The Effect of Gliclazide use on BDNF and NGF Levels in Rats with Diabetes Mellitus

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Objective: In this study, the effects of gliclazides, a second generation sulfonylurea group, on BDNF and NGF plasma levels, which are considered neurodegeneration biomarkers, will be examined. When designing our study, we assumed that gliazides might have positive neuronal effects. Thus, the possible positive effects of gliclazide will be emphasized in our study.

Methods: In the experiment, 21 adult male Wistar-Albino rats were used. Serum BDNF and NGF levels were determined by analyzing with enzyme-linked immunosorbent assay kit in accordance with the recommendations.

Results: BDNF levels were significantly lower in gliclazide-treated diabetic rats and non-medicated diabetic rats compared to the healthy control group (p=0.017, p<0.001, respectively). Although the BDNF level of rats with diabetes given gliclazide was increased compared to rats with and without diabetes, this difference was not significant (p=0.107). Similarly, NGF levels were significantly lower in rats given gliclazide (p=0.009) and diabetic rats not given gliclazide (p=0.001) compared to the healthy control group. When the diabetic groups were compared among themselves, although the NGF level was increased in the gliclazide group, this difference was not statistically significant (p=0.638). The differences between the groups were significant in cyclic AMP regulatory element binding (p<0.001), c-FOS (p<0.001), amyloid precursor protein (p<0.001), B-SECRETASE1 (p=0.004), and doublecortin (p<0.001) levels.

Conclusion: As a result, serum BDNF and NGF levels were significantly higher in non-diabetic healthy control group rats than in diabetic rats. While low serum levels of BDNF and NGF neurotrophins, which increase in neurodegeneration, were observed in diabetic rats, this level was observed to be higher in diabetic rats given gliclazide.

INTRODUCTION

Glucose metabolism is essential for normal brain function, and circulating glucose levels play an important role in learning and memory functions. Neurotrophins are important regulators in the development and functioning of the nervous system. Neurotrophin family members are nerve growth factor (NGF), brain-derived neurotrophic factor, neurotrophin-3, neurotrophin-4/5, neurotrophin-6, and neurotrophin-7.[1,2]

Brain-derived neurotrophic factor (BDNF) is an important neurotrophin that affects the survival, growth, and function of neurons in the central nervous system (CNS) and peripheral nervous system (PNS); stabilizes synapses; and regulates synaptic function, axon, and dendrite branching. While BDNF mainly helps neurons to develop and renew themselves in the CNS, it contributes to the structural health and maintenance of important nerve pathways in which neurotransmitters function.[3] NGF is an important neuropeptide of the neurotrophin family, as a complex composed of three non-covalently linked subunits. Other proteins such as cyclic AMP Regulatory Element Binding Protein (CREB) and cFOS are also a transcription factor involved in the development, maintenance, and neuronal plasticity of the nervous system, as well as learning and memory.[4]

Studies have shown that the deficiency in the synthesis of BDNF, which plays a critical role in the long-term potential based on learning and memory, may increase the susceptibility to neurological and neurodegenerative diseases such as type 2 diabetes mellitus (T2DM), Huntington’s disease, and Alzheimer’s disease in humans and animals.[5] NGF is a neurotrophic protein that has been shown to increase the growth, differentiation, and survival of nerve cells in mammals.[6]

Sulfonylureas act by increasing insulin secretion in pancreatic beta cells and are drugs used in the treatment of T2DM for more than 50 years. Its main effects are to re-
duce fasting hyperglycemia. Sulfonylureas are divided into two groups: First and second-generation sulfonylureas. Second-generation sulfonylureas (glyburide, glipizide, glimepiride, and gliclazide) are more potent and possibly safer than first-generation sulfonylureas. They are usually metabolized by the liver. Second-generation sulfonylureas are excreted both in the urine and in the feces. Second-generation sulfonylureas are non-ionically bound to plasma proteins and are more potent, and drug interactions are minimal.

Neurotrophins are synthesized from many cell types in the CNS, PNS, and peripheral tissues and are known to have biological effects both in the nervous system and in many tissues outside the nervous system. It is accepted that the deficiency in neurotrophin synthesis is related to the increased susceptibility to neurodegenerative diseases.

In this study, the effects of gliclazides, a second generation sulfonylurea group, on BDNF and NGF plasma levels, which are considered neurodegeneration biomarkers, will be examined. When designing our study, we assumed that gliclazides might have positive neuronal effects. Thus, the possible positive effects of gliclazide will be emphasized in our study.

MATERIALS AND METHODS

This study was carried out at Dicle University Health Sciences Application and Research Center. Ethical approval for the study was obtained from Dicle University Animal Experiments Local Ethics Committee (Date: April 17, 2023, Acceptance Number: 482040). Procedures were performed in accordance with standard experimental animal studies ethics.

Animals and Creation of Diabetes

In the experiment, 21 adult male Wistar-Albino rats, 8–10 weeks old, with an average weight of 250–300 g, obtained from Dicle University Health Sciences Application and Research Center, were used. Rats were fed in stainless steel cages at 22±2°C for 8 weeks with normal diet and tap water for 12 h in light and 12 h in darkness without any restriction.

