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Research Article

Assessment of physiological parameters in the application of a double adeno-associated virus 9 with a codon-optimized DYSF gene for limb girdle muscular dystrophy type R2

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Abstract

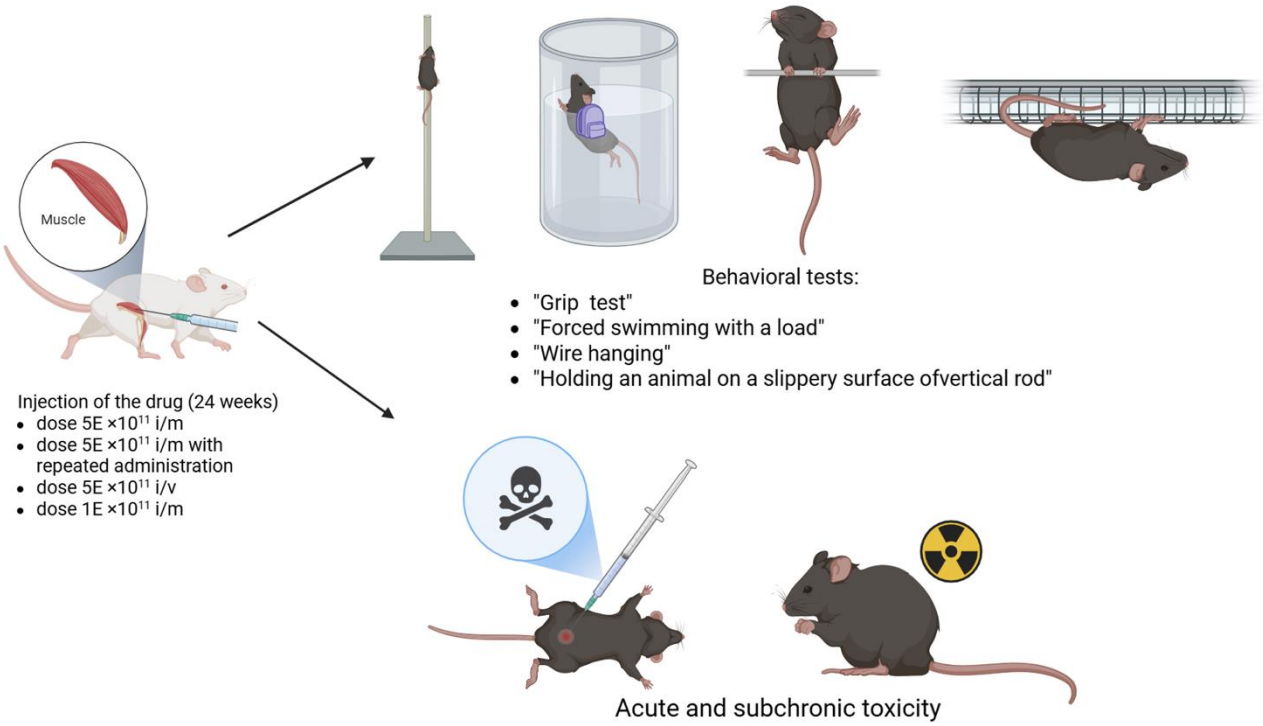
Introduction: Gene therapy for Myoshi myopathy is extremely relevant, as it may become the first pathogenetic treatment for dysferlinopathy. **The aim** of this study was to study the efficacy and safety of the use of a genetic construct, the AAV9-DYSF-DV3' virus, for the treatment of limb girdle muscular dystrophy LGMD) type R2.

Materials and Methods: Mouse models of limb girdle muscular dystrophy type R2 B6.A-Dysf^{prmd}/GeneJ were used to study the effectiveness of AAV9.DYSF drug and the corresponding C57BL/6J controls were used. During the study, muscle activity was determined on the basis of the following tests: "Grip test", "Holding an animal on a slippery surface of a vertical rod", "Forced swimming with a load", and "Wire hanging". In the course of acute and subchronic toxicity, hematological and biochemical blood tests of the rats, histological analysis and "Open field" behavioral testing were performed.

Results and Discussion: In this study, for the first time, a comprehensive investigation of the effectiveness of gene therapy using the two-vector system of adeno-associated AAV9-DYSF-DV3' virus with overlapping DYSF cDNA sequences was conducted in a mouse model of limb girdle muscular dystrophy type 2 R.

Conclusion: During the testing of the drug's effectiveness, it was discovered that drug AAV9.DYSF showed the best effectiveness in mice with the absence of the protein dysferlin in behavioral testing at the maximum dose ($5*10^{12}$) with a double intramuscular injection. In the "Grip test", the index in B6.A-Dysf^{prmd}/GeneJ mice increased by 29% (p=0.0026) relative to that in the K-group. In the tests "Forced swimming with a load", "Wire hanging", and "Holding an animal on a slippery surface of a vertical rod", the indicators also improved by 80% (p=0.0019), 104.8% (p=0.001) and 20% (p=0.025), respectively, relative to those of the negative control. During acute and subchronic toxicity, the administration of the drug to animals does not cause death or intoxication.

