DOI 10.1007/s10517-022-05446-x
Cell Technologies in Biology and Medicine, No. 4, March, 2022

CELL TECHNOLOGIES IN BIOLOGY AND MEDICINE

Application of a New Gene-Cell Construct Based on the Olfactory Mucosa Escheating Cells Transduced with an Adenoviral Vector Encoding Mature BDNF in the Therapy of Spinal Cord Cysts

E. K. Karsuntseva[1], G. A. Fursa[2], A. O. Sosnovtseva[1], A. D. Voronova[1], A. V. Chadin[1], A. S. Semkina[1,3], O. V. Stepanova[1,4], and V. P. Chekhonin[1,3]

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 4, pp. 211-216, December, 2021
Original article submitted December 6, 2021

A gene-cell construct based on rat olfactory mucosa ensheathing cells transduced with an adenoviral vector encoding a mature form of brain neurotrophic factor (mBDNF) was transplanted into post-traumatic cysts of rat spinal cord. Transplantation of the gene-cell construct improved motor activity of the hind limbs and reduced the size of cysts in some animals. However, comparison of the effects of transduced and non-transduced ensheathing cells revealed no significant differences. In parallel *in vitro* experiments, a decrease in the proliferation of transduced cells compared to non-transduced cells was observed. It is likely that mBDNF reduces proliferation of transduced cells, which can affect their efficiency. The therapeutic efficacy of the new gene-cell construct is most likely provided by the cellular component.

Key Words: *adenovector; olfactory mucosa ensheathing cells; gene therapy; cell therapy; spinal cord cysts*

Spinal cord injuries remain an important problem in modern medicine, because of the absence of effective treatment protocols [2]. Chronic complications in these injuries primarily result from the death of nerve cells and their low regenerative potential in the area of injury [22]. Cell therapy can be a promising solution to this problem, as evidenced by studies using mesenchymal, neural stem/progenitor, induced pluripotent stem, Schwann, and ensheathing cells (EC) obtained from various sources [1]. The use of olfactory mucosa ECs isolated from a patient with spinal cord injury seems to be optimal for many reasons [1,20]. This technology makes it possible to obtain autologous cell preparations without risk to patient's health [1], and their safety has been shown in clinical trials on the use of ECs for the treatment of spinal cord injuries [1]. In addition, the effectiveness of these cells was demonstrated in previous experiments in the treatment of spinal cord cysts in rats [19,20].

Another advanced trend in personalized medicine for various diseases of the CNS, including spinal cord injuries, is gene therapy based on adenovectors [6,7]. This approach can promote neuroregeneration through the point synthesis of various neurotrophic factors encoded in the adenovector genome [10]. Neurotrophic

[1]V. P. Serbsky National Medical Research Centre of Psychiatry and Narcology, Ministry of Health of the Russian Federation, Moscow, Russia; [2]M. V. Lomonosov Moscow State University, Moscow, Russia; [3]Pirogov Russian National Research Medical University, Moscow, Russia; [4]National Medical Research Center of Cardiology, Ministry of the Health of the Russian Federation, Moscow, Russia. ***Address for correspondence:*** nastyanastyav@mail.ru. A. D. Voronova

factors, the most important of which is brain neurotrophic factor (BDNF) [11], have certain prospects in the treatment of traumatic spinal cord injuries, because they have a neuroprotective effect, reduce neuronal atrophy, and have a positive effect on the dynamics of motor activity recovery [8,14].

The combination of cell and gene therapy is a promising trend in the treatment of spinal cord injuries. First, preliminary transduction of cells allows avoiding direct contact of the adenovector with the immune system [21] and can increase the efficiency of transgene delivery into the tissue and its expression. Second, the cellular component of the construct itself can also have a regenerative potential. Thus, we hypothesize that adenovector transduction of olfactory mucosa EC aimed at additional expression of neurotrophins can improve the efficiency of EC transplantation in experimental spinal cord cysts previously demonstrated by us [19,20].

The aim of this study was to evaluate the effectiveness of a new gene-cell construct based on rat olfactory mucosa EC transduced with an adenoviral vector encoding mature form of BDNF (mBDNF) in the treatment of spinal cord cysts.

MATERIALS AND METHODS

The study was approved by the Local Ethical Committee of the Pirogov Russian National Research Medical University and was carried out in accordance with Directive 2010/63/EU of the European Parliament and the Council of the EU (On the Protection of Animals Used for Scientific Purposes; September 22, 2010).

