Effect of INS37217, a P2Y2 Receptor Agonist, on Experimental Retinal Detachment and Electroretinogram in Adult Rabbits

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PURPOSE. To evaluate the effects of subretinal and intravitreal delivery of INS37217, a P2Y2 receptor agonist, on subretinal fluid reabsorption in experimentally induced retinal detachments in rabbits, and to characterize the effects of INS37217 on electroretinograms (ERG) in rabbits.

METHODS. A single retinal detachment was produced in New Zealand White rabbits by injecting approximately 50 µL of modified phosphate-buffered saline (MPBS) solution into the subretinal space (SRS). In all experiments, one eye served as the INS37217-treated eye and the contralateral eye served as the vehicle control. In the first series of experiments, each rabbit received a SRS injection of MPBS solution, with or without INS37217 (1 mM). In the second series of experiments, each eye received an SRS injection of MPBS solution, followed by an intravitreal injection of MPBS solution, with or without INS37217 (12, 1.4, and 0.15 mM). A masked observer determined the size of blebs by indirect ophthalmoscopy at 30-minute intervals for up to 3 hours after SRS injections. Optical coherence tomography (OCT) was conducted to provide cross-sectional images of the blebs. Cellular expression of P2Y2 receptor mRNA was localized by nonradioisotopic in situ hybridization in fresh rabbit retina–RPE tissue sections. Bilateral, full-field scotopic and photopic ERGs were made at 1, 7, and 14 days after a single intravitreal injection of 24 mM INS37217.

RESULTS. SRS administration of 1 mM INS37217 significantly enhanced subretinal fluid reabsorption when compared with vehicle controls (P < 0.05; repeated measures ANOVA). Intravitreal administration of INS37217 at 12 and 1.4 mM, but not at 0.15 mM, also significantly enhanced subretinal fluid reabsorption (P < 0.05). P2Y2 receptor mRNA was observed throughout the RPE and in discrete layers of the retina. INS37217 had no adverse effects on scotopic and photopic ERG amplitude and latency parameters at any of the postadministration time points evaluated.

CONCLUSIONS. These results demonstrate that INS37217 enhances subretinal fluid reabsorption in experimental retinal detachment in rabbits and support the development of INS37217 for stimulating subretinal fluid reabsorption in conditions that result in retinal detachment or retinal edema. (Invest Ophthalmol Vis Sci. 2002;43:3567–3574)

Fluid accumulates in the subretinal space or within the sensory retina in a wide variety of blinding conditions. In the wet form of age-related macular degeneration (AMD), subretinal and subretinal fluid is thought to result from neovascular choroidal blood vessels and is an important cause of decreased vision in this condition.1 Diabetic retinopathy is another blinding condition that results in loss of visual acuity due to diabetic macular edema, which is characterized by accumulation of intraretinal fluid and formation of cystoid edema.2 In rhegmatogenous, serous, and tractional retinal detachments, the retina is separated from the retinal pigment epithelium (RPE), and fluid accumulates in the subretinal space. To preserve vision in patients with retinal detachment, treatment must eliminate the extraneous subretinal fluid to reestablish a normal anatomic adhesion between the sensory retina and RPE.3 Similarly, the removal of extraneous fluid in intraretinal edema is thought to aid in the restoration of vision.4,5 Therefore, a pharmacologic approach to remove pathologic accumulation of subretinal and intraretinal fluid may help restore visual function in these diseased eyes.6,7

Previous work has shown that the RPE contains P2Y2 receptors that can be activated by endogenous ligands, such as adenosine 5′-triphosphate (ATP) and uridine 5′-triphosphate (UTP).8–10 Activation of the P2Y2 receptor at the apical membrane stimulates ion transport pathways and net apical-to-basolateral fluid absorption in bovine RPE.8–10 Previous work has shown that INS37217, a synthetic agonist for the P2Y2 receptor, stimulates fluid absorption in freshly isolated bovine and human fetal RPE.11,12 In addition, intravitreal administration of INS37217 enhanced subretinal fluid reabsorption in a rat model of experimentally induced retinal detachment.11,12

