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Research Article

First-Year Results of Subretinal Mesenchymal Stem Cell Implantation in Severe Retinitis Pigmentosa

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ABSTRACT

This study includes one-year results of 14 patients with severe Retinitis Pigmentosa (RP) who had subretinal mesenchymal stem cell (ADMSC) implantation. The highest Visual Acuity (VA) in the study was 20/2000 and 7 of the patients had severe VA loss. The patients received subretinal ADMSCs after total vitrectomy.

We observed no systemic complications. There were no ocular complications in 8 patients. Choroidal Neovascular Membrane (CNM) developed in one of the patients and intravitreal anti-VEGF injection was performed. The first six patients had Epiretinal Membrane (ERM) with peripheral tractional retinal detachment and received second vitrectomy. One of the patients experienced mild band keratopathy six months after the treatment and another patient had retrolental fibrous tissue at 1-year follow-up examination. Four patients showed VA gain during the first year. Subretinal implantation of ADMSCs may have some adverse effects and the patients should be followed up carefully. This study clarifies the side effects of the therapy which would enlighten future studies. Further studies with higher number of patients will be necessary to optimize the surgical procedure and to determine the benefits of this therapy.

Keywords: Mesenchymal stem cell; Subretinal; Retinitis pigmentosa

INTRODUCTION

Since their introduction by Thomson et al. human Embryonic Stem Cells (hESCs) have drawn attention to their potential clinical use [1]. Despite their great therapeutic potential, various concerns have risen regarding their limitless ability for self-renewal and plasticity, including hyperproliferation, tumor, and ectopic tissue formation. Although there have been various clinical trials using Stem Cells (SCs) in retinal diseases, their long term results are still in the process of discovery.

Retinitis Pigmentosa (RP) is a potentially blinding disease with severe vision loss by age 40-50 and characterized by the death of retinal cells [2]. It affects over 2 million patients worldwide [3]. Its clinical diagnosis is based on the presence of night blindness, visual field construction, bone spicule pigmentation and a reduction in electroretinograms (ERGs) [4]. Although there is no effective therapy for the disease up to date, new treatments including gene therapy and SC implantation to have been under investigation [5]. Finding an effective and safe treatment may reduce the economic burden on the patients and society [6].

There have been four types of stem/progenitor cells; retinal progenitor cells, ESCs, induced pluripotent SCs (iPSCs) and mesenchymal SCs (MSCs), that have been used in retinal diseases all having pros and cons [7]. Developmentally mature organs, such as bone marrow, adipose tissue, umbilical cord or amniotic fluid are the generation sources of MSCs, which have paracrine and immunosuppressive effects while having the disadvantages of low rate of cell migration and differentiation

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[7]. Compared to bone marrow-derived MSCs (BM-MSCs), adipose tissue-derived MSCs (ADMSCs) can be obtained and expanded easily; and also have a higher immunomodulatory capacity [8]. MSCs can secrete various cytokines, growth factors, and proteins and show anti-apoptotic, anti-inflammatory, immunomodulatory and angiogenic activity which are thought to be the mechanisms of retinal cell survival [8].

Here we aim to report first-year results of patients with severe RP who received subretinal stem cells and to discuss the safety and tolerability of the procedure.

MATERIAL AND METHODS

This single-center, prospective, phase 1 clinical safety study included 14 subjects of RP which are legally blind. The study followed the tenets of the Declaration of Helsinki, it was approved by the Institutional Review Board and the Review Board of Stem Cell Applications within the Ministry of Health according to the regulations in our country. Inclusion criteria of the patients, surgical technique, and postoperative follow-up procedures were described in our published data before. The production protocols of ADMSCs were previously mentioned by our study group [5]. Patients completed 12 months follow up period. Visual acuity results, the incidence, and variety of ocular and systemic side effects associated with ADMSCs treatment were evaluated.

RESULT

Morphology and phenotype of culture-expanded hADMSC and the demographic and clinical characteristics of the 14 study patients who attended to the study were presented in our previous report [5].

