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Zinc Supplementation Improves ZIP14 (SLC39A14) Levels in Cerebral Cortex Suppressed by icv-STZ Injection

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ABSTRACT

Introduction: Metabolic dysfunctions are critical in the pathology of Alzheimer's disease. Impaired zinc homeostasis, in particular, is a significant issue in this disease that has yet to be explained. Gene expression of ZIP14 in brain tissue has been previously reported. But to date, only one study has reported reduced ZIP14 levels in aged brain tissue. We investigated how dietary zinc deprivation and supplementation impact ZIP14 levels in the cerebral cortex in rats with sporadic Alzheimer's disease (sAH) produced by intracerebroventricular streptozotocin (icv-STZ). Impaired zinc homeostasis, in particular, is a significant issue with this condition that has yet to be elucidated.

Methods: Animals were divided into 5 groups in equal numbers (n=8): Sham 1 group: icv received artificial cerebrospinal fluid (aCSF); Sham 2 group: retrieved icv aCSF and intraperitoneal (ip) saline, STZ group: received 3 mg/kg icv-STZ; STZ-Zn-Deficient group: received 3 mg/kg icv-STZ and fed a zinc-deprived diet; STZ-Zn-Supplemented: It received 3 mg/kg icv-STZ and ip zinc sulfate (5 mg/kg/day ZIP 14 levels (ng/L) in cortex tissue samples taken from animals sacrificed under general anesthesia were determined by ELISA at the final stage of the experimental applications.

Results: Decreased ZIP14 levels in the sporadic Alzheimer's group were severely by zinc deficiency. Zinc supplementation treated the reduction in ZIP14 levels.

Conclusion: The results of the current study show that ZIP14 levels in cerebral cortex tissue, which are suppressed in the experimental rat Alzheimer model and are even more critically reduced in zinc deficiency, can be restored by zinc supplementation.

Keywords: Cerebral cortex, icv-STZ Injection, ZIP14, zinc deficiency, zinc supplementation

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INTRODUCTION

Various nutritional deficiencies, particularly disturbances in mineral and vitamin balance, have been associated with several neurological abnormalities (1). Supporting this report, it was pointed out that the disturbance in zinc balance may be important in many abnormalities in brain functions, especially depression (2–4). Zinc, the second most abundant trace element in the human body after iron, is required for a variety of biological processes including growth, development, reproduction, cellular immune reactions, and brain development and function (2). Zinc, which is necessary for the maintenance of brain functions, is also critical in the regulation of memory performance, learning and synaptic transmission (5). It has already been shown that a regression in the brain's zinc balance leads to deterioration in the mentioned functions (3,6). Zinc balance in cells is maintained by tight regulation of zinc transport and distribution to organelles. This regulation is mediated by zinc transport proteins responsible for zinc transport. Zinc carrier proteins are categorized into two main groups. These are ZIP (SLC39) and ZnT (SLC30) proteins. ZIP (SLC39) transporters regulate zinc distribution in the cytosol, while ZnT (SLC30) transporters provide zinc transport from the cytosol (7,8). The ZIP14 (SLC39A14) transporter is a zinc transporter protein that can be induced by proinflammatory pathways.

Highlights

- **Zinc affects ZIP14 levels in the cerebral cortex.**
- **ZIP14 levels change in experimental Alzheimer's.**
- **Dietary zinc status has critical effects in Experimental Alzheimer's.**

Mutations in this transporter protein have also been associated with symptoms of Parkinson's and dystonia in humans (9). Gene expression of ZIP14 in brain tissue has been previously reported (10). However, only one study (11) has found lower ZIP14 levels in aged brain tissue (11).

In this study, we investigated how dietary zinc deficiency and supplementation affect ZIP14 levels in the cerebral cortex in the sporadic form of Alzheimer's disease (sAH) induced by intracerebroventricular streptozotocin (icv-STZ) in rats.

