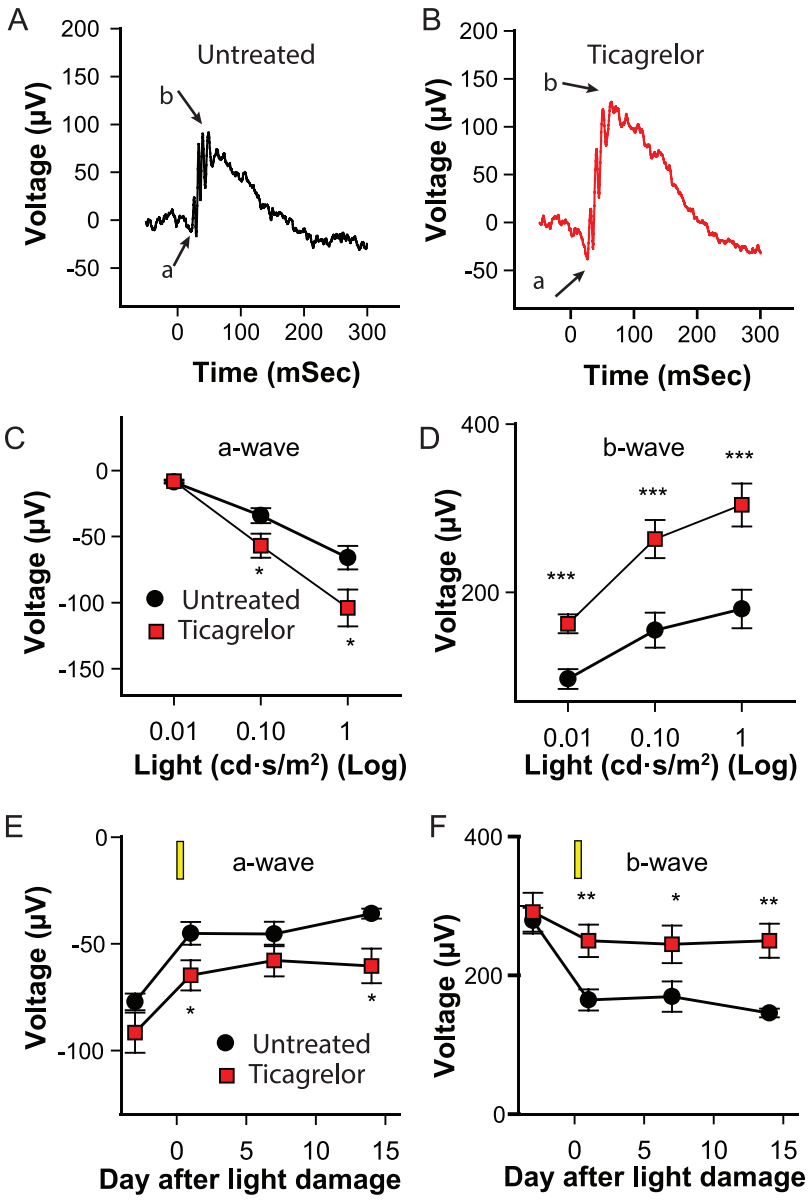

Figure 1. ERG traces from (**A**) untreated and (**B**) ticagrelor treated albino ABCA4$^{-/-}$ mice showing the scotopic response to 0.1 cd·s/m^2 light 1 day after exposure of mice to 10,000 lux for 6 hours. (**C**) The magnitude of the a-wave from untreated mice (black circles, $n = 20$) and mice treated with ticagrelor (red squares, $n = 14$) 1 day after the light exposure protocol was applied. Symbols represent the mean ± SEM of mouse eyes from batches **A–D**. $P = 0.664$, $^{*}P = 0.034$ and $^{*}P = 0.022$ for 0.01, 0.1, and 1.0 cd·s/m^2 respectively. (**D**) The mean peak b-waves from untreated and ticagrelor-treated mice from same conditions as in panel **C**; $^{***}P \leq 0.001$ for all. (**E**) The a-wave measured before and 1,7, and 14 days after application of light protocol (shown as a *yellow box*) in untreated (*black circles*, $n = 8$) and ticagrelor-treated (red squares, $n = 8$) mice. Results from 1.0 cd·s/m^2 flash from mouse batch (**D**) are $^{*}P = 0.043$ and $^{*}P = 0.011$ for 1 and 14 days, respectively. (**F**) The mean b-wave from the same mice as in panel **E** ($n = 8$, $^{**}P = 0.009$, $^{**}P = 0.049$, and $^{**}P = 0.001$ for 1, 7, and 14 days, respectively; all comparisons performed with 1-way ANOVA and Tukey's posthoc test, 0.1%–0.15% ticagrelor; see Methods).

with the transcription factor E-Box (TFEB) linking decreased lysosomal degradation with increased expression of lysosomal genes in a classic negative feedback loop.[20] Exposure of ABCA4$^{-/-}$ mice to the light damage protocol led to a negligible change in expression of *LAMP1* mRNA in the RPE/choroid. However, expression of *LAMP1* mRNA was decreased by over 40% in RPE cells from mice exposed to ticagrelor as compared to mice receiving untreated chow (Fig. 4A). Expression of a second gene regulated by TFEB, *LAMP2,* showed a similar decrease that approached significance (not shown). This supports previous direct measurements of lysosomal pH performed previously, which demonstrate acidification of lysosomes after oral delivery of ticagrelor.[14]

Additional experiments asked whether the loss of photoreceptors reflected changes specific to the ABCA4$^{-/-}$ mice. The pattern of photoreceptor nuclei loss in the albino ABCA4$^{-/-}$ mice exposed to light was compared to that in wild type BALB/c mice (Fig. 4B). The loss of nuclei was always greater in the ABCA4$^{-/-}$ mice, with the magnitude largest in the central superior region 0.5 mm from the optic nerve head. Overall, exposure to light led to a loss of >40% of photoreceptor nuclei in the ABCA4$^{-/-}$ mice as compared with BALB/c mice (Fig. 4C).

