Riluzole Inhibits VEGF-Induced Endothelial Cell Proliferation In Vitro and Hyperoxia-Induced Abnormal Vessel Formation In Vivo

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PURPOSE. The present study examined the effects of riluzole, a Food and Drug Administration–approved drug for amyotrophic lateral sclerosis, on VEGF-stimulated endothelial cell proliferation in culture, and on neovascularization in a rat model of retinopathy of prematurity (ROP).

METHODS. Human umbilical vein endothelial cell and bovine retinal endothelial cell cultures were treated with VEGF to induce endothelial cell proliferation in the presence or absence of riluzole. Activation of PKC βII was examined by quantifying its phosphorylated form on immunoblots. ROP was induced in 5-day-old rat pups by raising them in hyperoxic conditions for 7 days and in normoxic conditions for the next 5 days. Dextran fluorescence retinal angiography was used to quantitatively assess ROP.

RESULTS. Riluzole inhibited VEGF-stimulated PKC βII activation and cell proliferation in bovine retinal endothelial cell and human umbilical vein endothelial cell cultures. In addition, systemic administration of riluzole substantially ameliorated abnormal new vessel formation in the rat ROP model.

CONCLUSIONS. The present results suggest that riluzole is a potent inhibitor of VEGF-induced endothelial cell proliferation both in vivo and in vitro. Since long-term use of riluzole has already been proven safe in humans, the present data indicate that clinical trials of riluzole for proliferative retinopathies should be implemented expeditiously. (Invest Ophtalmol Vis Sci. 2005;46:4780–4787) DOI:10.1167/iovs.05-0376

The main stimulus for new vessel growth in proliferative retinopathies appears to be VEGF, a potent angiogenic protein.1–3 VEGF is produced by a variety of cells during developmental angiogenesis and in response to hypoxic conditions.4,6 Like many other growth factors, VEGF acts via its cognate tyrosine kinase receptors, Flt-1 and Flk-1.5,7–10 Flk-1 mediates VEGF-stimulated endothelial cell proliferation, while Flt-1 may promote cell migration or endothelial cell-cell or cell-matrix interaction. Consistent with the key role of VEGF in diabetic retinopathy and retinopathy of prematurity (ROP), the level of VEGF is upregulated in both conditions.1,11–15 Although the precise mechanism underlying VEGF upregulation is yet to be delineated, tissue hypoxia has been proposed as a main stimulus.12–15 In response to an increase in VEGF levels, endothelial cells proliferate to form new vessels. However, these new vessels are usually leaky and fragile, resulting in exudate formation and hemorrhage.5,16

Of the signaling molecules downstream of VEGF receptors, PKC has been demonstrated to be critical in mediating endothelial cell proliferation.17–19 Although inhibition of PKC may seem a reasonable approach to curtail endothelial cell proliferation in proliferative retinopathies, PKC serves diverse essential normal roles in intracellular signaling, indicating broad-spectrum inhibition of all PKCs may have harmful side effects, including cell apoptosis.22,23 However, recent studies have demonstrated that it is the PKC isozyme βII that is the critical family member in mediating VEGF effects on endothelial cells,17 indicating that selective inhibition of PKC βII may achieve the desired therapeutic effects. Indeed, ruboxistaurin (LY333531) was recently developed as a selective PKC βII inhibitor, and was found to reduce retinal endothelial cell proliferation in culture and in vivo with few side effects.21,24–26 Although a larger scale clinical trial found ruboxistaurin had only modest beneficial effects in diabetic retinopathy, this does not necessarily rule out all PKC inhibitors as drug candidates for diabetic retinopathy. The effectiveness of PKC βII inhibition in reducing VEGF-induced endothelial cell proliferation in preclinical studies suggests that other PKC inhibitors should be investigated.

