Inhibition of PVR with a Tyrosine Kinase Inhibitor, Dasatinib, in the Swine

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PURPOSE. We tested the efficacy of dasatinib, a Food and Drug Administration (FDA)-approved tyrosine kinase inhibitor, to prevent proliferative vitreoretinopathy (PVR).

METHODS. The effect of dasatinib on RPE sheet growth was determined by measuring enlargement of cultured RPE sheets in the presence or absence of dasatinib. Epithelial-mesenchymal transition (EMT) of RPE cells was assessed by expression of S100A4. A scratch wound assay, cell number count, and type I collagen contraction assay were used to examine the effect of dasatinib on migration, proliferation, and extracellular matrix (ECM) contraction, respectively. Our swine model of experimental PVR with green fluorescent protein–positive (GFP+) RPE cells was used to assess the efficacy of dasatinib in preventing traction retinal detachment (TRD) caused by PVR. Full-field electroretinography and histologic examination were used to determine the retinal toxicity of dasatinib.

RESULTS. Dasatinib prevented RPE sheet growth, cell migration, proliferation, EMT, and ECM contraction in a concentration-dependent manner. 0.1 μM dasatinib inhibited nearly 80% of vitreous fluid–stimulated collagen gel contraction. Dasatinib also prevented TRD caused by PVR in vivo. Only 1/11 eyes had a TRD in the presence of dasatinib, while all 11 controls eyes had a TRD. Dasatinib did not cause any detectable toxicity of the retina.

CONCLUSIONS. Dasatinib significantly inhibited PVR-related RPE changes in vitro and prevented TRD in an experimental PVR model in the swine without any detectable toxicity. Our data suggested that dasatinib may be effective in the prevention of PVR. *Invest Ophthalmol Vis Sci.* 2013;54:1150–1159 DOI: 10.1167/iovs.12-10418

Proliferative vitreoretinopathy (PVR) is the most common cause of failure in the surgical repair of rhegmatogenous retinal detachment (RRD), as well as a common complication of posterior segment ocular trauma.1,2 Despite recent advances in surgical techniques, nearly 10% of primary repairs for RRD fail due to PVR.3–5

The hallmark of PVR is the formation of an epiretinal membrane on the retinal surface consisting of cells and extracellular matrix (ECM). Several cell types, including RPE cells, glial cells, fibroblasts, myofibroblasts, and immune cells, have been detected in epiretinal membranes from human patients, as well as animal models of experimental PVR.6 RPE and glial cells can dedifferentiate and transform into fibroblasts and/or myofibroblasts, which are considered the contractile cellular phenotypes in epiretinal membranes; there also is clinical and experimental evidence that RPE and glial cells contribute to the final outcome of PVR.7–10 In the early stages of PVR the predominant cell type in epiretinal membranes is the RPE, and thus, is considered to have an important role in the final outcome of this disease.8,9,11,12 RPE cells can attach to the vitreous or the retinal surface, and undergo epithelial-mesenchymal transition (EMT) following detachment from their basement membrane and release from their monolayer on Bruch’s membrane. In this process RPE cells lose their epithelial morphology and transform into fibroblast-like cells.13–16 These cells start to migrate, proliferate, and deposit abnormal ECM, and thus, contribute to the formation of epiretinal membranes.8,17,18 Contraction of such epiretinal membranes results in a traction retinal detachment (TRD) leading to surgical failure and a poor visual results.

These key PVR-related cellular changes in RPE cells are hallmarks of other fibrotic diseases.19,20 Inhibiting one or more of these cellular processes is critical in preventing fibrosis. Tyrosine kinases provide an attractive target, since they are involved in regulation of fibrotic cellular changes. Furthermore, a variety of tyrosine kinase inhibitors (TKIs) are available. Imai et al. has shown that the TKI herbimycin A can reduce TRD significantly in a rabbit model of PVR.21 However, they also have reported localized anatomic damage to photoreceptors, as well as a transient decrease in retinal function. Herbimycin A covalently modifies thiol groups on target tyrosine kinases as well as other proteins.22,23 Using a more selective TKI without covalent modification could possibly reduce the toxic side effect of this drug without jeopardizing the ability to prevent PVR.