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METHODS

Animal Material and Groups

The study was carried out on 40 male Wistar rats obtained from the Experimental Medicine Research and Application Center of Selçuk University. The study protocol was approved by the Ethics Committee of Selçuk University Experimental Medicine Research and Application Center (decision no. 2019–44, dated 27.09.2019). Animals were divided into 5 groups in equal numbers (n=8).

Group 1, Sham 1: The animals in this group were injected with STZ solvent (artificial cerebrospinal fluid; aBOS) two days apart (on the 5th and 7th days) by icv injection. Animals were fed with standard rat chow.

Group 2, Sham 2: The animals in this group were injected with STZ solvent (artificial cerebrospinal fluid; aBOS) two days apart (on the 5th and 7th days) by icv injection. After the 7th day, these animals were given zinc sulfate solvent intraperitoneally (ip) saline for 21 days (5 mg/kg/day saline). Animals in this group were fed with standard rat chow.

Group 3, STZ: The rats in this group were injected with 3 mg/kg/STZ as icv on the 5th and 7th days. Animals were fed with standard rat chow.

Group 4, STZ+Zinc Deficient: The animals in this group were injected with 3 mg/kg/STZ as icv on the 5th and 7th days. Animals in this group were fed a zinc deficient (0.02 µg/g) diet after 7th day (for 21 days).

Group 5, STZ+Zinc Supplement: The animals in this group were injected with 3 mg/kg/STZ as icv on the 5th and 7th days. After the 7th day, the animals were supplemented with ip zinc zinc sulfate (5 mg/kg/day) for 21 days.

Experimental Procedures

Stereotaxic Procedure and icv-STZ Injection: Rats ip 70 mg/kg of ketamine and 8 mg/kg of xylazine were placed in a stereotaxic frame (Small Animal Stereotaxic System, ASI Instruments, USA). STZ was dissolved in aCSF and injected into the animals at a dose of 3 mg/kg. Each animal was injected with 10 µL of STZ or aCSF into each ventricle (injection coordinates: 4.8 mm dorsoventral, 0.8 mm anteroposterior, and 1.4 mm lateral to bregma), for a total of 20 µL at 48-hour intervals. After a 4-day adaptation period, icv injections were made on the 5th and 7th days.

Feeding Experimental Animals

Zinc sulfate was dissolved in saline and ip injected at a dose of 5 mg/kg to animals in the STZ-ZnSup group. Animals in the STZ-ZnDef group were fed a diet deficient in zinc (12). Zinc-deficient rat food was purchased from Arden Research and Experiment (Ankara, Turkey). Intraperitoneal injections of zinc and dietary changes were started on day 7 and continued for 28 days (4 weeks) until the end of the experiments.

The life of all animals was terminated by cervical dislocation under general anesthesia twenty-four hours after the experimental stages of the study were completed, and cerebral cortex tissue samples were taken (In order for the last zinc injection to be fully effective in the body, it was thought that it would be more appropriate to sacrifice the animals 24 hours after administration).

Determination of ZIP14 in Cerebral Cortex Tissue

Cerebral cortex tissue samples were first taken into liquid nitrogen. Then it was kept at -80^{0}C until ELISA analysis. ZIP14 level analyses were performed with ELISA technique in cerebral cortex tissue samples.

Cerebral cortex tissue sample homogenization was performed using the ultrasonic probe (BandelinSonopuls HD2200, BANDELIN electronic GmbH & Co. KG, Berlin, Germany). ZIP14 level in cortex homogenate samples was measured using Rat ZincTransporter ZIP14 ELISA Kit Catalaog Number E1786Ra (Bioassay Technology Laboratory, Shanghai CHINA) and spectrophotometer (SPECTROstar, BMG Labtech, Germany). Results are given in ng/L.

In our previously published study, it was determined that a sAH model was created in animals as a result of memory performance evaluated with the Morris water maze (12). This study was performed on Cerebral Cortex tissues from the same animals.

