Graefes Arch Clin Exp Ophthalmol
DOI 10.1007/s00417-011-1776-8

BASIC SCIENCE

Nicotine reduces VEGF-secretion and phagocytotic activity in porcine RPE

Alexa Karina Klettner · Johanna Doths · Johann Roider

Received: 31 January 2011 / Revised: 4 July 2011 / Accepted: 28 July 2011

Abstract
Background/Purpose Smoking is a strong environmental factor for the development of age-related macula degeneration. In this study, we investigated the effects of nicotine on RPE cell function in porcine in vitro models, focussing on cell death, VEGF secretion and phagocytotic ability.
Methods For these experiments, perfusion organ culture and primary RPE cell culture were used and exposed to nicotine up to 7 days. Survival was investigated in primary porcine RPE cells in an MTT and trypan blue exclusion assay. VEGF secretion was investigated in a porcine perfusion organ culture model using ELISA. A phagocytosis assay using FITC-labelled latex beads in primary RPE cells was used to assess the phagocytotic ability of the cells.
Results Nicotine does not induce cell death in the RPE at any time point up to 7 days of stimulation at any tested concentration. VEGF secretion, however, is diminished compared to untreated control already after 1 day of nicotine treatment and even more profoundly up to 7 days. Furthermore, phagocytotic ability of the RPE is diminished by nicotine in the highest concentrations tested (100 μM).
Conclusion Nicotine impedes RPE function (VEGF secretion, phagocytosis), which could be directly involved in the development of dry AMD and geographic atrophy.

Keywords RPE · Nicotine · VEGF · Perfusion culture · Phagocytosis

A. K. Klettner (✉) · J. Doths · J. Roider
Department of Ophthalmology, University of Kiel,
University Medical Center,
Arnold-Heller-Str. 3, Haus 25,
24105 Kiel, Germany
e-mail: aklettner@auge.uni-kiel.de

Introduction

Age-related macular degeneration (AMD) is the main cause for legal blindness in the elderly in the industrialized world [1], and due to the demographic development of the Western world, the number of patients is expected to increase considerably [2]. Due to this development, the understanding and prevention of the onset and progression of this disease should be of high priority. Several studies have shown that of all avoidable factors, smoking is the strongest environmental risk factor for developing AMD. Current smokers have a higher risk of developing early AMD, neovascular AMD and geographic atrophy. They exhibit worse AMD disease progression than non-smokers, and have a higher risk of developing a bilateral AMD [3–7]. The mechanisms of smoking as a risk factor may be attributed to several factors, such as oxidative stress and an abundance of toxic substances that are contained in cigarette smoke [8, 9]. A major component of cigarette smoke is nicotine, which has been shown to induce mitogenesis of smooth muscle cells [10] and aggravate choroidal neovascularisation in rats and mice [11, 12]. Nicotine can induce the release of vascular endothelial growth factor (VEGF) in vascular smooth muscle cells [13]. In a recent publication, nicotine-fed mice exhibited fewer pigment granules in RPE cells and a damaged photoreceptor-RPE interface and alterations in

Published online: 21 August 2011

RPE morphology in cell culture [14], most likely impeding the function of the RPE. In this study, we investigated the effect of nicotine on RPE function in primary and organ culture, focussing on cell survival, VEGF secretion and phagocytosis.

