Prolactin in Eyes of Patients with Retinopathy of Prematurity: Implications for Vascular Regression

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PURPOSE. Disruption of the anti-angiogenic environment of the retina leads to neovascular eye diseases, including retinopathy of prematurity (ROP). Prolactin (PRL), the hormone originally associated with milk secretion, is proteolytically processed to 16K-PRL, a fragment with potent antiangiogenic, proapoptotic effects. Whether 16K-PRL is produced in eyes of patients with ROP and promotes the regression of intraocular blood vessels associated with this disease was investigated.

METHODS. PRL was quantified in the aqueous humor, subretinal fluid, and serum from patients with stage 5 ROP and in patients with non-neovascular eye disorders. Intraocular expression of PRL was evaluated by RT-PCR, in situ hybridization, and Western blot analysis. Anti-PRL antibodies were injected intravitreally in neonatal rats, and apoptosis of hyaloid vessels determined by TUNEL and ELISA.

RESULTS. PRL was elevated in ocular fluids and serum from ROP patients. There was no correlation between PRL in ocular fluids and its level in serum, whereas PRL in aqueous humor and subretinal fluid were significantly correlated. PRL mRNA was expressed in blood vessels and leukocytes within retrolental fibrovascular membranes of ROP patients, and these membranes contained a 16 kDa immunoreactive PRL. The 16K-PRL isoform was more concentrated in subretinal fluid than in serum and was generated from PRL by subretinal fluid proteases. Intravitreal injection of neutralizing anti-PRL antibodies inhibited the apoptosis of hyaloid vessels in neonatal rats.

CONCLUSIONS. 16K-PRL derived from PRL internalized from the circulation or synthesized intraocularly can stimulate apoptosis-induced vascular regression and contribute to the development and progression of ROP. (Invest Ophthalmol Vis Sci. 2004;45:2049–2055) DOI:10.1167/iovs.03-1346

Retinopathy of prematurity (ROP) is a major cause of blindness in children throughout the world.1 Current treatment damages the retina and is frequently only partially effective at reducing blindness. ROP is initiated by delayed retinal vascular growth after premature birth, which leads to hypoxia-induced formation of surplus abnormal vessels and the attendant risk of retinal detachment and blindness.1,2 These alterations reflect an imbalance between factors that either stimulate or inhibit vessel growth. Vasoproliferative retinopathies are associated with elevated levels in the eye of multiple inducers of angiogenesis, including vascular endothelial growth factor,5,4 basic fibroblast growth factor (bFGF),5 insulin-like growth factor –1,5 angioptin,1 placental growth factor,9 and hepatocyte growth factor.10 Likewise, pigment epithelium-derived factor, a major inhibitor of angiogenesis in the eye, is downregulated during ocular neovascularization.10 However, in contrast to angiogenesis stimulators, few angiogenesis inhibitors are known to operate in the eye, and little attention has been given to the discovery of new inhibitors.11 This is surprising, considering that ocular tissues are maintained physiologically without the occurrence of neovascularization, and the ocular vasculature is highly restricted despite the presence of many angiogenesis stimulators in the eye.5,12,13

PRL, originally identified as a lactotrophic hormone secreted by the pituitary gland, is now known to be produced by numerous extrapituitary tissues, including endothelial cells,14–16 neuronal, and immune cells,17 and it is implicated in a vast array of physiological functions that range from reproduction and oomregulation to immunomodulation and angiogenesis.18,19 PRL can be proteolytically cleaved to 16K-PRL, a fragment that acts as a potent inhibitor of angiogenesis both in vivo and in vitro, inhibiting endothelial cell proliferation,20 and stimulating expression of the type-1 plasminogen activator inhibitor21 and endothelial cell apoptosis.22 The potential involvement of 16K-PRL in ocular angiogenesis is suggested by studies showing that 16K-PRL inhibits bFGF-induced corneal angiogenesis, and that implants containing anti-PRL antibodies induce angiogenesis in the cornea.23 In addition, PRL mRNA and PRL and 16K-PRL have been detected in the cornea, iris, and retina of rats.24 Furthermore, hypoxia, the main trigger of ocular neovascularization, decreases PRL synthesis and suppresses its conversion to 16K-PRL in rat pituitary tumor cells.25 Here, measurements were made of PRL and 16K-PRL in sera, ocular fluids and fibrovascular membranes
from patients with ROP. Evidence is presented that they are synthesized within the eye and that 16K-PRL can promote vascular regression in ROP. A preliminary report on some of these findings has appeared (Quiroz-Mercado H, et al. IOVS 2000;41:ARVO Abstract 1766).