Follow-up BCVA and eye examination

All 14 patients completed the one-year period and none of them had systemic side effects. Eight subjects had no ocular adverse effects regarding the SC treatment. Choroidal neovascular membrane (CNM) developed in one subject at the implantation site and treated with intravitreal anti-VEGF injection (Patient number 3). The first operated six patients including the patient with CNM, had epiretinal membrane (ERM) on the surface of the retina at the periphery with peripheral tractional retinal detachment, which needed second vitrectomy including total membrane peeling and silicon oil injection in five of them. One of the patients experienced mild band keratopathy six months after the treatment (Patient number 1) and another patient had retrolental fibrous tissue at a 1-year follow-up examination (Patient number 2) who had a normal posterior segment on the ultrasound (Figure 1). The development of these membranes and fibrous tissue is thought to be due to the vitreal reflux and undesirable preretinal proliferation of MSCs. To avoid the occurrence of this complication, the operation technique was modified as described in our previous report group [5]. This modification inhibited membrane formation in the remaining eight patients.

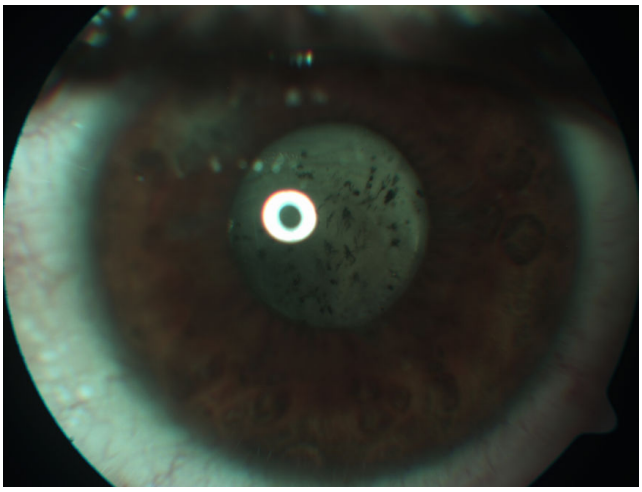

Figure 1: Anterior segment appearance of the patient with retrolental fibrous tissue.

We did not find a statistically significant difference in VA from baseline. Four patients had an increase in visual acuity during the first year (Patients numbered as 5, 9, 11, 14 in Table 1). This improvement persisted in three of them at the end of the first year (Table 1). The remaining ten had no change in VA (7 of them only had light perception before therapy).

Table 1: Demographic and visual acuity results according to the Snellen equivalent of enrolled subjects.

No/Age/Sex/Eye	BCVA Baseline	BCVA During the first year	BCVA at the end of the first year
1/44/male/OD	LP	LP	NLP
2/57/male/OD	LP	LP	LP
3/42/female/OS	LP	LP	LP
4/34/male/OS	20/20000	20/20000	20/20000
5/34/male/OS	20/2000	20/400	20/2000

6/30/female/OD	20/20000	20/20000	20/20000
7/29/male/OS	20/20000	20/20000	20/20000
8/47/male/OS	LP	LP	NLP
9/26/male/OD	20/20000	20/400	20/200
10/46/female/OS	LP	LP	LP
11/32/male/OS	20/20000	20/2000	20/2000
12/43/male/OS	LP	LP	LP
13/48/female/OS	LP	LP	LP
14/36/female/OD	20/20000	20/200	20/400

LP: Light perception; NLP: No light perception.

Perimetry

Improvements in the visual field were found in two subjects (Patient number 9 and 14) at 1-month follow-up examination, which persisted at the final one-year follow-up visit. The remaining subjects did not have any changes in the perimetry.

Electroretinography

There were no changes in the ERG recordings during the study period.

Fluorescein angiography: FFA of one subject (Patient number 3) showed CNM in the second month of the treatment. The remaining 13 patients revealed pathological findings on FFA. There was slight staining in the implantation area of MSCs in all patients (Figure 2).

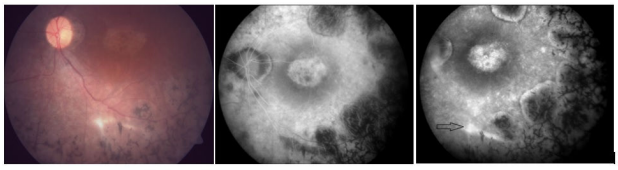

Figure 2: (A): Color fundus; (B): FFA images of a subject at the end of the first year including early; (C): late phases showing hyperfluorescent staining at the injection area.

