Perspectives in the Treatment of Hearing Loss with Stem Cells

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Article received on October 12, 2007. Article approved on January 9, 2008.

SUMMARY

Introduction: Damage and loss of hair cells in the inner ear is the most frequent cause of hearing loss, since mammalian hair cells are not replenished once lost. The treatment of hearing loss consists of hearing aids or cochlear implants, but both options restore hearing with limited success. Current research efforts are focusing on gene manipulation, gene therapy and stem cell transplantation for repairing or replacing damaged mammalian cochlear hair cells.

Objective: This review will discuss some of the latest findings and techniques of treatment with stem cells in the inner ear.

Conclusion: The recent discoveries about stem-cell-based therapy have opened an exciting new avenue for the development of strategies to restore hearing.

Key words: cochlea, hearing loss, stem cells, cultured cells, hair cells, biological therapy.

INTRODUCTION

Congenital deafness presents high incidence (1:1000 live born children and 1:1000 developing deafness during childhood). With the increase of population's life expectation, the prevalence of acquired deafness has been increasing. It is estimated that one third of adults who are older than 65 years old presents incapacitating hypoacusis. It is one of the most frequent chronic disorders, with more than 250 million affected people all over the world (1).

Most of acquired or congenital auditory losses are caused by damage or loss of cochlear cilliary cells or of their associated neurons. Deafness irreversibility in mammals is caused by its incapacitation of substituting lost cells, by cell division or by regeneration of endogenous cells in the internal ear epithelium. Clinically, the function of lost cilliary cells may be partially restored by electric stimulation of the audiotry nerve, which is possible with the use of cochlear implants. Other therapeutic alternatives biologically based are being explored (2,3).

One of the objectives of deafness therapy is the restoration of damaged cochlear or neural cells. Two possibilities have been currently considered: stimulation of endogenous cells by injection of exogenous cells (for example, growth factors or viral vectors manipulated by genetic engineering) or injection of progenitor cells or stem cells to substitute the lost cell (4).

In this review, the article selection was done through an electronic search of literature on MEDLINE data base and on the World Health Organization website. Studies which emphasized the potential terapeutical use of stem cells applied in the treatment of hearing loss were included.

LITERATURE REVIEW

Stem cells (SC) have self-replicating and differentiation properties in a variety of cell kinds. Such potential has been explored to pursue the regeneration of mammal cilliary cells. One important advance in the perspectives for the SC use to substitute the internal ear cells was the discovery of ciliary cells generation from embryonic SC, from adults SC of internal ear, SC of bone marrow and neural SC (5,6,7,8). Such cells are pluripotent, consequently, in theory, could originate all cell kinds of internal ear. In addition to this, such cells are structurally and functionally integrated to the embryo, when placed in initial phases of embryonic period. Therefore, one may think about the possibilities of clinical applications of such SC in deaf ears.

Currently, SC have been investigated in several diseases. They are experimentally used to generate muscle cells and coronary vessels for heart diseases, motor neurons for spinal cord lesion, insulin-secretory cells for diabetes and dopaminergic neurons for Parkinson's Disease (4).

There are three main SC sources for regeneration of ciliary cells: embryonic SC, SC which are isolated from the internal ear itself, and SC obtained from other organs such as the brain, skin and bone marrow.

Stem cells which are isolated from the internal ear itself

Two decades ago, the structural and functional recovery of audition has been observed after acoustic or medicine trauma in birds (9,10). After the death of cilliary cells, the non-sensory support cells receive molecular or genetic signals, which initiate proliferation or transdifferentiation in immature ciliary cells. Finally, there is reinnervation of cilliary cells causing the sensorial afference. The demonstration of regeneration of cilliary cells in birds has been inspiring and increasing the interest in the elucidation of such reparative process, once such phenomenon does not occur in mammals (11).

In 2003, LI et al. discovered that the utricular epithelium of adult mammals contain support cells with progenitor properties (6), while in the mature Corti organ, transdifferentiation of non-sensitive cells was only observed in cilliary cells with gene induction (12,13,14).

When cells are isolated from the sensitive epithelium of the utricle of adult rat, there is production of cell proliferator spheres. Such spheres are renewed and produce several kinds of cells with morphological characteristics and biological markers of cilliary cells, such as myosin VIIa and Brn-3.1; and of support cells, such as como $p27^{kip1}$. At light of current biological knowledge, such data suggest that the utricular support cells may be considered stem cells. When such cells were implanted in optical vesicles (developing ears) of chicken embryo, they were successfully integrated expressing ciliary cells features. In addition to this, when transplanted to muscles and liver of developing chicken embryo, such cells were totally incorporated, thus suggesting multipotent features of such stem cells (5,6).

In 2002, MALGRANE et al isolated cell forming spheres originated from Corti organs of just born rats, but the cochlea and the Conti organ were still immature, that is, had not completed their differentiation process yet. Such spheres had multipotent SC which were able to differentiate in all kinds of internal ear cells (12,13).

Embryonic Stem Cells

Such cells are originated from a cell group from inside the blastocyst. They are precursory of all embryologic lineage, once they are able to differentiate in mesodermic cells (bone, blood, muscles), endodermic cells (intestines) and ectodermic cells (skin, nerve, ciliary cells). Due to its power of generating multiple kinds of cells, they are considered pluripotent stem cells.

