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RESEARCH ARTICLE

Comparative analysis of three genetic constructs for delivery and expression of a modified single-domain antibody gene in rAAV

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Abstract

Objectives. To obtain and compare the efficiency of three recombinant adeno-associated virus (rAAV) variants expressing the gene of the modified single-domain antibody B11-Fc specific to botulinum toxin type A (BoNT/A): rAAV-DJ-CMV-B11-Fc, rAAV-DJ-CASI-B11-Fc, and scAAV-DJ-CMV-B11-Fc.

Methods. The AAV-DJ Packaging System (*Cell Biolabs*, USA) was used to create target constructs and obtain rAAV. Expression of the B11-Fc antibody gene in the obtained rAAV was assessed *in vitro* (HEK293, CHO-S, and C2C12 cell lines) and *in vivo* (BALB/c mice) using biolayer interferometry. The protective properties of the drugs were investigated on the model of lethal intoxication of mice with BoNT/A.

Results. The rAAV-DJ-CMV-B11-Fc drug demonstrated a high level of B11-Fc antibody production both *in vitro* and *in vivo* without a significant decrease in concentration for at least 6 months. Comparable levels of B11-Fc production were demonstrated by rAAV-DJ-CASI-B11-Fc and scAAV-DJ-CMV-B11-Fc drugs in both *in vitro* and *in vivo* studies, with the exception of C2C12 cells, where rAAV-DJ-CASI-B11-Fc demonstrated the highest efficacy. When investigating the protective activity of the drugs against a lethal dose of BoNT/A, it was found that rAAV-DJ-CASI-B11-Fc possessed more pronounced activity in the first two days following administration as compared to rAAV-DJ-CMV-B11-Fc. However, at later stages, starting from 3 months, the rAAV-DJ-CMV-B11-Fc drug product demonstrated the most pronounced protection against high doses of BoNT/A.

Conclusions. The obtained data show that rAAV-DJ-CASI-B11-Fc should be used for the induction of protection against BoNT/A at early stages (24–48 h) after administration, whereas for protection against the highest doses of BoNT/A in the long term, rAAV-DJ-CMV-B11-Fc should be used. Studies into the specific activity of the drugs at later stages after administration are still ongoing.

Keywords

rAAV, adeno-associated virus, single-domain antibodies, botulism, passive immunization, prophylaxis

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НАУЧНАЯ СТАТЬЯ

Сравнительный анализ трех генетических конструкций для доставки и экспрессии гена модифицированного однодоменного антитела в составе rAAV

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Аннотация

Цели. Получить и сравнить эффективность трех вариантов rAAV, экспрессирующих ген модифицированного однодоменного антитела B11-Fc, специфичного к ботулиническому токсину типа A (BoNT/A) — rAAV-DJ-CMV-B11-Fc, rAAV-DJ-CASI-B11-Fc и scAAV-DJ-CMV-B11-Fc.

Методы. Для создания целевых конструкций и получения rAAV использовали систему AAV-DJ Packaging System (*Cell Biolabs*, США). Экспрессию гена антитела B11-Fc в составе полученных rAAV оценивали *in vitro* (клеточные линии HEK293, CHO-S и C2C12) и *in vivo* (мыши линии BALB/c) при помощи биослойной интерферометрии. Защитные свойства препаратов изучали на модели летальной интоксикации мышей BoNT/A.

Результаты. Препарат rAAV-DJ-CMV-B11-Fc показал высокий уровень продукции антитела B11-Fc как *in vitro*, так и *in vivo* без значимого снижения концентрации в течение как минимум 6 месяцев. Препараты rAAV-DJ-CASI-B11-Fc и scAAV-DJ-CMV-B11-Fc показали сравнимый уровень продукции B11-Fc как *in vitro* и *in vivo*, за исключением клеток C2C12, где rAAV-DJ-CASI-B11-Fc показал самую высокую эффективность. При изучении защитной активности препаратов против летальной дозы BoNT/A продемонстрировано, что в первые двое суток rAAV-DJ-CASI-B11-Fc и scAAV-DJ-CMV-B11-Fc обладают более выраженной активностью по сравнению с rAAV-DJ-CMV-B11-Fc. Однако на более поздних сроках, начиная с 3 месяца, препарат rAAV-DJ-CMV-B11-Fc демонстрирует наиболее выраженную защиту против высоких доз BoNT/A.