Riluzole was initially developed as an antiglutamate agent, and it has several additional pharmacological effects,27–32 including PKC inhibition.33 Riluzole inhibited PKC activity in cortical cell cultures, and also inhibited the activity of purified PKC in vitro. Riluzole has been approved for human use by the Food and Drug Administration (FDA), is currently widely used in amyotrophic lateral sclerosis (ALS) patients, and has a favorable side effect profile. These characteristics of riluzole make it particularly attractive for studies on its potential use as a treatment for proliferative retinopathies.

In the present study, we examined the effects of riluzole on VEGF-induced proliferation in endothelial cell cultures. In addition, we tested the effects of riluzole on endothelial cell proliferation in a rat model of ROP.

MATERIALS AND METHODS

Human Umbilical Vein Endothelial Cell (HUVEC) Culture

HUVECs and media were purchased from Cambrex BioScience (Walkersville, MD) and plated onto fibronectin (Sigma-Aldrich, St. Louis, MO)-coated cell culture vessels (Nunc, Roskilde, Denmark) in endothelial basal medium (EBM) supplemented with human recombinant epidermal growth factor (10 ng/mL), hydrocortisone (1 μg/mL), gentamicin (50 μg/mL), bovine brain extract (12 μg/mL), and 10% fetal bovine serum (HyClone, Logan, UT). Two days later, medium was changed to medium containing 10% fetal bovine serum. After 2 days of culture, medium was changed to medium containing 10% fetal bovine serum and 20 ng/mL VEGF (R&D Systems, Minneapolis, MN). This procedure was repeated weekly.

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exchanged for endothelial cell growth medium (EGM). These cultures were maintained at 37°C in a humidified 5% CO₂ incubator. After 7 days in vitro, cells were used in experiments.

**Bovine Retinal Endothelial Cell (BREC) Culture**

BREC cultures were prepared using the *Lycopersicon esculentum*-coated Dynabead method originally developed for culturing choriocapillary endothelial cells.³⁹ Briefly, after removal of the conjunctiva, bovine eyes were incubated for 10 minutes in PBS containing 5% penicillin/streptomycin. A circular incision was made to remove the cornea and lens. The vitreous was removed using forceps. Under a dissecting microscope, the retina was gently isolated and then flushed out with PBS containing 0.1% bovine serum albumin. The tissue was centrifuged at 750 rpm for 2 minutes, and the supernatant was discarded. The pellets were digested by incubation in a collagenase/disparse mixture for 30 minutes at 37°C. After digestion, the cell suspension was filtered through a 70-μm mesh filter and then a 40-μm mesh filter. The supernatant was discarded, and the cell pellet was resuspended in 1 mL *L. esculentum*-coated bead/Bandetraea simplicifolia 4 mixture. The mixture was then incubated at room temperature for 1 hour with agitation, after which the bead and cell particles were immobilized using a magnet (MPC-1; Invitrogen Corp., Carlsbad, CA) and washed at least 10 times with media A (PBS containing 0.1% bovine serum albumin). Beads were removed and cells were cultured in EGM in six-well plates coated with fibronectin. BRECs were subcultured on fibronectin-coated plates in EGM containing 10% fetal bovine serum (HyClone). Two days later, the medium was changed to EGM. These cultures were maintained at 37°C in a humidified 5% CO₂ incubator. Cells between passages 3 and 5 were used.

**Assessment of Cell Proliferation**

Cells in 24-well plates were placed in a CO₂ incubator at 37°C for 4 days in EBM in the absence or presence of 100 ng/mL VEGF (R&D Systems, Minneapolis, MN), VEGF plus riluzole (Sigma-Aldrich), or VEGF plus GF109203X (Sigma-Adrich). After 4 days, cultured cells were stained with Hoechst 33258 (Molecular Probes, Eugene, OR) and photographed under a fluorescence microscope (IX70; Olympus, Tokyo, Japan). For cell counting, cultured cells were detached from the bottom with trypsin-EDTA treatment, and made into single cells by trituration with Pasteur pipettes. Cell numbers in each culture vessel were quantified by hemocytometer counting (four times for each vessel).

