MEDDOCS Open Access Publisher

Annals of Ophthalmology and Visual Sciences

Open Access | Research Article

The Secretome of Stem Cell from Apical Papilla Improves Hydrogen Peroxide Induced Retinal Toxicity in Rat, an Animal Study

Alireza Dehghani[1]; Mohammad Malekahmadi[1,2]; Fereshteh Karamali[2]; Sareh Soroushzadeh[2]; Heshmatollah Ghanbari[1]; Ardeshir Talebi[1]; Mohammad Hossein Nasr Esfahani[2]**

[1]Isfahan Eye Research Center, Department of Ophthalmology, Isfahan University of Medical Science, Isfahan, Iran.

[2]Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.

***Corresponding Authors:**

Alireza Dehghani[1]

Mohammad Hossein Nasr Esfahani[2]

[1]Isfahan Eye Research Center, Department of Ophthalmology, Isfahan University of Medical Science, Isfahan, Iran.

[2]Department of Animal Biotechnology, Cell Science Research Center, Royan Institute for Biotechnology, ACECR, Isfahan, Iran.

Tel: +98-913-119-9888; +98-913-310-9705;

Email: dehghani@med.mui.ac.ir,

mh.nasr-esfahani@royaninstitute.org

Received: Nov 10, 2022

Accepted: Nov 28, 2022

Published Online: Nov 30, 2022

Journal: Annals of Ophthalmology and Visual Sciences

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Copyright: © Naughton A (2022). *This Article is distributed under the terms of Creative Commons Attribution 4.0 International License*

Keywords: Conditioned medium; Neuroprotection; Retinal degeneration; Stem cells from apical papilla.

Abstract

Background: Retinal damage can lead to severe and permanent visual loss. Mesenchymal stem cell and their secretome containing stem cell-derived factors and have been shown to have proliferative and anti-inflammatory properties. Therefore, the aim of this study was to assay the conditioned medium derived from the stem cells from apical papilla (SCAP) on retinal toxicity induced by H_2O_2 in rats.

Methods: Thirty healthy 6-week-old rats were selected for this study. After two consecutive days of intra-vitreal H2O2 injection in in right eye, they were randomly divided into three groups: H2O2: which received no other intervention after H_2O_2 treatment, H2O2+SCAP-M and H2O2+SCAP-CM groups that received intra-vitreal injection of basic and conditioned culture medium, respectively, three days after H2O2 treatment. The Left eye was considered as the control group. After two weeks, all rats were euthanized and morphological and histo-chemical evaluations were performed.

Results: The retinal thickness was significantly better maintained in H2O2+SCAP-CM group compared to H2O2 and H2O2+SCAP-M groups. Further analysis of outer and inner nuclear layers showed a preservative effects of SCAP-CM group on the H2O2 induced toxicity. Evaluation of apoptosis by TUNEL assay and relative expression of pro-inflammatory (*IL-6* and *IL-1B*) and *BAX* genes, showed that they were attenuated following SCAP-CM administration whereas anti-apoptotic gene, *BCL-2*, was over expressed in SCAP-CM group.

Conclusion: Our data, for the first time, reported the neuro-protective effects of SCAP-CM on H2O2 induced retinal cell injury. Therefore, the SCAP secretome may have the potential for preservation of retinal damage.

Cite this article: Dehghani A, Malekahmadi M, Karamali F, Soroushzadeh S, Ghanbari H, et al. The Secretome of Stem Cell from Apical Papilla Improves Hydrogen Peroxide Induced Retinal Toxicity in Rat, an Animal Study. Ann Ophthalmol Vis Sci. 2022; 5(2): 1033.

Introduction

Various factors such as trauma, light, aging, drugs and systemic diseases leads to permanent retinal damage and visual loss. Cellular degeneration is one of the main pathologies of visual loss [1-3]. Different treatments, like cell-based regenerative therapies, are proposed and/or carried out for retinal degeneration with different degrees of success; but no completely and effective strategy has been proposed yet [4-8]. Several studies have shown that cell therapy could modulate the apoptosis process in retinal layer either by cell replacement or secretion of trophic factors and therefore improve the survival of the photoreceptors[1,5,6].

