Regenerative Stem Cell Therapy with Umbilical Cord Mesenchymal Stromal Cells In Deaf Animal Model

Sung Hun Lee, MD, Min Ji Kim, MD, Ju Eun Kim, MD, Dae Young Chung, MD, Kyoung Ho Park, MD, PhD.

Department of Otolaryngology Head & Neck Surgery,
The Catholic University of Korea, College of Medicine.

Abstract

Objectives: This study was performed to confirm the effect of transplantation of human umbilical cord blood mesenchylmal stem cells(UCB-MSCs) on hearing restoration in deaf animal model

Method: UCB was collected form pregnant women after obtaining consent, and mesenchylmal stem cells(MSCs) were extracted. We established deaf animal model and transplanted UCB-MSCs through the brachial vein of the guinea pigs. The animals were divided into 4 group : animals with normal hearing, animals with SNHL, animals with SNHL and injected with saline, and animals with SNHL and transplanted with UCB-MSCs. Hearing tests were conducted at 1,3, and 5 weeks, and the results were compared by grading auditory brainstem response(ABR) recordings and distortion product otoacoustic emissions(DPOAEs) for each treatment. Lastly, cochlear pathological features were examined.

Result: In SNHL group, decreased DPOAEs and increased ABR threshold were noted. And ABR hearing thresholds were unconverted and were similar to those observed in deafness. The transplanted UCB-MSC group showed a significant improvement in hearing threshold(40dB). Examination of the SNHL animals' cochlear morphological features demonstrated a noticeable lack of spiral ganglion cells and also showed degenerated outer hair cells. However, the transplanted UCB-MSCs showed an increase in spiral ganglion and hair cells.

Conclusions: Intravenous transplantaion of UCB-MSCs can enhance hearing thresholds, and regenerate inner ear hair cells and spiral ganglion neurons(SGNs)

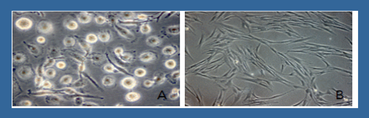
Introduction

Hearing loss is one of the most common diseases. The majority of human sensorineural hearing loss (SNHL) results from primary neuronal loss involving the degeneration of neurons in the absence of hair cell degeneration or secondary to the loss of hair cells that normally provide trophic support to spiral ganglion neurons (SGNs). Mesenchymal stem cells (MSCs) have the capacity for self-renewal and proliferation and are multipotent; thus, they can differentiate into various specific cell types such as muscle, epithelium, and liver cells. Recent studies have also demonstrated that neuronal cells such as Schwann cells (supporting cells) can differentiate from the endogenous stem cells in the inner ear and from MSCs. This study was performed to confirm the effect of transplantation of human umbilical cord blood mesenchymal stem cells (UCB-MSCs) on hearing restoration in a sensorineural hearing loss (SNHL) animal model.

Materials and Methods

1. Isolation of UCB-MSC

- centrifuge gradient method with the use of histopaque (Sigma-Aldrich, ST Louis, MO)
- 4℃ , for 20 minutes, at 2500 rpm.
- a layer of mononuclear cells between the serum and RBC layer was extracted.
- Flow Cytometry (FACS Caliber, Becton Dickson, San Diego, CA)
  Marker for hematopoietic stem cells ; CD34, CD45
  for mesenchymal stem cells ; CD73, CD90



2. Deaf animal Model & Transplantation of UCB-MSC

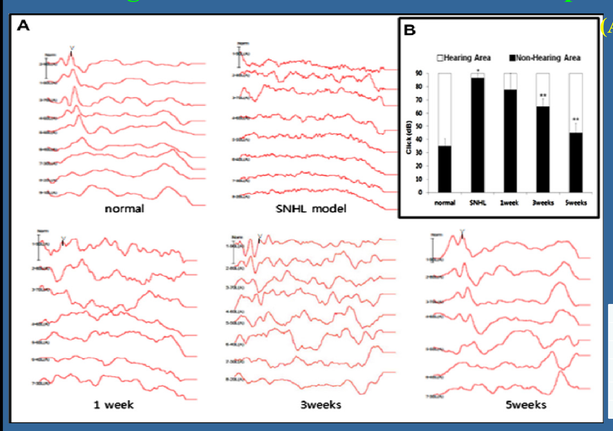
- SPF Guinea Pig (wt : 250 – 300 g) (5 cases in each group)
  (control, saline injection, MSC injection)
- Intra-tympanic Injection of 25mM ouabain octahydrate and neomycin solution in 10% of total volume
- Injection of UCB-MSC via brachial vein ($1x10^7$ cells in 100μL)
- Hearing test with ABR & DPOAE (HIS Smart EP System)
  (before deafening, after 1, 3, 5 weeks after MSC injection)
- *P –value* < 0.01

3. Morphologic Study

- Light Microscopy & SGN cell count
  - Toluidine blue staining
  - Image processing & analysis with Java (Image J; NIH)
- Hair Cell Regeneration
  - surface preparation
  - fluorescent image using
    Zeiss LSM510 META confocal microscope (Carl Zeiss)
  - Image processed using Zen 2009 Light Edition (Carl Zeiss)

