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Fibroblast growth factor-21: Preserving cell viability in diabetic neuropathy through the AKT/PI3K cellular pathway

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Abstract

Aim: This study aims to investigate the potential neuroprotective effects of fibroblast growth factor-21 (FGF21) on dorsal root ganglion (DRG) neurons under high glucose (HG) conditions, mimicking diabetic neuropathy. Specifically, we hypothesize that FGF21 enhances cell viability and reduces glucose-induced neuronal death via the activation of the phosphatidyl-inositol-3-kinase (PI3K)/AKT signaling pathway.

Materials and Methods: DRG neurons were cultured from 1-day-old to 2-day-old Wistar rats and exposed to HG concentrations to simulate diabetic conditions. Various concentrations of FGF21 were administered to the DRG neurons. Cell viability was assessed using the MTT assay (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), a widely used enzymatic method for determining cellular metabolic activity. The involvement of the PI3K/AKT signaling pathway in mediating the effects of FGF21 was also examined through biochemical assays and pathway inhibitors.

Results: Administration of FGF21 to DRG neurons exposed to HG conditions significantly protected cell viability and reduced glucose-induced neuronal death ($p<0.05$). The protective effects of FGF21 were found to be dose-dependent, with higher concentrations showing more pronounced benefits. Furthermore, the activation of the PI3K/AKT signaling pathway was confirmed to play a crucial role in the neuroprotective mechanism of FGF21, as inhibition of this pathway attenuated the protective effects.

Conclusion: This study demonstrates for the first time that FGF21 has a neuroprotective effect on DRG neuron survival in a diabetic neuropathy model. By activating the PI3K/AKT signaling pathway, FGF21 helps maintain cell viability and reduces glucose-related neuronal death. These findings provide a promising basis for the development of new therapeutic strategies for the treatment of diabetic neuropathy, leveraging the neuroprotective properties of FGF21.

Introduction

Diabetes is a public health issue with an increasing prevalence over time [1]. It not only increases countries' expenditure on patient care and costs, but also increases the risk of morbidity and premature death [2]. Diabetes, a chronic metabolic disease, causes numerous micro- and macrovascular complications [3]. Diabetic neuropathy is one of the most common of these complications. Diabetic neuropathy causes pain in patients and significantly reduces their quality of life [4]. Although the pathogenesis of diabetic neuropathy is not fully understood, some pathophysiological mechanisms are known to be responsible. Hyperglycemia can lead to mitochondrial damage in neurons, resulting in axonal injury, demyelination, and loss of nerve fibers [5-8]. High glucose (HG) inhibits axonal growth [9] and triggers apoptosis in Schwann cells [10]. Additionally, exposure to elevated glucose levels has been shown to directly adversely affect the morphology and function of Schwann cells [11]. It has been suggested that high serum glucose levels due to diabetes cause increased apoptosis and neuronal damage in sensory neurons. [12-14]. Studies have shown that dorsal root ganglion (DRG) neurons are more sensitive to oxidative stress and are more severely affected [15]. DRG neurons transmit the sensation of pain from the periphery to the central nervous system (CNS) [16], used in experimental model of diabetic neuropathy [17, 18] and become the target of therapeutic agents [16]. In this study, FGF21, which is used as a therapeutic agent, was identified by Nishimura T. and colleagues in the year 2000. FGF21 is a protein consisting of 181 amino acids (approximately 20 kDa) encoded on the 19th chromosome.

