Effects of Fresh Bone Marrow Mononuclear Stem Cells in Rat Model of Retinopathy of Prematurity

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Abstract

Background: This study was conducted to investigate the effects of intravitreal injection of bone marrow mononuclear stem cells (BMMNC) on neurological and vascular damages in a rat model of retinopathy of prematurity (ROP).

Methods: Ten newborn Wistar rats were divided randomly into the control and the oxygen-induced retinopathy (OIR) group. Animals in the OIR group were incubated in an oxygen chamber to induce retinopathy. One eye of animals in the OIR group received BMMNC suspension (treated eyes), and the contralateral eye received the same volume of saline injection. Then, all animals underwent funduscopy, electroretinography, and histopathological assessments.

Results: Compared to the saline injection group, eyes treated with BMMNC had less vascular tortuosity while veins and arteries had relatively same caliber, as revealed by fundus examination. Eyes in treatment group showed significantly elevated photopic and scotopic B waves amplitude. In histopathological assessments, nuclei count in inner retinal layer in the treatment group was significantly lower compared to untreated eyes. Also, BMMNC transplantation reduced apoptosis of neural retina cells.

Conclusions: Our results indicated that intravitreal injection of BMMNC reduces neural and vascular damages and results in recovered retinal function in rat model of ROP. Ease of extraction without in vitro processing, besides the therapeutic effects of BMMNCs, make this source of stem cells as a new choice of therapy for ROP or other retinal ischemic diseases in further biomedical research.

Background

Retinopathy of prematurity (ROP) is a vasoproliferative disease that alters retinal vascular patterns in preterm neonates with immature retinal vasculature. The condition is widely known as a potent cause of Permanent blindness in preterm neonates, and according to reports, 20000 neonates become blind or severely visually impaired from the disease annually (1). ROP pathophysiology consists of two phases; first, vascular growth arrest due to hyperoxia state and then, hypoxia phase. Preterm parturition (absence of physiologic hypoxia) (2) and oxygen therapy with high oxygen concentrations induces hyperoxic conditions that suppress normal retinal vascularization, resulting in poor and inadequate vasculature (3). In the second phase, increased metabolic demand of the retina with poor vascularization initiates the hypoxic phase. Following the hypoxic conditions and inflammation (due to oxidative stress), the retina runs several pathways to preserve and supply oxygen for the hypoxic tissue(4). Eventually, excessive vascularization and inflammation cause vascular leakage and then retinal detachment.

Current treatments include; laser therapy, vitrectomy, anti-vascular endothelial growth factor (VEGF) medications, and direct ablation of abnormal vessels (5). These conventional treatment options have several disadvantages vascular abnormalities, regression of the disease, and long-term systemic disease in neonates treated with anti-VEGF drugs, which is accompanied by limitations in the therapy (6) (7). despite the advantages of laser ablation for ROP treatment, its complications, including the cornea and

iris burn, anterior segment hemorrhage, and mild to moderate inflammation, affect the outcome (8). A better understanding of the condition's pathophysiology yields treatment modalities with lower complications and success rates. Regenerative medicine approaches have demonstrated promising results in conditions with similar inflammatory and pathophysiologic basis, mainly through stem cells. However, current practices such as regenerative medicine demonstrated promising treatments, especially by applying stem cells to similar pathologic inflammatory conditions (9).

Stem cells used in animal studies of ROP include; bone marrow stem cells of the mouse (Lin- or CD44-rich myeloid progenitor cells) and human peripheral stem cells (eEPCs (CD31 + CD45+) (10). These studies showed that stem cells could migrate to avascular regions of the retina and promote vascular repair by inhibiting angiogenesis and making the retina more flexible to oxygen concentration changes due to stimulating the hypoxia-inducible factor (HIF)-alpha pathway. These cells can differentiate into microglial cells that can play immunomodulatory roles in ischemia and phagocyte tissue debris and help retinal regeneration (11). Studies show that the stem cell's engraftment to preexisting astrocyte templates promotes retinal vasculogenesis during retinal development. Many cells migrate into the retina's deep layers and develop a deep retinal vascular network (12).

Among different sources of stem cells, bone marrow-derived mononuclear cells (BMMNC) are a heterogeneous population of cells consisting of stem cells (hematopoietic stem cells, mesenchymal stem cells, and endothelial progenitor cells) and immune cells (B cells, T cells, monocytes). Studies have illustrated BMMNC's anti-inflammatory effects and showed that it reduces neurodegeneration defects in ischemic conditions (13). In addition, previous studies showed that BMMNC is beneficial in retinal diseases such as optic nerve crush, retinal dystrophy, and age-related macular degeneration (14–16). The Fresh BMMNC without in vitro processing is cost-beneficial and reduces the chance of cell contamination and differentiation risk, and is considered a time-saving process(17). Based on the evidence mentioned above, we hypothesized that fresh bone marrow mononuclear cells could reduce neural and vascular damage to the retina during ischemia in the rat model of Retinopathy of prematurity; this study was designed to test BMMNC application in the rat model of ROP.

