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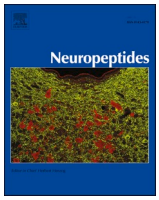

FGF-18 alleviates memory impairments and neuropathological changes in a rat model of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a multifactorial pathology marked by amyloid beta (Aβ) accumulation, tau hyperphosphorylation, and progressive cognitive decline. Previous studies show that fibroblast growth factor 18 (FGF18) exerts a neuroprotective effect in experimental models of neurodegeneration; however, how it affects AD pathology remains unknown. This study aimed to ascertain the impact of FGF18 on the behavioral and neuropathological changes in the rat model of sporadic AD induced by intracerebroventricular (ICV) injection of streptozotocin (STZ). The rats were treated with FGF18 (0.94 and 1.88 pmol, ICV) on the 15th day after STZ injection. Their cognitive function was assessed in the Morris water maze and passive avoidance tests for 5 days from the 16th to the 21st days. Aβ levels and histological signs of neurotoxicity were detected using the enzyme-linked immunosorbent assay (ELISA) assay and histopathological analysis of the brain, respectively. FGF18 mildly ameliorated the STZ-induced cognitive impairment; the Aβ accumulation was reduced; and the neuronal damage including pyknosis and apoptosis was alleviated in the rat brain. This study highlights the promising therapeutic potential for FGF18 in managing AD.

1. Introduction

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder marked by progressive loss of cognitive function. The main neuropathological AD features are the deposition of β-amyloid (Aβ) protein (Camargo et al., 2022) and hyperphosphorylated tau protein (Azarafrouz et al., 2022) in the brain. Further, intra- and extracellular accumulation of fibrillar aggregates leads to downstream pathologies such as cholinergic system dysfunction, oxidative stress, inflammation, and death of neurons by apoptosis(Anand et al., 2014; Eskandari et al., 2023). The severity of cognitive impairments in patients with AD is associated with the extent of the pathophysiological events mentioned. The two types of AD including familial AD and sporadic AD differ in the age of onset and course of the disease. Familial AD that has a genetic origin, accounts for less than 1% of cases, while sporadic AD that emerges at later ages represents the dominant part (Sirkis et al., 2022). Unfortunately, no disease-modifying treatments are available that would delay or prevent AD progression to date. Hence, identifying its underlying mechanisms and discovering more effective drugs remain an important issue for the definitive treatment of AD. To this end, experimental animal models of AD are extensively used. The intracerebroventricular (ICV) administration of streptozotocin (STZ) is a widely used animal model to mimic the pathology related to sporadic AD (Salkovic-Petrisic et al., 2013). ICV-STZ injection in rodents results in sporadic AD-like pathology, including oxidative stress, microglia activation, cholinergic damage, and synaptic dysfunction as well as memory and learning impairment, approximately two weeks after injection (Gao et al., 2014; Kamat, 2015; Rai et al., 2013). STZ causes insulin deficiency, which disrupts the insulin receptor signaling pathway, and a decrease in the GSK-3alpha/beta ratio (phosphorylated/total). This in turn stimulates NF-kB protein and increases Aβ accumulation and tau phosphorylation (Singh and Singh, 2023; Yamini et al., 2022). As a result, the integrity of neurons is altered, leading to neurodegeneration. Besides, neuroinflammation caused by high concentrations of proinflammatory cytokines such as TNF-α and IL-1β is associated with cognitive impairments in AD, and the same is

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acknowledged to occur in the ICV-STZ AD model (Masoumi et al., 2018; Yamini et al., 2018).

Fibroblast growth factor 18 (FGF18), a glycosylated secretory protein, is an anabolic growth factor involved in cartilage homeostasis (Lu and Lin, 2021). FGF18 has also been found to be expressed in the brain and is mainly involved in midbrain development (Liu et al., 2003). Earlier in vitro studies have shown that FGF18 stimulated neurite outgrowth and had a protective effect against 6-hydroxydopamine (6-OHDA) induced neurotoxicity (Guo et al., 2017b; Ohbayashi et al., 1998). In addition, animal models have shown that FGF18 has a neuroprotective effect against ischemic damage and parkinsonian neurodegeneration (Ellsworth et al., 2003; Guo et al., 2017b). Remarkably, Cavallaro et al. reported that exogenous FGF18 treatment enhanced rats' spatial learning abilities (Cavallaro et al., 2002). However, whether FGF18 exhibits neuroprotective effects have yet to be evaluated in animal models of AD. Therefore, the primary goal of the current study was to assess the effects of FGF18 on the behavioral and neuropathological changes in the rat model of STZ-induced AD.

2. Materials and methods

2.1. Animals

Male Wistar albino rats (275–300 g) were held under the typical settings with a 12:12 h light-dark cycle, controlled temperature (23 ± 2 °C), humidity (35–60%), and free access to food and water. All animal procedures were carried out following the National Institute of Health Guidelines for Principles of Animal Laboratory Care and approved by Sivas Cumhuriyet University's Animal Ethics Committee (Registry Number. 397 dated March 16, 2021).

2.2. Reagents

STZ was purchased from Sigma-Aldrich (St. Louis, MO, USA). FGF18 was from Genscript (New Jersey, USA). STZ and FGF18 dissolved in distilled water. All chemical solutions were freshly prepared before use.