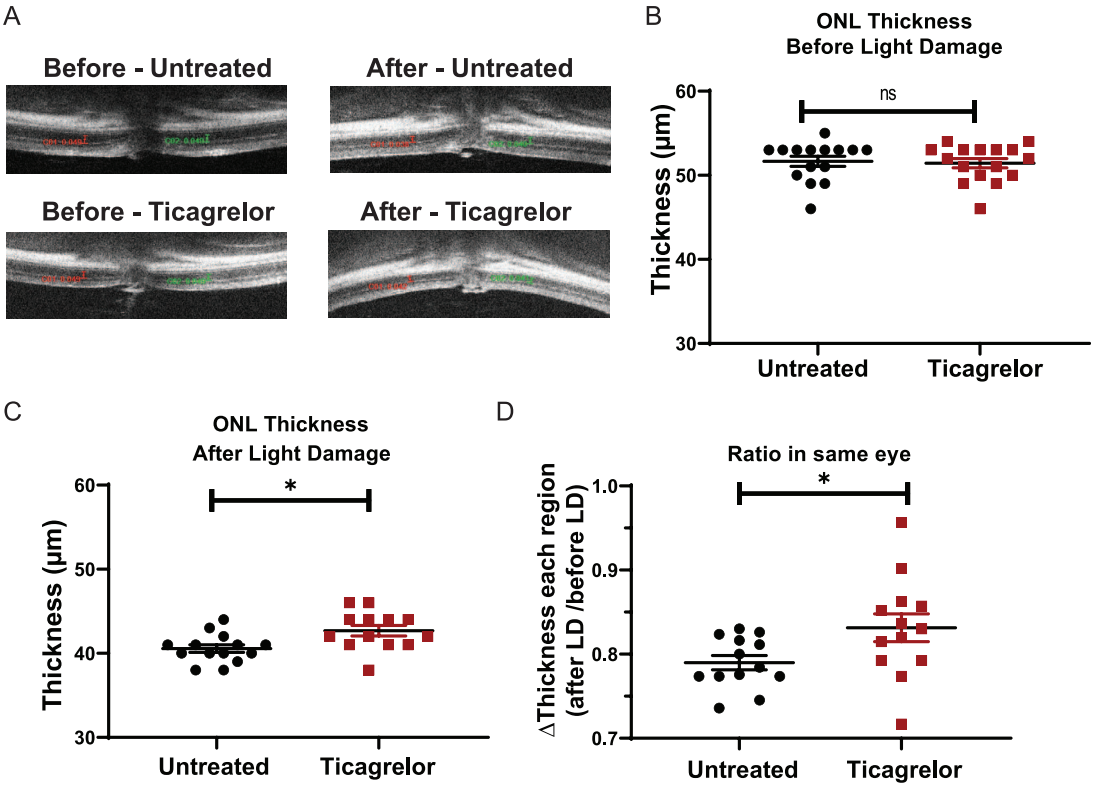

Figure 2. (**A**) OCT images of retina before and after light damage (LD) in untreated mice and those treated with 0.1 % ticagrelor for 8 months. (**B**) The ONL thickness was the same for untreated mice ($n = 15$) and those mice treated with ticagrelor ($n = 16$) before LD ($P = 0.78$). (**C**) When imaged 3 weeks after light exposure, the ONL was significantly thicker in mice treated with ticagrelor ($n = 14$) than untreated mice ($n = 13$; $^*P = 0.010$). (**D**) The ratio of ONL thickness within a particular region after light exposure compared to its thickness before light exposure (ΔThickness each region; after LD/before LD), showing relative loss within the same eye. This change in thickness was greater in eyes from untreated mice than eyes from ticagrelor-treated mice. ($^*P = 0.036$, $n = 13$; measurements from 4 untreated and 4 ticagrelor treated mice, with measurements from OS, OD, and nasal and temporal regions. All ANOVA with Tukey's posthoc test. Results from batch E mice; 17 months old at time of LD, 0.1% ticagrelor in food for 8 months before light exposure.

The effect of prolonged treatment with ticagrelor on the fundus was also examined. In ABCA4$^{-/-}$ mice exposed to 0.1% ticagrelor in chow for 7 months, fundus examination revealed no sign of retinal bleeding or other changes in gross morphology (Fig. 4D). In addition, no sign of hemorrhaging or angiogenesis was observed in the 18 ABCA4$^{-/-}$ mice treated with ticagrelor for an extended time used throughout the study.

Finally, the plasma concentration of ticagrelor in mice was determined. In mice with access to 0.15% ticagrelor in their chow for at least 6 weeks, the mean concentration of the drug in plasma was 0.42 ± 0.11 µM ($n = 7$).

Discussion

This study suggests that the $P2Y_{12}$ receptor antagonist ticagrelor may protect against the loss of photoreceptors in retinal degeneration. The use of three separate measures to demonstrate the benefits of ticagrelor adds considerable rigor to the conclusions; differences in photoreceptor function determined using the ERG are supported by the protection of ONL thickness determined using OCT and with nuclei counts obtained from histologic sections. As both the ERG and the OCT are measures used clinically to assess retinal health, these findings provide strong preclinical evidence that ticagrelor is protective.