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Physiologically, FGF21 is induced by various cellular stress factors such as fasting and exposure to cold. This condition enables it to act as a stress-responsive hormone [19]. FGF21 operates on a cell surface complex consisting of a traditional tyrosine kinase FGF receptor 1 (FGFR1) and a co-receptor protein named β-Klotho. It directly binds to both of these proteins for FGFR1 signal activation [20]. As a result, FGF21 facilitates adaptation to a stress environment by promoting gluconeogenesis, ketogenesis, and adaptive thermogenesis [21,22]. In metabolic diseases with elevated cellular stress factors such as diabetes, the potential role of FGF21 has been considered, and numerous studies have been conducted [23-25]. The administration of exogenous FGF21 to diabetic mice has resulted in a decrease in blood glucose and lipids, improvement in insulin sensitivity, and an increase in energy expenditure and fat utilization [26]. The relationship of FGF21 with pain has also been identified, and it has been found that FGF21 reduces the development of opioid tolerance acutely, minimizing both physical and psychological dependence [27]. Although the neuroprotective effects of FGF21 in peripheral nerve injury are known [28] its role in diabetic neuropathic pain and its mechanism have not yet been investigated.

Considering the anti-apoptotic, anti-oxidative, and neuroprotective effects of FGF21 in central nervous system diseases [29] and peripheral nerve damage [28], it is plausible to assume that FGF21 may exert neuroprotective effects on damaged DRG neurons due to diabetic neuropathy. To test this hypothesis, we applied FGF21 to primary DRG neurons, which were either healthy or part of a diabetes model, at different concentrations. We assessed the damage caused by hyperglycemia to the cells and the potential impact of FGF21 using cell viability analysis.

Materials and Methods

This study protocol was approved by the Local Animal Experiments Ethics Committee of Firat University in the session held on 26.12.2022 with approval number 2022/21-14.

Cell culture

DRG neurons were isolated from 1- to 2-day-old Sprague-Dawley rats. Neonatal rats were decapitated, the spinal cord was removed and DRGs were harvested pellet by pellet under a dissecting microscope (Olympus, Tokyo, Japan), all DRGs were isolated using forceps and temporarily transferred to a small petri dish containing neurobasal-A medium (Gibco, USA) and a culture medium containing B27 supplement (Gibco, USA). Collagenase (0.125% for 13 minutes) and trypsin (0.25% in PBS for 6 minutes) were used for enzymatic dissociation of the cells, which were then mechanically separated. Nerve growth factor (NGF 2.5S, Sigma-Aldrich, Germany) was added, and the obtained DRG cells were transferred to a humidified incubator (Heracell, Kendro Lab GmbH, Germany) at 37°C with 95% air and 5% CO_2 for use in MTT analyses DRG Culture Model of Hyperglycemia It is known that HG (above 35 mM) lead to the death of DRG neurons [30]. To create an in vitro model of diabetes, DRG cells were exposed to 45 mM glucose for 24 hours. The MTT test was initiated when the cell viability rate exceeded 90%. LY294002, an AKT/PI3K(Phosphatidylinositol-3-kinase) inhibitor, was used to assess the cellular pathway.

DRG culture model of hyperglycemia

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MTT assay

DRG cells were seeded in wells of 96-well plates for determining cell viability using the MTT method. FGF21 was dissolved in physiological saline. Firstly, four different doses of FGF21 (12.5 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL) [31,32] were applied to healthy cell groups. Then, the four different doses of FGF21 were applied in combination with HG. To create a diabetic neuropathy model, cells were exposed to HG (45 mmol/L) for 24 hours. The MTT method, one of the enzymatic test methods used to determine cell viability, was employed. The half-maximal effective concentration (EC_{50}) value for cell viability was calculated from the dose-response curve.

Statistical analysis

All statistical analyses of the data were performed using the SPSS program. Graphs were created using GraphPad software (GraphPad Prism version 9.2.0 for macOS, GraphPad Software, San Diego, California, USA), and Originpro 8.0 graph program. Figures were created using biorender.com. All data are presented as the mean and standard deviation (mean ± SD). The sample size estimates for this study are based on published research data and the results of our preliminary studies. To detect a significant difference in the relevant markers in our pilot study, the minimum required sample size was determined to be at least n = 7 per group in each experimental group. This calculation was conducted through power analysis, employing an alpha error of 0.05 and a beta error of 0.20 (achieving a power of 0.80). D'Agostino–Pearson test was used for the normal distribution of the data, one-way analysis of variance (one-way ANOVA) and post hoc Tukey's tests were used for differences between groups. A p-value of less than 0.05 was considered statistically significant in all analyses. MTT experiments were repeated at least 7 times on different days.