Recent studies have suggested that platelet-derived growth factor receptor (PDGFR), a receptor tyrosine kinase, and Src-family kinases (SFKs), a family of nonreceptor tyrosine kinases, have important roles in the development of PVR. PDGFRα has a critical role in PVR induced by fibroblasts and the human RPE cell line ARPE19.24,25 Furthermore, transactivation of PDGFRα by other growth factor receptors, is mediated by SFKs.26,27 It also has been shown that SFKs are important for collagen matrix contraction by ARPE19 cells.28 We chose to study dasatinib, since it can inhibit PDGFRs and SFKs, and is Food and Drug Administration (FDA)-approved for the treatment of chronic myeloid leukemia (CML).29–31 Dasatinib recently has been shown to prevent EMT, migration, and proliferation in...
various cell types, and fibrosis in vivo.32–36 Our studies showed that dasatinib can prevent PVR-related changes of RPE cells in vitro and inhibit TRD caused by PVR in vivo without any detectable toxicity to the retina.

**MATERIALS AND METHODS**

**Isolation of RPE Sheets for Primary Culture In Vitro**

All cell culture supplies were from Invitrogen (Carlsbad, CA) unless stated otherwise. The use of animals was approved by the University of Louisville Institutional Animal Care and Use Committee, and adhered to the ARVO Animal Statement for the Use of Animals in Ophthalmic and Vision Research. Porcine RPE sheets were isolated as reported previously.14 Swine vitreous fluid was obtained in a similar manner as described by Meitinger et al.37 Isolated RPE sheets were placed on porcine posterior capsule and cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) or 25% swine vitreous fluid (diluted 1:3 in DMEM without serum). RPE sheets were cultured in the presence or absence of TKIs listed in the Table. DMSO, used as solvent for the TKIs, served as a control. The concentration of DMSO did not exceed 0.1% in any sample.

**RPE Sheet Growth Assay**

Enlargement of RPE sheet cells were quantified to determine sheet growth over a period of 5 days. Two reference dots were marked on either side of RPE sheets on the bottom of culture dishes. Sequential images that contained the RPE sheet as well as these two reference dots were photographed on days 1 and 6. Photo-montages were prepared using Photoshop (Adobe Systems, Inc., San Jose, CA) for days 1 and 6, respectively. The two photo-montages were aligned using the reference dots, and a single line that went through the two reference dots and the RPE sheet was drawn. The distance from the one edge of the sheet to the other was measured. The difference in the distance between days 1 and 6 was calculated as sheet growth during this period.

**Immunocytochemical Staining**

RPE sheets cultured for 6 days on lens capsules were stained as described previously.14 Primary antibodies for S100A4 (Abcam, Cambridge, MA); an EMT marker,15 and RPE65, RPE differentiation marker (#MAB5482; Millipore, Billerica, MA), an EMT marker, 15 and RPE65, RPE differentiation marker (#MAB5482; Millipore, Billerica, MA) were added, and Alexa647-conjugated donkey anti-rabbit and Alexa488-conjugated donkey anti-mouse secondary antibodies (Invitrogen) were used for detection. Samples were mounted with Vectashield mounting media containing DAPI (Vector Lab, Burlingame, CA) and observed using a Zeiss microscope (Axiovert 200; Zeiss Microimaging, Thornwood, NY). In the absence of primary antibodies (negative control), only faint negligible staining was observed.

