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Gamma-Carboline Inhibits Neurodegenerative Processes in a Transgenic Model of Amyotrophic Lateral Sclerosis

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Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease caused by the selective death of motor neurons. An important component in the ALS pathogenesis is the aggregation of proteins prone to conformational changes and the formation of characteristic intracellular histopathological inclusions, on the basis of which this disease was attributed to the group of proteopathies [1]. In addition to the already known gene *SOD1*, recent medical genetic studies have identified a number of other genes whose mutations lead to the formation of pathogenic forms of their encoded proteins and development a neurodegenerative process with motoneuron lesions [2].

In the sporadic ALS forms, these proteins were also found in the histopathological inclusions of the autopsy material of ALS patients. Moreover, studies performed in experimental models of ALS in transgenic mice and cell cultures provided evidence that the pathological picture of proteopathy characteristic of ALS can be reproduced in the case of metabolic disorder of only one of the key proteins [3].

When studying the mechanisms of proteopathies associated with ALS and frontotemporal degeneration, a new type of molecular cellular pathology was described. This pathology is caused by the dysfunction of the DNA/RNA-binding proteins TDP43 and FUS (fused in sarcoma), which results in the inability of these proteins to form physiologically active, easily dissociating complexes with RNA (RNP). Instead, these proteins form stable RNA-free structures with stably deposited aggregated forms of TDP43 and FUS proteins. This process is accompanied by changes in the intracellular compartmentalization of TDP43 and FUS and their accumulation in the pathogenic inclusions in the cytoplasm [4–7].

To model the proteopathy with the FUS protein, the aberrant forms of the FUS protein that were able to effectively form protein inclusions in cell cultures, similar to those detected in the autopsy material of ALS patients, were obtained [8]. It was found that the removal of the nuclear localization signal together with the C-terminal region, which is significant for the conformational stability of the protein molecule, leads to the development of proteopathy with the involvement of the FUS protein [5, 6]. In the sequences of the gene encoding these protein regions, the largest number of mutations association with the hereditary forms of ALS and frontotemporal degeneration was detected. On the basis of such aberrant form of FUS, a transgenic model of proteopathy (fusopathy) was developed. Mice of this transgenic line were characterized by the formation of histopathological inclusions in the nervous system tissues and the development of neurodegenerative process accompanied by a progressive loss of motor neurons [9]. These transgenic mice were used in this work to study the neuroprotective effect of a compound of the gamma-carboline group on the progression of the model neurodegenerative process accompanied by primary lesion of motor neurons. It was previously shown that drug Dimebon is able to decelerate the progression of other model proteopathies [10–13]. We also found that Dimebon exhibits a geroprotective effect in the experiments with C57BL/6 mice [14]. For this reason, Dimebon was selected as the basic compound in the series of gamma-carbolines for testing in the fusopathy model.

The main criteria to evaluate the inhibition of progression of the neurodegenerative model disease were the increased lifespan of the transgenic mice, the transition time of the disease from the presymptomatic to