Graphical abstract



Keywords

dysferlin, LGMD R2, dual AAV vector, gene transfer, muscle regeneration

Introduction

Modern achievements in medicine and pharmacology have contributed to the development of new methods of pathogenetic therapy. According to statistics from the World Health Organization (WHO), congenital and hereditary diseases occur in 10–15% of newborns (Polikarpova et al. 2022). Neuromuscular diseases, which are caused by the irreversible accumulation of conformationally altered proteins, occupy a special place among them (Berezhnova et al. 2023). This group of pathologies includes dysferlinopathies, diseases associated with a violation of the structure and functions of the dysferlin protein (Zorin et al. 2017; Begam et al. 2018).

The prevalence of this type of disease, according to the WHO, ranges from 2.3 to 10 cases per 100,000 (from 1: 44,000 to 1: 10,000). Limb girdle muscular dystrophy type R2 is the fourth most common type in terms of prevalence among muscular dystrophies (Fanin and Angelini 2016; Wang et al. 2024). More than 25 forms of LGMD have been identified, which differ in severity of the disease, age of manifestation, phenotypic manifestations, and pathological changes in muscle tissue (Reash et al. 2022; Bouchard et al. 2023).

The division of subtypes of LGMD R2 disease depends on the resulting mutation: calpain, dysferlin, collagen VI, sarcoglycans, anoxamine 5 and 6, and protein bound to fucutin (Patel et al.

2017; Moore et al. 2021). In Eurasia, the calpain-related mutation is the main cause of LGMD R2, followed by the mutation associated with the fucutin-related protein (Khodabukus et al. 2024).

Dysferlin plays an important role in sarcolemmas, but it also has several other functions. First, it participates in membrane repair, and second, its function in phagocytosis has been proven. Third, it participates in the regulation of myogenin (Nagy et al. 2017; Park et al. 2021).

One of the approaches for treating this disease can be gene therapy. One of the safest vectors for muscle tissue at the moment is the vector of the adeno-associated virus (AAV). To create the drug, the researchers used a strategy with two independent AAV vectors with an overlapping sequence, one containing the 5' end of the cDNA and the other containing the 3' end. The introduction of both vectors into protein-deficient mouse muscles leads to the expression of a full-size protein; therefore, this approach can be applied to create a gene therapy drug from LGMD R2 (Yakovlev et al. 2023).

The purpose of this study was to investigate the effectiveness of the a genetic construct, AAV9.DYSF for the correction of LGMD R2. During this study, physiological tests were conducted to confirm the effectiveness of the treatment, and also toxicological studies of gene therapy were conducted.

Materials and Methods

Experimental animals

A mouse subline was used to study the effectiveness of the drug B6.A-Dysf^{prmd}/GeneJ (Bla/J) (n=96). C57BL/6J healthy control mice (n=10) were used. The animal cohorts were obtained by crossing A/J mice (#:000646), in which a spontaneous insertion in intron 4 was accidentally detected, with wild-type C57BL/6J mice. The colony was maintained and propagated by crossing mutant animals from the same litter. The presence of the mutant gene was confirmed via PCR analysis (Korokin et al. 2022).

For subchronic toxicity, Wistar rats of both sexes were used (n=240). BALB/c mice of both sexes were used in the acute toxicity study (n=40).

The experimental and control animals were kept in a pathogen-free vivarium at Belgorod State National Research University (NRU BelSU) in artificially regulated daylight hours (12 hours of dark and 12 hours of daylight) at temperatures ranging from +22°C to +26°C and had free access to food and water.

The study was conducted at the Research Institute of Pharmacology of Living Systems (Belgorod). The experiment was performed according to the Rules of Laboratory Practice, approved by Order No. 708n of the Ministry of Health of the Russian Federation of August 23, 2010, in strict compliance with the European Convention for the Protection of Vertebrate Animals Used for Experiments or for Other Scientific Purposes (Directive 2010/63/EU). The experimental studies were approved by the Bioethical Commission of Belgorod State National Research University (Minutes No. 15/10 dated Oct 29, 2021). Vivisection was performed in compliance with the ethical principles of treating laboratory animals outlined in the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes (CETS No. 123).

Drugs under study

To create AAV9. DYSF, a previously described codon-optimized sequence of the DYSF gene (Starostina et al. 2012), was used. Plasmid constructs containing the 5′ and 3′ sections of the DYSF gene were generated. The 5′ cassette included the MHCK7 promoter, a chimeric intron, and the first 3370 bp of the dysferlin cDNA, flanked by inverted terminal repeats. The 3′ cassette contained 3866 bp of dysferlin cDNA and the native 3′ nontranslated region, including a poly(A) sequence. The plasmid cassettes were used for the separate assembly of the double AAV vectors. The double AAV vector included DNA samples with a 1387 bp overlapping region serving as a substrate for recombination to create the codon-optimized dysferlin cDNA (of the canonical dysferlin isoform O75923-1). The pAAV.5′ and pAAV.3′ plasmids were used to generate two separate adeno-associated viruses of serotype 9 following the standard triple-transfection protocol. The quality of AAV9.DYSF. OVERLAP5′ and AAV9.DYSF. OVERLAP3′ virus levels were determined by staining on a denaturing polyacrylamide gel (SDS–PAGE) (Yakovlev et al. 2023).

