Int Ophthalmol (2019) 39:2757–2766
https://doi.org/10.1007/s10792-019-01120-w

ORIGINAL PAPER

Effect of intracameral human cord blood-derived stem cells on lasered rabbit trabecular meshwork

Ramanjit Sihota · Seema Sen · Sujata Mohanty · Mohammad Ahmad · Alok Ravi · Viney Gupta · Neerja Bhatla

Received: 1 May 2018 / Accepted: 22 May 2019 / Published online: 28 May 2019
© Springer Nature B.V. 2019

Abstract

Background This study aimed to investigate the effect of intracameral human cord blood stem cells on lasered rabbit trabecular meshwork.

Methods Immediately following diode laser application to the trabecular meshwork, human cord blood stem cells were injected intracamerally, in one eye of 12 albino rabbits. The other eye of ten rabbits was lasered controls and two eyes were normal controls. Rabbits were killed after 4, 8 and 12 weeks.

Results Lasered control rabbit eyes showed significant disruption of trabecular architecture, loss and pleomorphism of trabecular endothelial cells and progressive narrowing of trabecular spaces till 12 weeks. In contrast, lasered eyes, concurrently injected with human cord blood stem cells, showed relatively preserved endothelial cellularity and structure of the trabecular meshwork, at all time points. Human CD34- and CD44-positive cells were identified in 7/8 eyes treated with stem cells, at 4 and 8 weeks, and 2 of 3 at 12 weeks. Many PKH26-labeled human cord blood cells were visible throughout the trabecular area at 4 weeks. They gradually decreased in number by 8 weeks, and at 12 weeks, they appeared to be oriented along trabecular beams.

Conclusions There was a relative preservation of cellularity and architecture of the trabecular meshwork in eyes injected with human cord blood stem cells, as compared to lasered control eyes up to 12 weeks, without significant inflammation. This suggests a probable role for such stem cells in eyes with glaucoma, having trabecular dysfunction.

Keywords Intracameral cord blood cells · Trabecular meshwork · Stem cells

R. Sihota (✉) · A. Ravi · V. Gupta
Glaucoma Research Facility and Clinical Services, Dr Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi 110029, India
e-mail: rjsihota@hotmail.com

S. Sen · M. Ahmad
Ocular Pathology, All India Institute of Medical Sciences, New Delhi 110029, India

S. Mohanty
Stem Cell Facility, All India Institute of Medical Sciences, New Delhi 110029, India

N. Bhatla
Obstetrics and Gynaecology, All India Institute of Medical Sciences, New Delhi 110029, India

Introduction

Glaucoma is one of the leading causes of irreversible blindness in the world, with raised intraocular pressure being a major risk factor [1]. Currently, lowering intraocular pressure, IOP, is the only possible therapy, either by medications which require lifelong

compliance of the patient, or surgery that has risks of complications and failure [2].

Trabecular meshwork (TM) histopathology in glaucomatous eyes has shown a significant loss of trabecular endothelial cells, thickening and fusion of trabecular beams, and a consequent narrowing/loss of spaces through which aqueous may flow [3–7]. The trabecular meshwork is known to be a major site of aqueous outflow resistance.

Regenerative therapy to repopulate the trabecular meshwork with appropriate cells to restore function would help reduce intraocular pressure and preserve vision in glaucoma patients. Mesenchymal stem cells are thought to work through cellular differentiation as well as paracrine signaling to reestablish function. Efforts to repair or replace TM endothelial cells could prevent/correct or delay outflow dysfunction and thereby glaucomatous optic neuropathy; however, there are few such studies that have studied the induction of TM-like cells and their effect on ex vivo perfused human outflow pathway organ culture [8, 9]. Other studies have used fibroblast-derived pluripotent stem cells co-cultured with TM cells and have shown the cells to develop a phenotype similar to TM cells [10]. Zhu et al. [11] injected such induced pluripotent stem cells and fibroblasts into a mouse model of glaucoma and were able to show that these cells survived for 9 weeks at the TM and caused a proliferative response of endogenous TM endothelium. Roubeix et al. [12] injected bone marrow-derived mesenchymal stem cells into the anterior chamber of a glaucoma model of cauterized episcleral vein rat eyes. They demonstrated the stem cells on the TM and iridocorneal angle, with a fall in IOP of about 5 mmHg recorded in hypertensive eyes but not in controls. However, Roubeix et al. found that the stem cells were undetectable after 4 days. Snider et al. [13] used magnetic nanoparticles to direct injected pluripotent mesenchymal stem cells toward the entire TM, allowing targeted delivery. Thus, it is possible that stem cells delivered to the anterior chamber would be able to reach the trabecular meshwork and regenerate this tissue.

These studies have used animal models of glaucoma, genetically modified or by cauterization of episcleral vessels, where the human glaucoma trabecular endothelial cell loss/damage of trabecular endothelial cells is not replicated. The use of diode laser to the TM has been shown to cause damage and loss of TM cells by Levkovitch-Verbin et al.[14], and additionally, this would encourage the injected stem cells to 'home' to the TM site of inflammation, damage with associated cytokine release.

This study was therefore designed to evaluate the presence, persistence and effect of intracamerally injected human cord blood-derived stem cells, on the lasered TM of rabbits in the medium term.

