

Somatotopic principle of perineural implantation of stem cells in patients with brain injuries

Abstract

Background: Neuro destructive processes of any etiology are related to problematic and socially important diseases due to ineffective therapeutic strategy and need to search for new successful ways of treatment and rehabilitation of patient with cerebral infarctions and brain attacks

Aims: Authors plant overify hypothesis on viability of additional use of perineural implantation of autologous mesenchymal stem cells (MSC) in order to optimize standard therapy of patients with brain attacks. Such combined technology is aim datextra activation of brain plasticity mechanisms during development of neuro destructive processes.

Methods: The technique of MSC perineural migration to injured brain regions was experimentally verified on rats (n=40) paying attention to somatotopic organization of cranial nerves.

This technique was clinically tested in pilot project. Phenotyping of autologous MSC from adipose tissue (AT) was performed in 23 patients with brain attacks. These 23 patients received standard treatment as per international guidelines together with three perineural implantations of autologous MSC from AT with 5-9days intervals. The other group of patients (n=7) received only standard therapy as per international guidelines.

Results: Additional use of cell therapy resulted in more rapid and effective recovery of disordered neurological functions in all cases compared to those who received standard therapy. The phenomenon of abrupt recovery of neurological functions was established during first 24hours after each injection of autologous MSC. Cumulative recovery of functions progressed after each implantation.

Discussion and conclusion: Experimentally developed technique of perineural implantation of autologous MSC was successfully verified in clinical conditions in accordance with certified cell therapy guideline (The Ministry of Health of the Republic of Belarus) in combination with standard treatment of patients with cerebral infarctions. Cell therapy with autologous MSC from AT by means of perineural delivery to injured brain regions is the basis for activation of reparative potential of nerve tissue and progressive recovery of neurological functions in patients with cerebral infarctions.

Keywords: brain attack, stem cells, perineural migration, somatotopic principle, brain plasticity

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Abbreviations: SC, stem cells; MSC, mesenchymal stem cells; AT, adipose tissue; GDNF, glial cell derived neuro trophic factor [*Homo sapiens* (human)]; NGFR, nerve growth factor receptor; i/p, intraperitoneally

Introduction

Injuries, strokes and cerebral infarctions hold a specific place in the range of socially important diseases due to high level of mortality in all countries.¹⁻³ Current situation initiates development to completely new methods of early diagnostics, therapy and rehabilitation in neuro destructive processes management. Small success was achieved on the way of increasing effectiveness of therapy at this stage. In particular, indications for SC use are broadened along with standard therapy of patients with neuro destructive processes. The search for “cerebral infarction, stroke, and stem cell” revealed 637 articles in PubMed on July 27, 2018. Promising results have been obtained at earlier stages of cell technologies combination with standard methods of therapy.⁴⁻⁷ However, the number of articles describing side effects of cell technologies also increased.⁸⁻¹¹

Disadvantages of system injection of stem cells (SC) in patients with strokes and brain injuries have been established.^{10,11} Scientists revealed that intravenously or intraarterially administered of SC have extremely low ability to penetrate through blood-brain barrier from blood stream to brain tissue. Administration of SC into cerebrospinal fluid is complicated by craniocaudal flow of liquor. Neurosurgeons inject SC directly into brain tissue, but these manipulations are accompanied with additional surgical intervention (skull trepanation) worsening patient’s state. Perineural administration of mesenchymal stem cells (MSC) into brain appeared to be a kind of way out.¹¹⁻¹³ Enhancement of this technique allowed using somatotopic principle of cranial nerves’ organization for targeted migration of SC to specific brain region.^{11,12,14} In particular, intranasal perineural SC injection mainly through the system of olfactory nerves can be used in case of stroke located in anterior or middle cranial fossa. Localization of destructive focus in posterior cranial fossa requires SC injection into the area of trigeminal nerve endings in inferior nasal concha or directly into Meckel’s cave. The study was aimed at pilot clinical testing of SC perineural transport technique

with attention to somatotopic principle of exogenous SC distribution in the area of brain injuries.

Material and methods

Experimental stage

Wistar rats (n=40) weighing 210-250grams was subjected to brain tissue removal (100µl) by aspiration with micro pipette in a stereotaxic device under ketamine-xylazine-acepromazine anesthesia (55.6, 6.6 and 1.1mg/kg, respectively, i/p). Two groups of rats had bilateral destructions in somatosensory (n=20) and cerebellar (n=20) cortices. MSC suspension (30000 cells labelled by PKH67 green fluorescent linker (Sigma-Aldrich, St. Louis, MO, USA), in 50µl of phosphate-buffered saline) was injected into sub mucosa of nasal cavity or into Meckel's cave in 10min after surgery. 10 rats from each group received microinjection of MSC into sub mucosa of nasal cavity (Figure 1), another 10 received microinjection of MSC into Meckel's cave (Figure 1). The method details are described.¹¹⁻¹⁴ Animals were sacrificed in 0.5, 1.0, 4.0, 8.0, 24.0 and 72.0 hours and in 7, 14 and 21 days after procedures. Brain was extracted, frozen and sliced for confocal laser microscopy (Zeiss AxioVert 200M fluorescent microscope with Zeiss Axio Cam HRm CCD camera, Carl Zeiss, Germany).