Material and methods

Cell culture and treatment of cells

RPE were isolated as described elsewhere [15]. In brief, eyes of freshly slaughtered pigs were cleaned, the anterior part of the eye was removed and trypsin added to the eye cup, removed and substituted with trypsin-EDTA. The RPE cells were gently pipetted of the choroid, collected in media and washed. Cells were cultivated in Dulbecco's modified Eagle's medium (DMEM, PAA, Cölbe, Germany) and Ham F12 medium (PAA, Cölbe, Germany) (1:1) supplemented with penicillin/streptomycin (1%), L-glutamine, amphotericine B (0.5 μg/ml), HEPES (25 mM), sodium-pyruvate (110 mg/ml) and 10% porcine serum (PAA, Cölbe, Germany). For microscopy, cells were cultivated to confluence on collagen-coated (Collagen A, Biochrome, Berlin, Germany) cover slips (21×26 mm, Menzel GmbH, Braunschweig Germany). Confluent RPE cells were treated with indicated concentrations of nicotine (Sigma, Deisenhofen, Germany) (1 μM, 10 μM, 100 μM) for indicated time periods. For all experiments, cell of passages 2 and 3 were used.

Organ culture and treatment of culture

Organ culture was prepared as described elsewhere [16]. In brief, eyes of freshly slaughtered pigs were cleaned of adjacent tissue, the anterior part of the eye was removed and retina-RPE-choroid sheets were separated from sclera. Prepared tissue was fixed between the lower and upper part of a fixation ring and the ring was placed in culture chamber (Minucells & Minutissue, Bad Abbach, Germany). The chamber was placed on a heating plate and perfused with medium, (Dulbecco's modified Eagle's medium (DMEM) and Ham F12 medium (1:1) supplemented with penicillin/streptomycin (1%), L-glutamine, HEPES (25 mM), sodium pyruvate (110 mg/ml) and 10% porcine serum for up to 7 days. On the second day of cultivation, the tissue sheets were exposed to indicated concentrations of nicotine (1 μM, 10 μM, 100 μM). The perfusion of the tissue was interrupted, medium was removed from the chamber with a syringe and transferred to a falcon tube, where the respective VEGF-antagonist was added to the medium. The medium was transferred back into the chamber and incubated for 20 min. For long-term experiments, the appropriate concentration of nicotine was additionally applied to the medium container. The perfusion was restarted, and the supernatant was collected at indicated time points (4 h, 1 day, 3 days, 5 days, 7 days) for 1 h.

MTT-assay

Cell survival was evaluated with methyl thiazolyl tetrazolium (MTT) (Sigma, Deisenhofen, Germany) assay as described elsewhere [15]. In brief, 4 hours, 24 hours, 3 days or 7 days after treatment with indicated concentrations (1 μM, 10 μM, 100 μM) of nicotine (Sigma); culture media was discarded and the cells were washed three times with PBS and incubated for 2 h with 0.5 mg/ml MTT in DMEM at 37°C. After incubation, the MTT solution was discarded and DMSO was added to the cells. Cells were shaken at 200 rpm for 5 min on an orbital shaker, DMSO was collected and the absorbance was measured at 555 nm wavelength. Untreated control was defined as 100% survival.

Trypan blue exclusion assay

After stimulation, cell viability was determined by trypan blue exclusion assay as previously described [17]. In brief, cells treated for 24 h or 7 days with indicated concentrations of nicotine (1 μM, 10 μM, 100 μM) were washed with PBS and cells were detached with trypsin-EDTA. After centrifugation, supernatant was discarded, cell pellet resuspended in PBS and viable cells were counted in a Neubauer's cell chamber, using trypan blue (Sigma, Deisenhofen, Germany). The result was related to the number of cells in an untreated control, which was considered 100%.

VEGF secretion

The collected supernatant was diluted, and the VEGF content was measured using a Quantikine ELISA kit (R&D Systems) according to the manufacturer's instructions.