Methods

The study was carried out on healthy albino rabbits, after clearance from our Institutional Animal Ethics and Stem Cell Ethics Committees. The animals were housed in pathogen-free conditions and treated as per the ARVO statement on the use of animals in ophthalmic and vision research.

Twelve albino, New Zealand rabbits underwent a thorough clinical examination including intraocular pressure evaluation, to exclude any prior ocular or systemic pathology.

Stem cell isolation

Cord blood from volunteers, who had given informed consent, was processed at our Stem Cell Facility. Cord blood mononuclear cells (MNC) were separated by Ficoll density separation method. Briefly, cord blood diluted with normal saline was layered over lymphocyte separation medium (Bio-Whittaker) and centrifuged at 800 g for 25 min. MNC were aspirated and washed thrice in heparinized normal saline, so as to remove any traces of Ficoll. Cell numbers were assessed by counting cells in a Neubauer chamber under a microscope. The viability of mononuclear cells was evaluated by trypan blue dye exclusion test. Cell morphology was observed under a microscope after staining with Giemsa stain. Hematopoietic stem cell marker CD 34 was used to enumerate the absolute CD 34+ cells by flow cytometer (Fig. 1) as per the ISHAGE protocol, International Society of Hematotherapy and Graft Engineering [15].

Cord blood MNC were cryopreserved in liquid nitrogen at − 196 °C. The cryopreservation mixture contained 10% of dimethyl sulfoxide (DMSO), (Sigma-Aldrich, Merck KGaA, Germany) and 40% of human serum albumin (Reliance Life Science,

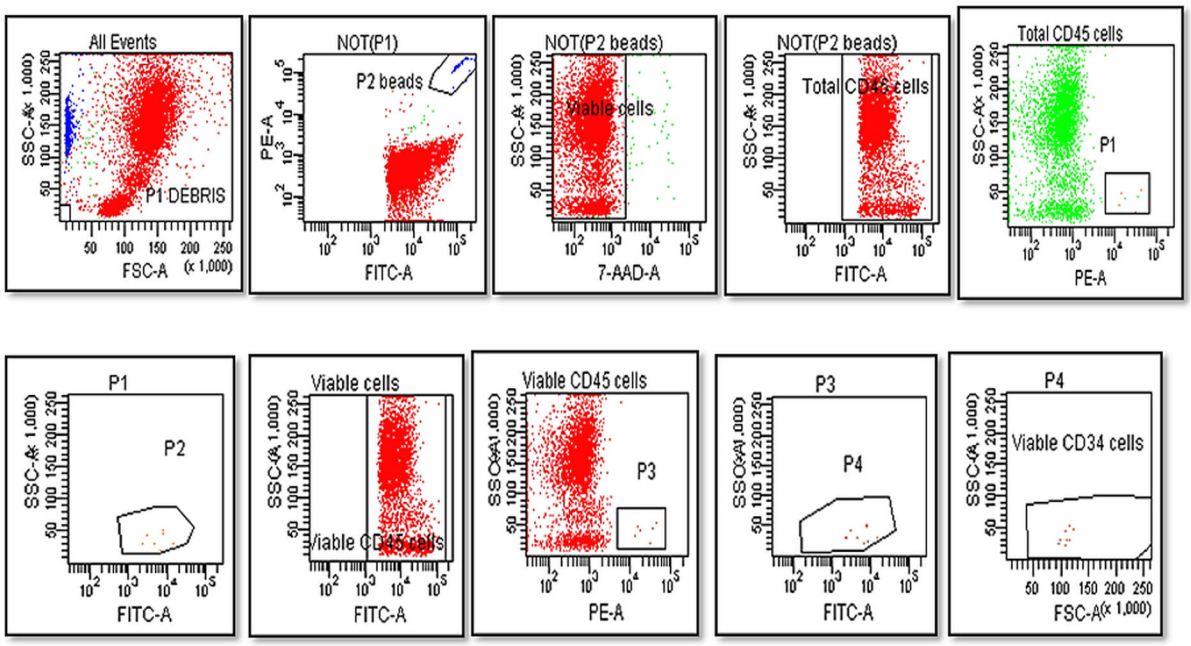

Fig. 1 Flowcytometry graph depicting the enumeration of CD 34+ cells from cord blood MNC. CD 34+ cell = 0.07%

India). Mononuclear cells were thawed and washed to remove traces of DMSO on the day of injection.

To better identify and track the transplanted stem cells in six rabbits, partial cord blood MNCs were stained with PKH-26 stain, as per manufacturer's instructions. Briefly, the cord blood MNCs were washed with Dulbecco's PBS by centrifugation at 400 g for 5 min at room temperature (RT). The pellet was suspended in 1 ml Diluent C with gentle pipetting, and an equal volume of 2× dye solution was added and immediately mixed by pipetting. This was incubated for 1–5 min with intermediate mixing at RT. The staining process was stopped by adding an equal volume of 1% BSA. The pellet was washed twice with 10 ml of complete media and the pellet suspended in 1.0 ml of complete media.

