Assessment of the Integrin α5β1 Antagonist JSM6427 in Proliferative Vitreoretinopathy Using In Vitro Assays and a Rabbit Model of Retinal Detachment

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PURPOSE. To explore the role of integrin α5β1 in proliferative vitreoretinopathy (PVR) pathogenesis by evaluating the expression of α5β1 on ARPE-19 cells and patient proliferative membranes, quantifying the inhibitory effects of JSM6427 (a small molecule α5β1 inhibitor) on ARPE-19 cell adhesion and migration, and assessing the therapeutic potential of JSM6427 in a rabbit retinal detachment model.

METHODS. Expression of α5β1 was evaluated on activated ARPE-19 cells by flow cytometry and on PVR membranes by immunohistochemistry. ARPE-19 cells were used in fibronectin-dependent adhesion and migration assays with various concentrations of JSM6427; IC50 was calculated. In the rabbit model, eyes were intravitreally injected with vehicle or JSM6427 on day 0 or 1 after retinal detachment; BrdU was administered intravitreally on day 3, and retinal tissues were harvested on day 3 (4 hours later) or 7. Retinal scarring, cellular proliferation, and inflammatory responses were quantified, and retinal morphology was analyzed in retinal sections.

RESULTS. Activated ARPE-19 cells and PVR membranes expressed high levels of α5β1; expression was low in control eyes. JSM6427 provided a dose-dependent blockade of ARPE-19 cell adhesion to fibronectin (IC50, 7.1 ± 2.5 μM) and inhibition of migration (IC50, 6.0 ± 4.5 μM). In the rabbit model, intravitreal injection of JSM6427 provided significant inhibition of proliferation of retinal cells (Müller cells, microglia, and macrophages) on days 3 and 7 after detachment and inhibition of inflammatory response and retinal scarring on day 7 after detachment.

CONCLUSIONS. JSM6427 is a promising treatment for PVR, with data suggesting that inhibition of α5β1-fibronectin interactions addresses multiple pathways involving retinal pigment epithelial, glial, and inflammatory cells. (Invest Ophthalmol Vis Sci. 2010;51:1028–1035) DOI:10.1167/iovs.09-3575

Proliferative vitreoretinopathy (PVR) is an aberrant wound-healing process characterized by intraocular inflammation, cellular proliferation, and scarring.1 A common complication of primary rhegmatogenous retinal detachment with an estimated risk ranging from 5% to 12%, PVR also is associated with other conditions such as penetrating ocular trauma and retinal translocation surgery.2 The management of PVR by vitrectomy has limited success in terms of visual benefits,2 even when adjunctive treatments, such as daunorubicin or 5-fluorouracil plus heparin,4,5 are administered. Current experimental pharmacologic treatments for retinal detachment and PVR based on nonspecific inhibition of cell proliferation have been unsuccessful so far, indicating that there is a great need for new therapeutic approaches.

The pathogenesis of PVR involves a wide variety of cell types, including retinal pigment epithelial (RPE) cells, monocyte/macrophages, microglial, glial cells (astrocytes, Müller cells, and perivascular glia), and fibroblasts/myofibroblasts.1,6–8 Evidence suggests that transdifferentiation of migrating RPE cells is a critical early step in PVR pathogenesis,1,9 with Müller cell activation10 and hypertrophy onto the retinal surface also being centrally involved in the formation of contractile epiretinal membranes.11 Migration and adhesion of dislocated RPE cells have been shown to be mediated primarily through interactions between fibronectin (FN) and the FN-specific integrin, α5β1.12 Both α5 and β1 integrin subunits13 and the corresponding ligand FN14–16 have been detected in proliferative retinal membranes, including those associated with PVR. The concentrations of FN in eyes of patients with PVR tended to increase with the severity of clinical stage, suggesting that elevated levels of FN in the subretinal fluid and in the vitreous may represent an early event in the pathogenesis of PVR.17