**ImmunobLOTS and ImmunoprecipitAtion**

Equal amounts of protein were separated using 8% SDS-PAGE, then transferred to PVDF membranes (Millipore Corp., Bedford, MA). Membranes were incubated overnight at 4°C with anti-phospho-PKC (9571S; Cell Signaling Technology, Beverly, MA), anti-phospho-FKl-1 (SC-16629-R; Santa Cruz Biotechnology Inc., Santa Cruz, CA), or anti-PKC antibody (06870; Upstate Biotechnology Inc., Lake Placid, NY). Chemiluminescence substrate (SuperSignal West Dura Substrate; Pierce Biotechnology Inc., Rockford, IL) was used to visualize the immunoreactive bands.

For immunoprecipitation, cells were lysed and centrifuged (12,000 rpm × 10 min). Aliquots of supernatant were incubated with PKC isoform-specific antibodies (α: sc-208, β: sc-8069, γ: sc-211; Santa Cruz Biotechnology, Inc.) overnight and then precipitated on Protein A- and G-agarose beads (Upstate Biotechnology Inc.) at 4°C for 1 hour. The immunoprecipitates were then washed three times with lysis buffer, followed by immunoblot analyses.

**Treatment of Cells with Small Interfering RNA (siRNA) Targeting PKC β**

An siRNA oligonucleotide that targeted PKC was chemically synthesized by a commercial supplier (Invitrogen). The target sequence was 5′-AACGCUGGUGUCAUGAAGTT-3′.³⁵ A fluorescein-labeled double-stranded RNA oligonucleotide (Invitrogen) was used for negative control. siRNA (150 pmol) was added to Opti-MEM (Invitrogen) (total volume, 40 μL). Oligofectamine reagent was made in Opti-MEM. A mixture of 40 μL siRNA solution and 10 μL oligofectamine reagent was then incubated for 15 minutes at room temperature. For transfection, 50 μL mixed solution was added to each 15-mm culture vessel containing 200 μL serum-free media. After 4 hours, 125 μL serum-containing growth media lacking antibiotics was added. At 20 hours after the addition of siRNA, cultures were exposed to 100 ng/mL VEGF or VEGF plus 1 μM riluzole. To check the effect of the siRNA on the level of PKC βII, at 24 hours after treatment, cells were harvested with sample buffer and subjected to Western blotting. At 96 hours after beginning VEGF treatment, cell proliferation was assessed as described above.

**Rat Model of ROP**

Pregnant Sprague–Dawley rats were obtained from Charles River Laboratories (St. Constant, Quebec, Canada). All experiments were performed in accordance with the Guidelines for Care and Use of Laboratory Animals (University of Ulsan, Seoul, Korea) and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. From postnatal day (P)5 to P12, rat pups were raised in a 75% ± 5% oxygen chamber with their mother for feeding. The accuracy of oxygen tension in the chamber was monitored twice daily with an oxygen analyzer (Hudson Ventronics, Temecula, CA). After exposure to hypoxia, rat pups were raised for another 5 days (P12–P17) in a room-air environment.⁴⁺° Control rat pups were raised in room air throughout the period (P5–P17). Riluzole (10 mg/kg) or osmolality-matched saline was intraperitoneally injected daily from P12 to P16. At P17, rat pups were killed for analysis.

**Fluorescein Dextran Angiography of Retinal Blood Vessels**

At P17, all rat pups were anesthetized with an intraperitoneal injection of 80 mg/kg ketamine and 15 mg/kg xylazine. After thoracotomy, the left ventricle was perfused with 0.5 mL of 20 mg/mL fluorescein isothiocyanate-labeled dextran (MWt 2 × 10⁶) dissolved in ultrapure water. The eyes were placed in 4% paraformaldehyde in PBS, and the retinas were removed under a dissecting microscope. Retinas were flat-mounted on slides using gelatin, then overlaid with cover slips, and the assemblies were sealed with nail polish. Fluorescein angiography was performed using a fluorescence microscope (BX60; Olympus) and a digital camera (C-2000Z; Olympus).