The rabbits were anaesthetized using sodium pentobarbitone, 60 mg/kg, and all procedures were carried out after the application of topical paracaine 1% drops. Laser injury to the TM was produced by the method described by Levkovitch-Verbin et al. [14] in mice, using 50–60-diode translimbal diode laser applications with the glaucoma probe, glaucoma laser system, Iridex Corporation, USA, of 0.5 s duration and power 1 W. The G probe has a fiber optic that protrudes 0.7 mm from the footplate. The probe was placed such that the indentation formed by the fiber optic was seen to be just posterior to the clear cornea, at the limbus, and for the next application, the probe was placed with the fiber optic adjacent to the indentation. Confluent laser photocoagulation of the trabecular meshwork was performed over 360 degrees.

Stem cell injection

Immediately after the laser, 1×10^5 cord blood stem cells in 0.1 ml were injected intracamerally using a 26G needle, after aspiration of 0.1 ml of fluid in one eye of 12 rabbits (Fig. 1). In six of the injected eyes, stem cells were also stained with PKH-26 prior to intracameral injection.

The second eye of two rabbits did not have any surgical intervention and served as normal controls. The second eye of ten rabbits underwent limbal diode laser photocoagulation alone. Four rabbits were killed at 4 weeks, four at 8 and three at 12 weeks. One rabbit died due to heat stress in the month of June, 11 weeks post-laser.

All rabbits were treated with ciprofloxacin drops qid for 1 week after the laser and were reviewed weekly for 1 month, and monthly thereafter, recording

ciliary congestion, anterior chamber flare or cells, synechiae and tonometry. The rabbits were killed using IV phenobarbitone 100 mg/kg.

Sixteen enucleated eye balls were formalin fixed and transported in 10% buffered formalin. The six eyeballs injected with PKH-26-stained stem cells had half the eyeball cryopreserved and half formalin fixed. Four-micron-thick, serial paraffin sections at the limbus including cornea, TM, iris and ciliary body were examined under light microscope after staining with hematoxylin and eosin. They were examined and analyzed by a pathologist who was not aware of which eyes had received the injection of stem cells. A note was specifically made of the type of cells in the TM, pleomorphism of trabecular endothelial cells, arrangement and alignment of trabecular beams, and spaces in the TM and any other significant abnormalities as compared to the two normal control rabbit eyes.

Unstained silane-coated sections were also cut from the paraffin-embedded blocks and subjected to immunohistochemical analysis using Avidin–Biotin indirect method. In brief, the deparaffinized tissue sections underwent antigen retrieval with citrate buffer for 20 min and were incubated with 0.3% H_2O_2 in methanol to inactivate endogenous peroxidase, followed by overnight incubation at 4 °C in a humidified chamber with Monoclonal Mouse Anti-Human CD34 Class II (Clone QBEnd 10), antibody, to confirm the presence of human hemopoietin stem cells in the sections. Anti-CD44, (CLONE f10-44-2), from Abcam, USA, was used as a marker for mesenchymal stem cells. In addition, antihuman *T* cell marker (CD3, Clone LN-10) from Leica Novocastra, India, was also used to detect any differentiation of stem cells to *T* cells in all eyes, both with and without injected human cord blood stem cells. Sequential incubations with biotinylated-linked secondary antibody and peroxidase-labeled streptavidin were done according to manufacturer's protocol (Dako cytomation LSAB+System-HRP kit, Denmark). The sections were visualized using 3, 3′-diaminobenzidine (DAB) peroxidase substrate for 2–3 min and were counterstained with hematoxylin and examined by light microscopy. The primary antibody was replaced by Tris-buffered saline in negative controls.

For eyes injected with PKH-26-stained stem cells, 4–5-micron-thick sections were cut on a cryostat and the red fluorescence was observed using a using an upright fluorescent microscope (Nikon Eclipse 80*i,* USA.).

Because of the range and individual variation among tissues that have been laser treated, at least 50 slides, two per clock hour, from each enucleated eye were studied.

Results

On clinical evaluation, there was mild ciliary congestion in the lasered control rabbit eyes for up to 2 weeks, while the eyes that received stem cells along with laser showed congestion for only the first 3–4 days. One lasered control eye that did not receive stem cells developed a significant inflammation, and posterior synechia, 1 week post-laser, and a rise of IOP by 4 mmHg after 4 weeks which returned to baseline by 8 weeks. All other laser-only eyes had an IOP within ± 2 mmHg of baseline. No evidence of inflammation, change in IOP > ± 2 mmHg or lenticular opacities were seen in any of the rabbit study eyes that were lasered, with simultaneously injected human cord blood-derived stem cells.

Histopathology and immunohistochemistry: (Table 1)

Normal rabbit trabecular meshwork

Two rabbit eyes, which were not lasered or injected with HUCB cells (normal control eyes), showed an anterior chamber angle with pectinate ligaments seen across the ciliary cleft. Part of the intracameral surface of the ciliary cleft was seen to be covered by a thin, endothelial membrane. Behind this membrane, narrow, well-defined, radial trabecular beams were seen. The beams were lined by a single layer of endothelial cells, having elongated nuclei and oriented parallel to the beam (Fig. 2). There was a loose reticular arrangement of the beams, enclosing many intertrabecular spaces. Some attachments between the trabecular beams and the sclera were also seen. Staining by antihuman CD34, CD44, or T and B-cells was all negative.