In the present study, we investigated the effects of both subretinal and intravitreal delivery of INS37217 on subretinal fluid reabsorption in the young adult rabbit eye, the size of which is similar to that of the human eye. These findings were evaluated by indirect ophthalmoscopy and confirmed with optical coherence tomography (OCT) imaging techniques. We also sought to determine the minimum dosage required for an efficacious effect, the presence and cellular localization of P2Y2 receptor mRNA in rabbit retina and RPE, and whether intravitreal INS37217 affects retinal function, as measured by electroretinography (ERG).

METHODS

All animal experiments were conducted according to the guidelines of the Duke University Animal Care and Use Committee under an approved protocol. The animals were maintained and treated in accor-
dance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Surgical Procedure for Inducing Subretinal Blebs

New Zealand White rabbits weighing approximately 1.5 kg (aged 2–3 months) were anesthetized with an intramuscular injection of 0.3 mL/kg body weight ketamine hydrochloride (100 mg/mL) and 0.5 mL/kg body weight xylazine hydrochloride (100 mg/mL). Ketamine hydrochloride was added as needed. For experiments requiring observation of the fundus, the pupil was dilated with 0.25% scopolamine hydrobromide, 1% cyclopentolate, and 2.5% phenylephrine hydrochloride eye drops.

One local retinal detachment was created in each eye. A wire lid speculum was placed and a segmental conjunctival peritomy (of approximately 2 clock hours) was made at the 3 and 9 o’clock positions. Two scleral incisions were made with a 19-gauge microvitreoretinal (MVR) blade 0.5 mm posterior to the limbus through the ciliary body. A self-retaining planoconcave contact lens was placed on the corneal surface. A chandelier light, which was used for illumination (Grieshaber & Co., AG, Schaffhausen, Switzerland), was carefully guided through one of the sclerotomy sites into the vitreous cavity to avoid touching the lens. Retinal detachments were made with a beveled 36-gauge retinal needle (Grieshaber & Co., AG) attached by an extension tube to a 1-mL syringe that was driven by a calibrated, mechanical syringe pump (model 351; Sage Instruments, Cambridge, MA). Under direct observation with an operating microscope, the retinal needle was inserted through the second sclerotomy and slowly advanced to either the nasal or temporal myelin wing. These sites were selected for injection, because the myelin wing gives additional structural support when compared with the adjacent areas of thin, avascular retina. The intraocular pressure was maintained at a low level to allow a slow hydrodissection of the fragile retina from the RPE. The tip of the 36-gauge needle was carefully inserted under the myelin wing. A localized dome-shaped detachment of the retina was created by using a mechanical syringe pump to inject approximately 50 μL phosphate-buffered saline (PBS) into the subretinal space. The instruments were removed from the eye, and the sclerotomy sites remained open to keep the intraocular pressure constant.

Injection Solution

Modified PBS (MPBS) solution, used for all subretinal and intravitreal injections, was composed of 13.6 mM Na₂HPO₄, 6.2 mM NaH₂PO₄, 130.5 mM NaCl, and 5 mM KCl, and had an osmolality of approximately 300 mOsm (pH 7.2). INS37217 (molecular weight [MW] 862) was added to the MPBS solution to achieve a target drug concentration of 12, 1.4, 1.0, or 0.15 mM. The experimental and control solutions were kept at equal osmolality. For concentrations of 1 mM INS37217 or less, an appropriate amount of NaCl was added to the MPBS solution to compensate for the osmolarity contribution of INS37217 (1 mM INS37217 contributes approximately 4-5 mOsm). For concentrations greater than 1 mM INS37217, solution isotonicity was maintained by reducing an equal osmolal of NaCl in the MPBS solution in place of the addition of INS37217. All experimental and control solutions for each dose cohort were formulated so that the resultant value as a dimensionless ratio relative to the mean reference diameter of the optic disc is approximately 1 mm, as previously determined under a microscope in 10 enucleated eyes from albino rabbits. Bleb size at each evaluation time point was first quantified by multiplying the vertical and horizontal dimensions, and then expressing the resultant value as a dimensionless ratio relative to the initial size of each bleb. This dimensionless (normalized) bleb size at each evaluation time point was then plotted and analyzed as a function of time.

