Differential Effects of Bactericidal/Permeability-Increasing Protein (BPI) Analogues on Retinal Neovascularization and Retinal Pericyte Growth

Differential Effects of Bactericidal/Permeability-Increasing Protein (BPI) Analogues on Retinal Neovascularization and Retinal Pericyte Growth

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Purpose. Bactericidal/permeability-increasing protein (BPI), an antibacterial and lipopolysaccharide-neutralizing protein, also has an antiangiogenic effect. To evaluate the therapeutic role of BPI in ischemic retinopathies, the antiangiogenic activity of a human recombinant 21-kDa modified N-terminal fragment of BPI ($rBPI_{21}$), which has the biological properties of the holoprotein, and a peptidomimetic (XMP.Z) derived from BPI were examined.

Methods. The effects of $rBPI_{21}$ and XMP.Z on VEGF-induced growth of bovine retinal microvascular endothelial cells (BRECs) and on serum-induced growth of bovine retinal pericytes (BRPs) and retinal pigment epithelial cells (BRPECs) were evaluated by determining total DNA content. The neonatal mouse model of retinopathy of prematurity (ROP) was used to study the effect of XMP.Z in vivo. Intraperitoneal injections of the peptidomimetic (10 mg/kg) were administered every 24 hours for 5 days (postnatal [P]12–P17) during induction of neovascularization. Retinal neovascularization was evaluated using flatmounts of fluorescein-dextran–perfused retinas and quantitated by counting retinal cell nuclei anterior to the internal limiting membrane.

Results. VEGF (25 ng/mL) increased the total DNA per well of BRECs by 120% ± 50% ($P < 0.001$), which was inhibited by addition of $rBPI_{21}$ or XMP.Z, with decreases of 77% ± 15% ($P < 0.05$) and 107% ± 19% ($P < 0.01$) at maximum effective doses of 75 and 15 μg/mL $rBPI_{21}$ and XMP.Z, respectively. In contrast, $rBPI_{21}$ at 75 μg/mL enhanced the total DNA per well of BRP 53% ± 14% ($P < 0.001$) in the presence of 5% fetal bovine serum (FBS), whereas XMP.Z enhanced BRP growth by 27% ± 7% ($P < 0.001$) at 5 μg/mL. In the presence of 10% FBS, $rBPI_{21}$ and XMP.Z increased BRP growth by 91% ± 35% ($P < 0.001$) and 43% ± 18% ($P < 0.01$), respectively. In the oxygen-induced ROP neonatal mouse model, retinal neovascularization was decreased by 40% ± 16% ($n = 5$, $P < 0.01$) when animals were treated with XMP.Z.

Conclusions. Two BPI-derived compounds, $rBPI_{21}$ and XMP.Z, significantly suppressed VEGF-induced BREC growth in vitro, while conversely enhancing the growth of BRPs, even above that induced by 20% FBS. When tested in animals, XMP.Z also suppressed ischemia-induced retinal neovascularization in mice. These data suggest that BPI-derived compounds may have unique therapeutic potential for proliferative retinal diseases such as diabetic retinopathy, if physiological levels can be achieved in clinical settings. (*Invest Ophthalmol Vis Sci.* 2002;43:503–509)

Ischemic retinal diseases, such as diabetic retinopathy, retinopathy of prematurity, and central retinal vein occlusion, are major causes of blindness in the United States.[1] Diabetic retinopathy remains the leading cause of visual loss and new-onset blindness among working-age U.S. citizens.[2] One of the earliest and most specific histologic changes in diabetic retinopathy is the loss of pericytes,[3,4] which leads to capillary loss and retinal ischemia, finally resulting in extensive retinal neovascularization and severe visual loss.[5,6] The neovascularization in ischemic retinal diseases is thought to be mediated primarily by growth factors such as vascular endothelial growth factor (VEGF),[7–9] whose expression is increased by hypoxia.

VEGF is a potent endothelial cell mitogen[10–12] and vasopermeability factor[10,13,14] that mediates its effect through high-affinity, cell-surface transmembrane receptors such as fms-like tyrosine kinase (VEGFR1, previously Flt) and fetal liver kinase 1 (VEGFR2, previously Flk-1).[15–18] Retinal endothelial cells possess numerous VEGF receptors,[16,18] and a variety of retinal cells produce VEGF, including retinal pigment epithelial cells (RPECs), pericytes, endothelial cells, Müller cells, and astrocytes.[7,19] One of the most potent inducers of VEGF is hypoxia, which increases VEGF mRNA expression by up to 30-fold.[7] Intraocular VEGF concentrations are increased during periods of active proliferation,[20] and its intraocular concentration decreases after successful laser therapy, which induces regression of neovascularization.[20] Inhibition of VEGF activity accomplished by a variety of methods prevents ischemia-induced retinal and iris neovascularization in animal models.[21–23]