Table 1 Summary of antihuman immunohistochemistry results after trabecular laser with or without HUCB stem cell injection in rabbit eyes

S. no.	Duration (weeks)	Rabbits (n = 12, 24 eyes)	CD34	CD44	T cells
1	4	Normal (2 eyes)	Nil	Nil	Nil
2	4	Lasered (2 eyes)	Nil	Nil	Nil
		Lasered + stem cell (4 eyes)	4/4	4/4	2/4
3	8	Lasered (4 eyes)	Nil	Nil	Nil
		Lasered + stem cell (4 eyes)	3/4	3/4	1/4
4	12	Lasered (4 eyes)[a]	0/3[a]	0/3[a]	Nil
		Laser + stem cell (4 eyes)	2/3[a]	2/3*	1/4

[a]One rabbit died of heat stress

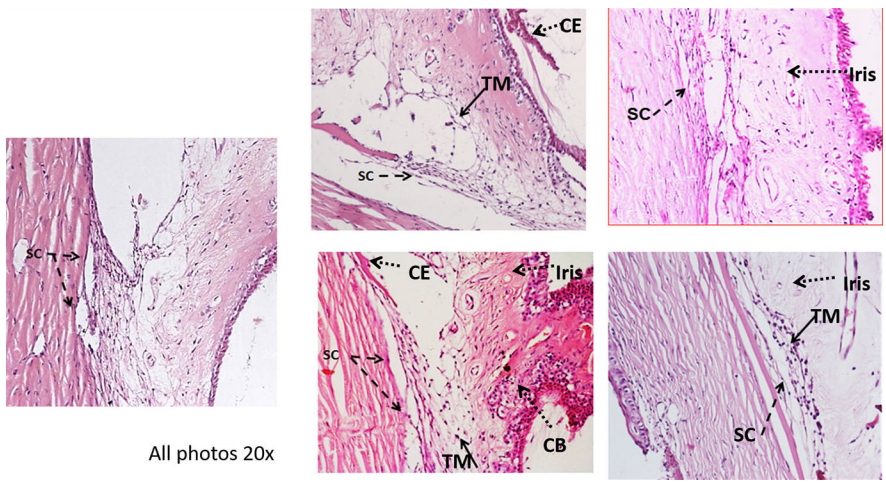

Fig. 2 *Left* Control rabbit eye, to show orderly, parallel arrangement of trabecular beams, spaces and Schlemm's canal, SC. *Upper middle* Lasered rabbit eye without stem cell injection at 4 weeks, showing architectural disruption of the trabecular meshwork, TM, damaged Schlemm's canal, SC and inflammatory cells. *Lower middle* Lasered rabbit eye with concomitant stem cell injection at 4 weeks, showing relative preservation of TM architecture and enlarged trabecular spaces. *Upper right* Lasered rabbit eye without stem cell injection at 12 weeks, showing fusion and compaction of the TM, and Schlemm's canal. *Lower right* Lasered rabbit eye with concomitant stem cell injection at 12 weeks, showing a narrower but relatively preserved TM and Schlemm's canal but fewer trabecular spaces than controls. Black arrow—trabecular spaces, dashed arrow—Schlemm's canal, dotted arrow—fused trabecular beams. (H&E 20×). CE: corneal endothelium, TM: trabecular meshwork, CB: ciliary body, SC: Schlemm's canal

4 weeks after trabecular laser

Lasered control eyes

Light microscopy showed disruption of the anterior pectinate ligaments and uveal trabecular beams in all four rabbit eyes. There were fewer intertrabecular spaces anteriorly and larger, irregular spaces posteriorly and in the uveal meshwork. The wall of Schlemm's canal was disrupted in areas. Desquamated trabecular endothelial cells and cellular debris could be seen in the anterior chamber and within trabecular spaces, while those still lining the beams were polymorphic. Mononuclear cells could be seen within the TM and iris which were negative for antihuman CD34 and CD44, (Fig. 2). The thickened, peripheral Descemet's membrane showed endothelial cell vacuolation and loss (Fig. 2).

Lasered study eyes with intracameral human cord blood-derived stem cells

The four study eyes showed some disruption of the anterior pectinate ligament; however, the overall architectural pattern of trabecular beams was maintained, with enlarged trabecular spaces. Schlemm's canal was similar to that of normal rabbit eyes. There was desquamation of a few trabecular endothelial cells, but cellularity was significantly preserved as compared to lasered eyes without stem cell injections. Large, mononuclear cells with darkly stained nuclei were seen on light microscopy. Antihuman CD34-positive cells were 3–4 cells/slide, with a few CD44-positive cells in all four eyes, within trabecular beams. Antihuman *T* cell positivity was seen in two out of four of the rabbits (Fig. 2). PKH-26-stained, fluorescent HUCB cells, 50–60 cells at 20×, were visible around edematous trabecular beams.

Eight weeks after trabecular laser

Lasered control eyes

Trabecular architecture showed fused trabecular beams and fewer spaces. Schlemm's canal was seen, with compacted juxtacanalicular beams. There was also increased mononuclear cellularity in all four such rabbits. Antihuman CD34, CD44 staining was negative.

Lasered study eyes with intracameral human cord blood-derived stem cells

All the four studied test eyes showed a relatively preserved architecture of trabecular beams, with increased cellularity on light microscopy, (Fig. 2). Immunostaining revealed the persistence of human CD34, 1–3 cells per slide and a few CD44-positive stem cells in 3/4 test eyes, lining trabecular beams and intrascleral channels. Thirty–forty PKH-26-labeled, pleomorphic cells were present in all eyes where injected, within trabecular spaces and along beams (Fig. 3a).