Cytokines and growth factors, such as interleukin-1β, interleukin-6, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), and basic fibroblast growth factor (bFGF), also play an important role in promoting the pathogenesis of PVR.12,18–23 Notably, bFGF, EGF, and PDGF have been found to promote RPE cell proliferation,20,21 and TNF-α has been shown to induce adhesion, migration, and upregulation of α5β1 integrin expression on RPE cells.12 Furthermore, bFGF has been shown to stimulate Müller cell proliferation and epiretinal membrane formation in an animal model,22 whereas PDGF, TGF-β, and insulin-like growth factor have been shown to promote tractional force generation by Müller cells in vitro.23

JSM6427, 3-2-[1-alkyl-5-[(pyridin-2-ylamino)-methyl]-pyrrolidine-3-yloxy]-[acetylamino]-2(alkylamino)-propionic acid, is a
small (590-Da) molecule inhibitor of the α5β1-FN interaction, with good tissue penetration and stability in physiological buffers. JSM6427 has a selectivity at least 1,700-fold greater for α5β1 than for other integrins (αvβ3, 1,700-fold; αvβ5, 7,500-fold; α3β1, 100,000-fold) expressed in normal RPE. and has been shown to result in a dose-dependent reduction in FN-mediated ERK-1/2 phosphorylation in ARPE-19 cells. The present study was designed to explore the role of integrin α5β1 in PVR pathogenesis by evaluating the expression of α5β1 on ARPE-19 cells and on proliferative membranes from patients with PVR, by quantifying the inhibitory effects of JSM6427 on ARPE-19 cell adhesion and migration, and by assessing the effects of JSM6427 on retinal scarring, cellular proliferation, inflammation, and Müller cell hypertrophy in a rabbit model of retinal detachment.

### Materials and Methods

#### Flow Cytometric Analysis of Integrin Expression on ARPE-19 Cells

Antibodies were purchased from BD Pharmingen (Heidelberg, Germany) (α5, BD555651, clone VCl; αvβ3, BD555504, clone 23C6; αv, BD555501, clone 9E10; secondary antibody, BD550874) or from Milipore (Schwalbach, Germany) (β1, MAB1951F, clone P4G11; αvβ5, MAB1961Z, clone PF16). The integrin β1 antibody was directly conjugated with fluorescein isothiocyanate. ARPE-19 cells (LGC Promochem ATCC, Wesel, Germany) were incubated in phosphate-buffered saline (PBS)/1% heat-inactivated fetal calf serum (FCS)/50 nM phorbol myristate acetate (PMA), and primary anti-integrin antibodies (0.4–1 μg/mL) for 30 minutes; negative control wells were incubated without a primary antibody. The cells were incubated with allophycocyanin-conjugated secondary antibody (1 μg/mL, BD550874), washed with PBS/1% FCS after each incubation step, and analyzed by flow cytometry (FACS Calibur; BD Bioscience, San Jose, CA).

#### ARPE-19 Adhesion Assay

ARPE-19 cells were grown to 80% confluence in Dulbecco’s modified Eagle’s medium (DMEM)/Ham’s F12 medium (Biochrom AG, Berlin, Germany) supplemented with 10% FCS and 2 mM glutamine on 96-well plates coated with 10 μg/mL FN (Millipore) and blocked with 1% bovine serum albumin (BSA). The cells were detached with cell dissociation solution (Sigma-Aldrich, Taufkirchen, Germany) and incubated in 25 mM HEPES/150 mM NaCl/2 mM EDTA (pH 7.4). They were transferred to EDTA-free buffer with 50 nM PMA and 2 mM MgCl2, and incubated for 15 minutes. Serial dilutions of JSM6427 or antibodies IIA1 (integrin α5β1; BD Pharmingen), MAB1976Z and MAB1961Z for αvβ3 and αvβ5 (Millipore), respectively, were added. A stock solution of JSM6427 (Jenri AG, Berlin, Germany) was prepared in dimethyl sulfoxide (DMSO). DMSO concentrations were adjusted for all samples and did not exceed 0.5%. Cells (5 × 104 cells/well) were plated on FN-coated plates for 45 minutes at 37°C and washed once with PBS. Adherent cells were fixed (5% glutaraldehyde), rinsed with PBS, and stained with 0.1% crystal violet. After the plates were rinsed with PBS, 10% acetic acid was added and the plates were analyzed at 570 nm (Spectra Max M5 microplate reader; Molecular Devices, Sunnyvale, CA). The experiments were performed in triplicate. The concentration required for 50% inhibition (IC50) was determined by computer (XLfit software; IDBS, Guildford, UK). The data were analyzed by one-way analysis of variance and are presented as the mean ± SD.

