Published in final edited form as:

Adv Exp Med Biol. 2018 ; 1074: 457–464. doi:10.1007/978-3-319-75402-4_56.

Pigment Epithelium-derived Factor Protects Retinal Pigment Epithelial Cells Against Cytotoxicity “In Vitro”

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Abstract

Oxidative stress has been implicated in neurodegenerative diseases, such as age-related macular degeneration. Hydrogen peroxide and sodium iodate can mediate oxidative injury. Sodium iodate induces a selective retinal degeneration targeting the RPE. We describe a method of chronic sodium iodate-mediated injury on RPE cells that may serve to evaluate protective factors against oxidative stress. Cytotoxicity and cell viability curves of ARPE-19 cells with sodium iodate were generated. The antioxidant pigment epithelium-derived factor decreased sodium iodate-mediated cytotoxicity without affecting ARPE-19 cell viability. A cell culture system to evaluate protection against oxidative stress injury with PEDF is discussed.

Keywords

Pigment epithelium-derived factor (PEDF); Retinal pigment epithelium (RPE); ARPE-19 cells; Toxicity; Oxidative stress; Sodium iodate (NaIO₃); Viability; Age-related macular degeneration

56.1 Introduction

The retinal pigment epithelium (RPE), a monolayer located at the back of the eye, plays a critical role in the development and maintenance of photoreceptors in the vertebrate retina. The importance of the RPE resides in the nearby relationship with the light-sensitive photoreceptors, which allows transmission of information, transfer of nutrients to photoreceptors, and phagocytosis of metabolic waste (Bok 1993; Rizzolo 1997). Thus, structural or functional injury of RPE can trigger pathologic events in retinal diseases. In fact, age-related macular degeneration (AMD), the most common cause of vision loss in patients over 60 years old, is characterized by RPE degeneration that eventually leads to blindness (VanNewkirk et al. 2000; Friedman et al. 2004). Although the pathophysiology of AMD is not well understood, one of the risk factors associated with it is oxidative stress injury of the RPE (Cai and McGinnis 2012). Thereby, cumulative and prolonged oxidative damage of the RPE may contribute to development of AMD. Administration of sodium iodate (NaIO₃) in rabbit and mouse eyes causes specific and selective RPE cell damage resulting in a reproducible AMD model (Sorsby 1941; Wang et al. 2014; Obata et al. 2004;

Hariri 2013; Bonilha et al. 2015). NaIO_3 retinal toxicity has also been studied in cultured RPE cells (Wang et al. 2014; Balmer et al. 2015; Hanus et al. 2016).

The RPE secretes PEDF into the interphotoreceptor matrix (Wu et al. 1995; Becerra et al. 2004) to act in neuronal differentiation, survival, inhibition of angiogenesis, tumorigenesis, and metastasis in retinal cells (Becerra 1997; Becerra and Notario 2013; Barnstable and Tombran-Tink 2004; Bouck 2002; Bullock and Becerra 2015; Polato and Becerra 2016). Of more interest to this study are the reported antioxidant effects of PEDF on retinal pericytes, granulosa cells, and cardiomyocytes (Amano et al. 2005; Bar-Joseph et al. 2014; Zhuang et al. 2016). Tsao et al. (2006) have reported that PEDF attenuates oxidative stress-induced cell death in ARPE-19 cells using hydrogen peroxide (H_2O_2) as an oxidative agent. However, it is not known whether PEDF can protect RPE cells against other oxidative agents.

The aim of this study is to investigate the effects of PEDF on ARPE-19 cells after exposure to prolonged nonlethal oxidative damage. Toxicity and viability of the RPE cells subjected to treatments with oxidative agents were determined.

56.2 Material and Methods

56.2.1 Materials

Freshly prepared stock solutions of NaIO_3 (90 mg/ml, Sigma, St. Louis, MO) in deionized water were filter-sterilized and was diluted in media for the assays. Stock solution of H_2O_2 (30%; Sigma, St. Louis, MO) was diluted in media for assays.

56.2.2 Cell Culture

Human ARPE-19 cells, obtained from the American Type Culture Collection (Manassas, VA, USA), were cultured in Dulbecco's modified eagle medium (F:12, cat.11330-032) supplemented with 10% fetal bovine serum (FBS, cat.26140-079) and 1% penicillin/streptomycin (cat.15070-063) at 37 °C with 5% CO_2 . All reagents were purchased from Gibco, Grand Island, NY. Assays were performed with ARPE-19 cultures between passages 30 and 38.