12 weeks after trabecular laser

One rabbit died from probable heat stress.

Lasered control eyes

There were fusion and derangement of trabecular beams, toward the uveal side, and very few, narrow intertrabecular spaces as compared to normal rabbit eyes. The corneoscleral TM retained some orientation parallel to the sclera, with thicker beams and fewer spaces. Endothelial cells could be seen within fused beams. Schlemm's canal was smaller and fibrosed in areas (Fig. 2).

Lasered study eyes with intracameral human cord blood-derived stem cells

The TM was narrow, with fewer beams as compared to controls, but almost normal-sized spaces. Fusion of beams and thickening of iris columns were noted in uveal TM. Trabecular endothelial cells were attached to beams. Schlemm's canal was similar to that in control rabbit eyes. Two–four cells per slide ×20 stained positive for human CD34, (Fig. 4a) and a few for CD44, in two of the three eyes. Human *T* cells were noted in two of three eyes (Fig. 4b). Twenty-five–thirty PKH-26-labeled linear cells were present per slide at ×20, in all eyes where injected, and were arranged regularly along trabecular beams (Fig. 3b).

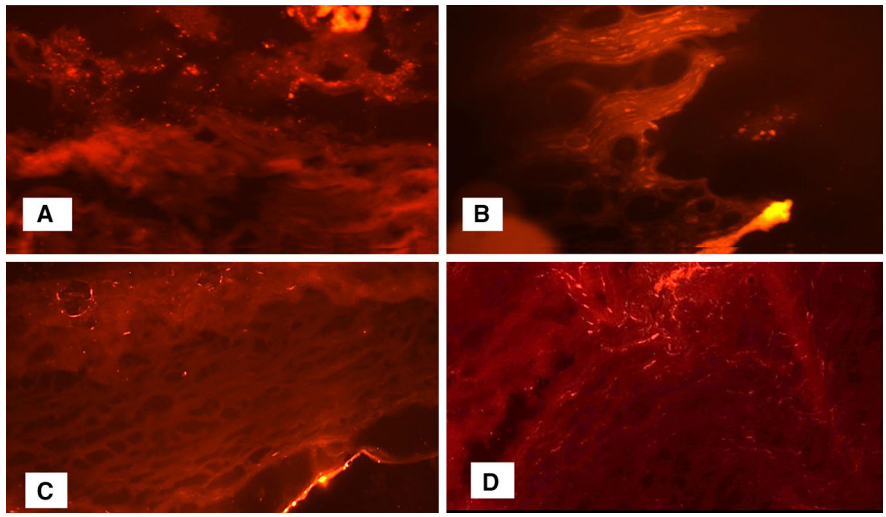

Fig. 3 Trabecular meshwork of lasered rabbit eyes observed under red fluorescence. **a** Lasered rabbit eye with concomitant stem cell injection at 8 weeks, showing many fluorescent cells within and around swollen trabecular tissue**. b** Lasered rabbit eye with concomitant stem cell injection at 12 weeks, showing fluorescent cells arranged regularly along trabecular beams (40×). **c** Lasered rabbit eye with concomitant stem cell injection at 8 weeks, showing fluorescent cells within and around swollen trabecular tissue (20×). **d** Lasered rabbit eye with concomitant stem cell injection at 12 weeks, showing fluorescent cells arranged regularly along some trabecular beams (20×)

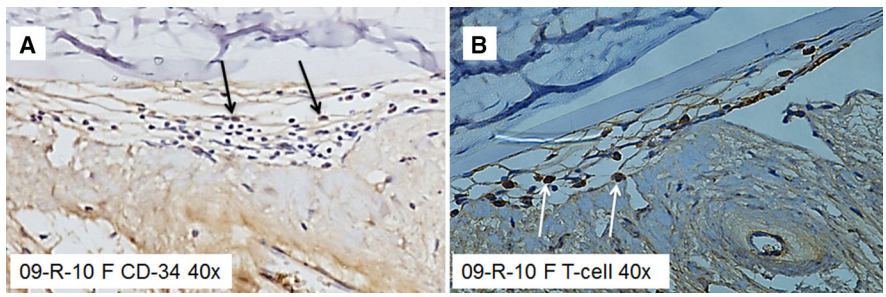

Fig. 4 Rabbit trabecular meshwork after trabecular laser and human cord blood cell injection. *Left* Relatively preserved rabbit trabecular architecture with increased cellularity. Antihuman CD34 immunostain showing the presence of CD-34-positive stem cells (40×). *Right*. Human-immunostained *T* cells seen at 12 weeks (Avidin biotin 40×)

Discussion

Stem cell therapy in ophthalmology has concentrated on diseases of the retina, with some work on neuroregeneration in glaucoma [16, 17], and the TM [18].. Targeting the TM is relatively easy and could influence a major risk factor for glaucoma. In this study, the lasered TM of rabbit eyes, with or without simultaneous injection of human cord blood stem cells, HUCB, was studied at 4, 8 and 12 weeks.