**Retinopathy Scoring System**

The retinopathy scoring system for animal models,³⁶–³⁹ modified from the International Criteria for Retinopathy of Prematurity system for humans,⁴⁰ was used to quantify the severity of retinopathy. Blood vessel growth, extraretinal neovascularization, central vasoconstriction, and vessel tortuosity were given scores from 0 to 3. Blood vessel tufts were scored from 0 to 4. Hence, the maximum possible score was 16. Retinopathy was scored by two independent evaluators in a condition-blinded manner.

**Retinal Sections**

Rats were killed using xylazine and ketamine. The eyes were frozen in liquid nitrogen, and 10-μm-thick sections were made through the cornea parallel to the optic disc using a cryostat (CM1850; Leica Instruments GmbH, Wetzlar, Germany). Tissue sections were stained with hematoxylin and eosin. Eight sections, 50 μm apart, per eye were used for counting the number of nuclei beyond the inner limiting membrane in a condition-masked fashion.⁴⁰·⁴¹
Statistical Analyses
All data are presented as means ± SD. To compare multiple sets of data, a one-way ANOVA with post-hoc Fisher's LSD test was used. For paired data sets, a two-tailed *t*-test was used. Values of *P* < 0.05 were considered to represent a significant difference.

**RESULTS**

**Inhibition of VEGF-Induced Endothelial Cell Proliferation by Riluzole**

Two types of cultured endothelial cells, BRECs and HUVECs, were incubated with VEGF in the presence or absence of riluzole, after which they were stained with Hoechst 33342, and cell numbers were determined as described in Materials and Methods. We found that BRECs incubated for 96 hours with 100 ng/mL VEGF (Fig. 1B) proliferated more than sham-washed control cells (Fig. 1A). Quantification of cell numbers showed that 96-hour incubation with 100 to 500 ng/mL VEGF increased cell numbers 2- to 2.5-fold compared with control cells (Fig. 1E). A similar concentration-dependent effect of VEGF on proliferation was observed in HUVECs (data not shown). Addition of riluzole (1 or 10 μM) inhibited VEGF-induced proliferation in both BRECs (Fig. 1C, 1F) and HUVECs (Fig. 1G) cultures. Consistent with the necessary role for PKC in VEGF-induced cell proliferation, a pan-PKC inhibitor, GF109203X (5 μM), also completely blocked VEGF-induced endothelial cell proliferation in both culture types (Fig. 1F, 1G). In addition, cell proliferation during the 4-day treatment period was quantified daily (Fig. 1H). Whereas 4-day treatment with 10 μM riluzole alone was not cytotoxic, compared with no treatment, the addition of 0.1 to 10 μM riluzole inhibited VEGF-induced proliferation in a concentration-dependent manner.