Выводы. Полученные в ходе исследования данные показывают, что для индукции защиты от BoNT/A на ранних сроках (24–48 ч) после введения следует применять препарат rAAV-DJ-CASI-B11-Fc, тогда как для индукции защиты от максимально высоких доз BoNT/A в долгосрочной перспективе следует использовать rAAV-DJ-CMV-B11-Fc. Исследования специфической активности препаратов на более поздних сроках после введения продолжаются.

Ключевые слова

rAAV, аденоассоциированный вирус, однодоменные антитела, ботулизм, пассивная иммунизация, профилактика

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INTRODUCTION

Recombinant adeno-associated virus (rAAV) vectors are an excellent tool not only for developing gene therapy agents, but also for creating preventive measures against infectious diseases based on passive genetic immunization. This principle is based on the delivery and long-term expression of neutralizing antibody genes specific to a particular pathogen. This approach has proven effective in creating preventive measures for influenza, COVID-19, HIV infection and other diseases [1, 2]. The effectiveness of rAAV-based passive genetic immunization drugs depends significantly on the design of the genetic construct used to ensure the expression of the neutralizing antibody gene.

In a previous study, we obtained an rAAV that provides long-term expression of the gene for the neutralizing modified single-domain antibody B11-Fc[1] (rAAV-B11-Fc). It was experimentally established that a single administration of rAAV-B11-Fc provides complete protection to animals from a lethal dose of botulinum neurotoxin serotype A for at least 120 days [3]. This study is a continuation of the aforementioned article and focuses on selecting the optimal genetic construct for delivering and expressing the B11-Fc antibody gene within an rAAV and developing a prototype candidate drug for genetic passive immunization and botulism prevention.

Thus, the aim of the present study is to obtain and compare the effectiveness of three rAAV constructs expressing the B11-Fc antibody gene, which is specific for botulinum toxin type A (BoNT/A): rAAV-DJ[2]-CMV-B11-Fc, rAAV-DJ-CASI-B11-Fc, and scAAV-DJ-CMV-B11-Fc. For convenience and clarity, rAAV-DJ-CMV-B11-Fc is used to refer to the previously used construct [3] for carrying the gene for the modified B11-Fc single-domain antibody [4] under the control of the CMV promoter (Cytomegalovirus promoter). rAAV-DJ-CASI-B11-Fc is a construct similar in structure but including the CASI[3]-promoter, representing a synthetic hybrid promoter that contains elements from CMV, chicken β-actin, and the UbC (Ubiquitin C) intron, as well as the woodchuck hepatitis virus (WHV) posttranscriptional regulatory element (WPRE), which enhances mRNA stability and expression levels. scAAV-DJ-CMV-B11-Fc is a self-complementary form of the vector expressing the B11-Fc gene under the control of the CMV promoter.

MATERIALS AND METHODS

Obtaining rAAV drugs. The rAAV-DJ-CMV-B11-Fc drug was obtained using plasmids as previously described [3]. The rAAV-DJ-CASI-B11-Fc drug was obtained using pAAV-CASI-B11-Fc, pAAV-DJ-vector (*Cell Biolabs*, USA), and pHelper Vector (*Cell Biolabs*, USA) plasmids. The pAAV-CASI-B11-Fc construct was obtained by synthesizing the CASI promoter sequence along with cloning sites and the WPRE sequence and polyadenylation signal at *Eurogen* (Russia), followed by cloning of the synthesized sequence between the left and right inverted terminal repeats (ITR), with replacing the existing expression cassette. The scAAV-DJ-CMV-B11-Fc drug was obtained using the plasmids pscAAV-CMV-B11-Fc, pAAV-DJ-vector (*Cell Biolabs*, USA), and pHelper Vector (*Cell Biolabs*, USA). The pscAAV-CMV-B11-Fc plasmid was obtained by cloning the B11-Fc gene sequence into the pscAAV-MCS plasmid (*Cell Biolabs*, USA).

To obtain rAAV vectors, the Human Embryonic Kidney 293 (HEK293) cell line (from the cell culture collection of the N.F. Gamaleya National Research Center for Epidemiology and Microbiology of the Ministry of Health of Russia) was used. Cultivation and transfection were performed as previously described under adherent conditions at 37°C and 5% CO_2 [3, 5]. Viral products were purified using affinity chromatography (AC) on the POROS CaptureSelect AAVX Affinity Resin sorbent (*Thermo Fisher Scientific*, USA) according to the manufacturer's instructions. Further purification and buffer exchange were performed using size exclusion chromatography (SEC) on an XK 26/100 column packed with Superdex 200 sorbent (*Cytiva*, USA). Final formulation of the drugs was performed using 100 kDa Amicon Ultra-15 centrifugal concentrators (*Merck*, USA). The quantity and quality of the obtained drugs were studied as described in [5].