Optical Coherence Tomography

OCT was used to confirm the clinical observations in selected cases. After maximal pupillary dilation and lubrication of the cornea, transpupillary OCT (Humphrey Instruments, San Leandro, CA) was performed in the area of the bleb. The area was systematically examined with multiple vertical and horizontal scans. The technique is based on low-coherence interferometry and uses back-scattering of laser light in a manner analogous to B-scan ultrasound to create a cross-sectional image of tissue. The back-scattering from the retina is captured by an interferometer, which measures the delay of the echo signals from various distances with a depth of 2 mm (maximum resolution 10–17 μm). The length of each OCT scan was adjusted from 1.9 to 2.5 mm, depending on the bleb size (maximum resolution, 25 μm). The intensity of optical scattering is represented as false-color images. Areas of high reflectance are represented by red-to-white colors, whereas low reflective areas are represented by blue-to-black colors. The hyper-reflective boundaries of the inner limiting membrane, myelin wing, and RPE were clearly delineated by OCT to outline the borders of the subretinal bleb. Scans were obtained every 30 minutes in selected cases.

Statistical Data Analysis: Bleb Size

For each treatment concentration, mean ± SEM bleb size at each time point for treatment versus control was plotted graphically as a function of time. A repeated-measures ANOVA was performed that included concentration, time, the interaction of concentration and time, and subjects nested within concentration in the model (SAS ver. 8.1; SAS, Cary, NC). The repeated measure was time (30, 60, and 90 minutes). Although observations were made for as long as 3 hours, in most cases the blebs that were treated with drug disappeared (relative area of 0) by 120 minutes. Inclusion of these time points in the analysis would violate assumptions of equal variance. The covariance structure that provided the best fit was first-order autoregressive (Akaike’s information criterion). Estimates of differences and their 95% confidence interval between active and placebo treatments at each time point and over all time points were made and compared by t-tests uncorrected for multiple comparisons. For the analysis of data produced by subretinal administration, each bleb was treated as an independent observation. All data are plotted as the mean ± SEM, unless otherwise noted.

In Situ Hybridization

Nonisotopic in situ hybridization (ISH) techniques were used to localize the cellular expression of P2Y₂ mRNA on fresh-frozen retina-RPE

(12, 1.4, and 0.15 mM) was administered into the vitreous cavity with a 100-μL syringe (Hamilton, Reno, NV) adjacent to the subretinal bleb. The surgeon (CHM or KH) was unaware of the content of the administered solutions.

Postoperative Observation

The corneal epithelium was protected with a layer of methylcellulose to maintain corneal clarity. Fundus photographs were obtained with a fundus camera (TRC-W; Topcon, Tokyo, Japan) in selected cases. The observer determined by indirect ophthalmoscopy the initial bleb size and the bleb size at 30-minute intervals for 3 hours. The vertical and horizontal dimensions of each subretinal bleb were recorded in disc diameters, with the adjacent optic disc used as a reference marker. (The mean reference diameter of the optic disc is approximately 1 mm, as previously determined under a microscope in 10 enucleated eyes from albino rabbits). Bleb size at each evaluation time point was first quantified by multiplying the vertical and horizontal dimensions, and then expressing the resultant value as a dimensionless ratio relative to the initial size of each bleb. This dimensionless (normalized) bleb size at each evaluation time point was then plotted and analyzed as a function of time.

