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Antibodies to Glutamate Reversed the Amnesic Effects of Proinflammatory S100A9 Protein Fibrils in Aged C57Bl/6 Mice.

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Abstract

Chronic intranasal administration of fibrillar structures of proinflammatory S100A9 protein impaired passive avoidance learning in old C57Bl/6 mice. Combined treatment with S100A9 fibrils and antibodies to glutamate was followed by an increase in horizontal locomotor activity of animals in the open-field test and did not disturb spatial memory.

Key Words: amnesia; S100A9 fibrils; glutamate antibodies; spatial memory; locomotor

The increase in the incidence of cognitive disorders in elderly and old people is an urgent problem of modern society. Alzheimer's disease (AD) is the most common type of dementia. The major symptoms of this chronic neurodegenerative disease include memory, thinking, and behavioral disturbances [1]. Little is known about the mechanisms of cognitive deficit in AD [3]. Disturbed conformation of some proteins and peptides is a

molecular mechanism leading to apoptotic death of neurons and subsequent development of dementia. Some misfolded proteins can form pathological aggregates that are deposited in brain parenchyma (e.g., senile plaques of β-amyloid protein [2,5]) and induce degeneration of neurofilaments with the formation of neurofibrillary tangles. These changes contribute to activation of microglia, induction of the endosomal-lysosomal signaling system in cells [2], inhibition of tissue oxidative systems [7], neuroinflammation [12], development of glutamate excitotoxicity [10], and apoptosis [1]. Along with $A\beta_{1-42}$ and tau protein playing a key role in the mechanisms of AD, much attention is paid to proinflammatory S100A9 protein whose expression increases in the microglia (e.g., in the temporal cortex of the brain) during inherited and sporadic AD [14]. S100A9 folding is sometimes impaired with the formation of toxic oligomeric and fibrillar structures of S100A9 (S100A9-FS) and their accumulation (aggregation) in the brain [13,14]. These changes can serve as a differential marker of early cognitive deficit during AD [6]. Previous studies showed that intranasal administration of monospecific antibodies to glutamate (AB-GL) produces an antiamnesic effect in the model of AD induced by injection of a neurotoxic fragment $A\beta_{25-35}$ into the Meynert nucleus of adult rats [4]. This work was designed to study the effect of in vitro synthesized amyloidogenic S100A9-FS on spatial memory formation during passive avoidance conditioning and locomotor activity of animals. We also evaluated the influence of combined treatment with AB-GL and S100A9-FS (intranasally) on old C57Bl/6 mice.

MATERIALS AND METHODS

Experiments were performed on 12-month-old male C57Bl/6 mice (n=60) weighing 31.0±1.0 g. The animals were maintained under standard conditions (12:12-h light/dark regimen) and had free access to water and food. To obtain S100A9-FS, native protein was dissolved in 10 mM sodium-phosphate buffer (pH 7.4), sonicated for 15 min, and centrifuged on an Eppendorf Centrifuge 5417R at 14,000 rpm. The supernatant was filtered through 0.22-μ filters (Millex) and incubated (concentration 2 mg/ml) in 10 mM sodium-phosphate buffer (pH 7.4) at 37 ^{o}C and constant agitation (Eppendorf Thermomixer Compact) for 24 h. The formation of amyloid S100A9-FS was estimated from variations in fluorescence of a staining agent (thioflavin T). Morphological characteristics of S100A9-FS were evaluated by atomic-force microscopy [11]. Polyclonal monospecific AB-GL were obtained by immunization of Chinchilla rabbits with a conjugate of glutamate and BSA. This conjugate was obtained with a bifunctional reagent glutaraldehyde [13]. The γ-globulin fraction of antibodies was precipitated from

