Effect of Ultralow Doses of Antibodies to S-100 Protein in Animals with Impaired Cognitive Function and Disturbed Emotional and Neurological Status under Conditions of Experimental Alzheimer Disease

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Course administration of ultralow doses of antibodies to S-100 protein restores cognitive functions and neurological status, improves emotional state, and reduces anxiety in animals with modeled Alzheimer disease based on cholinergic system deficiency caused by subchronic treatment with scopolamine.

Key Words: *S-100 protein; ultralow doses; Alzheimer disease; scopolamine*

Alzheimer disease (AD) is a slowly progressing neurodegenerative disease characterized by pronounced disturbances of cognitive functions with the loss of memory and learning capacity. Changed emotional status, anxiety, nervousness, agitation, and depression are also typical of AD patients.

Cholinergic system deficiency is a pathogenetic mechanism of AD development. The deficiency of cholinergic system is responsible for cognitive and behavioral deficit in AD. That is why cholinergic receptor agonists, primarily acetylcholinesterase inhibitors, are widely used for the treatment of AD [3,8]. Nootropic preparations are also used for correction of mnestic disorders in dementias [5,6,9].

Under experimental conditions, antagonists of the cholinergic system, *e.g.* scopolamine, induce memory disturbances similar to those observed in AD [4,9]. Therefore, repeated administration of scopolamine is widely used for AD modeling [1].

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Here we studied the effect of ultralow doses of antibodies to S-100 protein (ULD of anti-S100) on disturbed cognitive functions and emotional and neurological status under conditions of modeled AD.

MATERIALS AND METHODS

Experiments were carried out on 90 outbred male rats. The animals were maintained in plastic cages in an illuminated room and had free access to water and food. Muscarinic receptor blocker scopolamine was administered daily for 20 days for modeling cognitive dysfunction and after 10 days rat behavior and general state were evaluated [1].

The rats were randomly divided into 3 equal groups. Group 1 animals received intraperitoneal injections of distilled water, animals of groups 2 and 3 received scopolamine in a daily dose of 1 mg/kg. On days 21 through 30, groups 1 and 2 received distilled water *per os* and group 3 rats received ULD of anti-S100 in a volume 0.25 ml per 100 g body weight twice a day.

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Passive avoidance response (PAR) was conditioned in a shuttle box consisting of 2 similar compartments with electrode floor [1]. The strength of electrical painful stimulation was chosen individually for each animal. Escape (unconditioned response) was a transition to the adjacent compartment in response to electrical stimulus; avoidance (conditioned response) was a transition to the adjacent compartment in response to conditioned stimulus before electrical painful stimulation. The rats were trained for 7 days (10 presentations per day). Seven consecutive avoidance reactions per 10 presentations were considered as learning criterion.

Rat anxiety was evaluated in an elevated plus maze (EPM) [7] consisting of crossing arms (50×10 cm) elevated by 50 cm above the floor. Two opposite arms had 40-cm vertical walls. The time spent in open arms and the number of entries into light and dark arms were recorded. The number of crossings the central platform (total number of entries into light and dark arms of the maze) and the number of rearing postures were used for evaluation of the effect of the test compounds on motor activity of experimental animals. The latency of the first entry into the arm, the time spent on the central platform, and the number of grooming episodes and boluses were also recorded. The total time of observation was 5 min for each animal.

Orientation and exploratory behavior was evaluated using the open field test in a chamber (60×60 cm) with transparent cover. The floor of the chamber was divided into 9 squares and had 16 holes with a diameter of 4 cm. Rearing postures (vertical motor activity), crossed squares (horizontal motor activity), explored holes, grooming episodes, and boluses were counted.

Neurological status was evaluated using McGrow Stroke Index with modifications [2]. The number of rats with mild (score 0.5-2.5) and severe (score 3-10) neurological symptoms was determined.

TABLE 1. Effect of ULD of Anti-S100 on PAR Conditioning (Number of Conditioned Responses, % of the Number of Presentations; *M*±*m*)

Days of training	Group 1	Group 2	Group 3
Day 1	3.3±2.4	2.0±1.3	2.2±1.5
Day 2	12.2±4.7	4.0±3.1	3.3±2.4
Day 3	14.5±3.8	16.0±4.5	14.5±4.8
Day 4	22.2±6.0	14.0±6.9	32.2±12.1
Day 5	46.7±12.9	25.0±9.9	46.7±15.3
Day 6	55.6±10.7	18.0±8.0*	52.2±13.6^{+}
Day 7	67.8±12.3	20.0±10.3*	48.9±15.1^{+}

Note. Here and in Tables 2 and 3: $p<0.05$ compared to *group 1, $^{+}$group 2.