56.2.3 Cell Toxicity Assay

Cells were seeded in 96-well plates (1.3×10^4 cells/well) and incubated at 37 °C for 50 h to allow them to reach confluence. Culture media were replaced with serum-free media containing NaIO_3 or H_2O_2 at indicated concentrations and incubated at 37 °C for 16 h. Toxicity was determined by measuring the cytoplasmic lactate dehydrogenase enzyme (LDH cytotoxicity assay, Pierce™, Life Technologies, Carlsbad, CA) in conditioned media following the instructions of the manufacturer. The absorbance was measured using a microplate reader (SpectraMax5, Molecular Devices, Downingtown, PA).

56.2.4 Cell Viability Assay

Intracellular ATP levels, as an indicator of metabolically active cells, determined cell viability using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI) following the instructions of the manufacturer. Medium was removed from cells plated in

opaque 96-well plates, and 100 μ l of each PBS and CellTiter-G lo® Reagent were added, mixed, and incubated at 25 °C for 10 min before measuring luminescence using an automated plate reader (Envision, Perkin Elmer, MA).

56.2.5 Recombinant PEDF

Full-length human PEDF purified from conditioned medium of baby hamster kidney cells stably transfected with pBK-PEDF vectors was as previously described (Stratikos et al. 1996).

56.2.6 Statistical Analysis

Data are expressed as the average $\pm$ SD. The one-way ANOVA and Tukey post hoc test were used to determine the statistically significant differences, and $p < 0.05$ was considered significant (*).

56.3 Results

56.3.1 Toxicity and Viability of ARPE-19 Cells Treated with NaIO₃ and H₂O₂

ARPE-19 cells were exposed to toxic agents to examine concentration-response curves after chronic damage. Increasing concentrations of NaIO₃ or H₂O₂ in media without serum for 16 h were added before cell toxicity and viability were determined (Fig. 56.1a). Both toxicity and viability curves with NaIO₃ concentrations ranging between 0 and 15 mM were sigmoidal and inverse (Fig. 56.1b). The curves revealed a similar estimated concentration value for half-maximal effect (EC₅₀ = 6.5 mM) at which both curves seemed to intersect. While detectable levels of toxicity were observed with >6 mM NaIO₃, cell viability decreased with 5 mM NaIO₃ (70%). Cytotoxicity reached 80% with >9 mM NaIO₃. We compared these results with those obtained with H₂O₂ treatments. Curves for toxicity and viability were also sigmoidal and inverse to each other (Fig. 56.1c). The estimated EC₅₀ for toxicity and viability were ~450 μ M H₂O₂ and ~600 μ M H₂O₂, respectively. H₂O₂ at 200–600 μ M increased toxicity and was maximum at 600 μ M H₂O₂. The cell viability curve had a minimum decrease in values between 0 and 500 μ M H₂O₂, which decreased drastically with 700 μ M H₂O₂, *i.e.*, there were an estimated 85% viable ARPE-19 cells with 500 μ M and only 8% with 700 μ M H₂O₂. Similar results were obtained with at least two independent experiments.

56.3.2 Protection of ARPE-19 Cells Against NaIO₃-induced Cytotoxicity

PEDF protects ARPE-19 cells against acute H₂O₂ injury (Tsao et al. 2006). To evaluate its potential protective effect against chronic NaIO₃-induced cytotoxicity, we exposed ARPE-19 cells to PEDF (10 nM) during treatments with 6–8 mM NaIO₃ before determining cell toxicity and viability (Fig. 56.2a). PEDF decreased ARPE-19 cytotoxicity with 6 mM and 7 mM NaIO₃, while there was insignificant change with 8 mM NaIO₃ (Fig. 56.2b). PEDF protection efficacy against cytotoxicity decreased significantly with NaIO₃ concentration from 75% to 12% for 6 to 8 mM NaIO₃ (Fig. 56.2c). PEDF did not increase the cell viability in response to 6–8 mM NaIO₃ (Fig. 56.2d). Similar results were obtained with at least two independent experiments.