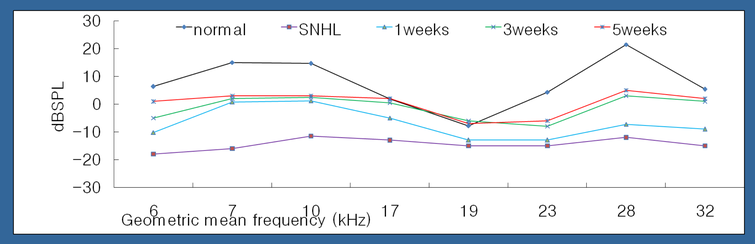
Results

1. Hearing restoration with UCB-MSCs transplantation



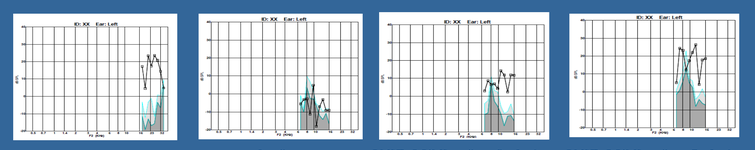
(A) Auditory brain response (ABR) results compared between normal hearing, SNHL, and UCB-MSC transplantation groups.
Click-evoked ABR waves were recorded up to 10 dB in guinea pigs with normal hearing. After intravenous injection, the UCB-MSC group showed a significant improvement in hearing threshold compared to that for the SNHL group.

	ABR thresholds
normal	35±5.5
SNHL*	87±5.2
1week	78±12.6
3weeks*	65±5.8
5weeks*	45±7.1



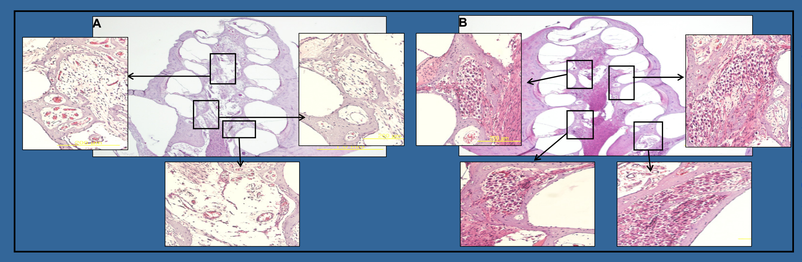
The SNHL group showed disappearance of DPOAE expression.

After UCB-MSC transplantation, the DPOAE re-expressed.



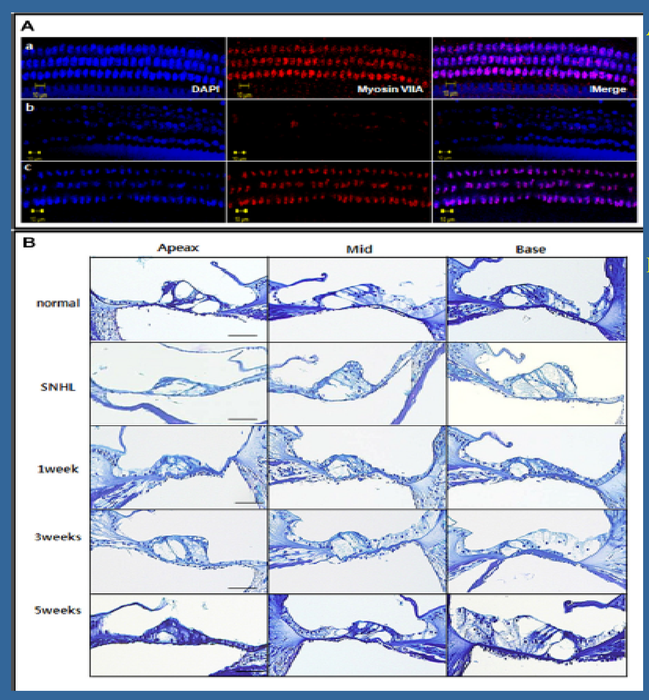
Normal hearing | deaf | UCB-MSCs after 3wks* | UCB-MSCs after 5wks*

2. Morphologic Regeneration after UCB-MSC Transplantation



Regeneration of SGNs after UCB-MSCs transplantation.
(A) Severe loss of SGNs from the basal to the apical turn of the cochlea was observed in the SNHL group.
(B) Five weeks after transplantation of UCB-MSCs, SGNs were regenerated in all the turns of the cochleae

Total average (n=5)	normal	SNHL model	1 week*	3 weeks*	5 weeks*
basal turn	30.3	5.0	15.0	20.0	24.7
2nd turn	44.8	8.8	19.5	25.3	37.7
3nd turn	39.8	9.5	25.0	31.7	34.7
4th turn	35.3	8.5	22.5	31.0	31.7



A. Surface preparation of the organ of Corti.
Normal hearing (a). In the SNHL group, multiple hair cells were lost from the basal to the apical turn of the cochleae (b). Five weeks after transplantation of UCB-MSCs, hair cells were visible (c).
(×400, n = 3, bars, 100 μm).
B. Serial microscopy sections of the organ of Corti from normal hearing versus SNHL and transplanted UCB-MSCs groups from the basal, middle, and apical regions of the cochleae.
In normal hearing, the morphological features of OC did not indicate damage. However, the SNHL group showed damage from ouabain and neomycin application and degeneration of outer hair cells at all turns of the cochlea. However, after transplantation of UCB-MSCs, the OC was regenerated in comparison with the SNHL state
(×400; n = 5; bars, 100 μm)

Conclusion

- Based on these results, intravenous transplantation of UCB-MSC *can restore hearing of deaf animal.*
- Transplantation of UCB-MSC can *regenerate the damaged SGN & hair cells*
- In the current study, it was demonstrated that *UCB-MSC* could be used *in stem cell based therapy for the deaf* in the future.