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cross-sections from adult New Zealand White rabbits. P2Y<sub>2</sub> receptor nucleotides 272 to 627, which were used for sense and antisense riboprobe synthesis, were amplified with polymerase chain reaction (PCR) primers designed to eliminate flanking plasmid sequences and incorporate either an upstream T3 promoter or a downstream T7 promoter. The resultant PCR products were used to synthesize digoxigenin-labeled riboprobes by in vitro transcription. Antisense and sense riboprobes were synthesized using T7 and T3 RNA polymerases, respectively, in the presence of digoxigenin-11-UTP (Roche Molecular Biochemicals, Indianapolis, IN) using an in vitro transcription kit (Maxiscript; Ambion, Austin, TX) according to the manufacturer’s instructions. After in vitro transcription, template DNA was degraded with DNase-I, and unincorporated digoxigenin was removed by ultrafiltration. Riboprobe integrity was assessed by electrophoresis through a denaturing polyacrylamide gel. Apparent molecular size was estimated by comparison with the electrophoretic mobility of a 100- to 1000-bp RNA ladder. Probe yield and labeling were evaluated by blot immunocytochemistry. Riboprobes were dispensed in 5-μL aliquots and stored at −80°C until used.

Tissue blocks were snap frozen in optimal cutting temperature embedding medium, cut sagittally into 5-μm-thick sections, and mounted on slides (SuperFrost Plus; Fisher Scientific, Pittsburgh, PA). Tissue sections were postfixed for 15 minutes in 4% paraformaldehyde in PBS (pH 7.4) and treated with active diethylpyrocarbonate for 30 minutes to inactivate tissue ribonucleases. Sections were incubated overnight at 58°C in hybridization buffer containing 0.5 to 1.0 g/mL of either antisense or sense probe. After hybridization, slides were subjected to a series of posthybridization stringency washes to reduce nonspecific hybridization. Tissue labeling was visualized by immunohistochemistry with alkaline phosphate–conjugated anti-digoxigenin antibody and nitroblue tetrazolium chloride-bromochloroindolyl phosphate (Roche Molecular Biochemicals), according to the manufacturer’s instructions. Tissue sections were counterstained with nuclear fast red. Assay controls included use of the P2Y<sub>2</sub> receptor sense probe, omission of probe, and omission of probe and anti-digoxigenin antibody. The purple reaction product corresponds to cytoplasmic and/or perinuclear staining of the riboprobe to P2Y<sub>2</sub> receptor mRNA. Cross-sections were also counterstained with nuclear fast red, which yields a pink stain. Each cell type was compared with replicate sections that were hybridized with the sense P2Y<sub>2</sub> receptor probe. Results were considered positive if staining was observed with the antisense probe and no staining or weak background was observed with the sense probe.

**FIGURE 1.** Effect of subretinal INS37217 on reabsorption of subretinal blebs. Subretinal blebs were created by injecting MPBS solution into the subretinal space, with or without INS37217 (1 mM). Results show that INS37217 increased the rate of clearance of subretinal blebs when compared with vehicle control.

**FIGURE 2.** Effects of intravitreal INS37217 on reabsorption of subretinal blebs at doses of (A) 12, (B) 1.4, and (C) 0.15 mM. MPBS solution was injected into the subretinal space to create blebs, followed immediately by an intravitreal injection of MPBS solution, with or without INS37217. Results show that INS37217 administered at 12 and 1.4 mM, but not at 0.15 mM, increased the rate of clearance of subretinal blebs when compared with vehicle control.
The effects of a single 50-μL intravitreal administration of INS37217 (24 mM) on scotopic and photopic ERG parameters were evaluated in 5- to 6-month-old Dutch Belted (pigmented) rabbits and compared with an intravitreal administration of the vehicle alone in the contralateral eye. In addition, a separate cohort of control rabbits was given an intravitreal administration of vehicle in both eyes (vehicle-only group). Therefore, there were two different types of vehicle controls used in the ERG analysis: contralateral eye vehicle control and vehicle-only control animals. The effects of INS37217 were compared with vehicle by using both types of vehicle controls (see the following section). For the ERG experiments, INS37217 was formulated with saline to achieve a final tonicity of approximately 300 mOsm. Simultaneous full-field, bilateral ERGs were made with ERG electrodes (Jet; LKC Technologies, Gaithersburg, MD) with an ERG recording apparatus (UTAS-2000; LKC Technologies) and Ganzfeld dome (LKC Technologies) at baseline (1 week before administration of the drug), and at 1, 7, and 14 days after injection of INS37217. Rabbits were dark adapted for at least 1 hour before ERG recording and then anesthetized with intramuscular injection of ketamine (40 mg/kg) and xylazine (4 mg/kg). Pupils were dilated with tropicamide (1%) and eyelids retracted with a lid speculum. Rod-only recordings were made by averaging the ERG responses from five consecutive flashes of dim stimuli (1.0 × 10\(^{-5}\) cd/s·m\(^{-2}\) at 15 seconds between flashes). Mixed rod-cone responses were determined by averaging ERG responses from three consecutive flashes of bright stimuli (2.5 cd/s·m\(^{-2}\) at 1 minute between flashes). Photopic responses were made by first light adapting animals (25 cd/cm\(^2\) background) for 5 minutes and then averaging 20 consecutive bright flash stimuli (1.0 × 10\(^{-5}\) cd/s·m\(^{-2}\) at 1 Hz).

