Research Article

Gene-modified leucoconcentrate for personalized *ex vivo* gene therapy in a mini pig model of moderate spinal cord injury

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Abstract

We previously demonstrated that gene-modified umbilical cord blood mononuclear cells overexpressing a combination of recombinant neurotrophic factors are a promising therapeutic approach for cell-mediated gene therapy for neurodegenerative diseases, neurotrauma, and stroke. In this study, using a mini pig model of spinal cord injury, we proposed for the first time the use of gene-modified leucoconcentrate prepared from peripheral blood in the plastic blood bag for personalized *ex vivo* gene therapy. Leucoconcentrate obtained from mini pig peripheral blood was transduced with a chimeric adenoviral vector (Ad5/35F) that carried an enhanced green fluorescent protein (EGFP) reporter gene in the plastic blood bag. The day after blood donation, the mini pigs were subjected to moderate SCI and four hours post-surgery they were intravenously autoinfused with gene-modified leucoconcentrate. A week after gene-modified leucoconcentrate therapy, fluorescent microscopy revealed EGFP-expressing leucocytes in spinal cord at the site of contusion injury. In the spleen the groups of EGFP-positive cells located in the lymphoid follicles were observed. *In vitro* flow cytometry and fluorescent microscopy studies of the gene-modified leucoconcentrate samples also confirmed the production of EGFP by leucocytes. Thus, the efficacy of leucocytes transduction in the plastic blood bag and their migratory potential suggest their use for temporary production of recombinant biologically active molecules to correct certain pathological conditions. This paper presents a proof-of-concept of simple, safe and effective approach for personalized *ex vivo* gene therapy based on gene-modified leucoconcentrate autoinfusion. The animal protocols were approved by the Kazan State Medical University Animal Care and Use Committee (approval No. 5) on May 27, 2014.

Key Words: chimeric Ad5/35F virus; enhanced green fluorescent protein; gene-modified leucoconcentrate; mini pig; peripheral blood; personalized *ex vivo* gene therapy; plastic blood bag; spinal cord injury

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Introduction

Cell-mediated gene therapy is an attractive approach for treatment of hereditary genetic diseases. Thus transduction of peripheral blood lymphocytes with a retroviral vector carrying gene encoding adenosine deaminase was successfully employed in the 1990s for *ex vivo* gene therapy of severe combined immunodeficiency (Blaese et al., 1995). The method was improved to use hematopoietic stem cells (HSCs) in 2002, and it was the first approved approach in Europe for the correction of inherent gene dysfunction with 100% survival and established safety and efficacy (Aiuti et al., 2017). In clinical trials, a similar approach was used to treat X-linked adrenoleukodystrophy. The method is based on autotransplantation of genetically modified HSCs in which the mutated ABCD1 gene was genetically corrected using a lentiviral vector (Cartier et al., 2009). Recently, *ex vivo* gene engineering of a patient's T-lymphocytes to express chimeric antigen receptor (CAR) was approved for cancer cells targeting (Zheng et al., 2018).

Another strategy of cell-mediated gene therapy suggests using cells as gene carriers and as producers of secretory therapeutic molecules (Qu et al., 2019). Gene modified stem (Neirinckx et al., 2015; Manley et al., 2017; Dalamagkas et al., 2018) and mature differentiated (Li et al., 2016; Papapetrou, 2017) cells are widely used for treatment of somatic diseases in pre-clinical (Mooney et al., 2017; Chellappan et al., 2018) and clinical trials (Ha et al., 2015).