Various types of mesenchymal stem cell (MSCs) such as adipose tissue, bone marrow, umbilical cord, dental, cartilage and skin have been used in treatment of retinal injury [9]. Researchers have revealed that most of mesenchymal stem cells injected into the vitreous didn't incorporate to the retina and their therapeutic effect is mainly due to secretion of paracrine factors which can release various growth factors to promote recovery of the retinal cells after injuries [4,7,8]. Besides, cell therapy also has disadvantages such as xenozoic infection, poor bioavailability, survival and function of transplanted cells in damaged area as well as being costly and time-consuming procedure. Also, the fate of transplanted cells in the injection site is another important issue which can't be controlled after in vivo transplantation [8-10].

On the other hand, anti-inflammatory and immunomodulatory effects of stem cells on injured tissues, are mainly mediated by paracrine factors released into extracellular environment [8-12]. According to recent studies, intra-vitreal injection of conditioned medium (CM) containing stem cell-derived factors such as growth factors, cytokine and chemokine has better therapeutic outcomes than stem cell injection. In addition, CM as a cell- free therapeutic approach has been considered to be both cost and time efficient [13].

Dental Stem Cells (DSCs) originated from apical papilla (SCAP), dental pulp stem cells (DPSC) and periodontal ligament stem cells (PDLSC), has been recently introduced because of its easy accessibility. The secreted factors by DSCs revealed higher levels of neurotrophic proteins when the results were compared to other MSCs [14]. As mentioned, different DSCs also secret a variety of growth factors and chemokine. DPSCs have more effective factors related to odontoblast differentiation in compare to proliferative chemokine secreted by SCAPs. On the other hand, SCAPs showed greater neuroprotective effect than other DSCs because they released more neurotrophic factors such as brain derived neurotrophic factors (BDNF) [10-12]. Interestingly, CM of SCAPs were more effective in axon regeneration than CM of other dental stem cells, like dental pulp and periodontal ligament [10].

Oxidative stress due to reactive oxygen species (ROS), initiates the caspase-mediated apoptosis pathway. Therefore, hydrogen peroxide (H2O2) as a source of ROS is commonly used for inducing retinal cell degeneration. Therefore, *in vivo* model, chemical -induced retinal toxicity using H2O2 is a useful method for evaluation of retinal diseases mechanisms and new treatment options [15].

In this study, we evaluated the protective effect of SCAP-CM on H2O2 induced retinal toxicity in a rat model

Methods

Culture and preparation of SCAP conditioned medium (SCAP-CM)

The SCAP were provided by Royan institute [16]. They were cultured in DMEM medium supplemented by 10% FBS (Gibco), 1% NEAA (Gibco) and 1% glutamax (Gibco). To prepare SCAP-CM, the 10^4 cell/cm^2 were cultured at tissue culture plate. One day later, the cells reached 80% confluency; then, the culture medium was removed and the cells were washed with PBS$^+$ twice. Then, FBS free medium was introduced to cells for one day. Lastly, the CM was collected and filtered (0.2 µm filter) for further analysis.

Protein quantification

To assay the concentration of proteins, SCAP-CM was lysed by adding an equal amount of Radioimmunoprecipitation (RIPA) buffer at 4° C for 30 min followed by 30 seconds sonication in an ice-cold bath. Protease and phosphate inhibitors (Sigma Aldrich, Missouri, USA) were added to lysis buffer at 1:10 concentration. Following lysis, total protein contents were measured using BCA protein assay kit (KIAZIST, Kermanshah, Iran) according to manufacture instruction.

Induction of neurotoxicity in retinal layers

The 6-week-old hooded rats (~150gr, n=10 for each group) were housed in rooms under standard laboratory conditions at temperature between 20-25 °C, regular daylight exposure and free access to food and water, according to the Association for Research in Vision and Ophthalmology (ARVO) [17]. All experimental protocols were approved by Institutional Research Ethics Committee at Isfahan University of Medical Science (IR.MUI.MED.REC.1399.053). All procedures were also carried out in compliance with the ARRIVE guidelines. To induce oxidative stress in retina layer, the rats were anesthetized by intra-peritoneal injection of ketamine (50 mg/kg) and xylazine (5mg/kg). The right eyes were enrolled in this study and the site of injection was disinfected by Povidone-iodine 5%. Then, intra-vitreal injection of 10µg/µl of Hydrogen Peroxide (H2O2) was performed (1.5 mm behind the limbus) using Hamilton syringe for each rat [15]. The H2O2 injection was repeated for one more day. The left eyes were considered as the control.