Current therapies designed to control this aberrant angiogenesis, such as panretinal photocoagulation and cryotherapy, are only partially effective and are inherently destructive to the retina.[24,25] VEGF inhibition has the potential to modulate the neovascular response in a nondestructive manner and therefore may have significant therapeutic value.[21,22]

Bactericidal/permeability-increasing protein (BPI) and its derivative compounds have antiangiogenic properties.[26] Recent reports indicate that BPI can inhibit angiogenesis through induction of apoptosis in human umbilical vein–derived endothelial cells.[27] BPI is a 55-kDa cationic protein present in the azurophillic granules of neutrophils,[28] also known as the cationic antibacterial protein of 57 kDa (CAP57)[29] and the bactericidal protein of 55-kDa molecular mass (BP55).[30,31] The high-affinity binding of BPI with the structurally conserved lipid A region[32] of lipopolysaccharide (LPS, or endotoxin, a glycolipid

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structural component of the bacterial cell wall) makes it specifically bactericidal to Gram-negative organisms. Moreover, the high-affinity interaction of BPI with lipid A also results in inhibition of LPS-dependent biological responses in vitro and in vivo.[33]

In this study, we examined a recombinant modified 21-kDa N-terminal fragment of human BPI ($rBPI_{21}$), which has equivalent or greater activity than the holoprotein in bactericidal and LPS binding assays,[32,34] and a 1.4-kDa peptidomimetic (XMP.Z)[35] derived from BPI. These studies suggest that $rBPI_{21}$ and XMP.Z can effectively inhibit bovine retinal endothelial cell (BREC) growth at low doses, leading to inhibition of angiogenesis both in vitro and in vivo. Further, both $rBPI_{21}$ and XMP.Z appear to exhibit the unusual and unique property of also stimulating the proliferation of bovine retinal pericytes (BRPs).

Materials and Methods

Materials

$rBPI_{21}$, which is a 21-kDa human recombinant modified, N-terminal BPI protein fragment, and XMP.Z, a 1.4-kDa peptidomimetic[35] of synthetic amino acids derived from domain II (residues 65-99) of human BPI were provided by Xoma (US), LLC (Berkeley, CA). Human recombinant $VEGF_{165}$ was obtained from R&D Systems (Minneapolis, MN).

Cell Culture

Fresh calf eyes were obtained from a local abattoir. Primary cultures of BRECs and BRPs were isolated by homogenization and a series of filtration steps as described previously.[36] BRPECs were isolated by gentle scraping after removal of the neural retina and incubation with 0.2% collagenase, as previously published.[37] BRECs were subsequently propagated with 10% plasma-derived horse serum (Sigma Chemical Co., St. Louis, MO), 50 mg/L heparin (Sigma) and 50 μg/mL endothelial cell growth factor (Roche Molecular Biochemicals, Indianapolis, IN) and grown on fibronectin (isolated by collagen affinity column)-coated dishes (Costar, Cambridge, MA). BRPs were cultured in Dulbecco's modified Eagle's medium (DMEM) with 5.5 mM glucose and 20% fetal bovine serum (FBS; GibcoBRL, Grand Island, NY) and BRPEs in DMEM with 5.5 mM glucose and 10% calf serum (GibcoBRL). Cells were cultured in 5% CO_2 at 37°C and media were changed every other day. Cells were characterized for their homogeneity by immunoreactivity with anti-factor VIII antibody for BRECs, monoclonal antibody 3G5 for BRPs,[38] and anti-cytokeratin antibody for BRPECs. BRECs from passages 2 through 7, and BRPs and BRPEs from passages 2 through 5 were used in these experiments. Cells remained morphologically unchanged under these conditions, as confirmed by light microscopy.

Cell Growth Assay

Cells (BRECs, ≈10,000 cells/well; BRPs, ≈20,000 cells/well; BRPECs, ≈15,000 cells/well) were seeded onto 12-well culture plates and allowed to settle overnight, after which they were treated with $rBPI_{21}$, XMP.Z, VEGF (25 ng/mL), serum or combinations thereof. BRECs and BRPECs were incubated for 4 days and BRPs for 6 days at 37°C, after which the cells were lysed in 0.1% SDS, and DNA content was measured by fluorometer (model TKO-100; Hoefer Scientific Instruments, San Francisco, CA), using Hoechst 33258 fluorescent dye. Total DNA content measured using this method correlated with actual cell number, as determined by hemocytometer counting of trypsinized retinal endothelial cells.[16] A similar direct relationship between DNA content per well and cell number per well for pericytes was also observed with 5%, 10%, and 20% FBS, used as growth stimulant (data not shown).