Disturbances in movement coordination were studied using rotarod test [6,9]. Inability of maintaining the balance over 2 min on a rod with a diameter of 4 cm rotating at a rate of 3 rpm was considered as disturbed coordination of movements [1].

Muscular tone was evaluated using the pull-up test on a horizontal bar elevated at a height of 20-30 cm above the floor. Pull-up failure attested to myorelaxant effect [1].

The data were processed statistically using one-way Kruskal—Wallis rank test. If the differences were significant ($p\leq0.05$), paired comparison was performed using nonparametric Mann—Whitney *U* test, Student *t* test; for the analysis of alternative form of registration χ^2 test was used.

RESULTS

In series I, the effect of ULD of anti-S100 on disturbed cognitive functions of rats was studied. In group 1, the

TABLE 2. Effect of ULD of Anti-S100 on Rat Behavior in EPM (*M*±*m*)

Parameter	Group 1	Group 2	Group 3
Latency of the first movement	17.8±5.1	11.1±1.5	10.4±2.0
Time spent on the central platform	31.6±9.0	13.3±3.6*	28.3±7.6^{+}
Time spent in the dark compartment	244.5±9.8	273.9±5.3*	258.2±9.3
Time spent in the light compartment	6.1±3.4	0.6±0.6*	3.1±0.2^{+}
Total number of transitions	5.9±1.3	2.6±0.8*	6.4±0.9
Rearings	6.5±1.3	3.3±0.8*	4.5±1.1
Grooming	2.1±0.6	3.0±0.9	2.8±0.7
Boluses	2.3±0.8	1.4±0.6	1.6±0.4

TABLE 3. Effect of ULD of Anti-S100 on Orientation and Exploratory Activity of Rats with Cholinergic System Deficiency in the Open Field Test (Score, *M*±*m*)

Parameter	Group 1	Group 2	Group 3
Horizontal activity	15.2±4.0	15.6±5.6	18.3±4.0
Vertical activity	6.7±1.9	3.1±1.1	5.4±0.8
Explored holes	11.3±2.4	4.7±0.8*	10.5±3.1+
Washing episodes	2.0±0.3	2.2±0.4	1.6±0.3
Defecation	1.7±0.2	1.5±0.2	1.2±0.2

number of conditioned avoidances on day 1 was 3.3%, but increased to 67.8% by day 7 (Table 1).

In group 2, PAR conditioning was significantly delayed. The dynamics of PAR conditioning during the first 3 days was similar to that in group 1 animals, but on days 5-7 the number of conditioned avoidances did not decrease and by day 7 learning criterion was attained in only 20% rats.

The dynamics of PAR conditioning in group 3 rats did not differ from that in group 1. By day 7, the learning criterion in group 3 was attained in 55.5% animals.

Subchronic administration of scopolamine leading to deficiency of the cholinergic system increased anxiety in experimental rats, which manifested in a 10-fold decrease in the time spent in open arms and prolonged stay in closed arms of EPM. Motor activity evaluated by the number of crossed squares and rearing postures also decreased (Table 2).

These changes attest to increased anxiety of rats with cholinergic deficiency. Administration of ULD of anti-S100 increased the time spent in open arms and on the central platform by 5 and 2 times, respectively ($p<0.05$).

Open-field testing of rats with cholinergic deficiency showed that the number of explored holed decreased by 2.2 times ($p<0.05$, Table 3). Administration of ULD of anti-S100 prevented the development of these disturbances and increased the number of explored holed to a level observed in group 1.

In rats receiving scopolamine, evaluation of the neurological status by McGrow scale revealed changes attesting to neurological deficit: weakness and flaccidity of movements, unilateral half-ptosis and unilateral ptosis were observed in 50% animals. In group 3 these abnormalities were observed in only 2 rats.

The rats treated with scopolamine demonstrated a tendency to weakening of the muscular tone: rotarod performance was disturbed in 30% rats and 30% rats were unable to pull up hind limbs in the test on a horizontal bar. In the group receiving ULD of anti-S100, only 20% rats failed to stay on the rotating rod and only 10% animals were unable to pull up hind limbs.

Thus, 10-day course treatment with ULD of anti-S100 improved cognitive functions by accelerating PAR conditioning, normalized emotional state, improved parameters of neurological status according to McGrow scale and in the rotarod test, and reduced anxiety in EPM test.

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