Intra-vitreal medium injection

To assay the SCAP-CM neuro-protective effects, three days after H2O2 treatment, the animals were anesthetized again by intra-peritoneal injection of ketamine and xylazine, as mentioned earlier. Then, 2µl of the medium was administrated into right eyes. All injections were performed using Hamilton syringe and after each injection, the syringe was hold in the same position for 30 seconds to prevent any leakage. The animals which revealed complications such as massive intraocular or retinal hemorrhage or endophthalmitis after intra-vitreal injections, were excluded from the study. The rats were kept under controlled conditions for 14 days. Finally, they were euthanized in a CO2 gas chamber and right eyes were enucleated for further analysis.

Experimental groups

All animals were randomly divided into three groups with 10 rats in each group (H2O2, H2O2+SCAP-M and H2O2+SCAP-CM). The H2O2 group only received H2O2 injection without further injection. The other two groups, in addition to H2O2, also re-

ceived medium collected from the SCAP (H2O2+SCAP-CM) or unconditioned media (H2O2+SCAP-M).

Histological assessments

Enucleated eyes were fixed in 4% paraformaldehyde for 24 hours, then they were embedded in paraffin. The left eyes were assigned as control group.

Then, 8µm sections from the eyeball were prepared using a microtome (Leica, RM2255, USA) and stained with hematoxylin and eosin. The thickness of retina selected via whole eye was measured under a light microscope (Olympus, USA) with AnalySIS LS Starter 5.0 software (Olympus, Japan). For accurate assessment of retinal thickness, the head of optic nerve has been considered as a standard point and retinal thickness was measured at a distance of 600 µm from the disc.

Apoptosis analysis

Evaluation of apoptosis rate was carried using the TUNEL method coupled with fluorescein (DeadEnd™ Fluorometric TUNEL System kit, Promega, Madison, WI) and PI counterstain (Sigma-Aldrich, USA). To quantify immunostaining results, apoptotic cells in all experimental groups were visualized under Olympus DP70 camera equipped with an Olympus DP70 camera and were calculated in at least 5 random fields from each experimental group.

qRT-PCR

To analysis the apoptotic inflammatory mediated genes, the retinal layer from enucleated eyes were isolated mechanically under dissecting stereomicroscope. Subsequently, they were washed by PBS^- and total RNA were extracted by TRIzol reagent from all four experimental groups in three independent experiments. Then, it transcribed to cDNA by Biotechrabbit™ Kit according to the manufacturer's protocol. Expression levels were normalized to *GAPDH* housekeeping gene and relative expression was calculated using the comparative threshold cycle method (ΔΔCT) and healthy control group was considered for calibration. The sequence of specific primer pairs has been listed below:

BCL-2: **FP**: 5`-ACTTCTCTCGTCGCTACCGTC-3`; **RP**: 5`-AAGAGTTCCTCCACCACCGT-3`; *BAX:* **FP**: 5`-GGATCGAG-CAGAGAGGATGG-3`; **RP**: 5`-ACACTCGCTCAGCTTCTTGG-3`; *IL-6:* **FP**: 5`-GAACAACTTACAAGATAACA-3`; **RP**: 5`-GACTCTA-ACTTCTCCATTA-3`; *IL-1β:* **FP***:* 5`-AGCAGGTGAAGAATGATT-3`; **RP**: 5`-GCAGTTGATGAAGATGTC-3`; *GAPDH:* **FP**: 5`-TGCCGCCTG-GAGAAACC-3`; **RP**: 5`-TGAAGTCGCAGGAGACAACC-3`.

Statistical analysis

Statistical analysis was performed via SPSS 25 (IBM SPSS, Inc. Chicago; IL) and data were presented as mean ± standard error (SE). The Kolmogorov–Smirnov Z test was used to assess the normal data distribution. Comparison between groups was done using one-way analysis of variance (ANOVA) with a LSD post-hoc test. The means were compared using Independent sample t-test and p<0.05 was considered significant.

Results

Maintenance of SCAP cells

In accordance with our previous reports, the cells were maintained and cultured according to routine protocol. The workflow of the study was presented in **figure 1A**. As it shown, the cells (passage 6-10) with around 80% confluency were used to collect SCAP-CM.

Protein concentration of SCAP-CM

In order to quantify the proteins secreted by the SCAP, the serum free conditioned medium was collected after 24 hours incubation. Based on BCA assay results, the protein concentration of the SCAP-CM was 527 µg/ml.