Methods

Study Design

All animal procedures in this study are carried out under the national animal care guidelines and approved by local Ethics committee. Animals were kept in 12-hour light/dark cycles and ad libitum access to food and water. In this study, ten newborn Wistar rats were divided randomly into two groups, including; the control group (n = 5) and the oxygen-induced retinopathy (OIR) group (n = 5) (Fig. 1). Based on previous studies subjecting cellular therapy in ROP animal models, the intervention was done at the initiation of phase 2 (hypoxia) of ROP modeling on day 12 p. in animals in the OIR group, one eye received BMMNC suspension (treatment group), and the contralateral eye received the same volume of saline injection (vehicle injection group). At the age of 17, all animals underwent funduscopy,

electroretinography examinations were euthanized by Co_2 inhalation, and their eyes were harvested for histopathological assessments (18).

Rat model of Oxygen Induced Retinopathy (OIR)

In the current study, we used the conventional protocol of preparing an animal model of ROP described in detail by Smith *et a*l. (19). Briefly, seven-day-old Wistar rats with their nursing dams were incubated for five days in a hand-made isolate chamber with oxygen concentration adjusted to 70–75%. During the incubation period, animals were provided free access to standard food and water, and The Oxygen concentration was monitored continuously by using an oxygen indicator (CityCell. the UK). We placed a heated pad under the chamber to maintain the chamber's temperature at 25 ℃. On date 12 p, animals were returned to free room air (21% O_2) for five days (Fig. 1).

BMMNC extraction and Autologous Transplantation

In order to extract bone marrow mononuclear cells, we dissected long bones of one 8- month-old male rat after euthanasia by placing the anesthetized rat in a CO_2 chamber. Bone marrow was extracted from the dissected humerus, femur, and tibia from the donor rat with cold sterile Dulbecco's modified eagle medium: nutrient mixture F-12 (DMEMF-12) (Gibco, Thermofisher Scientific, MA) and then filtered through 70 µm cell strainer (BD Biosciences, CA). Then the suspension was centrifuged at 500 g for 5 minutes (Eppendorf, Germany) at room temperature. The supernatant was removed, and cells were resuspended in 5 ml of 1 X RBC Lysis Buffer (Thermofisher Scientific, MA) and incubated for 5 min at room temperature. The lysis reaction stopped by adding 20 ml of Phosphate-buffered saline (PBS), and then the solution was centrifuged again and removed the supernatant. The final solution was achieved by adding 1 ml sterile PBS to the cell pellet. The availability of bone marrow cells was approved by trypan blue staining, and then one syringe was filled with final suspension, ready for the injection procedure.

After general anesthesia with an intraperitoneal injection of ketamine/Xylazine and saline mixture (85/10 mg/kg), we made a small incision on the fissure between the eyelids with a lancet blade (NO 24) to separate them. After enough exposure to the eyes, appropriate ocular surface analgesia was induced by tetracaine eye drops (Anestocaine 0.5%). 10 µl of the BMMNC suspended in sterilized PBS, containing 1.2 x 10^5 cells, was slowly injected intravitreally with a 30-gauge needle to one eye of each neonate rat in the OIR group and equivolume of sterile PBS was injected into the contralateral eyes.

Fundus examination

We captured fundus photographs from control, ROP and treatment groups and compared retinal vasculature and optic disc properties to evaluate ischemic damage to the eyes. All animals on day 17 underwent fundus examination by a slit lamp 78 diopter lens. Vascular tortuosity, variation in veins and arteries caliber, no polygonal vascular pattern, and the hazy appearance of the fundus were considered retinal vascular damage. Optic disc ischemic damage was defined by; edema of the optical disc (papilledema) and; enlarged, pale, and noneven boundaries of the optic disk (20).To capture fundus

images, we used smartphone fundus photography. The pupil was dilated using mydriatic eye drop (atropine 0.5%). Examiner sat in a chair while holding a slit lamp 78 diopter lens in his hand, wearing a headlight. Proper alignment of the light source, lens, and animal's eye resulted in authentic aerial images recorded using a smartphone camera. This method captured fundus images containing well demonstrable optic disc and retina vasculature.

Electroretinography (ERG)

Full-field ERGs were recorded using the RETIanimal system (Roland Consult, Germany) and a Ganzfeld (Q 450 SC, Roland Consult, Germany). B and A waves amplitude were measured in both dark and adapted eyes. Briefly, neonatal rats in P17 were dark-adapted for 2 hours' and anesthetized by injection of ketamine/Xylazine and saline mixture (85/10 mg/kg). The pupils of rats were dilated with 1% atropine sulfate ophthalmic solution, and the mouse body temperature was maintained at 35°C using an electric heat pad. After sufficient anesthesia of the corneal surface with tetracaine 0.5% eye drop, 12mm Golden electrodes were placed on the cornea, skin, and tail.

ERG responses under dark-adapted conditions were evoked by 9 flashes ranging from 0. 01 Log cd.s/m^2 to 10 Log cd.s/m^2 in distinct low/high pass filters (Low-pass filter was 0.05 Hz and high-pass was 500 Hz). In order to prevent attenuating dark adaptation, flash series were shined from the lowest to the highest intensity. A and B waves were evaluated in scotopic and photopic states, respectively. The waveforms were averaged across each flash series, and the A-wave, and b-wave, were taken at 3 Log cd.s/m^2 intensity for A and B waves in photopic conditions. Moreover, A-wave, B-wave recorded in scotopic condition with different intensities (-2, 0, 1 Log cd.s/m^2 for B wave and 0, 1 Log cd.s/m^2 for A wave). The A-wave values were calculated as the absolute value of the minimum amplitude following the flash stimulus, while the B-waves amplitude was calculated from baseline voltage (0 µV) to the peak of the response. Recorded waves were automatically analyzed, and waves' amplitude was measured by RETIanimal software.