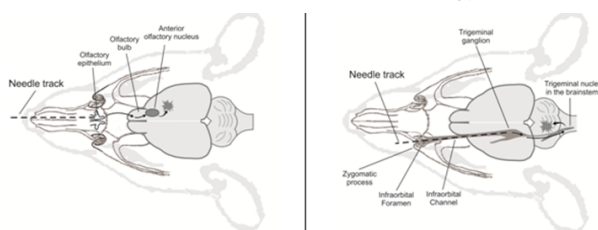


Figure 1 Scheme of stem cells injection in rat using intranasal way (left) and infraorbital channel in to area of Gasser's ganglion in Meckel's cave (right).

Clinical stage early passages of cell cultures of autologous MSC from AT have been obtained from 23 patients with brain strokes aged 47.4 (28.3±65.0) at the stage of cell material preparation for implantation. Viability of cell cultures, proliferative potential, expression of standard and additional phenotypic MSC markers have been comparatively assessed. Concentration of gill cell-derived neurotrophic factor (GDNF) was estimated in MSC AT cultures of patients with brain strokes.

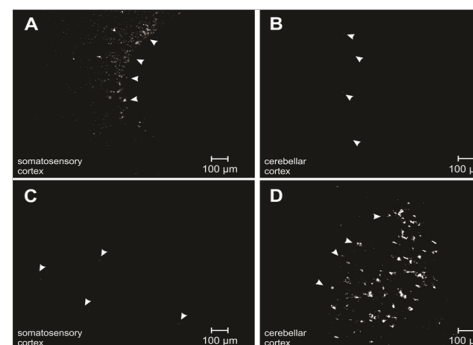
It was revealed that MSC AT cultures of patients with brain strokes correspond to main morpho-phenotypic criteria defined by International Society for Cellular Therapy, express nerve growth factor CD271, maintain proliferative potential and are characterized by spontaneous production of GDNF at early stages of cell cultures pass aging. Preparation was followed by three subsequent perineural endoscopic implantations of autologous MSC from adipose tissue in the amount of 5×10^6 up to 12×10^6 cells to 23 patients with 5-9 days intervals.

Results

Experimental stage

PKH67 signal was observed in olfactory bulb in 0.5 hours and in somatosensory zone in 24.0 hours after intranasal application in rats with somatosensory cortex lesion with the peak on 14th-21st days (Figure 2A). The signal was vague in rats with cerebellar cortex lesion (Figure 2B). Conversely, the signal was detected in rats with

cerebellar cortex lesion in caudal brainstem in 4.0 and 8.0 hours and in cerebellar cortex in 24.0 hours after microinjection into Meckel's cave with the peak on 21st day (Figure 2D). Microinjection into Meckel's cave was followed by weak signal in the damaged area in animals with somatosensory cortex lesion (Figure 2C). Our data demonstrate targeted migration of mesenchymal stem cells towards brain tissue of



anterior and posterior cranial fossa depending on the way of delivery (olfactory or trigeminal routes).

Figure 2 Fluorescent photographs showing the PKH67 labelled cells on 21st day after surgery in the lesioned brain areas contralateral to microinjection site. A and B following intranasal microinjection, C and D following microinjection into Meckel's cave.

Clinical stage

Good to tolerability of cell therapy and absence of toxic reactions and other side effects were registered in all 23 cases of perineural injection of MSC AT. Stable and pronounced recovery of neurological functions was noted during first 24 hours after each MSC injection, and it preserved in future. Initial assessment of neurologic deficiency according to NIHSS scale¹⁵ in 14 patients with initial cerebral infarctions who received both perineural MSC AT and ongoing standard therapy was 10.1 points and 1.9 points in 6 months; no one had re-infarction. Initial assessment of neurologic deficiency according to NIHSS scale in 9 patients with multiple secondary cerebral infarctions after old intracranial hemorrhages was 27.8 points and 14.2 points in 6 months after standard therapy combined with perineural injection of MSC AT.

Initial assessment of neurologic deficiency according to NIHSS scale in 7 patients who received only standard therapy was 11.6 points and 10.2 points in 6 months; two of them (28.6%) had cerebral re-infarctions.

Conclusion

Therefore, cell therapy with autologous MSC from AT by means of perineural delivery to brain has pronounced positive effect on recovery of neurological functions in patients with brain attacks. This effect is based on the principle of somatotopic migration of SC along cranial nerve fibers to brain injuries in specific regions. Less traumatic delivery of exogenous autologous MSC to injured brain regions contribute to activation of reparative processes in nerve tissue and effective recovery of neurological functions in patients with cerebral infarctions.

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Conflict of interest

All listed authors concur with the submission of the manuscript; all authors have approved the final version. The authors have no financial or personal conflicts of interest.

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