Results

The effect of FGF21 on DRG cell viability

a) The effect on healthy cells

Firstly, the effect of FGF21 on the viability of healthy DRG cells was examined. Different doses of FGF21 (12.5 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL) were applied to DRG cells. It was determined that the application of FGF21 alone had no effect on the viability of DRG cells when compared to the control group (Figure 2).

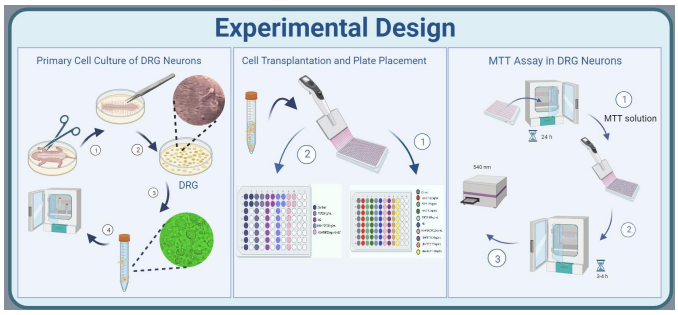

Figure 1. Experimental desing.

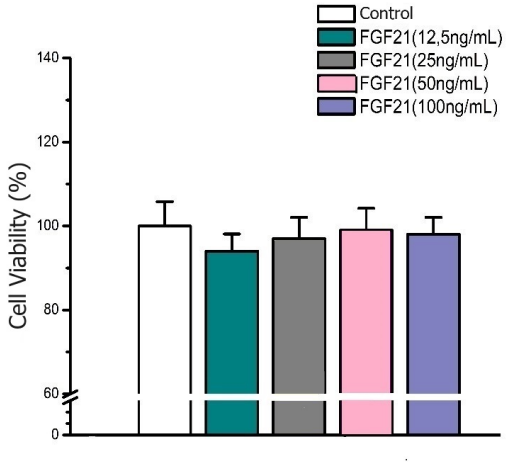

Figure 2. Dose-dependent effect of FGF21 on cell viability of healthy DRG neurons.

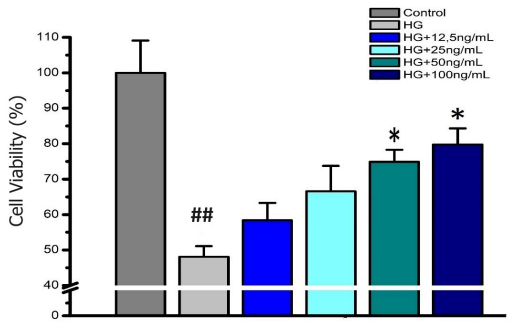

Figure 3. Dose-dependent effect of FGF21 on cell viability of DRG neurons ##p<0.01; High glucose (HG) vs control, *p<0.05; HG vs HG+FGF21 (50ng/mL, 100ng/mL), (one-way analysis of variance followed by post-hoc Tukey HSD test).

b) The effect on diabetic neuropathy model

The effect of applying different doses of FGF21 (12.5 ng/mL, 25 ng/mL, 50 ng/mL, 100 ng/mL) to DRG cells in conjunction with HG was investigated. The cell viability in the group treated with HG (48.08 ± 9.04) significantly decreased compared to the control group (100 ± 9.10 %) without any treatment (p<0.01). In the groups where FGF21 at different doses was applied along with HG, cell viability was found to increase in a dose-dependent manner when compared to the HG group. A statistically significant protective effect of FGF21 was observed at doses of 50 ng/mL (74.92 ± 8.89) and 100 ng/mL (79.76 ±12.89) (p<0.05) (Figure 3).