To determine the concentration curve of PEDF protection against NaIO₃-mediated injury, we treated ARPE-19 cells with 6 mM NaIO₃ in combination with PEDF ranging 0–10 nM, as above. The cytotoxicity curve shows a well-defined decrease in injury with increasing concentrations of PEDF (Fig. 56.3a). Additions of PEDF at 5 nM and 10 nM decreased 50% the levels of LDH cytotoxicity. PEDF had minor effects on viability at the concentrations tested. Similar results were obtained with at least two independent experiments.

56.4 Discussion

The study establishes an in vitro model system to induce cytotoxicity injury to ARPE-19 cells with NaIO₃, an oxidative toxic agent that can be applied to evaluate protective effects of PEDF against RPE cell injury. We chose agents known to generate oxidative stress and found that cell toxicity and viability occurred in a concentration-dependent fashion for both H₂O₂ and NaIO₃. Moreover, PEDF can protect against cytotoxicity from NaIO₃ and H₂O₂ (Fig. 56.3; Tsao et al. 2006). This model system may be useful in studies to elucidate mechanisms that attenuate NaIO₃-mediated injury of RPE cells. The results are of interest given that oxidative stress is an important mediator of neuronal cell death that contributes to the pathogenesis of retinal degeneration (Cai et al. 2000; Cai and McGinnis 2012; Bonet-Ponce et al. 2016).

The addition of H₂O₂ to cultured RPE cells is a classic model to study oxidative stress (Geiger et al. 2005; Kaczara et al. 2010). NaIO₃ toxicity of retinal cells appears to be dependent on serum level, as serum-free media condition elicited a much higher and reliable LDH release (Wang et al. 2014). Our results indicate that ARPE-19 cells are vulnerable to chronic NaIO₃- and H₂O₂-mediated cell injury, in agreement with previous reports (Fig. 56.1; Wang et al. 2014; Balmer et al. 2015).

We chose PEDF for protection against NaIO₃-mediated injury because it is a naturally occurring protein of the interphotoreceptor matrix that has demonstrable antioxidant properties in ARPE-19, specifically against H₂O₂-mediated apoptosis (Wu et al. 1995; Becerra et al. 2004; Reinoso et al. 2008; Mukherjee et al. 2007; Tsao et al. 2006). The PEDF-mediated protection against cytotoxicity contrasts with its lack of cell viability increase, which may be due to differences in mechanisms of action of PEDF to protect against NaIO₃-mediated injury. In this regard, ARPE-19 cells exposed to H₂O₂ concentrations between 400 and 600 μ M die by apoptosis, whereas necrosis is triggered at higher concentrations (Kim et al. 2003). Cell viability may require that the cells recover after the acute H₂O₂ damage (2 h), as Tsao et al. (2006) observed that PEDF decreases apoptotic nuclei numbers with an increase in cell viability after a recovery time of 16 h in media with 10% FBS. In contrast, our model system uses a chronic induction of injury in which the cells are exposed to NaIO₃ for more than 16 h with no serum. Although PEDF may not be protective when NaIO₃-mediated injury of ARPE-19 is devastating (*e.g.*, at 8 mM NaIO₃), PEDF protection efficacy with NaIO₃ at concentrations around the EC₅₀ (*e.g.*, 6 mM) clearly suggests that cell toxicity or death can be overcome with this factor.

In summary, the NaIO₃-mediated injury of ARPE-19 cell model can serve to study RPE degeneration and protection by relevant survival and antioxidant factors.

Acknowledgments

We thank Dr. Preeti Subramanian for cell culture assistance. This work was supported by the NEI, NIH Intramural Research Program.