**PKC βII Inhibition by Riluzole**

Since PKC activation is believed to be a crucial signaling event in VEGF-induced endothelial cell proliferation, we examined whether the PKC activator phorbol 12-myristate acetate (PMA) had a similar effect as VEGF on endothelial cell proliferation. Incubation of BRECs with 20 nM PMA for 96 hours increased cell numbers to a similar extent as 4-day treatment with 100 ng/mL VEGF (Fig. 2A). Addition of 0.1 to 10 μM riluzole inhibited this PMA-induced cell proliferation (Fig. 2A). To confirm PKC activation by VEGF, we measured levels of phospho-PKC by testing cell lysates in immunoblot assays using an anti-phospho-PKC antibody. We found that 24-hour incubation of HUVECs with 20 nM PMA or 100 ng/mL VEGF for 24 hours increased levels of phospho-PKC. Addition of riluzole or GF109203X (5 μM) inhibited the increase in phospho-PKC levels by either stimulant (Fig. 2B, C). We examined which PKC isoforms were activated by VEGF by immunoprecipitating with specific antibodies and then performing immunoblotting using an anti-phospho-PKC antibody. We found that VEGF increased the level of phospho-PKC βII, but had minimal effect on the phosphorylation of PKC α, PKC βI, or PKC γ (Fig. 2D). Addition of riluzole substantially reduced the VEGF effect on phospho-PKC βII levels, whereas addition of GF109203X with VEGF decreased levels of all phospho-PKC isoforms to below those in control cultures (Fig. 2D, E). To further test the possibility that PKC βII inhibition was the main mechanism of the riluzole effect on VEGF-induced endothelial cell proliferation, we turned to siRNA technology. Treatment with siRNA targeting PKC β markedly reduced PKC βII levels in HUVEC cultures, both VEGF-treated and untreated (Fig. 2F). As expected, in the presence of the siRNA, VEGF treatment did not induce cell proliferation (Fig. 2G). Control RNA used to assess the transfection efficiency of siRNA did not inhibit VEGF-induced proliferation (not shown). In the presence of siRNA, the inhibitory effect of riluzole on HUVEC proliferation disappeared. On the other hand, in sister cultures that were not treated with siRNA, riluzole markedly inhibited VEGF-induced proliferation.

**Amelioration of the ROP Pathology by Riluzole**

We examined the efficacy of riluzole in a rat model of ROP. To induce retinopathy, newborn rat pups were raised in a 75% ± 5% O2 environment for 7 days (P5–P12), and were then placed in a normoxic environment for 5 days (P12–P17). Fluorescein

**Figure 1.** Riluzole attenuates VEGF-induced proliferation in BRECs and HUVECs. (A–D) Fluorescence photomicrographs of Hoechst 33342-stained BRECs cultures after 96-hour incubation: (A) no agent added (control sham wash), (B) VEGF (100 ng/mL), (C) VEGF (100 ng/mL) plus riluzole (10 μM), and (D) riluzole only (10 μM). Scale bar, 100 μm. (E) Fold increase in nuclei numbers in BREC cultures after 96-hour exposure to various concentrations of VEGF, compared with untreated control (*n* = 6). (F) Effect of riluzole (0.1–10 μM) and GF109203X (a pan-PKC inhibitor; 5 μM) on VEGF-stimulated BREC proliferation (*n* = 6). (G) Effect of riluzole (0.1–10 μM) and GF109203X (5 μM) on VEGF-stimulated HUVEC proliferation (*n* = 4). (H) Time course (4 days) of changes in HUVEC cell numbers on treatment with VEGF (100 ng/mL), VEGF (100 ng/mL) plus riluzole (0.1–10 μM), or riluzole alone (10 μM) (*n* = 4). Values are means ± SD. *Significantly different from control, *P* < 0.001; †significantly different from VEGF alone, *P* < 0.001.
angiography data from retinas obtained from control rats raised continuously in normoxic conditions (P5–P17) appeared normal, with few tufts and a low degree of vascular tortuosity (Fig. 3A, 3B). In contrast, saline-treated pups exposed to hyperoxia exhibited significant vascular tuft formation, central vasoconstriction, and a greater degree of vessel tortuosity (Fig. 3C, 3D). The effect of riluzole was determined after daily intraperitoneal administration from P12 to P16 of either saline (control) or 10 mg/kg riluzole. Compared with saline-treated pups raised in hyperoxic conditions, riluzole-treated rat pups exhibited markedly attenuated tuft formation and vascular tortuosity (Fig. 3E, 3F). Identical administration of riluzole to normoxic control rat pups did not alter the retinal vasculature (data not shown). To quantify the severity of retinopathy, two evaluators scored fluorescein angiographs of whole-mount retinal sections in a condition-blinded manner. Control pups raised in normoxic conditions and given either saline or riluzole, had average scores of 1.21 and 1.46, respectively. Pups raised in hyperoxic conditions and given either saline or riluzole, had average scores of 8.75 and 4.84, respectively (Fig. 3G). Scores in each subcategory of retinopathy are presented in Table 1.