2.3. Experimental design and animal treatments

The experimental schedule of the study is shown in Fig. 1. A total of 24 rats were randomly assigned into four groups (Control, STZ, L-FGF18, and H-FGF18) and treated as follows:

(1) Control group, received an ICV injection of 10 µL distilled water.
(2) STZ group, received an ICV injection of 10 µL STZ (3 mg/kg).
(3) L-FGF18 group, received an ICV injection of 10 µL STZ and then treated an ICV of 0.94 pmol FGF18 on day 15.
(4) H-FGF18 group, received an ICV injection of 10 µL STZ and then treated an ICV of 1.88 pmol FGF18 on day 15.

After treatments, rats experienced the Morris water maze (MWM) test from days 16 to 20 and the passive avoidance (PA) test from days 20 to 21. All rats were anesthetized and sacrificed on day 21 after 1 h following the PA test. Under sterile surgical conditions, their whole brains were carefully excised from the skull. After the brains were divided into two with the sagittal plane, the cortex and hippocampus were evaluated for biochemical and histopathological changes.

2.4. ICV injection of STZ

Ketamine (85 mg/kg) and xylazine (12 mg/kg) intraperitoneal (i.p.) injections were used to anesthetize the rats. The rats were placed in the stereotaxic apparatus after their scalps were shaved and cleaned. After a sagittal midline incision in the scalp, burr holes were drilled on both sides of the skull with the help of the following coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to the sagittal suture and 3.6 mm below the brain surface, based on the previously published method (Zhu and Hou, 2021). One single ICV injection of STZ (3 mg/kg) dissolved in 10 µL of distilled water was slowly injected into the right lateral ventricle (Guo et al., 2017a). Control rats were treated similarly but with distilled water. The whole procedure was performed under sterile conditions.

2.5. Behavioral procedures

Memory function was assessed in two behavioral paradigms including MWM and PA tests. Behavioral procedures were performed in an isolated room with no disturbances during testing.

2.5.1. Morris water maze

The MWM test, as a reliable measure of hippocampal-dependent learning, was used to assess spatial memory (Cigel et al., 2021). MWM consisted of a 4-day learning period (acquisition trials) and an evaluation of spatial memory retention (probe trial) on day 5. This test was realized in a circular pool (160 cm in diameter, 55 cm high) filled with water (the temperature at 22 ± 1 °C) that was conceptually divided into four equal quadrants by imaging lines. The pool also contained a black cylindrical platform (10 cm in diameter) positioned in the center of the target quadrant, 1 cm below the water surface. The acquisition phase was carried out in four trials per day for 4 consecutive days. For each trial, rats entered the water facing the wall of the pool by changing the starting quarter every day. The rats had to swim during the acquisition trials until they found the platform under the water using distal extra-maze cues. If a rat did not find the hidden platform within 60 s, it was guided with the experimenter's help and allowed to stay on it for 10 s.

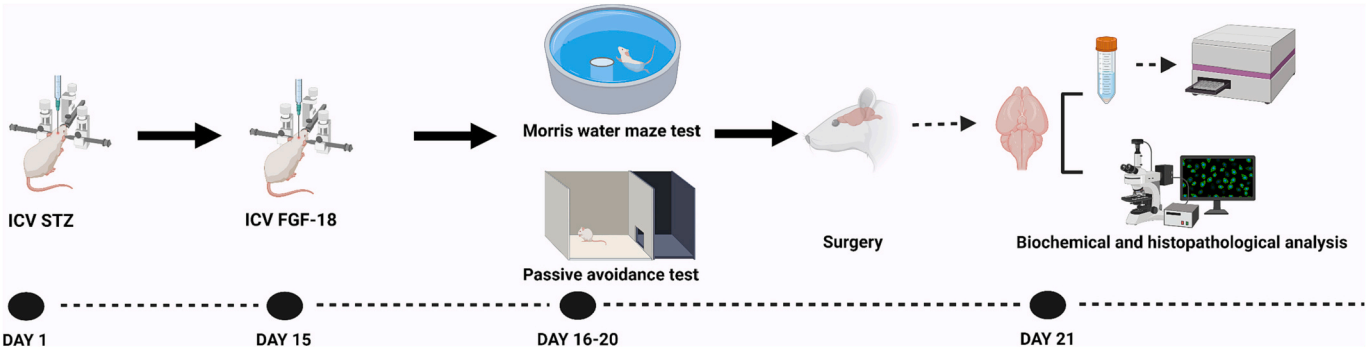

Fig. 1. Experimental design. Sporadic AD-like pathology was induced in rats by bilateral ICV infusion of STZ (3 mg/kg). The rats were treated with FGF18 (0.94 and 1.88 pmol, ICV) on the 15th day after STZ injection. Their cognitive function was evaluated in the behavioral tests. All rats were sacrificed after the behavioral tests for ELISA and immunohistochemical analysis (created with BioRender app, www.biorender.com).

The time to find the platform (escape latency) was recorded to assess the effect of FGF18 on learning activity. In the probe test on day 5, after the platform was removed, the rats were given 60 s to swim in the pool, and memory consolidation was defined by the percentage of time spent in the target quadrant. All these activities were recorded and analyzed automatically by the computerized video tracking system (Smart 3.0, Panlab, Barcelona, Spain).