Retinal degeneration occurred following intravitreal injection of H_2O_2

In accordance to previous report [15], injection of H2O2 by concentration of 10µg/µl could significantly attenuate the survival of neural retinal cells in compare to control group which has been observed by decrease thickness of retinal layers as well as increase the amount of apoptosis cells **(Figures 1B and 2)**.

The thickness of retinal nuclear layers was preserved by SCAP-CM

The histological analysis of retinal layer in different groups showed the retinal atrophy and retinal thinning was significantly lower when the SCAP-CM was administered **(Figure 1B)**.

This protective effect was not detected in SCAP-M in compared with SCAP-CM. (202.2 ± 32.2 and 236.8 ± 20.8 µm, respectively). Injection of SCAP-M didn't provide any improvement in contrast with H2O2 group (199.9 ± 65.3 µm) **(Figure 2A)**. Detailed analysis of thickness layers demonstrated that SCAP-CM could rescue the cells of both Outer Nuclear Layer (ONL) and inner nuclear layer (INL) **(Figures 2B and C)**.

On the other hand, histopathological analysis of the sections showed no stem cell morphology with high ratio of nuclear to cytoplasm. There were also no detected nuclear morphology changes from slender and cigar-shaped to eventually round characteristics of tumor cells or cell aggregates such as rosette like structures between cell layers of retina.

SCAP-CM inhibited the induced cell death in nuclear layers

The apoptotic rate of retinal cells was also investigated using TUNEL assay kit. As it shown in figures 2D and 3, the intra-vitreal injection of H2O2 induced apoptotic cells and the SCAP-M didn't reverse this effect. Although, the retinal cells of the eyes treated with SCAP-CM were protected from cell death.

To confirm this attenuation by SCAP-CM, the expression level of anti-apoptotic gene BCL-2 and regulatory apoptotic gene BAX were also evaluated at RNA level. The results demonstrated that SCAP-CM induced high level of BCL-2 expression while apoptosis regulator BAX decreased significantly after treatment by SCAP-CM **(Figure 4A and B)**.

SCAP-CM modulated the inflammatory response induced by H2O2

Surprisingly, our data showed that administration of SCAP-CM decreased pro-inflammatory genes *IL-6* and *IL-1β.* Although, this difference was just significant for *IL-1β* **(Figure 4C and D)**.

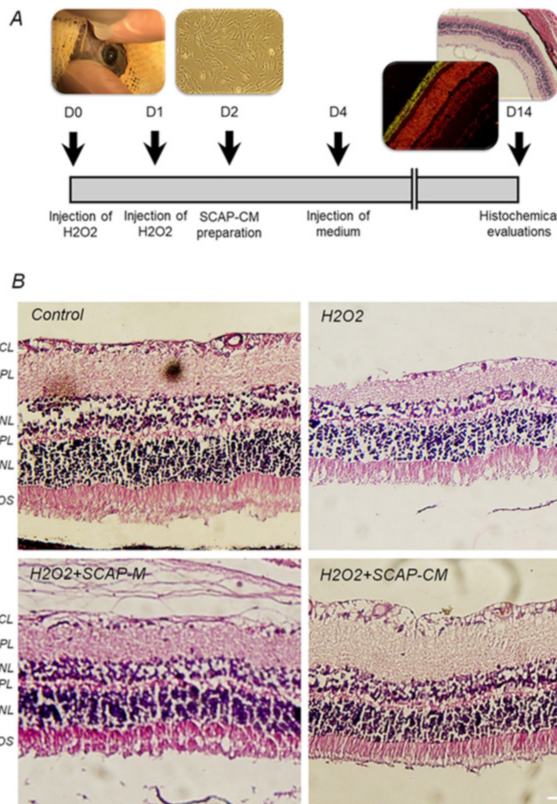

Figure 1: TUNEL staining of retinal tissue. Micrograph representing apoptosis of the retinal cells between the different experimental groups. Apoptosis was detected by TUNEL assay and TUNEL positive cells appear in green (middle column). Nuclei were stained with propidium iodide (PI; left column). The Merge figures were presented in right column. Scale bar: 100 µm.

GCL: Ganglion Cell Layer; IPL: Inner Plexiform Layer; INL: Inner Nuclear Layer; OPL: Outer Plexiform Layer; ONL: Outer Nuclear Layer; OS: Outer Segment.