Bibliography

- Amano S, Yamagishi S, Inagaki Y, Nakamura K, Takeuchi M, Inoue H, Imaizumi T (2005) Pigment epithelium-derived factor inhibits oxidative stress-induced apoptosis and dysfunction of cultured retinal pericytes. *Microvasc Res* 69(1–2):45–55 [PubMed: 15797260]
- Balmer J, Zulliger R, Roberti S, Enzmann V (2015) Retinal cell death caused by Sodium Iodate involves multiple Caspase-dependent and Caspase-independent cell-death pathways. *Int J Mol Sci* 16(7):15086–15103 [PubMed: 26151844]
- Bar-Joseph H, Ben-Ami I, Ron-El R, Shalgi R, Chuderland D (2014) Pigment epithelium-derived factor exerts antioxidative effects in granulosa cells. *Fertil Steril* 102(3):891–898.e3 [PubMed: 25064413]
- Barnstable CJ, Tombran-Tink J (2004) Neuroprotective and antiangiogenic actions of PEDF in the eye: molecular targets and therapeutic potential. *Prog Retin Eye Res* 23(5):561–577 [PubMed: 15302351]
- Becerra SP (1997) Structure–function studies on PEDF: a non-inhibitory serpin with neurotrophic activity. *Adv Exp Med Biol* 425:223–237 [PubMed: 9433504]
- Becerra SP, Notario V (2013) The effects of PEDF on cancer biology: mechanisms of action and therapeutic potential. *Nat Rev Cancer* 13(4):258–271 [PubMed: 23486238]
- Becerra SP, Fariss RN, Wu YQ, Montuenga LM, Wong P, Pfeffer BA (2004) Pigment epithelium-derived factor in the monkey retinal pigment epithelium and interphotoreceptor matrix: apical secretion and distribution. *Exp Eye Res* 78(2):223–234 [PubMed: 14729355]
- Bok D (1993) The retinal pigment epithelium: a versatile partner in vision. *J Cell Sci Suppl* 17:189–195 [PubMed: 8144697]
- Bonet-Ponce L, Saez-Atienzar S, da Casa C et al. (2016) Rotenone induces the formation of 4-hydroxynonenal aggregates. Role of ROS-mediated tubulin hyperacetylation and autophagic flux disruption. *Mol Neurobiol* 53(9):6194–6208 [PubMed: 26558631]
- Bonilha VL, Bell BA, Rayborn ME et al. (2015) Loss of DJ-1 elicits retinal abnormalities, visual dysfunction, and increased oxidative stress in mice. *Exp Eye Res* 139:22–36 [PubMed: 26215528]
- Bouck N (2002) PEDF: antiangiogenic guardian of ocular function. *Trends Mol Med* 8:330–334 [PubMed: 12114112]
- Bullock J, Becerra SP (2015) PEDF in the retina In: Geiger M (ed) *The Serpin Family. Proteins with multiple functions in health and disease*. Springer, Cham, pp 197–212
- Cai X, McGinnis JF (2012) Oxidative stress: the achilles' heel of neurodegenerative diseases of the retina. *Front Biosci (Landmark Ed)* 17(1):1976–1995 [PubMed: 22201850]
- Cai J, Nelson KC, Wu M, Sternberg P, Jr, Jones DP (2000) Oxidative damage and protection of the RPE. *Prog Retin Eye Res* 19:205–221 [PubMed: 10674708]
- Friedman DS, O'Colmain BJ et al. (2004) Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol* 122:564–572 [PubMed: 15078675]
- Geiger RC, Waters CM, Kamp DW, Glucksberg MR (2005) KGF prevents oxygen-mediated damage in ARPE-19 cells. *Invest Ophthalmol Vis Sci* 46:3435–3442 [PubMed: 16123449]
- Hanus J, Anderson C, Sarraf D, Ma J, Wang S (2016) Retinal pigment epithelial cell necroptosis in response to sodium iodate. *Cell Death Discov* 2:16054 [PubMed: 27551542]
- Hariri S (2013) Noninvasive imaging of the early effect of sodium iodate toxicity in a rat model of outer retina degeneration with spectral domain optical coherence tomography. *J Biomed Opt* 18:026017
- Kaczara P, Sarna T, Burke JM (2010) Dynamics of H₂O₂ availability to ARPE-19 cultures in models of oxidative stress. *Free Radic Biol Med* 48:1064–1070 [PubMed: 20100568]