Phagocytosis assay

The phagocytosis of the RPE under nicotine stimulation was evaluated with a phagocytosis assay, as previously described [18]. In brief, photoreceptor outer segments (POS) were prepared from porcine retinas and POS preparation was used to opsonize FITC-labeled latex beads (1 μm in diameter) (Sigma, Deisenhofen, Germany). RPE cells of passage 2 were grown to confluence on collagen-coated cover slips in 12 well dishes. Cells

were treated for 24 h with indicated concentrations of nicotine, and incubated for 4 h with FITC-covered, POS-opsonized latex beads. For fixation, cells were briefly washed with PBS 0.1% azide, and fixed in paraformaldehyde, followed by an acetone–ethanol treatment. Nuclear staining was performed using Hoechst stain. Cells were washed and mounted using Slow Fade Mounting Medium (Invitrogen). Uptake of beads was visualized using a fluorescence microscope (Leica, Germany). Eight pictures per slide were analyzed. Pictures were taken randomly, provided that the pictures exhibited distinguishable nuclei and beads. Beads and nuclei were counted and the ratio determined. Per slide, the mean of all ratios was calculated.

Statistics

Each experiment was independently repeated at least three times. Significances were calculated with *t*-test, calculated with Sigma Plot software. A p value less than 0.05 was considered significant.

Results

Nicotine does not alter RPE survival

When treated with different concentrations of nicotine (1 μM, 10 μM ,100 μM), no cell death could be detected after 4 h, 24 h, 3 days or 7 days in MTT assay (Fig. 1 a). In order to validate the data and to exclude cell death that is masked by higher physiological activity of the cells, cell death at 24 h and 7 days was additionally evaluated with trypan blue exclusion assay. No decrease in cell number could be detected at either time point with any concentration tested (1 μM, 10 μM,100 μM) (Fig. 1b).

Nicotine reduces VEGF secretion

Nicotine alters the VEGF secretion in perfusion organ culture in a time- and concentration-dependent manner. Compared to untreated control, nicotine significantly reduces VEGF secretion at 1 day when used at a concentration of 10 μM (p=0.016) and 100 μM (p=0.013). At day 3 and day 5,