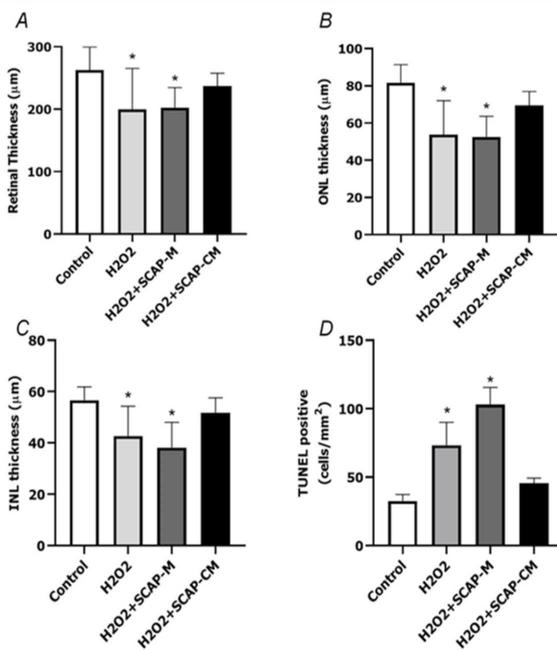

Figure 2: The quantitative results of retinal thickness and apoptosis rate in retinal layers. **(A)** Retinal, **(B)** ONL and **(C)** INL thickness of layers in different groups **(D)** numbers of apoptotic cells per square millimeter. Statistical significances are indicated with *P < 0.05 in compare to Control group.

ONL: Outer nuclear layer; IN: Inner nuclear layer.

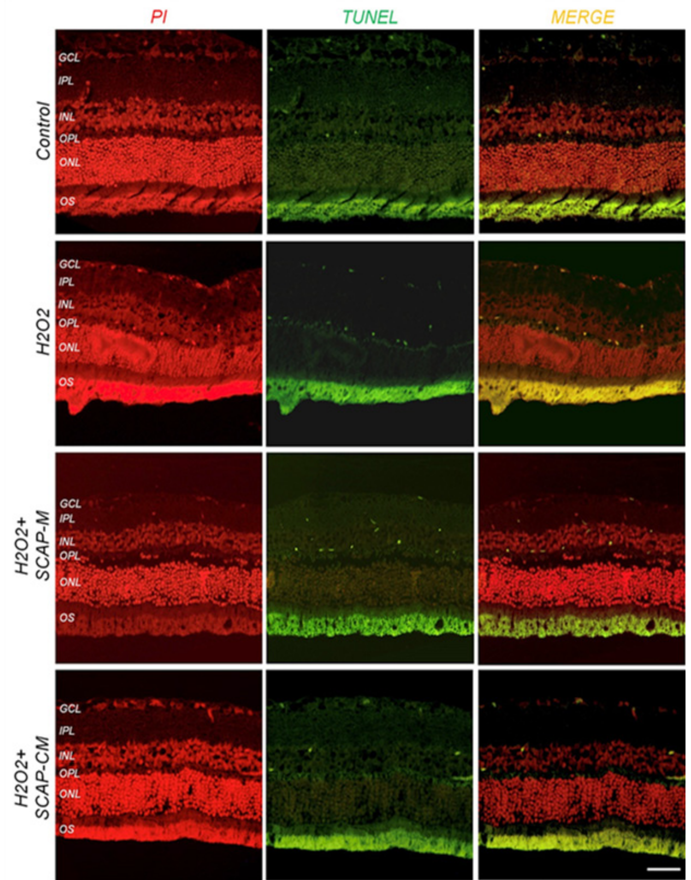

Figure 3: TUNEL staining of retinal tissue. Micrograph representing apoptosis of the retinal cells between the different experimental groups. Apoptosis was detected by TUNEL assay and TUNEL positive cells appear in green (middle column). Nuclei were stained with propidium iodide (PI; left column). The Merge figures were presented in right column. Scale bar: 100 µm.

GCL: Ganglion Cell Layer; IPL: Inner Plexiform Layer; INL: Inner Nuclear Layer; OPL: Outer Plexiform Layer; ONL: Outer Nuclear Layer; OS: Outer Segment.