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In this study, we for the first time proposed the use of peripheral blood gene-modified leucoconcentrate (GML) for autotransplantation to allow the temporary production of recombinant biologically active molecules to correct certain pathological conditions. Earlier we suggested (Islamov et al., 2017a) using umbilical cord blood mononuclear cells (UCB-MCs) as carriers simultaneously for three genes encoding glial cell line-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), and neural cell adhesion molecule (NCAM) to stimulate neuroregeneration in the central nervous system (CNS). We intravenously infused gene-modified UCB-MCs into amyotrophic lateral sclerosis transgenic mice (Islamov et al., 2017a) and intrathecally injected *ex vivo* transduced UCB-MCs into a rat model of stroke (Sokolov et al., 2018) and mini pigs with spinal cord contusion injury (SCI) (Islamov et al., 2017b). Collectively, these studies established the rationale of using leucocytes for targeted delivery of recombinant genes that encode therapeutic molecules in the CNS. The significant disadvantages of using UCB-MCs as a cell carrier for gene therapy are a relatively small amount of these cells for a single infusion, impossibility of repeated infusions of cells from the same donor, and the risks associated with allotransplantation. All this imposes a severe limitation for wide use of UCB-MCs for *ex vivo* gene therapy in adults. Thus the employment of the leukocytes (leucoconcentrate) obtained from the patient's peripheral blood may be an attractive alternative for delivery of recombinant genes, supporting short time production of the biologically active molecules for pathogenic therapy of various somatic disorders. Since the mononuclear cells in GML have a long period of life, the production of the therapeutic molecules depends on the effective expression for adenovirus vectors which is limited to about 2 weeks in humans (Crystal, 2014).

The other important question for delivery of a recombinant gene into the leucocytes is the design of a viral vector. Adenoviral vectors (serotype 5, Ad5) are widely used because of thier short term expression in different types of eukaryotic cells (Qu et al., 2019). The level of cells transduced with Ad5 depends on cell types and transduction methods (*in vivo* or *ex vivo* approaches) (Denby et al., 2004; Nicklin et al., 2004). For more effective transduction of the peripheral blood leucocytes, we used a chimeric Ad5/35F virus with modified fiber, which has high affinity to cluster of differentiation 46 (CD46) that is expressed on all nuclear blood cells (Adams et al., 2011).

The goal of this pilot trial was to present the new strategy for personalized cell-mediated gene therapy based on autoinfusion of gene-modified leucoconcentrate prepared from peripheral blood and transduced with adenoviral vectors with modified fibres (Ad5/35F) in a plastic blood bag. In this study, the potential of GML therapy was examined using a mini pig model of spinal cord injury (SCI).

Materials and Methods

Animals and treatments

We employed mature 8-month-old Vietnamese pot-bellied miniature female pigs (30 kg; n = 3) obtained from Federal Center for Toxicological, Radiation and Biological Safety (Kazan, Russia). Two weeks prior to the experiment, animals were maintained separately in a housing area with a 12-hour light/dark photoperiod, controlled temperature (24–25°C) and air conditioning with organized access to food and water. The animal protocols were performed according to ethical procedures for the use of animals in laboratory experiments and conducted in strict compliance with the guidelines established by the Kazan State Medical University Animal Care and Use Committee (approval No. 5, approved on May 27, 2014).

The day before the surgery, animals were anesthetized with an intramuscular injection of Zoletil 100 (Virbac Laboratoires, Carros, France; 10 mg/kg) and Xyla (Interchemie werken "De Adelaar" B.V., Castenray, the Netherlands; 40 mg/kg) in the back of the neck, and 100 mL of blood was collected from the subclavian vein into a plastic blood bag. Subsequently, animals were re-infused with 100 mL of saline via the auricular vein. The animals were housed separately and allowed to recover. On the next day, a moderate SCI was performed as described previously (Islamov et al., 2017b). In brief, under deep anesthesia with Zoletil 100 (10 mg/kg) and maintained with 2.5% isoflurane (Laboratorios Karizoo, SA, Barcelona, Spain) in mixture with oxygen, animals underwent a laminectomy at the T8–9 vertebral level, followed by a contusion injury with a custom made weight-drop device (Jones et al., 2012). Specifically, a 50-g weight fell on the spinal cord from a 50-cm height. Four hours after SCI, mini pigs received intravenous autoinfusion of 30 mL of GML via the auricular vein, prepared as described below (**Figure 1**).