Experimental groups

Groups of mice were formed from the same litter with synchronous birth dates, the selection was carried out according to weight, and the animals were distributed into groups in random order (Table 1).

Table 1. Distribution of animals by group

Group	AAV1	AAV2	*K_1-	AAV3	*K_2-	AAV4	*K_3-	*K_4-	K+ (healthy mice)
Mode of introduction	$1*10^{12}$	$5*10^{12}$	0.9% NaCl	$5*10^{12}$ (reintroduction)	0.9% NaCl	$5*10^{12}$	0.9% NaCl	0.9% NaCl	0.9% NaCl
	50 mcL i/m for each muscle, hind limbs					150 mcL i/v		i/v	150 mcL i/m
No of animals in the group	14	14	10	14	10	14	10	10	10

Note: * – Mice with a DYSF gene knockout.

Behavioral testing was performed 3 months after the initial injection of the drug.

Analysis of motor function in mice

"Grip test"

The installation consists of a stainless steel mesh connected to a sensor for measuring the grip force (in grams) of the mouse's forelimbs. The animal was allowed to grab onto a horizontal grid with its front paws, and then the mouse was pulled back by the tail until its grip loosened, while the hind paws of the mouse did not touch the grid. The force sensor maintained the peak value of the thrust force. The test is used to study the functions of the neuromuscular system. The average values of 5 successful measurements of forelimb strength were used for the analysis. (García-Campos et al. 2020; Nagaraju et al. 2000).

"Wire hanging"

The test is based on the instincts of the mice to avoid falling. Each mouse was placed on a horizontally stretched wire with all four limbs gripped (wire diameter 3 mm, height above the surface 60 cm). The ability to stay on the wire is measured via a stopwatch by estimating the time the mouse keeps from falling until it falls. The best result of two attempts was taken as the final value, with a 20-minute pause between them (Shi et al. 2021).

"Forced swimming with a load"

The physical performance of the animals in this test was assessed by the duration of swimming with a load that was 5% of the body weight (the weight of the load was determined experimentally), which was attached to the root of the tail of the animal via a rubber bandage. The weight of the animals was determined with an accuracy of 0.1 g, and the load was selected with an accuracy of 0.01 g. The duration of the test (swimming) was recorded via a stopwatch with an accuracy of 1 second. The end of the experiment was considered to be the moment of animal fatigue onset, the sign of which was the animal's inability to float to the surface of water for 5 seconds or refusal to swim (dive to the bottom for more than 5 seconds). A sign of animal fatigue is a violation of motor coordination (rotation around its axis and rolling on its side in the water column) (Patel et al. 2023).

Swimming was carried out in organic glass vessels with an inner diameter of 30 cm and a height of 60 cm. The height of the water column was 30 cm, and the water temperature was 23±1°C.

"Holding an animal on a slippery surface of a vertical rod"

In studies on laboratory animals, this technique has been used mainly for screening and assessing the safety of physical performance. The installation consists of a tripod with a rod (diameter of 7 mm and a height of 60 cm) with a plastic fence mounted on top. For the experiment, the animal was placed at the same distance from the top of the rod, strictly upside down, and at least 1 m above the floor. The time at which the animal fell from the rod was recorded via a stopwatch. The safety of physical performance was assessed via comparisons with the control group.

"Open field"

The animal under study was placed in an installed IR Actimeter (Panlab, Harvard apparatus). The following indicators were evaluated: total locomotor activity (the number of crossed squares), the number of wall posts, the number of urinations and defecations, and autogrooming. Each animal was tested for 5 minutes under home lighting.

Acute toxicity

Acute toxicity has been studied in mature laboratory mice. This test system was selected under the Guidelines for Conducting Preclinical Drug Trials. The acute toxicity study was performed with a single intravenous injection of the test drug at a maximum allowable volume of 0.2 mL into the tail vein of a mouse. The detection of mortality, serious conditions, assessment of general health, and signs of toxicity was carried out continuously for two hours after drug administration and then every hour up to 12 hours after drug administration. In the following days, the data were recorded daily. The animals were weighed before drug administration and during the observation period on the 2^{nd}, 8^{th} and 15^{th} days of the experiment. The animal's body weight, which was determined after a night of fasting before euthanasia and necropsy, was used to calculate the ratio of organ mass to body weight. On the eve of the necropsy day, at 16:00, the animals were deprived of food, but had free access to water. All the animals were subjected to complete necropsy, which included an external examination, an examination of the injection site, all the openings, body cavities and their contents.