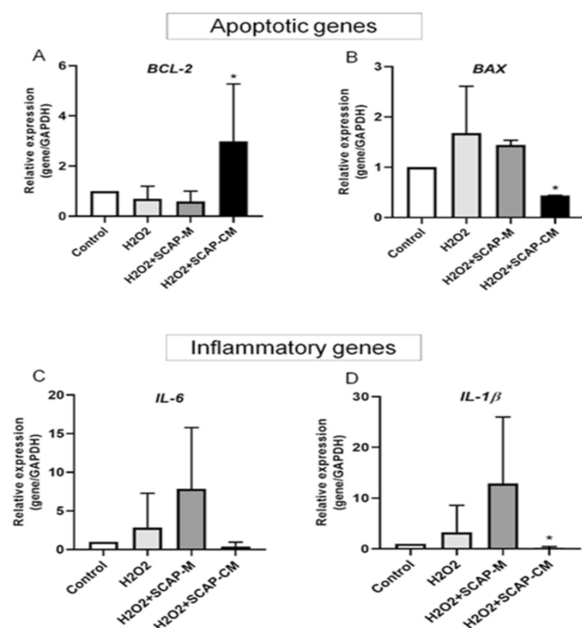

Figure 4: Analysis of relative gene expression in different groups. **(A)** Anti-apoptotic gene BCL-2 and **(B)** Regulatory pro-apoptotic gene BAX. The immune-modulatory genes **(C)** IL-6 and **(D)** IL-1B. Data are shown as mean ± SE. Data have been calculated from n = 3 individual experiment per group. * show relative significance at *P < 0.05 in compare to H2O2 group.

Discussion

As many injuries and diseases lead to permanent visual loss via irreversible retinal damages, discovery of the effective treatments to improve the visual abilities are very important [2,3]. The final effects of disorders are usually abridged to apoptosis and/ or inflammation responses [18]. Reactive oxygen species (ROS) are the main factors involved in many visual disorders. Hydrogen peroxide (H2O2) as a ROS source is commonly used for inducing retinal cell toxicity via oxidative stress [15]. Previous studies have demonstrated the intraocular injection of H2O2 could induce animal model of oxidative stress [15,18,19]. In accordance with that, in the current study, the intra-vitreal H2O2 injection was used to induce retinal damage via oxidative stress. The model was confirmed by reduced retinal thickness, increased number of apoptotic cells and high expression of pro-inflammatory genes *IL-6* and *IL-1β* in compare to control.

Some tissues such as intestine or skin have high potential to replace the lost cells; although the retina is one of the structures with limited regenerative potential. Therefore, a large number of apoptotic or dead cells during damage cannot be replaced by healthy ones [9]. Several experimental studies and clinical trials have assessed the effect of stem cell therapy in retinal diseases [9]. Evidence suggests that MSCs survive for a short period of time after transplantation in a pro-inflammatory environment. Therefore, their loss may lead to a poor therapeutic response . On the other hand, anti-inflammatory and immunomodulatory effects of MSCs are mainly mediated by paracrine factors, released into extracellular environment [12,13]. Duarte *et al*., found that both early-outgrowth bone marrow stem cells and their CM are equally effective to prevent diabetic retinopathy symptoms in animal model [4]. It seems that intra-vitreal injection of CM containing stem cell-derived factors such as growth factors and chemokine has better therapeutic results than direct injection of the stem cells [7.8].

The protein concentration assay of SCAP-CM showed a high level of secretion in our study. This result is in accordance with previous reports regarding the secretion of MSCs, even more than 100-1000 times extracellular vesicles compared with other cell lines [20].