2.5.2. Passive avoidance test

The PA test, emotional learning with long-term memory, was carried out as described earlier (Taskiran et al., 2021). The PA test device (Ugo Basile, Comerio, Italy) consisted of a light room connected to a dark chamber by an automatically retractable room. Twenty-four hours after habituating to the apparatus, an acquisition trial was performed. The rats were placed in the light room separately, and the automatic door was opened after 10 s.

When the rat entered the dark room, the door was automatically closed, and an electric shock (0.25 mA, 2 s, once) was delivered via the ground grids. In this trial, the latency time of the animal's dark room was recorded. After twenty-four hours, each rat was placed in the light room for the retention trial, and the latency time the rats entered the dark room was recorded.

2.6. ELISA assay

The brain tissues were homogenized in a sterile phosphate-buffered saline (PBS) through a beat beater device (HT 24 Bead Beating Homogenizer, OPS Diagnostics, USA). Then homogenates were centrifuged at 4000 rpm for 10 min at 4 °C. The centrifuged homogenate's supernatant was collected, and the Bradford protein assay was used to determine the amount of protein (Ernst and Zor, 2010). The content of $A\beta_{1-42}$ in the hippocampus and cortex was measured using sandwich ELISA kits (BT Lab, Shanghai, China) according to the manufacturer's protocol. Briefly, after adding 40 μL samples to the sample wells, 10 μL mouse $A\beta_{1-42}$ antibody was added. Then added 50 μL streptavidin-HRP to the sample wells and standard wells, and then mixed thoroughly. After covering the plate with a sealer, it was incubated for 60 min at 37 °C. Then the sealer was removed and the plate was washed with a washing buffer. After adding 50 μL substrate solution A and 50 μL substrate solutions to each well, respectively, it was incubated with a new seal for 10 min at 37 °C. Finally, a 50 μL stop solution was added to each well and the optical density (OD value) of each well was determined by using a microplate reader (Thermo Fisher Scientific, Altrincham, UK) set to 450 nm.

2.7. Histopathological examination

After sacrificing the anesthetized rats, all the removed brain tissues were divided into two with the sagittal plane, and the right hemispheres were put in a 10% buffered formalin solution. After tissues were taken into paraffin blocks, 4-μ tissue sections were stained with hematoxylin-eosin (H-E) for histopathological examination through a light microscope. Histopathological evaluations were blindly determined by two expert pathologists in all tissue samples. Pyknotic and degenerative changes in neurons in the cortex and cornu ammonis (CA1/CA2, CA3) regions of the hippocampus were evaluated semi-quantitatively as absent (0), mild (1), moderate (2), and severe (3).

2.8. Immunohistochemical examination

After 4-μm tissue sections were passed through xylene and alcohol series and washed with PBS, they were kept at H_2O_2 (3%) for 10 min to inactivate endogenous peroxidase. To reveal the antigen in the tissues, they were treated with antigen retrieval solution in a microwave oven at 500 W for 2 × 5 min. Tissues were washed with PBS and were incubated at +4 °C overnight with 8-hydroxy-2′ -deoxyguanosine (8-OhDG), a marker of oxidative DNA damage, monoclonal antibody (Catalog no. sc-66,036) and anti- microtubule-associated protein 1 light chain 3 (LC3β), an autophagy-related protein, (Catalog no. sc-271,625) at 1/200 dilution. Subsequently, the large volume detection system: anti-polyvalent, HRP (Thermo Fisher, Catalog no: TP-125-HL) was used as recommended by the manufacturer. DAB (3,3′-diaminobenzidine) was used as the chromogen. Immunopositivity in the cortex, CA1/CA2, and CA3 regions were evaluated semi-quantitatively as absent (0), mild (1), moderate (2), and severe (3).

2.9. Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM) and analyzed by one-way or two-way analysis of variance (ANOVA) with Tukey's post hoc test. For histopathological examinations, the differences between the groups were determined using the Kruskal–Wallis test and Mann–Whitney test. *P* values ($p < 0.05$) were regarded as significant. Statistical analyses were performed using GraphPad Prism software version 7 (GraphPad Software, San Diego, CA, USA).

3. Results

3.1. FGF18 treatment restores ICV-STZ-induced cognitive dysfunction in rats

First, we elucidated the effect of FGF18 on the long-term memory of ICV-STZ-treated rats through PA and MWM tests. During the acquisition trial, no significant difference in the delay in entering the dark room was observed between the study groups in the PA test ($p > 0.05$, Fig. 2). On the contrary, the STZ and L-FGF18 rats showed decreased step-through latency than the control rats in the retention trial ($p < 0.05$, Fig. 2). Additionally, the H-FGF18 rats demonstrated significantly increased step-through latency than the STZ rats.

