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Research Article

Neuroprotective Effects of Thermal Stimulation of the Retina and Selective Retina Therapy Implications for the Treatment of Macular Disease

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Abstract

Objectives: Thermal Stimulation of the Retina (TSR, a photothermal sublethal continuous wave laser) as well as Selective Retina Therapy (SRT, a photodisruptive selective RPE regenerating micro pulsed laser) have shown therapeutic effects on age-related macular degeneration (AMD), in AMD mouse models. Both laser modalities act on the RPE regenerative processes. In this study, we investigate a possible neuroprotective effect of TSR and SRT.

Methods: Neuronal SH-SY5Y cells were stressed by H2O2 to simulate oxidative stress on neuroretinal cells, like seen in AMD. Cell viability was measured by methylthiazolyl-tetrazolium (MTT), assay. Cells were incubated with apical supernatants from organ cultures (retinal pigment epithelium (RPE), Bruch's Membrane (BrM) and choroid), treated by TSR or by SRT and compared with cells incubated with supernatants from controls to evaluate neuroprotective effects.

Apical secretion of Nerve Growth Factor (NGF), Brain Derived Neurotrophic Factor (BDNF), Vascular Endothelial Growth Factor (VEGF), and Pigment Epithelium Derived Factor (PEDF), were measured quantitatively by ELISA in untreated or irradiated porcine organ cultures.

Results: Viability of oxidatively stressed SH-SY5Y cells declined by about 50 %. Supernatants from TSR treated organ cultures had a protective effect, in contrast to cells incubated with supernatants from SRT treated organ cultures.

No NGF, nor BDNF secretion could be detected in untreated or irradiated controls in the apical compartment of organ cultures. Apical VEGF secretion was significantly reduced, PEDF significantly increased after SRT treatment. This was not the case after TSR treatment.

Conclusions: TSR-treated organ cultures exerted neuroprotective, by so far unidentified mediators. There is no RPE derived NGF or BDNF secretion to the apical compartment and this is not altered by laser therapy. Apical VEGF decrease and PEDF increase in SRT treated organ cultures did not affect neuronal cell survival. TSR might have neuroprotective properties which need to be examined in further studies.

ABBREVIATIONS

AMD: Age-Related Macular Degeneration; BDNF: Brain Derived Neurotrophic Factor; BrM: Bruch's membrane; DMSO: Dimethyl Sulfoxide; ELISA: Enzyme-Linked Immunosorbent Assay; FCS: Fetal Calf Serum; H2O2: Hydrogen Peroxide; IL: Interleukin; MTT assay: methylthiazolyl-tetrazolium assay; NADH+ : Nicotinamide Adenine Dinucleotide; NADPH: Nicotinamide Adenine Dinucleotide Phosphate; NGF: Nerve Growth Factor; PEDF: Pigment Epithelium Derived Factor; RPE: Retinal Pigment Epithelium; SH-SY5Y: Name of the Neuronal Cell-Line; SRT: Selective Retina Therapy; TBHP: tert-Butyl hyperoxide; TSR: Thermal Stimulation of the Retina; VEGF: Vascular Endothelial Growth Factor

INTRODUCTION

Human vision is characterized by a great central visual resolution. The ability to read, which is essential in our modern visual world, mostly depends on our visual acuity that is achieved at the fovea region of retina. Here, each photoreceptor is connected to a single bipolar cell and a single ganglion cell. This one to one to one connection is the key to high resolution and only present at fovea centralis in the middle of the macular region. Any disease that affects the macula is a direct threat to central vision. Maculopathies, most of all age-related macular degeneration (AMD), are the most common cause for legal blindness in the industrialized world [1,2]. Degenerating retinal pigment epithelium (RPE), followed by photoreceptor deterioration, or

direct photoreceptor cell death or dysfunction cause central vision loss. It is therefore beneficial to find neuroprotective therapies that save photoreceptor cells and function, especially at the macular region.