Subchronic toxicity

Subchronic toxicity and local irritant effects were studied in mature laboratory rats. This test system was selected under the Guidelines for Conducting Preclinical Drug Trials. The drug at various doses was injected once intravenously into the tail vein of each rat. The choice of doses for the study of subchronic toxicity was made so that the studied doses were $1x10^{12}$/L, $3x10^{12}$/L and $30x10^{12}$/L, which was equivalent to the therapeutic, maximum therapeutic and ten times the maximum therapeutic doses for humans, respectively.

Histological examination

For histological examination, biological material was poured into paraffin wax in a carousel-type machine in standard mode STP-120 (Microm International GMbH, Germany) via a battery made of ethyl alcohol and xylene. The blocks with the standard orientation of the pieces were filled at a station for pouring biological material into paraffin EC 350 (Microm International GMBH, Germany). To ensure standardization, they were filled with paraffin in the form of multiblocks of 5-6 pieces. Sections for histological examination of 5 microns thickness were made on a semi-automatic rotary microtome with a system for transporting and straightening the sections HM 340E (Microm International GMbH, Germany). Hematoxylin and eosin (HE) staining was performed with a machine to stain histological sections and smears (Microm International GMbH, Germany).

Hematological and biochemical blood examination

For hematological analysis, blood was collected in EDTA tubes. The measurements were performed on an ABBOTT CELL-DYN 3700 automatic hematology analyzer. For biochemical analysis, blood was collected in tubes with a coagulation activator. After coagulation, the blood was centrifuged and analyzed on an Olympus AU 640 biochemical analyzer.

Statistical processing

Statistical processing was performed using GraphPad Prism Software 8.0 (GraphPad Software Inc., USA). Depending on the feature distribution type and equality of variances, the significance of the results obtained was assessed via a parametric (ANOVA) or nonparametric (Kruskal–Wallis test) one-way analysis of variance, and an unpaired Student's t test was used as a post hoc analysis to identify differences in intergroup comparisons, the Mann–Whitney test, with Benjamini–Hochberg correction for multiple hypothesis testing. The results were considered significant at $p \leq 0.05$.

Results and Discussion

The results of the present study revealed that the maximum effect in the "Grip test" (Fig. 1) was observed in the AAV3 i/m group after intramuscular repeated administration of the maximum dose of the virus 2 months after initial administration (p=0.0026) compared with the group of mice with *DYSF* gene knockout (B6.A-Dysf^{prmd}/GeneJ), which did not differ from that of healthy mice in the K+ group, indirectly indicating the restoration of muscle fiber volume. The muscle strength index also increased in the groups that received a single administration of the maximum dose of the drug ($5 * 10^{12}$), regardless of the route of administration. In the AAV2 i/m and AAV4 i/v groups, the indices increased by 24.5% (p=0.0021) and 17% (p=0.0239), respectively, compared with those in the group negative control. The lowest efficacy in this test was shown by the group receiving the lowest dose of the drug via the intramuscular route of AAV1 i/m (p=0.0426).

The best results in the ”Forced swimming with a load” test (Fig. 2) were shown by the groups with the maximum dose of intramuscular injection of the drug ($5*10^{12}$) AAV2 i/m or AAV3 i/m, and there were no differences in efficacy between the groups with a single injection of the drug and those with repeated administration, and differences between the experimental groups and the *DYSF* (K-) gene knockout group accounted for 85% and 80% (p=0.0043; p=0.0019), respectively. An intermediate increase in the duration of swimming was shown by the group with a minimum ($1*10^{12}$) intramuscular dose of AAV1 i/m, which was 59% (p=0.054) greater than that of the group negative control. Compared with the negative control group, the intravenous group showed no significant differences.