Histopathological Assessment

We approved death by the absence of heartbeat in all animals euthanatized in p17 by an overdose of ketamine injection. Both animals' eyes were enucleated and immediately fixed in 10% neutral-buffered formalin with the fixative solution, which was replaced every two days until the tissues hardened. In addition, each eye was embedded in a paraffin block, and five µm sections parallel to the sagittal axis of the optic nerve were prepared and stained with hematoxylin and eosin (H&E). According to Wilkinson-Berka *et al.* Study (21), to score neovascular nuclei in the internal limiting surface of the retina, Slides were examined qualitatively in 40X magnification under a light microscope (Nikon. Japan). Endothelial cells or blood vessels were counted with full lumen, retina layers' thickness (inner nuclear layer, outer nuclear layer, and ganglion cell layer), apoptosis, and organization of layers were compared Qualitatively (22).

Statistics

We used GraphPad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California, USA) for statistical analysis and graphing. Kolmogorov–Smirnov test was used to confirm the normal distribution of data, and differences between groups were calculated using Anova and T-test. P values less than 0.05 were considered to indicate statistical significance. All values were expressed as the mean ± SEM.

Results

Establishment of ROP Rat models

Fundus examination in the control group revealed radial straight vascular branches with no tortuosity. Also, Veins and arteries were unicaliber (Fig. 2.A). However, fundus examination in the ROP group showed abnormal vessel architecture, distorted distribution and tortuosity, non-polygonal structure, and different veins and artery caliber. In addition, optic disc injury was indicated by papilledema, pale and noneven optic disc boundaries (Fig. 2.B).

In ROP group, ERG data indicate significantly weak B waves, with much more difference in lower intensities, in both scotopic ($P \leq 0.05$ in 0 Log cd.s/m^2 and $P \leq 0.01$ in -2 Log cd.s/m^2 intensity) and photopic ($P \leq 0.01$ in + 3 Log cd.s/m^2 intensity) conditions, comparison to with control group (Fig. 3.A_C). In addition, there is a considerable significant difference in photopic A waves between the two groups ($P \leq 0.0001$) (Fig. 3.D). Although, no significant difference was reported between control and ROP groups in scotopic A waves (Fig. 3.E).

Histopathological results in the control group showed that the retina's internal limiting surface was clear, with well-organized retinal layers (intact outer plexiform layer and outer nuclear layer). The neovascular nucleus was rarely seen (Fig. 4.A). On the other hand, the retina in the ROP group was disorganized in layers and had an uneven internal limiting surface of the retina. Besides, extensive hydropic degeneration and apoptosis were seen in the inner nuclear layer (Fig. 4.B). It is notable that in the ROP group, the neovascular nuclei count was significantly higher compared with the control group ($P \leq 0.005$) (Fig. 3.F).

Evaluation of BMMNC transplantation effects on ROP-affected retina

Fundus examination

Fundus examination in the ROP group showed notable disturbed vascular tortuosity with engorged veins and arteries with a different caliber and completely non-polygonal. We also detected papilledema, noneven boundaries, and an enlarged pale appearance of the optic disc (Fig. 2.B).

On the other hand, fundus images from BMMNC-treated eyes show more vessel branches than untreated ROP eyes (vehicle injection). The number of Engorged vessels in BMMNC-treated eyes is much lesser than in untreated ROP (vehicle injection), and they have the same caliber. Vascular radial branches were relatively straight and had a polygonal appearance. No papilledema was seen in the treatment group, and the appearance of the optic disc was normal (Fig. 2.C).

Electroretinography

BMMNC treatment elevated response to stimuli of ischemia-affected retinas versus vehicle injection in rat puppies (Fig. 3.A). In the BMMNC treatment group, B waves amplitudes significantly increased in -2 Log cd.s/m^2 and + 3 Log cd.s/m^2 Intensities compared to the vehicle injection group in scotopic and photopic

conditions ($P \leq 0.05$) (Fig. 3.B, C). Although the in-vehicle injection group average of A waves amplitude was reported to be lower compared to BMMNC treated group, there is a more significant decrease in A wave amplitude in-vehicle injection group versus BMMNC treatment in photopic ERGs. However, both groups reported no significant difference among A waves (Fig. 3.D, E).

Histopathology

Inner and outer nuclear layer cell apoptosis and hydropic degenerations were relatively lower in BMMNC-treated eyes than in the vehicle-injected group. In the BMMNC treatment group, retinal layers remained organized and were not dissociated like retinal layers in the untreated ROP group (Fig. 3.B, C). Also, neovascular nuclei in the internal limiting surface of the retina were significantly lower in number ($P \leq 0.05$) compared to the vehicle injection group (Fig. 3.F).

Discussion

In this study, we examined the hypothesis of whether intravitreal injection of BMMNC could reduce neurological and vascular damage in retinal ischemia in an animal model of ROP. Compared to the vehicle injection group, Fundus examinations in BMMNC treated eyes revealed less vascular tortuosity, relatively same veins, and arteries caliber with a polygonal vasculature pattern. Moreover, optic disc appearance improved after the treatment, and no papilledema was seen in that group. Additionally, in the BMMNC treatment group, optic disc boundaries were even in fundus exams (Fig. 2). BMMNC transplantation in the animal model of ROP could significantly improve response to visual stimuli in the neural retina, as indicated by elevated dark-adapted photopic and scotopic B waves amplitude (Fig. 3.A-D). As shown in Fig. 3.F, nuclei cont. in the vehicle injection and BMMNC treatment groups revealed that BMMNC transplantation could significantly suppress neovascularization in the rat model of ROP. Also, histopathological findings showed that BMMNC transplantation to ROP-affected eyes could reduce apoptosis of neural retina cells.