Thermal stimulation of the retina (TSR) is a photothermal temperature increase within RPE induced by continuous wave laser irradiation [3,4]. Selective retina therapy (SRT) is a photodisruptive selective RPE cell damage induced by micro-pulsed laser irradiation [5,6]. Both laser modalities do not damage the neuroretina and therefore do not affect retinal function [3,7-9]. We could show before that both TSR and SRT have positive therapeutic effects on AMD-like alterations of murine retina in AMD mouse models [3,7]. Pathologically thickened Bruch's membrane (BrM), becomes thinner most likely due to an increased expression of matrix metallo proteases (MMP) [4,10]. Dysmorphic RPE cells become a more physiological phenotype, most likely due to replacement and regeneration [3,7]. Inflammatory processes are suppressed by TSR [11]. Neither long-lasting inflammatory process nor damage to neuroretina have been seen.

For successful treatment of macular disease, a neuroprotective component would be beneficial. Neuroprotection has been attributed to macular xanthophylls, lutein and zeaxanthin, having their highest concentration at the fovea region [12,13]. Therefore, supplementation of lutein and zeaxanthin has been proposed as preventive measure for AMD, however with no success [14]. Dysregulation of neurotrophins, like brain derived neurotrophic factor (BDNF), and nerve growth factor (NGF), in AMD and glaucoma [15], a progressive disease of peripheral visual field deficiency due to ganglion cell loss, has been suggested as important feature of these diseases. Both neurotrophins have become a target for novel therapeutic strategies to induce neuronal regeneration, especially in glaucoma [16]. Angiogenesis is regulated by vascular endothelial growth factor which is counteracted by pigment epithelium derived factor (PEDF) [17]. Both VEGF and PEDF have protective properties in the retina, especially on RPE cells [18,19]. An induction of neuroprotective factors could be a therapeutic option for macular disease, especially degenerative diseases like AMD.

We herein examine the possible neuroprotective effect of TSR and SRT on neuronal cells in vitro. Neuronal cells (SH-SY5Y), were placed in cell-culture medium with supernatants either of laser-treated or untreated organ-cultures. Then cells were put under oxidative stress, like seen in AMD. Viability was evaluated comparing laser-treated with untreated supernatants to investigate a possible neuroprotective effect on neuronal cells of proteins secreted by laser treated RPE cells. In addition, the apical secretion of neurotrophins and cell mediators with neuroprotective properties was measured quantitatively and compared to the apical secretion of these factors in laser-treated organ cultures to evaluate possible protective mediators.

MATERIALS AND METHODS

Porcine Organ Culture

Fresh porcine eyes were acquired from a local abattoir. The preparation has been described in detail before [10]. Briefly, the eye bulbs were cut at the limbus removing the anterior segment including lens and vitreous body. The bulbs were opened by longitudinal incisions and neuroretina was removed. The complex of RPE, BrM and choroid was removed carefully from sclera. A plastic ring-system was inserted, and the RPE/BrM/choroid complex fixed into it. Rings were placed into 12-well-culture plate and kept warm at 37°C in 1.5 ml organ culture medium (Dulbecco's Modified Eagle Medium (DMEM) High Glucose (Biochrom, Berlin, Germany) and Ham´s F-12 with L-glutamine (PAA Laboratories, Pasching, Austria) enriched with 10 % fetal calf serum (FCS) (10592F, Linaris, Wertheim-Bettingen, Germany) and penicillin/steptomycin 10000 U/ml (Biochrom, Berlin, Germany).