**Statistical Data Analysis: ERG**

The ERG (amplitude and implicit times of the a- and b-wave) data were subjected to statistical group mean comparisons. The homogeneity of group variances for the treated eye data were assessed with the Bartlett test. If the group variances were heterogeneous (P ≤ 0.001), log or rank transformation was attempted to obtain the homogeneity. If group variances were homogeneous (P > 0.001), an analysis of covariance (ANCOVA) was performed with the control (vehicle-treated) eye data used as a covariate, provided that the slopes between groups were not shown to be heterogeneous at the 0.01 level of significance. When the ANCOVA overall F test revealed significant group differences (P ≤ 0.05), t-tests on least-square means were used to perform pair-wise mean comparisons between control and treated groups. When either the variances or the slopes were heterogeneous among groups, the percentage difference (control eye − treated eye/control eye) was computed for each animal, and the group comparisons were performed with the Kruskal-Wallis test. If the Kruskal-Wallis test indicated significant group differences (P ≤ 0.05), the pair-wise group comparisons (control versus treated groups) were performed with the Wilcoxon rank sum test.

**Results**

**Effects of Subretinal and Intravitreal Injections of INS37217**

In the first series of experiments, an isotonic MPBS solution containing 1 mM INS37217 was injected directly into the subretinal space. The contralateral eye received a subretinal injection of isotonic MPBS solution alone. Figure 1 shows that INS37217-containing subretinal blebs resolved significantly faster than blebs containing MPBS solution alone. Reattachment of the retina was observed at 120 to 150 minutes in the INS37217-treated blebs, whereas the control subretinal blebs did not resolve over the 3-hour observation period. There was a significant difference in subretinal reabsorption at 30, 60, and 90 minutes, according to repeated-measures ANOVA (P < 0.05).

In the second series of experiments, subretinal blebs were first induced with MPBS solution, which was followed immediately by a 50-μL intravitreal injection of MPBS solution, with or without INS37217 (12, 1.4, and 0.15 mM), just adjacent to the bleb. Figures 2A and 2B show that the two higher doses of
INS37217 significantly enhanced the rate of reabsorption of the subretinal bleb and retinal reattachment when compared with vehicle. There was a statistically significant difference in the reabsorption rate between the eye treated with INS37217 and the vehicle alone at 30, 60, and 90 minutes ($P < 0.05$). INS37217-treated eyes showed near complete retinal reattachment by approximately 90 minutes, whereas control blebs had not completely reabsorbed at 180 minutes.

Figure 2C shows the reabsorption rate of subretinal bleb in eyes treated with 0.15 mM intravitreal INS37217 or vehicle alone. There was no statistically significant difference in the reabsorption of subretinal fluid at any time point in eyes with 0.15 mM INS37217, compared with vehicle alone.

