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Research Article

Effect of Antiangiogenic Treatments about Biomarkers of Oxidative Stress in Patients with Age-Related-Macular Degeneration

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Abstract

Objective

To analyze the effect of antiangiogenic treatments about biomarkers of oxidative stress in patients with age related macular degeneration (AMD).

Material and Methods

Total of 73 patients with exudative AMD (no previous anti-VEGF treatment) were selected. 36 patients were received ranibizumab 0.5 mg/4 weeks and 37 patients were received pegaptanib 0.3 mg/6 weeks. The follow up was 6 months. The parameters were determined before and after antiangiogenic therapy: total antioxidant activity (TAS), reduced and oxidized glutathione (GSH/GSSH), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD) and protein carbonyl groups.

Results

Average value of TAS in patients treated with Pegaptanib was 166.6±20.4 μM Trolox and in patients treated with Ranibizumab was 202.4±27.4 μM Trolox. Average value of GSH/GSSH in patients treated with Pegaptanib was 8.2±1.4μM and in patients treated with Ranibizumab was 6.2±1.1 μM. The values of carbonyl groups in patients treated with Pegaptanib was 72.1±7.0 μmol/mg and in patients treated with Ranibizumab was 68.3±4.1 μmol/mg. After antiangiogenic therapies, average values of total antioxidant activity, GSH/GSSH and endogenous antioxidant enzymes decreased slightly and values of carbonyl groups were increased (there were not significant differences).

Conclusions

There was not statistically significant difference but Pegaptanib and Ranibizumab may disturb the homeostatic maintenance of oxidative stress.

Keywords: Age-Related-Macular Degeneration; Antioxidant Enzymes; Antioxidant Activity; Pegaptanib; Protein Carbonyl Groups; Oxidative Stress; Ranibizumab.

Introduction

Age-related macular degeneration (AMD) has become the leading cause of irreversible central vision loss in the elderly, affecting approximately 7.2 million people in United States over the age of 40 (6.5%) [1]. There are two main types of AMD: atrophy and neovascular. Neovascular AMD is characterized by the presence of choroidal neovascular membranes that are often associated with subretinal fluid, hemorrhage and eventually subretinal fibrosis with loss of vision [2]. Furthermore, the subsequent upregulation of inflammatory cytokines and growth factors, including vascular endothelial growth factor (VEGF), induces the growth of neovascular membranes from the choriocapillaris into the subretinal or subretinal pigment epithelium spaces [3].

Neovascularization (CNV) is a common pathological process in various retinal vascular disorders including diabetic retinopathy and AMD. The development of neovascular vessels may lead to complications such as vitreous hemorrhage, fibrovascular tissue formation, and traction retinal detachments. Various proangiogenic factors are involved in these complex processes. To counter this, drugs administered intravitreal (Pegaptanib, Bevacizumab and Ranibizumab) that specifically target VEGF have become the standard treatment for exudative AMD in an attempt to treat these vascular disorders [4]. In the presence of abnormal CNV, oxidative stress defense mechanisms may no longer be adequate which cause cell death. Moreover, cell death in the region of intraretinal vascular was associated with an increased presence of markers associated with oxidative stress [5], [6].

AMD has been linked to the stress engendered by radical oxygen species in macular photoreceptor cells and proximate retinal pigment epithelium cells (RPE). There are numerous mechanisms in the retina for preventing and forestalling oxidative damage, but many of these anti-oxidative mechanisms have begun to break down which increases the susceptibility of the retina to accumulate this damage with increasing age [7].

Currently many laboratories are investigating drugs in the field about VEGF. Enzymes are strong therapeutic target to treat AMD for example: the signal transducer and activator of transcription-3 is a direct transcriptional activator of VEGF gene [8] or Jun kinases (mitogen-activated protein kinase family) which are activated in response to growth factors and variety of stresses, including oxidative stress [9].