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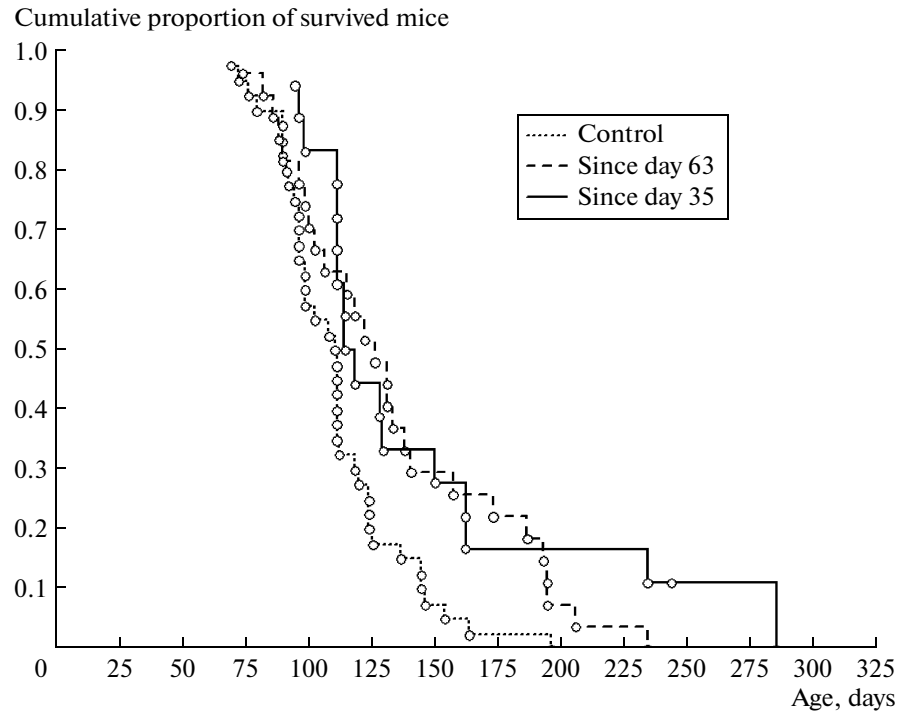

Fig. 1. Comparison of survival curves (according to Kaplan–Meir) between the groups of model animals administered with Dimebon at a dose of 11 mg/kg body weight starting from the age of 35 days ($n = 18$) and 63 days ($n = 27$) and the control group ($n = 40$).

symptomatic stage, and the symptomatic stage duration.

In this study, we used representative groups of hybrid male mice (CBA × C57BL/6J)F_1, in the genome of which the transgenic cassette encoding the pathogenic form of the FUS protein under a neurospecific promoter was in a hemizygous state [9].

Animals were genotyped by the conventional PCR using the primers 5'-TCTTTGTGCAAGGCCTGGGT-3' and 3'-AGAAGCAAGACCTCTGCAGAG-5' under the following conditions; preliminary denaturation for 2 min at 94°C followed by heating at 94°C for 15 s, at 58°C for 30 s, and at 72°C for 40 s (30 cycles) with detection of the 255-bp fragment.

The effect of Dimebon was studied in two phases of the model disease: in the early (presymptomatic) phase with the beginning of the drug administration on postnatal day 35 day and in the near-symptomatic phase, when the nervous system of the transgenic mice contained FUS-reactive histopathological inclusions (postnatal day 63). The experimental animals received the drug with drinking water ad libitum, and the control group did not receive the drug. Mice in the control and experimental groups were housed under the same conditions (12-h day/night cycle). The work with animals were performed in accordance with the "laboratory Practices in the Russian Federation" (2003). Data were statistically processed using Student's t test; differences were considered significant at $p < 0.05$.

A statistically significant increase in the mean lifespan of the mice treated with the drug at a dose of 11 mg/kg body weight was observed in both experimental groups. For example, the mean lifespan of the mice that received the drug starting from the age of 35 days ($n = 18$) was 143 ± 13 days, which is approximately 29% higher than in the control animals ($n = 40$).

Thus, the chronic administration of Dimebon, started in the early presymptomatic phases of the fusopathy model, increased the lifespan of transgenic animals (Fig. 1, $p = 0.012$). Moreover, a similar effect of Dimebon was observed in the group of mice that received the drug in the late presymptomatic phase of the model disease (i.e., immediately before the manifestation of the first clinical symptoms of the neurodegenerative process). In this group, when the drug was used as a dose of 11 mg/kg body weight ($n = 27$), the mean lifespan increased by 20% compared to the control ($r = 0.047$, Fig. 1).