**Optical Coherence Tomography**

OCT scanning was performed in selected cases to provide a cross-sectional image of the subretinal bleb and adjacent regions and to provide an independent, qualitative confirmation of the topographic observations made by indirect ophthalmoscopy. Figure 3 shows representative, time-lapse OCT images of subretinal blebs in an animal treated with 12 mM INS37217 in one eye (treatment eye) and MPBS solution in the fellow eye (control eye). Initially, after the creation of the subretinal bleb, a dome-shaped elevation of the retina can be observed in both cases. During the early postoperative period, the bleb lost the convex contour and the surface became irregular. The reduced diameter of the bleb observed by indirect ophthalmoscopy was confirmed by OCT. As the subretinal fluid continued to reabsorb, the retinal surface developed small folds. The OCT images clearly demonstrated the point at which subretinal fluid completely reabsorbed and correlated in all cases with the findings made with indirect ophthalmoscopy.

**In Situ Hybridization**

P2Y$_2$ receptor mRNA expression was determined by ISH with antisense riboprobe (Fig. 4, left) and sense riboprobe (Fig. 4, right) from fresh-frozen ocular sections from an albino rabbit. The purple stain indicates positive ISH signal for P2Y$_2$ receptor mRNA. The antisense probe produced a strong positive ISH signal for P2Y$_2$ receptor mRNA over the entire RPE monolayer. There was some positive ISH signal in scattered cell bodies along the inner nuclear layer, inner segment, and ganglion cell layer. Some labeling was also detected in cell bodies in the choroid along the vascular endothelium and in smooth muscle cells. No specific signal was detected with the sense probe.

**Electroretinography**

To evaluate the effects of P2Y$_2$ receptor activation on ERG function, INS37217 was administered as a single intravitreal injection and full-field, bilateral scotopic and photopic ERGs were made at multiple time points after injection. The experimental eye received a 50-μL injection of 24 mM INS37217, which corresponds to a dose of approximately 1 mg/eye, and the contralateral eye received the same volume of vehicle. Normal, healthy eyes were used in these ERG studies, and retinal detachments were not induced as part of the procedure. This dose of INS37217 was more than twice that of the highest efficacious intravitreal dose used in our efficacy study. Figure 5A shows representative waveforms from a single animal at 1 week before treatment and at 1, 7, and 14 days after injection. Figure 5B summarizes the effects of INS37217 versus vehicle on scotopic and photopic a- and b-wave ERG amplitudes. There were no significant differences in ERG amplitudes between the two groups at any of the time points evaluated. In addition, there was no significant difference in a- and b-wave ERG latencies between the two groups (results not shown).

These ERG findings demonstrate that at higher-than-eficacous concentrations, INS37217 had no adverse effects on measured scotopic and photopic parameters.

**Discussion**

In our study, the synthetic P2Y$_2$ receptor agonist INS37217, when given intravitreally or subretinally, stimulated subretinal fluid reabsorption in an experimentally induced model of nonrhegmatogenous retinal detachment in young adult rabbit eyes. P2Y receptors belong to the superfamily of G-protein-coupled receptors and are activated by extracellular nucleo-
The endogenous ligands for P2Y_2 receptors are ATP and UTP. In eyes given an efficacious amount of INS37217, retinal reattachment was clinically evident between 90 and 120 minutes after administration, as evaluated with indirect ophthalmoscopy. In vehicle-treated eyes, the retina failed to reattach completely, even at the final evaluation time point (3 hours after administration). The onset of INS37217-induced stimulation of subretinal fluid reabsorption was clearly visible as early as the first evaluation time point (30 minutes after administration) for both modes of administration. These clinical findings were confirmed with OCT cross-sectional images of the retina-choroid. INS37217 is a small molecule with a molecular weight of 770 in free solution and 861 in its salt-conjugated form. It is unlikely that INS37217 diffused to the subretinal space through the retinal hole, because retinal holes made in a similar manner have previously been shown to be...
too small to be a conduit for fluid. Therefore, these results strongly suggest that intravitreally administered INS37217 remains sufficiently intact throughout its diffusion across the retina to activate P2Y2 receptors at the apical membrane of the RPE.