TSR and SRT in organ cultures

Organ cultures were placed under a slit-lamp adapted laser system in organ culture medium in 12-well plates. Organ cultures were irradiated by either TSR (100 ms duration, 200 µm spot size, power titrated to no instant cell-death and a cell death rate of ~ 2 % one day after TSR), or by SRT (300 ms duration, 100 Hz, 1.4 µs pulse duration, 200 µm spot size, energy titrated to an initial cell-death rate of 80 to 100 % within the spot area). Calcein-assays were performed afterwards to confirm cell-death rates, as described previously [10]. Organ cultures were then placed in a modified Ussing chamber to separate the apical from basal compartment [10]. Six organ cultures each were cultivated for one or three days. Organ culture medium was refreshed every 24 hours. Medium was collected, cooled at 4°C, centrifuged at 13000 rpm for 5 minutes and then kept as supernatant at -20°C until further use.

Cytokine detection in ELISA

For determination of neurotrophin secretion to the apical compartment of organ cultures, cultivation of 6 organ cultures was carried out for three days. Every 24 hours, supernatants, as described above, were collected and exchanged with fresh culture medium. ELISAs were conducted in commercially available kits for the detection and quantification of BDNF (BDNF Quantikine ELISA, R&D systems, Minneapolis, MN, USA), NGF (Pig NGF ELISA Kit, Cusabio, Houston, TX, USA), VEGF (VEGF Quantikine ELISA, R&D systems, Minneapolis, MN, USA), and PEDF (PEDF Human Recombinant, Novatein Biosciences, Woburn, MA, USA). After three days of cultivation supernatants were placed in 96 well-plates of the ELISA kits. Wells were washed automatically by Elx50 (Biotek, Winooski, VT, USA) or manually, depending on the recommendation in the respective manuals. Photometric analysis was done by Elx800 microplate photometer (Biostep, Jahnsdorf, Germany) at λ= 450 nm. Protein concentration was calculated automatically by Gen5 (Biostep).

SH-SY5Y Cells

SH-SY5Y (Leibniz-Institute DSMZ, German Collection of Microorganisms and Cell Cultures GmbH) is a thrice-cloned sub-line of bone marrow biopsy-derived line SK-N-SH. It is a human neuronal cell line frequently used in neuroscience. For cell culture experiments, deep frozen cells (-80°C) were defrosted at room temperature. They were then placed in 10 ml culture medium, composed of RPMI with phenol red (Merck, Darmstadt, Germany), FCS 10 % (10592F, Linaris, Wertheim-Bettingen, Germany), penicillin/streptomycin 10,000 U/ml (Biochrom,

Berlin, Germany). After twice centrifugation at 900 rpm, the cell pellet was suspended in 10 ml culture medium and cultivated at 37°C in cell culture flasks until confluence of 75 to 80 % was achieved, after which the cells were passaged (Figure 1).

For passaging, cells were removed from cell culture flask enzymatically by biotase (1 ml for T25 flasks, at 37°C for 3 minutes). Cells were then suspended in basic cell culture medium (BM: composed of RPMI without phenol red, L-Glutamine, penicillin/ streptomycin). After centrifugation at 900 rpm, cell pellet was re-suspended in 4 ml culture medium no. 1 (CM1: composed of RPMI with phenol red, FCS 10 %, penicillin/ streptomycin). Cells were separated every 48 hours (1:4). In T25 flasks with 9 ml CM1, 1 ml of the cell suspension was added. Cell culture flasks were incubated at 37°C with 5 % CO2.

For cell suspension in 96 well plates, cells were placed in culture medium no. 2 (CM2: composed of RPMI, L-Glutamine, penicillin/streptomycin, FCS 5 %), at 10,000 cells per well. To achieve this, only cell culture flasks with 80 % confluence were used. Cell pellets were suspended in CM2. Cell number was determined in a Neubauer chamber. Cells were seeded at a concentration of10,000 cells per well and cultivated at 37 °C for 96 hours. Cell morphology (Figure 1), was evaluated every 24 hours and medium was exchanged every 48 hours.