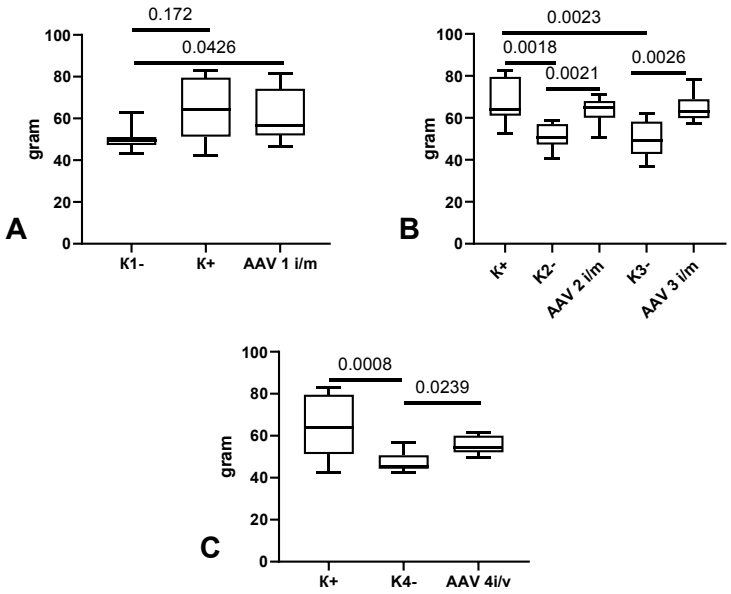


Figure 1. Analysis of the grip strength of the forelimbs in the ”Grip test”. ***Note:*** **A** – results of the physiological Bla/J test of mice with *DYSF* gene knockout after intramuscular administration of $1*10^{12}$ units of virus with the *DYSF* gene (AAV1 i/m); **B** – results of the physiological test of Bla/J mice with *DYSF* gene knockout after i/m of $5*10^{12}$ units of *DYSF* gene virus (AAV2 i/m) and Bla/J mice with *DYSF* gene knockout after i/m of $5*10^{12}$ units of *DYSF* gene virus with repeated drug administration (AAV3 i/m); **C** – results of the physiological Bla/J test of mice with *DYSF* gene knockout after i/v of $5*10^{12}$ units of virus with the *DYSF* gene (AAV4 i/v).

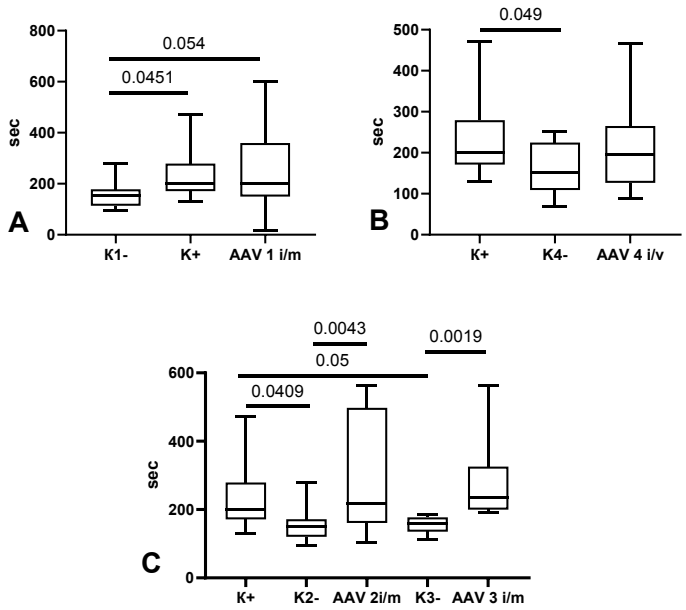


Figure 2. Analysis of the endurance score in the test “Forced swimming with a load”. ***Note:*** **A** – results of the physiological Bla/J test of mice with *DYSF* gene knockout after intramuscular administration of $1*10^{12}$ units of virus with the *DYSF* gene (AAV1 i/m); **B** – results of the physiological test of Bla/J mice with *DYSF* gene knockout after i/m of $5*10^{12}$ units of *DYSF* gene virus (AAV2 i/m) and Bla/J mice with *DYSF* gene knockout after i/m of $5*10^{12}$ units of *DYSF* gene virus with repeated drug administration (AAV3 i/m); **C** – results of the physiological Bla/J test of mice with *DYSF* gene knockout after i/v of $5*10^{12}$ units of virus with the *DYSF* gene (AAV4 i/v).

The test is mainly used to assess physical activity and changes in the working capacity of mice while modeling the conditions of the labor process. However, limb girdle muscular dystrophy type 2B occurs, and there is no repair of muscle fibers mediated by dysferlin, which leads to progressive muscle degeneration due to necrosis of muscle fibers. This particular test is the most sensitive test for assessing changes in animal endurance and indirectly indicates the state of muscle strength.

When muscle tone was assessed in the "Wire hanging" test (Fig. 3), intramuscular administration of both the minimum and maximum doses resulted in a 2-fold increase in muscle tone in the AAV1 i/m, AAV2 i/m and AAV3 i/m groups with repeated drug administration 2 months after the initial injection ($p=0.0197$; $p=0.0331$; $p=0.001$) compared with that in the negative control group. The group with intravenous administration did not show efficacy in this test. Statistically significant differences between the negative and positive controls were found in all the groups ($p<0.0001$).