#### ARPE-19 Migration Assay

The undersides of cell migration filters (8 μm pore; Transwell BD Falcon HTS FluoroBloc; VWR International, Darmstadt, Germany) were coated with 10 μg/mL FN (120 kDa fragment; Millipore) and blocked with 2% BSA. ARPE-19 cells grown to 80% confluence in DMEM/Ham’s F12 medium (Biochrom AG) and to quiescence in DMEM/Ham’s F12 medium/0.1% BSA without FCS (assay medium) were trypsinized and washed. The cells (2.5 × 104) were preincubated in assay medium containing 10 ng/mL bFGF with the same concentrations of JSM6427 as in wells, plated in the upper compartment, allowed to migrate through the filters for 5 hours, and then stained with 4 μM calcein AM (Invitrogen, Carlsbad, CA) in Hanks’ balanced salt solution. All experiments were performed in triplicate. Fluorescence was measured at 485/538 nm (SpectraMax M5 reader; Molecular Devices). The mean fluorescence for cells cultured in the absence of bFGF was set at 100%. For some experiments, photographs were taken by a masked observer, and the total area of fluorescence was quantified with image-analysis software (ImageJ software), and IC50 was determined (XLfit software; IDBS). The value for cells incubated with bFGF was set at 100% and the background of noncoated, BSA-blocked wells was subtracted. For cytotoxicity assays, ARPE-19 cells were grown for 2 days in the presence of serial dilutions of JSM6427. The cells were subsequently fixed, stained with crystal violet, and quantified as described for adhesion assays.

### Evaluation of Integrin Expression on Human PVR Membranes

Procedures were conducted in conformance with the principles of the Declaration of Helsinki, and the patients gave informed consent. Thirteen patients with disorders associated with pathologic preretinal membranes (PVR associated with retinal detachment, n = 8; idiopathic epiretinal membranes, n = 5) underwent surgical removal of the membranes. The membranes were fixed (4% paraformaldehyde), transferred to PBS, and paraffin embedded. Similarly processed retinal tissues from three pyogenic granulomas served as a positive control for α5β1 labeling, whereas sections from a glaucomatous eye and an eye with a uveal melanoma (taken from an area distinct from the tumor) were used as normal controls. Sections were deparaffinized and rehydrated, microwaved in 0.01 M citrate buffer (pH 6.0), washed twice in purified water, blocked with 5% Tris-buffered saline (TBS)/1% BSA, and washed three times with TBS/1% BSA. Immunohistochemistry was performed with mouse monoclonal anti-human (1:700, MAB1969, Millipore) or polyclonal rabbit anti-α5β1 (1:6000, AB1928 or AB1921; Millipore) primary antibodies diluted in TBS/1% BSA. All antibodies showed similar staining patterns. Vasculature was labeled with polyclonal rabbit anti-human von Willebrand factor antibody (1:50,000, A0082; Dako, Hamburg, Germany), macrophages were labeled with mouse monoclonal anti-human, CD68 (1:50, PG-M1, Dako), gial cells were labeled with mouse monoclonal anti-human glial fibrillary protein (1:400, GFAP; 6F2, Dako), and RPE cells were labeled with monoclonal mouse anti-human CK18 (1:500, Cy-90; Sigma-Aldrich). Primary antibodies were detected with an ABC assay (StreptABComplex/HRP Duet Mouse/Rabbit kit; A0492; Dako) and sections were counterstained with Mayer’s Haemalaun.