SH-SY5Y Oxidative Cell Stress

In preliminary examinations cell stress was induced by tert-Butyl hyperoxide (TBHP), Erastin and H2O2. H2O2 induced the most reproducible reduction of activity in MTT assay. Stress titration revealed a concentration of 3500 µmol for stress induction over a time period of 24 hours. Therefore, cell stress was induced by 24 h incubation with 3500 µmol H2O2 to achieve an approximate 50 % reduction in MTT assay. Oxidative stress is a key component of AMD pathogenesis.

Methylthiazolyl-tetrazolium (MTT)- Assay

MTT is a yellow dye that can be ingested by vital cells and metabolically reduced to blue/ violet formazan. The color change is measured photometrically at λ= 550 nm. The reduction ability depends on the amount of reduction equivalents like NADH+ and NADPH. Thereby, MTT assay is a measure for the rate glycolysis rate of cells [20]. MTT assay provides a measurable feedback about the metabolic activity and viability of the cell.

MTT powder was diluted in Dulbecco's Modified Eagle Medium at 5 mg/ml and stored at -20°C. Shortly before the assay, this solution was diluted in BM (see M&M section SH-SY5Y Cells) at 1:10 concentration. For MTT assay, 10 µl of this solution were given to each well and wells were incubated at 37°C for 4 hours. Afterwards, medium was removed. 100 µl Dimethylsulfoxide (DMSO) were added to enable a better release of violet dye from the cells. Plates were shaken at 200 rpm at 37°C for 10 min. Photometric analysis followed this procedure. Blank value of pure BM was measured in unused wells at the rim of the plate after 10 µl MTT solution was added and after removal replaced by pure DMSO.

Supernatant in Cell Culture Medium

TSR and SRT supernatants, as described above, were collected every 24 hours over one or three days after laser irradiation. To evaluate a possible neuroprotective effect of these supernatants, SH-SY5Y cells were cultivated as described above on 96 well plates. Ninety-six hours after seeding, culture medium was removed, and supernatants were applied to the wells. Organ culture medium of untreated organ cultures was used as control medium. Incubation with supernatants was done for 24 hours. After the incubation, oxidative cell stress was applied as described above. MTT assay was then carried out to determine cell viability. The experiments were divided into four treatment groups (TSR and SRT each incubated over one or three days). Each group was compared to a control group incubated over the same period. Each group consisted of supernatants from 6 organ cultures.

Statistics

Both study parts are explorative studies: For the determination of MTT activity, the photometric median values of 7 wells incubated with normal organ culture medium (10 % FCS), were determined 100 %. Photometric values of unstressed cells in 12 wells incubated with supernatants of 6 untreated organ cultures were compared with this 100 % control and determined untreated and unstressed control. Photometric values of stressed cells in 12 wells also incubated with supernatants of 6 untreated organ cultures were compared to this control. Then,

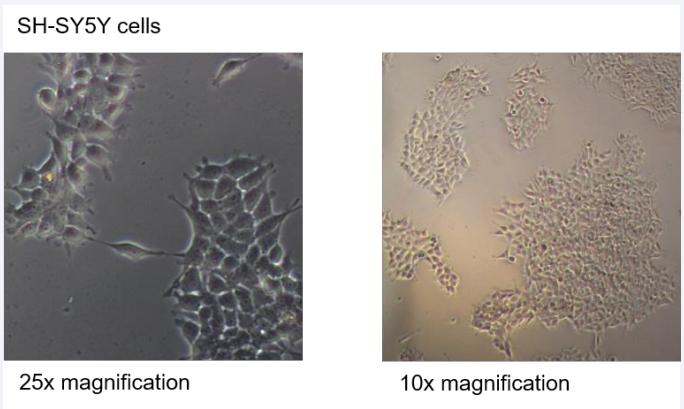

Figure 1 Representative example of SH-SY5Y cells. These cells connect and build neuronal networks. The phenotype displayed represent intact neuronal cells.

photometric values of stressed cells in 12 wells incubated with supernatants from 6 laser-treated organ cultures each laser modality were compared to stressed cells in 12 wells incubated with supernatants from 6 untreated organ cultures. Statistical analysis was done by independent t-test with confidence interval of 95 % and p-value of 5 % as significance level.

