Graefes Arch Clin Exp Ophthalmol (2016) 254:2277–2279
DOI 10.1007/s00417-016-3445-4

LETTER TO THE EDITOR

Expression of cyclooxygenases and trophic and growth factors in epiretinal membranes at late stages of proliferative vitreoretinopathy

Marina Tikhonovich[1] · **Pavel Lyskin**[2] · **Elena Ioyleva**[2] · **Svetlana Gavrilova**[1]

Received: 25 April 2016 / Revised: 12 July 2016 / Accepted: 19 July 2016 / Published online: 2 August 2016

Keywords Proliferative vitreoretinopathy · Membrane · Cyclooxygenase · VEGF · BDNF · NGF

Dear Editor,

Proliferative vitreoretinopathy (PVR) is one of the main causes of failure after rhegmatogenous retinal detachment (RRD) surgery. Inflammation is a key factor in the development of PVR. This pathology is characterized by proliferation of retinal pigment epithelial (RPE) cells, macrophages, glial cells, fibroblasts, and vitreous cells on the retinal surface, which leads to avascular membranes formation. Growth factors and inflammatory mediators stimulate cell proliferation.

Cyclooxygenases (COX) catalyze the production of prostaglandins from arachidonic acid; this is one of the mechanisms of initiation and maintenance of the inflammatory response. The first and the second types of cyclooxygenase are normally present in the retina. COX-2 expression has been found in human choroidal neovascular membranes [1], in vascularized epiretinal membranes in diabetic retinopathy, and in idiopathic epiretinal membranes [2]. COX-1 was not detected in membranes.

Brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), and vascular endothelial growth factor (VEGF) regulate the viability of retinal cells and their normal functioning. However, retinal detachment and its ischemia are factors that promote the proliferation of glial and RPE cells [3, 4] which actively take part in the development of membranes during PVR.

The purpose of this research was to study the level of expression of pro-inflammatory COX-1 and COX-2, VEGF, BDNF, and NGF in epiretinal membranes from patients with severe PVR.

Eleven patients suffering from RRD complicated by PVR at late stages were included in the study (Table 1). Patients with other ocular and systemic pathologies were excluded. The localization of retinal breaks was peripheral in all patients.

All vitreoretinal surgeries were performed with standard 25-gauge instruments by a single surgeon. During the surgery, the epiretinal membrane of all the eyes was made more visible with triamcinolone acetonide (Kenalog®; KRKA, Slovenia). Membranes were immediately placed in liquid nitrogen, after which they were transferred to 500 ml TRI reagent (Sigma-Aldrich, St. Louis, MO, USA) for a period of 12 h.

The standard method of semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) was used. We used specific primers for β-actin (forward: 5'-AGGCCAACCGCGAGAAGATGAC-3'; reverse: 5'-TCGGCCGTGGTGGTGAAGC-3'), COX-1 (forward: 5'-GCTCCAACCTTATCCCCAGTCCC-3'; reverse: 5'-CATCAACACAGGCGCCTCTTCTAC-3'), COX-2 (forward: 5'-CCTGATGATTGCCCGACTCCC-3'; reverse: 5'-ATACATCATCAGACCAGGCACCAGAC-3'), VEGF (forward: 5'-GGAGGAGGGGGAGGAGGAAGAAGA-3'; reverse: 5'-AGCCCCCGCATCGCATCAG-3'), NGF (forward:

✉ Marina Tikhonovich
marina.tikhonovich@gmail.com

Pavel Lyskin
plyskin@yahoo.com

Elena Ioyleva
med.elioileva@yahoo.com

Svetlana Gavrilova
sgavrilova@mail.ru

[1] Faculty of Medicine, M. V. Lomonosov Moscow State University, 35/5 Lomonosov ave, Moscow 119192, Russia

[2] The S.N. Fyodorov "Eye Microsurgery" Federal State Institution, 127486 Moscow, Russia

Table 1 Clinicopathological profile of patients with PVR and mRNA expression of COX-1, COX-2, VEGF, BDNF, and NGF (in standard units)