In recent years, scientists have been attracted to the different linage of bone marrow cells due to the high expression of the HIF 1-alpha gene and its mentioned profits in ROP treatment (Fig. 5), (23). In 2006, Ritter *et al.* used myeloid progenitor cells in a murine OIR model, revealing those cells differentiated into microglia and promoted vascular repair (11). Another study by Zhao *et al.* shows that Bone marrow mesenchymal cells reduced apoptosis in a rat model of Retinopathy of prematurity (24). In 2019 Li Calzi *et al.* used *a* combination of human progenitor cells, including bone marrow-derived CD34 + cells and vascular wall–derived endothelial colony-forming cells (ECFCs), to treat the murine OIR model. He suggested that intravitreal injection of the cell combination may provide some protection to the neural

retina (25). In addition, some of the studies used modified bone marrow stem cells to enhance their therapeutic effect. For example, Qian Ma *et al.* used bone marrow mesenchymal stem cells modified by angiogenin-1 in the mice model of ROP and revealed that modified cells could reduce neovasculogenesis, and protect retinal vasculature during the disease (26). As described previously, many of those cell lines demonstrated a therapeutic role in an animal model of OIR. Its expected BMMNC treatment have all therapeutic characteristic of Its cellular constituent or even synergically protect the developing retinal vasculature and neural retina in an animal model of retinal disease.

Interestingly, successful studies reported that intravitreal administration of whole BMMNC could benefit retinal injuries. In 2011 and 2014, Zaverucha-do-Valle *et al.* used the intravitreal injection of the entire BMMNC fraction containing all its cell subtypes for a rat model of optic nerve crush. They reported that bone marrow mononuclear cell therapy improves axonal outgrowth and increases retinal ganglion cell survival (27, 28). In The other attempt to use whole BMMNC for retinal disease, Di Pierdomenico *et al.* reported that intravitreal and subretinal injection BMMNCs, improve photoreceptor survival in a rat model of retinal degeneration (29).

A potential explanation for retinal function recovering after this treatment is the reservation of cellular origins b waves (rods, cones, and bipolar cells) in the retina from cellular death. As we illustrated in histopathological results, hydropic degeneration and cell death in the neural retinal layer were reduced in the treatment group, and BMMNC saved the population of cells. Scotopic ERGs show rod-originated signals recovered after the treatment; photopic ERGs with the saturation of rod photoreceptors revealed that cone-originated b waves also recovered. BMMNC treatment recovers retinal function by saving cone or rod photoreceptors in this disease. Our study confirms Di Pierdomenico *et al*. results, which used BMMNC for retinal degeneration, except that Di Pierdomenico *et al.* reported no significant improvement in ERG signals after BMMNC treatment. It seems that conflicting results come from different pathological conditions between two diseases. In the rat model of ROP, when BMMNC was applied, retinal neural cells (rod, cone, and bipolar cells) were not destroyed and injected BMMNC just saved photoreceptor cells from further ischemic damage. At the same time, in Di Pierdomenico *et* al. research, affected photoreceptors got lost, and injected BMMNC did not penetrate the retina and thus could not replace the lost photoreceptors' function he mentioned (30). Reservation of optic disc degeneration and vasoprotective effects of BMMNC treatment might be due to two main reasons, first, as Zaverucha-do-Valle *et al.* showed, BMMNCs transplantation promotes neuroregeneration in the damaged optic nerve, and second, BMMNC containing cells protect neural retina forming cells (MSCs and HMSCs) from ischemic injury by several mechanisms including; downregulating proinflammatory cytokines, secretion of anti-inflammatory substance, upregulating anti-apoptotic gens, rebalancing stimulators and inhibitors (VEGF, PEDF) (18, 31).

We assessed the effects of BMMNC therapy in several aspects (neural-vascular) that improve comprehensive sight in such a complex situation. As a study limitation, this research did not evaluate retinal vasculature quantitively. It cannot make a definitive claim about BMNNC treatment benefits on retinal vasculature during ROP progression, but it can be confirmed based on qualitative fundus exams.

Another limitation of this study includes a non-quantitative evaluation of apoptosis of retinal cells. Also, our evaluation shows that BMMNC administration could reduce apoptosis in the neural retina, but more accurate tests should be performed.

For further studies, we suggest quantitative analyses of retinal vasculature methods, including Isolectin staining of the flat-mounted retina for more accurate vascular assessments. Also, quantitative evaluation methods of apoptosis-like TUNEL immunoassay are better to be performed. We have no definitive information on the underlying mechanism of properties of BMMNCs, and more studies should be performed focused on specific gene expression (VEGF) to elucidate the protective mechanism of application of whole BMMNCs in retinal ischemia.

Conclusions

Nowadays, only a few treatments can prevent or recover neural and vascular retina from retinal degenerative and vasproliferating diseases. However, bone marrow cell transplantation-based treatments show promising effects in such conditions. BMMNCs have more availability and feasibility without time-consuming and expensive procedures for sorting specific cell lines. Also, intravitreal injection of BMMNCs represented an acceptable safety profile and was used in one clinical trial, and no detectable structural or functional toxicity was reported over ten months (32). In conclusion, as the first research in fresh BMNNC application in a rat model of Retinopathy of prematurity, this study confirmed that intravitreal injection of BMMNC could reduce neural and vascular damage and recover retinal function during the disease. BMMNCs therapy could be considered a new treatment choice for ROP or another retinal ischemic disease in further biomedical research.