The MWM test was used to confirm the results observed in the PA test. In the MWM test, the escape latency was analyzed using two-way ANOVA for repeated measures. The results indicated significant effects of the day ($F = 9.612$; $p < 0.001$) and group ($F = 10.370$; $p < 0.001$) but no significant effect of day × group ($F = 1.542$; $p = 0.1476$). The Tukey test further revealed that the STZ rats consistently exhibited a significantly longer escape latency than the control rats on days 3 and 4 and

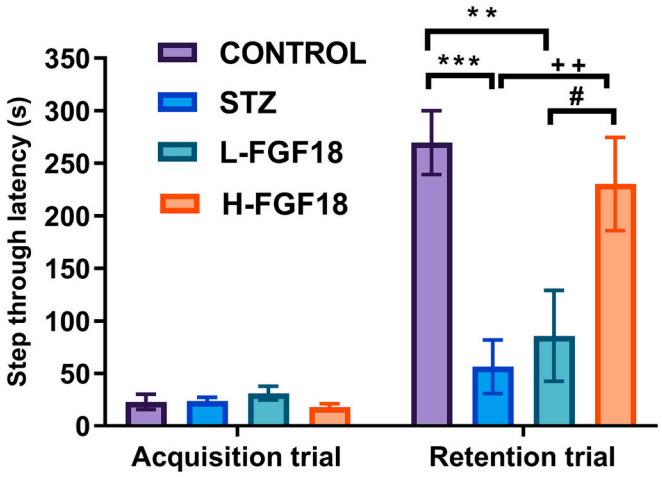

Fig. 2. Effect of the treatment of FGF18 in ICV-STZ rats on emotional learning with long-term memory in the PA test. Values are presented as mean ± SEM. ($n = 6$ rats in each group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus the Control group; $^{++}p < 0.01$ versus the STZ group; $^{\#}p < 0.05$ versus the L-FGF18 group. Abbreviations: ICV-STZ, intracerebroventricularly streptozotocin; PA, passive avoidance.

that the H-FGF18 significantly decreased the escape latency than the STZ rats on day 4. The percentage of time spent in the target quadrant during the probe trial demonstrated that all the rats preferred the target quadrant except the STZ rats, which displayed a considerable decrease in the percentage of time in the target quadrant compared to the control rats ($p < 0.05$, Fig. 3B). However, there was no significant difference in swimming speed among the study groups ($p > 0.05$, Fig. 3C).

3.2. FGF18 treatment reduces brain Aβ levels in ICV-STZ rats

To investigate whether FGF18 impacts the Aβ formation, we calculated the Aβ levels in the brain using an ELISA assay. Fig. 4A demonstrated that the cortical $A\beta_{1-42}$ content remarkably increased in the STZ rats compared to the control rats ($p < 0.05$). Treatment with FGF18 reduced the $A\beta_{1-42}$ levels in the cortex compared to that of the STZ rats, but decreases in the $A\beta_{1-42}$ content in the L-FGF18 rats did not achieve statistical significance (Fig. 4A). Similarly, the hippocampal $A\beta_{1-42}$ content remarkably increased in the STZ rats compared to the control and L-FGF18 rats ($p < 0.05$), however, treatment with FGF18 only reduced the $A\beta_{1-42}$ levels in the hippocampus in the H-FGF18 rats ($p < 0.05$, Fig. 4B).

3.3. FGF18 treatment alleviates the brain histopathological changes in ICV-STZ rats

We also investigated histopathological changes to provide further evidence of whether FGF18 improves ICV-STZ-induced pathological changes in the brain. While the control group had a normal histological appearance, pyknotic/degenerative changes were observed in the cortex and CA1/CA2 and CA3 areas of the hippocampus of other groups. As expected, in the STZ rats, pyknotic/degenerative changes were severe in the cortex, but moderate in the CA1/CA2 and CA3 areas. These histopathological findings were moderate in the cortex and mild in the CA1/CA2 and CA3 areas in the L-FGF18 ratsHowever, in the H-FGF18 rats, pyknotic/degenerative changes were mild in both the cortex and the CA1/CA2 and CA3 areas (Fig. 5). The nuclei of the pyknotic cells were observed to be dark and shrunken, while the nucleus was separated from the cytoplasm in the degenerative components.

Moreover, immunohistochemical staining for 8-OHdG and LC3β revealed significant differences among the study groups. 8-OHdG expression in the control and the experimental rats is illustrated in Fig. 6. While 8-OHdG immunopositivity was not observed in the cortex, CA1/CA2, and CA3 sections in the control rats, moderate immunopositivity in the cortex and severe immunopositivity in the CA1/CA2 and CA3 areas were observed in the STZ rats. In the L-FGF18 rats, moderate immunopositivity was observed in the cortex, CA1/CA2, and CA3 regions. In the H-FGF18 rats, immunopositivity was decreased in both cortex and CA1/CA2 and CA3 areas.

4. Discussion

AD is a common neurodegenerative disease that causes irreversible cognitive impairment. However, the discovery of drugs that delay or prevent AD progression is fraught with challenges because pathological pathways that govern AD are complex. Here, using ICV-STZ rats as an animal model of sporadic AD, we examined the impact of FGF18 on cognition and the pathological alterations in their brains. The results of our study demonstrated that treatment with FGF18 mildly improved spatial memory, reduced brain Aβ levels, and ameliorated brain histopathological changes in ICV-STZ rats.

FGFs are a family of polypeptide growth factors that control a broad spectrum of cellular activity processes involved in growth, development, metabolism, and apoptosis (Guillemot and Zimmer, 2011). Some of these regulatory peptides also exert neuronal functions in the brain, such as neural outgrowth and neurogenesis (Mudò et al., 2009). FGF18 is expressed within different regions in the brain both during development as well as in adults. Previous reports have demonstrated that FGF18 promotes neurite outgrowth and has mitogenic effects on glial cells (Hoshikawa et al., 2002; Ohbayashi et al., 1998). Several studies have also displayed the neuroprotective effects of FGF18 in cerebral ischemia and Parkinson's disease rat models (Ellsworth et al., 2003; Guo et al., 2017b), but to our knowledge, the present study is the first to report the role of FGF18 in improving the pathology and symptoms of AD.

