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New avenues for treatment with trehalose of mice with pharmacological and transgenic models of Alzheimer's disease

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Motivation and Aim: Recently, the treatment of animal models of Alzheimer's disease (AD) using autophagy activation has been actively developed. It can attenuate the accumulation of aberrant proteins, activate mitophagy, reduce oxidative stress and neuroinflammatory process [1, 2]. Positive results have been obtained for both inducers of mTOR-dependent autophagy (rapamycin and its analogs) [3, 4] and activators of mTOR-independent autophagy (lithium, curcumin, metformin) [5]. Induction of mTOR-independent autophagy, in contrast to mTOR-dependent autophagy, does not cause concomitant pleiotropic effects of suppression of biosynthetic processes, inhibition of cell growth and proliferation, and suppression of immunity [6]. The extent to which these effects are therapeutically beneficial or detrimental for the treatment of neurodegeneration is not known. Among mTOR-independent inducers, the disaccharide trehalose has attracted particular attention for its ability to reduce Aβ accumulation, produce antioxidant and anti-inflammatory effects, and restore hippocampal neurons and cognitive function [7–9]. Therapeutic effects have been identified for both pharmacological and transgenic models of the disease [9–11]. The predominant mode of treatment is the addition of trehalose in drinking (2% solution) [12, 13]. Nevertheless, it is of interest to find the optimal regimens for trehalose administration [7, 9] and reveal the efficacy of trehalose treatment depending on the age of mice [4, 14], since it is important to know the effectiveness of treatment at late stages of disease course, close to the translational transition.

Methods and Algorithms: A dose-dependent therapeutic effect of trehalose (2, 4, and 4% in the intermittent mode) was studied in a pharmacological model of AD induced by intracerebroventricular injection of aggregated amyloid-β fragment Aβ (25–35) [15]. To elucidate the efficacy of age-dependent trehalose treatment, mutant mice of 5xFAD strain with progressive course of the disease were used. Three groups of mice were studied: 1. Trehalose treatment (2 months) of 2-month-old mice. 2. Trehalose treatment (5 months) of 2-month-old mice. 3. Trehalose treatment (2 months) of 5-month-old mice. In mice of the 5xFAD strain, behavioral abnormalities have appeared at the age of 5–6 months. Behavioral testing was performed using open-field, plus-maze, and conditioned passive avoidance response tests. At the end of testing, brain material was collected for immunohistochemical analysis (IHC) using transcardiac perfusion of the brain with saline solution containing 4 % paraformaldehyde. In the frontal cortex and three hippocampal regions (CA1, CA3, and dentate gyrus) affected in AD, the expression

of autophagy marker LC3-II and neuroinflammatory marker IBA1, Aβ accumulation, and neuronal density (Nissl staining) were evaluated [15, 16].

Results: Pharmacomodeling of AD by administration of Aβ (25–35) caused a marked increase in the levels of the disease marker Aβ in the frontal cortex and all hippocampal areas studied, while all variants of two-week trehalose treatment sharply reduced the index ($p < 0.01$ for the CA1 hippocampal area), restoring it almost to baseline. In parallel, a significant activation of autophagy was noted, especially for 4% trehalose ($p < 0.01$). Trehalose substantially attenuated Aβ-induced inflammation of the hippocampus measured by the expression of microglial marker IBA1 ($p < 0.05$), and restored the density of Nissl-stained neurons in the frontal cortex and CA1 hippocampal area. All trehalose treatment regimens resulted in cognitive improvement of the fear-associated long-term memory and learning in the passive avoidance in the with the maximal effect of complete recovery for 4 % trehalose solution at continuous mode.

The disadvantage of the pharmacological model of AD is the short-term effect of neurointoxication, less pronounced signs of pathology, and spontaneous recovery due to the compensatory processes. At the same time, in humans, AD is a progressive disease which obviously needs prolonged treatment at different periods of disease course. We studied mice of the 5xFAD strain, which carry five mutations in two AD-related genes *App* and *Psen1* (*App* gene: K670N/M671L; I716V; V717I; P *Psen1* gene: M146L; L286V). At the age of 2 months, 5xFAD mice are characterized by the Aβ accumulation in the brain; at the age of 6 months they demonstrate phenotypic cognitive disorders [17]. In the T-maze and open field tests, no significant differences in the treatment of the three age-dependent groups of 5xFAD mice were observed. It is worth noting the normalizing effect on anxiety during prolonged (5 months) trehalose treatment. The core results of the therapy efficacy were obtained in the passive avoidance test. The main index (step-through latency) was sharply reduced in 5xFAD mice of all ages studied compared to the wild-type. Trehalose treatment was effective for all groups of mice of different ages ($p < 0.01$), the step-through latency was almost restored to the level of wild-type control mice. We can highlight the high results of behavioral recovery in the 2-month treatment group at the later period of the disease (5 months). Apparently, this is due to the beginning of the formation of phenotypic differences in the behavior of mice of this strain at this age [17].

Conclusion: 1. Elucidation of the dose-dependence of the therapeutic effect of trehalose showed that conventional treatment of Aβ-induced AD-like pathology in mice by drinking of 2 % trehalose solution could be improved by increasing the trehalose concentration (up to 4 %) in a regimen of constant (but not intermittent) trehalose consumption. In the subsequent experiments, the effective concentration of trehalose was increased to 3 %.

2. In an age-dependent study, the feasibility of treating a transgenic “progressive” model of AD with trehalose in young “latent” 2-month-old mice (corresponding to the onset of accumulation of the disease marker Aβ) was demonstrated. Similarly, treatment was also successful in mice of the mature 5-month-old age known for the appearance of phenotypic behavioral abnormalities. This latter result may be useful for considering the translational transition.

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