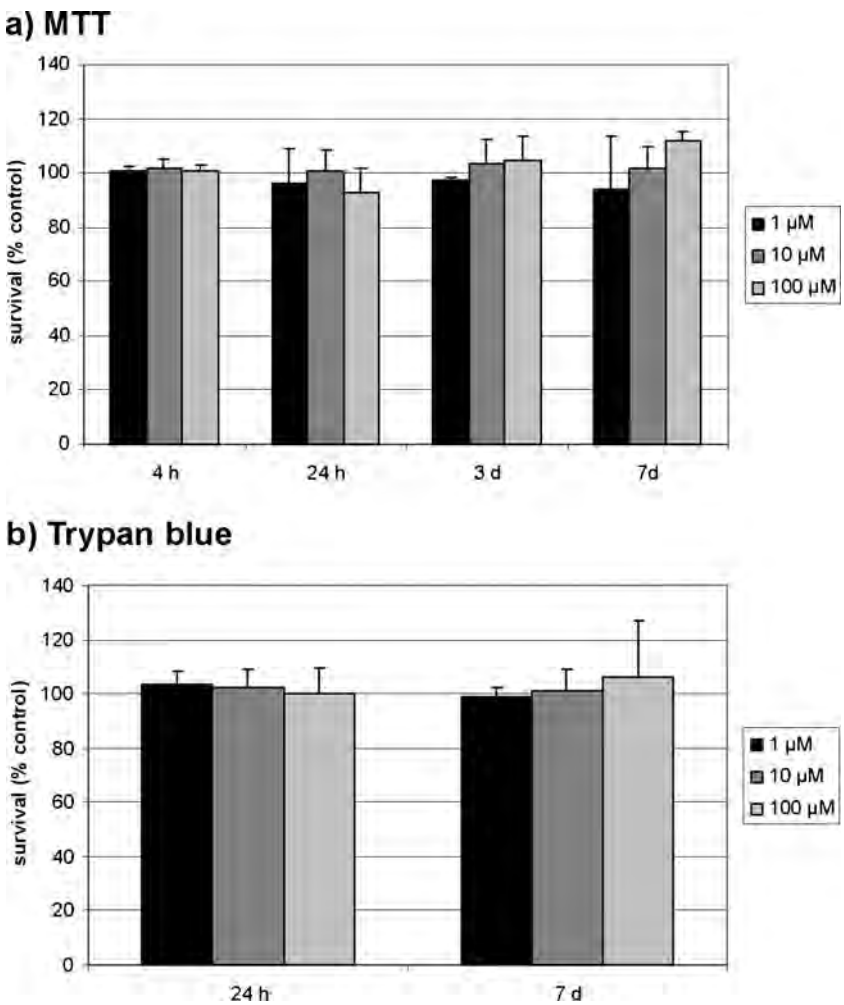

Fig. 1 Cell survival. RPE cells were cultured to confluence and treated with indicated concentrations of nicotine (1 μM, 10 μM, 100 μM) for (**a**) 4 h, 24 h, 3 days or 7 days, and cell viability was detected in MTT assay or (**b**) for 24 h and 7 days, and cell viability was detected in trypan blue exclusion assay. *Error bars* represent standard deviation. No significant changes in survival were detected. Significance was determined with Student's *t*-test

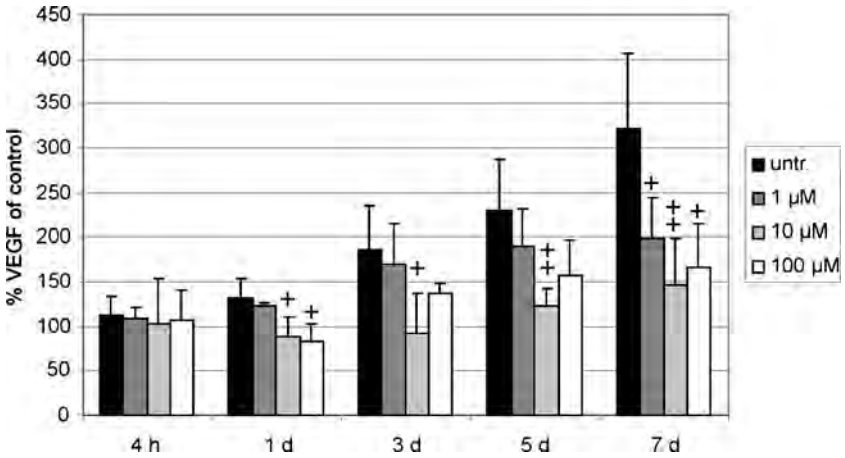

Fig. 2 VEGF content. Perfusion organ cultures of retina/RPE/choroid was stimulated with indicated concentrations of nicotine (1 µM, 10 µM, 100 µM) or served as untreated control (*untr.*). Medium was obtained after indicated period of time (4 h, 1 day, 3 days, 5 days, 7 days) and VEGF content was evaluated in ELISA. *Error bars* represent standard deviation. VEGF content was significantly diminished compared to control Significance was determined with Student's *t*-test. + $p<0.05$; ++ $p<0.01$

10 µM nicotine significantly reduces VEGF (3 days: $p=0.018$; 5 days: $p=0.006$), while the effect of 1 µM and 100 µM do not reach statistical significance. At day 7 of culture, all tested concentrations significantly diminished VEGF secretion (1 µM: $p=0.04$; 10 µM: $p==0.007$; 100 µM $p=0.019$) (Fig. 2).

Nicotine impedes phagocytotic function of RPE cells

RPE cells readily ingest latex beads that have been opsonized with POS [18]. When incubated with nicotine for 24 h, 100 µM highly significantly reduces the number of uptaken beads to 60.6% (SD 6.9%; $p<0{,}001$). Lower concentrations (1 µM 97.4% SD 27.9%; 10 µM 86.9% SD 13.9%) did not exhibit a statistically significant effect (Fig. 3).