OCT findings: OCT images of six patients with ERM formation showed disorganization of the retinal layers and retinal edema which improved after second vitrectomy and ERM peeling (Patient number from 1 to 6). All patients showed varying numbers of white spots within the retina, which decreased during the first year follow-up period (Figure 3). Three of the patients showed cystoid lesions, which differ in size and number on OCT during follow-up.

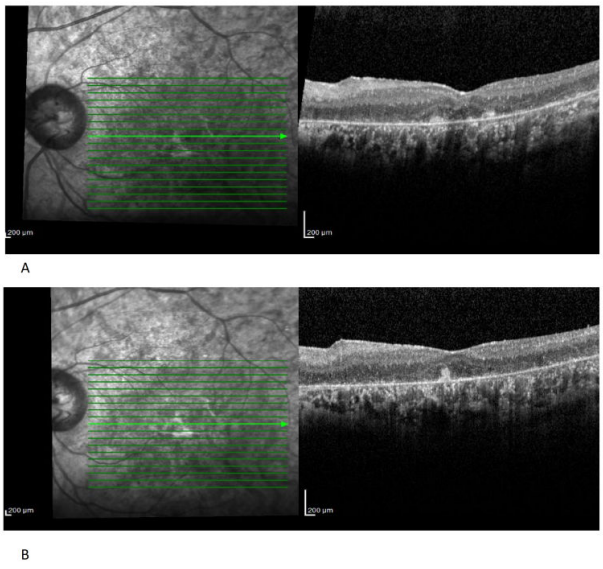

Figure 3: (A): OCT images of a subject at the first month and at the end of the first year; (B): The number of hyperreflective dots within the retina decreased in number and size.

DISCUSSION

Retinitis pigmentosa is a potentially blinding, hereditary progressive neurodegenerative disease with no approved and effective treatment making these patients search to try new treatment modalities. Recent investigations include nutritional supplementation, light reduction and gene therapy; valproic acid and vitamin A supplementation with modest benefits and potential side effects [9]. Surgical interventions other than stem cell-based therapies for the potential treatments for RP include use of retinal prosthetics and intravitreal delivery of encapsulated cells secreting neurotrophic factors [10-13]. Very few studies exist in the literature regarding stem cell-based treatment modalities in RP. Park et al reported the phase 1 clinical trial results of intravitreal autologous bone marrow

CD34+ cell injection in various retinal disorders including one patient with RP with the baseline VA of 20/640 [14]. The patient reached 20/250 at 2 weeks as the best follow-up BCVA, declined to 20/400-2 letters at the final examination. The patient also had recovery on visual function evaluated by Goldmann perimetry, which persisted at the final visit. He had flat ERG readings through the follow-up period of the study. Their findings showed that the procedure was effective and safe. In the Reticell-clinical trial, the investigators analyzed the quality of life of 20 subjects with RP who received intravitreal use of autologous, bone marrow-derived, stem cell implantation [9]. All patients had improvement in the quality of life evaluated with National Eye Institute Visual Function Questionnaire-25 (NEI-VFQ) 3 months after the injection; unfortunately, the improvement disappeared in the 12th month. The paper did not mention any adverse effects. In a recent phase 1, prospective open-label study conducted in Iran, the investigators studied the safety of single intravitreal implantation of autologous bone marrow-derived MSC in patients with severe RP [15]. All patients in their study group had visual acuity of slight light perception bilaterally. There were no side effects in eyes of 2 out of 3 patients who also described increase in the perception of light after 2 weeks of the injection, which persisted for 3 months. However the fundus findings of the third patient revealed extensive preretinal and vitreal fibrosis starting in the second week of the therapy, which caused tractional retinal detachment and vision loss to no light perception in the first month. The patient had the symptoms of pain and showed further ciliary injection, cyclitic membrane formation, retrolental fibrous tissue, shallow anterior chamber, ocular hypotony and partial tractional retinal detachment at 3-month follow-up. Ocular examination of the patient showed vision of no light perception, mature cataract, iris neovascularization, ocular hypotony and shallow anterior chamber at 1-year follow-up. The investigators injected this patient's MSCs intravitreally to a mouse, which also caused fibrosis on day 60 while the other two patients' cells injection to mice did not cause any fibrous tissue formation. MSCs were not observed in the affected animal eyes after two months, and the researchers suggested that the reaction was an inflammatory response to donor MSCs. The outcome of this study offers an assessment of stem cells in animals prior to implantation in humans as a solution, and the authors conclude that intravitreal implantation of MSCs for RP necessitates animal studies in order to clarify the possible side effects of this treatment.