METHODS
Study Subjects
Undiluted samples of aqueous humor, subretinal fluid, serum, and retrolental fibrovascular membrane (FVM) were obtained from patients with stage 5 ROP undergoing open-sky vitrectomy. For comparison, PRL determinations in aqueous humor and serum were taken from age-matched infants undergoing intraocular surgery for congenital cataracts. The protocol for sample collection followed the tenets of the Declaration of Helsinki, was approved by the Ethics Committee of the Hospital ‘Luis Sánchez Bulnes,’ and informed consent was obtained from the infants’ parents. Age at surgery ranged from 6 months to 3 years. Medical histories were taken of these patients to exclude any underlying systemic disease including diabetes mellitus, congestive heart failure, hypertension, renal or hepatic insufficiency, and seizure disorders. The duration and extent of retinal detachment were recorded. Blood samples were obtained from the anesthetized infants immediately before surgery, and sera were immediately separated by 5 minutes microfuge centrifugation, and stored at −70°C until assayed.

Bioassay
Bioactive PRL was determined using the Nb2-cell bioassay as detailed previously.28 Incubations were carried out for 48 hours in the absence or presence of different dilutions of aqueous humor, subretinal fluid, serum, or of the human PRL standard purchased from A. F. Parlow (National Hormone and Pituitary Program, Torrance, CA) with or without a 1:500 dilution of PRL antiserum. The human PRL antiserum (HC-1) was generated in our laboratory and characterized as described.15 Proliferation of Nb2 cells is linear in the range of 0.05 to 1 ng/mL PRL and is a standard procedure used to determine PRL levels in serum samples.27

Immunoprecipitation–Western Blot Analysis
Size heterogeneity of PRL was determined in subretinal fluid and serum samples by immunoprecipitation–Western blot analysis using antiPRL antisemur (HC-1), and the previously reported technique.15 Optical density values were determined using 1D image analysis software, version 3.5 (Eastman Kodak Company, Rochester, NY).

Reverse Transcriptase–Polymerase Chain Reaction (RT-PCR)
Total RNA from FVM of ROP patients was obtained and RT-PCR performed essentially as described by Clapp and colleagues.28 Two primers complementary to human PRL cDNA were synthesized: upstream primer from exon 2 (5′-GATGCCAGGTGACCCTTCGAGA-3′) and downstream primer from exon 5 (5′-AGATGTGTGTGGATGATG-3′). RT-PCR products were confirmed by Southern blot analysis.

In Situ Hybridization
Sense and antisense PRL mRNA probes were transcribed in vitro from a linearized plasmid (pcDNA3; Invitrogen, Carlsbad, CA) containing the cDNA for human PRL with T7 and SP6 polymerases and labeled with Digoxigenin-UTP (Boehringer Mannheim, Manheim, Germany). FVM from ROP patients were washed in PBS, embedded in Tissue-Freezing Medium (Leica Instruments, Nussloch, Germany), sectioned (10 μM), and subjected to in situ hybridization performed as previously described.16

PRL Cleavage Analysis
The activity of the enzymes that cleave PRL to 16K-PRL was assayed by the reported method29 with the following modifications. Briefly, 10 μL of subretinal fluid diluted 1:5 in water were mixed with 10 μL of the human PRL standard (20 ng per μL of 0.1 M Tris-HCl, pH 7.4) and with 20 μL of reaction buffer (0.1 M citrate-phosphate, 0.15 M NaCl, pH 5.0) for 24 h at 37°C. The PRL cleavage products were separated by SDS-PAGE under reducing conditions and subjected to Western blot analysis.

Intravitreous Injection
An antirat PRL polyclonal antibody able to neutralize the activity of PRL and 16K-PRL in vivo35 and in vitro was used.16,30 Animals were maintained and treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Wistar rats at postnatal day 10 (P10) were anesthetized with intraperitoneal pentobarbital (20 ng per g of body weight) and their eyes intravitreally injected with 2 μL (2 μg) of purified antiPRL polyclonal antibodies, control antibodies (purified from normal serum), or vehicle only (PBS). At P15, eyes were enucleated and the hyaloid tissue removed.