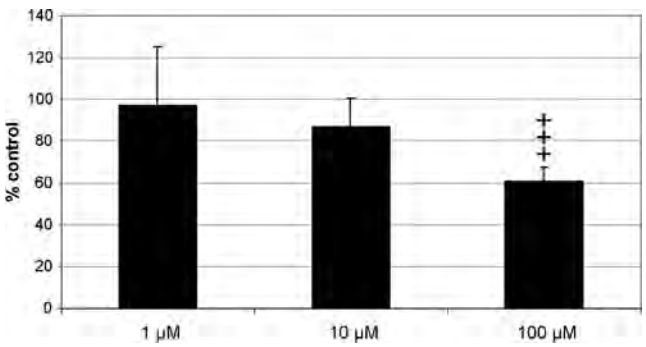

Fig. 3 Phagocytosis. RPE cells were cultured and treated for 24 h with indicated concentrations of nicotine. After 24 h, cells were incubated for 4 h with latex beads that had been opsonized with photoreceptor outer segment fragments. *Error bars* represent standard deviation. Treatment with 100 µM, but not with 1 µM of 10 µM, highly significantly diminished phagocytotic uptake of beads. Significance was determined with Student's *t*-test. +++ $p<0.001$

Discussion

Smoking is an avoidable environmental risk factor for the development of age-related macular degeneration. Cigarette smoke extract (CSE) induces dose-dependent cell death in human RPE and ARPE-19 cells [19], and for some components of cigarette smoke, direct apoptotic effects have been found [20, 21]. Our data show, however, that nicotine itself has no toxic effect on RPE cells. These data correlates well with the findings of Patil et al. [22], which showed a resistance of ARPE-19 cells to nicotine-induced toxicity after different concentrations of nicotine for 24 h, in contrast to endothelial or neurosensory retinal cells, which are susceptible for nicotine-induced cell death. We were able to show that the resistance of the RPE against nicotine is not limited to cell lines but holds true for primary RPE cells, and is valid after even after prolonged exhibition (up to 1 week). The authors suggested the resistance of ARPE-19 cells to be explained by an absence of nicotine receptors on these; however, RPE cells do express nicotinic acetylcholine receptors, and as such are able to specifically respond to nicotine [23, 24].

Cigarette smoke extract (CSE) has been shown to induce VEGF in RPE cells [19]. When using nicotine in various concentrations, however, the VEGF in retina/RPE/choroid organ cultures was not elevated compared to untreated control. In fact, as early as 1 day of exposition to nicotine, VEGF was significantly reduced compared with untreated control. This effect was even stronger after 7 days of culture. This finding correlates well with data recently obtained in the mouse model [25], where the authors find that chronic exposure to nicotine impairs angiogenesis and reduces the serum levels of VEGF. In a paper published while our manuscript was under submission, a contradicto-

ry finding was obtained, where nicotine induced an increase of VEGF in primary rat RPE cells and in ARPE-19 cells [23]. Both the mouse model [25] as an in vivo model and our perfusion organ culture model differ profoundly from classical cell culture experiments used in the cited study, as these models allow an interaction of the involved tissues, in our case of RPE, choroid, and retina. In our study, the secretion of VEGF in untreated cultures increases over time. As the tissue deteriorates during cultivation, the induction of VEGF-A is most likely a survival factor which is induced as the organ culture ages [26]. VEGF-A has recently been shown to be an autocrine survival factor for RPE cells [27], where the inhibition of VEGF or VEGF-R2 signaling results in a higher susceptibility towards oxidative stress. Furthermore, VEGF is an important factor for the vascularization of the choroid [28] and has protective effects on neurons [29]. It is upregulated after traumatic nerve injury [30] or seizure [31]. In our system, the choroid and, more importantly, the retina are present in culture, so especially the deterioration of the retina over time might be a strong stimulus for the RPE to secrete VEGF. Considering that smoking itself increases the oxidative stress burden and reduces the presence of antioxidants, an inhibition of VEGF by nicotine could be detrimental, as the increase of the oxidative burden would go along with a decrease in protection. This effect would not be seen in cell culture experiments, as no additional noxes or interacting tissue are present.