For the measurement of neurotrophin secretion, supernatants of 4 organ cultures for control, NGF and BDNF, respectively, collected every 24 hours over 3 days beginning with the start of cultivation (with or without laser irradiation), were analyzed by ELISA. Neurotrophin examinations were stopped then, for no NGF or BDNF was found in controls or in lasered organ cultures. For the measurement of VEGF and PEDF expression, supernatants of 9 organ cultures each group were included. Protein secretion level of controls was defined 100 %. Means were compared with controls by two-tailed dependent t-test with confidence interval of 95 %, p-value of 5 % as significance level.

RESULTS

The first part of the experiment was to evaluate a possible neuroprotective effect of laser treated supernatants on neuronal cells. SH-SY5Y cells were stressed by H2O2 inducing oxidative stress with impaired cell metabolism, vitality, or even cell death, like seen in AMD. Cell viability and metabolic activity were measured by MTT assay. H2O2 induced oxidative cell stress, applied for 24 hours, induced a mean reduction in MTT to a level of 59 (+/- 9) % (absolute photometric values in arbitrary units: unstressed= 0.191 ± 0.01; stressed= 0.112 ± 0.01). TSR lasered organ cultures produced supernatants that led to significant less cell viability reduction in MTT than untreated control supernatants (p= 0.02 for 1 day supernatant and p< 0.01 for 3 days supernatant), indicating that the treated SH-SY5Y cells were less susceptible to H2O2 induced oxidative cell stress (absolute photometric values in arbitrary units of SH-SY5Y cells incubated with TSR supernatants: day 1 unstressed= 0.201 ± 0.06 ; H2O2= 0.170 ± 0.017; day 3 unstressed= 0.189 ± 0.013; H2O2= 0.142 ± 0.007). Conversely, SH-SY5Y cells incubated with SRT treated organ culture supernatants did not show any changes in their susceptibility to H2O2 induced oxidative cell stress (absolute photometric values in arbitrary units of SH-SY5Y cells incubated with SRT supernatants: day 1 unstressed= 0.172 ± 0.048 ; H2O2= 0.101 ± 0.021; day 3 unstressed= 0.184 ± 0.014; H2O2= 0.107 ± 0.027) (Figure 2).

The second part of the study was to evaluate the laser induced apical secretion of potentially neuroprotective neurotrophins BDNF and NGF, as well as angiogenesis controlling, potentially neuroprotective VEGF and PEDF in RPE cells. Porcine organ cultures placed in modified Ussing chambers allowed for the analysis of protein secretion to the apical compartment. There was no secretion of BDNF or NGF to the apical compartment of RPE/BrM/choroid organ cultures. In supernatants of 4 organ cultures each group, collected every 24 hours over three days after either TSR or SRT, there was also no secretion of BDNF or NGF to the apical compartment in ELISA analysis.

Conversely, VEGF and PEDF were constitutively secreted to the apical compartment of the organ cultures. The secretion level of VEGF (490.56 pg/ml ± 80.68 for the TSR control and 573.67 pg/ml ± 83.5 for the SRT control), and PEDF (82.32 pg/ml ± 20.29 for the TSR control and 59.99 pg/ml ± 22.15 for the SRT control) to the apical compartment in untreated organ cultures was set 100 %. Protein secretion from laser treated organ cultures, groups of 9 organ cultures each, was compared to these controls and statistically evaluated by t-test (Figure 3). TSR did not alter apical VEGF (448.94 pg/ml ± 272.12) or PEDF (96.21 pg/ml ± 25.51) expression. SRT led to a decrease in VEGF (472.47 pg/ml ± 107.74; p= 0.03) and an increase in PEDF (91.42 pg/ml ± 29.44; p= 0.01) secretion.