In the current study, we demonstrated the memory-restorative effects of FGF18 in the MWM test, one of the most accepted testing paradigms to evaluate hippocampus-dependent spatial learning in rodents (Garthe and Kempermann, 2013). We found that STZ injection led to typical spatial memory impairments but modestly reversed after FGF18 treatment. Similarly, PA testing showed that STZ-induced deterioration in long-term memory was reversed in response to FGF18 treatment. Corroborating our data, a previous study reported that a single exogenous administration of FGF-18 (0.94 pmol) enhanced spatial learning behavior (Cavallaro et al., 2002). The same researchers reported that FGF18 is up-regulated in the learning and memory process and has promising for treating neurodegenerative diseases such as Alzheimer's. FGF18 also improved motor impairment in a 6-OHDA-induced rat model of Parkinson's Disease (PD) and prevented dopaminergic neuron loss in the substantia nigra (Guo et al., 2017b) In addition, Ellsworth et al. reported that infusion of FGF18 reduced deficits in tests of working memory and general exploratory behavior in the cerebral ischemia animal model MCAo rats (Ellsworth et al., 2003). Moreover, in the same study, these authors reported that FGF18 increases regional cerebral blood flow in MCAo rats (Ellsworth et al., 2003). Considering that there is a decrease in the cerebral blood flow stimulated by the oligomeric Aβ in AD (Korte et al., 2020), the neuroprotective effects of FGF18 may be related to its role in the maintenance of cerebral blood flow.

It is worth noting that oxidative stress plays an essential role in AD, as it is associated with neuronal degeneration (Vedagiri and Thangarajan, 2016) The brain is susceptible to an imbalance between the

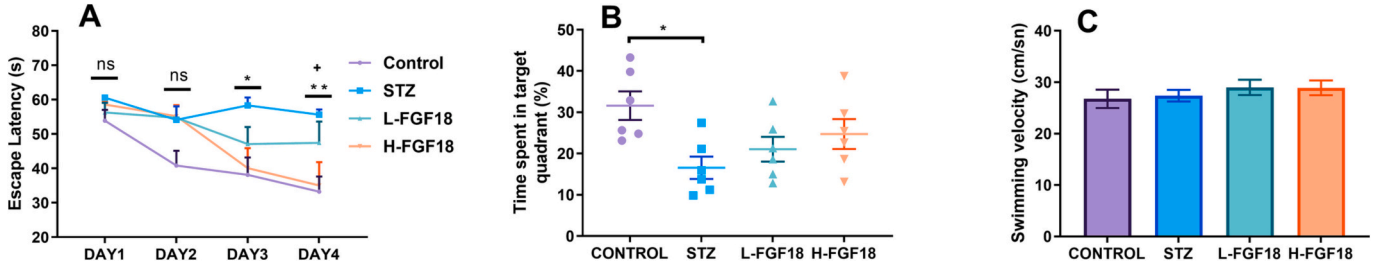

Fig. 3. Effect of the treatment of FGF18 in ICV-STZ rats on spatial learning with long-term memory in the MWM test. (A) Analysis of latencies to the platform during the training days of the MWM test. (B) Comparisons of the percentage of time spent in the target quadrant during the probe trial. (C) Comparisons of the average swim speeds. Values are presented as mean ± SEM. ($n = 6$ rats in each group). *$p < 0.05$, **$p < 0.01$ versus the Control group; $^{+}p < 0.05$ versus the STZ group. Abbreviations: n.s. not significant, ICV-STZ, intracerebroventricularly streptozotocin; MWM, Morris water maze.

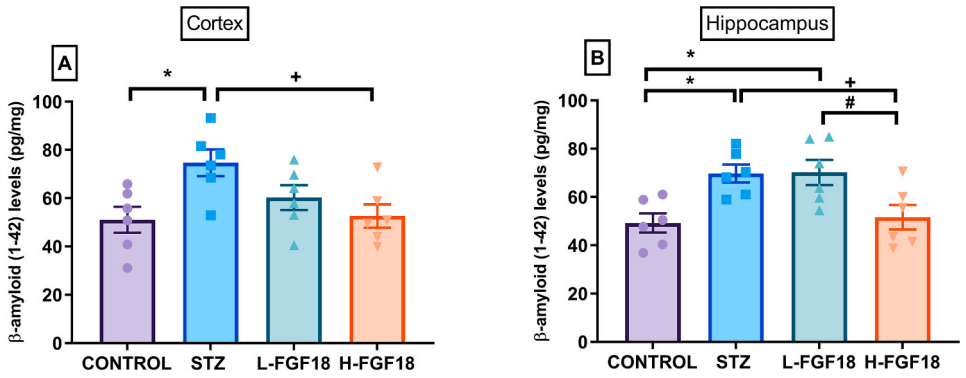

Fig. 4. Effect of the treatment of FGF18 in ICV-STZ rats on cortical (A) and hippocampal (B) Aβ accumulation. Values are presented as mean ± SEM. ($n = 6$ rats in each group). *p < 0.05 versus the Control group; $^{+}$p < 0.05 versus the STZ group; $^{\#}$p < 0.05 versus the L-FGF18 group. Abbreviations: ICV-STZ, intracerebroventricularly streptozotocin; Aβ, amyloid-beta.

