Gene Therapy of Amyotrophic Lateral Sclerosis

I. A. Zavalishin, N. P. Bochkov*, Z. A. Suslina, M. N. Zakharova, V. Z. Tarantul, B. S. Naroditskiy***, N. A. Suponeva, S. N. Illarioshkin, M. M. Shmarov***, D. Y. Logunov***, I. L. Tutyhina***, L. V. Verkhovskaya***, E. S. Sedova***, A. V. Vasiliev, L. V. Brylev, and A. L. Ginzburg*****

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 145, No. 4, pp. 467-470, April, 2008
Original article submitted February 28, 2008

Two-year experiments were performed to evaluate the neurotrophic effect of hypoxia-inducible factors (vascular endothelial growth factor and angiogenin) expressed in recombinant human adenoviruses in amyotrophic lateral sclerosis. Randomized placebo-controlled trial demonstrated safety and good tolerability of the recombinant antiviral drugs. The life span of patients under conditions of hypoxia increased after treatment with the test drug, which was probably related to improved resistance of motoneurons. The presence of virus-neutralizing antibodies decreases the effectiveness of adenoviral vectors, which necessitates differential approach to the selection of patients and continuous monitoring of gene therapy.

Key Words: *amyotrophic lateral sclerosis; gene therapy; adenoviral vector; VEGF; angiogenin*

Amyotrophic lateral sclerosis (ALS) is a fatal disease of the central nervous system. This disease is characterized by progressive selective degeneration of motoneurons, which results in the development of atrophic paralysis, progression of respiratory failure, and death of the patient.

The development of therapeutic approaches is based on modern data on the mechanisms of motoneuron death obtained *in vitro* on cell cultures and *in vivo* on transgenic animals.

Neurotrophic factors hold much promise for the therapy of ALS [1,2].

It was shown that well-known factor of angiogenesis, vascular endothelial growth factor (VEGF), possesses neurotrophic function. A new class of hypoxia-inducible neurotrophic factors was identified.

Previous experiments showed that the decrease in VEGF concentration results not only in the impairment of neuronal perfusion, but also in a deficiency of VEGF-mediated neuroprotection. Deletion of the promoter of VEGF gene determining the response to hypoxia is followed by damage to lower motoneurons in transgenic mice. The observed changes are similar to those in ALS [7].

Genetic study showed that some polymorphisms of the human VEGF gene are associated with reduced expression of VEGF and higher risk of ALS (by 1.8 times) [6]. Blood VEGF concentration in these patients decreases by 50%. Moreover, the concentration of VEGF in the cerebrospinal fluid from patients correlates with the type and progression of ALS.

Previous observations showed that chromosome 14 in 2 patients with sporadic ALS carries mutant angiogenin gene [4]. These data confirm the

Research Center of Neurology, Russian Academy of Medical Sciences; *I. M. Sechenov Moscow Medical Academy; **Institute of Molecular Genetics, Russian Academy of Sciences; ***N. F. Gamaleya Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences, Russia

0007-4888/08/1454-0483

involvement of angiogenic factors in neurodegenerative processes during ALS. Several polymorphisms of the angiogenin gene are associated with the increased risk of ALS.

The discovery of neuroprotective functions of angiogenic factors opens new prospects for the therapy of ALS. Administration of VEGF-expressing lentiviral vector to transgenic mice with G93A mutation in the SOD1 gene increased the life span of animals by 30%. Published data show that expression of the VEGF gene remains high for 12 months after intramuscular injection of viral vector into extremities, tongue, diaphragm, *etc*. [5].

Here we studied the neurotrophic effect of angiogenic factors in ALS patients with neurodegenerative processes of the central nervous system. We evaluated the safety, tolerability, and effectiveness of a recombinant adenoviral expression vector with encoding sequences for VEGF and angiogenin (ANG). This vector was injected intramuscularly to ALS patients.

MATERIALS AND METHODS

Recombinant adenoviral vector was used to study neurotrophic function of angiogenic factors. VEGF and ANG genes were retrogradely transported to spinal motoneurons after intramuscular injection of recombinant adenoviruses.

A recombinant adenovirus Ad5-VEGF was obtained by the method of homologous recombination in *E. coli* cells. The human VEGF gene was cloned in a shuttle plasmid vector pShuttle-CMV (Stratagene). pShuttle-VEGF plasmid construct included genomic regions of human adenovirus serotype 5 (essential for homologous recombination) and expression cassette with the *vegf* gene. Homologous recombination in *E coli* strain BJ5183 cells was performed between pShuttle-VEGF and pAd-Easy plasmids (Stratagene). A pAd5-VEGF plasmid construct contained the full-length genome of Ad5. The expression cassette with the *vegf* gene was inserted into E1 genomic deletion. Cell line 293 was transfected with the pAd5-VEGF plasmid after hydrolysis by the PacI restrictase site (method of lipofection, Lipofectamin-2000, Invitrogen).

