The Antiangiogenic Effects of Integrin α5β1 Inhibitor (ATN-161) In Vitro and In Vivo

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PURPOSE. Integrin α5β1 is involved in the development of choroidal neovascularization (CNV). Thus, the inhibition of integrin α5β1 may provide an alternative to the current standard of CNV therapy, which involves inhibition of vascular endothelial growth factor (VEGF) and is not effective in all patients. This study evaluated the antiangiogenic effects of ATN-161, a small peptide inhibitor of integrin α5β1, in human choroidal endothelial cells (hCECs) and in laser-induced CNV in rats. Furthermore, the utility of spectral-domain optical coherence tomography (SD-OCT) for dynamic observation of the development of CNV in animal models was assessed.

METHODS. The antiangiogenic potential of ATN-161 was evaluated in VEGF-stimulated hCECs by MTS proliferation assays, migration assays, and synthetic matrix capillary tube formation assays. To evaluate the antiangiogenic effects of ATN-161 in vivo, CNV was induced in rats by laser photocoagulation. ATN-161, scrambled peptide, or AF564 anti-VEGF antibody, were injected intravitreally immediately after photocoagulation. Eyes were examined by SD-OCT and fluorescein angiography on days 1, 7, and 14 after injection, and the areas of CNV were measured by analysis of choroidal flatmounts at day 14.

RESULTS. ATN-161 inhibited VEGF-induced migration and capillary tube formation in hCECs, but did not inhibit proliferation. In vivo, injection of ATN-161 after laser photocoagulation inhibited CNV leakage and neovascularization to an extent similar to AF564. Furthermore, SD-OCT and histologic examinations indicated that ATN-161 significantly decreased the size of laser-induced lesions.

CONCLUSIONS. Integrin α5β1 inhibition by ATN-161 may be a promising alternative therapy for CNV-related angiogenesis. In addition, SD-OCT technology allows excellent visualization of experimentally induced CNV in vivo. (Invest Ophthalmol Vis Sci. 2011;52:7213–7220) DOI:10.1167/ iovs.10-7097

CHOROIDAL neovascularization (CNV), the hallmark of exudative age-related macular degeneration (AMD), leads to low vision or blindness in many elderly patients.1–5 The clinical consequences of CNV are determined by the relative activities of angiogenic activators and inhibitors. Vascular endothelial growth factor (VEGF) and its receptors are among the most validated signaling pathways involved in angiogenesis.4

In recent years, the antibody-based inhibitors of VEGF signaling have been regarded as breakthrough therapies for treating CNV, and significant improvement in visual acuity has been achieved in AMD patients receiving these treatments.5–8 However, clinical success rates of VEGF inhibitors are lower than success rates in experimental CNV models. Study results showed that only 30% to 40% of patients receiving standard anti-VEGF treatment gained more than 15 Early Treatment Diabetic Retinopathy Study (ETDRS) letters, and roughly 18% of patients continued to lose their visual acuity.9–11 Moreover, it has been found that factors other than VEGF are likely to play important roles in established exudative AMD.12–14 Thus, despite the substantial improvement offered by current VEGF inhibitor-based therapies, the potential for further improvement remains.

An increasing number of studies support the significant role of integrin family molecules, particularly integrin α5β1, in the pathophysiology of CNV.15,16 Previous reports showed that integrin α5β1 expression increased dramatically in vascular endothelial cells treated with growth factor in vitro17 and in laser-induced CNV lesions in rats and mice.18 The results of a phase II clinical trial in which cancer patients were treated intravenously with the integrin α5β1 inhibitor volociximab suggest that integrin α5β1 inhibition has remarkable antiangiogenic efficacy.19 Systematic administration of the integrin α5β1 inhibitor JSM6421 has also been shown to alleviate both inflammatory corneal neovascularization and hypoxia-induced retinal neovascularization.20,21 However, intravitreal injection of JSM6421 in rat CNV models showed little antiangiogenic efficacy.22 In fact, various preclinical studies have indicated that the biological function of integrin mainly depends on its specific sequence or structure after ligand occupancy.23,24 Thus, further investigations into the therapeutic potential of integrin α5β1 inhibitors in clinical trials of AMD appear necessary.