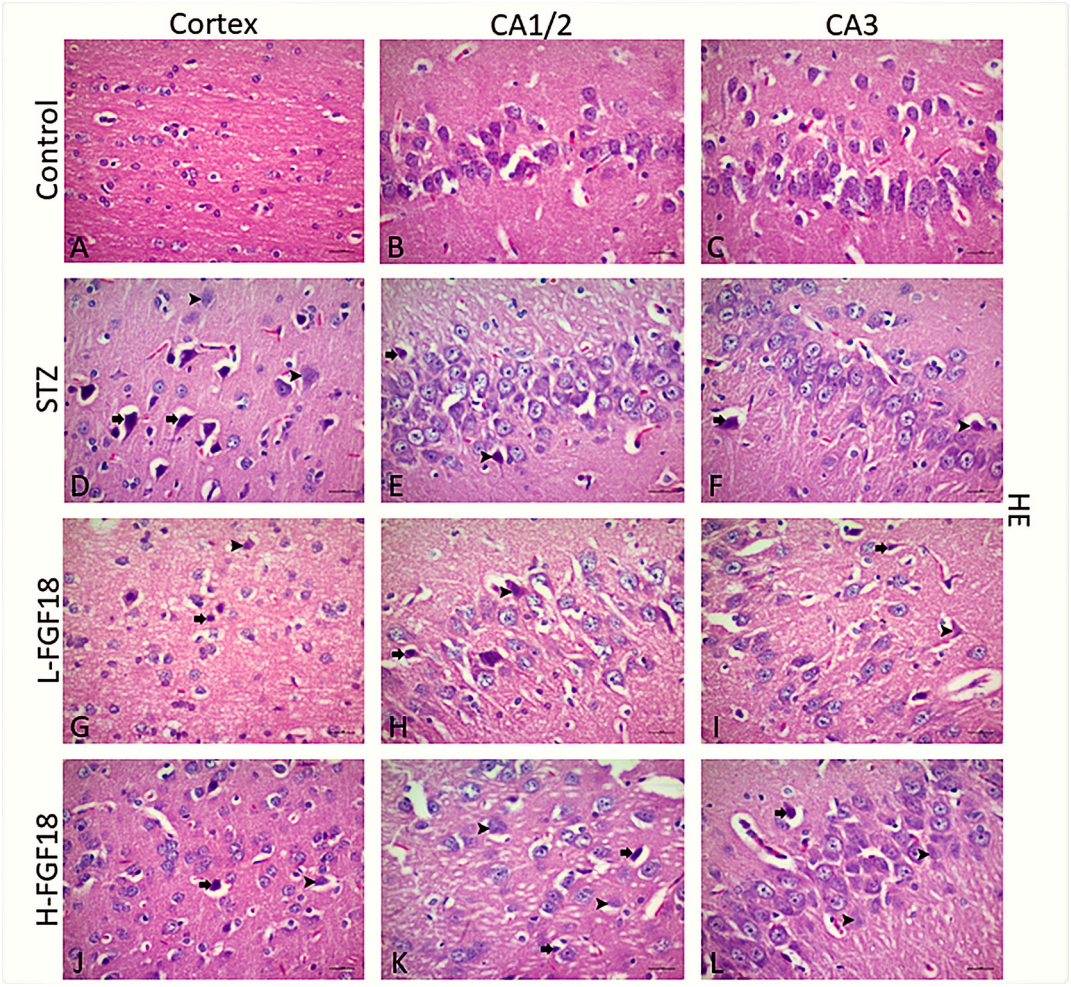

Fig. 5. Histological analysis of pyknotic and degenerative neuron with hematoxylin & eosin (HE) staining. Control Group (A, B, C). Normal histological appearance. STZ Group (D, E, F). Severe pyknotic and degenerative neurons in the cortex and moderate in CA1/2 and CA3. L-FGF18 Group (G, H, I). Moderate pyknotic and degenerative neurons in the cortex and mild in CA1/2 and CA3. H-FGF18 Group (J, K, L). Mild pyknotic and degenerative neurons in the cortex, CA1/2, and CA3. (→= Pyknotic neuron, ▶= Degenerative neuron). HE stain, ×40.

production and clearance of reactive oxygen species (ROS) because it has intense energy demand and high oxygen consumption. ROS can cause oxidative damage to nucleic acids and occur as an increase in 8-OHdG levels, widely used as an oxidative DNA biomarker (Peña-Bautista et al., 2019). Previous studies that measured the level of 8-OHdG in AD animal models and post-mortem brain tissues showed an abnormal increase of 8-OHdG in AD (Cimini et al., 2009; Mecocci et al., 1993; Wang et al., 2005). In agreement with previous studies, we observed that ICV-STZ injection leads to 8-OHdG immunopositivity in the brain, specifically in the hippocampus, which is the brain area most affected by the disease. We also observed that STZ-induced oxidative DNA damage could be ameliorated by FGF18 administration. Since $A\beta_{1-42}$ has DNA-