Construction of the chimeric Ad5/35F virus

The recombinant plasmid pAd5/35F-EGFP containing a full Ad5 genome, the chimeric type 5 and 35 fiber gene and an enhanced green fluorescent protein (EGFP) reporter gene under the control of a cytomegalovirus (CMV) immediate early promoter was generated by homologous recombination in *Escherichia coli* as described previously (Rogozhin et al., 2011). Briefly, the pZ35 plasmid contained the full Ad5 genome with deletion of the E1 region and the chimeric fiber gene. pZ35 was linearized with *Pac*I and mixed with pShuttle-CMV-EGFP plasmid (Stratagene, La Jolla, CA, USA) digested with *Pme*I. *E. coli* BJ5183 cells were electroporated with the DNA mixture using the MicroPulser electroporation apparatus (Bio-Rad, Hercules, CA, USA) according to manufacturer's instructions. Bacterial colonies obtained after 18–24 hours of incubation at 30°C were analyzed by polymerase chain reaction (PCR) and restriction endonuclease digestions. The resulting molecular clone was tested by multiple restriction enzyme digestions and then sequenced. The recombinant chimeric Ad5/35F-EGFP virus was obtained via lipofection of the *Pac*I-linearized pAd5/35F-EGFP plasmid into human embryonic kidney cell line 293 (HEK-293 cells, ATCC CRL-11268) using Lipofectamine 2000 (Thermo Fisher Scientific, Carlsbad, CA, USA) according to the product protocol. An assembly of the recombinant Ad5/35F-EGFP virus in HEK-293 cells was detected visually by the development of the characteristic cytopathic effect. Identity of the chimeric virus was verified by PCR and diagnostic restriction enzyme digestions. The recombinant

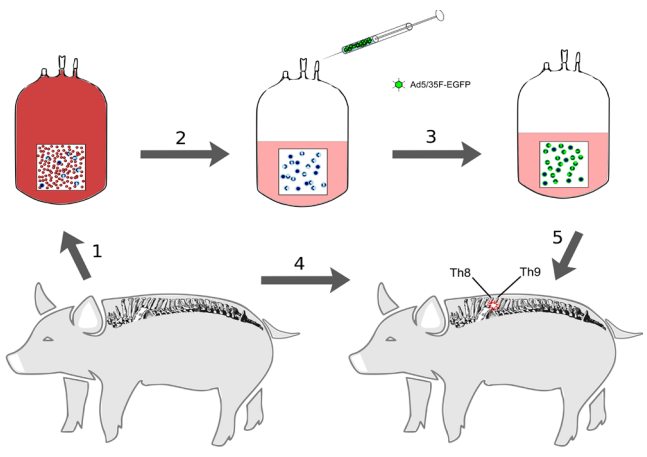

Figure 1 | **Experiment design.**
In mini pigs, 100 mL of blood was collected from *v.subclavia* for preparation of leucoconcentrate (1). The obtained leukoconcentrate was transduced using Ad5/35F-EGFP (2) and after 14 hours, the prepared GML-EGFP was stored at room temperature until use (3). A day after blood sampling from the animal, contusion injury of the spinal cord was performed (4) and GML-EGFP was administered via v.auricularis 4 hours after modeling neurotrauma (5).

chimeric Ad5/35F-EGFP virus was purified and concentrated using size-exclusion and anion exchange chromatography. The viral sample titre was determined by a viral particle (vp) absorbance at 260 nm using a conversion factor of 1 OD unit equavelent to 1.12×10^{12} vp/mL (Maizel et al., 1968). Specific infectivity of the chimeric Ad5/35F-EGFP virus was assessed by a plaque formation assay using HEK-293 cells.