A purified fragment of the fibronectin cell-binding domain, which binds integrin α5β1 and contains the PHSRN sequence but not the RGD sequence, has been found capable of stimulating cell invasion in vitro.12,25 ATN-161 (Ac-PSHRN-NH2) is derived from the PHSRN sequence, but contains a cysteine residue in place of the original arginine residue.13 Capping the ends of the PHSRN peptide by acetylation and amidation has been shown to increase its stability and bioactivity, enhancing the ability to inhibit cell invasion by 30-fold.27 A recent study28 found that PHSRN can inhibit fibronectin binding to integrin α5β1 and thus block the angiogenesis in tumors, suggesting...
that ATN-161 may also have therapeutic potential as a CNV treatment.

In this study, we examined whether integrin α5β1 plays an important pathologic role in the development of CNV by examining the effects of ATN-161 in cultured hCECs and in laser-induced CNV animal models. Currently, histologic examination remains the accepted method for quantitative assessment of retinal morphology in rodent models of laser-induced CNV. In humans, however, special-domain optical coherence tomography (SD-OCT) has become the new standard for non-invasive in vivo ophthalmic imaging and is widely used for diagnosing and monitoring various retinopathies. Thus, we also investigated the feasibility of using SD-OCT for dynamic observation of the development and production of laser-induced CNV in rats.

MATERIALS AND METHODS

The present study conforms to the Guide for the Care and Use of Laboratory Animals of the United States National Institutes of Health and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All chemicals were of reagent grade.

Peptide Synthesis

The Ac-PHSCN-NH2 peptide (ATN-161) and scrambled peptide Ac-HSPNC-NH2 were synthesized by ChinaPeptides Co., Ltd. (Shanghai, PR China) by a high-efficiency, solid-phase method, using an automatic peptide synthesizer (Symphony; Protein Technologies, Tucson, AZ). The peptide purities were 98% for ATN-161 and 95% for Ac-HSPNC-NH2, as assessed by reversed-phase high-performance liquid chromatography (HPLC, analytical; Shimadzu, Kyoto, Japan). The products were stored at −20°C. ATN-161 was diluted with phosphate-buffered saline (PBS) to concentrations of 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, and 100 μM, and Ac-HSPNC-NH2 was diluted to 100 μM for use.

In Vitro Efficacy Studies

Cell Culture and Drug Treatment. Human choroidal endothelial cells (hCECs) were cultured as previously described for three to eight passages before use. Before each experiment, hCECs at 70% confluence were incubated for 30 minutes in serum-free endothelial cell medium supplemented with 0.1% heparin and 0.1% endothelial cell growth supplement (ECGS; SciCell Research Laboratories, San Diego, CA). The cells were then seeded on plates coated with 10 μg/mL IV collagen (Sigma-Aldrich, St. Louis, MO) and allowed to grow for 8 to 24 hours in the presence of VEGF (20 ng/mL; human VEGF-165; R&D, Minneapolis, MN) alone or in combination with ATN-161 (1 nM–100 μM), Ac-HSPNC-NH2 (100 μM), or the anti-VEGF-A antibody bevacizumab (Bev) 0.025, 0.25, and 2.5 mg/mL; Avastin; Genentech/Roche, South San Francisco, CA).