The specific activity of rAAV drugs *in vitro*. HEK293 cells, CHO-S (serum-free suspension-adapted Chinese hamster ovary cells) (*Thermo Fisher Scientific*, USA, Cat. No. R80007), and C2C12 (a mouse C3H myoblast cell line) (ATCC® CRL-1772™) were transduced at a dose of 10^5 vg/cell. Culture fluid samples were collected at 96 and 144 h. The concentration of B11-Fc in the culture medium was determined by biolayer interferometry (BLI) using an OctetRED96e System (*Fortebio*, USA) and Anti-Human IgG Fc sensors (*Fortebio*, USA).

[1] B11-Fc is a single-domain antibody modified with a human IgG1 Fc fragment.

[2] rAAV-DJ is recombinant Adeno-Associated Virus (rAAV) with a DJ-type capsid, which is a synthetic (chimeric) serotype created by DNA shuffling from eight different natural AAV serotypes.

[3] CASI-promoter is a synthetic (chimeric) promoter. It consists of the CMV enhancer, the chicken β-actin promoter (CAG), and the UbC enhancer.

Specific activity of rAAV drugs *in vivo*. Female BALB/c inbred mice (6 weeks old, 18–20 g) (Scientific Center for Biomedical Technologies of the Federal Medical Biological Agency of Russia, Andreevka branch) were administered rAAV at a dose of 10^{11} vg/animal intramuscularly. Blood was collected at key time points. The concentration of B11-Fc in serum was determined similarly to the *in vitro* experiment. The protective activity of the drugs against BoNT/A was studied as described in [3, 6]. The study was conducted in a vivarium under standard conditions: polycarbonate cages, stocking density up to 5 animals per cage. Feeding was carried out with complete pelleted feed (*ad libitum*) with access to water. All procedures were performed in accordance with the protocol approved by the local ethics committee of the N.F. Gamaleya National Research Center for Epidemiology and Microbiology (protocol No. 11 dated June 25, 2021).

Statistical analysis. Statistical analysis was performed using GraphPad Prism 9.0 software (*Dotmatics*, USA). Survival was analyzed using the Kaplan–Meier method with the log-rank test. Significance level $p < 0.05$.

RESULTS AND DISCUSSION

Design of genetic constructs and production of rAAV drugs

The desired level, stability, and specificity of tissue expression are determined by *cis*-acting elements of rAAV vectors, including promoters. In this study, we investigated the efficiency of B11-Fc antibody expression within rAAV vectors carrying three different genetic constructs: rAAV-DJ-CMV-B11-Fc, rAAV-DJ-CASI-B11-Fc, and scAAV-DJ-CMV-B11-Fc.

The CMV promoter is widely used due to its high expression in various cells (e.g., human colon carcinoma HCT116, colorectal adenocarcinoma DLD-1, and brain and liver cells) [7]. However, it is subject to epigenetic silencing *in vivo*, particularly in muscle and liver cells, which limits its long-term activity and can trigger an immune response [8–11].

The CASI promoter is a synthetic hybrid type (CMV, β-actin, UBC), whose methylation resistance ensures stable expression. In studies of induced pluripotent stem cells differentiated into retinal pigment epithelium cells (iPSC-RPE) [12], as well as in gene therapy for colorectal cancer using the drug AdC68-cetuximab [13], the CASI promoter demonstrated high and stable expression both *in vivo* and *in vitro* [14]. Thus, according to the literature, the CASI promoter is an effective means for delivering and expressing a transgene in muscle tissue.

Self-complementary adeno-associated virus (scAAV) vectors contain double-stranded DNA, which, according to the literature data, allows for faster and higher levels of transgene expression [15–17]. However, scAAV elicits a more pronounced immune response against the transgene [18, 19]. It should also be noted that the scAAV-DJ-CMV-B11-Fc construct carries the core CMV promoter sequence without an enhancer, as the effective packaging capacity of AAV is limited to approximately 5000 nucleotides; when packaging self-complementary scAAV particles, this capacity is effectively reduced by almost half due to the packaging of both sense and antisense DNA together, which necessitates the shortening of regulatory element sequences in the construct.