Li et al, in 2003, were able to develop internal ear cells in vitro from rat SC embryonic cells. Such SC, created with certain growth factors, expressed specific marker genes which are characteristic of cilliary cells (5).

Recently, Coleman et al did a xenotransplantation of embryonic SC of rats in deaf laboratory animals. The implantation of such cells was done through tympanic scale through a cochleostomy. The survival and tissue compatibility were evaluated in the first four weeks. In addition to cells floating in the perilymph and adhered in the cochlear cytoarchitecture, migration of transplanted cells to Rosenthal canal was observed. Another interesting finding was the expression of neural markers NF-L, proving a differentiation of embryonic SC (15).

An intriguing characteristic of such experiment was the absence of inflammatory response of the cochlea in relation to xenotransplant. Due to such peculiar property, some authors consider cochlea an immunological sanctuary (15).

Neural stem cells

The partial integration of SC introduced in injured tissues, like in the Parkinson's Disease and spinal cord injury in animal models, justified the application of such technique to treat the neurossensorial hearing loss.

Neural SC are able to differentiate in the main kinds of neural cells: neurons, astrocyte and oligodendrocyte. Rat neural SC were xenotransplanted to the internal ear of adult laboratory animals to evaluate them in terms of survival level and differentiation degree. Neural SC survived for at least 4 weeks after the implantation and migrated to functionally important regions: Corti organ, spiral ganglion and auditory nerve. There was a neuron differentiation for specific marker expression TUJ1, however there was no expression of markers of ciliary cells myosin VIIa (16).

Neural SC is usually gathered in the lateral wall of the lateral ventricle and dentate gyrus, but the olfactory epithelium may also be considered a source of such cells. The olfactory epithelium has a unique characteristic, once it is the only junction between the central and peripheral nervous system which contains neural stem cells. Patel, in 2006, introduced such neural SC in rat cochlea and observed that they survived and grew in the three cochlear gyrus and in the peri- and endolymphatic fluids (17).

Mesenchyme stem cells of bone marrow

Mesenchyme SC of bone marrow are also possible candidates to treat neurossensorial deafness. They are able to differentiate several cell kinds (osteoblasts, chondrocyte, myoblasts, adipocyte and neurons) and are easily obtained.

Such SC may be used in the internal ear in three possible strategies: restoration of lost cells, production of growth factor and gene introduction.

In 2004, Naito et al, after implanting autologous cells of bone marrow in internal ear of rats carrying induced deafness, demonstrated survival and migration of injected cells in several cochlea regions: vestibular and tympanic scales, spiral ligament, strias vascularis and modiolo, including spiral ganglion and cochlear nerve. In addition to implantation, the cells are differentiated in neurons due to the observation of expression of neural markers such as NF200 (18). In another similar experiment, some of the implanted cells expressed conexin-26 protein, which belongs to the intercellular junction of support cells and fibroblasts of Corti cells and essential for the maintenance of endocochlear potential. Such finding indicates a possible use of transplant of bone marrow SC to restore intercellular cells in the cochlear conjunctive tissues (19).

Discussion

The experimental use of stem cells to treat deafness shows promising results for further clinical application. Despite of good expectations, there are several obstacles to such therapy, and many questions are raised: which is the best SC? May there be immunological rejection in cell transplants? What is the best cell implant way without injuring the internal ear cytoarchitecture? How is it possible to remedy the tumor growth of SC? Are the new ciliary cells reinnervated? Will the potential SC therapy substitute the current implants and hearing aids? Will the reconstruction of the whole tonotopic map of the cochlea be possible?

When choosing the most proper SC to the cellular restoration of internal ear, embryonic SC are, in theory, good candidates, once they are able to generate cells of the three germination capsules and have been successfully used in experiments with other diseases (diabetes, Parkinson

and spinal cord injuries). However, there are evidences that some embryonic SC lineages are able to become tumors, creating undesirable tissues after the cell transplant (20). In addition to this, embryo destruction, after SC capitation, is questioned under the ethical point of view.

Neural SC are taken from organs, thus its use would be ethically plausible. Due to the close anatomical and embryological relation between internal ear and brain, neural SC may be used to restore cochlear ciliary cells. Other application would be to treat auditory neuropathies (21).

However, the limited access to neural SC would harm its clinical use, once they are found in deep regions of the brain. On the other hand, mesenchymes SC of bone marrow are easy to obtain and also generate neurons in their differentiation process. Together with produced neurotrophyns, they would increase the number of neurons and would provide support to spiral ganglion, with potential improvement of the cochlear implant efficiency (7).

In vitro comparisons of differentiation potential in ciliary cells of neural SC with the use of adult SC of internal ear evidenced two differences. First, the internal ear SC differentiate 100 times more than neural SC (10% against 0.1%). Second, internal ear CT differentiate more completely in cilliary cells than neural SC (6).

Before such evidences, the use of SC of the organ itself is considered proper as biological repair instrument. Consequently, internal ear SC would be the best choice.

Conclusion

The use of stem cells to treat neorossensorial deafness still seems a distant reality. There are many questions to be answered. However, the initial steps have already been taken when trying to understand the mechanisms involved in the cellular regeneration of internal ear.

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