Several lines of evidence implicate changes in lysosomal function in the photoreceptor protection associated with ticagrelor. RPE cells express $P2Y_{12}$ receptors, and the lysosomal pH of isolated RPE cells was elevated by $P2Y_{12}$ receptor agonists and decreased by $P2Y_{12}$ receptor antagonists.[14] Treatment with ticagrelor significantly lowered lysosomal pH and partially reduced autofluorescence in RPE cells of ABCA4$^{-/-}$ mice when measured ex vivo.[14] The decreased expression of lysosomal marker *LAMP1* in RPE cells isolated from ticagrelor-treated mice described in the present study is consistent with improved lysosomal function; *LAMP1* expression is controlled by TFEB and regulated by the degree of lysosomal degradation.[21–23] The decreased expression of *LAMP1* is consistent with the more acidic lysosomal pH and lipofuscin clearance detected in our previous study.[14]

The precise cellular mechanisms linking improved lysosomal function with increased cellular health are still being determined, although several possibilities have been identified. For example, blue light acting on lipofuscin in lysosomal-related organelles can activate the NLRP3 inflammasome, trigger secretion of proinflammatory interleukin-1β, and kill RPE cells.[24–26] The efflux of Ca^{2+} through the lysosomal cation channel TRPML1 is needed for autophagy and lysosomal function,[27] but channel activity is blocked by lipofuscin accumulation in RPE cells.[27,28] The ABCA4 protein was recently identified on endolysosomal membranes[17]; it will be interesting to determine whether its function is altered by luminal pH or lipid accumulations.

The ABCA4$^{-/-}$ mouse is a good model of A2E accumulation in the lysosomes of RPE cells, but the mice show little