Western Blot Analysis. Equivalent amounts of protein (50 μg, determined by Bradford assays) were resolved on 10% SDS-polyacrylamide gels. The protein was electrotransferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA), which was then blocked in a solution of 5% (wt/vol) skim milk powder in PBS (pH 7.5) for 1 hour at room temperature and probed overnight at 4°C with anti-integrin α5 (H-104, 1:300; Santa Cruz Biotechnology, Fremont, CA), anti-integrin β1 (EP1041Y, 1:200; Abcam, Cambridge, MA) or anti-β-actin (1:1000; Cell Signaling Technology, Danvers, MA) antibodies. After membranes were washed with PBS, horseradish peroxidase (HRP)-conjugated goat anti-rabbit secondary antibodies (1:2000; Cell

![Image](image_url)
Signaling Technology) were incubated with the membranes for 1 hour at room temperature. Finally, proteins were detected by enhanced chemiluminescence (GE Healthcare, Piscataway, NJ) and visualized after exposure to autoradiographic film (Kodak, Rochester, NY). Protein levels were quantified by densitometry and normalized to \(\beta\)-actin levels.

**MTS Cell Proliferation Assay.** After serum starvation, hCECs in 96-well cell culture plates were incubated with each drug treatment (described above) at 37°C for 24 hours. The plates were then washed twice with PBS, and 20\(\mu\)L MTS (3-(4,5-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium; Cell Titer 96 Aqueous One Solution; Promega, Madison, WI) was added to each well. MTS color change was monitored at 490 nm with a universal plate reader (model 680; Bio-Rad, Hercules, CA).

**Cell Migration Assay.** The migration capacity of hCECs was examined in 24-well plates with a modified Boyden chamber consisting of a membrane insert (Transwell; Corning Inc., Corning, NY) containing a membrane with 8.0-\(\mu\)m pores in the top chamber. The bottom chamber contained 600 \(\mu\)L of serum-free endothelial cell medium and human VEGF-165 (20 ng/mL). Briefly, hCECs were preincubated with the drug treatments (described above) at 37°C for 30 minutes, then \(5 \times 10^4\) cells/well were placed in the top chamber and allowed to migrate for 8 hours in the presence of the applied drugs. The membranes were then fixed and stained with hematoxylin, and the number of migrated cells on the bottom side of the membrane was counted in five randomly chosen fields. The experiments were performed in triplicate.

**Capillary Tube Formation Assay.** Synthetic matrix (Matrigel; BD Biosciences, Bedford MA) assays were performed to investigate the effects of ATN-161 and Bev on the formation of VEGF-induced capillary-like structures, as previously described.\(^{54}\) The basement membrane matrix (Matrigel, growth factor reduced; BD Biosciences) was thawed on ice to prevent premature polymerization, then 50 \(\mu\)L was plated into each well of a 96-well plate and allowed to polymerize at 37°C for 60 minutes. The cells were then incubated with the distinct drug treatments (described above) for 30 minutes, and 100 \(\mu\)L of cells were immediately seeded to the matrix-coated plates and incubated with the same treatments for 18 hours at 37°C in a 5% CO\(_2\) humidified atmosphere. A semiquantitative measurement of the tube formation of hCECs was performed as previously described.\(^{35}\) Experiments were repeated three times.

**In Vivo Efficacy Studies**

**Laser-Induced CNV and Drug Injections.** Laser photocoagulation was performed on 12-week-old male Brown-Norway (BN) rats, each weighing 200 to 250 g. The rats were anesthetized throughout the procedure by intraperitoneal injection of 1% sodium pentobarbital (45 mg/kg), and the pupils were dilated with tropicamide (5 mg/mL) and phenylephrine hydrochloride (5 mg/mL) during optical manipulation. Only one eye of each animal was subjected to laser injury. Five to seven spots (50 \(\mu\)m diameter) per eye were applied between the major retinal vessels with the following laser parameters:
532 nm, 200 mW, and 100 ms. Only the laser spots in which a bubble was produced without hemorrhage, indicating perforated Bruch’s membranes, were considered effective and included in the study.