Anti-VEGF therapies have been shown to be effective in preventing vision loss from neovascular and complications of retinal diseases particularly in AMD. Sloan et al. [10] observed that the introduction of anti-VEGF therapy reduced vision loss by 41% and onset of severe vision loss and blindness by 46%. However, inhibition of VEGF may disturb the homeostatic maintenance of normal vasculature and ROS [11]. Anti-VEGF therapy may have negative effects on cells in the retina. Despite encouraging results in halting the disease and improving the vision, intravitreal injection of anti-VEGF agents may be associated with systemic adverse events and devastating ocular complications [12]. Animal studies have shown that systemic neutralization of VEGF with soluble VEGF receptors resulted in reduced thickness of the inner and outer nuclear layer in the adult mouse retina. Given the expression of VEGF and VEGF receptor 2 in the adult retina, and the constitutive activation of the receptor, investigators hypothesized that VEGF plays a role in maintenance and function of the adult retina and anti-VEGF agents may negate the physiologic function and neutralize VEGF mediated neuroprotection [13]. For this reason, the monitoring of therapies is very important and Zafrilla et al. [5] has shown that systematic oxidative stress in the wet AMD can be measured by different biomarkers.

The aim of this study was to analyze the effect of antiangiogenic treatments about biomarkers of oxidative stress in patients with exudative AMD.

Materials and Methods

Patients

Total of 73 patients with exudative AMD (no previous anti-VEGF treatment) were recruited for the study. The average age was 71 years (55-82) and there were 40 women and 33 men. Patients were selected in the Ophthalmology Service at the University Hospital Morales Meseguer of Murcia (Spain).

Study Design

AMD patients were diagnosed and underwent an eye examination consists of the following tests: Corrected visual acuity - far/near; biomicroscopy of anterior segment; intraocular pressure measurement; retinography, angiography and OCT (Optical coherence Tomography).

Patients with exudative AMD had no received previous anti-VEGF treatment, and started treatment with three charge doses of antiangiogenic therapy. 37 patients received 0.3 mg of Pegaptanib (every 6 weeks) and 36 patients received 0.5 mg of Ranibizumab (every 4 weeks). Patients will be randomized to receive intravitreal injections of Pegaptanib sodium or Ranibizumab and the plasmatic parameters were determined before and after the treatment. Patients will be randomized to receive intravitreal injections of Pegaptanib or Ranibizumab and the plasmatic parameters were determined before and after the treatment.

The follow up was 6 months. The treatment was performed in accordance with the Guidelines of Clinical Practice of The SERV (Spanish Society of Retina and Vitreous) [14].

Off-label anti-VEGF therapy was administered in the operating room observing a sterile protocol for intravitreal injection that included the use of 5% povidone-iodine solution, topical anesthesia, eyelid-speculum application, intravitreal injection of 0.3 mg of ranibizumab or pegaptanib via pars plana in the infero-temporal quadrant at 4 mm from the limbus in the phakic eye and at 3.5 mm in the pseudophakic eye followed by postoperative topical antibiotic eye-drops medication. Patients were given topical netilmicin + dexamethasone four times daily for 2 weeks after injection.

Subjects with history of diabetes mellitus, renal disease, hypertension, cardiovascular disease, smokers, alcoholics, and subjects who are on antioxidant supplements were excluded from the study. The physician and ophthalmologist ruled out any systemic/ophthalmic diseases from the control subjects before recruitment after a detailed check up. Informed consent was obtained from all the participants in the study. The authors' institutional research and ethical committee approved the study (5 October 2010, register number: 3280). All procedures pertaining to human subjects strictly adhered to tenets of Helsinki declaration.

Biochemical Determinations

Blood samples were collected from the median cubital vein and placed in EDTA-containing vials. Blood is centrifuged (3000 rpm for 15 min) to obtain serum at room temperature within 1 h of collection and stores at -80º C until the assays were performed.

Measurement of Total Antioxidant Activity

Total antioxidant status in plasma was measured by ORAC method. The oxygen radical absorbance capacity was determined as described by Davalos et al. [15] with slight modifications. The ORAC analyses were carried out on a Synergy HT multi-detection microplate reader, from Bio-Tek Instruments, Inc. (Winooski, Vt, USA), using 96-well polystyrene microplates with black sides and clear bottom, purchased from Nalge Nunc International. Fluorescence was read through the clear bottom, with an excitation wavelength of 485/20 nm and an emission filter of 528/20 nm. The plate reader was controlled by KC4, version 3.4, software. The reaction was carried out in 75 mM sodium phosphate buffer (pH 7.4), and the final reaction mixture was 200 µL. Fluorescein (FL) (100 µL; 3 nM, final concentration) and serum (70 µL), were placed in the wells of the microplate. The mixture was preincubated for 30 min. at 37 ºC, before rapidly adding the 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) solution (30 µL; 19 mM, final concentration) using a multichannel pipette. The microplate was immediately placed in the reader and the fluorescence recorded every 1.14 min. for 120 min. The microplate was automatically shaken prior to each reading. A blank with FL and AAPH using sodium phosphate buffer instead of the antioxidant solution, and eight calibration solutions using Trolox C as antioxidant were also used in each assay. All reaction mixtures were prepared in triplicate and at least three independent assays were performed for each sample.