Furthermore, all three constructs contain different introns that contribute to increased transgene expression: the human β-globin intron in the pAAV-DJ-CMV-B11-Fc construct, the human ubiquitin C intron in the pAAV-DJ-CASI-B11-Fc construct, and the SV40 small intron in the pscAAV-DJ-CMV-B11-Fc construct. The WPRE located downstream of the transgene in the pAAV-DJ-CASI-B11-Fc construct helps to stabilize mRNA and increase transgene expression.

Thus, we selected the three most promising genetic constructs for delivering and effectively expressing the B11-Fc antibody gene within rAAV. The study used the synthetic serotype rAAV-DJ, which is capable of transducing a wide range of different cells and tissues [10, 20] and which have been previously used for delivering and expressing neutralizing antibody genes [2, 3].

The corresponding plasmid constructs pAAV-DJ-CMV-B11-Fc, pAAV-DJ-CASI-B11-Fc, and pscAAV-DJ-CMV-B11-Fc were used to obtain three rAAV drugs. Schematic diagrams of the genetic constructs are shown in Fig. 1.

The rAAV-DJ-CMV-B11-Fc, rAAV-DJ-CASI-B11-Fc, and scAAV-DJ-CMV-B11-Fc drugs obtained from HEK293 cell culture were purified using affinity and size exclusion chromatography. The quality of the drugs was assessed using SDS-PAGE electrophoresis in polyacrylamide gel, Western blot, and electron microscopy. The rAAV preparation scheme and typical quality control results are presented in Fig. 2.

Comparison of B11-Fc expression in the obtained rAAV drugs *in vitro* and *in vivo*

To assess B11-Fc expression, HEK293, CHO-S, and C2C12 cell lines were transduced with rAAV-DJ-CMV-B11-Fc, rAAV-DJ-CASI-B11-Fc, scAAV-DJ-CMV-B11-Fc, and rAAV-DJ-CMV-EGFP (as a control). The drug was added at a rate of 100000 genomic copies per cell. Culture fluid samples were collected 96 and 144 h after transduction.

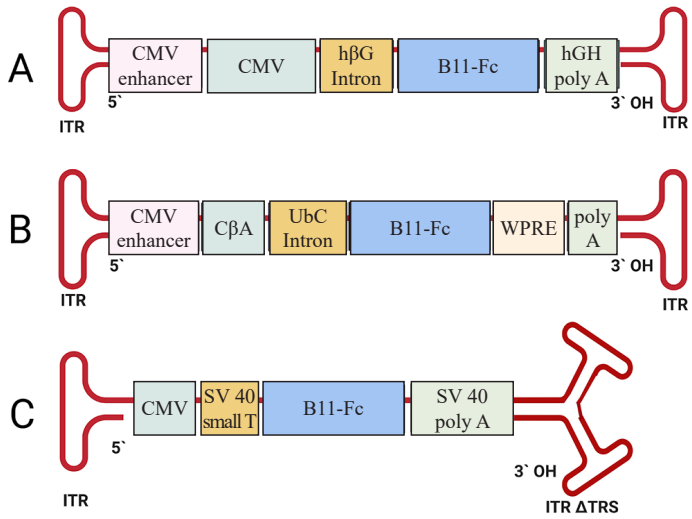

Fig. 1. Schematic representation of the obtained genetic constructs containing the gene of the modified antibody B11-Fc.

A: pAAV-DJ-CMV-B11-Fc scheme, CMV is a sequence of the full-length CMV promoter with enhancer; hβG intron is an intron of the human β-globin gene; B11-Fc is a sequence encoding the antibody B11-Fc; hGH poly A is a polyadenylation signal of human growth hormone; ITR are inverted terminal repeats.

B: pAAV-DJ-CASI-B11-Fc scheme; CASI is a synthetic hybrid promoter containing the CMV enhancer and CβA (chicken β-actin); UbC intron is an intron of ubiquitin C; B11-Fc is a sequence encoding the antibody B11-Fc, fused with the Fc fragment; WPRE is a woodchuck hepatitis virus post-transcriptional regulatory element; poly A is a synthetic polyadenylation signal; ITR are inverted terminal repeats.