Animal Model

This study adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. A reproducible model of hypoxia-induced neovascularization was used that has been described previously.[39] Litters of 7-day-old (postnatal [P]7) C57BL/6J mice and their nursing mothers were exposed to 75% ± 2% oxygen for 5 days. At P12, the mice were returned to ambient air. Intraperitoneal injections of either vehicle alone (phosphate-buffered saline) or XMP.Z (10 mg/kg body weight) were administered every 24 hours for 5 days (P12–P17) after the return to normoxic conditions.

Retinal Flatmounts

Eyes were enucleated at P17 after intracardiac perfusion with fluorescein-dextran in 4% paraformaldehyde, as described previously.[39] Retinas were isolated, flatmounted (Vectashield; Vector Laboratories, Burlingame, CA), and observed under a fluorescence microscope (model AX70TRE; Olympus, Tokyo, Japan).

Quantification of Neovascularization

As described previously,[21] mice at P17 ($n = 5$) were deeply anesthetized by 100 mg/kg pentobarbital sodium (Abbott Laboratories, Chicago, IL) and killed by cardiac perfusion of 4% paraformaldehyde in phosphate-buffered saline. Eyes were enucleated and fixed in 4% paraformaldehyde overnight at 4°C before paraffin embedding. Over 50 serial 6-μm paraffin-embedded axial sections were obtained, starting at the optic nerve head. After staining with periodic acid–Schiff reagent and hematoxylin, 10 intact sections of equal length, each 30 μm apart, were evaluated for a span of 300 μm. All retinal vascular cell nuclei anterior to the internal limiting membrane were counted in each section by a fully masked protocol. The mean of all 10 counted sections yielded average neovascular cell nuclei per 6-μm section per eye. No vascular cell nuclei anterior to the internal limiting membrane are observed in normal unmanipulated animals.[40]

Statistical Analysis

All experiments were performed in triplicate and repeated at least three times unless otherwise noted. Results are expressed as mean ± SD, unless otherwise indicated. Analyses of in vivo results were performed by Student's *t*-test. For statistical analysis of in vitro study results, analysis of variance and the Tukey test were used to compare quantitative data populations with normal distribution and equal variance. $P < 0.05$ was considered statistically significant.

Results

Effect on VEGF-Induced BREC Growth

The effects of $rBPI_{21}$ and XMP.Z on VEGF-induced growth of BRECs were evaluated. Stimulation of BRECs with recombinant human VEGF (25 ng/mL) produced a 120% ± 50% ($P < 0.001$) increase in cellular DNA content after 4 days, compared with control cells (Fig. 1). This VEGF-induced cell growth was suppressed by the addition of either $rBPI_{21}$ or XMP.Z. The magnitude of the suppressive effect on cell growth was dose dependent, with maximum inhibition of 77% ± 15% ($P < 0.05$) and 107% ± 19% ($P < 0.01$) observed, respectively, when the doses of 75 μg/mL $rBPI_{21}$ (Fig. 1A) and 15 μg/mL XMP.Z (Fig. 1B) were used.

Effect on the Growth of BRPs and BRPECs

BRPs grew by 220% ± 10% ($P < 0.001$) and 489% ± 38% ($P < 0.001$) after 6 days in the presence of 20% FBS, as measured by total DNA content (Fig. 2A, filled bars) and cell number (Fig. 2A, shaded bars), respectively. In the presence of 5% FBS, the addition of 5, 25, and 75 μg/mL $rBPI_{21}$ increased the total amount of DNA in BRPs by 34% ± 1% ($P < 0.01$), 45% ± 11% ($P < 0.01$), and 53% ± 14% ($P < 0.001$), respectively, and the total BRP cell number by 38% ± 46%, 72% ± 65%, and 94% ± 64% ($P < 0.01$), respectively.

