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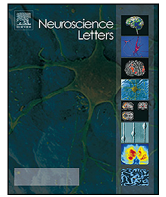

Research article

A pilot study of cell-mediated gene therapy for spinal cord injury in mini pigs

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HIGHLIGHTS

- Intrathecal injection of the gene-cell constraction was used for the first time for SCI treatment in large animal mini-pig SCI model.
- Results provide evidence of feasibility and potential efficacy of proposed UCBC-based delivery of three therapeutic genes therapy.
- Observed recovery in treated animals support the positive effect of the gene-cell constriction after SCI.

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ABSTRACT

Currently, in clinical practice there is no efficient way to overcome the sequences of neurodegeneration after spinal cord traumatic injury. Using a new experimental model of spinal cord contusion injury on miniature pigs, we proposed to deliver therapeutic genes encoding vascular endothelial growth factor (VEGF), glial cell line-derived neurotrophic factor (GDNF) and neural cell adhesion molecule (NCAM) to the damaged area, using umbilical cord blood mononuclear cells (UCBC). In this study, genetically engineered UCBC (2×10^6 cells in 200 ml of saline) were injected intrathecally to mini-pigs 10 days after SCI. Control and experimental mini pigs were observed for 60 days after surgery. Histological, electrophysiological, and clinical evaluation demonstrated significant improvement in animal treated with genetically engineered UCBCs. Difference in recovery of the somatosensory evoked potentials and in histological findings in control and treated animals support the positive effect of the gene-cell constriction for recovery after spinal cord injury. Results of this study suggest that transplantation of UCBCs simultaneously transduced with three recombinant adenoviruses Ad5-VEGF, Ad5-GDNF and Ad5-NCAM represent a novel potentially successful approach for treatment of spinal cord injury.

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1. Introduction

Spinal cord injury (SCI) is classified as a neurotrauma, although it is followed by disturbance in blood supply, neurodegenerative and other pathological consequences [1]. Regardless numerous animal studies and clinical trials, to this time there is no successful clinical protocol for SCI treatment. To enhance the efficacy of SCI therapy along with experiments on rodents and clinical trials, experiments

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on large animals models appear to be highly important. Most of the previous studies on neuroregeneration were performed on rodents and reported positive results require further investigations on large animal models similar in anatomy and physiology to human [2–4]. Between others available experimental models mini pigs are seem to be one of the optimal because of anatomic, physiologic and ethical reasons [2]. The importance of performing experiments on swine is dictated by several reasons, such as (1) anatomical considerations, thus, the length of neuritis in human is more then 100 times longer compare to rodent, (2) sharing with human common physiological characteristics, (3) pharmaceutical reasons, such the dosage and the way of drug delivery is close to human. These details are vary from model to model and particularly between the small and large animals and the positive results obtained on rodents may not be directly applicable on patients [5,6]. Current progress in gene therapy for spinal cord injury aimed to protect neurons and stimulate the growth of neuritis [7]. Both the direct gene therapy, as well as a cell-mediated gene therapy for spinal cord injury, is actively studied during the last years. The therapeutic genes and types of cells for genes delivery are diverse, although the best gene or gene-cell constriction for stimulation of neuroregeneration is still has to be identified [8]. Up to date a big variety of cell types and viral vectors are employed for cell-based gene delivery into CNS in rodents. Direct gene therapy or cell-based gene delivery following the SCI was successful in preservation of neurons and neuronal fibers and in improving locomotion in rats [9–11]. In our previous experiments, using the model of spinal cord injury in rats and transgenic G93A mice with amyotrophic lateral sclerosis (ALS), we successfully developed the gene-cell construction based on umbilical cord blood mononuclear cells (UCBC) and adenoviral vectors expressing neurotrophic factors (VEGF and GDNF) and neural cell adhesion molecule (NCAM) [12]. Adenoviral vector carrying GDNF for direct gene or UCBC-mediated GDNF gene therapy of spinal cord contusion in rat showed advantages of both methods. Co-expression in UCBC of recombinant VEGF and NCAM or GDNF and NCAM also showed symptomatic improvement, increased life span and sustained cell homing after transplantation into ALS mice. Our results suggest that the tandem overexpression of VEGF, GDNF (as a therapeutic molecules) and NCAM (as a molecule for survivability and addressed homing of UCBC) in genetically engineered UCBC can be useful in treatment of SCI in mini pigs. Based on our previous results with UCBC-mediated gene therapy on SCI rat model, we proposed a pilot experiment on mini pigs with genetically engineered UCBC. The goal of this study was to develop and evaluate methodological aspects of a new treatment strategy of UCBS transplantation combined with three therapeutic genes in a large animal model of the mini-pig. To address this goal, we investigated the therapeutic efficacy of UCBC transduced with VEGF, GDNF and NCAM after intrathecal transplantation in mini-pigs with spinal cord contusion.