In these medium-term results of our study, we found that no serious ocular or systemic complications were experienced by the patients between the 6-month endpoint of our previous report and 1-year follow-up of the patients [5]. In addition to reported previous complications of our study, one patient developed mild band keratopathy and one patient developed retrolental fibrous tissue. The latter patient was pseudophakic and did not show any signs of inflammatory reaction and retrolental fibrous tissue developed after 6 months and the posterior segment revealed normal on the ultrasound. Unfortunately these two patients whose initial visual acuity was light perceptions lost their vision to no light perception at 1 year. We think that the vitreal reflux and undesirable preretinal proliferation of MSCs were the reasons behind the formation of these membranes and fibrous tissue proliferation.

Of the 3 patients who showed improvement in their visual acuity between their initial and 6-month follow-up, one of them improved from 20/400 to 20/200 between the 6 months and 1-year follow-up. The patients with visual acuity improvement were young (36, 32, 26 years old) with a relatively short duration of disease. They also had better visual acuities than the rest of the patients before treatment. Both of the patients who developed improvement in their visual field testing sustained it throughout the year. When we evaluate the functional results of our study we may conclude that SC treatment is beneficial in the early stages of the disease.

We noted a decrease in size and number of cell accumulations within the retina during the 12 months follow-up period, which was initially noted after surgery on OCT in all patients. Contrary to our findings, Schwartz et al. reported in their study that during the median 22 month follow-up of their patients with Age-related Macular Degeneration (AMD) and Stargardt's macular dystrophy that underwent subretinal implantation of hESC-derived retinal pigment epithelium, they observed an increase in subretinal pigmentation in 13 of 18 patients [16]. However no correlation existed between postoperative pigmentation and visual improvement. These correlations are consistent with our observations that although the decrease of the cell accumulations was noted throughout the follow-up period, some of the patients showed improvement in their visual acuities. Based on the interpretations of their study Schwartz et al. thought that subretinal pigmentation may not have represented the transplanted retinal pigment epithelium, but may have represented macrophages or native cells ingesting pigment [16]. We did not use retinal pigment epithelium in our study, so we did not expect any hyperpigmented tissue within the retina, we also think that the cells accumulations could also represent activated macrophages and/or microglial cells in our study rather than the MSCs themselves, because although they showed a decrease in size and number, some of our patients showed improvement in their visual acuities [17]. In preclinical studies also, immunohistochemistry was shown to be the only method to show the survival and integration of the transplanted hESC-retinal pigment epithelium cells rather than a pigment increase [18,19].

One of the exceptional properties of the subretinal space is that it is immune privileged [20]. Thus instead of the classical findings of acute graft rejection such as uveitis or lymphocyte infiltration, rejection can be demonstrated by cell loss or progressive loss of function in the absence of inflammation [16]. Since 2 patients with perception of light at the initial exam lost their vision to no light perception and we noticed decrease of the cell accumulations and white dots within the retina we do not know if this loss of vision was whether due to the natural course of the disease or because of graft rejection. We also observed cystoid lesions with varying sizes and numbers on OCT of two patients, which may be due to the natural course of the disease or due to the graft rejection itself.

CONCLUSION

Subretinal implantation of ADMSCs may have some adverse effects and the patients should be followed up carefully. The result of this study clarifies the complications of the therapy which would be beneficial for future studies. Further studies with larger groups will be necessary to optimize the surgical technique and to determine the effects of this therapy.

AUTHORS' CONTRIBUTION

AO: Study design, patient selection and follow-up, surgical intervention, data collection, manuscript preparation; ZBG: Study design, preparation of stem cells, laboratory tests, manuscript preparation; DGS: Data collection, manuscript preparation; NS: Patient follow-up, data collection, manuscript preparation; MC: Study design, preparation of stem cells; YO: Study design, laboratory tests.

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