The BN rats with CNV were randomly divided into six groups: sham-injected (n = 9), vehicle-injected (n = 9), ATN-161_{10\text{ug/ml}}-injected (0.1 \mu g/mL; n = 9), ATN-161_{10\text{ug/ml}}-injected (10 \mu g/mL; n = 9), Ac-HSPNC-NH2-injected (10 \mu g/mL; n = 9), and AF564-injected (R&D Systems; 25 \mu g/mL; n = 9). Immediately after laser photocoagulation, animals received intravitreal injections (5 \text{L}) of the designated treatments.

Fluorescein Angiography. On days 1, 7, and 14 after laser photocoagulation, CNV lesions were studied by fluorescein angiography (FA) with a confocal scanning laser ophthalmoscope (Heidelberg Retinal Angiograph; Heidelberg Engineering, Heidelberg, Germany). The anesthetized rats were intraperitoneally injected with fluorescein sodium (10%, 0.1 mL/kg), and late-phase angiograms were obtained 5 minutes after the injection. The formation of CNV was evaluated according to the presence or absence of dye leakage, as described previously. The guidelines for CNV scoring were as follows: no leakage (score 0); minimum leakage or a staining of tissue with no leakage (score 1); small but evident leakage of less than one quarter disc area (score 2); and large evident leakage (score 3).

Spectral-Domain Optical Coherence Tomography. Images were obtained with the clinically used SD-OCT (Spectralis OCT; Heidelberg Engineering) in the same session as FA. The OCT images were used to evaluate CNV thickness, defined as the subretinal hyperreflective material above the RPE layer. To evaluate the cross-sectional size of each lesion in the OCT images, the sections that included the center of the CNV were selected for analysis. To minimize observer variations, all scans were obtained by one experienced physician. CNV thickness was quantified by computer-assisted manual segmentation analysis using the proprietary software for SD-OCT data.
**Choroidal Flatmounts.** Two weeks after laser photocoagulation, RPE-choroid-sclera complexes were microsurgically isolated and fixed with 4% paraformaldehyde for 1 hour and then incubated with blocking buffer (PBS, 0.5% Triton X-100, and 5% bovine serum albumin [BSA]) for 4 hours at 4°C. The complexes were then incubated with AlexaFluor-594-conjugated isolectin (GS-IB4; Invitrogen, Carlsbad, CA) for 1 hour at room temperature, to visualize vessel formation. Quantification of CNV complex lesions was performed with a confocal microscope (FV 1000; Olympus, Tokyo, Japan), and the area of CNV-related fluorescence was measured (Image-Pro Plus; Media Cybernetics, Bethesda, MD).

**Enzyme-Linked Immunosorbent Assay.** RPE-choroid complexes were microsurgically isolated from eyes on days 1, 3, 7, and 14 after laser photocoagulation and sonicated in 300 μL of lysis buffer supplemented with protease inhibitors (Roche, Dee Why NSW, Australia). The lysates were centrifuged at 12,000 rpm for 15 minutes at 4°C, and the levels of VEGF were determined with a rat VEGF ELISA kit (R&D Systems), according to the manufacturer’s protocol. The total protein concentration was determined by Bradford assay.

**Statistics**

All the results are expressed as the mean ± SD. The statistics analyzed by one-way analysis of variance (ANOVA) followed by a t-test assuming unequal variances (statistical analysis software; SPSS 15.0 for Windows, SPSS; Chicago, IL). All the P values were two-sided, and the differences were considered statistically significant at P < 0.05 in all cases.

**RESULTS**

**Integrin α5β1 Expression in VEGF-Treated Endothelial Cells**

Western blot analysis with anti-integrin antibodies revealed that the expression levels of integrin α5β1 were increased in hCECs treated with VEGF (P < 0.001 vs. no VEGF group, Fig. 1). Further analysis revealed that the VEGF-mediated increase in integrin α5β1 expression was inhibited by both ATN-161 and Bev (P < 0.001 vs. VEGF group, Fig. 1)

**The Effects of ATN-161 on VEGF-Induced Endothelial Cell Migration and Proliferation**

As illustrated in Figure 2, VEGF-treatment enhanced hCEC proliferation after 24 hours, and this increase was not affected by Ac-HSPNC-NH2 scrambled peptide. Similarly, ATN-161 did not affect the proliferation of hCECs at any concentration tested. In contrast, Bev inhibited the VEGF-induced proliferation of hCECs in a dose-dependent manner (Fig. 2).