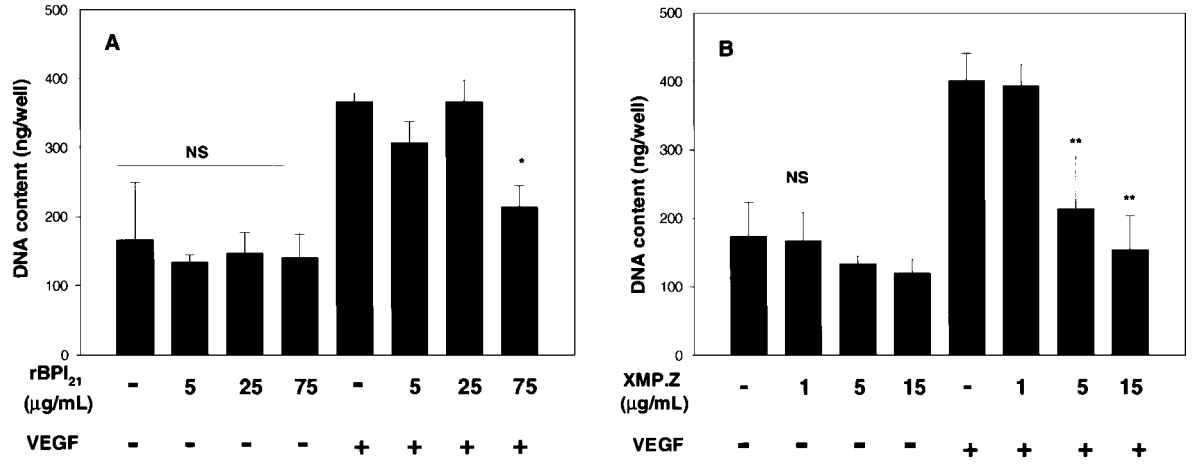

FIGURE 1. The effect of $rBPI_{21}$ and XMP.Z on VEGF-induced BREC growth. Sparsely plated BRECs were exposed for 4 days to the indicated concentrations of $rBPI_{21}$ (**A**) or XMP.Z (**B**) in the presence or absence of 25 ng/mL VEGF. DNA content was measured using Hoechst 33258 fluorescent stain after cell lysis in 0.1% SDS. Experiments were performed in triplicate and repeated three times. Significant differences indicated at $^{*}P < 0.05$ and $^{**}P < 0.01$ versus control containing VEGF (25 ng/mL) alone.

In the presence of 10% FBS, DNA content of BRP increased by 192% ± 19% ($P < 0.001$) after 6 days (Fig. 2B). The addition of 5, 25, and 75 μg/mL $rBPI_{21}$ in the presence of 10% FBS increased DNA content by 46% ± 33% ($P < 0.01$), 74% ± 39% ($P < 0.001$), and 91% ± 35%($P < 0.001$), respectively, above 10% FBS alone. Of particular interest, the addition of 25 and 75

