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Latanoprost rescues retinal neuro-glial cells from apoptosis by inhibiting caspase-3, which is mediated by p44/p42 mitogen-activated protein kinase

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Abstract

The purpose of this study was to investigate whether latanoprost, a prostaglandin F2α analogue, has a direct anti-apoptotic effect both in retinal neuro-glial cells in culture and in diabetic retina. R28 cells, immortalized retinal neuroglial progenitor cells, were induced apoptosis by 24 h serum deprivation. Serum withdrawal made up to 15% of R28 cells pyknotic and activated caspase-3 immunoreactive, and latanoprost acid suppressed apoptosis with dose dependency at an optimum concentration of 1.0 μM ($P < 0.001$). UO126, a mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase kinase (MEK) 1 and 2 inhibitor reversed this effect. Streptozotocin induced one- or three-month diabetic rats received balanced-salt-solution (BSS) in the left eye and latanoprost eye drops in the right for 5 days. Retinal wholemount was subjected to terminal dUTP nick end labeling (TUNEL) staining, whereas eyeballs were enucleated for cleaved caspase-3 immunofluorescence. Retinal homogenates were probed for phospho- or total p44/p42 MAPK and Akt. One- and three-month diabetic retina had 30.2 ± 15.3 and 23.6 ± 9.0 TUNEL positive cells per 0.5 cm^2, respectively, whereas control retina had few TUNEL positive cells. Latanoprost instillation significantly reduced these cells (10.0 ± 3.1 and 11.3 ± 3.1 cells per 0.5 cm^2 for 1 M and 3 M, respectively, $P < 0.01$), whereas BSS did not. Latanoprost also significantly reduced cleaved caspase-3 immunoreactive cells in ganglion cell and inner nuclear layers ($P < 0.05$). Latanoprost increased phosphorylated to total protein ratio of p44/p42 MAPK ($P < 0.05$), but not of Akt. Taken together, the present findings suggest that latanoprost rescues retinal neurons and/or glial cells from apoptosis, which is probably mediated by p44/p42 MAPK through caspase-3 inhibition.

Keywords: retinal neurodegeneration; apoptosis; latanoprost; mitogen-activated protein kinase; diabetic retinopathy

1. Introduction

Latanoprost is one of the prostaglandin-related anti-glaucoma medications with powerful ocular hypotensive effects (Hotehama and Mishima, 1993; Van der Valk et al., 2005) through facilitated uveoscleral outflow (Toris et al., 1993; Stjernschantz et al., 1998). Latanoprost is a prostaglandin F2α (PGF2α) analogue, has the high affinity to the specific receptor FP (Sharif et al., 2003), and is an isopropyl esterified prodrug, which is converted to latanoprost acid (LA) by endogenous esterase (Bito and Baroody, 1987). In addition to the intraocular pressure (IOP) lowering effect, a prior paper demonstrated the potential neuroprotective ability in retina of latanoprost (Drago et al., 2001). Latanoprost decreased lactate accumulation in the in vivo retina given ischemia/reperfusion injury, whereas LA reduced lactate dehydrogenase (LDH) release from primary cultures of human retinal cells in vitro exposed to glutamate or hypoxia/re-oxygenation (Drago et al.,

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2001). PGF2α is known to inhibit apoptosis of some types of cells and tissues including cortical neurons (Cazevieille et al., 1994). In addition, the retina expresses FP receptor (Ocklind et al., 1996, 1997; Davis and Sharif, 1999). However, precise mechanism by which latanoprost and its active metabolite LA exert the neuroprotective ability in retina is still open to debate.

Evidence is mounting that diabetic retinopathy affects not only vascular permeability and growth but also the neuro-glial metabolism and function in retina (Barber, 2003; Nakamura et al., 2005), including increased apoptosis of retinal inner neurons (Hammes et al., 1995; Barber et al., 1998; Agardh et al., 2001; Kanamori et al., 2004a; Martin et al., 2004), altered expression of glial fibrillary acidic protein (GFAP) in astrocytes and Müller cells (Lieth et al., 1998; Mizutani et al., 1998; Barber et al., 2000; Rungger-Brändle et al., 2000), microglia activation (Zeng et al., 2000), and impaired glutamate metabolism (Ambati et al., 1997; Lieth et al., 2000; Kowluru et al., 2001). Streptozotocin (STZ)-induced diabetic pathology in rat retina, which is an IOP irrelevant neurodegenerative disease unlike glaucoma, is a useful model for assessing the neuroprotective ability of anti-glaucoma agents such as latanoprost, because one should distinguish the direct neuroprotective effects in the retina from IOP-lowering effects of the anti-glaucoma agents.