serum samples of immunized rabbits with ammonium sulfate (40% saturation) and dialyzed. The γ-globulin fraction was purified from anti-BSA antibodies by means of affinity chromatography (CNBr-Sepharose 4B [Sigma] as a sorbent with immobilized BSA), lyophilized, and stored at 4 ^{o}C. The content of AB-GL in serum samples from immunized rabbits was estimated by solid-phase ELISA. The conjugate of glutamate and horse γ-globulin (heterologous protein carrier) was used as a test antigen. The titer of AB-GL was 1:1024±1:16. C57Bl/6 mice were divided into 5 equal groups. Control animals received physiological saline (4 µl) intranasally for 14 days. Mice of two treatment groups intranasally received S100A9-FS (in vitro individual preparation, 0.48 mg/kg in 4 µl). Animals of the two other groups were treated with S100A9-FS and ABGL (250 µg/kg in 4 µl). In series I, the behavior of mice was studied in the open-field test. We recorded the distance of running, time of locomotion and rest, and number of vertical rearing postures. Locomotor activity was evaluated in an automated test with AutoTrack software (OptoVarimex system, Columbus Instruments) for 0-6 min. In series II, passive avoidance reflex was conditioned in mice. The experimental chamber consisted of light (15.5×15.5×19 cm) and dark (9×9×17 cm) compartments and metal grid floor consisting of rods 0.3 cm in diameter and spaced 0.9 cm apart. Both chambers were connected through a gate with a guillotine door. On day 1, the mice were individually placed into a light compartment illuminated with a 100-W lamp and allowed to familiarize with the chamber for 60 sec. By the end of this session, the door between two compartments was opened. Due to innate hole exploratory behavior typical of rodents, the animal moved to a dark compartment. The latency of transition (LT1) was recorded. When the animal appeared in a dark compartment (with all four limbs and 2 / 3 of the tail), the door was closed and electrocutaneous stimulation (0.3 mA, 1.0 s) was applied, after which each animal was immediately removed and placed in the home cage. The acquired skill was tested after 24 h. The animal was placed in an illuminated compartment for 30 sec to familiarize with the conditions. The door was opened, and the latency of transition into a dark compartment was recorded (LT2). The behavior of animals was studied for 300 sec (beginning from the door opening on days of training and testing). The difference between LT of animal transition into the dark compartment during learning and 24 h after training (ΔLT) reflected the memory retention. The results were analyzed by Statistica 6.0 software. Several independent samples were compared by one-way nonparametric analysis of variance (Kruskal-Wallis H test) followed by post-hoc analysis (Mann-Whitney U test). The data are presented as M±m. The critical level of statistical significance in null hypothesis testing was taken as 0.05.

RESULTS AND DISCUSSION

The size of S100A9-FS was ~3 nm. The intensity of ThT fluorescence during their formation was elevated by 5.6 times. The time of locomotion in the open-field test for S100A9-FS-receiving mice was 20.4% greater than in controls. However, the mean free path length (locomotor distance) did not differ from the controls. Intranasal co-administration of S100A9 fibrils with AB-GL led to a 32.0% increase in open field free path length, an elevation of ambulation time by 35.7% but a 57.0% reduction in the immobility time compared with controls. There was also a 11.3% increase in ambulation time (time of locomotion) and decreased immobility time by 26.0% in these animals compared with the group treated with S100A9 fibrillar structures alone (Table 1).

TABLE 1. Effect of Intranasal Administration of S100A9-FS and AB-GL on Locomotor Activity of Old C57Bl/6 Mice in the Open-Field Test (n=12; $M \pm m$)

Parameter	Control	S100A9-FS	S100A9-FS+AB-GL
Locomotor distance, cm	1401.8±116.2	1642.6±79.6	1851.1±51.2*
Resting, sec	88.6±10.2	76.4±5.5	56.4±4.0*+
Time of locomotion, sec	107.0±7.9	128.9±5.1*	145.2±3.5*+
Speed, cm/sec	13.0±0.2	12.6±0.2	12.7±0.2
Number of vertical rearing postures	26.8±6.2	29.7±3.1	25.6±3.5

Note. $p<0.05$ in comparison with the *control, +S100A9-FS-receiving mice.