### Rabbit Model of Retinal Detachment

All experimental procedures performed in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and to the guidelines of the facility Animal Resource Center. Before the procedure, rabbits were sedated with an intramuscular injection of xylazine (3 mg/kg)/ketamine (15 mg/kg; Henry Schein, Inc., Melville, NY) and proparacaine was applied topically to the eye (Henry Schein, Inc.). Approximately one half of the inferior retina was detached in the right eyes of adult New Zealand Red pigmented rabbits by infusing hyaluronate sodium (Healon; Pharmacia, Piscataway, NJ) in 0.25% balanced salt solution via a glass pipette between the neural retina and RPE (sodium hyaluronate was used to prevent retinal reattachment). In three eyes with retinal detachments, 1 mg JSM6427 in 50 μL PBS was
intravitreally injected with a 30-gauge needle via the same hole as the pipette; three control rabbits were injected with PBS (vehicle) alone. Both 3- and 7-day time points were examined. In the 3-day group, JSM6427 or vehicle injections were administered on either day 0 (immediately after creating the detachment) or 24 hours later (day 1). On postdetachment day 5, 10 µg BrdU (Sigma-Aldrich) in 50 µL BSS was injected intravitreally and euthanatization (120 mg/kg intravenous sodium pentobarbital; Henry Schein, Inc.) was performed 4 hours later, after which retinal tissues were harvested. These experiments established that injection on postdetachment day 1 was more effective than on day 0; therefore, in the 7-day experiment, injections were administered on postdetachment day 1, bromodeoxyuridine (BrdU) was again administered on day 3, and euthanatization was performed on day 7.

The rabbit eyes were enucleated and fixed (4% paraformaldehyde/0.1 M sodium cacodylate buffer, pH 7.4; Electron Microscopy Sciences, Fort Washington, PA). Retinal tissue (0.1 M sodium cacodylate buffer, pH 7.4; Electron Microscopy Sciences, Fort Washington, PA). Retinal tissue was excised from three regions from the nondetached control eyes. Tissues were rinsed three times with PBS or lectin were added in PBTA overnight at 4°C. Anti-BrdU (1:200; Accurate Chemical and Scientific Corp., Westbury, NY) was used to label dividing cells, anti-vimentin (1:500; Dako) was used to label Müller cells, and isolectin B4 Griffonia simplicifolia (1:50; Vector Laboratories, Burlingame, CA) was used to label microglia and macrophages. After the sections were washed in PBTA, they were incubated with pooled secondary antibodies (streptavidin CY5, donkey anti-rat CY3, and donkey anti-mouse CY2, all at 1:200 in PBTA; Jackson Immunoresearch, West Grove, PA) then washed in PBTA. They were mounted with 5% n-propyl gallate/glycerol, and single-plane images were taken from at least four sections from three different regions within each eye (Fluoview 500 laser scanning confocal microscope; Olympus, Tokyo, Japan) to quantify BrdU-labeled cells or subretinal scars and to measure the length of subretinal scars. Labeled cells (either anti-BrdU/vimentin or isolectin B4) were counted per millimeter of retina from the stored images by using an embedded magnification bar. For each experimental group, at least 24 section areas were quantified. To reveal the morphology of the retina, high-quality images were taken as a Z series of five planes and collapsed as a projection. All values are expressed as the mean ± SEM. Statistical comparisons between treatment groups were analyzed using the paired Student’s t-test, P < 0.05 was considered significant.

RESULTS

In Vitro Characterization of ARPE-19 Cells

After retinal detachment, RPE cells have been found to migrate from the basement membrane and, in contacting the vitreous, exhibit a more motile phenotype that is accompanied by altered integrin expression.26 In our assays, PMA-activated ARPE-19 cells revealed high surface expression of α5 integrin and its corresponding β1 subunit as determined by semiquanitative flow cytometry (Fig. 1). Other FN-binding integrins (αvβ3, αvβ5, and α4β1) were also expressed on ARPE-19 cells but at lower levels than α5.