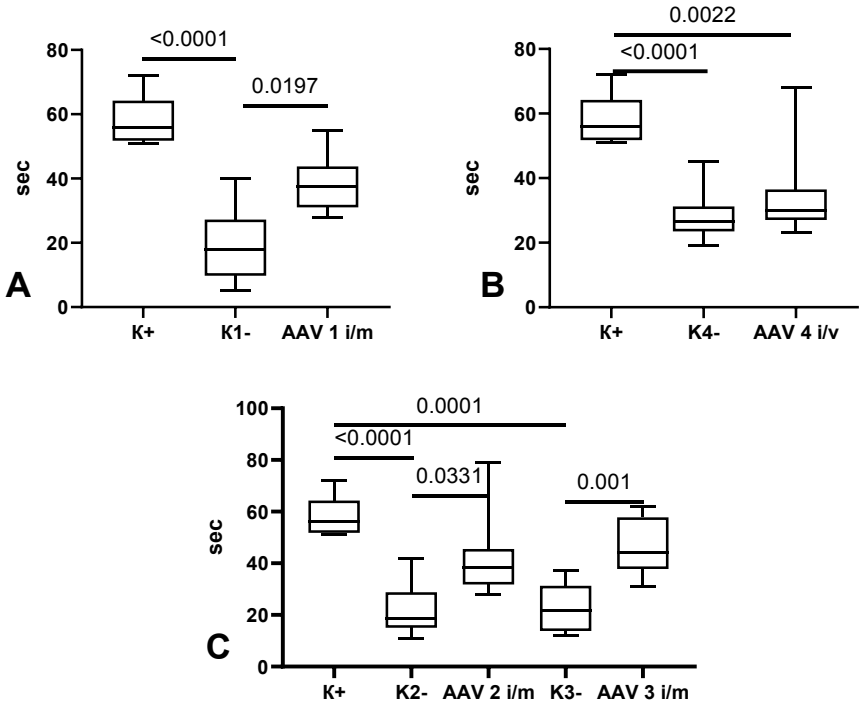


Figure 3. Analysis of the assessment of physical performance in the test "Wire hanging". ***Note:*** **A** – results of the physiological Bla/J test of mice with *DYSF* gene knockout after intramuscular administration of $1*10^{12}$ units of virus with the *DYSF* gene (AAV1 i/m); **B** – results of the physiological test of Bla/J mice with *DYSF* gene knockout after i/m of $5*10^{12}$ units of *DYSF* gene virus (AAV2 i/m) and Bla/J mice with *DYSF* gene knockout after i/m of $5*10^{12}$ units of *DYSF* gene virus with repeated drug administration (AAV3 i/m); **C** – results of the physiological Bla/J test of mice with *DYSF* gene knockout after i/v of $5*10^{12}$ units of virus with the *DYSF* gene (AAV4 i/v).

In the physical performance safety test "Holding an animal on a slippery surface of a vertical rod" (Fig. 4), the effectiveness of the drug was shown only at the maximum dose ($5*10^{12}$) when it was administered intramuscularly, regardless of the frequency of use. Compared with that of the control K- group, the retention time on the rod increased by ≈20% ($p= 0.0041$; $p=0.0251$) in the AAV2 i/m and AAV3 i/m groups with repeated administration of the drug 2 months after the initial injection. The drug did not show efficacy at the minimum dose ($1*10^{12}$) with intramuscular injection to the AAV1 group or at the maximum dose ($5*10^{12}$) with intravenous administration to the AAV4 group in this test.

During acute and subchronic toxicity, a single intravenous injection of the drug AAV9.DYSF in mice at a dose of 0.2 mL does not cause death or intoxication in experimental animals.

The results of the clinical observation of the experimental animals revealed no differences between the experimental group and the control group. Thus, the general condition of the animals did not change during the entire period of observation. No animal mortality was noted. There were no behavioral features in the experimental animals (Table 2) that were different from those of the control animals.

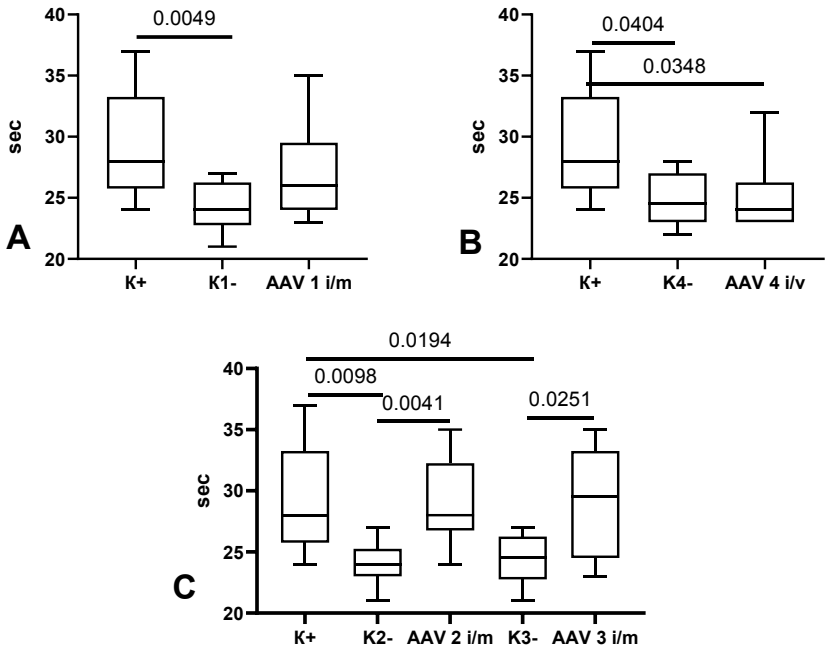


Figure 4. Physical performance analysis in the test "Holding an animal on a slippery surface of a vertical rod". ***Note:*** **A** – results of the physiological Bla/J test of mice with *DYSF* gene knockout after intramuscular administration of $1*10^{12}$ units of virus with the *DYSF* gene (AAV1 i/m); **B** – results of the physiological test of Bla/J mice with *DYSF* gene knockout after i/m of $5*10^{12}$ units of *DYSF* gene virus (AAV2 i/m) and Bla/J mice with *DYSF* gene knockout after i/m of $5*10^{12}$ units of *DYSF* gene virus with repeated drug administration (AAV3 i/m); **C** – results of the physiological Bla/J test of mice with *DYSF* gene knockout after i/v of $5*10^{12}$ units of virus with the *DYSF* gene (AAV4 i/v).