Abbreviations

ROP: retinopathy of prematurity

OIR: oxygen induced retinopathy

HIF: hypoxia inducible factor

VEGF: vascular endothelial growth factor

BMMNC: bone marrow mononuclear cell

ERG: electroretinography

Declarations

Ethics approval and consent to participate: All animal procedures in this study are carried out under the national animal care guidelines and approved by the Ethics committee of the children's medical center, Tehran, Iran.

Availability of data and materials: All data generated or analysed during this study are included in this published article.

Competing interests: All authors declare that they do not have any competing intereset.

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Authors' contributions: Saman Behboodi Tanourlouee: Methodology, writing the proposal, Study design, Study performance (Animal, Cellular, udusphotography, ERG and Histopathology), writing the manuscript. Masoumeh Majidi Zolbin: Methodology, Study performance(Cellular), supervision, approval of the final manuscript. Ashkan Azimzadeh: Study performance (Animal, Cellular), Methodology. Javad Fahanik Babaei: Study performance (ERG), approval of the final manuscript Masoud Bitaraf: Study performance (Animal, Cellular), writing the manuscript, approval of the final manuscript. Kayvan Mirnia: Conceptualization, Study design, Methodology, approval of the final manuscript, supervision. Abdol-Mohammad Kajbafzadeh: Conceptualization, approval of the final manuscript, supervision

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References

1. Blencowe H, Lawn JE, Vazquez T, Fielder A, Gilbert C. Preterm-associated visual impairment and estimates of retinopathy of prematurity at regional and global levels for 2010. Pediatr Res. 2013;74(Suppl 1(Suppl 1):35–49.
2. Alon T, Hemo I, Itin A, Pe'er J, Stone J, Keshet E. Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. Nat Med. 1995;1(10):1024–8.
3. Hakeem AHAA, Mohamed GB, Othman MF. Retinopathy of prematurity: a study of prevalence and risk factors. Middle East Afr J Ophthalmol. 2012;19(3):289–94.
4. Rivera JC, Holm M, Austeng D, Morken TS, Zhou T, Beaudry-Richard A, et al. Retinopathy of prematurity: inflammation, choroidal degeneration, and novel promising therapeutic strategies. J Neuroinflamm. 2017;14(1):165.
5. Mutlu FM, Sarici SU. Treatment of retinopathy of prematurity: a review of conventional and promising new therapeutic options. Int J Ophthalmol. 2013;6(2):228–36.
6. Quinn GE, Darlow BA. Concerns for Development After Bevacizumab Treatment of ROP. Pediatrics. 2016;137(4).
7. Gotz-Więckowska A, Chmielarz-Czarnocińska A, Pawlak M, Gadzinowski J, Mazela J. Ranibizumab after laser photocoagulation failure in retinopathy of prematurity (ROP) treatment. Sci Rep.

2017;7(1):11894.

8. Kaiser RS, Trese MT. Iris atrophy, cataracts, and hypotony following peripheral ablation for threshold retinopathy of prematurity. Archives of ophthalmology (Chicago, Ill: 1960). 2001;119(4):615-7.
9. Adak S, Magdalene D, Deshmukh S, Das D, Jaganathan BG. A Review on Mesenchymal Stem Cells for Treatment of Retinal Diseases. Stem cell reviews and reports. 2021;17(4):1154–73.
10. Medina RJ, O'Neill CL, Humphreys MW, Gardiner TA, Stitt AW. Outgrowth Endothelial Cells: Characterization and Their Potential for Reversing Ischemic Retinopathy. Investig Ophthalmol Vis Sci. 2010;51(11):5906–13.
11. Ritter MR, Banin E, Moreno SK, Aguilar E, Dorrell MI, Friedlander M. Myeloid progenitors differentiate into microglia and promote vascular repair in a model of ischemic retinopathy. J Clin Invest. 2006;116(12):3266–76.
12. Otani A, Kinder K, Ewalt K, Otero FJ, Schimmel P, Friedlander M. Bone marrow-derived stem cells target retinal astrocytes and can promote or inhibit retinal angiogenesis. Nat Med. 2002;8(9):1004–10.
13. Giraldi-Guimardes A, Rezende-Lima M, Bruno FP, Mendez-Otero R. Treatment with bone marrow mononuclear cells induces functional recovery and decreases neurodegeneration after sensorimotor cortical ischemia in rats. Brain Res. 2009;1266:108–20.
14. Zaverucha-do-Valle C, Mesentier-Louro L, Gubert F, Mortari N, Padilha AB, Paredes BD, et al. Sustained effect of bone marrow mononuclear cell therapy in axonal regeneration in a model of optic nerve crush. 2014;1587:54–68.
15. Siqueira RC, Messias A, Voltarelli JC, Scott IU, Jorge RJR. Intravitreal injection of autologous bone marrow–derived mononuclear cells for hereditary retinal dystrophy: a phase I trial. 2011;31(6):1207–14.
16. Cotrim CC, Toscano L, Messias A, Jorge R, Siqueira RCJCO. Intravitreal use of bone marrow mononuclear fraction containing CD34 + stem cells in patients with atrophic age-related macular degeneration. 2017;11:931.
17. Du F, Wang Q, Ouyang L, Wu H, Yang Z, Fu X, et al. Comparison of concentrated fresh mononuclear cells and cultured mesenchymal stem cells from bone marrow for bone regeneration. Stem Cells Transl Med. 2021;10(4):598–609.
18. Xu W, Cheng W, Cui X, Xu G. Therapeutic effect against retinal neovascularization in a mouse model of oxygen-induced retinopathy: bone marrow-derived mesenchymal stem cells versus Conbercept. BMC Ophthalmol. 2020;20(1):7.
19. Smith LE, Wesolowski E, McLellan A, Kostyk SK, D'Amato R, Sullivan R, et al. Oxygen-induced retinopathy in the mouse. Investig Ophthalmol Vis Sci. 1994;35(1):101–11.
20. Levkova EA, Lugovskoy S, Peresypkina A, Bashuk V. Correction of retinal ischemic injuries by using non-selective imidazoline receptor agonists in the experiment. 2019.
21. Wilkinson-Berka JL, Alousis NS, Kelly DJ, Gilbert RE. COX-2 Inhibition and Retinal Angiogenesis in a Mouse Model of Retinopathy of Prematurity. Investig Ophthalmol Vis Sci. 2003;44(3):974–9.