EC were obtained from the olfactory mucosa of sexually mature male Wistar rats (n=40) weighing 250-300 g according to the previously described method [19]. Enriched cultures of EC (passage 3-4) were used in further experiments. The cells were cultured using Gibco and HyClone reagents.

In CNS neurons, mBDNF is the predominant form of the neurotrophic factor BDNF [4]. In the Department of Fundamental and Applied Neurobiology of the V. P. Serbsky National Medical Research Centre of Psychiatry and Narcology, we have developed a new adenoviral vector encoding mBDNF (Ad5/35-CAG-mBDNF). Adenovector Ad5/35-CAG-Fluc was constructed as a control vector [18]. To create the adenoviral vector, the nucleotide sequence of rat cDNA encoding mBDNF was used. Synthesis of the adenovector containing this transgene, its amplification, purification, storage, and titration were carried out according to the protocols described by us earlier [21].

For further *in vivo* and *in vitro* experiments, we used the minimum effective multiplicity of infection equal to 50 virus particles per cell. Infection of EC with viral particles was carried out in a minimal volume of medium while maintaining the selected multiplicity of infection. In 2 days, the transduced cells were passaged and prepared for transplantation into rat spinal cord cysts.

Post-traumatic cysts of the spinal cord were modeled according to the developed in the Department of Fundamental and Applied Neurobiology of the V. P. Serbsky National Medical Research Centre of Psychiatry and Narcology [23]. In sexually mature female Wistar rats weighing 200 g (n=45), contusion injury at the Th3-Th4 level was modeled. Cyst formation was confirmed 4 weeks after surgery using an MRI scanner for small rodents. Images of the injury site were obtained in sagittal and axial projections and analyzed using MultiVox Dicom Viewer software. Repeated imaging of the spinal cord in the area of injury was performed 4 weeks after cell transplantation.

Cell preparations were transplanted 4 weeks after injury to rats with MRI-confirmed post-traumatic cysts and indices of motor activity of hind limbs not exceeding 13 points according to the 21-point BBB open field scale [3]. The cell preparations were stereotaxically injected into the cyst cavity on the basis of MRI data using an injector in 10 μl DMEM/F-12 medium (1:1). According to our previous study, transplantation of 1.5×10^6 cells is sufficient to provide the therapeutic effect [19]. Therefore, the rats were injected with 1.5×10^6 non-transduced EC (n=7), or 1.5×10^6 cells transduced with Ad5/35-CAG-mBDNF(n=6), or 1.5×10^6 cells transduced with Ad5/35-CAG-Fluc (n=6). Control animals (n=9) were injected with the same volume of DMEM/F-12 medium (1:1).

To assess the effectiveness of cell transplantation, motor activity of the hind limbs was assessed according to the BBB scale weekly over 4 weeks.

In parallel, *in vitro* experiments were carried out to assess the proliferation of EC after transduction with adenovectors Ad5/35-CAG-mBDNF and Ad5/35-CAG-Fluc. To this end, the cells were seeded at a seeding density of 10^4 per 1 cm^2. On the next day, the cells were infected according to the transduction protocol. On days 1, 2, 4, 6, 8, and 11, the cells were harvested with 0.1% trypsin and counted using a TC20 automatic counter (Bio-Rad).

The results were statistically analyzed by the Tukey's test and Dunn's test using GraphPad Prism 8 (GraphPad Software, Inc.). The data are presented as $M\pm SD$. The differences were significant at $p<0.05$.

RESULTS

The effectiveness of recovery of hind limb mobility after transplantation of cell preparations was assessed

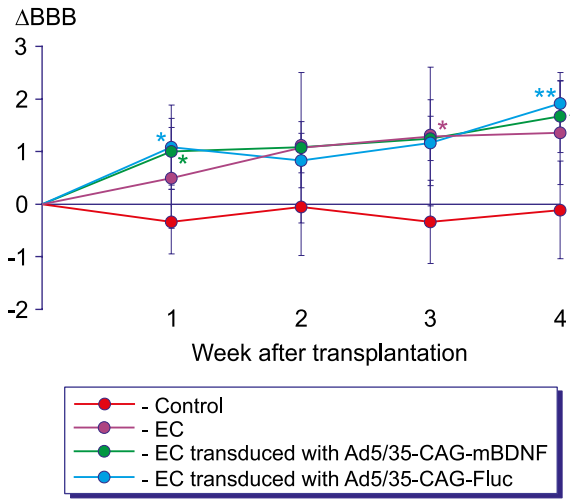

Fig. 1. Changes in the parameters of motor activity of the hindlimbs of rats after transplantation of 1.5×10^6 rat EC into spinal cord cysts. Changes in BBB scores (ΔBBB) are presented. *p<0.05, **p<0.01 in comparison with the control (Dunn's test).