**Migration Assay**

The scratch wound assay was used to assess the effect of dasatinib on RPE cell migration. Primary cultured porcine RPE cells at passages 2 and 3 were used. RPE cells (5 × 10⁴) in 150 µL of 10% FBS-DMEM were seeded onto the middle of 35 mm dishes. Following attachment (5–4 hours), 1.5 mL of 10% FBS-DMEM were added and the cells were allowed to grow for 3 days. Cells were pretreated with dasatinib or DMSO (control) in serum-free DMEM for 1 hour before making a scratch in the middle of a confluent patch using a sterile 200 µL pipette. Photograph of the scratch was immediately (time [t] = 0) taken and the medium was replaced with 25% vitreous-DMEM in the presence of dasatinib or DMSO (control). Then, 5 µM aphidicolin (Sigma-Aldrich, St. Louis, MO) were added to the media to inhibit cell proliferation.38 Images of the same region photographed at t = 0 were recaptured after 24 hours in culture. Images were merged using Photoshop, and the migration distance was analyzed at five points with even intervals. Data were normalized to control for each experiment. Each experiment included four replicates, and at least three independent experiments were performed with comparable results.

**Collagen Matrix Contraction Assay**

Collagen matrices (1.0 × 10⁷ cells in 0.1 mL) were placed in 12-well culture plates and allowed to gel at 37°C for 1 hour. Subsequently, matrices were pre-incubated with serum-free DMEM in the presence of dasatinib or DMSO at 37°C for 1 hour. Media was replaced with 25% vitreous-DMEM in the presence of dasatinib or DMSO. Then, the matrices were released gently from the underlying culture plates and photographed immediately. The gels were incubated for 24 hours and photographed. The area of the matrices was quantified using NIH image software. Each experiment included at least three replicates, and at least three independent experiments were performed with comparable results.

**Assays for Proliferation and Apoptosis**

Cultured swine RPE cells (passage 2 or 3) were seeded at 2.0 × 10⁵ per 35 mm dish in 10% FBS–DMEM for 24 hours. Cells were pretreated with dasatinib or dimethyl sulfoxide (DMSO, control) in serum-free DMEM for 1 hour, and subsequently placed in 25% vitreous-DMEM in the presence of dasatinib or DMSO (control). RPE cells were cultured further for 48 hours.

For the proliferation assay, each dish was trypsinized and the cell number counted using a hemocytometer. Each experiment included at least four replicates, and at least three independent experiments were performed with comparable results.

For apoptosis, cells were fixed with 4% paraformaldehyde for 10 minutes, and TUNEL staining was performed using a commercially available kit (DeadEnd Fluorometric TUNEL System; Promega, Madison, WI). Three separate sections were photographed for each dish, and the ratio of TUNEL-positive (TUNEL+) cells determined by analyzing the number of TUNEL+ cells and total nuclei using Image J software (available in the public domain at http://rsbweb.nih.gov/ij/). Three independent experiments with at least three replicates were performed with comparable results.

**TKIs Used in This Study and Their Targets**

<table>
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<tr>
<th>TKI</th>
<th>Dose, µM</th>
<th>Tyrosine Kinases Inhibited</th>
<th>References</th>
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<td>51</td>
</tr>
<tr>
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Fetal RPE Isolation and Culture

Fetal RPE cells, used for induction of PVR in vivo (see below), were isolated from embryonic week 12 swine eyes and cultured as described previously. Normal RPE cells without GFP expression as well as GFP-positive RPE cells (GFP-RPE) were used in this study. In short, RPE cells/partial sheets isolated using dispase were cultured in tissue culture flasks for 2 to 6 weeks, and then plated on transwell and cultured further for 1 to 7 weeks. Cells isolated and cultured by this method were >95% cytokeratin-positive. Transwells with cultured RPE cells were treated with dispase to obtain RPE cell clusters, which were used for PVR induction in vivo.

Effect of Dasatinib on PVR Formation In Vivo

PVR was induced in female domestic swine (Oak Hill Genetics, Ewing, IL) as previously described. In short, PVR was induced using a three-step procedure involving pars plana vitrectomy, total retinal detachment by subretinal balanced salt solution (BSS; Alcon Laboratories, Fort Worth, TX) injection in each of four quadrants using a 39-gauge needle, and injection of RPE cells (8 \times 10^4 cells) into the vitreous cavity.

Clinical examinations were performed postoperatively on days 3, 7, 10, and 14. PVR grading was based on the Silicone Study Classification System for PVR. A single investigator evaluated the retinal status in a masked fashion using indirect ophthalmoscopy. Fundus color photographs were taken using a Topcon fundus camera (Oakland, NJ).