Apoptosis Determination
Samples were evaluated for evidence of DNA fragmentation associated with apoptosis by the following techniques.

TUNEL assay. FVM from ROP patients or the hyaloid tissue from rats was fixed in 4% paraformaldehyde in PBS, pH 7.4 for 10 minutes or 20 minutes, respectively. Subsequently, the tissues were washed in PBS, embedded in Tissue-Tek, sectioned (10 μm), and analyzed for apoptotic cells by terminal deoxynucleotidyltransferase-mediated UTP end labeling (TUNEL) staining, using a Detection Kit (Roche Diagnostics, Mannheim, Germany) and a fluorescence microscope (Olympus BX60, Lake Success, NY). Each section was visually scanned with a high power (40X) objective in a serpentine manner to record the total number of TUNEL positive cells in the entire section. The total number of TUNEL-positive cells per unit area was calculated after determining the area by tracing the outline of each section with an image analysis system attached to the microscope.

ELISA. The apoptotic cell death detection ELISA (Boehringer Mannheim, Indianapolis, IN) was used to quantitatively determine fragmented nucleosomal DNA associated with apoptotic cell death, according to manufacturer’s instructions.

Statistics
Experiments were replicated at least three times. Means were compared using Student’s t-test, and correlations were evaluated by linear regression analysis. When comparing more than three groups, ANOVA with post-hoc analysis was used. The significance level was set to 5%.

RESULTS
PRL Levels in Ocular Fluids and Serum from Patients with ROP
Samples were obtained from 48 patients (21 females and 27 males) with stage 5 ROP. The mean age of the patients was 1.2 years (range, 0.5 to 3 years). The ELISA measured PRL in all samples of subretinal fluid and serum with mean values of 23.3 ± 1.9 ng/mL and 43.0 ± 5.6 ng/mL, respectively (Fig 1A). The concentration of PRL in serum was significantly higher (P < 0.05) than in subretinal fluid. In the aqueous humor, average immunoreactive PRL (4.8 ± 1.1 ng/mL) was lower (P < 0.05) than in the subretinal fluid, and was detected only in 51.3% of the samples (19 of 37 patients) (Fig 1A). There was no significant correlation between PRL concentration and age (P = 0.3, ANOVA) or sex (P = 0.4, P = 0.2, and P = 0.3, for the aqueous humor, subretinal fluid, and serum, respectively).
Elevation of PRL Levels in ROP Patients

PRL levels in ROP patients were compared to those in 18 patients (6 females and 12 males) with a non-neovascular eye disorder (congenital cataracts). The mean age of these patients was 5 years (range, 1 to 6 years). Because there is no retinal detachment in these patients, the subretinal fluid is not amenable to sampling. As in the ROP group, patients with congenital cataracts showed no correlation between PRL levels and the age or sex status. The mean PRL values measured in both the aqueous humor and serum of these patients were significantly lower than corresponding values in ROP patients.

Lack of Correlation between Ocular and Circulating PRL Levels

To investigate whether ocular PRL could originate from systemic PRL, the concentrations of PRL in ocular fluids and those in serum were compared in the same individuals. However, no correlation was found between PRL concentration in aqueous humor and PRL concentration in serum ($r = 0.0103, P < 0.96$, Fig. 3), nor between PRL in subretinal fluid and PRL in serum ($r = 0.0993, P < 0.53$, Fig. 3). However, the concentration of PRL in aqueous humor was significantly correlated with its concentration in subretinal fluid ($r = 0.6791, P < 0.01$, Fig. 3).

PRL Synthesis in FVM of ROP Patients

RT-PCR—Southern blot analysis detected the expression of PRL mRNA in FVM from patients with ROP. RT-PCR amplified a single product with a corresponding length of 348 bp (Fig. 4A, lane 3), which is similar to the one amplified from the PRL cDNA positive control (Fig. 4A, lane 1). To localize the expression of the PRL message, FVM were subjected to in situ hybridization using an antisense PRL RNA probe. The membranes were comprised of a fibrous component with interspersed blood vessels. PRL mRNA was detected in the vascular portions within vessel walls and in infiltrated leukocytes (Figs. 4B and 4D). In contrast, no apparent hybridization signal was found in FVM hybridized with the sense probe (Figs. 4C and 4E).