No.	Age (years)	Sex	PVR stage	Lens	Preoperative VA	Postoperative VA	Follow-up period (months)	Number of recurrences of RD	COX -1	COX -2	VEGF	BDNF	NGF
1	56	M	C2	Pseudophakia	20/400	0	10	2	0.44	0.06	0.53	0.63	+
2	66	M	D3	Pseudophakia	20/2000	Light perception	18	6	0.43	0.14	0.52	0.71	−
3	58	M	C2	Cataract	20/1000	20/2000	36	0	1.01	0.29	0.55	1.13	−
4	59	M	D3	Pseudophakia	20/2000	0	4	2	0.86	0.07	0.86	1.78	−
5	31	F	D2	Pseudophakia	20/320	Finger counting	15	3	0.51	0.05	0.38	0.46	−
6	74	F	C2	Cataract	Finger counting	20/200	15	1	1.66	0.13	0.72	2.96	−
7	67	M	C1	Clear	Finger counting	20/400	5	1	0.35	0.09	0	1.53	−
8	60	F	D1	Cataract	20/400	20/1000	10	1	1.79	0.76	1.66	2.17	−
9	61	F	D2	Pseudophakia	20/2000	0	6	2	1.94	0.30	0.69	6.92	−
10	54	M	C3	Pseudophakia	20/500	20/400	12	2	0.35	0.17	0.61	0.62	+
11	74	F	B	Pseudophakia	20/640	20/640	14	0	0.60	0.24	0.62	0.67	+

PVR proliferative vitreoretinopathy, *COX* cyclooxygenase, *VEGF* vascular endothelial growth factor, *BDNF* brain-derived neurotrophic factor, *NGF* nerve growth factor, *M* male, *F* female, *RD* retinal detachment, *VA* visual acuity. We used the classification of retinal detachment with PVR developed by the Retina Society in 1983. Stage B wrinkling of the inner retinal surface, rolled edge of the retinal break, retinal stiffness, vessel tortuosity. Stage C full thickness fixed retinal folds: C1, one quadrant; C2, two quadrants; C3, three quadrants. Stage D fixed retinal folds in four quadrants: D1, wide funnel shape; D2, narrow funnel shape; D3, closed funnel (optic nerve head not visible)

5'-CAGCGTCCGGACCCAATAACAG-3'; reverse: 5'-GTGGAAGATGGGATGGGATGATG-3'), and for the ninth encoding exon of the BDNF gene (forward: 5'-TGGCTGGC GATTCATAAGGATAGAC-3'; reverse: 5'-GGCAACGG CAACAAACCACAA-3'), which were synthesized by CJSC Syntol, Moscow, Russia. The results of PCR were normalized to the RNA content in a sample (Table 1).

Expression of COX-1, COX-2, and trophic and growth factors was observed in all membranes in any of the late stages of the disease and for any duration of membrane existence (Fig. 1).

Dependencies of the expression of pro-inflammatory factors on patients' gender, their age, frequency of disease recurrences and treatment outcomes have not been identified.

Spearman's correlation analysis of the data showed that the more the membrane expressed COX-1, the more it produced VEGF (Spearman's $\rho = 0.71$; $p < 0.05$) and BDNF (Spearman's $\rho = 0.74$; $p < 0.01$). Production levels of mRNA VEGF and BDNF were also positively correlated with each other (Spearman $\rho = 0.61$; $p < 0.05$).

It is believed that at a later stage of PVR, the intensity of inflammation in the eye decreases. However, we have shown

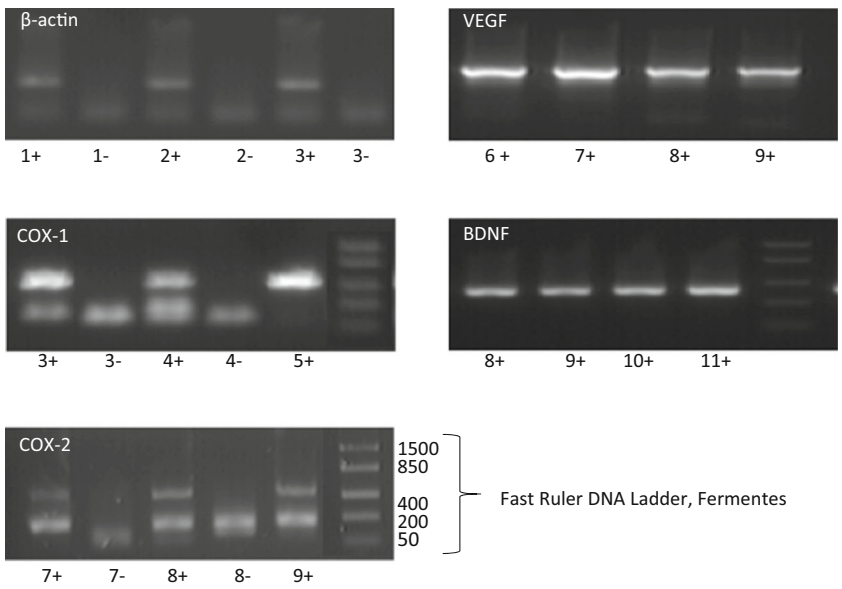

Fig. 1 Examples of gel pictures of RT-PCR results. Numbers refer to the number of the sample; "+" denotes the sample after the reverse transcription, and "−" denotes the negative control sample (without the reverse transcription)