In order to avoid a temperature effect, only the inner 60 wells were used for experimental purposes, while the outer wells were filled with 200 µL of distilled water. The antioxidant abilities were expressed as µM Trolox equivalents.

Measurement GSH/GSSH

Reduced and oxidized Glutathione (GSH/GSSH) were determined by colorimetric determination (Oxis Research TM Bioxytech GSH/GSSH-412 TM Burlingame, USA) according to the manufacturer's instructions.

Determination of Enzymatic Activity

For the quantitative determination of glutathione peroxidase (GPx) in the blood the Ransel commercial kit was used. This method is based on that of Plagia and Valentine [16]. Absorbance reduction was measured al 340 nm for 2 min and the GPx activity was expressed as U/L of sample.

Determination of glutathione reductase (GR) was achieved using the procedure described by Anderson et al. [17] 100 µL of serum was added to the mixture of 0.1 EDTA, 0.1 mM NADPH in 50 mM HEPES/KOH (pH=8) buffer. The reaction was initiated by adding 100 µL reduced glutathione 1 mM (final volume 1mL) and the reduction of NADPH at 340 nm was monitored for 3 min. The enzymatic activity was expressed as U/L.

The analysis of erythrocyte superoxide dismutase (SOD) activity was performed using Randox (Randox, Crumlin, UK). The activities were measured enzymatically at 37ºC on a Varian spectrometer (mod. Cary Bio-50 UV-Vis) at 505 nm, as previously reported Delmas-Beauvieux et al [18], [19]. Randox provided standards. SOD activity was expressed as U/g Hb of sample.

Measurement of Protein Carbonyl Groups

Protein carbonyl groups were determined by an ELISA kit (Biocell Corporation Ltd, New Zealand) according to the instructions.

Statistical Analysis

All data were analyzed by using SPSS 17.0 statistical software (SPSS Inc., Chicago, IL). Descriptive statistic is presented as mean ± standard deviation. Means were compared by the variance test of repeated means. Analysis of variance (ANOVA) was used to examine significant differences in the protein carbon-

yl groups, GSH/GSSH ratio, TAS, SOD, GPx and GR of the two groups of study. A probability of less than 0.05 ($p<0.05$) was considered statistically significant.

Results

The 6-month results from two treatments realized were analyzed retrospectively. After 6 months, no serious adverse ocular and systemic side effects were reported from any of the patients in the study during the entire period of treatment; there was no intraocular pressure elevation, inflammatory reaction, retinal detachment, retinal tear, vitreous hemorrhage, traumatic cataract, cardiovascular or cerebrovascular thromboembolic events.

Average values of **total antioxidant activity** at baseline in patients treated with Pegaptanib are 166.6±20.4 μM Trolox and 202.4±27.4 μM in patients treated with Ranibizumab; significant differences were observed. After anti-VEGF therapy, the dates were not significantly different (151.2 ±16.5 μM Trolox) in patients treated with Pegaptanib and (193.7±122.1 μM Trolox) in patients treated with Ranibizumab.

According to the **GSH/GSSH** results, initial average values were higher in patients treated with Pegaptanib (8.2 ±1.4 μM) than in patients treated with Ranibizumab (6.2 ±1.1μM); significant differences were found. After antiangiogenic therapies these values decreased slightly but there were no significant differences (7.9±1.6μM) in patients treated with Pegaptanib and (5.8±2.1 μM) in patients treated with Ranibizumab.

Average values of **GPx** at baseline was 7149.1±2120 U/L in patients treated with Pegaptanib and at 6 months these values didn't show significant changes (6549.1±1511 U/L). In the same way the patients treated with Ranibizumab showed no changes in average values of GPx (7328.1±1954 U/L) and after intravitreal therapy with Ranibizumab (6728.1± 1846 U/L).