GML preparation

Mini pig peripheral blood was collected into a plastic blood bag with 35 mL of anticoagulant-preservative solution (CPDA). Leucoconcentrate preparation included three steps. (1) An equal volume of 6% hydroxyethyl starch was added to 100 mL of blood; the bag was placed upside down, centrifuged (DP-2065 R PLUS, Centrifugal Presvac RV; Presvac, Buenos Aires, Argentina) at 34 × *g* for 10 minutes at 10°C, and the erythrocyte layer was discarded. (2) The bag was again centrifuged as above, and the supernatant was expressed into a new plastic blood bag using an FK-01 manual plasma extractor (Leadcore, Ekaterinburg, Russia). (3) Saline was added to the bag at a 1:9 ratio, and the bag was centrifuged at 490 × *g* for 10 minutes at 10°C. After this, the supernatant was discarded from the bag, and the remaining cells in the bag were considered as leukoconcentrate. The obtained leucoconcentrate was immediately added to the Ad5/35F-EGFP virus that carried the EGFP reporter gene (multiplicity of infection [MOI] = 3), according to the white blood cell (WBC) count in the leucoconcentrate. After 12-hour transduction, saline was added to the bag at a 1:9 ratio, the mixture was centrifuged at 290 × *g* for 10 minutes at 10°C, and the supernatant was squeezed out of the bag. The remaining solution in the bag (approximately 30 mL) was considered GML that carried the EGFP gene (GML-EGFP) and was used for the *in vitro* study and autoinfusion.

In vitro GML study

A complete blood count (CBC) was performed to evaluate naïve peripheral blood and leucoconcentrate transduced with adenoviral vector carrying reporter green fluorescent protein (Ad5/35F-EGFP) before intravenous autoinfusion into mini pig was performed using the Sysmex XP-300 (Sysmex Corporation, Kobe, Japan). To assess EGFP expression, GML-EGFP were seeded on 10-cm untreated culture dishes and incubated for 72 hours in RPMI-1640 medium (PanEco, Moscow, Russia) supplemented with 10% fetal bovine serum (FBS; Biosera, Nuaille, France) and a mixture of 100 U/mL penicillin and 100 µg/mL streptomycin (Thermo Fisher Scientific, Waltham, MA, USA). The cells were cultured at 37°C in a humidified incubator with 95% O_2 and 5% CO_2. EGFP-positive cells were observed with an inverted fluorescence microscope (Ziess Z1AxioObserver, Ziess, Oberkochen, Germany) and evaluated by flow cytometry using a BD FACS Aria III (BD Biosciences, San Jose, CA, USA). Flow cytometry data were expressed as a percentage of EGFP-positive cells.

In vivo GML study

One week after SCI, mini pigs were intramuscularly injected with Zoletil 100 (10 mg/kg) and Xyla (40 mg/kg) via the back of the neck. Under deep anesthesia maintained with 2.5% isoflurane, animals were infused with potassium chloride (150 mg/kg) via the auricular vein. A 5-cm-long spinal cord segment centered at the site of contusion and a fragment of spleen were removed and fixed in 4% paraformaldehyde (Sigma). Afterwards, samples of spinal cord and spleen were cryoprotected in 30% sucrose, embedded in tissue freezing medium, and frozen 20 µm sections were prepared using a Microm HM 560 cryostat (Thermo Fisher Scientific). Slide-mounted sections were incubated in DAPI solution (10 µg/mL in PBS; Sigma), embedded in antifade mounting medium and studied under a laser scanning confocal microscope (LSM 510-Meta) (Zeiss, Jena, Germany).

Statistical analysis

To analyze the obtained complete blood count values, the R 3.5.3 (R Foundation for Statistical Computing, Vienna, Austria) software was used. Sample mean was used to represent central tendency and unbiased sample standard deviation was applied as a variability measure. *T*-test was used for comparison between GML and peripheral blood assay data (cell counts were log2 transformed to estimate fold changes), and $P < 0.05$ were considered statistically significant.

Results

Complete blood counts

For analysis, the samples of naïve peripheral blood were obtained from the plastic blood bag immediately after blood collection and the samples of GML-EGFP were collected from the plastic blood bag 12 hours after transduction of the leucoconcentrate with Ad5/35F-EGFP. Complete blood counts of the naïve peripheral blood and GML-EGFP preparation are presented in **Table 1**. The resulting volume of GML (30 mL) was more than three times less than the collected blood volume. Notably, GML had three times lowered the number of WBCs ($P = 0.002$) which may be due to five times reduced count of granulocytes ($P = 0.015$) and six times of monocytes ($P = 0.003$) when compared to the collected peripheral blood. The number of lymphocytes was approximately equal in GML and in the blood from the plastic bag. Red blood cells (RBCs) were 19-fold lowered ($P = 0.001$) in the GML compared to the peripheral blood. In our opinion, the highly reduced number of RBC allows for effective WBC transduction in the plastic blood bag.