To evaluate the inhibitory potential of the integrin α5β1-specific inhibitor JSM6427 in preventing RPE cell attachment to FN, PMA-activated ARPE-19 cells were allowed to bind to FN in the presence of various concentrations of JSM6427. JSM6427 provided a dose-dependent blockade, with a mean IC50 of 7.1 ± 2.5 µM calculated from four independent experiments; a representative experiment is shown in Figure 2. Blocking with an α5-specific antibody provided a 42% reduction in ARPE-19 adhesion to FN (P < 0.01) while blocking with antibodies specific for αvβ3 and αvβ5 did not prevent cell adhesion (Fig. 2), suggesting that α5 is a predominant integrin involved in FN-mediated binding by ARPE-19 cells.

Boyden chamber migration assays (Transwell; VWR International) were used to evaluate the effects of bFGF in inducing
migration of ARPE-19 cells toward FN. No migration was seen in uncoated wells (Fig. 3) in contrast to FN-coated wells, which strongly induced migration that was enhanced with bFGF. JSM6427 dose-dependently inhibited migration of ARPE-19 cells in the presence of FN/bFGF, with effective concentrations in the low micromolar range (IC50, 6.0 ± 4.5 µM). JSM6427 did not affect cell viability at concentrations of up to 50 µM excluding that the observed effects were caused by cytotoxic effects of JSM6427 on ARPE-19 cells.

Integrin Expression in Surgically Excised Human Proliferative Membranes

To determine the level of expression of α5β1 on patient eyes with retinal proliferative diseases, 14 membranes from 13 patients were evaluated by immunohistochemistry (PVR after retinal detachment, n = 8; idiopathic epiretinal membranes, n = 5). Of these 14 membranes, 9 were suitable for semiquantitative analysis of all cell markers; the results of this analysis are presented in Table 1.

Photographs of a membrane from a patient with PVR (case 5) are shown in Figures 4A–F and from a patient with an idiopathic epiretinal membrane (case 2) are shown in Figures 4G–L. All tissues had a consistent and prominent immunoreactivity with all three anti-α5β1 antibodies tested (two polyclonal and one monoclonal). Representative images for one of the polyclonal antibodies and the monoclonal antibody are shown in Figures 4A, 4G, 4B, and 4H. The immunoreactivity primarily colocalized with cells that were also positive for CK18 (Figs. 4C, 4D) and CD68 (Figs. 4E, 4K), respectively, indicating that the cells were most likely of RPE and/or macrophage origin, and occasionally with cells that were positive for factor VIII (Figs. 4F, 4L). Numerous cells, particularly in the membrane from the patient with the idiopathic epiretinal membrane, were also positive for GFAP (Figs. 4D, 4J), indicating the glial cell origin of these cells. Generally, there was little reactivity with the factor VIII antibody in these membranes (Figs. 4F, 4L). In contrast, positive-control retinal tissues from three pyogenic granulomas showed a highly vascularized inflammatory condition characterized by strong α5β1 expression on endothelial and inflammatory cells within the stroma and intense labeling of vascular channels (Fig. 4M). Negative-control retinal tissues from the glaucomatous eye (Fig. 4N) and an eye with uveal melanoma showed no appreciable labeling of RPE cells for α5β1 and only minor immunoreactivity in choroidal vessels but not in the choriocapillaris. Retinal vessels also had only minor immunoreactivity with the α5β1 antibodies.

Rabbit Model of Retinal Detachment

To investigate the effects of JSM6427 (1 mg) in preventing the proliferative and scarring changes resulting from retinal detachment, a rabbit model was used. Approximate intraocular levels of JSM6427 were 500 µM immediately after injection and 70 µM at 24 hours after injection (unpublished data). In this model, BrdU was administered on postdetachment day 3, and the eyes were harvested 4 hours later to examine the effects of JSM6427 on cell proliferation. Another group of similarly treated eyes was harvested on day 7 to examine the effects of JSM6427 on proliferation, inflammation, and subretinal glial scar formation. Representative images from normal, untreated (control) detached, and treated detached rabbit retinal tissues are shown in Figure 5. No glial scarring was observed on day 3 in either vehicle- or JSM6427-treated eyes.

Intravitreal injection of JSM6427 resulted in a significantly greater inhibition of total cellular proliferation (Müller cells, photoreceptors, and retinal pigment epithelial cells).