C: pscAAV-DJ-CMV-B11-Fc scheme, CMV is a CMV core promoter sequence without enhancer; SV40 small T is a small intron of SV40 virus; B11-Fc is a sequence encoding B11-Fc antibody; SV40 poly A is an SV40 polyadenylation signal; ITR ΔTRS is a modified ITR with nickase site deletion, required for formation of self-complementary structure of vector DNA

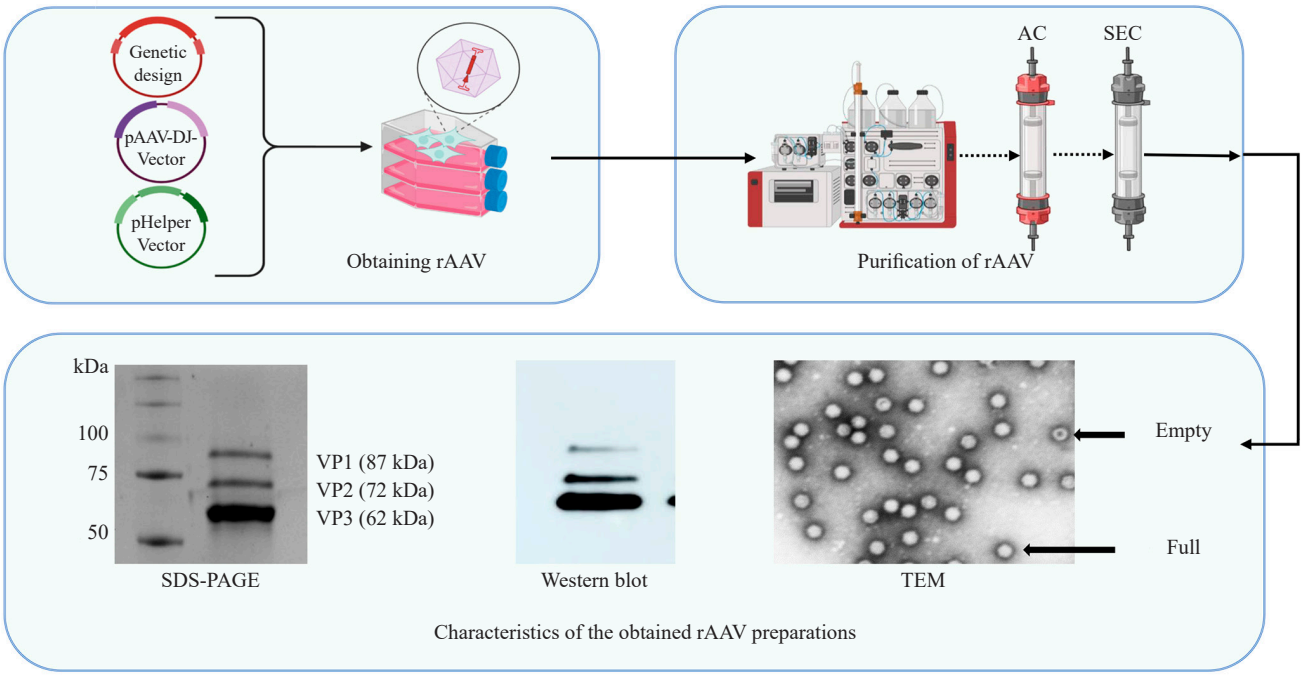

Fig. 2. Brief scheme of the rAAV preparation process. AC is affinity chromatography, SEC is size-exclusion chromatography, SDS-PAGE is polyacrylamide gel electrophoresis, VP1, VP2, VP3 are structural proteins of adeno-associated virus, TEM is transmission electron microscopy

No cytotoxic effect was observed when the drugs were transduced at the selected dose. Analysis using biolayer interferometry (BLI) showed that the production level of B11-Fc depends on the promoter and cell line (Fig. 3a).

The rAAV-DJ-CMV-B11-Fc construct provided high expression in CHO-S cells (up to 69.2 μg/mL by 144 h), while the level was lower in C2C12 cells (6.13 μg/mL at 96 h). The CASI promoter (rAAV-DJ-CASI-B11-Fc) provided stable expression (Fig. 3a), particularly in C2C12 myoblast cells (up to 19.5 μg/mL), which indirectly confirms its effectiveness for muscle tissue. scAAV-DJ-CMV-B11-Fc provided a moderate level of antibody expression in CHO-S and HEK293 cells, but the lowest level in C2C12. However, antibody expression was not shown when transduced with the control vector (rAAV-DJ-CMV-EGFP). Thus, the rAAV-DJ-CMV-B11-Fc drug provides a high level of B11-Fc expression in CHO-S and HEK293 cells, and the rAAV-DJ-CASI-B11 drug provides a high level of expression in C2C12 cells, while the scAAV-DJ-CMV-B11-Fc drug provides a moderate level of expression in CHO-S and HEK293 cells and a low level of expression in C2C12 cells.