Administration of Dasatinib In Vivo

Dasatinib was administered at a final estimated concentration of 0.3 μM by injecting 12 μM dasatinib in 0.1 mL of 2% DMSO/BSS into the vitreous cavity through a 30-gauge needle attached to a 1 mL syringe. For controls, only 0.1 mL of 2% DMSO/BSS was injected. The amount of dasatinib injected was based on the assumption that the volume of the pig vitreous cavity is approximately 4 mL, and thus, the final concentrations of dasatinib and DMSO were estimated to be 0.3 μM and 0.05%, respectively. Dasatinib was administered on days 0, 3, 7, and 10 for the PVR induction and retinal toxicity studies.

Assessment of Retinal Toxicity of Dasatinib

A subset of control animals (n = 5), that is, swine in which PVR was not induced, was used to assess potential retinal toxicity of dasatinib in vivo using the full-field ERG (fERG) and histology. The induction of PVR was avoided purposely to avert a confounding variable on retinal electrophysiology and structure—namely, a localized retinal detachment.

Electrophysiology. Animals were anesthetized and pupils dilated using the protocol described previously. The fERG recordings were performed following the ISCEV standard for full-field clinical electrophotography using a UTAS ERG System with a BigShot Ganzfeld Stimulator (LKC Technologies, Inc., Gaithersburg, MD) and an ERG-jet electrode (Fabrinal SA, La Chaux de Fonds, Switzerland). After dark adaptation (20 minutes), 0.01 and 3 cd·s·m⁻² stimulations were used for the rod and rod-cone response, respectively. After light adaptation (10 minutes), 3 cd·s·m⁻² stimulation was used for the cone response and flicker ERG.

ERG recordings were performed on days 0, 7, and 14. Fundus exams with indirect ophthalmoscopy were performed on days 0, 3, 7, 10, and 14.

Histologic Evaluation. Following fERG and fundus exam on day 14, the animals were euthanized. Eyes were enucleated and fixed in 4% paraformaldehyde for 48 hours. Plastic sections were prepared and photographs taken as described previously.

Statistical Analysis

The difference in PVR grading was analyzed with the Mann-Whitney U test, and Student’s t-test was used for analysis of in vitro assays (sheet growth, migration, proliferation, and contraction) as well as ERG analyses. MedCalc statistical software Version 12.1.1 (MedCalc

Figure 1. Effect of tyrosine kinase inhibitors on RPE sheet growth stimulated by 10% FBS-supplemented media. (A) Effect of dasatinib on RPE sheet growth. Dasatinib concentration dependently inhibited RPE sheet growth. (B) Effect of SFK inhibitors PP2 and SU6656 on RPE sheet growth. PP3, an inactive analogue of PP2, was used as control. At 10 μM, SFK inhibitors significantly inhibited RPE sheet growth. (C) Effect of imatinib mesylate, an Abl-kinase inhibitor, on RPE sheet growth. Imatinib mesylate had no significant effect even at 10 μM. *P < 0.05, significantly different from control.

Figure 2. Effect of dasatinib on RPE sheet growth stimulated by 25% vitreous fluid-supplemented media. Dasatinib concentration dependently inhibited RPE sheet growth. *P < 0.05, significantly different from control.
Expression of EMT marker S100A4 is reduced in the presence of dasatinib. Representative images of RPE sheets cultured in the presence or absence of dasatinib immunostained for EMT marker S100A4 (red) and RPE differentiation marker RPE65 (green). In the absence of dasatinib (control), RPE cells undergo EMT and strongly express S100A4. Dasatinib concentration dependently reduced the expression of S100A4.
Software, Mariakerke, Belgium) was used for statistical analysis. A P value of less than 0.05 was considered significant.