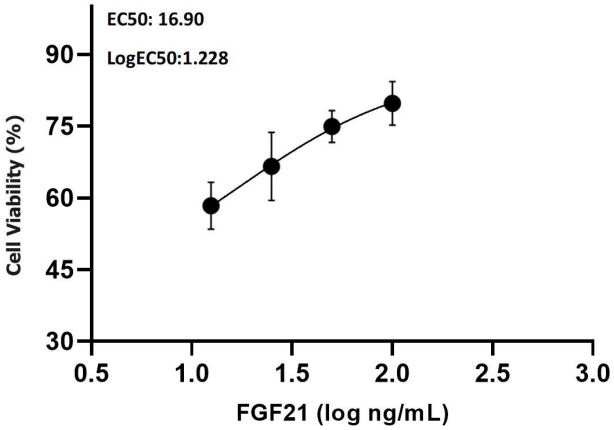

Figure 4. Dose-response curve in cell viability.

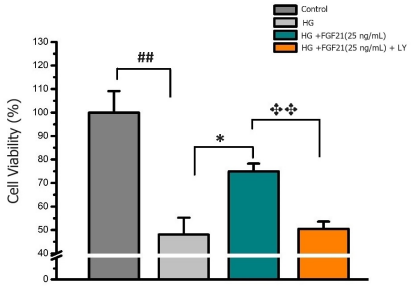

Figure 5. Effect of FGF21 on viability of DRG cells via AKT signaling pathway. ##p<0.01; High Glucose (HG) vs control, *p<0.05 HG vs HG+FGF21 (25 ng/mL), ✣✣ p<0.01 HG+FGF21 (25 ng/mL) vs HG+FGF21(25 ng/mL)+LY (one-way analysis of variance and then the post-hoc Tukey HSD test).

The effect of FGF21 on cell viability via the AKT/PI3K pathway

The calculations performed to determine the effective dose of FGF21 in improving DRG cell viability (EC_{50}) revealed that approximately 25 ng/mL of FGF21 is required (log EC_{50} = 1.228) (Figure 4). The effectiveness of FGF21 on DRG cell viability was interrogated through the AKT/PI3K pathway. The application of LY(50.45 ± 8.83) to DRG cells eliminated the effect of FGF21, leading to a statistically significant decrease in DRG cell viability

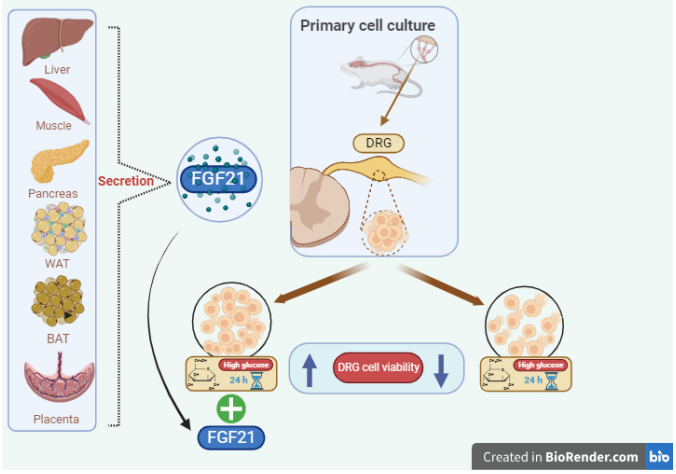

Figure 6. FGF21 preserves cell viability in DRG neurons and that this effect occurs through the AKT/PI3K signaling pathway.

($p<0.05$) (Figure 5).

Discussion

This study has revealed that FGF21 preserves cell viability and that this effect occurs through the AKT/PI3K signaling pathway (Figure 6). According to the current literature, there are no studies examining the effect of FGF21 on cell viability in DRG neurons. However, various studies have explored the beneficial effects of FGF21 treatment on diabetes [33-35]. Our study addresses this gap in the literature by thoroughly investigating the effects of FGF21 on cell viability in DRG neurons. The findings contribute to a better understanding of FGF21's potential therapeutic applications and the underlying mechanisms involved.