**Reduction of Extraretinal Vessel Formation by Riluzole**

Extraretinal vessel formation was examined in retinal sections stained with hematoxylin and eosin. In normoxic-environment control pups, extraretinal vessel formation was rare (Fig. 4A). However, in pups raised in hyperoxic conditions, extraretinal endothelial cells were numerous, and some had formed vascular tubes (Fig. 4B). Treatment with riluzole markedly reduced the number of extraretinal endothelial cells (Fig. 4C). To quantify the degree of extraretinal vessel formation, the number of endothelial cell nuclei over the internal limiting membrane was counted. We found that in hyperoxia-exposed pups, riluzole markedly reduced the number of extraretinal endothelial cells, compared with saline-treated control pups (Fig. 4D).

**DISCUSSION**

The central findings of the present study were that riluzole effectively inhibited VEGF-stimulated cultured endothelial cell proliferation and reduced abnormal vessel formation in a rat model of ROP. Since diabetic retinopathy and ROP share up-regulation of VEGF as a common pathogenic mechanism for abnormal vascular proliferation, it may be anticipated that riluzole will have a positive effect in patients with diabetic retinopathy.

Riluzole treatment given after the hyperoxic period ameliorated central vasoconstriction. This effect is not easily explained if central vasoconstriction is a permanent pathology at the end of the hyperoxic period. Our results suggest the possibility that at the end of the hyperoxic period, central vaso-
constriction remains partially reversible with anti-VEGF treatments. Central retinal vessels were well formed at P7. Therefore, the initial change during the hyperoxic period is likely vasospasm, which then gradually turns into permanent occlusion. Since little knowledge is available as to when vasoconstriction completes, it seems possible that the beginning of the normoxic period lies within the time window of opportunity for therapeutic intervention. In fact, squalamine given at the beginning of the normoxic period also significantly reduced central vasoconstriction.41

Riluzole is a benzthiazole compound originally shown to inhibit glutamatergic transmission.27,28 Probably due to its antiexcitotoxic effect, riluzole protects against neuron death in a variety of conditions, including ischemia and chronic neurodegenerative conditions.29,30 In 1996, the FDA approved riluzole for treatment of ALS.42 Given that riluzole is routinely administered to ALS patients for prolonged periods and is not known to cause any serious side effects, it is now regarded as safe for long-term use.

Although widely known as an antiexcitotoxic drug, riluzole does not act directly on glutamate receptors. Rather, it decreases the release of neurotransmitters such as glutamate and acetylcholine from presynaptic terminals.43,44 In addition, riluzole inhibits inward currents via voltage-gated
Na channels and voltage-gated calcium channels.\textsuperscript{31,32,45,46} All these effects may contribute to its neuroprotective effect in ALS. We recently showed that riluzole inhibits PKC activity in cortical cell cultures, as well as the activity of purified PKC in vitro.\textsuperscript{33} Consistent with the possibility that inhibition of PKC by riluzole underlies its inhibitory effect on endothelial cell proliferation, riluzole reduced not only VEGF-induced but also PMA-induced endothelial cell proliferation. Moreover, activation of PKC, in particular the PKC \(\beta\) II isoform, was also inhibited by riluzole at the same concentrations. When PKC \(\beta\) II was downregulated by siRNA, however, riluzole did not decrease endothelial cell proliferation. The effective concentrations of riluzole that inhibited endothelial cell proliferation (0.1–10 \(\mu\)M) were a little lower than those that inhibited PKC in cortical cell culture (1–30 \(\mu\)M).\textsuperscript{53} The difference may be due to a difference in cellular uptake or in PKC isoform composition. In humans, administration of 100 mg/d riluzole results in peak plasma levels ranging from 30 to 1552 \(\mu\)g/L (0.13–6.6 \(\mu\)M),\textsuperscript{47} which lie within the effective riluzole concentration range that inhibited endothelial cell proliferation as well as PKC \(\beta\) II in endothelial cell cultures.