An overall reduction in VEGF in nicotine-treated tissues in the presence of additional noxes such as oxidative stress could be involved in the deterioration of RPE, choroid, and retina. In this context, nicotine might be especially involved in the onset of early, dry AMD [32, 33]. This overall reduction does not exclude the possibility of localized increased VEGF production, which might be present in border areas in which the RPE is still intact, as found in CNV in geographic atrophy [34].

Phagocytosis of shed photoreceptor outer segments is one of the most important functions of the RPE, which is indispensable for sustaining vision [35]. The serious consequences of malfunctioning phagocytosis can be observed in RCS rats, which are blind due to a malfunction of RPE phagocytosis [36]. The deterioration of phagocytotic processes can be a sign of aging, as reduced phagocytotic ability can be observed in Arpe cells when cultured on Bruch's membrane of aged donors [37]. Furthermore, the accumulation of waste products in the subretinal space is a hallmark of AMD, and disorders in phagocytosis can result in the accumulation of subretinal debris [38, 39]. We were able to show that nicotine reduces the phagocytotic ability of RPE cells, thereby interfering with an important function of the RPE and possibly directly promoting changes which might induce premature aging, leading to deterioration of the tissue. Additionally, if debris is accumulated due to impaired phagocytosis because of nicotine, macrophages might be attracted, which might contribute to inflammatory processes and thus contribute to the development of AMD. One has to consider, however, that macrophages employed in phagocytosis in the retina generally are not pro-inflammatory [40, 41].

Taken together, we have shown a direct effect of nicotine on RPE cells, and the cellular effects of nicotine that we present here can increase the risk of developing dry age-related macular degeneration. Our data offer a logical explanation as to why smoking increases the AMD risk so much.

Acknowledgements The authors would like to thank Elisabeth Richert for her help with this study.

Financial relationships None.

References

1. Fine SL, Berger JW, Maguire MG, Ho AC (2000) Age-related macular degeneration. N Engl J Med 3427:483–492
2. Pauleikhoff D, Scheider A, Wiedmann P, Gelisken F, Scholl HP, Roider J, Mohr A, Zlateva G, Xu X (2009) Neovaskuläre altersabhängige Makuladegeneration in Deutschland. Ophthalmologe 106:242–251
3. Smith W, Assink J et al (2001) Risk factors for age-related macular degeneration: pooled findings from three continents. Ophthalmology 108:697–704
4. Tomany SC, Wang JJ, Van Leeuwen R, Klein R, Mitchell P, Vingerling JR, Klein BE, Smith W, De Jong PT (2004) Risk factors for incident age-related macular degeneration: pooled findings from 3 continents. Ophthalmology 111:1280–1287
5. Khan JC, Thurlby DA, Shahid H, Clayton DG, Yates JR, Bradley M, Moore AT, Bird AC, Genetic Factors in AMD Study (2005) Smoking and age related macular degeneration: the number of pack years of cigarette smoking is a major determinant risk for both geographic atrophy and choroidal neovascularisation. Br J Ophthalmol 90:75–80
6. Chakravarthy U, Augod C, Bentham GC, de Jong PT, Rahu M, Seland J, Soubrane G, Tomazzoli L, Topouzis F, Vingerling JR, Vioque J, Young IS, Fletcher AE (2007) Cigarette smoking and age-related macular degeneration in the EUREYE Study. Ophthalmology 114:1157–1163
7. Klein R, Knudtson MD, Cruickshanks KJ, Klein BE (2008) Further observations on the association between smoking and the long-term incidence and progression of age-related macular degeneration: the Beaver Dam Eye Study. Arch Ophthalmol 126:115–121
8. Cano M, Thimmalappula R, Fujihara M, Nagai N, Sporn M, Wang A, Neufeld A, Biswal S (2010) Handa J. Cigarette smoking, oxidative stress, the anti-oxidant response through Nrf2 signaling, and age-related macular degeneration. Vis Res 50:652–664
9. Hecht SS (2006) Cigarette smoking: cancer risks, carcinogens and mechanisms. Langenbecks Arch Surg 391:603–613
10. Pestana IA, Vazquez-Padron RI, Aitouche A, Pham SM (2005) Nicotinic and PDGF-receptor function are essential for nicotine-stimulated mitogenesis in human vascular smooth muscle cells. J Cell Biochem 96:986–995
11. Kaliappan S, Jha P, Lyzogubov VV, Tytarenko RG, Bora NS, Bora PS (2008) Alcohol and nicotine consumption exacerbates choroi-