It has been shown that multipotent mesenchymal stem cells, MSC, from bone marrow or cord blood, can differentiate into various lineage cells, allowing them to repair and regenerate cells, including endothelial cells of the blood vessel, neural cells and even retinal cells [9, 19, 20]. Umbilical cord blood stem cells have the advantage that they are less likely to undergo rejection, graft versus host disease, differentiate spontaneously or form teratomas [21–24]. They also do not require retroviral vectors or gene transmission. UCB contains not only hematopoietic progenitors, but also several other types of stem/progenitor cells, from very primitive embryonic-like cells to relatively mature neuronal and endothelial progenitor cells, some of which express CD 34 and CD 44, and a few a leukocyte-like morphology. In this study, we

therefore used freshly isolated multipotent stem cells from human cord blood.

Our study induced trabecular meshwork damage by a single, low-dose translimbal diode laser application to the TM, so that injected stem cells would be attracted to the damaged TM. Immunosuppressants were not used. Mild ciliary congestion was seen for a few days, and only one lasered rabbit eye without HUCB cells had a significant rise in IOP, with evidence of uveitis. Johnson et al. [16] similarly did not report ocular hypertension in rabbits they lasered externally at the limbus. A raised IOP was only seen after an average of three sittings of trabeculoplasty in monkeys and rabbits [25–27].

In the present experimental study, histopathology revealed that lasered control eye TM showed abnormal architecture, endothelial desquamation, fusion of beams and fewer intertrabecular spaces. In rabbit eyes having simultaneous HUCB cell injection with laser, there was relative preservation of trabecular architecture, up to 12 weeks. The remaining trabecular beams returned to their orientation parallel to the sclera, but with fewer spaces. There was a loss of trabecular endothelial cells in all lasered rabbit eyes, but less where stem cells had been injected. Johnson et al. showed inflammation and damage to the TM after externally lasering the limbus in rabbits. Levkovitch et al. [14] described closure of intertrabecular spaces and major outflow channels in rats following diode laser photocoagulation of the TM. Similar changes have been described after argon laser trabeculoplasty in humans [27, 28].

Our experimental rabbit study was able to show the presence of some HUCB cells lining trabecular beams, similar to trabecular endothelial cells, up to 12 weeks after stem cell injection, both on immunostaining and by PKH fluorescence. A few cells positive for human CD44 were also seen up to 12 weeks. These were therefore persistent human cord blood-derived cells, detected within the trabecular spaces for 4 weeks and lining beams and some aqueous channels thereafter. The induction of TM cells from induced pluripotent stem cells has been reported [10], with a high probability of survival in HLA disparate recipients of UCB [29, 30] and expression of CD34 [31]. CD44 positivity of hUCMSCs is described [32]. Manuguerra-Gagné et al. [9] injected MSCs into mouse anterior chambers, which were not detectable after 96 h, probably due to stem cell escape through the TM or early mortality in areas of severe cellular stress. However, Johnson et al. [16] showed 5-week persistence of intravitreally injected stem cells in the retina of rats. Siqueira et al. [33] reported an improved QOL in patients of RP receiving intravitreal stem cells for a period of 3 months. Park et al. [34] described intravitreal autologous BM CD34+ cell therapy degenerative retinal conditions. CD34+ hematopoietic cells have also been used in spinal cord injury [35] and peripheral vascular disease [36].

In our study, none of the lasered rabbit eyes injected with HUCB stem cells showed either clinical or histopathological evidence of rejection, up to 12 weeks. Cells staining with antihuman *T* cell antibody were seen in rabbit eyes where HUCB stem cells were injected at the time of laser up to 12 weeks. CD34 cord blood cells are multipotential and can generate endothelial cells and also lymphohematopoietic lineages such as *B* and *T* cells. No such *T* cells were seen in rabbit eyes that only underwent a laser. *T* cells of umbilical cord blood have an inherent immunological immaturity [19, 37]. This may be due to the unique properties of the neonatal immune system, which permit the development of tolerance to alloantigens or xenoantigens. While donor *T* cells are undesirable as effector cells of graft versus host disease, they are valuable for engraftment by preventing the recipient's immune system from rejecting the donor cells. Coulson Thomas et al. [38] showed that human UMSCs survive xenograft transplantation in the mouse model and also induce the maturation of T-regulatory cells and inflammatory cell death. Engraftment implies functionality, which we did not assess in this study [39]. Ishikawa et al. [40] reported that human cord blood CD34+ progenitor cells can generate cells across allogeneic and xenogeneic barriers. Inflammation from attendant graft versus host disease may also contribute to the changes seen. Stem cells appear to help tissues in many ways, by homing into injured tissue, transdifferentiation, cell fusion and secretion of paracrine factors such as antiapoptotic and angiogenic cytokines 11, 35.

The limitations of this study were the small number of eyes for which ethical clearance was given to assess safety. The acute model of trabecular damage was used to serve only as a 'focus' for stem cell homing, unlike the chronic trabecular changes in glaucoma. Injected stem cells can go into 360° of the TM, and also many could escape through this over time. Despite studying 50 slides per eye, it is possible that

the stem cells could have been in lasered areas that were not sampled. The presence of stem cells was not a random phenomenon, as they were not seen in control eyes and were positively stained with antihuman antibodies and PKH. We evaluated over two slides per clock hour per eye, in which a similar distribution of laser spots, 50–60 overall, was applied; however, it is possible that similar areas—lasered and unlasered—may not have been compared over time. Objective evaluation of cell density and trabecular area with appropriate software in larger number of animals would permit statistical analysis. This study shows structural preservation; however, functional efficacy needs to be confirmed. In a rat retinal dystrophy model by Lund et al. [41], most of the photoreceptors degraded before 100 days of age because of a defect in RPE; however, both structure and function of the retina were preserved after transplantation of human UTCs.