In diabetic neuropathy, HG has been found to damage axons and Schwann cells [36-38], inhibit distal axon growth [39], and increase apoptosis [40]. Studies have revealed an association between cell viability and apoptosis in DRG neurons affected by this condition [8,41]. Our study, in line with the literature, demonstrated a decrease in cell viability in HG-treated DRG neurons compared to the control. The observed decrease in cell viability emphasizes the adverse effects of HG on neuronal health. Existing studies report an association between mitochondrial damage, apoptosis, and cell death due to HG [42,43]. Recent researches have also highlighted the significant effects of FGF21 in neuronal apoptosis, and preserve mitochondrial stability. In the same study, FGF21 neurological disorders [29]. In a study conducted in a model of aging mice, FGF21 administration was found to alleviate hippocampal damage, inhibit was found to protect neurons against inflammation by increasing mitochondrial function through the mitogen-activated protein kinase (MAPK)/AKT pathways [29]. Recent studies also indicate the presence of FGF21 receptors in the brain and its ability to cross the blood-brain barrier, suggesting its potential as a therapeutic target in CNS diseases [44,45]. Chen et al.'s study demonstrated that FGF21 improves neurodegeneration in in vivo and in vitro models of Alzheimer's disease, reduces apoptosis, and has a neuroprotective effect through the MAPK pathway [46]. Recent studies have found that FGF21 significantly improves oxidative stress, reduces apoptosis, and alleviates peripheral nerve damage [28]. Our findings reveal that FGF21 exhibits a dose-dependent increase in cell viability in DRG neurons exposed to HG levels, demonstrating an in vitro neuroprotective effect. This effect is attributed to FGF21 reducing the death rate caused by glucose in these neurons. Through our study, we provide the first evidence of the neuroprotective effect of FGF21 in DRG neurons subjected to a diabetic model, complementing existing research demonstrating the neuroprotective effects of FGF21 in various cell types. In this study, we speculate that the preservation of cell viability following FGF21 administration is likely achieved through the mechanism of restoring mitochondrial damage and reducing apoptosis. However, to confirm these claims and gain a better understanding of the neuroprotective mechanism of FGF21, more detailed research and experimental studies are required.

AKT is known as a significant metabolic regulator among cellular pathways and can be phosphorylated and activated in diabetic mice [47,48]. PI3K activates AKT by promoting the conversion of diphosphoinositide to triphosphoinositide on the plasma membrane [49]. The intracellular signaling pathway AKT/PI3K regulates the transition from G1 to S phase in the cell cycle and plays a crucial role in regulating cell viability, proliferation, and apoptotic processes [50]. Previous studies have shown that FGF21 activates the PI3K/AKT signaling pathway, facilitating autophagy in prostate cancer cells [51], and regulating apoptosis, autophagy, and oxidative stress in human endothelial cells [32]. Our study also demonstrated that FGF21 utilizes the PI3K/AKT pathway to preserve cell viability. This suggests that the PI3K/AKT signaling pathway may play a critical role in the mechanisms through which FGF21 supports cellular health.

Conclusion

In conclusion, this finding provides an opportunity to obtain new data that could pave the way for novel approaches in the development of therapeutic agents for diabetic neuropathy. Furthermore, elucidating the mechanisms through which FGF21 affects cell viability and supporting experiments in vivo could contribute to the development of targeted treatments for diabetic neuropathy.

Acknowledgement

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Disclosure statement

We, the undersigned authors, hereby declare that we have no conflicts of interest related to this article. We affirm that our research is free from any financial, personal, or professional conflicts of interest, thereby ensuring the preservation of scientific integrity.

Data availability statement

Data presented in this manuscript file will be available upon reasonable request to Mete Özcan (mozcan@firat.edu.tr), it is not made publically avail-able be-

cause the software required to process raw data has access limi-tations and license requirement.

Ethical approval

This study protocol was approved by the Local Animal Experiments Ethics Committee of Firat University in the session held on 26.12.2022 with approval number 2022/21-14.

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