dal neovascularisation by modulating the regulation of complement system. FEBS Lett 582:3451–3458
12. Suner IJ, Espinosa-Heidmann DG, Marin-Castano M, Hernandez EP, Pereira-Simon S, Cousins SW (2004) Nicotine increases size and severity of experimental choroidal neovascularisation. Invest Ophthalmol Vis Sci 451:311–317
13. Kanda Y, Watanabe Y (2007) Nicotine-induced vascular endothelial growth factor release via the EGFR-ERK pathway in rat vascular smooth muscle cells. Life Sci 80:1409–1414
14. Yang L, Gong H, Wang Y, Wang Y, Yin H, Chen P, Zhang H, Wang Y (2010) Nicotine alters morphology and function of retinal pigment epithelial cells in mice. Toxicol Pathol 38:560–567
15. Klettner A, Kruse ML, Meyer T, Wesch D, Kabelitz D, Roider J (2009) Different properties of VEGF-antagonists: bevacizumab but not ranibizumab accumulates in RPE cells. Graefes Arch Clin Exp Ophthalmol 247:1601–1608
16. Klettner A, Roider J (2008) Comparison of bevacizumab, ranibizumab and pegaptanib in vitro: efficiency and possible additional pathways. Invest Ophthalmol Vis Sci 49:4523–4527
17. Klettner A, Koinzer S, Waetzig V, Herdegen T, Roider J (2010) Deferoxiamine mesylate is toxic for retinal pigment epithelium cells in vitro, and its toxicity is mediated by p38. Cutan Ocul Toxicol 29:122–129
18. Klettner A, Möhle F, Lucius R, Roider J (2011) Quantifying FITC-labeled latex beads Opsonized with photoreceptor outer segment fragments: an easy and inexpensive method of investigating phagocytosis in retinal pigment epithelium cells. Ophthalmic Res 46:88–91
19. Bertram K, Baglole C, Phipps RP, Libby RT (2009) Molecular regulation of cigarette smoke induced oxidative stress in human retinal pigment epithelial cells: implications for age-related macular degeneration. Am J Physiol Cell Physiol 297:1200–1210
20. Sharma A, Neekhra A, Gramajo AL, Patil J, Chwa M, Kuppermann B, Kenney MC (2008) Effects of benz(e)pyrene, a toxic component of cigarette smoke, on human retinal pigment epithelium cells in vitro. Invest Ophthalmol Vis Sci 49:5111–5117
21. Jia L, Liu Z, Sun L, Miller SS, Ames BN, Cotman CW, Liu J (2007) Acrolein, a toxicant in cigarette smoke, causes oxidative damage and mitochondrial dysfunction in RPE cells: protection by (R)-alpha-lipoic acid. Invest Ophthalmol Vis Sci 48:339–248
22. Patil AJ, Gramajo AL, Sharma A, Seigel GM, Kuppermann BD, Kenney MC (2009) Differential effects of nicotine on retinal and vascular cells in vitro. Toxicology 259:69–76
23. Pons M, Marin-Castano ME (2011) Nicotine increases the VEGF/PEDF ratio in retinal pigment epithelialiu: a possible mechanism for CNV in passive smokers with AMD. Invest Ophthalmol Vis Sci 52:3842–3853
24. Maneu V, Gerona G, Fernandez L, Cuenca N, Lax P (2010) Evidence of alpha 7 nicotine acetylcholine receptor expression in retinal pigment epithelial cells. Vis Neurosci 27:139–147
25. Konishi H, Wu J, Cooke JP (2010) Chronic exposure to nicotine impairs cholineric angiogenesis. Vasc Med 15:47–54