RESULTS

Effect of Dasatinib on RPE Sheet Growth

Since the growth (enlargement) of the RPE sheet over time involves EMT, migration, and proliferation of RPE cells, it is useful in testing the efficacy of drugs on prevention of these cellular events. Dasatinib significantly reduced RPE sheet growth in DMEM supplemented with 10% FBS (Fig. 1A). This was not due to cell death, since dasatinib, up to 1 \( \mu \)M, had no significant effect on cell viability (data not shown). Three other TKIs with different inhibitory profiles (see Table) were tested to identify which tyrosine kinase inhibited by dasatinib contributed to RPE sheet growth. SFK inhibitors PP2 and SU6656 significantly inhibited growth compared to PP3, an inactive analogue of PP2 (Fig. 1B). In contrast, imatinib mesylate, an Abl kinase inhibitor, had no inhibitory effect on RPE sheet growth even at 1 \( \mu \)M (Fig. 1C).

Vitreous contains a variety of factors that can stimulate RPE cells, and past studies have shown that RPE cells can respond differently to vitreous fluid compared to serum. Thus, the effect of dasatinib on vitreous fluid–stimulated RPE sheet growth also was tested. When cultured in 25% vitreous-DMEM, similar to cells cultured in FBS-supplemented media, dasatinib concentration dependently inhibited RPE sheet growth (compare Figs. 1A and 2).

Effects of Dasatinib on RPE Cell EMT, Migration, Proliferation and Apoptosis

Cells at the edge of RPE sheets cultured under control conditions (without an inhibitor) undergo dedifferentiation, migration, and proliferation. S100A4, an EMT marker also known as fibroblast-specific protein 1, is expressed only in cells that have migrated away from the sheet (Fig. 3A). In contrast, the expression of RPE65, an RPE differentiation marker, was limited to the epithelioid cells within the sheet. The addition of dasatinib to the culture media concentration dependently decreased the expression of S100A4 (Fig. 3).

To examine the effect of dasatinib separate from its effect on EMT and proliferation, a migration assay was done using cultured RPE cells that already had undergone EMT, in the presence of aphidicolin, a proliferation inhibitor. In the absence of dasatinib, vitreous-fluid supplemented media stimulated migration of cells into the open scratched area. Migrating cells were elongated and fibroblast-like. In contrast, migration was prevented significantly in the presence of dasatinib compared to controls in a concentration-dependent fashion (Fig. 4).

The effect of dasatinib on proliferation separate from EMT and migration was determined using sparsely plated cells that already had undergone EMT. Under control conditions, vitreous-supplemented media stimulated proliferation with a significant increase in the cell number during the two-day culture period. Dasatinib inhibited this proliferation in a concentration-dependent manner (Fig. 5). This effect of dasatinib on cell number was not due to increased apoptosis, since even at 1 \( \mu \)M, the highest concentration tested, the percentage of apoptotic cells did not change (Fig. 6).

Effects of Dasatinib on RPE Cell Contraction

ECM contraction has an important role in the development of TRD from PVR. Since contraction is difficult to measure in the RPE sheet culture model, an in vitro collagen gel contraction assay was used to measure the inhibition of contraction by dasatinib. Vitreous-supplemented media induced gel contraction by cultured RPE cells, with gel shrinking by 36% on average during 24 hours incubation in the absence of dasatinib. In contrast, dasatinib inhibited gel contraction in a concentration-dependent manner (Fig. 7).

Efficacy of Dasatinib in Preventing PVR In Vivo

The effect of dasatinib on prevention of PVR was tested in vivo using a swine model. We injected 0.3 \( \mu \)M of dasatinib in the
vitreous cavity, since that concentration inhibited RPE sheet growth, migration, and contraction by more than 80% in our in vitro assay. Dasatinib was injected on days 0 (immediately after RPE cell injection), 3, 7, and 10. Consistent with previous findings, all control eyes (12/12), in which DMSO was injected instead of dasatinib, suffered PVR with TRDs (grade 2 PVR) by day 14 (Fig. 8). A representative fundus photograph demonstrates the TRD that develops within 14 days in a control eye (Fig. 8A, control). In contrast to control eyes, eyes injected with dasatinib did not suffer a TRD in all but one animal (1/12, Fig. 8B). Another animal had retinal wrinkling (PVR grade 1), but did not progress to retinal detachment. Postenucleation analysis of GFP-RPE injected eyes treated with dasatinib showed that despite the lack of TRD, the majority of eyes formed epiretinal membranes (Fig. 8C).