Treatment of hCECs with VEGF also increased the number of migrating cells (P < 0.001 vs. nontreated; Figs. 3A, 3K). ATN-161 decreased the number of cells migrating in response to VEGF in a dose-dependent manner starting at 100 nM (P < 0.001 vs. VEGF group, Figs. 3B, 3C, 3K), and Bev application resulted in similar inhibitory effects on VEGF-induced cell migration at concentrations of 0.25 and 2.5 mg/mL (P < 0.001 vs. VEGF group, Figs. 3D, 3K). The scrambled peptide did not have a significant effect on hCEC migration (Figs. 3E, 3K).

As demonstrated in Figure 3, VEGF strongly stimulated endothelium tube formation by hCECs plated in synthetic matrix (P < 0.001 vs. nontreated). The VEGF-induced capillary tube formation was inhibited by ATN-161 in a dose-dependent manner (Figs. 3G, 3H, 3L). Bev (0.25 and 2.5 mg/mL) also inhibited VEGF-induced tube formation (P < 0.001 vs. VEGF group, Figs. 3I, 3L). Treatment with the scrambled peptide (100 μM) did not affect VEGF-induced endothelium tube formation (Figs. 3J, 3L).

**FIGURE 6.** Visualization of the effects of ATN-161 and AF564 on laser-induced CNV. Representative images of retinal structures on day 14 after laser photoocoagulation and the specified injections are presented. (A–F) FAs, (G–L) choroidal flatmounts stained with isolectin B4, (M–R) SD-OCT, and (S–X) H&E staining.
Detection of Laser-Induced CNV by SD-OCT and FA

Images obtained with SD-OCT on day 1 after laser photocoagulation provided visualization of detailed structures, including epiretinal hemorrhage, retinal edema, pigment epithelial detachment (PED), and subretinal fluid (Fig. 4A). On day 7, the hemorrhage or fluid within the retina was absorbed, and a densely packed mass appeared at the center of the lesions (Fig. 4). By day 14, a robust CNV complex extended toward the subretinal space in the eye subjected to laser photocoagulation, but not in the untreated eye (Figs. 4B, 4C).

SD-OCT images of eyes subjected to laser photocoagulation followed by sham injection showed retinal edema and hemorrhage on day 1, evidence of CNV on day 7, and progression of CNV on day 14 (Figs. 5F–H). SD-OCT images of eyes subjected to laser photocoagulation followed by ATN-16110μg/mL injection showed retinal hemorrhage and PED, but a remarkable reduction of CNV thickness on days 7 and 14, with no obvious PED (Figs. 5N–P). An autofluorescent ring was detected with FA by day 1 after laser photocoagulation in both groups (Figs. 5A, 5I), and in the late phase (5 minutes after injection of FA), a uniformed hyperfluorescent area appeared in both groups (Figs. 5B, 5J). Seven days after laser photocoagulation, vascular tubes were visible in all lesions (Figs. 5C, 5K). Similarly, at 14 days, leakage was less evident in the laser-induced lesions that received ATN-16110μg/mL injection (Figs. 5D, 5L).

Effects of ATN-161 on Choroidal Neovascularization In Vivo

FA images obtained on day 14 after laser photocoagulation (Fig. 6) were scored and revealed a significant reduction of leakage in the ATN-16110μg/mL-, ATN-16110μg/mL-, and AF564-injected groups, compared with the scrambled peptide-, sham-, and vehicle-injected groups (Fig. 7A; Table 1). The CNV area, measured in choroidal flatmounts on day 14 (Fig. 6), was smaller in both ATN-161-injected groups compared with the scrambled peptide-injected group (Fig. 7B, Table 1). Similarly, the CNV area measured in the AF564-injected group was smaller than the CNV area measured in the vehicle- and sham-injected groups (Fig. 7B, Table 1).