In experiments on passive avoidance behavior 15 days after initiation of daily S100A9 fibril administration, the aged mice developed a spatial memory deficit which was manifested by a reduction of ΔLT to 28.9 ± 21.6 ($P<0.01$), compared with a control group (ΔLT = 144, 24.6 + 0. In the animal group intranasally treated for 14 days with S100A9 fibrillar structures combined with AB-GL, the degree of reminding (ΔLT= 117.9 ± 34.2 s) did not differ from that displayed by the controls (Fig 1.)

These findings have disclosed an amnestic effect expressed on passive avoidance behavior incited by S100A9 fibrillar structures in aged mice. This action potentially stems from the development of glutamate excitotoxicity and subsequent apoptotic neuronal death in relevant brain structures. Hence, intranasal co-administration of AB-GL with S100A9 fibrils reversed the passive avoidance behavioral disruption through a

protective antiamnestic action probably originating from AB-GL binding any glutamate excess thereby reducing the prospect of neuronal apoptosis [8, 9]. The marked increase in horizontal locomotor activity in the open field following co-administration of AB-GL with S100A9 fibrils reflected unhindered orienting-exploratory activity. This would therefore serve as additional support for reduced toxic effects of both glutamate and S100A9 fibrillar structures themselves.

In conclusion, the results of the current study demonstrated on one hand, that amyloidogenic fibrillar species of the pro-inflammatory protein S100A9 are capable of instigating spatial memory impairment. This may be a component mechanism in the molecular sequence leading to cognitive deficits implicated in neurodegenerative diseases. On the other hand, intranasally administered monospecific antibodies to glutamate displayed protective effects and reversed spatial amnestic activity in aged mice.

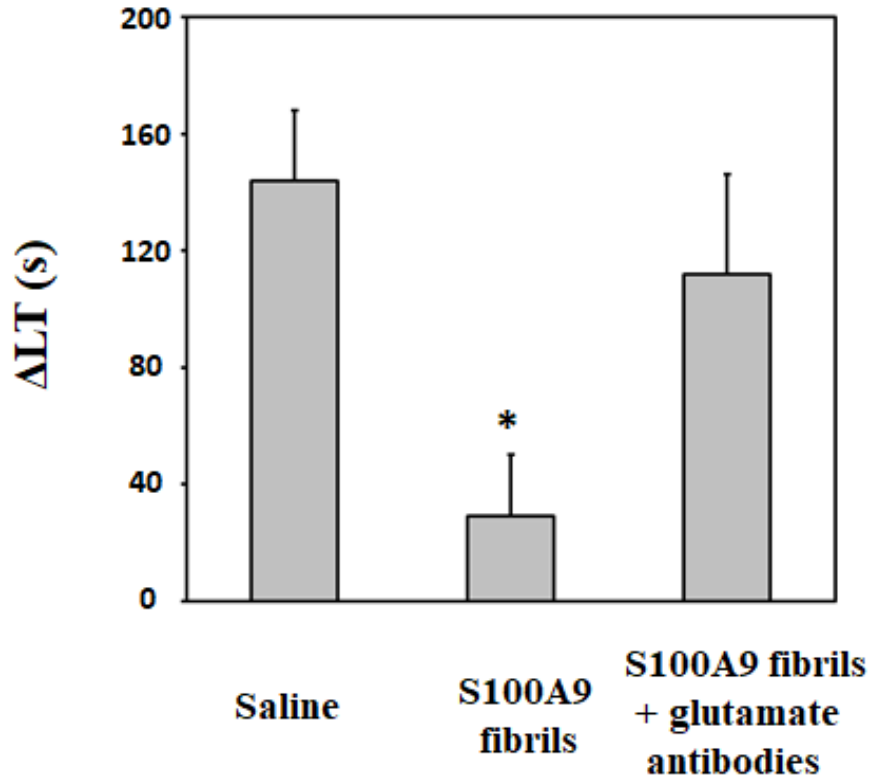

Figure 1. Effects of intranasal administration of the pro-inflammatory protein S100A9 amyloidgenic fibrillar structures individually or in co-administration with nonspecific antibodies to glutamate on passive avoidance behavior in comparison with control aged C57BL/6 mice.