The choice of cell lines was based on the fact that, due to the low mitotic activity of muscle tissue, genetic passive immunization drugs based on rAAV are usually administered intramuscularly to ensure long-term antibody production in the body. The less intensely the cells divide, the longer rAAV is able to persist as episomes and express the transgene [12, 16, 17].

Thus, C2C12 myoblast cells were chosen as a model for muscle tissue for the indirect assessment of the effectiveness of the constructs *in vitro*. The HEK293 cell line was used as an alternative model to study the expression level of the obtained rAAV constructs in other cell types, particularly epithelial cells. CHO-S cell line was selected based on its high efficiency for antibody production, including modified single-domain antibodies [6, 21].

During the experiment, it was shown that the rAAV-DJ-CASI-B11-Fc construct had the highest efficiency in myoblast cells, which is consistent with the literature data [14, 18]. At the same time, despite the expectation of higher expression for the scAAV-DJ-CMV-B11-Fc construct due to more efficient transduction, the rAAV-DJ-CMV-B11-Fc construct produced the highest expression in HEK293 and CHO-S cells. Since the scAAV-DJ-CMV-B11-Fc construct provided the lowest level of B11-Fc antibody production, the hypothesis about the advantage of self-complementary rAAV in our experiment was not confirmed; however, this outcome may be due to the absence of an enhancer sequence in the construct.

To assess B11-Fc expression *in vivo*, BALB/c mice were injected with 10^{11} vg of rAAV constructs: rAAV-DJ-CMV-B11-Fc, rAAV-DJ-CASI-B11-Fc, scAAV-DJ-CMV-B11-Fc, as well as a negative control rAAV-DJ-CMV-EGFP (8 animals per group). Serum was collected on days 1, 2, 3, 7, 14, 21, 28, 56, 62, 84, 112, 120, 140, 149, 168, and 184 after injection with various rAAV variants. The dosage of the drugs was chosen based on data from previous studies [3]. B11-Fc expression began within 24 h in all groups. The maximum concentration was observed in the CMV vector group and was more than 250 μg/mL on day 120, with approximately 230 μg/mL maintained up to day 184. The CASI vector provided up to 120 μg/mL (peak on day 56), followed by stabilization at a level of ~80 μg/mL. The concentration of scAAV reached ~75 μg/mL by day 120 and remained stable (Fig. 3b). In the group of animals injected with the control vector (rAAV-DJ-CMV-EGFP), no specific BLI signal was detected (not shown in the graph).

Thus, *in vitro* data from C2C12 cell studies may not fully reflect transgene expression from rAAV following

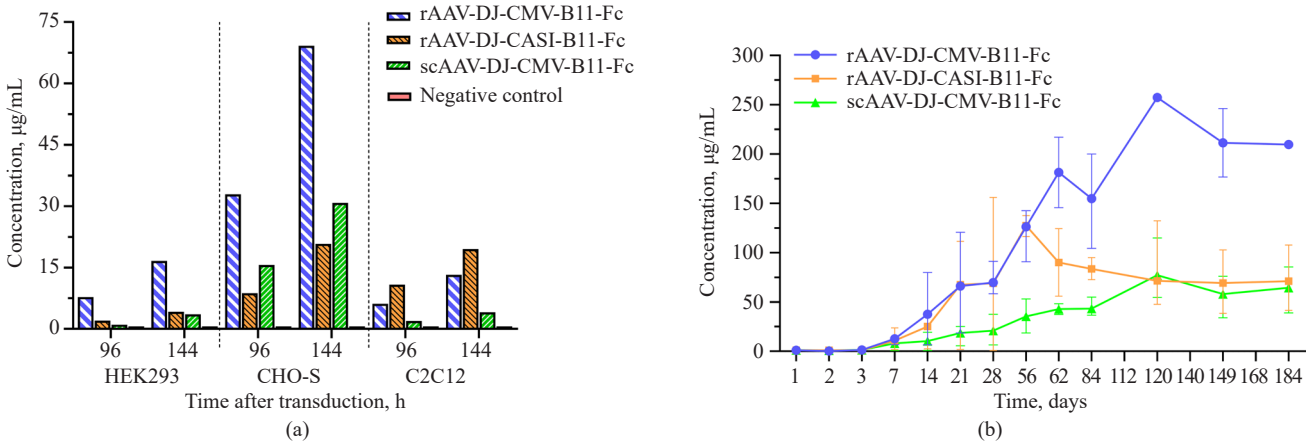

Fig. 3. Concentration of B11-Fc antibodies (μg/mL): (a) in culture fluid after rAAV transduction; (b) in mouse serum, at different time points after rAAV injection

intramuscular administration *in vivo*. However, based on the combined *in vivo* data presented above, a preliminary conclusion can be drawn about the promising nature of the rAAV-DJ-CMV-B11-Fc construct for delivering and expressing the B11-Fc antibody gene.