Measurement of the CNV areas in SD-OCT images (Fig. 6) showed that the CNV thickness on day 14 was significantly smaller in the ATN-16110μg/mL-, ATN-16110μg/mL-, and AF564-injected groups than in the vehicle-injected and scrambled peptide–injected groups. The CNV thickness in the ATN-16110μg/mL-injected group was smaller than the CNV thickness in the sham-injected group during the entire course (Fig. 7C, Table 1). The measured CNV thickness was not significantly different between the ATN-16110μg/mL- and AF564-injected groups (Fig. 7C, Table 1).

Figure 7. Quantification of the effects of ATN-161 and AF564 on laser-induced CNV. (A) Fluorescein leakage on day 14 was quantified by scoring FAs. (B) Quantification of CNV area on day 14 measured on choroidal flatmounts. (C) Quantification of CNV thickness on day 14 measured on SD-OCT images. Mean values ± SD are shown (n = 5–6 eyes per group). *P < 0.05; †P < 0.001 versus the sham-injected group. (D) ELISA analysis of VEGF expression at the indicated time points after laser photocoagulation. (P < 0.001 vs. sham-injected group).
DISCUSSION

The angiogenic effects of integrin α5β1 have been well documented.26–30,57 However, the effects of the integrin α5β1 inhibitor ATN-161 on CNV induction and progression have not been fully elucidated. In this study, treatment with ATN-161 reduced angiogenesis, especially in proliferating endothelial cells and prevented the formation of laser-induced CNV at an early stage. The angiogenic effects of ATN-161 appeared to be mediated by direct inhibition of capillary tube formation. Moreover, SD-OCT was a reliable technique for imaging the retinal structures in the rodent eyes, and the data obtained by SD-OCT allowed for precise comparison of the effects observed at different time points within different treatment groups.

Extracellular matrix proteins and their integrin receptors play important roles in modulating CNV and, in the eye, integrin α5β1 is highly expressed in experimentally induced CNV membranes and corneal neovascularization.18,21 In the present study, integrin α5β1 expression was upregulated in hCECs after incubation with VEGF, and the increase was prevented by simultaneous treatment with an anti-VEGF antibody (Bev) or ATN-161. Although ATN-161 did not inhibit VEGF-induced hCEC proliferation, it did suppress VEGF-induced hCEC migration and capillary tube formation in vitro. Furthermore, a single intravitreal injection of ATN-161 reduced VEGF expression levels and prevented CNV development with an efficacy similar to that of the VEGF antibody AF564, when administered immediately after photoocoagulation in vivo.

These results suggest that application of anti-integrin α5β1 agents at the early stages of CNV could reduce the production of VEGF and progression of CNV. This angiogenic activity of ATN-161 was attributable to the specific spatial arrangement of its amino acid side chains, since the scrambled peptide Ac-HSPNC-NH2 failed to inhibit capillary tube formation in vitro or laser-induced CNV in vivo. The expression of α5β1 integrin is upregulated in response to angiogenic factors,58 and the interaction between α5β1 integrin and fibronectin has been found to promote adhesion, proliferation, and cell signaling in human RPE59,60 and vascular endothelial cells.41 Indeed, a notable function of integrins is to provide a link between extracellular molecules and the actin-based microfilament system, and proliferative responses to soluble growth factors often depend on integrin-mediated adhesion to extracellular substrates.

The results presented here suggest a level of cross-talk between integrin α5β1 and VEGF-regulated pathways, as well.