Intravitreal INS37217 was administered at a dose volume of 50 μL and at concentrations of 0.15, 1.4, and 12 mM to determine efficacy in the present study. These three concentrations correspond to intravital delivery of approximately 0.006, 0.06, and 0.5 mg of INS37217, respectively. Assuming homogeneous distribution across the entire vitreous (~1.5 mL in young adult rabbits), these three doses of INS37217 correspond to intravitreal concentrations of approximately 3.5, 35, and 300 μM, respectively. We found intravitreal INS37217 to be efficacious at administered concentrations of 12 and 1.4 mM, but not at 0.15 mM. The range of efficacious doses is consistent with the pharmacology of the P2Y2 receptor, which has an apparent EC50 for INS37217 of approximately 5 μM in electrophysiological recordings made from freshly isolated bovine RPE (Miller SS, personal communications, 2001).

INS37217's nonisotopic ISH techniques, P2Y2 receptor mRNA was localized in specific layers of the retina, including the RPE; in discrete cells in the inner nuclear layer; and in ganglion cells. To evaluate any potential adverse effects of intravitreal INS37217 on electroretinography, we conducted full-field, bilateral ERG recordings in Dutch belted rabbits before and at multiple time points up to 14 days after a single INS37217 administration at a higher-than-ef facious concentration (24 mM). In intravitreal toxicology studies, regulatory considerations strongly recommend that pigmented animals be used to evaluate any potential toxic effects of a pharmaceutical compound. Therefore, pigmented rabbits are preferred to albino rabbits in evaluating the tolerability of an intravitreally administered drug, which explains the choice of pigmented Dutch Belted rabbits rather than albino rabbits for the ERG studies. Our ERG results indicated no significant alterations of scotopic and photopic a- and b-wave amplitudes and latencies up to 14 days after intravitreal administration of INS37217. These results indicate that intravitreal INS37217 does not adversely affect ERG function, at least under the conditions of the present study.

Clinical Implications

Retinal detachment occurs in many ocular conditions and often results in permanent loss of visual acuity if the macula is involved. Photoreceptors are metabolically very active, and detachment separates them from their primary source of oxygen and glucose. Even a shallow detachment can lead to extreme photoreceptor hypoxia and glucose deprivation, as well as photoreceptor remodeling, degeneration, and apoptosis. In experimental models of retinal detachment, Müller cells have been shown to undergo reactive gliosis, hypertrophy, and proliferation, and RPE cells have been shown to proliferate in the subretinal space and along the vitreous-retinal interface. In experimental retinal detachments, the severity of these changes in retina and RPE are correlated with the height and duration of detachments. Similarly, in clinical macula-off detachments, visual recovery after successful surgical reattachment is highly correlated with preoperative visual acuity (generally a function of the height of the detached macula), as well as the duration of the detachment.

These known experimental and clinical findings suggest that a pharmaceutical approach that stimulates subretinal fluid reabsorption may be therapeutically useful if it is able to reduce the height, duration, and extent of a detachment. Such an approach may improve visual outcomes after retinal reattachment surgery. Scleral buckling surgery is the most commonly used procedure for repairing rhegmatogenous retinal detachments, and a routine adjunct procedure that vitreoretinal surgeons use to enhance retinal reattachment consists of using a small needle that is inserted posteriorly through the sclera to drain the accumulated subretinal fluid. Serious complications are more frequently associated with this adjunct procedure than with any component of scleral buckling surgery. Therefore, a pharmacologic approach for stimulating subretinal fluid reabsorption could obviate the need for routine external subretinal fluid drainage, thereby decreasing a significant source of complications associated with scleral buckling surgery. Carbonic anhydrase inhibitors such as acetazolamide and brinzolamide have been shown to enhance subretinal fluid reabsorption in experimental retinal detachment in rabbits, and acetazolamide is clinically used for resolving some forms of macular edema.

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