Average values of **GR** are higher in patients treated with Pegaptanib than in patients treated with Ranibizumab (54.1±3.4 U/L versus 50.6±2.9 U/L) and significant differences were observed. After antiangiogenic therapies these values decrease slightly although not found significant differences (52.6±2.4 U/L Pegaptanib versus 48.7±2.7 U/L Ranibizumab).

Average values of **SOD** are higher in patients treated with Pegaptanib than in patients treated with Ranibizumab (885.8±25.4 Ug/Hb versus 815.8±75.8 Ug/Hb) although significant differences were not observed. After antiangiogenic therapies, these values decrease slightly but there were not significant differences (845.8±22.1 Ug/Hb Pegaptanib versus 795.8±75.8 Ug/Hb Ranibizumab).

Average values of **carbonyl groups** were higher in patients in patients treated with Pegaptanib than in patients treated with Ranibizumab (72.1±7.0 μmol/mg versus 68.3±4.1 μmol/mg) and significant differences were found. After antiangiogenic therapies these values increased but significant differences were not observed (75.1±8.1 μmol/mg Pegaptanib versus 71.8±5.8 μmol/mg Ranibizumab).

Discussion

The anti-VEGF therapy can negate the protective role of endogenous VEGF and the long-term intravitreal usage of anti-VEGF may have collateral effects on retinal cells, although these results have been borne out in animal models only [20], [21]. Furthermore, antioxidant enzymes that form part of the complex system that protects the retina from oxidative damage, and all three of these enzymes (GPx, GR, SOD) are found in the photoreceptors and the RPE [22], [23]. Currently, studies [5], [24], [25] were agreed with our results before the treatment but it had never measured these parameters after anti-VEGF therapy, so this article is a pioneer in this research. We have wanted to know how Pegaptanib and Ranibizumab affected these biomarkers because the anti-VEGF may disturb the homeostatic maintenance of the ROS (antioxidant enzymes were decreased).

Firstly, the values before therapies were compared with other studies. Mendoza [26] studied probability to develop AMD was higher in older individuals with lower values of antioxidant parameters for example TAS was small. Colak [22] observed a value of TAS significantly associated with AMD compared to controls. GSH is a major non-enzymatic antioxidant that is effective in protecting cells against to reactive oxygen products and toxins [27]. The prior study demonstrated that plasma GSH levels were lower in an AMD patient group than in a control group [28]. Furthermore, a pilot study aimed to investigate systemic and retinal vascular function and their relationship to circulatory markers of cardiovascular risk in early AMD patients without any already diagnosed systemic vascular pathologies and observed that blood revealed that AMD patients exhibited higher oxidized glutathione [29],[30]. A previous study demonstrated that activities of GPx in the plasma and erythrocytes were lower in an AMD patient group than in a control group [31], [32] and other were unchanged [9]. The latest articles demonstrated values of SOD, GR and GPx are higher in control group than in patients with wet AMD [5], [33], and carbonyl groups are higher in wet AMD [31],[33] (the intensity of lipid peroxidation was higher with the progression of AMD). Finally, oxido-reduction disturbance may be involved in the pathogenesis of AMD. After therapy, we cannot compare with others articles because nothing is published.

In a cell culture model, VEGF was shown to protect motor neurons [34]. This mechanism was confirmed in human RPE cells

in culture, in which treatment with VEGF-A protected RPE cells from hydrogen peroxide–induced apoptosis [35]. In summary, VEGF appears to protect against oxidative stress through induction of antioxidant agents [36], [37] and pathways associated with cell survival [38]. Repeated treatment with anti-VEGF agents may negate the physiologic function and neutralize VEGF mediated neuroprotection [39]. Furthermore, VEGF has been shown to induce mitochondrial SOD (major enzyme in the defense against oxidative stress). Subsequently, enhanced VEGF expression was shown to protect neuronal cells from oxidative stress and treatment with anti-VEGF antibodies abrogated this effect [40].

Conclusion

Pegaptanib and Ranibizumab may disturb the homeostatic maintenance of the ROS to decrease the values of antioxidant enzymes. However, results were slightly smaller after therapies, and the fact may contribute the negative effect of Pegaptanib and Ranibizumab about stress oxidative.

References

1. Klein R, Chou CF, Klein BE, Zhang X, Meuer SM et al. Prevalence of age-related macular degeneration in the U.S. population. Arch Ophthalmol. 2011, 129: 75-80.