Table I. Summarized Data for Treatment of Rats

<table>
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<tr>
<th>Treatment</th>
<th>CVN Leakage Score</th>
<th>CVN Area (μm²)</th>
<th>CVN Thickness (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>2.5 ± 0.6</td>
<td>35.272 ± 1.301</td>
<td>0.9 ± 0.6</td>
</tr>
<tr>
<td>Vehicle</td>
<td>2.4 ± 0.7</td>
<td>35.835 ± 1.293</td>
<td>10.0 ± 12.6</td>
</tr>
<tr>
<td>ATN-161</td>
<td>1.1 ± 0.6†</td>
<td>16.890 ± 1.384</td>
<td>81.3 ± 16.8*</td>
</tr>
<tr>
<td>ATN-161</td>
<td>0.7 ± 0.6†</td>
<td>12.186 ± 1.270</td>
<td>72.3 ± 3.8†</td>
</tr>
<tr>
<td>AF564</td>
<td>0.9 ± 0.6†</td>
<td>15.801 ± 1.422</td>
<td>86.0 ± 8.3*</td>
</tr>
<tr>
<td>Ac-HSPNC-NH2</td>
<td>2.5 ± 0.7</td>
<td>34.541 ± 2.014</td>
<td>104.0 ± 8.3</td>
</tr>
</tbody>
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Data are expressed as the mean ± SD. *P < 0.05 vs. the sham-injected group. †P < 0.001 vs. the sham-injected group.

The exact interactions underlying the relationship between VEGF-A and integrin α5β1 remain unclear, but integrins and growth factor receptors appear to share several common downstream signaling molecules, many of which have also been implicated in angiogenesis, including FAK, Rho, Src, and PAK.42–44 Similar to results in the present study, Lee et al.45 observed VEGF-A-induced upregulation of α5β1 expression during the early phase of vessel sprouting in fibronectin-rich multipotent mesenchymal stromal cells, and VEGF stimulation of endothelial cells upregulates expression of α1β1 and α2β1.46 Another study found that VEGFR-1-mediated cell adhesion and migration could be inhibited by anti-α5β1 antibodies through direct antibody binding to VEGFR-1.45 Furthermore, Wijelath et al.47 reported that VEGF165 could stably bind to fibronectin and induce capillary growth. These data provide the basis for a tantalizing, novel hypothesis that not only are integrins and growth factor receptors coordinated in angiogenic responses, but direct interaction between the integrins and the growth factors could contribute to angiogenesis. Further studies are necessary to determine whether α5β1 integrin inhibition in conjunction with anti-VEGF therapies could result in a synergistic therapy. Of note, α5β1 integrin expression is very limited in normal tissue but upregulated in response to angiogenic factors in CNV lesions.18 Therefore, therapeutic targeting of α5β1 integrin expressed in CNV lesions may be more specific than targeting of VEGF, and could thereby prevent unintended damage to normal tissue.

To investigate potential CNV therapies such as α5β1 integrin inhibition completely in preclinical models requires a reliable method for imaging in live animals. Currently, only a few studies have reported application of OCT setups for noninvasive, high-quality, repeatable imaging in vivo imaging of the rodent retina.29,48 In our study, the SD-OCT image allowed for precise spatial registration of the retina and illustration of CNV histopathology in live animals. By combining the information obtained with SD-OCT and FA, we were able to more precisely observe CNV morphology and its correlation with microvessel leakage. Thus, it may be even more important and efficient to use SD-OCT for evaluating the therapeutic or adverse effects of experimental interventions in a time-course experiment in live animals, such as a rodent CNV model.

In conclusion, the antiangiogenic effects of ATN-161 in laser-induced CNV observed in this study suggest a new method that may improve treatment of AMD. Our results, together with those reported previously, provide conclusive evidence that integrin α5β1 is involved in the development and progression of CNV and is a strong candidate for therapeutic targeting. Since ATN-161 may be an effective therapeutic candidate for ocular neovascular diseases, further studies to determine the underlying mechanism of action and the ocular pharmacokinetics are warranted.

References

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