Intraocular Generation of 16K-PRL

Consistent with the translation of PRL mRNA and the posttranslational cleavage of the protein, a 16 kDa immunoreactive PRL was detected by Western blot analysis in lysates from FVM (Fig. 5A). In addition, immunoprecipitation-Western blot analysis revealed PRL-immunoreactive proteins of 23 and 16 kDa in subretinal fluid and sera from three different ROP patients (Fig. 5B). The ratio of 16K-PRL to full-length protein (23K-PRL) was significantly higher ($P < 0.05$) in subretinal fluid than in serum from the same patients ($1.0 \pm 0.2$ vs. $0.3 \pm 0.08$, respectively). This difference may result from the local proteolysis of PRL by subretinal fluid proteases. To determine whether the proteolytic activity responsible for generation of 16K-PRL is present in subretinal fluid, exogenous PRL (200 ng) was incubated with 2 μL of subretinal fluid, and PRL proteolysis was evaluated by Western blot analysis (Fig. 5C). Because the concentration of endogenous PRL in 2 μL of subretinal fluid is not enough to be detected by Western blot analysis (Fig. 5C), any PRL-immunoreactive protein should derive from the exogenous hormone. After incubation, in addition to PRL, a second immunoreactive protein was seen that corresponded in size to 16K-PRL (Fig. 5C). The generation of the 16 kDa product seemed to be attributable to the action of a protease in the subretinal fluid, because no 16K-PRL was evident when the 23K-PRL standard was incubated with heat-inactivated subretinal fluid or in the absence of subretinal fluid (Fig. 5C).
Apoptosis in FVM

Several TUNEL-positive cells were visualized throughout the FVM from ROP patients, many of which were associated with vascular structures (Figs. 6A, 6B, and 6C).

AntiPRL Antibodies Prevent Apoptosis in Hyaloid Vessels from Neonatal Rats

To investigate whether PRL-derived peptides could play a role in promoting apoptosis of blood vessels in FVM of ROP, evaluation was made of the effect of the intravitreous injection of neutralizing antiPRL antibodies on apoptosis of the hyaloid vascular system in neonatal rats. The hyaloid system is a transient network of intraocular vessels present during development; in humans it normally regresses by apoptosis before birth, whereas in the rat it regresses mainly between P10 and P20.31 We observed that at P13 the hyaloid tissue removed from rat eyes treated with control antibodies (Figs. 6D and 6E) or vehicle (not shown) showed prominent TUNEL staining in association with blood vessels and in cells interspersed within the stroma. In contrast, very few TUNEL-positive cells were detected in the hyaloid system from eyes injected with antiPRL antibodies (Figs. 6F and 6G). These differences were found to be statistically significant ($P < 0.01$) after counting the number of TUNEL-positive cells (Fig. 7A). Similarly, measurement of fragmented nucleosomal DNA by cell death ELISA showed that intravitreous administration of PRL antibodies significantly ($P < 0.01$) reduced apoptosis of the hyaloid tissue (Fig. 7B).

DISCUSSION

ROP is an ischemia-induced proliferative retinopathy that shares pathological characteristics with other common ocular diseases including diabetic retinopathy and age-related macular degeneration. While different etiologies lead to insufficient perfusion of the retina, once a critical level of ischemia has been achieved, the different forms of retinopathy converge on the common hypoxia-induced formation of blood vessels that may invade the vitreous and lead to retinal detachment and blindness. Current laser therapy has reduced the incidence of blindness by suppressing neovascular growth leading to retinal detachment; however, visual outcome after treatment is often poor. Extending the knowledge of natural angiogenesis inhibitors within the eye is key to developing more effective approaches for the treatment and prevention of these diseases. 16K-PRL, a potent inhibitor of angiogenesis, is produced locally within the eye of patients with ROP, and may be involved in the regression of intraocular blood vessels.

**FIGURE 3.** Lack of correlation between PRL levels in serum and PRL levels in aqueous humor (AH) or in subretinal fluid (SF), and significant positive correlation between PRL levels in AH and PRL levels in SF of patients with ROP. Determinations were by ELISA. The number of patients analyzed in each group is indicated in parentheses. Bars show the mean ± SEM of the respective groups whose individual values were correlated. The correlation coefficient ($r$) and significance level ($P$) are indicated.