EGFP reporter gene expression *in vitro*

To study EGFP gene expression *in vitro*, the GML-EGFP samples were cultured for 72 hours after transduction. Fluorescent microscopy using the green channel revealed EGFP-positive WBCs with intensive green fluorescence surrounded by negative erythrocytes. Round shaped EGFP-positive cells by their size may be classified as monocytes and lymphocytes (**Figure 2A**). Flow cytometry analysis demonstrated that 0.6% of WBCs in gene-modified leucoconcentrate (GML-EGFP) efficiently express green fluorescent protein. Thus, 3 days after transduction of mini pig leucoconcentrate with Ad5/35F-EGFP with MOI = 3 the obtained gene-modified leucoconcentrate (GML-EGFP) contained 0.6% EGFP-positive WBCs (**Figure 2B**).

EGFP reporter gene expression *in vivo*

EGFP reporter gene expression was studied using fluorescent microscopy in the post-traumatic spinal cord and spleen 7 days after intravenous GML-EGFP autoinfusion to the mini pigs

Table 1 | **Complete blood count for peripheral blood and GML**

Sample	WBC ($\times10^9$/L)	RBC ($\times10^{12}$/L)	HGB (g/L)	HCT (%)	PLT ($\times10^9$/L)	LYM ($\times10^9$/L)	MON ($\times10^9$/L)	GRAN ($\times10^9$/L)	Volume (mL)
Peripheral blood	8.30±1.77	4.54±1.52	74.75±24.60	25.00±7.93	265.75±55.72	4.21±2.08	0.48±0.23	3.57±2.34	130±17.32
GML	14.8±2.07*	1.18±0.84*	19.25±12.84*	8.10±5.68*	246.00±67.46*	10.88±1.63	0.38±0.21*	3.54±1.37*	26±7

Evaluation of the naïve mini pig peripheral blood and leucococentrate transduced with adenoviral vector carrying reporter green fluorescent protein (Ad5/35F-EGFP) before intravenous autoinfusion into mini pig. Results are presented as the mean ± standard deviation. *$P < 0.05$, *vs*. peripheral blood. GML: Gene-modified leucoconcentrate; GRAN: granulocytes; HCT: hematocrit; HGB: hemoglobin; LYM: lymphocytes; MON: monocytes; PLT: platelet; RBC: red blood cell; WBC: white blood cell.

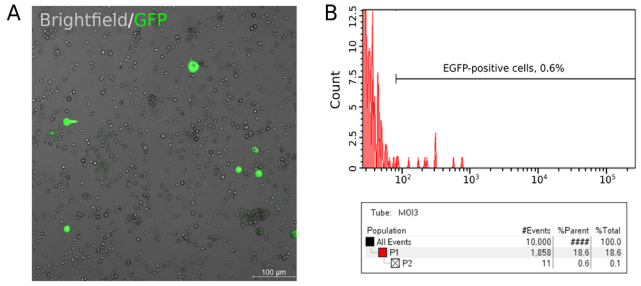

Figure 2 | ***In vitro* study of the EGFP reporter gene expression in gene-modified leucoconcentrate GML-EGFP 72 hours after transduction of the leucoconcentrate with Ad5/35F-EGFP (multiplicity of infection = 3).**
(A) Fluorescent microscopy using the green channel demonstrates EGFP-positive white blood cells. (B) Flow cytometry analysis demonstrates that 0.6% of white blood cells in gene-modified leucoconcentrate (GML-EGFP) efficiently express green fluorescent protein. EGFP: Enhanced green fluorescent protein; GML: gene-modified leucoconcentrate.