Table 1. Semiquantitative Analysis of Immunohistochemical Assay of Membranes from Patients with PVR and Idiopathic Epiretinal Membranes

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(a) Polyclonal rabbit anti-α5β1 antibody (AB1928), (b) polyclonal rabbit anti-α5β1 antibody (AB1921), (c) monoclonal mouse anti-α5β1 antibody MAB1968. Staining: ++, strong; +, medium; and (+), weak; --, negative; ND, not determined; iERM, idiopathic epiretinal membrane.
greater inhibition of the proliferation of Müller cells (Fig. 6B), microglia (Fig. 6C), and macrophages (Fig. 6D) compared with vehicle when injections were administered at day 1 postdetachment. In this model, Müller cells comprised the majority of proliferating cells (Fig. 6B). Both JSM6427 and vehicle injections resulted in significantly greater inhibition of proliferation of total cells and Müller cells when injections were administered on day 1 compared with those administered on day 0 (Figs. 6A, 6B).

At 7 days postdetachment, retinal sections of eyes injected with JSM6427 on day 1 had significant reductions in the total number of anti-BrdU-positive cells (Fig. 6A), anti-BrdU-positive Müller cells (Fig. 7A), isoclitin B4-positive cells (macrophages and microglia; Fig. 7B), subretinal scars (Fig. 7C), and length of subretinal scars (Fig. 7D) compared with vehicle-injected eyes. JSM6427 significantly inhibited the inflammatory response and glial scarring as demonstrated by the reduction in the number of microglia and macrophages (Fig. 7B) and by the reductions in the number and length of subretinal scars (Figs. 7C, 7D).

**DISCUSSION**

The present studies demonstrated that JSM6427 provided dose-dependent inhibition of adhesion and migration of RPE cells in response to FN. This provides further support of the important role of α5β1-FN-mediated signaling in the pathogenesis of PVR. Similar results were obtained with JSM6427 in assays using primary human fetal RPE cells (Li R, et al., manuscript in preparation). Our comprehensive evaluation of 14 membranes from 13 patients demonstrated that α5β1 was highly expressed on proliferative membranes but not on normal retinal tissues or RPE cells. This extends the findings of an early study demonstrating that α5 and β1 were expressed in two eyes with PVR but were not expressed on the normal RPE cell layer. Based on our results, the α5β1-positive cells in proliferative membranes were shown to be not only RPE cells and/or macrophages but also of glial cell origin. These findings are consistent with studies demonstrating glial cells in both PVR membranes and idiopathic epiretinal membranes. In addition, our results of in vitro experiments demonstrating that α5β1 was upregulated on activated ARPE-19 cells are consistent with a study showing that α5β1 is expressed at low levels on quiescent primary human RPE cells and is upregulated on proliferating cells. Since RPE cells appear to mediate the contraction of epiretinal membranes in PVR, the upregulation of α5β1 during transdifferentiation and its interaction with FN may be an important step in the disease pathogenesis by leading to the fibrotic response and contraction of membranes after retinal detachment.

In addition to RPE cells, the pathogenesis of PVR involves a wide variety of other cell types such as monocyte/macrophages, microglia, glial cells such as Müller cells, and fibroblasts/myofibroblasts. In our immunohistochemical analysis, we demonstrated co-localized expression of α5β1 with these cell types, supporting their role in PVR pathogenesis. In the study of retinal detachment in the rabbit, JSM6427 significantly reduced the intraretinal proliferation of Müller cells and the presence of inflammatory cells (microglia and macrophages) while also reducing the number and length of subretinal glial scars. Previous studies in animal models have demonstrated that the gliotic response leading to the formation of subretinal scarring begins within 1 day of a retinal detachment and is associated with increased expression of GFAP and vimentin and that the glial membranes continue to expand for as long as the retina remains detached. Experimental retinal detachment was also shown to promote increased proliferation of all nonneuronal cell types that begins within a day after detachment, peaks between days 3 and 4, and continues at low levels for
weeks after detachment. It is not known what role, if any, this proliferation plays in the formation of glial scars. Also, it is not known if JSM6427 affects glial scar formation through its antiproliferative effects or by some other mechanism, perhaps by inhibiting the expansion of Müller cells into the subretinal space. The fact that integrins mediate proliferation and migration may indicate, however, that JSM6427 affects both events.