22. Mezu-Ndubuisi OJ, Macke EL, Kalavacherla R, Nwaba AA, Suscha A, Zaitoun IS, et al. Long-term evaluation of retinal morphology and function in a mouse model of oxygen-induced retinopathy. Mol Vis. 2020;26:257–76.
23. Rivera JC, Holm M, Austeng D, Morken TS, Zhou TE, Beaudry-Richard A, et al. Retinopathy of prematurity: inflammation, choroidal degeneration, and novel promising therapeutic strategies. 2017;14(1):1–14.
24. Zhao Y-S, Zhao K-X, Wang X-L, Chen Y-X, Wang L, Mu Q-J. [Effects of bone marrow mesenchymal stem cell transplantation on retinal cell apoptosis in premature rats with retinopathy]. Zhongguo Dang Dai Er Ke Za Zhi. 2012;14(12):971–5.
25. Li Calzi S, Shaw LC, Moldovan L, Shelley WC, Qi X, Racette L, et al. Progenitor cell combination normalizes retinal vascular development in the oxygen-induced retinopathy (OIR) model. JCI Insight. 2019;4(21):e129224.
26. Ma QQ, Liu FY, Shi M, Sun CH, Tan Z, Chang XD, et al. Bone marrow mesenchymal stem cells modified by angiogenin-1 promotes tissue repair in mice with oxygen-induced retinopathy of prematurity by promoting retinal stem cell proliferation and differentiation. J Cell Physiol. 2019;234(11):21027–38.
27. Zaverucha-do-Valle C, Gubert F, Bargas-Rega M, Coronel JL, Mesentier-Louro LA, Mencalha A, et al. Bone marrow mononuclear cells increase retinal ganglion cell survival and axon regeneration in the adult rat. Cell Transpl. 2011;20(3):391–406.
28. Zaverucha-do-Valle C, Mesentier-Louro L, Gubert F, Mortari N, Padilha AB, Paredes D. B, et al. Sustained effect of bone marrow mononuclear cell therapy in axonal regeneration in a model of optic nerve crush. Brain Res. 2014;1587:54–68.
29. Di Pierdomenico J, Gallego-Ortega A, Martínez-Vacas A, García-Bernal D, Vidal-Sanz M, Villegas-Pérez MP, et al. Intravitreal and subretinal syngeneic bone marrow mononuclear stem cell transplantation improves photoreceptor survival but does not ameliorate retinal function in two rat models of retinal degeneration.n/a(n/a).
30. Di Pierdomenico J, Gallego-Ortega A, Martínez-Vacas A, García-Bernal D, Vidal-Sanz M, Villegas-Pérez MP, et al. Intravitreal and subretinal syngeneic bone marrow mononuclear stem cell transplantation improves photoreceptor survival but does not ameliorate retinal function in two rat models of retinal degeneration. Acta Ophthalmologica. 2022;n/a(n/a).
31. Mathew B, Poston JN, Dreixler JC, Torres L, Lopez J, Zelkha R, et al. Bone-marrow mesenchymal stem-cell administration significantly improves outcome after retinal ischemia in rats. Graefe's Archive for Clinical and Experimental Ophthalmology. 2017;255(8):1581–92.
32. Singh MS, Park SS, Albini TA, Canto-Soler MV, Klassen H, MacLaren RE, et al. Retinal stem cell transplantation: Balancing safety and potential. Prog Retin Eye Res. 2020;75:100779.