The p44/p42 mitogen-activated protein kinase (MAPK) is known to mediate the neurotrophic ability of several types of growth factors in various kinds of cells or tissues (Cobb, 1999). Upon binding of growth factors to their specific receptors, a GDP bound form of small molecular weight G protein Ras is transformed to a GTP bound one, which subsequently activates Raf, mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK) 1/2, and eventually p44/p42 MAPK by dual phosphorylation of threonine and tyrosine residues in the TEY motif. The activated p44/p42 MAPK phosphorylates and thus regulates downstream targets to modulate cellular function and gene expression (Cobb, 1999).

The purposes of this study were: 1) to test whether latanoprost and LA prevent retinal neurons and/or glial cells in vivo and in vitro, respectively, from apoptosis, which is the fundamental process of the various neurodegenerative diseases in retina including glaucoma (Levin, 2003) and diabetic retinopathy (Barber, 2003); and 2) if so, to elucidate which intracellular pro-survival pathway mediates the cytoprotective ability of latanoprost. In the present study, we tested whether LA and latanoprost eye drops could reduce apoptosis of serum-deprived R28 cells, an immortalized retinal neuro-glial progenitor cell line (Seigel, 1996), and that of cells in retinal ganglion cell layer (GCL) in STZ-induced diabetic rats, respectively, through the p44/p42 MAPK pro-survival signaling and eventual caspase-3 inactivation.

2. Methods

2.1. Specific reagents

LA was purchased from Cayman Chemical (Ann Arbor, MI). Latanoprost eye drop (Xalatan®) was from Pfizer (Tokyo, Japan). LY 294002 was from Upstate Biotechnology (Lake Placid, NY). UO126, KT 5823, KT5720, and KN-93 were from Calbiochem (San Diego, CA). ApopTag Peroxidase In Situ Apoptosis Detection Kit was purchased from Chemicon International (Temesula, CA).

2.2. Cell culture and Induction of apoptosis

R28 cells, retinal progenitor cells that were immortalized by transfection with adenovirus 12S E1A into the neonatal rat retinal tissue (Seigel, 1996), were used in this study. These cells express both glial and neuronal, but not vascular, phenotypes such as GFAP, vimentin, S-100, GluR1 through 3, Thy-1, and NMDA and GABA receptors (Seigel et al., 1996, 2004; Sun et al., 2002). Because of their easiness to handle and established characterization as retinal non-vascular cells, R28 cells have been widely used to elucidate the molecular mechanisms of apoptosis and neurotrophic factor effects over the limitation due to the gene alteration via adenovirus-transfection (Seigel and Liu, 1997; Seigel et al., 2000; Tezel and Wax, 2000; Barber et al., 2001; Nakamura et al., 2001; Mukuno et al., 2004; Narayanan et al., 2006). R28 cells were seeded on glass coverslips at 2×10^5 cells/cm^2 density and fed with Dulbecco's modified Eagle's medium supplemented with 10% newborn bovine serum (GIBCO Inc., Rockville, MD) as described previously (Barber et al., 2001; Nakamura et al., 2001; Mukuno et al., 2004). When grown to 60% confluency, they were deprived of serum for 24 h with or without varying concentrations of LA or DMSO (10 μl/ml medium) as a vehicle. Although glutamate toxicity is thought to be involved in pathogenesis of various neurodegenerative conditions, R28 cells are quite resistant to glutamate toxicity. The glial cell nature that R28 cells potentially hold may facilitate extracellular gutamate uptake. At least in part, for this reason, we chose the serum-deprivation as apoptosis induction, which is another established method (Seigel and Liu, 1997; Barber et al., 2001). Each one of the following inhibitors with indicated concentrations was added 15 min prior to addition of LA or DMSO in some groups of experiments. The inhibitors were LY294002, a phosphatidylinositol 3-OH kinase (PI3K) inhibitor; UO126, an MEK1/2 inhibitor; KT5823, a protein kinase G (PKG) inhibitor; KT5720, a protein kinase A (PKA) inhibitor; or KN-93, a Ca^{2+}/calmodulin kinase II (CaMK II) inhibitor.

2.3. CM-1 immunocytochemistry

CM-1 immunocytochemistry against activated caspase-3 in a combination with Hoechst nuclear staining was performed as previously described (Barber et al., 2001; Nakamura et al., 2001; Mukuno et al., 2004). In brief, the coverslips were fixed in 1% paraformaldehyde for 10 min, and blocked in 10% goat serum at room temperature for 1 h. They were then incubated with a rabbit polyclonal antibody against activated caspase-3 (1:1000, Idun Pharmaceuticals, La Jolla, CA) at room temperature for 1 h. The cells were washed and incubated with tetramethyl rhodamine (TRITC)-conjugated goat anti-rabbit IgG (1:2000, Jackson ImmunoResearch, West Grove, PA) and