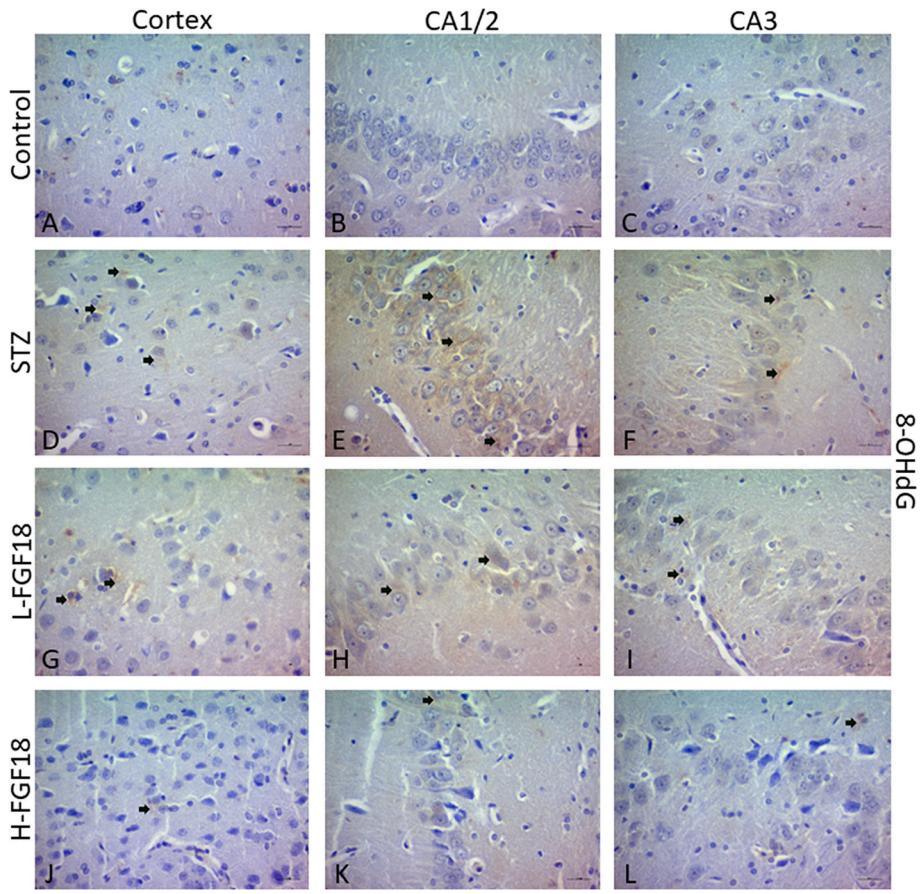

Fig. 6. A. Histological analysis of 8-OHdG expression with immunohistochemical (IHC) staining. Control Group (A, B, C). Immune negativity. STZ Group (D, E, F). Moderate immunopositivity in the cortex and severe immunopositivity in CA1/2 and CA3. L-FGF18 Group (G, H, I). Moderate immunopositivity in the cortex, CA1/2, and CA3. H-FGF18 Group (J, K, L). Mild immunopositivity in the cortex, CA1/2, and CA3. (→= Immunopositivity) IHC stain, ×40. The LC3β expression in the control and the experimental rats is illustrated in Fig. 6B. While LC3β immunopositivity was not found in the cortex of the control rats, mild immunopositivity was observed in the CA1/CA2 and CA3 areas. In the STZ rats, immunopositivity was found to be moderate in the cortex and severe in the CA1/CA2 and CA3 sections. Besides, LC3β immunopositivity was found to be mild in both the cortex and CA1/CA2 and CA3 sections in the L-FGF18 rats; it was observed that the immunopositivity in the H-FGF18 rats was mild in the cortex and moderate in the CA1/CA2 and CA3 areas. B. Histological analysis of LC3β expression with immunohistochemical staining. Control Group (A, B, C). Immune negativity in the cortex. Mild immunopositivity in CA1/2 and CA3. STZ Group (D, E, F). Moderate immunopositivity in the cortex and severe immunopositivity in CA1/2 and CA3. L-FGF18 Group (G, H, I). Mild immunopositivity in the cortex, CA1/2, and CA3. H-FGF18 Group (J, K, L). Mild immunopositivity in the cortex, moderate immunopositivity in CA1/2 and CA3. (→= Immunopositivity) IHC stain, ×40.

nicking activity similar to nucleases and can contribute to DNA damage (Suram et al., 2007), we suggest that the reduction of brain abnormal Aβ with FGF18 may contribute to its inhibiting effect on oxidative DNA damage.

Previous studies demonstrated that STZ-induced neurotoxicity involves neuronal apoptosis (Biswas et al., 2016; Park et al., 2020). The cytosolic microtubule-associated protein LC3β is recruited to form autophagosomes during autophagy and is therefore used to identify autophagosomes as cytoplasmic puncta when autophagy is induced (Lee and Lee, 2016). Further, a previous study reported that autophagic vacuole accumulation may precede apoptotic cell death (González-Polo et al., 2005). We observed that STZ-induced neuronal apoptosis in the brain of ICV-STZ rats could be attenuated by FGF18 administration as evaluated by LC3β immunopositivity. Taken together with apoptosis is seen in Alzheimer's disease secondary to oxidative stress (Smith et al., 1997), we suggest that FGF18 reduces ICV-STZ-induced apoptosis and oxidative stress, resulting in protective effects on neurodegeneration.