The presented data revealed that intra-vitreal injection of SCAP-CM could rescue the ONL and INL by reduresented in retinal thickness. Sugitani et al. revealed that human adipose stem cells conditioned medium has neuro-protective effect against light-induced retinal damage *in vitro* and *in vivo* in animal models [21]. Mead et al., reported that dental pulp stem cells (DPSCs) have a significant regenerative and protective effect on axotomized retinal ganglion cells when the results were compared to other MSCs [22]. Also, Histological assessment of retinal thickness in sodium iodate –induced (NaIO3) retinal degeneration, confirmed the protective role of DPSCs in retinal thickness of treated rats after one month. It has been found that rat DPSCs can apply neurotrophin-mediated neuro-protection as well as axonogenesis in damaged retinal glial cells [23]. As mentioned before, BDNF secretion from SCAP plays a key role in neurite outgrowth in vitro [14,24]. Recently, a research study reported that BDNF secretion was significantly higher in SCAP compared with other dental stem cells and SCAP-CM was more effective in axon regeneration than CM of other dental stem cells [10]. Li XX et al. showed that stem cells from human exfoliated deciduous teeth and their derived conditioned medium have anti-apoptotic effect and can improved retinal function by decreasing photoreceptor degeneration in mouse model of retinitis pigmentosa [25]. Our recent study also established the neuroprotective effects of SCAP extracellular vesicles in RCS model [26]. Our results, in consistent with others, reinforced this hypothesis that injection of SCAP-CM not only provided a neuroprotective effect on retinal layers after treatment with H2O2 via secretion of a wide variety of trophic factors such as BDNF, GDNF and some neurotrophins, but also didn't regenerate the formation of any stem or tumor cells following SCAP-CM injection. We found significant difference in retinal thickness and degree of apoptosis between SCAPs-CM treated rats in comparison with other groups. The expression analysis of inflammatory gene IL-6 didn't show any significant changes compared with other groups. It might be speculated the inadequate concentration of immunomodulatory factors to stimulate the immune response in presence of SCAP-CM. Although, the expression level of pro-inflammatory factor IL-1β was decreased in retinal cells by SCAP-CM injection. These results highlighted the protective effect of the SCAP-CM in the apoptosis rate of the retinal cells. In accordance with the decrease of the apoptosis, the improvement of retinal thickness was detected in CM treated groups in comparison with other groups. Taken together, our results indicated the potent neuroprotective and anti-apoptotic effect of SCAP-CM.

Conclusion

Our data, for the first time, reported the effects of SCAP-CM on survival and protection of damaged retinal cells. The secretion of the SCAP can be used as a new source for retinal protection and repair. However, high CM potential can be derived from concentrated CM or SCAP extracellular vesicles, which should be evaluated in future studies.

Declarations

Ethics approval

All experimental protocols were approved by Institutional Research Ethics Committee at Isfahan University of Medical Science (IR.MUI.MED.REC.1399.053). All procedures were conducted according to ARRIVE guidelines.

Consent for publication: Not applicable.

Competing interests: The authors declare that they have no competing interests.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' contributions

All authors contributed to the study conception and design. Experimental procedures, data collection and analysis were performed by A.D, M.M, F.K and S.S. All authors read and approved the final manuscript.

Acknowledgements

We thank the Royan institute and Isfahan University of medical science to provide experimental support.

References

1. Chang H-M, Hung K-H, Hsu C-C, Lin T-C, Chen S-Y. Using induced pluripotent stem cell-derived conditional medium to attenuate the light-induced photodamaged retina of rats. Journal of the Chinese Medical Association. 2015; 78: 169-176.

2. Krigel A, Berdugo M, Picard E, Levy-Boukris R, Jaadane I, et al. Light-induced retinal damage using different light sources, protocols and rat strains reveals LED phototoxicity. Neuroscience. 2016; 339: 296-307.

3. Tsang SH, Sharma T. Drug-induced retinal toxicity. Atlas of Inherited Retinal Diseases. 2018: 227-232.

4. Duarte DA, Papadimitriou A, Gilbert RE, Thai K, Zhang Y, et al. Conditioned medium from early-outgrowth bone marrow cells is retinal protective in experimental model of diabetes. PLoS One. 2016; 11: e0147978.

5. Ezquer M, Urzua CA, Montecino S, Leal K, Conget P, et al. Intravitreal administration of multipotent mesenchymal stromal cells triggers a cytoprotective microenvironment in the retina of diabetic mice. Stem cell research & therapy. 2016; 7: 1-17.

6. Jin Z-B, Gao M-L, Deng W-L, Wu K-C, Sugita S, et al. Stemming retinal regeneration with pluripotent stem cells. Progress in retinal and eye research. 2019; 69: 38-56.

7. Kumar P, Kandoi S, Misra R, Vijayalakshmi S, Rajagopal K, et al. The mesenchymal stem cell secretome: a new paradigm towards cell-free therapeutic mode in regenerative medicine. Cytokine & growth factor reviews 2019; 46: 1-9.

8. Jha KA, Pentecost M, Lenin R, Klaic L, Elshaer SL, et al. Concentrated conditioned media from adipose tissue derived mesenchymal stem cells mitigates visual deficits and retinal inflammation following mild traumatic brain injury. International journal of molecular sciences. 2018; 19: 2016.

9. Öner A. Stem cell treatment in retinal diseases: recent developments. Turkish journal of ophthalmology. 2018; 48: 33.