using BBB test. After transplantation of all cell preparations, a positive dynamic of hind limb recovery was observed throughout the experiment (4 weeks), while in the control group, no positive dynamics was found (Fig. 1). Significant differences after administration of non-transduced EC were observed in 3 weeks (p=0.0177), Ad5/35-CAG-Fluc-transduced EC after 1 and 4 weeks (p=0.023 and p=0.0055, respectively), and EC transduced with Ad5/35-CAG-mBDNF also after 1 and 4 weeks (p=0.023 and p=0.0315, respectively). Thus, the revealed effect is apparently due to the influence of the cellular component of the new adenovector construct Ad5/35-CAG-mBDNF. We have previously shown that olfactory mucosa EC survive in the area of chronic injury over 30 days and migrate into the tissue of the spinal cord [19]. Therefore, these cells could influence neuroregeneration throughout the entire experiment.

Another criterion for the efficiency of transplantation of Ad5/35-CAG-mBDNF-transduced EC was the size of the cysts before and after EC transplantation into the damaged area (Fig. 2). MRI showed that the percentage of spinal cord cyst reduction in the experimental groups was practically the same. Significant differences from the control were observed after transplantation of EC transduced with Ad5/35-CAG-mBDNF (p=0.0404) and Ad5/35-CAG-Fluc (p=0.0101). These findings suggest that the effect was produced by the cellular component of the developed adenovector construct, which corresponds to the results obtained in our previous study [19].

The data of MRI scans of the spine at the Th3-Th4 level 4 weeks after contusion injury and after transplantation of Ad5/35-CAG-mBDNF transduced cells are shown in Figure 3. MRI images in other experimental groups demonstrated the same trend (data not shown).

The revealed therapeutic effects were probably due to the cell component of the construct. *In vitro* experiments were performed to determine the cause of the ineffectiveness of mBDNF. The autocrine effect of mBDNF on the proliferation of transduced cells was assessed, because it is the number of cells that is an important criterion for successful cell therapy [13]. On day 7 of the experiment, a tendency towards a decrease in the proliferation of Ad5/35-CAG-mBDNF-transduced cells was observed, while non-transduced and Ad5/35-CAG-Fluc-transduced cells produced no such an effect (Fig. 4). At the same time, significant differences were observed on day 11 during culturing of Ad5/35-CAG-mBDNF-transduced EC in comparison with non-transduced EC (p=0.0032) and EC transduced with Ad5/35-CAG-Fluc (p=0.0029). Thus, we believe that mBDNF present in the gene-cell construct slows down the proliferation of transduced cells, and the new gene-cell construct studied by us is not the best for cell therapy, where the number of cells is undoubtedly an important factor.

According to published data, administration of neurotrophic factor BDNF has a positive effect in the treatment of various spinal cord injuries [9], though some researchers do not confirm its effectiveness [8]. Based on our *in vitro* experiments, we believe that transduced cells divide more slowly than non-transduced ones. Hence, after transplantation into the site of injury, the number of transduced cells may also be lower than that of non-transduced cells. This can negate the expected effect of mBDNF, which is why the Ad5/35-CAG-mBDNF-transduced cells are similar in their effect to non-transduced EC.

In addition to the negative influence of the autocrine effect on the rate of cell proliferation, the absence of a pronounced effect of Ad5/35-CAG-mBDNF transduced cells can be related to other mechanisms.

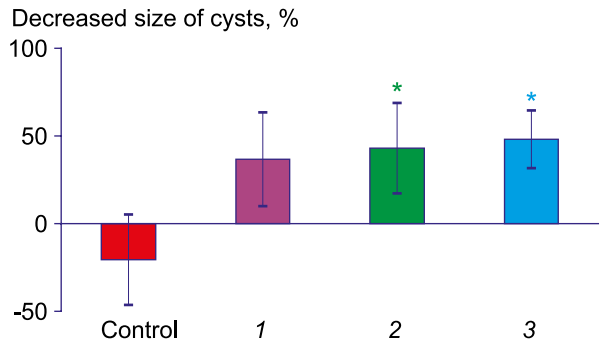

Fig. 2. Change in the size of post-traumatic cysts in rat spinal cord after transplantation of non-transduced EC (*1*) or EC transduced with Ad5/35-CAG-mBDNF (*2*) or Ad5/35-CAG-Fluc (*3*) in a dose of 1.5×10^6 per rat. *p<0.05 in comparison with the control (Dunn's test).