As a result, all the constructs studied provide a significant level of gene expression and B11-Fc antibody production both *in vitro* and *in vivo*. However, the rAAV-DJ-CMV-B11-Fc construct shows the best results, which correlates with the data on the protective activity of the drugs *in vivo*, described in the next section.

Evaluation of the protective capacity of rAAV-B11-Fc *in vivo*

The experiment evaluated the ability of various rAAV constructs expressing the B11-Fc single-domain antibody to protect mice from BoNT/A toxin. The effectiveness of the therapeutic antibodies was evaluated based on the survival rate of the animals after toxin administration. Mice were injected with 10^{11} vg of rAAV, followed by different doses of BoNT/A toxin administered intraperitoneally at various intervals. The research results are presented in Fig. 4.

Within 24 h, rAAV-DJ-CASI-B11-Fc and scAAV-DJ-CMV-B11-Fc provided 100% survival from 5 and 10 LD_{50}, while rAAV-DJ-CMV-B11-Fc showed ~60% survival. After 48 h, all constructs, including rAAV-DJ-CMV-B11-Fc, provided 100% protection at doses up to 20 LD_{50}, but at 30 LD_{50}, rAAV-DJ-CMV-B11-Fc only protected 80% of the animals. On the 10th day after administration, all drugs provided complete protection at doses up to 150 LD_{50}. After 78 days, the CMV vector maintained 100% protection even at 1000 LD_{50}, while