The results suggest that Dimebon is able to decelerate the progression of the neurodegenerative process in fusopathy and that its efficiency was highest when the drug began to be used in the early presymptomatic phase of the disease. These data are consistent with the results obtained in the study of another proteopathy type, where a selective loss of motor neurons was also observed, though with the involvement of the γ-synuclein protein. Dimebon in this case also inhibited the progression of neurodegeneration both in the early

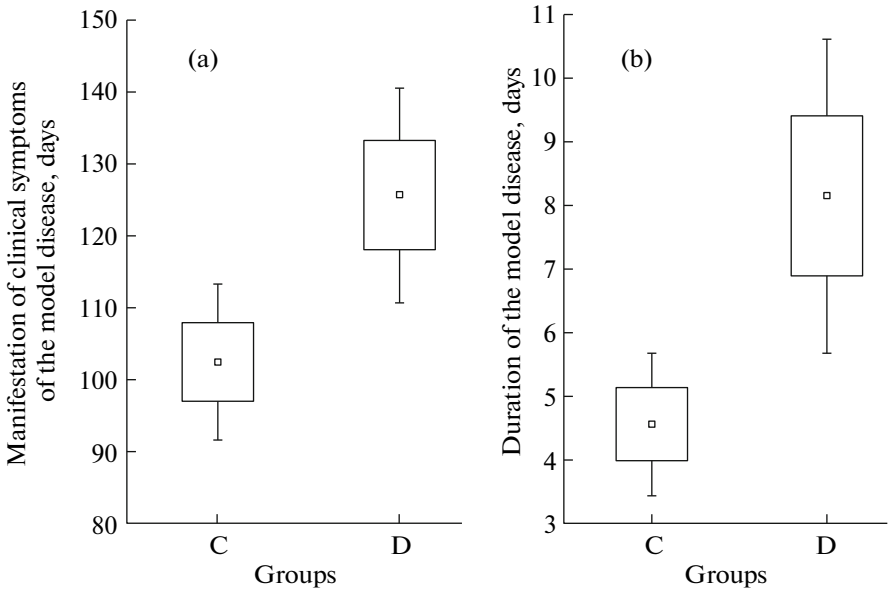

Fig. 2. Manifestation of clinical symptoms in transgenic animals. The mean age of (a) onset and (b) duration of the symptomatic period in the mice administered with the drug starting from the age of 63 days (D, $n = 27$) and in the control group (C, $n = 40$). Data are represented as $M \pm m$.

presymptomatic and in late symptomatic stages of the model disease [10, 15].

Another important index of the efficacy of Dimebon in fusopathy is the delayed beginning of manifestation of the clinical symptoms of the neurodegenerative process (Fig. 2). In the group of the transgenic animals that were administered with the drug starting from day 63, the mean age of the beginning of the model disease was 126 ± 7 days, which is 22% later than in the control group ($r = 0.023$). This delay was accompanied by a statistically significant decrease in the rate of progression of the neurodegenerative process in the terminal stage of the model disease, which was expressed in an increase in the symptomatic phase duration (Fig. 2b).

Thus, our findings suggest that Dimebon decelerates the progression of the neurodegenerative process that is accompanied by the death of motor neurons in the model fusopathy. On the basis of the results of this study, Dimebon and a number of other carboline compounds can be consider as a basis for the development of pathogenetic therapy of ALS, the development of which is extremely important in view of the limited number of treatment methods. The only drug used to increase the life expectancy of patients with ALS is Riluzole, which prolongs the life of patients for at most 10% without changing the quality of life of the patient. Currently, new approaches to the treatment of ALS based on the use of modern biotechnological methods and replacement therapy for the lost motor neurons in patients with advanced stages of the disease are developed. The creation of drugs that have a therapeutic effect in ALS is regarded as an essential component of complex therapy. Compounds of the γ-carboline series (Dimebon and its fluorinated derivatives), which exhibit an improved pharmacokinetics, can decelerate the progression of proteopathy due to activation of own intracellular systems of controlled degradation of pathogens protein complexes, reducing the amount of intracellular deposits and their intermediate toxic aggregation products. Possibly, this may explain the effect of Dimebon on the lifespan of model animals, which manifests itself as a delayed onset of the model disease.

Our data are the first evidence that the compounds of the γ-carboline series can be considered as the basis for the development of drugs for the treatment of neurodegenerative diseases characterized by a specific loss of motor neurons.

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