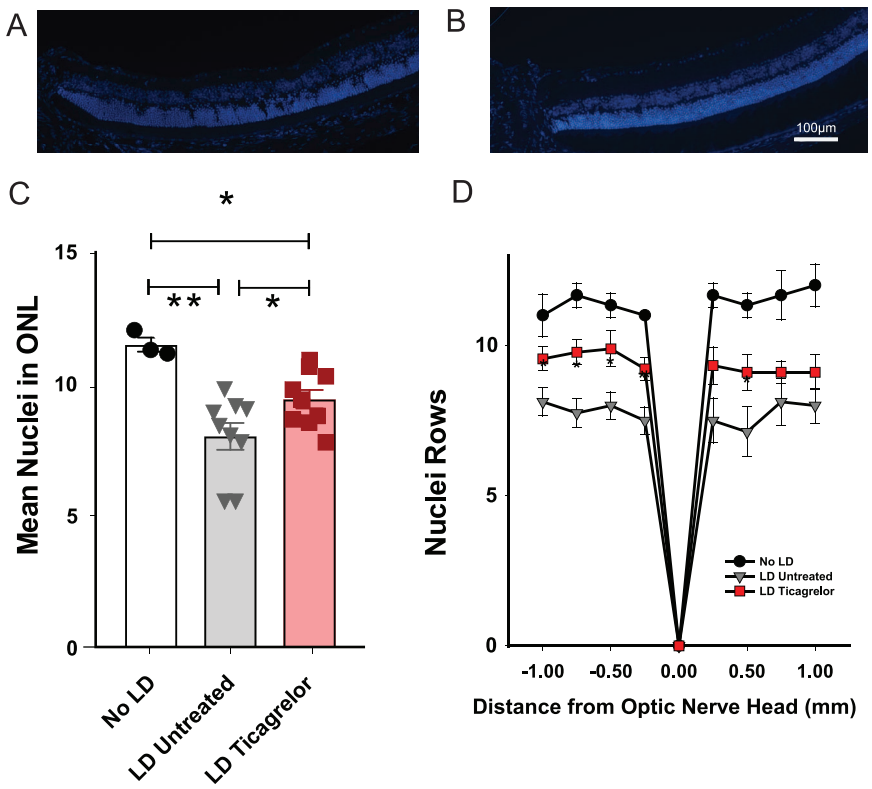

Figure 3. Images of retinal sections from (**A**) untreated and (**B**) ticagrelor-treated ABCA4$^{-/-}$ mice exposed to LD and stained with 4′,6-diamidino-2-phenylindole to show the number of surviving nuclei. (**C**) Mean ± SEM of nuclei counts from mice with no LD, untreated mice with LD, and ticagrelor-treated mice with LD (*$P < 0.05$, **$P < 0.01$ ANOVA, Holm-Sidak posthoc test from the mean counts across all regions from 3, 9, and 9 mice, respectively). (**D**) Spider graph from counts of the number of nuclei rows with distance from the optic nerve head either side, in mice with no LD ($n = 3$), untreated mice with LD ($n = 8$), and ticagrelor-treated mice with LD ($n = 9$; *$P < 0.05$, ANOVA, Holm-Sidak post hoc test, mice from batch A and D with 0.1%–0.15% ticagrelor, as described in Methods).

photoreceptor loss, even though humans with mutations in *ABCA4* frequently show loss of sight in Stargardt's disease.[29] The addition of moderate light exposure killed more photoreceptors in albino ABCA4$^{-/-}$ mice than albino BALB/c wild type controls, indicating a role for the ABCA4 absence in the photoreceptor loss measured in the current study. This supports the findings of the Sparrow group, where moderate light damage also killed more photoreceptors in the albino ABCA4$^{-/-}$ mouse than in wild types.[18] The excessive accumulation of lipofuscin was identified as a contributing factor to the cell death in their study, as models without lipofuscin did not lose photoreceptors. Although the ability of ticagrelor to protect photoreceptors in ABCA4$^{-/-}$ mice exposed to light is promising, confirmation in patients with Stargardt's disease is needed.