Figures

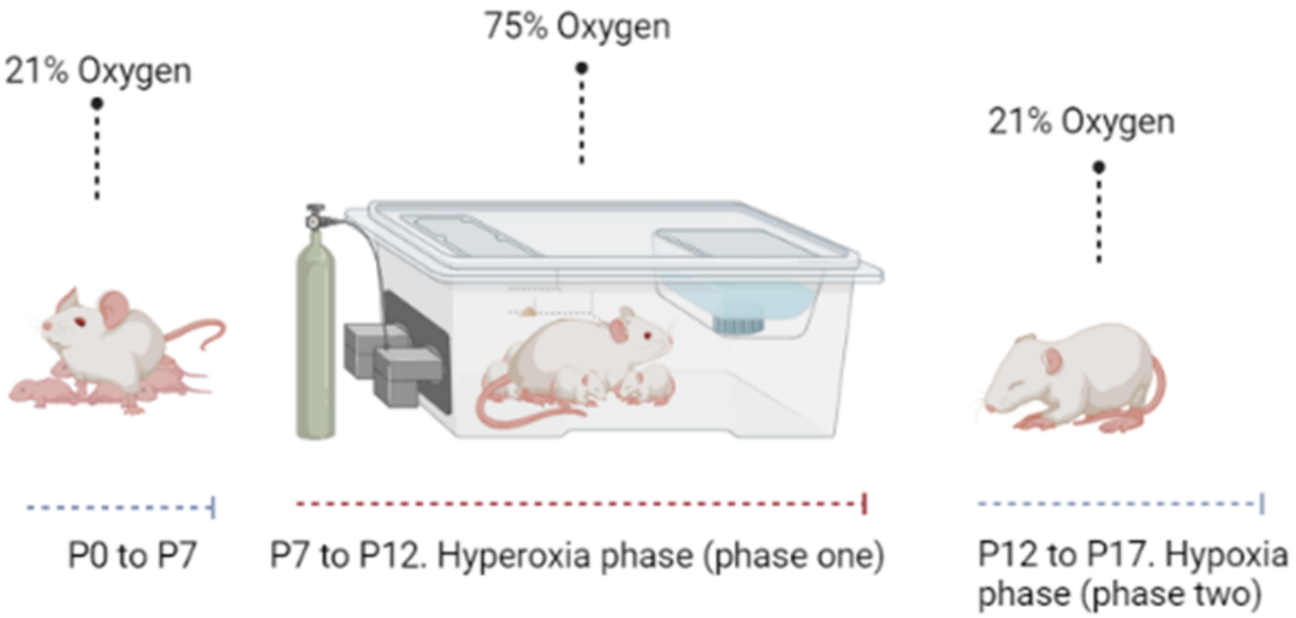

Figure 1

oxygen Induced Retinopathy procedure. Created with BioRender.com

Figure 2

fundus photography. A; Control, B; Vehicle injection, C; BMMNC treatment. A: intact retina with straight radial branches without tortuosity and with a polygonal shape. Note the same caliber of veins and arteries and normal appearance of the optic disc with no edema and well even boundaries. B: ischemic retina with engorged vessels and vascular tortuosity(flesh). Veins and arteries do not have the same caliber ad there is no polygonal appearance in vascular structure. Note papilledema(star) enlarged and pale optic disc with no even boundaries which indicates optic disc injury. C: there are more vessels that are detectable in the treatment group retina, veins and arteries are relatively unicaliber, vascular tortuosity is detectable but radial branches are relatively straight and make a polygonal shape. The optic nerve is normal, there is no enlargement and papilledema detected and appearance of the optic nerve is pink with even boundaries.