The ordinate shows ΔLT (s); * - $P < 0.05$ compared to control.

REFERENCES

1. Acute neuronal injury. The Role of Excitotoxic Programmed Cell Death Mechanisms. Fujikawa DG, ed. New York, 2010.

2. Cataldo AM, Hamilton DJ, Barnett JL, Paskevich PA, Nixon RA. Abnormalities of the endosomal-lysosomal system in Alzheimer's disease: relationship to disease pathogenesis. Adv. Exp. Med. Biol. 1996;389:271-280.

3. De-Paula VJ, Radanovic M, Diniz BS, Forlenza OV. Alzheimer's disease. Subcell Biochem. 2012;65:329-352.

4. Gorbatov VY, Trekova NA, Fomina VG, Davydova TV. Antiamnestic effects of antibodies to glutamate in experimental Alzheimer's disease. Bull. Exp. Biol. Med. 2010;150(1):23-25.

5. Gruden MA, Davudova TB, Malisauskas M, Zamotin VV, Sewell RD, Voskresenskaya NI, Kostanyan IA, Sherstnev VV, Morozova-Roche LA. Autoimmune responses to amyloid structures of Abeta(25-35) peptide and human lysozyme in the serum of patients with progressive Alzheimer's disease. Dement. Geriatr. Cogn. Disord. 2004;18(2):165-171.

6. Horvath I, Jia X, Johansson P, Wang C, Moskalenko R, Steinau A, Forsgren L, Wågberg T, Svensson J, Zetterberg H, Morozova-Roche LA. Pro-inflammatory S100A9 protein as a robust biomarker differentiating early stages of cognitive impairment in Alzheimer's disease. ACS Chem. Neurosci. 2016;7(1):34-39.

7. Keller JN, Kindy MS, Holtsberg FW, St Clair DK, Yen HC, Germeyer A, Steiner SM, Bruce-Keller AJ, Hutchins JB, Mattson MP. Mitochondrial manganese superoxide dismutase prevents neural apoptosis and reduces ischemic brain injury: suppression of peroxynitrite production, lipid peroxidation, and mitochondrial dysfunction. J. Neurosci. 1998;18(2)687-697.

8. Kolobov VV, Davydova TV, Fomina VG. Protective action of glutamate antibodies on increased expression of genes of programmed death of rat brain cells induced by injection of a β-amyloid fragment (25-35). Izv. Akad. Nauk. Ser. Biol. 014;(2):133-141

9. Kolobov VV, Fomina VG, Davydova TV. Antibodies to glutamate reduce the neurotoxic effects of $A\beta_{(25-35)}$ in prefrontal cortex cell transcriptome. Dokl. Biochem. Biophys. 2012;447:277-279.

10. Lewerenz J, Maher P. Chronic glutamate toxicity in neurodegenerative diseases-what is the evidence? Front. Neurosci. 2015;9:469.

11. Malisauskas M, Zamotin V, Jass J, Noppe W, Dobson CM, Morozova-Roche LA. Amyloid protofilaments from the calcium-binding protein equine lysozyme: formation of ring and linear structures depends on pH and metal ion concentration. J. Mol. Biol. 2003;330(4):879-890.

12. Minter MR, Taylor JM, Crack PJ. The contribution of neuroinflammation to amyloid toxicity in Alzheimer's disease. J. Neurochem. 2016;136(3): 457-744.

13. Vogl T, Gharibyan AL, Morozova-Roche LA. Pro-inflammatory S100A8 and S100A9 proteins: self-assembly into multifunctional native and amyloid complexes. Int. J. Mol. Sci. 2012;13(3)2893-2917.

14. Wang C, Klechikov AG, Gharibyan AL, Wärmländer SK, Jarvet J, Zhao L, Jia X, Narayana VK, Shankar SK, Olofsson A, Brännström T, Mu Y, Gräslund A, Morozova-Roche LA. The role of pro-inflammatory S100A9 in Alzheimer's disease amyloid-neuroinflammatory cascade. Acta Neuropathol. 2014;127(4)507-522.