VEGF is a potent angiogenic protein that is induced under hypoxic conditions. On secretion, VEGF acts at its cognate receptor tyrosine kinases, Flt-1 and Flk-1. It appears that Flk-1 mediates the cell proliferative effect of VEGF, whereas Flt-1 mediates cell migration and endothelial cell-cell or cell-matrix interaction.\textsuperscript{5} This endothelial cell proliferative signaling via Flk-1 in the retina involves downstream PKC \(\beta\) II. Selective PKC \(\beta\) II inhibitors such as LY333531 were found to reduce retinal vascular permeability and intraocular neovascularization.\textsuperscript{48} Although inhibiting PKC appears very promising as a treatment in proliferative retinopathies, a clinical trial of LY333531 in human diabetic retinopathy provided somewhat disappointing results, indicating a need for the continuing search for effective drugs.

While discovering new chemicals based on their ability to inhibit PKC is a traditional approach in drug discovery, uncovering new actions of clinically approved drugs is possibly a more economical approach. To further encourage that latter approach, we found in the present study that the FDA-approved, anti-ALS drug riluzole inhibited PKC \(\beta\) II activation as well as VEGF-stimulated proliferation in endothelial cell cultures. Furthermore, systemic administration of riluzole was highly effective in reducing abnormal vessel formation in a rat model of ROP.

Our data showing that riluzole inhibited VEGF-mediated endothelial cell proliferation and abnormal vessel formation

\begin{table}[h]
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\textbf{Condition} & \textbf{Blood Vessel Growth (0–3)} & \textbf{Blood Vessel Tufts (0–4)} & \textbf{Extra Retinal Neovascularization (0–3)} & \textbf{Central Vasoconstriction (0–3)} & \textbf{Vessel Tortuosity (0–3)} \\
\hline
Control/saline & 0 & 0.57 ± 0.51 & 0 & 0.07 ± 0.27 & 0.57 ± 0.58 \\
Control/riluzole & 0 & 0.64 ± 0.50 & 0 & 0 & 0.82 ± 0.40 \\
ROP/saline & 0.8 ± 1.06* & 1.65 ± 0.49* & 2.15 ± 0.37* & 1.9 ± 0.72* & 2.25 ± 0.44* \\
ROP/riluzole & 0.11 ± 0.32* & 1.11 ± 0.32* & 1.06 ± 0.54* & 1 ± 0.59* & 1.56 ± 0.51* \\
\hline
\end{tabular}
\caption{Retinopathy Scores for Each Category}
\end{table}

\textbf{FIGURE 4.} Riluzole reduces extraretinal neovascularization. (A–C) Hematoxylin- and eosin-stained retinal sections obtained from rat pups raised in normoxic or hyperoxic conditions, then subjected to the following treatments: (A) normoxic conditions, saline treatment (CTL); (B) hyperoxic conditions, saline treatment (ROP); and (C) hyperoxic conditions, riluzole treatment (ROP + Ril). \textit{Inset} in (B) shows extraretinal vessel formation above the internal limiting membrane (arrow-beads). (D) Number of endothelial cell nuclei above the internal limiting membrane (\(n = 18–20\) each). Values are means \(\pm\) SD. *Significantly different from controls, \(P < 0.01\); \# signifi-
cantly different from ROP, \(P < 0.01\). Scale bar, 100 \(\mu\)m.
in a rat ROP model suggest that riluzole may also be effective in human proliferative retinopathies, probably acting as a PKC inhibitor. The side-effect profile is of crucial importance in considering PKC inhibitors for therapeutic use since PKC serves diverse normal functions in a variety of tissues. The safety profile of riluzole has been extensively examined in humans, and the side effects reported thus far include somnolence, nervousness, anorexia, and asthenia, all of which are relatively minor. Hence, initiating clinical trials for riluzole is likely to be easier than for novel drugs. We propose that the FDA-approved drug riluzole be considered as a potential therapeutic agent for proliferative retinopathy.

References