10. Kolar MK, Itte VN, Kingham PJ, Novikov LN, Wiberg M, et al. The neurotrophic effects of different human dental mesenchymal stem cells. Scientific reports 2017; 7: 1-12.

11. Kang J, Fan W, Deng Q, He H, Huang F. Stem cells from the apical papilla: a promising source for stem cell-based therapy. BioMed research international. 2019; 2019.

12. Tachida Y, Sakurai H, Okutsu J, Suda K, Sugita R, et al. Proteomic comparison of the secreted factors of mesenchymal stem cells from bone marrow, adipose tissue and dental pulp. Journal of Proteomics & Bioinformatics. 2015; 8: 266.

13. Zhang M, Zhang F, Sun J, Sun Y, Xu L, et al. The condition medium of mesenchymal stem cells promotes proliferation, adhesion and neuronal differentiation of retinal progenitor cells. Neuroscience letters. 2017; 657: 62-68.

14. Yu S, Zhao Y, Ma Y, Ge L. Profiling the secretome of human stem cells from dental apical papilla. Stem cells and development. 2016; 25: 499-508.

15. Huang B, Liang J-J, Zhuang X, Chen S-W, Ng TK, et al. Intravitreal Injection of Hydrogen Peroxide Induces Acute Retinal Degeneration, Apoptosis, and Oxidative Stress in Mice. Oxidative Medicine and Cellular Longevity. 2018; 2018: 5489476.

16. Karamali F, Esfahani M-HN, Hajian M, Ejeian F, Satarian L, et al. Hepatocyte growth factor promotes the proliferation of human embryonic stem cell derived retinal pigment epithelial cells. Journal of Cellular Physiology. 2019; 234: 4256-4266.

17. Hookes L. Association for Research in Vision and Ophthalmology (ARVO)--2010 Annual Meeting. For Sight: The Future of Eye and Vision Research--part 1. IDrugs. 2010; 13: 427-429.

18. Reisenhofer MH, Balmer JM, Enzmann V. What Can Pharmacological Models of Retinal Degeneration Tell Us? Curr Mol Med. 2017; 17: 100-107.

19. Dinc E, Ayaz L, Kurt AH. Protective Effect of Combined Caffeic Acid Phenethyl Ester and Bevacizumab Against Hydrogen Peroxide-Induced Oxidative Stress in Human RPE Cells. Current Eye Research. 2017; 42:1659-1666.

20. Yeo RWY, Lai RC, Zhang B, Tan SS, Yin Y, et al. Mesenchymal stem cell: An efficient mass producer of exosomes for drug delivery. Advanced Drug Delivery Reviews 2013; 65: 336-341.

21. Sugitani S, Tsuruma K, Ohno Y, Kuse Y, Yamauchi M, et al. The potential neuroprotective effect of human adipose stem cells conditioned medium against light-induced retinal damage. Experimental Eye Research. 2013; 116: 254-264.

22. Mead B, Logan A, Berry M, Leadbeater W, Scheven BA. Paracrine-mediated neuroprotection and neuritogenesis of axotomised retinal ganglion cells by human dental pulp stem cells: comparison with human bone marrow and adipose-derived mesenchymal stem cells. PloS one. 2014; 9: e109305.

23. Alsaeedi HA, Koh AE-H, Lam C, Abd Rashid MB, Harun MHN, et al. Dental pulp stem cells therapy overcome photoreceptor cell death and protects the retina in a rat model of sodium iodate-induced retinal degeneration. Journal of Photochemistry and Photobiology B: Biology. 2019; 198: 111561.

24. De Almeida JFA, Chen P, Henry MA, Diogenes A. Stem cells of the apical papilla regulate trigeminal neurite outgrowth and targeting through a BDNF-dependent mechanism. Tissue Engineering Part A. 2014; 20: 3089-3100.

25. Li X-X, Yuan X-J, Zhai Y, Yu S, Jia R-X, et al. Treatment with Stem Cells from Human Exfoliated Deciduous Teeth and Their Derived Conditioned Medium Improves Retinal Visual Function and Delays the Degeneration of Photoreceptors. Stem cells and development. 2019; 28: 1514-1526.

26. Hadady H, Karamali F, Ejeian F, Soroushzadeh S, Nasr-Esfahani MH. Potential neuroprotective effect of stem cells from apical papilla derived extracellular vesicles enriched by lab-on-chip approach during retinal degeneration. Cellular and Molecular Life Sciences. 2022; 79: 350.