2. Brown GC, Brown MM, Sharma S, Stein JD, Roth Z. The burden of age-related macular degeneration: a value medicine analysis. Trans Am Ophthalmol.Soc. 2005, 103: 173-178.

3. Machegiani F, Marra M, Olivieri F, Cardelli M, James RW et al. Paraoxonase 1: genetics and activities during aging. Rejuvenation Res. 2008, 11: 113-127.

4. Agrawal S, Joshi M, Christoforidis JB. Vitreous inflammation associated with intravitreal anti-VEGF pharmacotherapy. Mediators Inflamm. 2013, 943409.

5. Zafrilla P, Losada M, Perez A, Caravaca G, Mulero J. Biomarkers of Oxidative stress in patients with wet age related macular degeneration. Published the journal of nutrition, health and aging. The journal of nutrition, health and aging. 2013, 17(3): 219-22.

6. Kinnunen K, Petrovski G, Moe MC, Berta A, Kaarniranta K. Molecular mechanisms of retinal pigment epithelium damage and development of age related macular degeneration. Acta Ophthalmol. 2012, 90: 99-309.

7. Kim YW, Kyzova T. Oxidative stress in angiogenesis and vascular disease. Blood. 2014, 123: 625-631.

8. Li X, Cai Y, Wang YS, Shi YY, Hou W, et al. Hyperglycaemia Exacerbates Choroidal Neovascularisation in Mice via the Oxidative Stress- Induced Activation of STAT3 Signalling in RPE Cells. PLoS One. 2012, 7(10): 47600.

9. Yildirim Z, Ucgun NI, Yildirim F. The role of oxidative stress and antioxidants in the pathogenesis of age-related macular degeneration. Clinics. 2011, 66.

10. Sloan FA, Hanrahan BW. The Effects of Technological Advances on Outcomes for Elderly Persons With Exudative Age-related Macular Degeneration. JAMA Ophthalmol. 2014.

11. Kerr BA, Byzova TV. The dark side of the oxidative force in angiogenesis. Nat Med. 2012, 18(8): 1184-1185.

12. Falavarjani KG, Nguyen QD. Adverse events and complications associated with intravitreal injection of anti-VEGF agents: a review of literature. Eye (Lond). 2013, 27(7): 787-94.

13. Saint-Geniez M, Maharaj AS, Walshe TE, Tucker BA, Sekiyama E et al. Endogenous VEGF is required for visual function: evidence for a survival role on müller cells and photoreceptors. PLoS One. 2008, 3(11): e3554.

14. Ruiz-Moreno JM, Arias-Barquet L, Armadá-Maresca F, Boixadera-Espax A, García-Layana A et al. Guidelines of clinical practice of the SERV: Treatment of exudative age-related macular Degeneration (AMD). Arch Soc Esp Oftalmol. 2009, 84: 333-344.

15. Dávalos A, Gómez-Cordovés C, Bartolomé B. Extending applicability of the oxygen radical absorbance capacity (ORAC-Fluorescein) assay. J. Agric. Food Chem. 2004, 52: 48-54.

16. Plagia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte gluthatione peroxidase. J. Lab Clin Med. 1967, 70: 158-169.

17. Anderson JW, Foyer CH, Walker DA. Light-dependent Reduction of dehydro-ascorbate and uptake of exogenous ascorbate by spinach chloroplasts. Planta. 1983, 158: 442-450.

18. Delmas-Beauvieux MC, Peuchant E, Couchouron A. The enzymatic antioxidant system in blood and glutathione status in human immunodeficiency virus (HIV)-infected patients: effects of supplementation with selenium or β-carotene. Am.J. Clin Nutr. 1996, 64: 101-107.

19. Wang Y, Zang QS, Liu Z, Wu Q, Maass D et al. Regulation of VEGF induced endotelial cell migration by mitocondrial reactive oxygen species. Am J Physiol Cell Physiol. 2011, 301(3): 695-704.

20. Marco A. Zarbin. Current concepts in the pathogenesis of age-related macular degeneration. Arch Ophthalmol. 2004,

122: 598-614.

21. Vikram S. Brar, K. V. Chalam. VEGF Inhibitor Induced Oxidative Stress in Retinal Ganglion Cells. Studies on Retinal and Choroidal Disorders. Oxidative Stress in Applied Basic Research and Clinical Practice. 2012, 585-593.