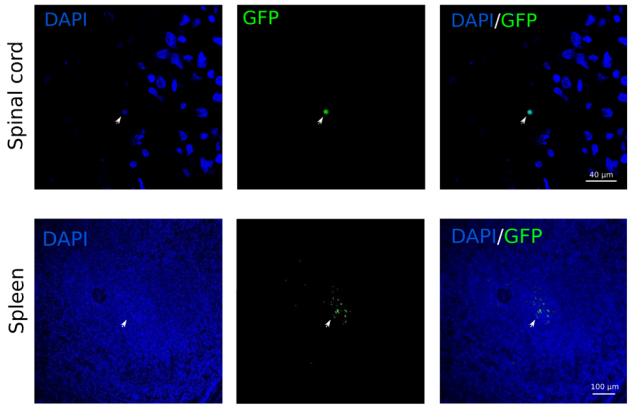

Figure 3 | **Fluorescent examination of the EGFP reporter gene expression in mini pig spinal cord (upper panel) and spleen (lower panel) 7 days after neurotrauma and subsequent intravenous autoinfusion of gene-modified leucoconcentrate GML-EGFP.**
In the spinal cord at the level of epicenter injury, arrows indicate the EGFP-positive cell which corresponds to the morphology of the mononuclear leucocytes with round nuclei. In the spleen, arrows indicate the group of EGFP-positive cells with the same morphology in lymphoid follicles. Colors: Green = EGFP; blue = DAPI. DAPI: 4′,6-Diamidino-2-phenylindole; EGFP: enhanced green fluorescent protein; GML: gene-modified leucoconcentrate.

with SCI. In the spinal cord, EGFP-positive cells were observed in rostral, epicentral and caudal segments (**Figure 3**). In each studied 20 µm-thick cross-section of the spinal cord, one or two EGFP-positive cells may be found. The morphology of EGFP-positive cells corresponds to the mononuclear leucocytes with round nuclei. In the spleen from mini pigs 7 days after GML-EGFP autoinfusion, groups of EGFP-positive cells located in the lymphoid follicles of white pulp were revealed (**Figure 3**). EGFP-positive cells had the same morphology as the cells found in spinal cord. Thus, gene-modified WBCs after intravenous autoinfusion may circulate in the bloodstream throughout the body, migrate into post-traumatic spinal cord tissue and lymphoid tissue of the spleen, and efficiently produce reporter green fluorescent protein.

Discussion

Contemporary gene and cell technologies are widely used in pre-clinical and clinical trials for the treatment of congenital and acquired pathology. An important factor in the development of cell-mediated gene therapy methods is employing cellular material from the patient for genetic modification and subsequent autotransplantation. We previously showed positive results in amyotrophic lateral sclerosis (Islamov et al., 2017a), SCI (Islamov et al., 2017b; Izmailov et al., 2017) and stroke (Sokolov et al., 2018) animal models with gene-modified UCB-MCs that produce recombinant VEGF, GDNF and NCAM. These results demonstrated the rationale for using the UCB mononuclear fraction for *ex vivo* gene therapy (Mukhamedshina and Rizvanov, 2016). Moreover, different populations of UCB cells have already been used in clinical trials for treatment of non-hematopoietic disorders (Ichim et al., 2010; Yang et al., 2010; Liu et al., 2013; Yao et al., 2013; Zhu et al., 2016). In spite of all mentioned advantages of UCB cells, there are currently no approved protocols to use gene-modified UCB-MCs for temporary production of recombinant biologically active molecules for pathogenetic treatment of somatic disorders.

For the first time, we proposed the use of WBCs obtained from patient's peripheral blood as a cell system for personalized *ex vivo* gene therapy. The simplicity of the method lies in obtaining a leucoconcentrate and its subsequent transduction in a standard blood bag without any risks in *in vitro* manipulations (culturing, using animal products and antibiotics). The maximum possible elimination of RBCs and the use of a chimeric Ad5/35F virus with modified fiber, which has high affinity to cluster of differentiation 46 (CD46) that is expressed on all nuclear blood cells (Adams et al., 2011), allows generation of GML in a plastic blood bag the day after blood donation.