Table 2. Results of studying the effect of AAV9.DYSF on indicators of motor activity and the integrity of the physiological response of rats before euthanasia

Indicator	Group							
	Control for 1 month	AAV9.DYSF 1 month			Control for 3 month	AAV9.DYSF 3 month		
		$1x10^{12}$/L	$3x10^{12}$/L	$30x10^{12}$/L		$1x10^{12}$/L	$3x10^{12}$/L	$30x10^{12}$/L
Activity	17.35±4.6	15.75±2.77	19.5±5	18±4.45	19.65±4.9	18±4.8	19±4.8	18.5±5
Wall racks	6.9±2.29	7.35±2.13	7±2.51	6.35±2.23	7.35±2.48	7.4±1.96	7.75±2.4	7.85±2
Urination	2.2±1.51	1.9±1.68	1.55±1.43	1.5±1.1	1.8±1.4	2.5±1.4	2±1.57	1.6±1.1
Bowel movements	3.5±1.4	3.4±1.6	2.65±1.46	3.55±1.28	2.7±1.38	3±1.3	2.75±1.7	3.4±1.47
Autogrooming	4.2±1.54	4.25±1.59	4.3±1.13	4.4±1.39	3.75±1.52	3.9±1.6	4.05±1.5	4±1.3

Note: Descriptive statistics have been applied to all the data. The data obtained as a result of the research are presented in the format M (arithmetic mean) ± SD (standard deviation). The data were checked for normality of the distribution via the Shapiro–Wilk test. Intergroup differences were analyzed via parametric methods due to the normal type of distribution. Student's unpaired t-test was used to identify differences in the intergroup comparisons. The differences were determined at a significance level of $p<0.05$.

The results of the hematological (Tables 3) and biochemical (Tables 4) blood tests demonstrated the absence of statistically significant differences in the studied parameters between the groups of animals receiving the studied drug AAV9.DYSF at doses of $1x10^{12}$/L, $3x10^{12}$/L and $30x10^{12}$/L, corresponding to those used for the follow-up of control animals. The data obtained indicate that the studied drugs at the studied doses do not have a negative effect on erythro-, leuko-, or thrombopoiesis; the leukocyte formula; or biochemical markers of damage to internal organs.

Table 3. Results of studying the effect of AAV9. Effects of DYSF on hematological parameters of rat blood

Indicator	Group							
	Control for 1 month	AAV9.DYSF 1 month			Control for 3 month	AAV9.DYSF 3 month		
		$1x10^{12}$/L	$3x10^{12}$/L	$30x10^{12}$/L		$1x10^{12}$/L	$1x10^{12}$/L	$3x10^{12}$/L
Hemoglobin, g/L	131.1±6.77	129±8.25	130.3±7.66	130±8.87	128.3±6.29	131±8.57	129.9±8.42	130.6±9.23
Erythrocytes $*10^{12}$/L	8.14±0.74	8.09±0.79	7.74±0.89	7.9±0.94	7.72±0.7	7.69±0.72	7.94±0.89	7.76±0.75
Platelets $*10^{9}$/L	617.1±55	616±56.46	612.5±56.17	594±44.7	606.8±53.41	593±57.89	592.8±53.97	597.2±59.51
Leukocyte $*10^{9}$/L	7.72±1.24	7.99±1.39	8.28±1.67	8.14±1.25	7.86±1.41	8.24±1.32	7.44±1.02	7.81±1.41
Neutrophils, %	32.7±6.13	31.35±6.58	32.75±5	30±5.25	32.7±5.5	32.1±7.35	32.15±5.99	34±6.44
Monocytes, %	2.3±2	3.1±1.86	2.95±2.35	2.9±1.92	2.7±2	3±2.38	3.5±1.82	3.8±1.85
Lymphocytes, %	65±6.77	65.55±7.03	64.30±6	67.1±5.19	64.6±5.56	64.9±7.62	64.35±6.47	62.2±7.02
Hematocrit, %	45.4±4.45	45.05±5.19	46.8±3.66	45.8±4.46	44.3±4.57	45.55±3.76	45.75±3.88	45.05±4.1

Note: Descriptive statistics have been applied to all the data. The data obtained as a result of the research are presented in the format M (arithmetic mean) ± SD (standard deviation). There were no statistically significant differences between the groups.

Gravimetric results and macroscopic and microscopic examination of the internal organs of rats 1, 3 and 6 months after intravenous administration of the investigational drug AAV9.DYSF at doses of $1x10^{12}$/L, $3x10^{12}$/L and $30x10^{12}$/L did not cause dystrophic, inflammatory or other pathological changes in internal organs in the studied dose range.