**FIGURE 4.** Expression of PRL mRNA in fibrovascular membranes of ROP patients. (A) Southern blot of the RT-PCR product obtained by using primers specific for PRL in fibrovascular membranes from patients with ROP (lane 3). PRL cDNA was used as a positive control (lane 1), and omission of reverse transcriptase served as a negative control (lane 2). (B) and (D) PRL mRNA is visualized by in situ hybridization with an antisense RNA probe in blood vessels (arrow heads) and in leukocytes (arrows) of fibrovascular membranes from patients with ROP. No positive signal follows hybridization with the sense probe (C, E). Scale bar = 50 μm (B–E).
PRL acts as a circulating hormone and as a cytokine in a wide variety of processes including angiogenesis. PRL can be posttranslationally modified by proteolytic cleavage to 16kDa-PRL, a fragment with antiangiogenic actions.19 PRL in the aqueous humor, subretinal fluid, and serum of patients with stage 5 ROP was determined by both ELISA and the specific Nb2-cell bioassay. The values did not vary with the gender or age of the patients, and were not influenced by emotional stress since samples were obtained from patients under general anesthesia. Actually, PRL values in serum of control patients (congenital cataracts) were equivalent to those reported in anesthetized children (24.4 ± 3.4 vs. 18.4 ± 11.4 ng/mL, respectively) and in conscious children (13.3 to 25.5 ng/mL) with matching ages (between 1 and 6 years).32,33 Likewise, PRL levels in aqueous humor of control infants were similar to those reported in adult patients (0.5 to 1.9 ng/mL) undergoing cataract surgery.34

Interestingly, higher PRL values were measured in serum and aqueous humor from ROP patients than from patients with a non-neovascular eye disorder (congenital cataracts). It is unclear whether this increase is functionally related to the disease. A functional connection between pituitary hormones and vasoproliferative retinopathies was hypothesized a long time ago after finding that regression of retinal neovascularization followed pituitary infarction.35 In fact, based on this observation, pituitary ablation was used as a form of therapy for proliferative diabetic retinopathy.36 Nevertheless, analysis of diabetes has either shown reduced circulating levels of PRL in association with severe retinopathy,37 or no change in connection with this disease.38 The reasons for these discrepancies are not immediately obvious, but may suggest that changes in systemic PRL relate to general conditions associated with the specific disease other than retinopathy itself. Diabetes can affect the secretion of PRL,39 and several studies describe hyperprolactinemia in preterm and term infants.40,41

![Figure 5](image5.png)

**Figure 5.** (A) Western blot analysis of immunoreactive PRL-like proteins in fibrovascular membranes (FVM) of patients with ROP. Human PRL standard marks the 23 kDa position. (B) Subretinal fluid (SF) and serum (S) samples from three different patients with ROP were immunoprecipitated and subjected to Western blot analysis. Immunoreactive proteins of 23 and 16 kDa are indicated (arrows). (C) Western blot analysis of PRL cleaved products generated after a purified human PRL standard was incubated in the absence (lane 1) or presence of subretinal fluid (SF) from patients with ROP, before (lane 2) and after heat inactivation for 30 minutes at 85°C (Δ, lane 3). SF incubated in the absence of PRL (lane 4). Blots are representative of three independent experiments.

![Figure 6](image6.png)

**Figure 6.** (A) TUNEL-positive cells in fibrovascular membranes from patients with ROP. Magnification of insert in (A) illustrates the association of TUNEL-positive cells with blood vessels (arrowbeads) under fluorescence (B) and light-field microscopy (C). TUNEL-positive cells in the hyaloid vascular system of neonatal rats injected intravitreously with control antibodies (D) or antiPRL antibodies (F). Same fields illustrating TUNEL-labeled cells in blood vessels (arrowbeads) under fluorescence (D, F) and light-field microscopy (E, G). Scale bar: (A) 400 μm, (B, C) 50 μm, and (D-G) 100 μm.