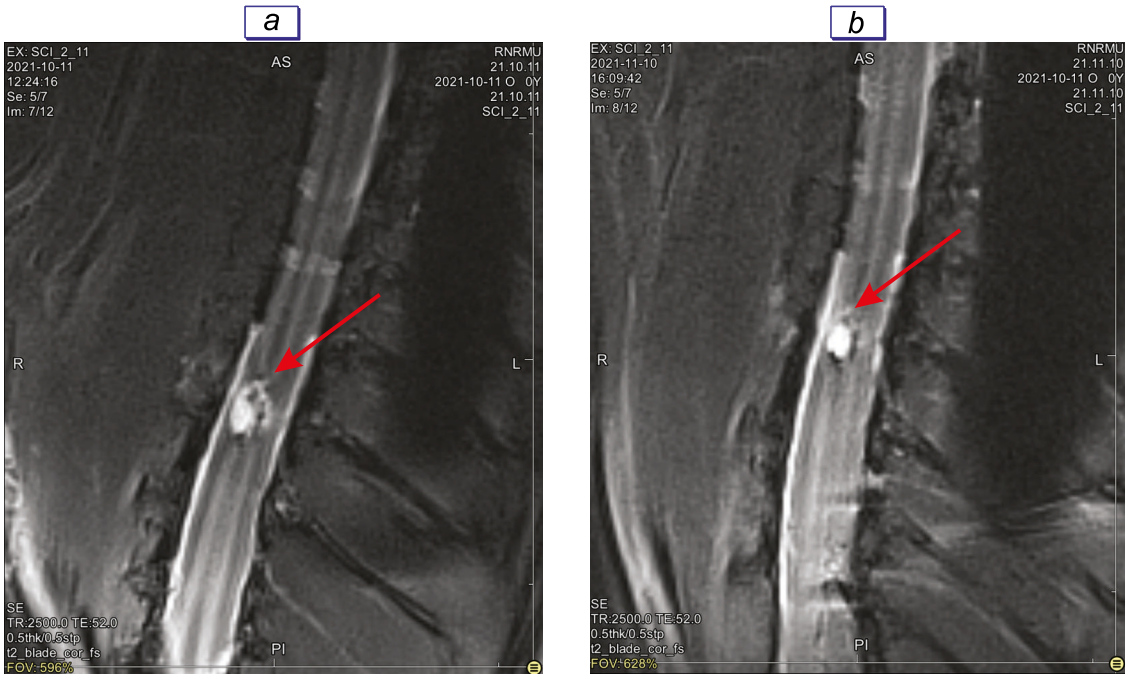

Fig. 3. MRI image of spinal cord cyst (arrow) in the sagittal projection before (*a*) and 4 weeks after (*b*) transplantation of EC transduced with Ad5/35-CAG-mBDNF in a dose of 1.5×10^6 per rat. A decrease in cyst size by 71% was revealed.

The neurotrophic factor BDNF can suppress debris phagocytosis by EC in the damaged area [5], which slows down the processes of neuroregeneration. Also, mBDNF binds only to the TrkB receptor expressed by not all neurons [15], which means that not all of them interact with mBDNF.

The therapy of various spinal cord injuries with gene-cell constructs is only developing part of regenerative medicine. Variable viral constructs consisting of neurotrophins and different types of cells are created in laboratories. For instance, a gene-cell construct developed from human umbilical cord blood mononuclear cells transduced with genes encoding VEGF and GDNF promotes remyelination of neuronal axons [16,17]. A construct was created from bone marrow stromal cells transduced with an adenoviral vector encoding BDNF, which supports trophic function and neuronal regeneration [12].

Our study showed that the efficiency of rat olfactory mucosa EC transduced with an adenoviral vector encoding mBDNF is probably determined by the cellular component. BDNF may not be the best therapeutic molecule for treating spinal cord injuries. Adenovector constructs based on EC with other neurotrophic factors may be more effective. Autologous EC have shown their effectiveness in the treatment of spinal cord injuries and can be further used in personalized medicine.

This work was supported by the Russian Science Foundation (grant No. 17-15-01133).