**Toxicity Studies of Dasatinib In Vivo**

Clinical examination did not reveal cataract formation, retinal detachment or vitreous hemorrhage after the intravitreal injection of dasatinib. Full-field ERGs demonstrated no significant effect of the injection of dasatinib on retinal function. Average b-wave amplitude of dark-adapted 0.01 and 3 cd·s·m⁻² stimulations (rod and rod-cone response, respectively) and light-adapted 3

![Figure 6](http://tvst.arvojournals.org/)

**Figure 6.** Effect of dasatinib on RPE cell apoptosis. RPE cells were cultured for 48 hours in the presence or absence of dasatinib in 25% vitreous fluid–DMEM. Following fixation, apoptotic cells were identified by TUNEL assay. (A, B) Representative images of TUNEL staining of cells in control and 1 μM dasatinib condition, respectively. Apoptotic (TUNEL+) cells are indicated by arrowheads. (C) Quantified data showed no difference between dasatinib and control. Data are from three different experiments with at least three replicates for each experiment.

![Figure 7](http://tvst.arvojournals.org/)

**Figure 7.** Effect of dasatinib on RPE cell contraction. Cell/collagen matrices containing 1.0 × 10⁵ cultured swine RPE cells in 1.5 mg/mL type I collagen were allowed to gel at 37°C for 1 hour. DMEM supplemented with 25% vitreous, in the presence or absence of dasatinib, was added to each well, and the matrices were released from the underlying culture plates. Photographs were taken immediately (t = 0) and after 24 hours incubation (t = 24 hours). (A) Representative images of gels before (t = 0) and after (t = 24 hours) contraction in the presence of varying doses of dasatinib. (B) Average gel contraction normalized to control (without dasatinib). Dasatinib prevented gel contraction in a concentration-dependent manner. Data are from at least three different experiments with minimum of three replicates for each experiment. *P < 0.05, significantly different from control.
cd·s·m⁻² stimulations (cone-response and flicker ERG) on days 7 and 14 of dasatinib-injected eyes did not change from baseline (day 0, before injection of dasatinib or DMSO, Fig. 9A). In addition, there was no significant change from DMSO-injected control eyes on day 14. Light microscopic evaluation of histologic sections showed no obvious change between dasatinib-injected and control eyes (Figs. 9B, 9C).

**DISCUSSION**

We examined the effect of dasatinib on PVR-related changes of RPE cells in vitro, as well as in an experimental model of PVR induced by RPE cells. RPE cells were used, since these cells are believed to have a critical role in PVR development, especially during early stages.¹¹,¹² Dasatinib prevented RPE sheet growth in vitro in a concentration-dependent fashion and inhibited the development of TRD in vivo. Dasatinib was developed originally as a dual inhibitor for SFKs and Abl kinases, and currently is known to inhibit a variety of tyrosine kinases, including PDGFR and c-Kit (see Table).²⁹,₃₀,₄₈-₅₃ To obtain an idea of which tyrosine kinases targeted by dasatinib contribute to RPE sheet growth, we tested three other TKIs. SU6656, a SFK-specific inhibitor,⁵⁴ and PP2, a SFK-selective inhibitor that also blocks Abl, PDGFR, and c-Kit,⁵⁴-⁵⁸ significantly inhibited RPE sheet growth. In contrast, imatinib mesylate, an Abl kinase inhibitor that also blocks PDGFR and c-Kit,⁵₀,⁵₉,₆₀ did not have any significant effect on RPE sheet growth. Taken together, our data suggested that SFKs, at least in part, have a role in RPE sheet growth. The lack of effect of imatinib mesylate seemingly contradicts the previously reported role of PDGFR in ARPE19 cell proliferation.²⁴ However, the same study showed that ARPE19 cells can, albeit to a reduced extent, proliferate in the absence of PDGFR. Since RPE sheet growth in our model involves not only cell proliferation, but also EMT and migration, the lack of an effect of imatinib mesylate may represent a combination of PDGFR-independent proliferation, as well as an absence of an effect on EMT and/or migration in RPE sheet growth. Studies will be conducted in the future to identify tyrosine kinases involved in each of these cellular responses.