26. Miura Y, Klettner A, Noelle B, Hasselbach H, Roider J (2009) The change of morphological and functional characteristics of RPE cells during cultivation of RPE-choroid perfusion tissue culture. Ophthalmic Res 43:122–133
27. Byeon SH, Lee SC, Choi SH, Lee HK, Lee JH, Chu YK, Kwon OW (2010) Vascular endothelial growth factor as an autocrine survival factor for retinal pigment epithelial cells under oxidative stress via the VEGF-R2/PI3K/Akt. Invest Ophthalmol Vis Sci 51:1190–1197
28. Bhisitkul RB (2006) Vascular endothelial growth factor biology: clinical implications for ocular treatments. Br J Ophthalmol 90:1542–1547
29. Zachary I (2005) Neuroprotective role of vascular endothelial growth factor: signalling mechanisms, biological function and therapeutic potential. Neurosignals 14:207–221
30. Vaquero J, Zurita M, de Oya S, Coca S (1999) Vascular endothelial growth/permeability factor in spinal chord injury. J Neurosurg 90:220–223
31. Nicoletti JN, Shah SK, McCloskey DP, Goodman JH, Elkady A, Atassi H, Hylton D, Rudge JS, Scharman HE, Croll SD (2008) Vascular endothelial growth factor is up-regulated after status epilepticus and protects against seizure-induced neuronal loss in hippocampus. Neuroscience 151:232–241
32. Epinosa-Heidmann DG, Suner IJ, Catanuto P, Hernandez EP, Marin-Castano ME, Cousins SW (2006) Cigarette smoke-related oxidants and the development of sub-RPE deposits in an experimental animal model of dry AMD. Invest Ophthalmol Vis Sci 47:729–737
33. Wang AL, Neufeld AH (2010) Smoking mice: a potential model for studying accumulation of drusen-like material on Bruch's membrane. Vision Res 50:638–642
34. Sunness JS, Gonzales-Baron J, Bressler NM, Hawkins B, Applegate C (1999) The development of choroidal neovascularisation in eyes with the geographic atrophy form of age-related macular degeneration. Ophthalmology 106:910–919
35. Nandrot EF, Dufour EM (2010) Mertk in daily retinal phagocytosis: a history in the making. Avd Exp Med Biol 664:133–140
36. Bok D, Hall MO (1971) The role of the pigment epithelium in the etiology of inherited retinal dystrophy in the rat. J Cell Biol 49:664–682
37. Sun K, Cai H, Tezel TH, Paik D, Gaillard ER, Del Priore LV (2007) Bruch's membrane aging decreases phagocytosis of outer segments by retinal pigment epithelium. Mol Vis 13:2310–2319
38. Zarbin MA (1998) Age-related macular degeneration: review of pathogenesis. Eur J Ophthalmol 8:199–206
39. Valter K, Maslim J, Bowers F, Stone J (1998) Photoreceptor dystrophy in the RCS rat: roles of oxygen, debris, and bFGF. Invest Ophthalmol Vis Sci 39:2427–2442
40. Joly S, Samardzija M, Wenzel A, Thiersch M, Grimm C (2009) Nonessential role of beta3 and beta5 integrin subunits for efficient clearance of cellular debris after light-induced photoreceptor degeneration. Invest Ophthalmol Vis Sci 50:1423–1432
41. Patel M, Chan CC (2008) Immunopathological aspects of age-related macular degeneration. Semin Immunopathol 30:97–110