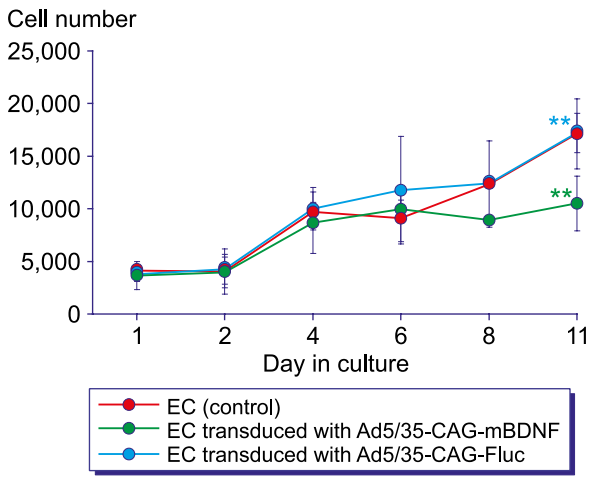

Fig. 4. Proliferation of rat EC *in vitro*. $^{**}p<0.01$ in comparison with the control (Tukey test).

REFERENCES

1. Voronova AD, Stepanova OV, Chadin AV, Reshetov IV, Chekhonin VP. The Cell Therapy in Traumatic Spinal Cord Injury. Vestn. Ross. Akad. Med. Nauk. 2016;71(6):420-426. doi: 10.15690/vramn731
2. Alizadeh A, Dyck SM, Karimi-Abdolrezaee S. Traumatic spinal cord injury: an overview of pathophysiology, models and acute injury mechanisms. Front. Neurol. 2019;10:282. doi: 10.3389/fneur.2019.00282
3. Barros Filho TE, Molina AE. Analysis of the sensitivity and reproducibility of the Basso, Beattie, Bresnahan (BBB) scale