Statistical Analysis

Statistical evaluation of the findings was made with the IBM Statistical Package for Social Sciences (SPSS) version 22.0 package program. Arithmetic means and standard error of all parameters were calculated. The results were expressed as the mean ± standard error of the meanThe "Shapiro-Wilk" test was used to determine the homogeneity of the data, and it was determined that the data had a normal distribution. A one-way analysis of variance test was used to determine the differences between groups, and the Bonferroni test was used to determine the origin of the difference. Differences at the level of $p < 0.05$ were considered significant.

RESULTS

In the current study, the lowest ZIP14 levels in the cerebral cortex were obtained in group 4, which received intracerebroventricular (icv) STZ injection and was fed zinc-deficient feed ($p<0.05$). ZIP14 levels in the cortex tissue of the icv-STZ group (group 3) fed with standard rat chow were higher than group 4 ($p<0.05$) and lower than all other groups ($p<0.05$). Zinc supplementation (group 5) reversed the suppressed ZIP14 levels in cortex tissue seen in the icv-STZ groups, bringing it to the values of the sham-control groups (Table 1).

DISCUSSION

ZIP14 (Slc39a14), which is highly expressed on the membrane of epithelial cells of the gastrointestinal tract, is a zinc transporter whose synthesis is stimulated mainly by proinflammatory factors (13). Deletion of the ZIP14 gene in experimental animals' results in suppression of the absorption of zinc in the digestive tract (14). This condition also produces dysfunctions such as impaired intestinal defense barrier, abnormalities in glucose metabolism, muscle wasting as well as skeletal defects with aging (14,15). Similarly, ZIP14 mutations in humans cause neurological defects (14,16).

In addition to known neuronal iron transporter proteins in hippocampal tissue, ZIP14, a zinc transporter protein, has been reported to contribute to iron transport, albeit to a limited extent (17). This information is important for the role of metals in the formation of β amyloid, which is one of the important pathologies in Alzheimer's disease (12). It has been reported that the expression of ZIP14, a zinc transporter protein, is decreased in aged brain tissue (11). Accordingly, the aging-related increase in zinc levels in the cerebral cortex and hippocampal tissue, ZIP 14, indicates that there may be a critical relationship between Alzheimer's and aging (18,19). Since zinc is a redox-active metal that plays an important role in β amyloid aggregation and extracellular plaque formation (20), it is very interesting to investigate zinc dysregulation as a mechanism in Alzheimer's and aging.

Table 1. ZIP14 levels in cerebral cortex tissue of study groups

Groups	ZIP14 (ng/L)
Group 1- Sham 1	462.44±26.63A
Group 2 - Sham 2	461.87±30.75A
Group 3 - STZ	426.41±22.35B
Group 4 - STZ-Zinc Deficient	404.46±20.63C
Group 5 - STZ-Zinc Supplement	476.31±28.22A
The difference between the means with different letters in the same column is statistically significant. (P<0.05) A>B>C STZ: streptozotocin; ZIP14: zinc transporter protein.	

In our study, cerebral cortex ZIP14 levels, which were decreased in the icv-STZ group, were radically decreased in the icv-STZ zinc-deficient group. Zinc supplementation treated the reduction in icv-STZ ZIP14 levels. We could not find a study in which we could compare our current study exactly in Med-line scans.

Gene expression of ZIP14 in brain tissue has been reported previously (17). However, only one study to date has reported reduced levels of ZIP14 in aged brain tissue (11).

Our findings reveal that zinc has a significant impact on ZIP14 levels in cerebral cortical tissue in an experimental rat Alzheimer model. This is the first study to investigate the link between zinc and ZIP14 levels in the cerebral cortex of an experimental rat Alzheimer's model.

Previous Presentations: This study was presented as an oral presentation at the 3rd International and 7th Congress of Medicines and Treatment (Rational Use of Medicines) held in Bafra-TRNC on September 21–25, 2022.

Research Data Policy and Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Explanation: The authors declare that all data were generated in-house and that no paper mill was used.

Ethics Committee Approval: This study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the Experimental Animals Ethics Board of Selçuk University's Experimental Medicine Research and Application Center (decision no. 2019-44, dated 27.09.2019). This research was performed on animals (rats).

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Conflict of Interest: The authors declared that there is no conflict of interest.

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