Dasatinib inhibited RPE cell EMT, migration, proliferation, and contraction in a concentration-dependent manner. Dasatinib at 0.1 µM inhibited migration by 60%, while proliferation was inhibited by 50%. Contraction was most sensitive with 0.1 µM dasatinib inhibiting nearly 80% compared to controls. Interestingly, a report by Lei et al. also showed vitreous fluid-stimulated gel contraction by ARPE19 cells were more sensitive than proliferation to PDGFR expression levels.²⁴ Src and FAK, another known target of dasatinib, also have been shown to

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**Figure 8.** The effect of dasatinib on PVR development in vivo. (A) Development of TRD and its inhibition by dasatinib. Eyes injected with RPE cells (non-GFP) are shown in this example, but similar results were obtained with eyes injected with GFP-RPE. In the absence of dasatinib (control), contraction over time leads to a localized TRD by day 14. In contrast, the eye treated with dasatinib does not suffer TRD. (B) PVR grading on day 14 in the presence or absence of dasatinib in a swine model of PVR. Dasatinib prevented TRD (PVR grade 2 or higher). Only one of 12 eyes injected with dasatinib resulted in TRD, while the retina was detached in all control eyes (12/12). There was a statistically significant difference between the control and experimental eyes on day 14. *P < 0.05, significantly different from control. Classification of PVR grade used in this study is shown below. (C) Photographs of the fundus on day 14 and the eyecup of an eye injected with GFP-RPE and treated with dasatinib. The images show the lack of retinal detachment despite the presence of a GFPþ epiretinal membrane.
contribute to collagen gel contraction by unstimulated (serum-free media) and stimulated (TGF-β/serum or PDGF) ARPE19 cells. Since dasatinib is known to inhibit PDGFR, SFKs, and FAK, it is possible that PDGFR and/or SFKs have a role in contraction in our in vitro assay.

Dasatinib significantly inhibited PVR, in particular TRD, in vivo. To our surprise, analysis of enucleated eyes showed that the majority of eyes injected with dasatinib formed epiretinal membranes consisting of GFP+ cells. Since dasatinib is known to inhibit PDGFR, SFKs, and FAK, it is possible that PDGFR and/or SFKs have a role in contraction in our in vitro assay.

Dasatinib significantly inhibited PVR, in particular TRD, in vivo. To our surprise, analysis of enucleated eyes showed that the majority of eyes injected with dasatinib formed epiretinal membranes consisting of GFP+ cells. This suggests that RPE sheets/cell clusters injected during the induction of PVR grew during the 14-day period of observation. Dasatinib was injected twice a week with an estimated intravitreal concentration of 0.3 µM, which should have prevented RPE sheet growth significantly based on our in vitro data. However, intravitreal concentrations of drugs are known to decrease over time, with the half-life ranging from 1 hour to several days. Importantly, except for 1 eye out of 12, TRD was not observed in the presence of dasatinib. While we do not know the half-life of dasatinib in our in vivo model, based on in vitro data showing contraction being more sensitive to dasatinib than cell growth, it is possible that a reduced concentration of intravitreal dasatinib allowed cell growth, but was still sufficient to prevent contraction and localized TRD. We plan to conduct experiments to determine the half-life of dasatinib, and to identify a method of intraocular delivery that can sustain the concentration of dasatinib in the eye at levels that would prevent formation of epiretinal membranes.

The swine, in contrast to other species, has an eye with many similarities to the human eye, such as size and anatomy, including a holangiotic retinal vasculature and a cone-enriched area centralis. Our study used a swine model of experimental PVR that reproduces the key features found in human PVR, and 0.3 µM dasatinib significantly reduced the incidence of TRD without any detectable toxic effect on the swine retina during the limited duration of the study. While further tests will be required to confirm that similar results are achieved in other cell types involved in PVR in man, this FDA-approved medication may have an important role in preventing PVR.

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References