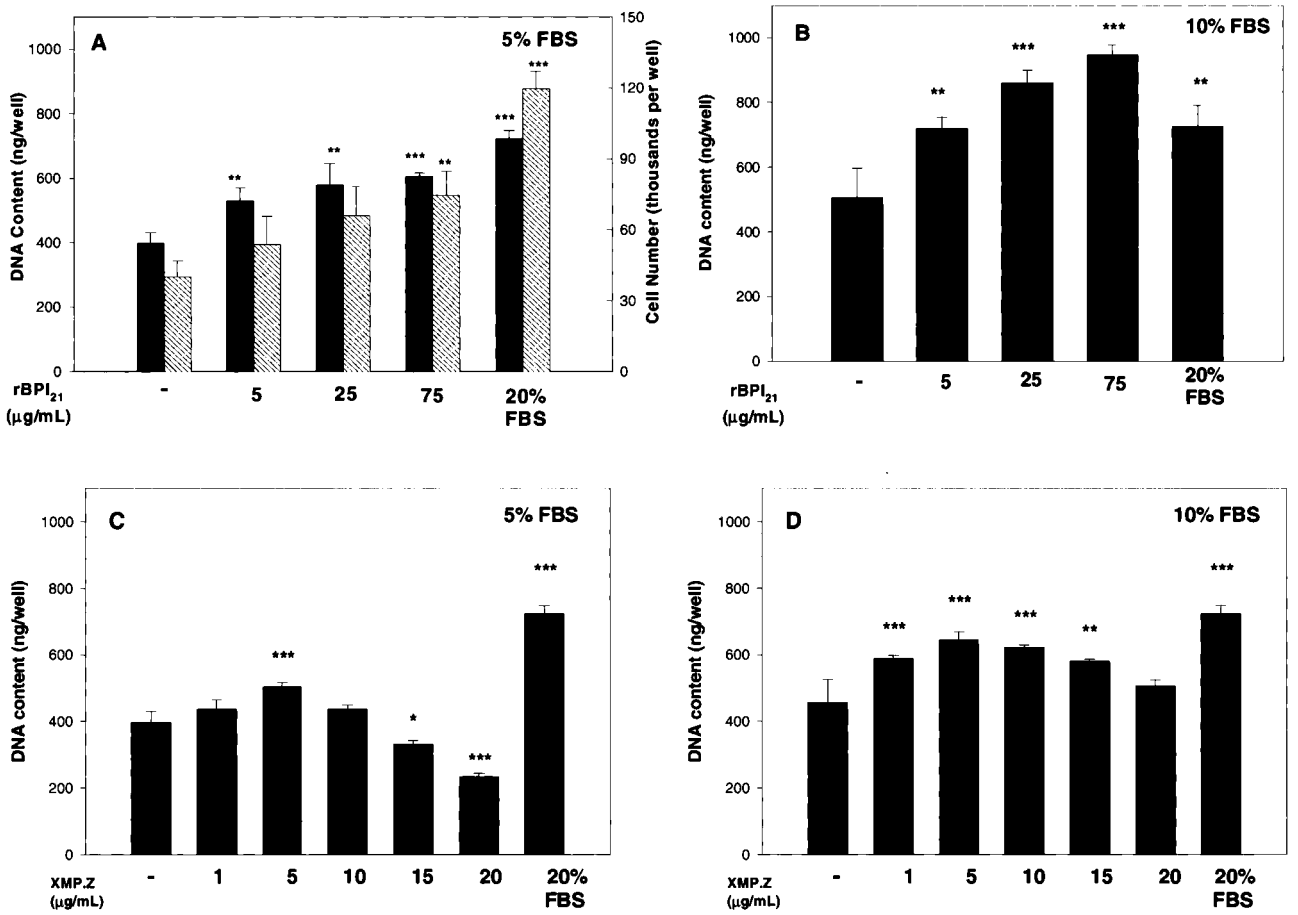

FIGURE 2. Effect of $rBPI_{21}$ and XMP.Z on serum-induced growth of BRPs. Sparsely plated BRPs were exposed for 6 days to the indicated concentrations of $rBPI_{21}$ (**A**, **B**) and XMP.Z (**C**, **D**) in either 5% FBS (**A**, **C**) or 10% FBS (**B**, **D**) in DMEM. DNA content (*filled bars*) was measured using Hoechst 33258 fluorescent stain after cell lysis in 0.1% SDS, and cell number (*hatched bars*) was measured with a hemocytometer. Individual experiments were performed in triplicate and repeated three times. Significant differences indicated at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus the basal level.

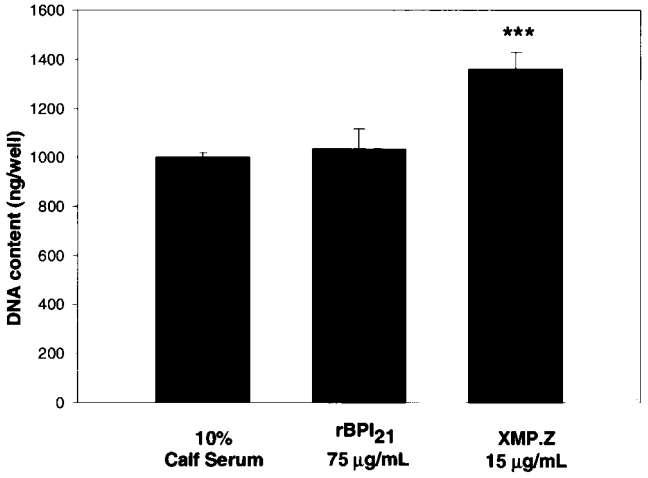

FIGURE 3. Study of the actions of $rBPI_{21}$ and XMP.Z on cultured BRPECs. BRPECs were plated and exposed for 4 days to the indicated concentrations of $rBPI_{21}$ and XMP.Z or to 10% calf serum. DNA content was measured using Hoechst 33258 fluorescent stain after cell lysis in 0.1% SDS. Individual experiments were performed in triplicate and repeated three times. Results are plotted as mean ± SD. ***Significant difference at $P < 0.001$ versus control cells in 10% calf serum.

μg/mL of $rBPI_{21}$ to 10% FBS increased total DNA content by 19% ± 7% and 31% ± 13% ($P < 0.01$; Fig. 2B), respectively, even above that stimulated by 20% FBS.