- Kim MH, Chung J, Yang JW, Chung SM, Kwag NH, Yoo JS (2003) Hydrogen peroxide-induced cell death in a human retinal pigment epithelial cell line, ARPE-19. *Korean J Ophthalmol* 17:19–28 [PubMed: 12882504]
- Mukherjee PK, Marcheselli VL, Barreiro S, Hu J, Bok D, Bazan NG (2007) Neurotrophins enhance retinal pigment epithelial cell survival through neuroprotectin D1 signaling. *Proc Natl Acad Sci U S A* 104(32):13152–13157 [PubMed: 17670936]
- Obata R, Yanagi Y, Tamaki Y, Hozumi K, Mutoh M, Tanaka Y (2004) Retinal degeneration is delayed by tissue factor pathway inhibitor-2 in RCS rats and a sodium-iodate-induced model in rabbits. *Eye* 19:464–468
- Polato F, Becerra SP (2016) Pigment epithelium-derived factor, a protective factor for photoreceptors in vivo. *Adv Exp Med Biol* 854:699–706 [PubMed: 26427478]
- Reinoso MA, Mukherjee P, Marcheselli V, Bergsma D, Hesse R, Bazan N (2008) PEDF promotes biosynthesis of a novel anti-inflammatory and anti-apoptotic mediator NPD1 in retinal pigment epithelial cells. *Ochsner J* 8(1):39–43 [PubMed: 21603555]
- Rizzolo LJ (1997) Polarity and the development of the outer blood–retinal barrier. *Histol Histopathol* 12:1057–1067 [PubMed: 9302567]
- Sorsby A (1941) Experimental pigmentary degeneration of the retina by sodium iodate. *Br J Ophthalmol* 25:58–62 [PubMed: 18169746]
- Stratikos E, Alberdi E, Gettins PG, Becerra SP (1996) Recombinant human pigment epithelium-derived factor (PEDF): characterization of PEDF overexpressed and secreted by eukaryotic cells. *Protein Sci* 5(12):2575–2582 [PubMed: 8976566]
- Tsao YP, Ho TC, Chen SL, Cheng HC (2006) Pigment epithelium-derived factor inhibits oxidative stress-induced cell death by activation of extracellular signal-regulated kinases in cultured retinal pigment epithelial cells. *Life Sci* 79(6):545–550 [PubMed: 16504212]
- VanNewkirk MR, Nanjan MB et al. (2000) The prevalence of age-related maculopathy: the visual impairment project. *Ophthalmology* 107:1593–1600 [PubMed: 10919916]
- Wang J, Iacovelli J, Spencer C, Saint-Geniez M (2014) Direct effect of sodium iodate on neurosensory retina. *Invest Ophthalmol Vis Sci* 55(3):1941–1953 [PubMed: 24481259]
- Wu YQ, Notario V, Chader GJ, Becerra SP (1995) Identification of pigment epithelium-derived factor in the interphotoreceptor matrix of bovine eyes. *Protein Expr Purif* 6(4):447–456 [PubMed: 8527930]
- Zhuang W, Zhang H, Pan J et al. (2016) PEDF and PEDF-derived peptide 44mer inhibit oxygen-glucose deprivation-induced oxidative stress through upregulating PPAR γ via PEDF-R in H9c2 cells. *Biochem Biophys Res Commun* 472(3):482–488 [PubMed: 26966066]

Fig. 56.1.

Cytotoxicity and viability of ARPE-19 cells with NaIO₃ and H₂O₂. (a) Timeline of experimental design on ARPE-19 cells. (b–c) The cells were incubated with NaIO₃ or H₂O₂ at indicated concentrations (*x-axis*) for 16 h. After treatment, the cytotoxicity and viability were determined by the LDH and CellTiter-Glo® assays, respectively. Plots show cytotoxicity values (right *y-axis*) and viability values (left *y-axis*) as a function of agent concentration. The dotted lines correspond to the estimated value for EC₅₀ for each activity: viability NaIO₃, 6.5 mM; cytotoxicity NaIO₃, 6.5 mM; viability H₂O₂, 600 μM; and cytotoxicity H₂O₂, 450 μM. Each data point is the average of four replicate assays ± SD. LU luminescence units

Fig. 56.2.

PEDF effects on NaIO₃-induced injury of ARPE-19 cell. **(a)** Timeline showing the experimental design. **(b)** Cytotoxicity of ARPE-19 cells treated with the indicated concentrations of NaIO₃ and PEDF (*x-axis*). Toxicity values (*y-axis*) are expressed as percentage being 100% the maximum LDH in lysed cells with Triton-X100. **(c)** Efficacy of PEDF protection is plotted as percentage of protection at each NaIO₃ concentration (*x-axis*), being 100% the toxicity value of cells not treated with PEDF. **(d)** Cell viability of ARPE-19 exposed to NaIO₃ (*x-axis*) with and without PEDF. Each bar is the average of four replicate assays $\pm$ SD. LU luminescence units, n.s. not significant

Fig. 56.3. PEDF concentration curve. Plot showing concentration-response of PEDF (*x-axis*) on cytotoxicity (right *y-axis*) and viability (left *y-axis*) of ARPE-19 cells treated with 6 mM NaIO₃. Each bar is the average of four replicate assays $\pm$ SD. LU luminescence units