It is interesting that the inhibitory effects of JSM6427 on cellular proliferation in our rabbit retinal detachment model were greater when the agent was administered 1 day after detachment than when given immediately after detachment. The reasons for this are unknown. One possibility is that the needle puncture on day 1 caused a “rescue” effect mediated by upregulation and release of endogenous survival factors. This possibility would also explain the slight inhibitory effect of the vehicle injection comparing day 0 and day 1. Another possibility is that JSM6427 is quite rapidly eliminated (GZ, unpublished data, 2008), as generally occurs for small molecules after intravitreal injection, as gener-

Although the current rabbit model did not permit a detailed analysis of RPE cells, it is likely that RPE cells had an effect on other cell types. During the pathogenesis of PVR, a shift from cell–cell to cell–substrate contacts promotes the transdifferentiation of RPE cells, resulting in morphologic changes and expression of growth factors and extracellular matrix proteins. RPE cell-derived growth factors can act not only in an autocrine manner but also in a paracrine manner to activate Müller cells and transdifferentiated RPE cells are proposed to

Figure 5. Representative images of rabbit retinal tissues on day 3 (A–C) or day 7 (D, E) after detachment: JSM6427 was administered intravitreally 1 day after detachment. (A) On day 3, no proliferating cells, as demonstrated by anti-BrdU immunoreactivity, were detected in the nondetached (control) retina. Anti-vimentin labeling of Müller cells (green) extended into the outer nuclear layer (ONL), and microglia (blue) were confined to the inner plexiform layer. (B) In the detached vehicle-injected retina, many Müller cell nuclei were labeled with anti-BrdU (red) in the inner nuclear layer (INL), whereas the anti-vimentin labeling of Müller cells extended to the outer limiting membrane (OLM, arrow). Many retinal regions contained fewer microglia. A macrophage is present in the subretinal space (blue). (C) In the detached JSM6427-treated retina, fewer Müller cell nuclei were labeled with anti-BrdU, whereas the morphology of Müller cells appeared similar to those of the untreated controls. (D) On day 7, in the vehicle-injected detached retina, many anti-BrdU-labeled Müller cells were seen in the retina and in regions where the Müller cell cytoplasm extended into the subretinal space forming a subretinal scar (see inside the white brackets). Many macrophages are present in the subretinal space associated with the glial scar. (E) The number of anti-BrdU-labeled Müller cell nuclei, microglia, and macrophages was reduced in the JSM6427-treated detached retina, as well as the number and size of Müller cells extending into the subretinal space (enclosed in brackets). Arrow: OLM. GCL, ganglion cell layer; OS, outer segments. Scale bar, 50 μm.
be the most important triggers of PVR. JSM6427 resulted in inhibition of multiple \( \alpha_5 \beta_1 \)-mediated effects in vitro and in vivo, suggesting that it provides therapeutic benefits in the treatment of PVR.

Acknowledgments

The authors thank Seike Gericke (Jerini AG) for technical assistance with the in vitro assays and Linda Whetter, DVM, PhD (Zola Associates), for assistance with manuscript preparation.

References

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**Figure 6.** Analysis of anti-BrdU-labeled cells in detached retinas at days 3 and 7 after intravitreal injection of JSM6427 and vehicle, respectively, on postdetachment days 0 (d0) and 1 (d1) (for tissues harvested on day 3) or postdetachment day 1 only (for tissues harvested on day 7). Labeled cells were quantified in at least four retinal sections from three different regions within each eye. Total cells (A), Müller cells (B), microglia (C), and macrophages (D). Data are expressed as the mean ± SEM.

**Figure 7.** Analysis of proliferation, inflammatory cells, and subretinal scarring in detached retinas at day 7 after intravitreal injection of JSM6427 and vehicle, respectively, on day 1 (d1). Labeled cells were quantified in at least four retinal sections from three different regions within each eye. (A) Müller cells, (B) macrophages and microglia, (C) subretinal scars, and (D) length of subretinal scars.