![Figure 7](image7.png)

**Figure 7.** Quantitative analysis of apoptosis in the hyaloid system of the neonatal rat injected intravitreously with control antibodies (control) or with antiPRL antibodies (α-PRL). (A) The total number of TUNEL-positive cells was counted in each section and expressed per 50 μm². (B) Apoptosis was quantified by cell-death ELISA. Absorbance was measured at 405 nm. Bars show the mean ± SEM from three independent samples. *P < 0.01 vs. control.
High circulating PRL could cause an increase of its concentration in ocular fluids. Radioautographic studies have shown that iodinated PRL injected intracardially is incorporated into ocular tissues, including the retina, choroid, and ciliary body. Such incorporation could be mediated by specific receptor-mediated transport, since PRL receptors are localized in the ciliary epithelium (Dueñas Z and Clapp C, unpublished observations, 2005). Also, impairment of the blood ocular barrier in ROP patients could favor ocular accessibility of the circulating hormone. However, against the latter, no correlation was found between the concentration of PRL in serum and PRL values in aqueous humor or in subretinal fluid of ROP patients. Nonetheless, the concentration of PRL in aqueous humor correlated significantly with that in subretinal fluid. This observation is consistent with subretinal fluid being derived mostly from the vitreous and the posterior aqueous humor flow, and suggests that PRL is internalized into the eye by receptor-mediated transport at the level of the aqueous humor-producing ciliary epithelium.

In addition to its active uptake from the circulation, PRL can be synthesized intraocularly. PRL mRNA has been detected in the cornea, iris, and retina of rats, and cultures of rat retinal capillary endothelial cells express and release PRL. Retrolental FVM of ROP patients express the PRL mRNA, localized within blood vessels and interspersed leukocytes. The cells expressing PRL in the blood vessels could be of endothelial origin, because endothelial cells from retinal capillaries and other vascular beds, in species including the human, synthesize PRL. Also, finding PRL mRNA in leukocytes is not surprising, since compelling evidence shows that immune cells express and respond to this hormone. Whereas the mRNA amplified by RT-PCR corresponded in size to the one encoding for the full-length protein (23K-PRL), Western blot analysis of homogenates from FVM only detected a 16 kDa PRL-like protein. Thus, in these membranes, as at other PRL-producing sites, 16K-PRL appears to be generated not by alternative splicing but by the proteolytic cleavage of PRL. The demonstration that subretinal fluid contains PRL, 16K-PRL, and PRL-cleaving enzymes, substantiates this conclusion. Also, the higher proportion of 16K-PRL found in subretinal fluid relative to that in serum provides evidence that the cleavage of PRL to 16K-PRL can take place intraocularly.

Whether derived from the cleavage of PRL internalized from the circulation or synthesized intraocularly, 16K-PRL may play an important role in the development and progression of ROP. 16K-PRL can halt angiogenesis by inducing the apoptosis of endothelial cells, and endothelial cell apoptosis is an important event mediating the regression of blood vessels in ROP, which in turn, can lead to the resolution of the disease. Investigators have found that two thirds of infants who are born weighing 1250 g or less develop ROP, but only ~6% require treatment, and that even severe ROP can undergo successful spontaneous involution. This report demonstrates that blood vessels in FVM from stage 5 ROP are undergoing apoptosis-mediated regression. Because pro-apoptotic 16K-PRL is present in these membranes, 16K-PRL may promote vascular regression in ROP patients.

The vascular system in ROP membranes includes portions of the hyaloid vasculature, a transient network of intravascular vessels that nourish the immature lens, retina, and vitreous. This system normally regresses before birth, but in premature infants, hyaloid vessels persist and exacerbate vascular vessels that nourish the immature lens, retina, and vitreous. Because the antibodies inhibit apoptosis of both vessel-associated and nonvessel-associated cells, it is clear that not only endothelial cells but also other cell types are targets of pro-apoptotic PRLs. In summary, PRL levels are elevated in the circulation and in the eyes of ROP patients, suggesting that PRL-derived peptides promote the regression of retinal neovascularization in ROP. In these patients, high levels of ocular PRL, originating from internalized systemic PRL or from the hormone synthesized locally, may lead to an increase in the ocular concentration of 16K-PRL. This increase would help counterbalance pathologic angiogenesis by stimulating the apoptosis of blood vessels, and thus, contribute to the favorable resolution of the disease. In this regard, high levels of circulating PRL could have a favorable impact on the prognosis of ROP, since they may enable greater production of 16K-PRL within the eye. In addition, the finding that 16K-PRL promotes vascular regression is clinically relevant, indicating a potential treatment for patients who already have established ocular neovascularization. Notably, the observation that human milk feeding reduces ROP, can be explained on the basis of the present work, since high PRL concentrations are found in milk, milk PRL can reach systemic circulation, and PRL in systemic circulation can reach ocular fluids (present results). In conclusion, these findings link PRL peptides with the underlying causes of ROP, and with the prevention and course of the disease, and they warrant further investigation.

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References