Table 4. Results of studying the effect of AAV9. Effects of DYSF on biochemical parameters of rat blood

Indicator	Group							
	Control for 1 month	AAV9.DYSF 1 month			Control for 3 month	AAV9.DYSF 3 month		
		$1x10^{12}$/L	$3x10^{12}$/L	$30x10^{12}$/L		$1x10^{12}$/L	$1x10^{12}$/L	$3x10^{12}$/L
Total protein, g/L	67.9±4.4	69.78±6	68.5±5.5	71.08±5.64	68.72±5.56	70±5.4	69.2±4.9	71.2±6.4
Urea, mmol/L	7.74±0.52	7.78±0.56	8.02±0.42	7.72±0.56	7.97±0.51	7.68±0.48	7.72±0.49	7.84±0.48
Creatinine, mg/dL	0.48±0.05	0.48±0.05	0.47±0.05	0.49±0.05	0.47±0.04	0.47±0.05	0.49±0.04	0.49±0.05
Triglycerides, mmol/L	1.29±0.22	1.29±0.23	1.39±0.18	1.24±0.21	1.23±0.24	1.28±0.24	1.29±0.27	1.34±0.19
Cholesterol, mmol/L	2.07±0.31	2.09±0.31	2.13±0.33	1.98±0.4	2.01±0.38	2.05±0.41	2.07±0.4	2.11±0.3
Total bilirubin, mg/dL	2.56±0.5	2.26±0.46	2.38±0.47	2.46±0.46	2.4±0.55	2.38±0.47	2.52±0.39	2.14±0.37
Glucose, mmolLl	4.34±0.3	4.22±0.36	4.26±0.44	4.2±0.36	4.19±0.35	4.3±0.45	4.26±0.33	4.25±0.4
Alkaline Phosphatase, Unit/L	328.2±15.48	319.3±15.32	318±19.9	333±17	327.3±18.21	326±19.1	320±17.3	332.6±17.39
AST, Unit/L	51.9±7.77	48.95±7.16	50.4±6.45	48.6±6.29	53.25±6.06	51.1±8.97	53.2±6.16	51.65±6
ALT, Unit/L	63.5±6.84	64.75±6	64.8±7.38	63.65±7.7	65.45±7.92	66.9±7.52	64.8±7.7	65.25±8.57
Albumin, g/L	30.43±2.87	29.68±2.96	30.7±2.7	30.95±3.04	29.3±3.45	28.8±2.73	30.3±2.98	30.09±3.2
*Globulin, g/L	37.45±5.5	40.1±5.15	37.8±6.18	40.13±5.91	39.42±7.11	41.2±6	38.9±5.44	41.11±6.38

Note: * – calculated indicators. Descriptive statistics were applied to all the data. The data obtained as a result of the research are presented in the format M (arithmetic mean) ± SD (standard deviation). There were no statistically significant differences between the groups.

As a result of the acute toxicity study, the animals in the experimental group did not differ from those in the control group. Thus, the general condition of the animals did not change during the entire observation period. No animal mortality was observed. No behavioral features were observed in the experimental mice other than the control animals. The intensity and nature of motor activity did not change during the entire observation period and did not differ from the indicators of control animals. Seizures were absent in all the animals under observation. The

response to tactile, painful, sound and light stimuli was adequate in all experimental animals. There were no differences between the experimental and control animals.

Conclusion

The highest efficacy in mice without the dysferlin protein (B6.A-Dysf^{prmd}/GeneJ) in behavioral testing was shown by the drug AAV9.DYSF at the maximum dose ($5*10^{12}$) with a double intramuscular injection.

Thus, in the ”Grip test”, the AAV3 i/m index in the group increased by 29% (p=0.0026) relative to that in the negative control. An increase in the indicators was also observed in the tests ”Forced swimming with a load”, ”Wire hanging”, and ”Holding an animal on a slippery surface of a vertical rod” and reached 80% (p=0.0019), 104.8% (p=0.001) and 20% (p=0.025), respectively, relative to those of the K group. The group with a single intramuscular dose showed lower efficacy in the $5*10^{12}$ test. The group that received AAV9.DYSF at a dose of $1*10^{12}$ showed average efficacy in all tests, except for the ”Holding an animal on a slippery surface of a vertical rod” test, where efficacy was not detected. Intravenous administration of AAV9.DYSF at a dose of $5*10^{12}$ had the least effect.

During acute and subchronic toxicity, a single intravenous injection of AAV9.DYSF at a dose of 0.2 mL does not cause death or intoxication in experimental animals.

Additional information

Conflict of interest

The authors declare the absence of a conflict of interests.

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Data availability

All of the data that support the findings of this study are available in the main text.

Ethics Statements

The experimental studies were approved by the Bioethical Commission of Belgorod State National Research University (Minutes No. 15/10 dated Oct 29, 2021).

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