DISCUSSION

In the presented study we investigated the effect of TSR and SRT on neuroprotective processes. First, we examined the neuroprotective effect of supernatants from laser-treated organ cultures on an oxidatively stressed neuronal cell line. Second, we examined the secretion of common neurotrophic factors, like BDNF and NGF, as well as angiogenic factors with neuroprotective properties, like VEGF and PEDF.

First, we examined, if supernatants collected apically have a neuroprotective influence on neuronal cells. Our neuronal cell line, SH-SY5Y cells (Figure 1), derives from human neuroblastoma. These cells are a widely used model for neuronal cells [21], with some limitations. SH-SY5Y cells are neuronal cells, but they are no retinal neurons. They derive from a neuroblastoma cell line, hence cell proliferation and possible mutation rates might be increased in these cell lines. However, this neuronal cell line, put under oxidative stress, is a widely used model to study neuroprotective agents [22,23]. To induce this oxidative stress, H2O2 is commonly used. It mimics oxidative stress in the retina [24]. Oxidative stress is a key component of AMD pathogenesis. It is pronounced in the macular and especially fovea region, for cell metabolism here, is the highest throughout retina. Cell viability was examined by MTT assay. This assay is widely used to determine metabolic activity of the cell [20] and a valuable tool to examine cell viability. Cells oxidatively stressed by H2O2 displayed a reduced viability, as assessed by MTT, to about 50 % in our experiment. Cells stressed by H2O2 and incubated with supernatants from TSR laser-treated organ cultures showed less decline of viability compared to cells incubated with supernatants from untreated organ culture medium. The TSR supernatant seemed to provide these cells with neuroprotective tools, making them more resistant to cell stress. So far, we have not identified which factors in TSR supernatant are responsible for the neuroprotective effect. We know that TSR leads to a suppression of inflammatory processes one day after laser irradiation [11]. Also, chaperones like heat shock proteins are expressed in temperature challenged RPE cells [25]. These protective properties may have the neuroprotective influence, we see in our experiment. SRT-treated organ culture supernatants, on the other hand, did not influence the viability of SH-SY5Y cells put under cell stress. It might be due to the short-time pro-inflammatory processes that follow RPE cell-death induced by SRT [11], or other wound-healing processes. Inflammation is needed to clear the RPE surroundings from cell debris within the laser-spot area. However, it might be a problem considering neuroprotection. Inflammatory processes, especially an increased expression of IL1b and IL-18 like seen after SRT [11], may harm neuronal cell viability through an overactive inflammasome, like seen in pyroptosis [26].