The stimulation of purinergic receptors is emerging as an important pathway through which to modulate lysosomal pH in RPE cells. Previous work has shown that stimulation of the $P2X_7$ receptor can raise the lysosomal pH of RPE cells.[10] Application of an antagonist for the $P2X_7$ receptor lowered the amount of autofluorescence in these cells, suggesting a baseline level of receptor stimulation by agonist ATP normally contributed to accumulation. Likewise, the ability of $P2Y_{12}$ receptor antagonist ARC66096 to reduce autofluorescence in the absence of added agonist suggests that endogenous receptor stimulation by agonist ADP is occurring.[14] As purinergic receptors have been implicated in multiple retinal diseases and basic visual functions,[30–33] a more thorough understanding of the endogenous signaling pathways will be of benefit.

Evidence suggests that ticagrelor given orally can reach RPE cells. The reduced expression of *LAMP1* mRNA in RPE cells of mice treated with ticagrelor in the present study, combined with previous work showing a reduction in the lysosomal pH of RPE cells after ticagrelor is delivered in food or water and the presence of $P2Y_{12}$ receptors on the cells,[14] suggests ticagrelor acts directly on RPE cells, although an indirect effect cannot be ruled out.

Ticagrelor as a Potential Treatment for Retinal Degenerations

Several observations suggest treatment with ticagrelor may have relevance for human disease. Ticagrelor is currently used to prevent thromboembolic events in patients.[34] The plasma exposure of ticagrelor observed in the present mouse study are at or below those found clinically; the standard patient dose of 180 mg/day led to plasma concentrations of ticagrelor in humans ranging from 770 ng/mL 2 hours after dosing to 227 ng/mL steady state.[35,36] Plasma concentrations in the current study of mice treated with chronic ticagrelor were equivalent of 220 ng/mL, similar to the lower range of concentrations found in humans. The lack of any signs of retinal hemorrhage in mice treated for 7 months with ticagrelor suggests that excessive retinal bleeding is unlikely to occur at concentrations capable of providing protection.