The evidence suggests that an increased accumulation of Aβ in the brain is linked to cognitive dysfunction (Ballard et al., 2011; Zhang et al., 2020). We observed increases in the $A\beta_{1-42}$ levels in the brain with memory impairment in the rats injected with STZ. However, a single high dose of FGF18 treatment effectively attenuated the accumulation of Aβ in the cortex and hippocampus of rats, suggesting that reducing Aβ deposition in the brain may be responsible for the memory protective effect of FGF18. Additionally, Aβ induces the generation of ROS and the appearance of oxidative stress in astrocytes, affecting among others glutamate uptake (raising the risk of excitotoxicity) and mitochondrial function (González-Reyes et al., 2017; Matos et al., 2008). Therefore, the reduction in Aβ accumulation in the brain by FGF18 treatment may have protected neuroglial cells from oxidative stress induced by amyloid peptides.

The current study has several limitations. One of the primary limitations of our study is that relatively few animals were used in the experimental groups in terms of behavioral experiments. Secondly, it is the absence of a group administered only ICV-FGF18. There is also a need to consider the lack of ICV-STZ-induced other neuropathological events such as cholinergic dysfunction, neuroinflammation, and tau hyperphosphorylation lost in our study settings. Furthermore, it would be useful to investigate the effects of chronic treatment with FGF18 after exposure to STZ rather than a single injection of FGF18. Finally, unlike the amyloid-β induced model, which is a more established paradigm, the ICV-STZ rat model does not entirely match sporadic AD.

In conclusion, we reported here for the first time that FGF18 treatment reversed STZ-induced cognitive impairments and neuronal

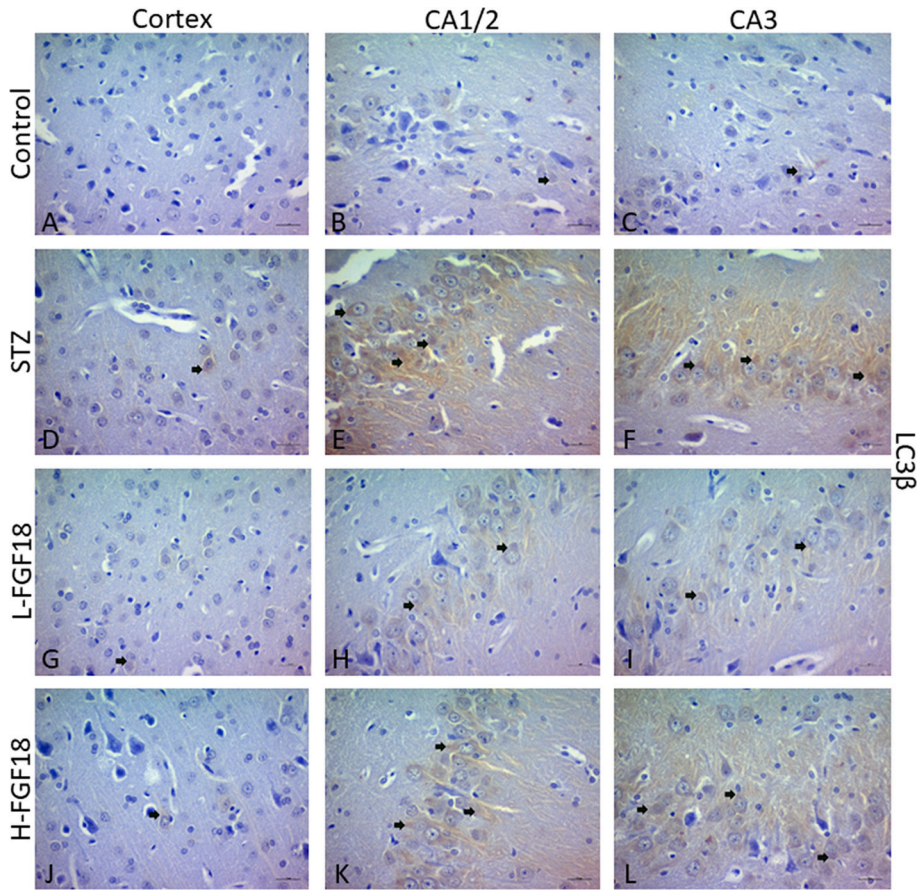

Fig. 6. (*continued*).

damage. Although our results identify a potential neuroprotective role of FGF18, its' therapeutic potential for AD need to be explored further.

CRediT authorship contribution statement

Arzuhan Cetindag Ciltas: Conceptualization, Investigation, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Sebahattin Karabulut:** Conceptualization, Investigation, Methodology, Resources, Writing – original draft, Writing – review & editing. **Bilal Sahin:** Investigation, Methodology, Visualization. **Ahmet Kemal Filiz:** Conceptualization, Investigation, Resources, Writing – review & editing. **Fatih Yulak:** Investigation, Methodology, Writing – original draft. **Mustafa Ozkaraca:** Investigation, Methodology, Formal analysis, Writing – original draft. **Ozhan Karatas:** Investigation, Methodology, Formal analysis, Writing – original draft. **Ali Cetin:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors have no competing interests.

Data availability

Data will be made available on request.

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