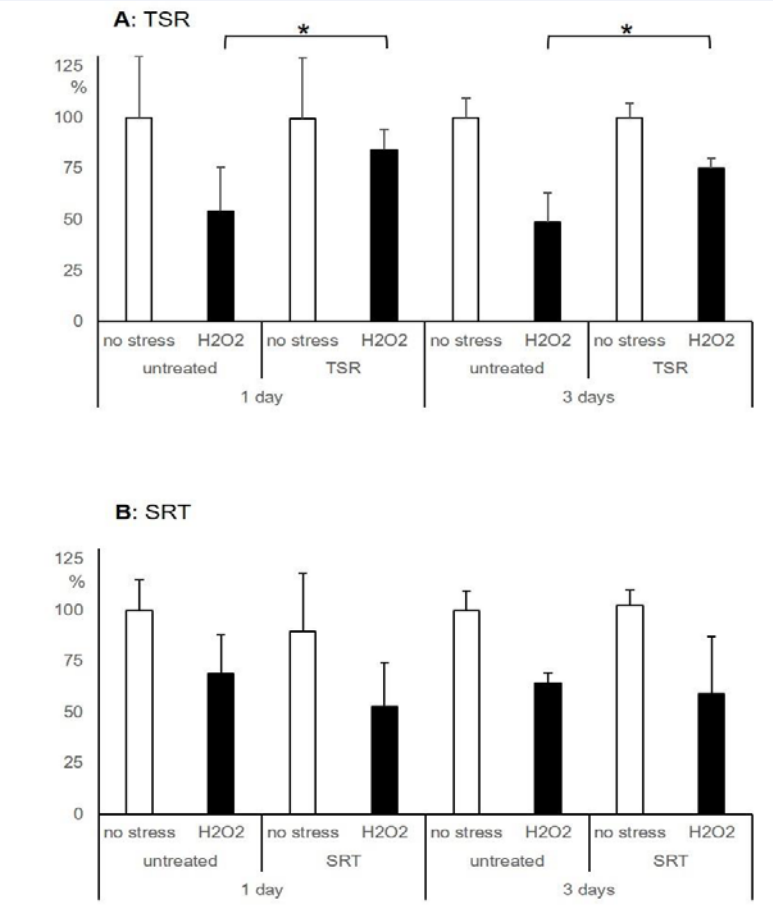

Figure 2 SH-SY5Y neuronal cells in 12 cell culture wells each group were stressed by H2O2 and compared with unstressed (no stress) cells in percentage of the untreated and unstressed control. Stressed cells (black bars) were significantly reduced in viability examination by MTT assay compared to the unstressed cells (empty bars). Then cells were incubated for 24 hours, with organ culture medium obtained from laser treated porcine organ cultures (TSR or SRT), collected every 24 hours over one or three days. Cell viability of stressed cells incubated with untreated organ culture medium compared with viability of stressed cells incubated with laser-treated organ culture medium were evaluated by t-test (95 % confidence interval). Those incubated with TSR-treated (A) supernatants (groups of six organ cultures and 12 wells each) were significantly ($p = 0.02$ for cells incubated with one day supernatant and $p < 0.01$ for cells incubated with three days supernatant) more active in MTT assay than those incubated with untreated organ culture medium. Stressed SH-SY5Y cells incubated with SRT-treated (B) supernatants (groups of six organ cultures and 12 wells each) did not show an enhanced activity in MTT assay compared with stressed cells incubated with untreated organ culture medium.

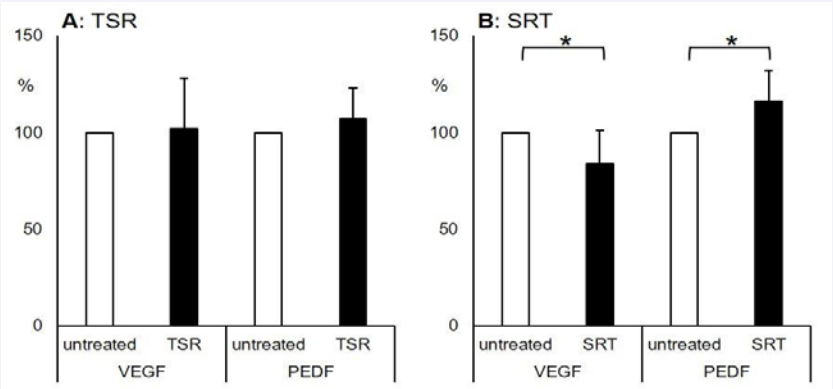

Figure 3 Apical secretion of VEGF and PEDF in supernatants of untreated organ cultures (empty bars, groups of 9 each) compared with the secretion in supernatants of laser treated organ cultures (A: TSR or B: SRT, groups of 9 each) during a three-day time period. Protein secretion in untreated organ cultures was set 100 %. Secretion in treated organ cultures was compared to untreated controls and statistically evaluated by t-test. There were no differences in TSR treated organ cultures compared with control. SRT led to a decrease in VEGF secretion (84 %, ± 17) and an increase in PEDF secretion (116 % ± 16) to the apical compartment.

Our finding, that TSR induced proteins that are neuroprotective, warrants further experiments and therefore part two of the study was conducted.