Similar studies were conducted with XMP.Z (Figs. 2C, 2D). Increasing FBS from 5% to 20% increased total DNA content by 83% ± 19% ($P < 0.001$) after 6 days. The addition of 5 μg/mL XMP.Z to 5% FBS increased DNA content of BRP by 27% ± 7% ($P < 0.001$). In contrast, the addition of 10 μg/mL XMP.Z did not enhance the growth effects of 5% FBS and 15 and 20 μg/mL decreased DNA content of pericytes by 16% ± 10% ($P = 0.05$) and 41% ± 4% ($P < 0.001$), respectively. Similarly, the total DNA content of BRP incubated with 20% FBS increased by 43% ± 5% ($P < 0.002$) above that of 10% FBS. The addition of 1, 5, 10, and 15 μg/mL XMP.Z to 10% FBS enhanced the DNA content by 31% ± 20% ($P < 0.001$), 43% ± 18% ($P < 0.001$), 38% ± 22% ($P < 0.001$), and 29% ± 20% ($P < 0.01$), respectively, compared with cells incubated with 10% FBS alone (Fig. 2D). The addition of 20 μg/mL XMP.Z to 10% FBS did not enhance the growth of BRPs.

The effect of $rBPI_{21}$ and XMP.Z on BRPECs was also studied (Fig. 3). The growth effect of 10% calf serum, in the presence or absence of 75 μg/mL $rBPI_{21}$ or 15 μg/mL XMP.Z on BRPECs was determined. The addition of 75 μg/mL $rBPI_{21}$ to 10% calf serum did not change the growth stimulation of 10% calf serum alone, whereas the addition of 15 μg/mL XMP.Z enhanced the growth of BRPEs by 36% ± 5% ($P < 0.001$) in the presence of 10% calf serum.

Effect of XMP.Z on Ischemia-Induced Retinal Neovascularization In Vivo

To evaluate whether XMP.Z suppresses retinal neovascularization in vivo, we used a murine model of ischemia-induced retinal vascularization.[39] C57BL/6J mice exposed to 75% ± 2% O_2 for 5 days (P7–P12) showed development of extensive retinal neovascularization after they were returned to room air on P12. Retinal neovascularization occurred in 100% of animals by P17.[40] As previously reported,[40] oxygen treatment induced vaso-obliteration, with extensive avascular retinal areas by P12. At P17 neovascular tufts were evident, extending above the internal limiting membrane into the vitreous, particularly in the midperiphery.

To investigate the effects of XMP.Z on retinal neovascularization, the compound was injected intraperitoneally at 10 mg/kg every 24 hours from P12 to P17. In preliminary studies, doses of XMP.Z in excess of 30 mg/kg · d for 5 days were well tolerated in newborn mice (Xoma, unpublished data, 2000). Retinal neovascularization was evaluated at P17 by examining retinal flatmounts (Fig. 4) and cross sections (Fig. 5). In flatmounted retinas from vehicle-treated mice, perfusion of retinal vasculature with fluorescein-dextran detected considerable areas of neovascularization in midperipheral retinas and at the optic nerve head (Fig. 4A). The area of neovascularization was reduced, and neovascularization was less evident around the optic nerve head in retinas from mice treated with XMP.Z (Fig. 4B). Examination of retinal cross sections showed a reduction in retinal neovascularization anterior to the internal limiting membrane in mice treated with XMP.Z (Fig. 5B) compared with control mice (Fig. 5A). Differences between treatment groups were not observed in neuronal retinal layers.

Quantitation of retinal neovascularization by masked counting of endothelial cell nuclei anterior to the internal limiting membrane indicated that administration of XMP.Z (10 mg/kg, intraperitoneal) from P12 to P17 reduced ischemia-induced retinal neovascularization by 40% ± 16% compared with that in untreated control mice (Fig. 6, $n = 5$, $P < 0.01$).

DISCUSSION

Full-length BPI (55 kDa) and compounds derived from it are known to have anti-infective and antiangiogenic properties.[26,28,33,34] More recently, the full-length BPI has been shown to induce apoptosis in human umbilical vein endothelial cells and to partially inhibit angiogenesis in a corneal pocket assay.[27]

In the present study, we characterized two compounds derived from BPI with regard to their actions on retinal vascular and nonvascular cells and retinal microvessels in vivo. Both of these molecules were derived from the aminoterminal region of the protein, because this part of the molecule exhibits high affinity binding to heparin[35] and inhibits angiogenesis in other systems.[26] One of the compounds ($rBPI_{21}$) is a recombinant 21-kDa protein, whereas the other (XMP.Z) is a 1.4-kDa peptidomimetic. The results from the mitogenic assays in retinal endothelial cells shows that both $rBPI_{21}$ and XMP.Z are able to inhibit VEGF-induced endothelial cell proliferation. The median effective doses (ED_{50}) for $rBPI_{21}$ and XMP.Z are similar (between 2 and 5 μM). This is a much higher effective dose than that for several other inhibitors of angiogenesis such as thrombospondin-1 (ED_{50}, 0.5 nM), endostatin (ED_{50}, 3 nM),[41] angiostatin (ED_{50}, 1 nM),[42,43] and PEDF (ED_{50}, 0.4 nM).[43] These other antiangiogenic factors appear to mediate their actions by binding to high-affinity cellular receptors, which typically bind in the nanomolar range. In contrast, the mechanism(s) of antiangiogenic action for the BPI-derived compounds is not yet fully understood but could involve binding to growth factors directly or to cellular receptors that have not been reported.