This study demonstrated the presence and persistence of human cord blood stem cells along trabecular beams in lasered TM of rabbit eyes, up to 12 weeks, without significant inflammation, and with preserved cellularity and architecture of the TM. Intracameral HUCB stem cell injections appear to be helpful in preserving trabecular architecture and cellularity after acute trabecular injury, however, further studies are necessary to evaluate the effect of such cells in chronic trabecular dysfunction, as seen in glaucoma.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving animals The study was carried out after clearance from our Institutional Animal Ethics and Stem Cell Ethics Committees. The animals were housed in pathogen-free conditions and treated as per the ARVO statement on the use of animals in ophthalmic and vision research. All applicable international, national and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Statement on human rights All procedures performed in studies involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments.

Informed consent Informed consent was obtained from all stem cell donors included in the study.

References

1. Kingman S (2004) Glaucoma is second leading cause of blindness globally. Bull World Health Organ 82(11):887–888
2. Coleman AL, Miglior S (2008) Risk factors for glaucoma onset and progression. Surv Ophthalmol 53(Suppl 1):S3–S10
3. Tektas OY, Lütjen-Drecoll E (2009) Structural changes of the trabecular meshwork in different kinds of glaucoma. Exp Eye Res 88:769–775
4. Sihota R, Lakshmaiah NC, Walia KB, Sharma S, Pailoor J, Agarwal HC (2001) The trabecular meshwork in acute and chronic angle closure glaucoma. Indian J Ophthalmol 49:255–259
5. Tripathi RC (1978) Aqueous outflow pathway in normal and glaucomatous eyes. Br J Ophthalmol 56:157–174
6. Spencer WH (1985) Glaucoma. In: Spencer WH (ed) Ocular Pathology, An Atlas and Textbook. WB Saunders, Philadelphia, pp 480–547
7. Alvarado JA, Murphy CG (1992) Outflow obstruction in pigmentary and open angle glaucoma. Arch Ophthalmol 110:1769–1772
8. Abu-Hassan DW, Li X, Ryan EI, Acott TS, Kelley MJ (2015) Induced pluripotent stem cells restore function in a human cell loss model of open-angle glaucoma. Stem Cells (Dayton, Ohio) 33(3):751–761. https://doi.org/10.1002/stem.1885
9. Manuguerra-Gagné R, Boulos PR, Ammar A, Leblond FA, Krosl G, Pichette V, Lesk MR, Roy DC (2013) Transplantation of mesenchymal stem cells promotes tissue regeneration in a glaucoma model through laser-induced paracrine factor secretion and progenitor cell recruitment. Stem Cells 31:1136–1148
10. Ding QJ, Zhu W, Cook AC, Anfinson KR, Tucker BA, Kuehn MH (2014) Induction of trabecular meshwork cells from induced pluripotent stem cells. Invest Ophthalmol Vis Sci 55:7065–7072
11. Zhu W et al (2016) Transplantation of iPSC-derived TM cells rescues glaucoma phenotypes in vivo. Proc Natl Acad Sci USA 113:E3492–E3500
12. Roubeix C et al (2015) Intraocular pressure reduction and neuroprotection conferred by bone marrow-derived mesenchymal stem cells in an animal model of glaucoma. Stem Cell Res Therapy 6:177
13. Snider EJ, Kubelick KP, Tweed K, Kim RK, Li Y, Gao K, Read AT, Emelianov S, Ethier CR (2018) Improving stem cell delivery to the trabecular meshwork using magnetic nanoparticles. Sci Rep 8(1):12251
14. Levkovitch-Verbin H, Quigley HA, Martin KR, Valenta D, Baumrind LA, Pease ME (2002) Translimbal laser photocoagulation to the trabecular meshwork as a model of glaucoma in rats. Invest Ophthalmol Vis Sci 43:402–410
15. Keeney M, Chin-Yee I, Weir K, Popma J, Nayar R, Sutherland DR (1998) Single platform flow cytometric absolute CD34+ cell counts based on the ISHAGE guidelines. Int Soc Hematother Graft Eng Cytom 34:61–70
16. Johnson TV, Bull ND, Hunt DP, Marina N, Tomarev SI, Martin KR (2010) Neuroprotective effects of intravitreal