We examined the apical secretion of cytokines in RPE/choroid organ cultures since the neuroretina is located apical to the RPE monolayer. We hypothesized a neuroprotective influence generated by the apical release of neurotrophins from laser treated RPE cells in this model. We did not find a secretion of BDNF or NGF to the apical compartment neither in untreated nor in laser-treated organ cultures. This was unexpected, since mRNA expression of neurotrophins has been shown before in RPE cells [27]. However, the neuroretina is removed from our organ cultures and neurotrophins are mainly expressed in neuroretina [28]. Also, the separation of the apical from basal compartment might be an explanation for this finding. This separation has not been part of former studies. The absence of NGF and BDNF in apical supernatants of RPE/BrM/choroid organ cultures may be due to a lack of secretion of these neurotrophins in RPE cells, although the expression of these has been shown before in cultured human RPE cells [27,28]. A different model, including neuroretina or even an animal model is needed to answer the question of the release of NGF and BDNF in ocular tissue after TSR or SRT. However, neuroprotection does not only depend on the secretion of BDNF and NGF, therefore, examinations should be expanded to other factors in further studies.

VEGF is known to be a major factor in angiogenesis and if expressed in the retina can lead to neovascularization [19,29]. On the other hand, VEGF is also known to have neuroprotective properties [30-32]. The apical secretion of VEGF may therefore protect the viability of neuroretina. We detected a decline in apical VEGF secretion only in SRT laser-treated organ cultures, while TSR did not show any influence on the apical secretion of VEGF. Hypothetically, this could be a useful tool in the treatment of neovascular AMD and maybe even more so in retinal angiomatous proliferation, where neovascularization derives from the retinal vascular system and not from choroid. If a decline in VEGF expression to the apical compartment shows any effect on neuroretinal viability or function remains unclear. There is evidence that VEGF deprivation leads to a lack of trophic support for neuroretina to sustain function and viability [33,34]. The decline in apical VEGF secretion after SRT might therefore explain, why there is no neuroprotective effect induced by SRT, at least in our model.

PEDF is known as the natural antagonist of VEGF and is therefore responsible for angiogenesis homeostasis [17]. It is also a known neuroprotective protein [18,35]. The apical expression of PEDF could therefore be a useful tool to protect neuroretina from degeneration. In our experiments, SRT led to an increase in PEDF expression to the apical compartment. This correlates with the downregulation of VEGF, thereby creating an anti-angiogenic milieu in the subretinal space. Whether this change in secretion has any effect on neuroretinal function and protection, is not known. Contrary to SRT, TSR did not have an influence on the apical expression of PEDF.

The presented data is limited by the fact that we did not conduct a whole secretome analysis of all proteins secreted in supernatants. We therefore could not determine the exact proteins that led to the neuroprotective effect seen in TSR. Our findings warrant further examinations, like whole secretome analysis. It would be of great interest to transfer the experiment to retinal neurons, microglia and Mueller cells, to get a better understanding of the processes involved.

CONCLUSION

We know that TSR and SRT have an effect on AMD-like alterations in murine models [3,7]. A neuroprotective effect would be useful to inhibit neurodegeneration, like seen in AMD or other macular disease. The presented data is preliminary in terms of neuroprotection achieved by TSR or SRT. However, the study provides valuable hints that TSR might have neuroprotective properties and that SRT does not have such an effect. This needs to be evaluated further in future studies.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

JP conducted the experiments, analyzed data and red proofs.

ER conducted experiments, red proofs.

IV supported statistical analysis and red proofs.

KH supported manuscripts preparation and red proofs.

PD supported laboratory work, especially cell cultures and experiments, red proofs.

JH supported the laboratory, red proofs.

AK supported the laboratory work, analysis of data and manuscript preparation, red proofs.

JT experiments, data analysis and presentation, wrote manuscript, corresponding author.

Ethics approval and consent to participate

The presented data are not based on human or animal experiments. Porcine organ cultures derive from eyes from the local abattoir.

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