Antiangiogenic effects of XMP.Z are confirmed in the neonatal hyperoxia model, which shows a 40% reduction in retinal neovascularization. These results indicate that XMP.Z has potent antiangiogenic properties. Because retinal neovascularization in this model may involve angiogenic factors other than VEGF, including other non–heparin-binding growth factors such as insulin-like growth factors (IGFs), complete inhibition of angiogenesis may be difficult. Further studies are therefore

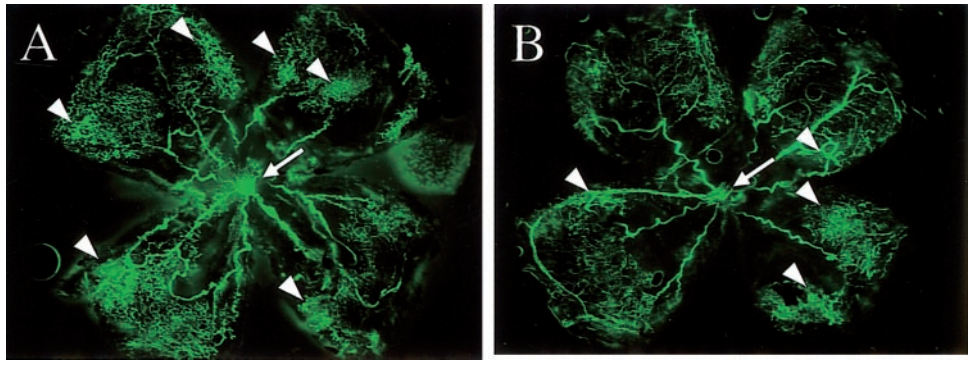

FIGURE 4. Retinal flatmount of neonatal mouse eye treated with vehicle or XMP.Z. Flatmounted fluorescein-dextran–perfused retinas are shown after 5 days of ambient air (P17) after hyperoxygenation and daily intraperitoneal injection from P12 to P17 with 10 mg/kg XMP.Z (**A**) or vehicle (**B**). Areas of neovascularization in midperipheral retinas (*arrowheads*) and optic head (*arrow*) in the treated mice (**B**) compared with control mice (**A**) are indicated.

needed to determine whether greater antiangiogenic effects can be achieved with higher doses of XMP.Z. In addition, correlative studies between plasma levels of XMP.Z and its retinal antiangiogenic effects are also needed. Comparative studies between $rBPI_{21}$ and XMP.Z may also identify whether there are other active domains within the BPI protein that have antiangiogenic actions.

The finding that compounds derived from BPI can enhance pericyte growth while inhibiting VEGF-induced proliferation of BRECs and exhibiting antiangiogenic activity in vivo is surprising for several reasons. Most antiangiogenic factors do not have growth-promoting actions on other types of nontransformed cells. In this case, $rBPI_{21}$ and, to a lesser extent, XMP.Z clearly enhanced pericyte proliferation, even above that observed with 20% FBS. This is the first growth-promoting or -inhibiting factor that has been reported to enhance pericyte growth equal to 20% FBS, while also inhibiting endothelial cell growth. This finding is even more interesting, because pericytes are very slow-growing cells, with a doubling time of several days rather than hours.

These stimulatory activities are located in the amino terminal region of BPI, as illustrated by the results with $rBPI_{21}$ and, to a lesser extent, the XMP.Z peptidomimetic. However, it is likely that the region of BPI that is responsible for the mitogenic actions of pericytes does not coincide exactly with XMP.Z, because at high concentration and low serum levels, XMP.Z had an inhibiting effect on the pericyte. This was not observed with $rBPI_{21}$ or in the presence of high levels of serum. Thus, the peptidomimetic with different structures in the same region must be studied to better define the region of pericyte growth.

The mechanism of $rBPI_{21}$'s unexpected stimulatory actions on pericytes is interesting, because no cellular receptor for BPI has been identified. BPI has, however, been shown to opsonize Gram-negative bacteria, suggesting that there may be receptors on phagocytic cells such as polymorphonuclear cells.[44] The antiangiogenic activity of BPI and its derivatives could also be the result of competition between its heparin-binding domain and the many growth factors that require heparin binding for activity. Some additional possibilities to explain these results include: (1) $rBPI_{21}$ binds to pericyte growth factors and enhances their stimulatory actions, (2) $rBPI_{21}$ could bind and inactivate inhibitory factors in the serum and thereby indirectly enhance the actions of serum, and (3) $rBPI_{21}$ may bind to high-affinity sites on the pericytes and induce stimulatory actions directly.