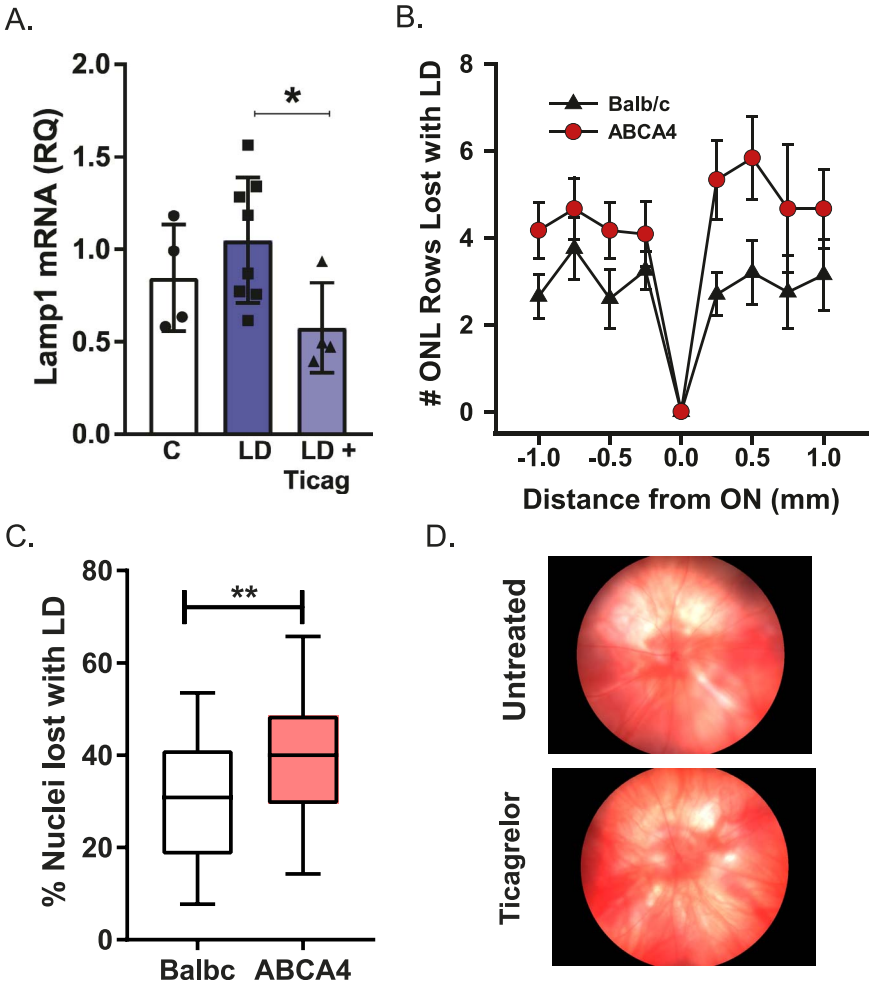

Figure 4. (**A**) Expression of *LAMP1* mRNA in RPE/choroid from control ABCA4$^{-/-}$ mice (**C**), those exposed to LD, and LD following pretreatment with ticagrelor (LD+ticag; $^{*}P = 0.049$, ANOVA, $n = 4$–8 mice). (**B**) Spider plot indicating the mean loss in the number of nuclei rows in the ONL 2 weeks after application of LD, showing increased sensitivity of photoreceptors from albino ABCA$^{-/-}$ mice compared to wildtype BALB/c ($n = 3$-4). (**C**) The percentage of nuclei lost 2 weeks after LD in BALB/c compared to albino ABCA4$^{-/-}$ mice ($^{**}P = 0.002$, $n = 32$–40 regions). (**D**) In ABCA4$^{-/-}$ mice exposed to 0.1% ticagrelor in the diet for 7 months, fundus examination revealed no sign of retinal bleeding or other changes in gross morphology. Upper images from mouse on control diet; lower images from ticagrelor treated mice. Similar responses were seen in three pairs.

The efficacy shown in the current study, combined with the apparently acceptable tolerability, suggest that further investigation into the use of ticagrelor to treat recessive Stargardt's disease and other neurodegenerative disorders linked with lysosomal accumulation may be warranted.

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