There are several rationales for using peripheral WBCs for *ex vivo* personalized gene therapy. (1) WBCs possess high synthetic and secretory activity. (2) WBCs have specific abilities to migrate from the bloodstream to body tissues. (3) The CD46 molecule on the WBC surface provides efficient transduction with adenoviral vectors with modified fibers (Ad5/35F). (4) WBCs may be transduced with one or more recombinant therapeutic genes. (5) *Ex vivo* WBC transduction prevents the direct toxic and immunogenic effects of the adenoviral vector on the recipient. (6) The GML provides temporary production of recombinant biologically active protein molecules in the recipient. (7) The genetic vector concentration during leucoconcentrate transduction or the amount of GML used for infusion allows researchers/clinicians to control the level of therapeutic molecules produced in the recipient. (8) Repeated GML autoinfusion is possible. (9) GML can be infused systemically (intravenous) or locally (intramuscular and subcutaneous). (10) Blood centers can produce GML using approved genetic constructs that contain human therapeutic genes.

Translational research about novel therapeutic protocols requires experimental studies on large laboratory animals that possess anatomical, physiological, and biochemical characteristics close to humans. Among the known model organisms, mini pigs are considered the most optimal for pre-clinical trials. Mini pigs for testing cell-mediated gene therapy protocols have several advantages, including pharmacological requirements related to the available cellular material, dosage and methods of delivering the drug to the target organ. Besides, mini pigs are highly suitable for the investigation of blood components for personalized therapy.

In the present study, the proposed concept of personalized *ex vivo* gene therapy based on an individual's GML was demonstrated in a mini pig model of SCI. GML individual for each mini pig was prepared the day before SCI and was intravenously infused four hours after surgery. Post-surgery recovery of the experimental animals corresponded to the severity of SCI (Islamov et al., 2017b) and GML autoinfusion did not reveal any specific reaction in the mini pigs. One week post-surgery, fluorescent microscopy of the spinal cord revealed WBCs that expressed EGFP. The presence of EGFP-positive cells in the white pulp of the spleen suggests that transduced lymphocytes migrated into their original site in the organism and effectively produced recombinant protein.

Thus the data of this study and our previous results

on therapeutic efficacy of gene engineered UCB-MCs overexpressing GDNF, VEGF, and NCAM for the treatment of amyotrophic lateral sclerosis, stoke, and SCI in animal models suggests that GML carrying the same combination of therapeutic genes may be as well efficient for stimulation of neuroregeniration in the CNS. Recombinant GDNF produced by WBCs will act as a neuroprotective factor (Sondell et al., 1999; Cheng et al., 2002). The expression of VEGF, as a neuroprotective and angiogenic factor (Zhang et al., 2000), and NCAM in WBCs is proposed to increase homing of the gene-modified leucocytes in the CNS (Islamov et al., 2017a). However, the use of GML is not limited to be used for the treatment of neurological disorders. The potential of GML therapy is determined by combinations of recombinant therapeutic human genes. For example, genes encoding VEGF and angiogenin may be useful for stimulation of neovascularization, lactoferrin, and defensin to enhance innate immunity in bacterial infections, bone morphogenic proteins (BMP2 and BMP7) for bone regeneration.

However, the present investigation should be considered as a pilot study, especially due to the use of the reporter gene (egfp) and not therapeutic genes for the preparation of GML. Additional experiments are also needed to optimize transduction efficiency when generating lecoconcentrate in plastic blood bags. Further pre-clinical studies are required to address the potential clinical problems such as the control of the produced recombinant therapeutic molecules level and their possible side effects.

Conclusion

In the present study, we demonstrated a proof-of-concept of personalized *ex vivo* gene therapy based on an individual's gene-modified leucoconcentrate. Our results demonstrated the simple, safe and effective approach for preparation of GML from peripheral blood and adenoviral vectors with modified fibres (Ad5/35F) in a plastic blood bag. Efficacy of transduction, migratory potential and secretory activity of WBCs obtained from the mini pig peripheral blood suggest their use for temporary production of the specific recombinant biologically active molecules for pathogenetic therapy of variable nosology, including trauma, ischemia, degeneration, autoimmunity, infection, and others.

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