22. Čolak E, Majkić-Singh N, Žoric L, Radosavljević A, Kosanović-Jaković N. The impact of inflammation to the antioxidantdefense parameters in AMD patients. Aging Clin Exp Res. 2012, 24(6): 588-94.

23. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. SurvOphthalmol. 2000, 45: 115–34.

24. Rybka J, Kupczyk D, Kędziora-Kornatowska K, Pawluk H, Czuczejko J et al. Age-related changes in an antioxidant defense system in elderly patients with essential hypertension compared with healthy controls. Redox Rep. 2011, 16(2): 71-7.

25. Uğurlu N, Aşık MD, Yülek F, Neselioglu S, Cagil N. Oxidative stress and anti-oxidative defence in patients with age-related macular degeneration. Curr Eye Res. 2013, 38(4): 497-502.

26. Mendoza-Núñez VM, Ruiz-Ramos M, Sánchez-Rodríguez MA, Retana-Ugalde R, Muñoz-Sánchez JL. Aging- Related Oxidative Stress in Healthy Humans. Tohoku J. Exp. Med. 2007, 213: 261-268.

27. Penninckx M. A short review on the role of glutathione in the response of yeasts to nutritional, environmental, and oxidative stresses. Enzyme Microb Technol. 2000, 26: 737–742.

28. Samiec PS, Drews-Botsch C, Flagg EW, Kurtz JC, Sternberg P et al. Glutathione in human plasma decline: in association with aging, age-related macular degeneration, and diabetes. Free RadicBiol Med. 1998, 24: 699–704.

29. Qin L, Mroczkowska SA, Ekart A, Patel SR, Gibson JM et al. Patients with early age-related macular degeneration exhibit signs of macro- and micro-vascular disease and abnormal blood glutathione levels. Graefes Arch Clin Exp Ophthalmol. 2014, 252(1): 23-30.

30. Uno K, Nicholls SJ. Biomarkers of inflammation and oxidative stress in atherosclerosis. Biomark Med. 2010, 4: 361-373.

31. Venza I, Visalli M, Cucinotta M, Teti D, Venza M. Association between oxidative stress and macromolecular damage in elderly patients with age-related macular degeneration. Aging Clin Exp Res. 2012, 24: 21-27.

32. Evereklioglu C, Er H, Doganay S, Cekmen M, Turkoz Y et al. Nitric oxide and lipid peroxidation are increased and associated with decreased antioxidant enzyme activities in patients with age-related macular degeneration. Doc Ophthalmol. 2003, 106: 129–136.

33. Shen XL, Jia JH, Zhao P, Fan R, Pan XY et al. Changes in blood oxidative and antioxidant parameters in a group of Chinese patients with age-related macular degeneration. J Nutr Health Aging. 2012, 16: 201-204.

34. Li B, Xu W, Luo C, Gozal D, Liu R. VEGF-induced activation of the PI3-K/Akt pathway reduces mutant SOD1-mediated motor neuron cell death. Brain Res Mol Brain Res. 2003, 111: 155-164.

35. Byeon SH, Lee SC, Choi SH, Lee HK, Lee JH et al. 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. 2010, 51: 1190-1197.

36. Takahashi T, Shimizu H, Morimatsu H, Inoue K, Akagi R et al. Heme oxygenase-1: a fundamental guardian against oxidative tissue injuries in acute inflammation. Mini Rev Med Chem. 2007, 7: 745-753.

37. Morse D, Lin L, Choi AM, Ryter SW. Heme oxygenase-1, a critical arbitrator of cell death pathways in lung injury and disease. Free RadicBiol Med. 2009, 47: 1-12.

38. Kim AH, Khursigara G, Sun X, Franke TF, Chao MV. Akt phosphorylates and negatively regulates apoptosis signal-regulating kinase 1. Mol Cell Biol. 2001, 21: 893-901.

39. Saint-Geniez M, Maharaj AS, Walshe TE, Tucker BA, Sekiyama E et al. Endogenous VEGF Is Required for Visual Function: Evidence for a Survival Role on Müller Cells and Photoreceptors. PLoS one. 2008, 3: e3554.

40. Madhavan L, Ourednik V, Ourednik J. Neural stem/progenitor cells initiate the formation of cellular networks that provides neuroprotection by growth factor-modulated antioxidant expression. Stem Cells. 2008, 26: 254-265.