2. Methods

This study was conducted in compliance with local IACUC protocol (institutional animal protocol at Kazan State Medical University (KSMU), # 5, from May 27, 2014) and IRB protocol based on the license of 'Stem Cell Bank' of KSMU. All pregnant women were signed informed consent for using their umbilical cord blood and were thoroughly examined before the delivery for possible contraindications for umbilical cord blood cell donation. Umbilical cord blood was collected by trained personnel of the maternity hospital according to the instruction of Stem Cell Bank of KSMU.

Preparation of genetically modified human umbilical cord blood mononuclear cells

2.1. Adenoviral vectors generation

The plasmids and the recombinant human adenoviruses of serotype 5 Ad5-VEGF165, Ad5-GDNF, and Ad5-NCAM1 were obtained using VEGF165, GDNF, and NCAM1 genes, respectively. The nucleotide sequences encoding VEGF165 (Gene Bank NM_001171626.1), GDNF (Gene Bank NM_019139.1) and NCAM1 (Gene Bank NM_001076682.2) were obtained by chemical synthesis in "Evrogen" JSC. The AdEasy Adenoviral Vector System was used to construct Ad5-VEGF165, Ad5-GDNF and Ad5-NCAM1 according to the manufacturer's instructions. rAds were grown in HEK-293 and purified by exclusion chromatography. The titers of Ad5-VEGF165, Ad5-GDNF and Ad5-NCAM1 (2×10^9 PFU/ml, 4×10^9 PFU/ml and 1×10^9 pfu/ml, respectively) were determined by the plaque formation technique in the HEK-293 cell culture.

2.2. Cell preparation

Umbilical cord blood was obtained from healthy women during normal delivery. The blood was processed in accordance to the protocol of the legitimate and ethical standards generally accepted in the stem cell bank of the Kazan State Medical University. Mononuclear fraction of human UCB was isolated by standard technique of sedimentation on to a density barrier (Ficoll, 1.077 g/ml) as described previously [13]. After purification UCBCs were cultivated in RPMI-1640 medium supplemented with 10% FBS and mixture of antibiotics penicillin and streptomycin (100 U/ml, 100 micrograms/ml) (Sigma, USA). UCBC were seeded in 10 cm culture dish and simultaneously transduced with three recombinant adenoviruses Ad5-VEGF, Ad5-GDNF and Ad5-NCAM at MOI 10. Afterwards cells were incubated for 12–16 h in a humid environment at a temperature of +37 °C with 5% CO_2 content, washed with DPBS solution and prepared for transplantation to experimental group of animals.

2.3. Animals and treatments

In the study 6-month-old female Vietnamese Pot-Bellied miniature pig weighing 5 kg were employed. For two weeks before the surgery animals were kept solely in a housing area with 12 h light/dark cycles, controlled temperature (24–25 °C) and water/food supply. All experimental procedures were performed in accordance with the Kazan State Medical University Animal Care and Use Committee guidelines.

2.4. Contusion spinal cord injury model

At the day of surgery pre-medication with intramuscular injection of ketamine (5 mg/kg) and diazepam (0.5 mg/kg) was performed. Antibiotic cefazolin (60 mg) was given intramuscular before surgery. Anesthesia was induced with propofol and afterwards animals were endotracheally intubated and maintained on isoflurane (1.3%). Rectal temperature was evaluated continuously during surgery and electrophysiological tests and regulated with thermal blankets. The operative area was cleaned with povidone-iodine, dressed with sterile material. Intravenous infusion and postoperative drug administration was performed via the venous catheter. An urinary catheter was placed during the surgery and was maintained post-operatively. Contusion model with weight-drop device was used in our group over last years and was designed based on spinal cord contusion rodent model described previously [3,14]. Briefly, for SCI a laminectomy was performed at the Th9–Th10 vertebral level and a custom modified weight-drop device ensured falling of the 50-g weight from the 50 cm height on the spinal cord causing the contusion injury [15].

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