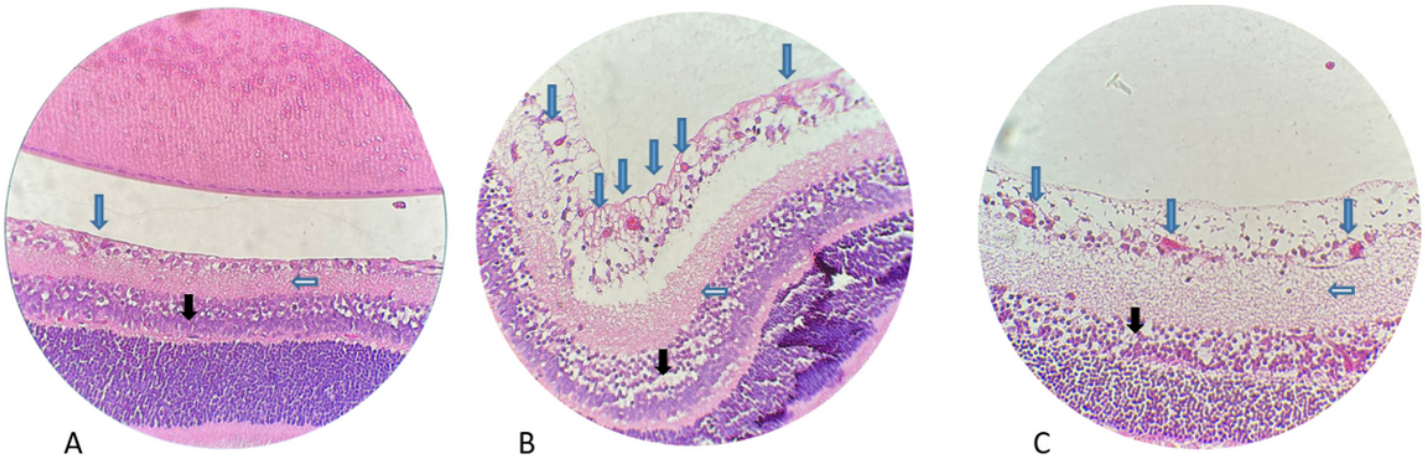

Figure 3

A; Simultaneously electroretinography waves of BMMNC treatment (right eye) and vehicle injection (left eye) of a rat. Note that high b wave amplitude in BMMNC treatment versus vehicle injection. B; an average scotopic B wave amplitude in BMMNC, vehicle injection, and control in three ascending intensities. There are significant differences between vehicle injection and BMMNC treatment groups in -2 log cd·s·m-2 intensity and also between the control group and vehicle injection in 0 log cd·s·m-2 intensity. There is no significant difference among groups in +1 log cd·s·m-2 intensity. C; Average photopic B Wave amplitude in BMMNC, Vehicle injection, and control groups in +3 log cd·s·m-2 intensity. The significant difference is detectable among BMMNC treatment and vehicle injection groups. Also, there is a notable difference between vehicle injection and control groups. D; Average photopic B Wave amplitude in BMMNC, Vehicle injection, and control groups in +3 log cd·s·m-2 intensity. The significant difference is detectable among BMMNC treatment and vehicle injection groups. Also, there is a notable difference between vehicle injection and control groups. E; average scotopic A Wave amplitude in BMMNC treatment, Vehicle injection, and control groups in 0 and +1 log cd·s·m-2 intensity. There is no Significant among groups. F; neovascular nuclei cont. on internal retinal surface in 5 eyeballs of each group. (*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.005$, ****: $P \leq 0.0001$).

Figure 4

Histopathology of retina in three groups, A; Control, B; Vehicle Injection, C; BMMNC Treatment (H&E. X40). A: normal vasculature of outer retina (blue arrow), intact outer plexiform layer and outer nuclear layer (white and black arrows) note the well-organized layers. B: numerus neovasculogenesis spots and engorged vessels (blue arrows), dissociated inerplexiform layer (white arrow) and extensive hydropic degeneration and apoptosis in innnernuclear layer (black arrow), note that disorganization of layers. C: neovasvular spots (blue arrows), organized inerplexiform layer (white arrows), hydropic degeneration and apoptosis in innnernuclear layers (black arrows), note the organization of layers.

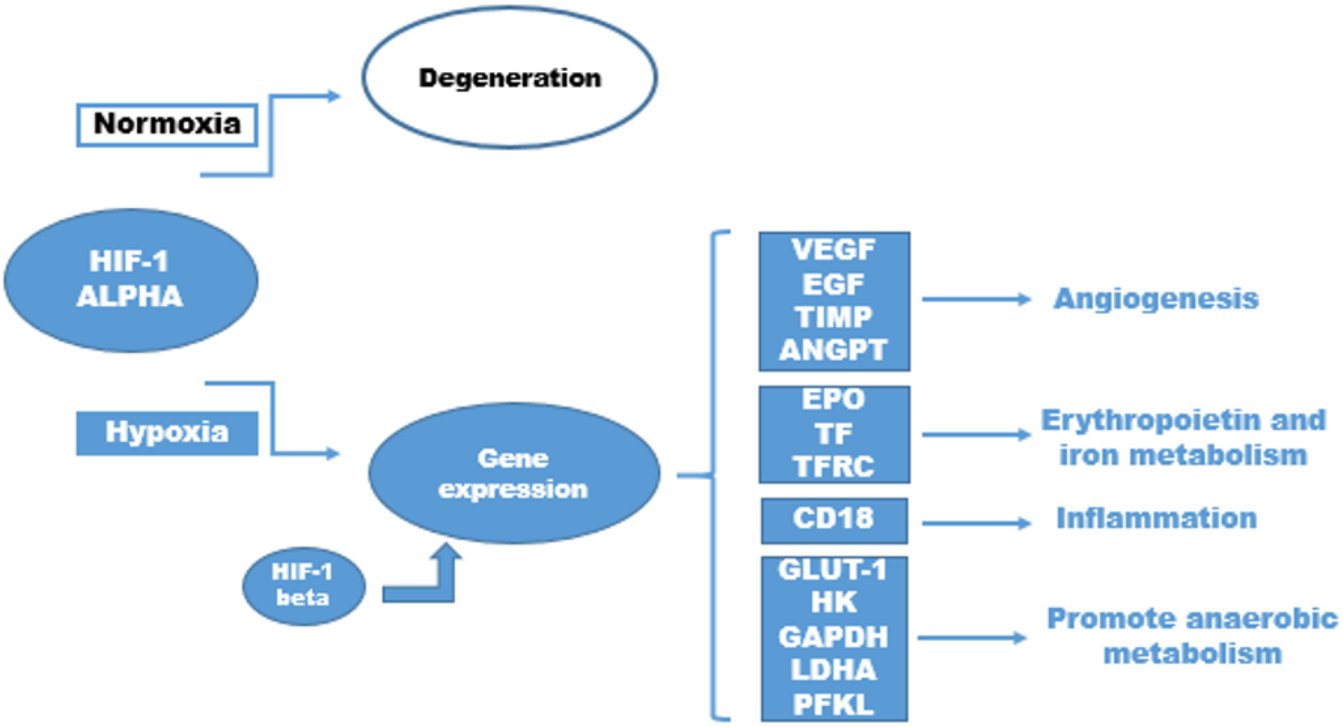

Figure 5

HIF-1alpha pathway Overview. HIF: Hypoxia-inducible factor. VEGF: Vascular endothelial growth factor. EGF: Epidermal growth factor. TIMP: Tissue inhibitor of metalloproteinase. ANGPT: Angiopoietin. EPO: erythropoietin. TF: Transferrin. TFRC: Transferrin receptor. GLUT-1: Facilitated glucose transporter. HK: Hexokinase. Glyceraldehyde 3-phosphate dehydrogenase. LDHA: L-lactate dehydrogenase. PFKL: 6-phosphofructokinase 1