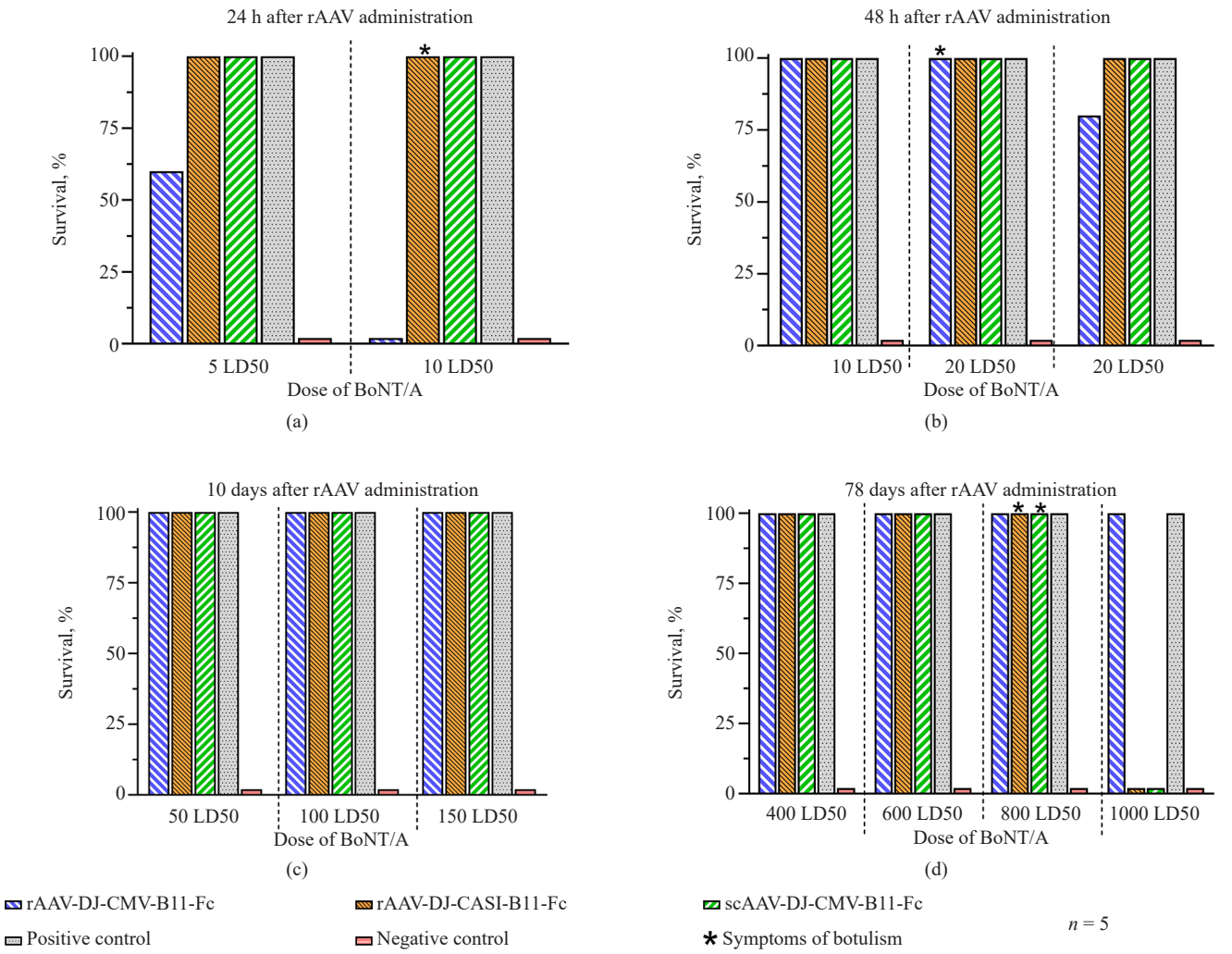

Fig. 4. Protective activity of rAAV drugs expressing neutralizing antibody B11-Fc when different doses of BoNT/A were administered at different times. The survival rate of animals (%) is shown when intoxicated with different doses of BoNT/A (in LD_{50}) 24 h (a), 48 h (b), 10 days (c), and 78 days (d) after rAAV administration. The groups with botulism symptoms are marked with an asterisk (*); the number of animals was $n = 5$ for each group

rAAV-DJ-CASI-B11-Fc and scAAV-DJ-CMV-B11-Fc were only effective against 800 LD_{50}.

Positive controls were animals that received purified B11-Fc intramuscularly at a dose that provided protection against BoNT/A toxin [6]. Administration of the rAAV drugs under study resulted in a pronounced protective effect against intoxication, as evidenced by animal survival and the absence of intoxication signs. At the same time, in the negative control groups (saline administration), there was 100% animal mortality.

The obtained data confirm the possibility of inducing long-lasting protection against BoNT/A with a single intramuscular administration of rAAV drugs expressing the B11-Fc antibody gene. At the same time, it has been shown that rAAV-DJ-CMV-B11-Fc exhibits more pronounced activity against extremely high doses of botulinum toxin starting from 2–3 months after administration. Less encouraging data on the protective activity of rAAV-DJ-CMV-B11-Fc [3] may be due to differences in the drug purification scheme, as described in the previous chapter. In turn, the rAAV-DJ-CASI-B11-Fc and scAAV-DJ-CMV-B11-Fc drugs can potentially be used, among other things, for emergency prophylaxis of botulism, since they provide protection as early as 24 h after administration.

CONCLUSIONS

In this study, a comparative analysis of three rAAV constructs expressing a modified single-domain antibody B11-Fc against botulinum neurotoxin type A (BoNT/A) was conducted.

The obtained data demonstrate that the rAAV vector design (including genome shape and promoter type) has a key influence on transgene expression levels and the duration of the protective effect. The rAAV-CMV-B11-Fc and rAAV-CASI-B11-Fc constructs are promising for long-term passive immunization. The scAAV-CMV-B11-Fc vector can be used in situations requiring early expression and protection in an emergency prophylaxis setting.

Authors' contributions

E.I. Ryabova—performing transfection and expression experiments in HEK293, CHO-S, and C2C12 cell lines; data collection and analysis; writing the manuscript text.

A.A. Derkaev—performing *in vivo* experiments on the botulism model; evaluating protective efficacy; data collection and analysis.

I.B. Esmagambetov—general supervision; revising the manuscript text; approval of the final version of the manuscript for publication.

M.A. Dovgiy—development and cloning of rAAV constructs expressing the B11-Fc single-domain antibody.

A.A. Blinov—analysis of prototypes using Western blot and SDS-PAGE; processing experimental data.

R.M. Hossain—quantitative evaluation of antibody production in culture supernatant; processing experimental data.

O.E. Dmitriev—chromatographic purification of vectors; analysis of drug properties.

D.S. Polyansky—preparation of figures, illustrations, and structural diagrams of constructs; participation in writing and formatting the manuscript.

A.N. Noskov—research concept; isolation and purification of botulinum toxin type A.

D.V. Shcheblyakov—design of the genetic construct expressing the single-domain antibody fused to an Fc fragment; research supervision.

D.Y. Logunov, **A.L. Gintsburg**—research supervision; approval of the final version of the manuscript for publication.

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