mesenchymal stem cell transplantation in experimental glaucoma. Invest Ophthalmol Vis Sci 51:2051–2059
17. Otani A, Dorrell MI, Kinder K, Moreno SK (2004) Rescue of retinal degeneration by intravitreally injected adult bone marrow-derived lineage-negative hematopoietic stem cells. J Clin Invest 114:765–774
18. Du Y, Yun H, Yang E, Schuman JS (2013) Stem cells from trabecular meshwork home to TM tissue in vivo. Invest Ophthalmol Vis Sci 54:1450–1459
19. Grewal SS, Barker JN, Davies SM, Wagner JE (2003) Unrelated donor hematopoietic cell transplantation: marrow or umbilical cord blood? Blood 101:4233–4244
20. Zigova T, Song S, Willing AE, Hudson JE, Newman MB, Saporta S, Sanchez-Ramos J, Sanberg PR (2002) Human umbilical cord blood cells express neural antigens after transplantation into the developing rat brain. Cell Transplant 11:265–274
21. Hao L, Zhang C, Chen XH, Zou ZM, Zhang X, Kong PY, Liang X, Gao L, Peng XG, Sun AH, Wang QY (2009) Human umbilical cord blood-derived stromal cells suppress xenogeneic immune cell response in vitro. Croat Med J. 50:351–360
22. Wang M, Yang Y, Yang D, Luo F, Liang W, Guo S, Xu J (2009) The immunomodulatory activity of human umbilical cord blood-derived mesenchymal stem cells in vitro. Immunology 126:220–232
23. Laughlin MJ (2001) Umbilical cord blood for allogeneic transplantation in children and adults. Bone Marrow Transplant 27:1–6
24. Jablonska A, Kozlowska H, Markiewicz I, Domanska-Janik K, Lukomska B (2010) Transplantation of neural stem cells derived from human cord blood to the brain of adult and neonatal rats. Acta Neurobiol Exp (Wars) 70:337–350
25. Gherezghiher T, March WF, Nordquist RE, Koss MC (1986) Laser Induced glaucoma in rabbits. Exp Eye Res 43:885–894
26. Wang RF, Schumer RA, Serle JB, Podos SM (1998) A comparison of Argon laser and Diode laser photocoagulation of trabecular meshwork to produce the Glaucoma Monkey Model. J Glaucoma 7:45–49
27. Johnson DH (2007) Histologic findings after argon laser trabeculoplasty in glaucomatous eyes. Exp Eye Res 85:557–562
28. Koller T, Stürmer J, Remé C, Gloor B (2000) Membrane formation in the chamber angle after failure of argon laser trabeculoplasty: analysis of risk factors. Br J Ophthalmol 84:48–53
29. Leow SN, Luu CD, Nizam MHH, Mok PL, Ruhaslizan R, Wong HS, Halim WHWA, Ng MH, Ruszymah BH, Chowdhury SR, Bastion ML, Then KY (2015) Safety and efficacy of human Wharton's Jelly-derived mesenchymal stem cells therapy for retinal degeneration. PLoS ONE 10:e0128973
30. Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, Goldman A, Kersey J, Krivit W, MacMillan ML, Orchard PJ, Peters C, Weisdorf DJ, Ramsay NK, Davies SM (2002) Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood 100:1611–1618
31. Lin CS, Ning H, Lin G, Lue TF (2012) Is CD34 truly a negative marker for mesenchymal stromal cells? Cytotherapy. 14:1159–1163
32. Zhang YJ, Yang YX, Hu MY, Ni QK, Li HG, Miao ZC (2015) Importance of CD44 on umbilical cord mesenchymal stem cells for expansion of hematopoietic cells. Cell Mol Biol (Noisy-le-grand) 61:18–25
33. Siqueira RC, Messias A, Messias K, Arcieri RS, Ruiz MA, Souza NF, Martins LC, Jorge R (2015) Quality of life in patients with retinitis pigmentosa submitted to intravitreal use of bone marrow-derived stem cells (Reticell -clinical trial). Stem Cell Res Ther 6:29
34. Park SS, Bauer G, Abedi M, Pontow S, Panorgias A, Jonnal R, Zawadzki RJ, Werner JS, Nolta J (2014) Intravitreal autologous bone marrow CD34+ cell therapy for ischemic and degenerative retinal disorders: preliminary phase 1 clinical trial findings. Invest Ophthalmol Vis Sci 56:81–89
35. Srivastava A, Bapat M, Ranade S, Srinivasan V, Murugan P, Manjunath S, Thamaraikannan P, Abraham S (2010) Autologous multiple injections of in vitro expanded autologous bone marrow stem cells for cervical level spinal cord injury—a case report. J Stem Cells Regen Med 6:175–176
36. Subrammaniyan R, Amalorpavanathan J, Shankar R, Rajkumar M, Baskar S, Manjunath SR, Senthilkumar R, Murugan P, Srinivasan VR, Abraham S (2011) Application of autologous bone marrow mononuclear cells in six patients with advanced chronic critical limb ischemia as a result of diabetes: our experience. Cytotherapy 13:993–999
37. Roncarolo MG, Bigler M, Martino S, Ciuti E, Tovo PA, Wagner J (1996) Immune functions of cord blood cells before and after transplantation. J Hematother 5:157–160
38. Coulson-Thomas VJ, Gesteira TF, Hascall V, Kao W (2014) Umbilical cord mesenchymal stem cells suppress host rejection: the role of the glycocalyx. J Biol Chem 289:23465–23481
39. Rieck B (2003) Unexpected durability of PKH 26 staining on rat adipocytes. Cell Biol Int 27:445–447
40. Ishikawa F, Shimazu H, Shultz LD, Fukata M, Nakamura R, Lyons B, Shimoda K, Shimoda S, Kanemaru T, Nakamura K, Ito H, Kaji Y, Perry AC, Harada M (2006) Purified human hematopoietic stem cells contribute to the generation of cardiomyocytes through cell fusion. FASEB J 20:950–952
41. Lund RD, Wang S, Lu B et al (2007) Cells isolated from umbilical cord tissue rescue photoreceptors and visual functions in a rodent model of retinal disease. Stem Cells 25:602–611

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.