Irrespective of its mechanism of action, BPI-derived compounds clearly exhibit antiangiogenic and anti-VEGF properties on retinal endothelial cells without inhibiting the growth of BRPs and BRPECs. In addition, BPI itself is stimulatory for the pericyte. Given that retinal pericytes replicate only slowly if at all in vivo, are preferentially lost early in the course of diabetic retinopathy, and may act to suppress retinal endothelial cell growth, the BRP-stimulatory action of BPI-derived compound could be important in the treatment of diabetic retinopathy and related disorders.[45] However, the amount of $rBPI_{21}$ needed for the suppression of endothelial cell growth in the range of 25 to 75 μg/mL is very high and may be difficult to achieve physiologically. The ideal approach would be to identify a peptidomimetic in the region of $rBPI_{21}$ that has both inhibitory actions on endothelial cells and mitogenic actions on pericytes without any inhibitory actions on nonendothelial cells, even at

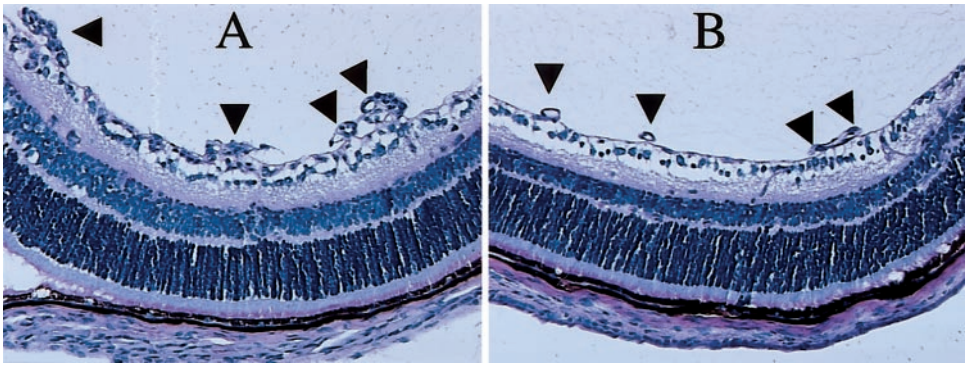

FIGURE 5. Cross section of retinas from neonatal mice at P13. After 5 days of hyperoxygenation, which had begun at P7, mice were placed in a normoxic environment from P12 to P17 and injected daily intraperitoneally with 10 mg/kg XMP.Z. Mice in the control group were treated with vehicle (phosphate-buffered saline). Vertical 6-μm sections of P17 retina, stained with periodic acid-Schiff reagent, showed extensive retinal neovascularization (**A**, *arrowheads*) anterior to the inner limiting membrane. Retinal neovascularization in mice treated with XMP.Z (**B**, *arrowheads*) was reduced.

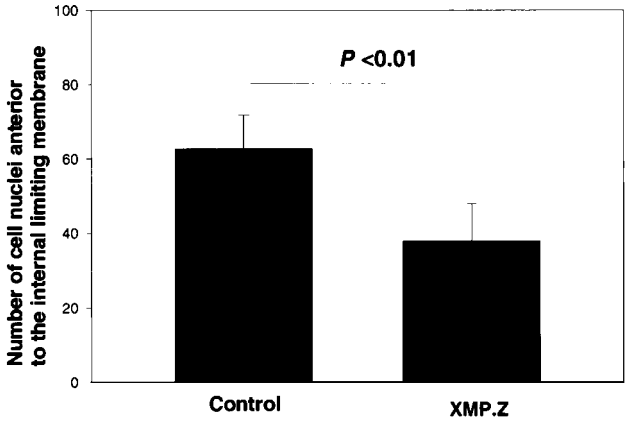

FIGURE 6. Quantitative analysis of cell nuclei anterior to the internal limiting membrane in the retina of neonatal mice. Quantitative evaluation was performed to determine the effect of XMP.Z or vehicle alone on retinal neovascularization. Neovascularization was quantitated by counting all nuclei anterior to the internal limiting membrane. Error bars, SD for all animals in each group ($n = 5$ per group, $P < 0.01$).

high concentrations. Thus, the spectrum of biological properties exhibited by the BPI-derived compounds represents a potentially ideal combination for a therapeutic agent directed at diabetic retinopathy. However, this remains a speculation that requires further study before it can even be considered as a candidate for clinical trial.

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