Recombinant adenovirus Ad5-ang contained expression cassette with the ANG gene in E1 genomic deletion. It was obtained after homologous recombination between pShuttle-ang and pAd-Easy plasmid constructs. Cell line 293 was transfected with the pAd5-ang plasmid.

Expression of the *vegf* gene was detected in the culture medium of 293 cells infected with recombinant adenovirus Ad5-VEGF in a dose of 5 PFU/cell. An enzyme immunoassay (EIA) was performed with the commercial kit Human VEGF QuantioGlo ELISA Kit (R&D Systems). Expression of the *ang* gene was detected in the culture medium of 293 cells infected with recombinant adenovirus Ad5-ang in a dose of 5 PFU/cell. An enzyme immunoassay was performed with the commercial kit Human Angiogenin Quantikine ELISA Kit (R&D Systems).

The results were analyzed by Statistica 7 software. The data are presented as means and standard deviations.

All patients were examined and selected according to international criteria for the inclusion and exclusion.

The present study was performed on 10 patients with cervicothoracic sporadic ALS. The patients were randomly divided into 2 groups (5 patients per group) and received AdvVEGF+ANG or placebo. They gave voluntary informed consent. This trial was approved by the Ethics Committee of the Research Center of Neurology.

The drug or placebo was injected bilaterally into the trapezius, deltoid, and quadriceps muscle. Injections were performed at 4-week intervals for 2 years. The single dose of study drug was 0.8 opt. U (2×10^9 viral infectious particles).

Each group consisted of 4 men and 1 woman. The age of patients in the treatment and placebo group was 41.8±4.1 and 52.0±10.8 years, respectively. The duration of the disease in patients of the treatment and placebo groups was 17.6±8.9 and 15.0±11.7 months, respectively. Both groups were of comparable sex, age, and type and duration of the disease.

The safety and tolerability of the test drug were estimated during monthly monitoring of physiological parameters (blood pressure, heart rate, body temperature, and body weight), laboratory tests of the blood and urine (clinical and biochemical tests and detection of oncology markers), recording of ECG, and evaluation of undesirable events.

The progression of the disease was estimated from the following parameters: neurological deficit (according to the Amyotrophic Lateral Sclerosis Functional Rating Scale Revised, ALSFRSR), vital lung capacity (VLC/TVLC, spirometry), muscle force of 36 muscles (according to the Manual Muscle Test [MMT] scale), and survival of ALS patients. The electromyogram was recorded to verify the diagnosis of ALS.

Blood VEGF concentration was measured under basal conditions and 2 weeks after injection of the test drug. EIA was performed with the commercial kit Human VEGF Quantikine ELISA Kit (R&D Sys-

tems). The amount of virus-neutralizing antibodies was measured monthly by the reaction of virus neutralization with wild-type human adenovirus serotype 5 in culture of 293 cells (human embryonic kidney cells) [3].

RESULTS

Five patients from the treatment group completed the first year of this trial. One patient refused participation in a further study after 1 year. One patient of this group died during the second year (22 months after the start of the trial). Three patients completed the trial.

Three patients of the placebo group completed the first year of this trial. One patient died 8 months after the start of the trial. One patient died 10 months after the start of the trial. One female patient died 23 months after the start of the trial. Two patients completed the trial.

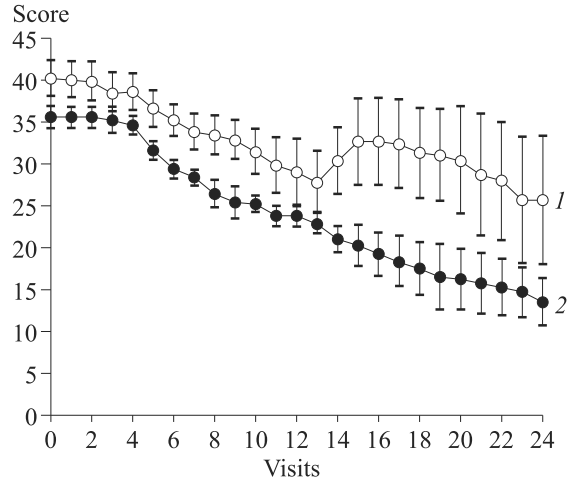

Fig. 1. Increase in the severity of neurological deficit in ALS patients (ALSFRSR) of the placebo (*1*) and treatment groups (*2*).

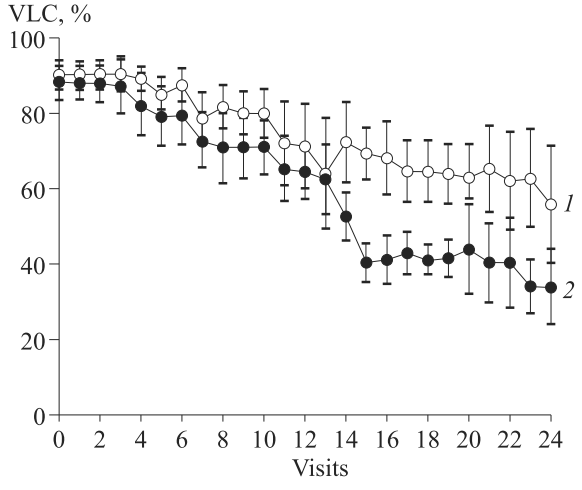

Fig. 2. VLC in ALS patients of the control (placebo, *1*) and treatment groups (*2*).