in Wistar rats. Clinics (Sao Paulo). 2008;63(1):103-108. doi: 10.1590/s1807-59322008000100018
4. Bathina S, Das UN. Brain-derived neurotrophic factor and its clinical implications. Arch. Med. Sci. 2015;11(6):1164-1178. doi: 10.5114/aoms.2015.56342
5. Baydyuk M, Wu XS, He L, Wu LG. Brain-derived neurotrophic factor inhibits calcium channel activation, exocytosis, and endocytosis at a central nerve terminal. J. Neurosci. 2015;35(11):4676-4682. doi: 10.1523/JNEUROSCI.2695-14.2015
6. Blits B, Dijkhuizen PA, Hermens WT, Van Esseveldt LK, Boer GJ, Verhaagen J. The use of adenoviral vectors and ex vivo transduced neurotransplants: towards promotion of neuroregeneration. Cell Transplant. 2000;9(2):169-178. doi: 10.1177/096368970000900204
7. Blits B, Oudega M, Boer GJ, Bartlett Bunge M, Verhaagen J. Adeno-associated viral vector-mediated neurotrophin gene transfer in the injured adult rat spinal cord improves hind-limb function. Neuroscience. 2003;118(1):271-281. doi: 10.1016/s0306-4522(02)00970-3
8. Fouad K, Bennett DJ, Vavrek R, Blesch A. Long-term viral brain-derived neurotrophic factor delivery promotes spasticity in rats with a cervical spinal cord hemisection. Front. Neurol. 2013;4:187. doi: 10.3389/fneur.2013.00187
9. Hassannejad Z, Zadegan SA, Vaccaro AR, Rahimi-Movaghar V, Sabzevari O. Biofunctionalized peptide-based hydrogel as an injectable scaffold for BDNF delivery can improve regeneration after spinal cord injury. Injury. 2019;50(2):278-285. doi: 10.1016/j.injury.2018.12.027
10. Ingusci S, Verlengia G, Soukupova M, Zucchini S, Simonato M. Gene therapy tools for brain diseases. Front. Pharmacol. 2019;10:724. doi: 10.3389/fphar.2019.00724
11. Koda M, Hashimoto M, Murakami M, Yoshinaga K, Ikeda O, Yamazaki M, Koshizuka S, Kamada T, Moriya H, Shirasawa H, Sakao S, Ino H. Adenovirus vector-mediated in vivo gene transfer of brain-derived neurotrophic factor (BDNF) promotes rubrospinal axonal regeneration and functional recovery after complete transection of the adult rat spinal cord. J. Neurotrauma. 2004;21(3):329-337. doi: 10.1089/089771504322972112
12. Koda M, Kamada T, Hashimoto M, Murakami M, Shirasawa H, Sakao S, Ino H, Yoshinaga K, Koshizuka S, Moriya H, Yamazaki M. Adenovirus vector-mediated ex vivo gene transfer of brain-derived neurotrophic factor to bone marrow stromal cells promotes axonal regeneration after transplantation in completely transected adult rat spinal cord. Eur. Spine J. 2007;16(12):2206-2214. doi: 10.1007/s00586-007-0499-3
13. Li J, Lepski G. Cell transplantation for spinal cord injury: a systematic review. Biomed. Res. Int. 2013;2013:786475. doi: 10.1155/2013/786475
14. Liu S, Sandner B, Schackel T, Nicholson L, Chtarto A, Tenenbaum L, Puttagunta R, Müller R, Weidner N, Blesch A. Regulated viral BDNF delivery in combination with Schwann cells promotes axonal regeneration through capillary alginate hydrogels after spinal cord injury. Acta Biomater. 2017;60:167-180. doi: 10.1016/j.actbio.2017.07.024
15. Liu X, Obianyo O, Chan CB, Huang J, Xue S, Yang JJ, Zeng F, Goodman M, Ye K. Biochemical and biophysical investigation of the brain-derived neurotrophic factor mimetic 7,8-dihydroxyflavone in the binding and activation of the TrkB receptor. J. Biol. Chem. 2014;289(40):27 571-27 584. doi: 10.1074/jbc.M114.562561
16. Mukhamedshina YO, Gilazieva ZE, Arkhipova SS, Galieva LR, Garanina EE, Shulman AA, Yafarova GG, Chelyshev YA, Shamsutdinova NV, Rizvanov AA. Electrophysiological, morphological, and ultrastructural features of the injured spinal cord tissue after transplantation of human umbilical cord blood mononuclear cells genetically modified with the VEGF and GDNF genes. Neural Plast. 2017;2017:9857918. doi: 10.1155/2017/9857918
17. Mukhamedshina YO, Rizvanov AA. Genetically modified human umbilical cord blood cells as a promising strategy for treatment of spinal cord injury. Neural Regen. Res. 2016;11(9):1420-1421. doi: 10.4103/1673-5374.191213
18. Stepanenko AA, Sosnovtseva AO, Valikhov MP, Chekhonin VP. A new insight into aggregation of oncolytic adenovirus Ad5-delta-24-RGD during CsCl gradient ultracentrifugation. Sci. Rep. 2021;11(1):16088. doi: 10.1038/s41598-021-94573-y
19. Stepanova OV, Voronova *A*D, Chadin AV, Valikhov MP, Abakumov MA, Reshetov IV, Chekhonin VP. Isolation of rat olfactory ensheathing cells and their use in the therapy of posttraumatic cysts of the spinal cord. Bull. Exp. Biol. Med. 2018;165(1):132-135. doi: 10.1007/s10517-018-4114-x
20. Stepanova OV, Voronova AD, Chadin AV, Valikhov MP, Semkina AS, Karsuntseva EK, Chekhonin IV, Shishkina VS, Reshetov IV, Chekhonin VP. Efficiency of human olfactory ensheathing cell transplantation into spinal cysts to improve mobility of the hind limbs. Stem Cells Dev. 2019;28(18):1253-1263. doi: 10.1089/scd.2019.0092
21. Stepanova OV, Voronova A, Sosnovtseva AO, Stepanenko AA, Chadin A, Karsuntseva E, Fursa GA, Valikhov M, Semkina A, Vorobyev PO, Reshetov I, Chekhonin V. Study of the therapeutic efficiency of transduced olfactory ensheathing cells in spinal cord cysts. Stem Cells Dev. 2021. doi: 10.1089/scd.2021.0265
22. Xu W, Chi L, Xu R, Ke Y, Luo C, Cai J, Qiu M, Gozal D, Liu R. Increased production of reactive oxygen species contributes to motor neuron death in a compression mouse model of spinal cord injury. Spinal Cord. 2005;43(4):204-213. doi: 10.1038/sj.sc.3101674
23. Zhang C, Morozova AY, Abakumov MA, Gubsky IL, Douglas P, Feng S, Bryukhovetskiy AS, Chekhonin VP. Precise delivery into chronic spinal cord injury syringomyelic cysts with magnetic nanoparticles MRI visualization. Med. Sci. Monit. 2015;21:3179-3185. doi: 10.12659/msm.895624