that pro-inflammatory enzymes COX-1 and COX-2 are expressed by membranes at later stages of PVR development. This indicates that the inflammatory processes in membranes remain active in the late stages of the disease.

Membranes also express mRNA of BDNF, VEGF, and in some cases NGF. An increase in the proliferative and secretory capacities of cells forming the membrane under the effects of VEGF, BDNF, and NGF can be seen as a negative effect [3, 4]. These factors also exert neurotrophic effects on neurons and glial cells in the retina. Studies have shown that VEGF reduces the rate of degradation and apoptosis of ganglion cells [5], photoreceptors, and Müller cells [6]. BDNF and NGF are powerful factors that increase the viability of retinal ganglion cells [7, 8] and reduce the degeneration of photoreceptors. These effects are undoubtedly positive.

Our data analysis showed a positive correlation between the expression of VEGF and BDNF in membranes formed in the late stages of PVR. In 2002, Louissaint et al. showed that endothelial cells were able to produce not only VEGF, but BDNF as well [9].

In addition, we found a direct correlation between the expression of VEGF and COX-1 and the expression of BDNF and COX-1 in tested membrane samples. Bryant et al. found that VEGF was able to stimulate the expression of COX-1 in endothelial cells [10]. TGF-β and IL-1β also stimulate the expression of both VEGF and COX-1.

Analysis of our own results and published data allows us to conclude that the presence of the membrane complicating RRD helps to maintain the inflammation in the eye and the growth of the membrane itself.

Acknowledgments This study was supported by RFBR grant no. 14-04-01318-A.

The patients have consented to the use of this information in this letter for submission to the journal.

References

1. Maloney S, Fernandes BF, Castiglione E et al (2009) Expression of cyclooxygenase-2 in choroidal neovascular membranes from age-related macular degeneration patients. Retina 29:176–180
2. Kase S, Saito W, Ohno S, Ishida S et al (2010) Cyclo-oxygenase-2 expression in human idiopathic epiretinal membrane. Retina 30: 719–723
3. Forstreuter F, Lucius R, Mentlein R (2002) Vascular endothelial growth factor induces chemotaxis and proliferation of microglial cells. J Neuroimmunol 132:93–98
4. Kaven CW, Spraul CW, Zavazava NK, Lang GK, Lang GE (2000) Growth factor combinations modulate human retinal pigment epithelial cell proliferation. Curr Eye Res 20:480–487
5. Böcker-Meffert S, Rosenstiel P, Röhl C et al (2002) Erythropoietin and VEGF promote neural outgrowth from retinal explants in postnatal rats. Investig Ophthalmol Vis Sci 43:2021–2026
6. Saint-Geniez M, Maharaj ASR, Walshe TE et al (2008) Endogenous VEGF is required for visual function: Evidence for a survival role on Muller cells and photoreceptors. PLoS One 3:1–13
7. Mansour-Robaey S, Clarke DB, Wang YC, Bray GM, Aguayo AJ (1994) Effects of ocular injury and administration of brain-derived neurotrophic factor on survival and regrowth of axotomized retinal ganglion cells. Proc Natl Acad Sci U S A 91:1632–1636
8. Sivilia S, Giuliani A, Fernández M et al (2009) Intravitreal NGF administration counteracts retina degeneration after permanent carotid artery occlusion in rat. BMC Neurosci 10:1–14
9. Louissaint A, Rao S, Leventhal C, Goldman SA (2002) Coordinated interaction of neurogenesis and angiogenesis in the adult songbird brain. Neuron 34:945–960
10. Bryant CE, Appleton I, Mitchell JA (1998) Vascular endothelial growth factor upregulates constitutive cyclooxygenase 1 in primary bovine and human endothelial cells. Life Sci 62:2195–2201