In all patients with ALS the disease progressed. We revealed an increase in the severity of neurological deficit and decrease in the total score by the ALSFRSR scale (Fig. 1). The patients exhibited increasing pulmonary failure and reduction of VLC (dynamics of VLC, Fig. 2), muscle strength (MTT scale), and body weight.

Despite progressive pulmonary failure in all 10 patients, the life span under hypoxia (VLC<45%) significantly differed in the treatment and placebo groups. The patients receiving AdvVEGF+ANG were more resistant to hypoxia than control patients (Fig. 3).

The content of virus-neutralizing antibodies in the blood significantly increased in patients of the AdvVEGF+ANG group, while the amount of antibodies in patients of the placebo group remained unchanged over 24 months. The content of antibodies increased most significantly in a patient dying by the 22nd month. The effectiveness of AdvVEGF+ANG in this patient decreased, which was probably related to a strong immune response and adenovirus neutralization.

Two-year study demonstrated the safety and good tolerability of adenoviral vector after intramuscular injection to ALS patients. No serious undesirable events were observed.

Short-term (2-3 h) mild systemic reactions in 2 patients were manifested in the influenza syndrome (fever, 38°C). AdvVEGF+ANG had little effect on the results of laboratory tests.

Taking into account possible oncogenicity of angiogenic factors, tumor markers in the blood of ALS patients were assayed before and 1 month or 1 year after the start of gene therapy. The following tumor markers were evaluated in female patients: epithelial cancer antigen (ECA), serum antigen (CA125), and dimeric tumor pyruvate kinase (TuM2-PK). The following tumor markers were evaluated in male patients: ECA, prostate-specific antigen (PSA, total and free), and TuM2-PK. The concentrations of ECA, PSA, and CA125 remained unchanged in patients of both groups. However, TuM2-PK concentration progressively increased in patients of the treatment and placebo groups. No intergroup differences were found in TuM2-PK concentration. The increase in dimeric pyruvate kinase activity with the progression of ALS probably reflects activation of metabolism in these patients. It cannot be excluded that TuM2-PK serves as a marker of progressive neurodegeneration during ALS.

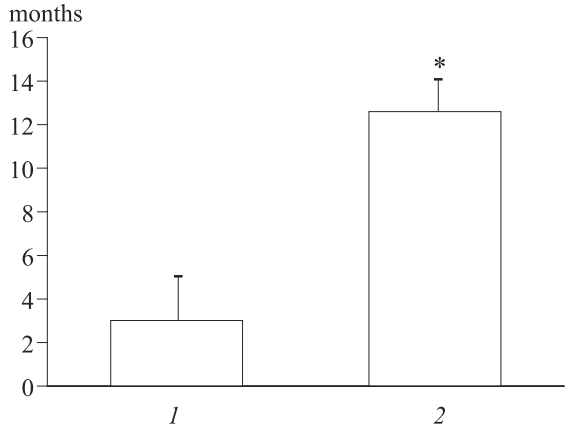

Fig. 3. Life span of ALS patients during hypoxia (VLC<45%): control (*n*=3, *1*) and treatment groups (*n*=3, *2*). **p*<0.05 compared to the control.

Two-year randomized placebo-controlled trial demonstrated the safety and good tolerability of adenoviral vector AdvVEGF+ANG in ALS patients. Administration of angiogenic factors increases the resistance of these patients to hypoxia. The presence of virus-neutralizing antibodies decreases the effectiveness of adenoviral vectors, which necessitates differential approach to the selection of patients and continuous monitoring of gene therapy.

REFERENCES

1. I. A. Zavalishin and M. N. Zakharova, *Neurodegenerative Diseases and Aging* [in Russian], Moscow (2001), pp. 354-399.
2. M. N. Zakharova, A. V. Vasiliev, and L. V. Brylev, *Amyotrophic Lateral Sclerosis* [in Russian], Ed. I. A. Zavalishin, Moscow (2007), pp. 354-423.
3. *Immune Diagnostics of Viral Infections* [in Russian], Eds. T. V. Peradze and P. Halonen, Moscow (1985).
4. M. J. Greenway, M. D. Alexander, S. Ennis, *et al*., *Neurology*, **63**, No. 10, 1936-1938 (2004).
5. B. Kaspar, J. Llado, and N. Sherkat, *Science*, **301**, 839-842 (2003).
6. D. Lambrechts, E. Strokebaum, M. Morimoto, *et al*., *Nature Gen*., **34**, No. 4, 383-394 (2003).
7. B. Oosthuyse, L. Moons, E. Strokebaum, *et al*., *Ibid*., **28**, No. 2, 131-138 (2001).