A single dose of nicotinamide (110 mg/kg) was administered to the abdominal cavity of rats to induce experimental diabetes. Fifteen minutes after nicotinamide administration, streptozotocin (STZ), which was prepared by dissolving 14 rats allocated for diabetic groups in 0.1 M citrate buffer (Ph 4.5), was administered to each rat intraperitoneally as a single dose of 60 mg/kg (Sigma-Aldrich, Co., St. Louis, MO, USA). To prevent hypoglycemia, a possible side effect of STZ, 5% glucose was added to the drinking water of the rats in the first 48 h after the injection. Animals with a blood glucose level above 250 mg/dL measured by glucometer from the tail vein after 72 h following the injection were considered as diabetic.

Twenty-one Wistar Albino rats were divided into 3 groups of 7 each. Group 1 (n=7): Control group without diabetes. These rats were given only placebo (tap water) in addition to the normal diet for 8 weeks. Group 2 (n=7): Control group with diabetes. Diabetes was induced in these rats and only placebo (tap water) was given for 8 weeks in addition to the normal diet. Group 3 (n=7): Diabetes was established and treated with gliclazide (10 mg/kg/day) for 8 weeks in addition to normal diet and tap water.

At the end of the 8-week experiment, blood was drawn from the heart of the rats by exsanguination under mild ketamine anesthesia and the rats were sacrificed. Glucose levels of blood samples were measured with Abbott Diagnostics original kits and Abbott Architect CI Photometric Autoanalyzer (Abbott Laboratories, Abbott Park, IL, USA). In addition, serum BDNF, NGF, TDP-43, amyloid precursor protein (APP), β-secretase 1, CREB, and c-FOS levels from blood samples were determined by analyzing in accordance with the recommendations of the enzyme-linked immunosorbent assay kit (Sunred Biological Technology, Shanghai, China) manufacturer. Results were read at 450 nm and expressed in pg/mL and ng/mL.

Statistical Analysis

Statistical analysis was performed using the SPSS 26.0 (SPSS Inc., Chicago, IL, USA) program. Shapiro–Wilk test was used for normal distribution evaluation. Arithmetic mean ± standard deviation will be used for continuous variables with a normal distribution, and frequency and percentage will be used for categorical variables. Analysis of variance (ANOVA) test was used for comparisons of our continuous data and Tukey’s test was used for in-group comparisons. Chi-square test will be used for categorical variables. The relationship between the two numerical variables was examined by Spearman correlation analysis and the p<0.05 value was considered significant for all evaluations.

RESULTS

The ANOVA test aggregated result of BDNF, NGF, and other neurodegeneration markers are shown in Table 1. BDNF was 0.22±0.03 ng/mL in healthy rats, 0.13±0.02 in non-medicated diabetic rats, and 0.16±0.04 ng/mL in glycoside-treated diabetic rats (Fig 1). The difference between the groups was significant (p<0.001). NGF levels were 4.39±1.13, 2.76±0.20, and 3.11±0.44 ng/mL in healthy rats, non-medicated diabetic rats, and glycoside-treated diabetic rats, respectively (Fig 2). The difference was statistically significant (p=0.001). In the ANAVO test analysis, the differences between the groups were significant in CREB (p<0.001), c-FOS (p<0.001), APP (p<0.001), B-SECRETASE1 (p=0.004), and doublecortin (DCX) (p<0.001) levels. However, TDP-43 level was similar in all three groups and the difference was not significant (p=0.166). Intra-group comparisons of BDNF and NGF levels are
shown in Table 2. The BDNF level was significantly lower in diabetic rats both given and not given gliclazide than the healthy control group (p=0.017, p<0.001, respectively). Although there was an increase in the BDNF level of rats with diabetes and those given gliclazide, this difference was not significant (p=0.107). Similarly, NGF levels were significantly lower in rats given gliclazide (p=0.009) and diabetic rats not given gliclazide (p=0.001) compared to the healthy control group. When the diabetic groups were compared among themselves, although the NGF level was increased in the gliclazide group, this difference was not statistically significant (p=0.638).

While BDNF has a positive correlation with CREB (R=0.642, p=0.002), c-FOS (R=0.740, p<0.001), and DCX (R=0.504, p=0.020), there was a negative correlation with

Table 1. Distribution of BDNF, NGF and other neurodegeneration markers in rats

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P for ANOVA test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>100.29± 9.34</td>
<td>541.29±21.27</td>
<td>421.29±10.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BDNF (ng/mL)</td>
<td>0.22±0.03</td>
<td>0.13±0.02</td>
<td>0.16±0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NGF (ng/mL)</td>
<td>4.39±1.13</td>
<td>2.76±0.20</td>
<td>3.11±0.44</td>
<td>0.001</td>
</tr>
<tr>
<td>CREB (ng/L)</td>
<td>375.43±36.22</td>
<td>218.29±25.80</td>
<td>253.57±13.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>c-FOS (ng/mL)</td>
<td>4.53±0.75</td>
<td>2.56±0.31</td>
<td>3.16±0.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>APP (ng/mL)</td>
<td>1.09±0.24</td>
<td>2.12±0.14</td>
<td>1.12±0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B-SECRETASE1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(ng/mL)</td>
<td>2.52±0.45</td>
<td>3.44±0.44</td>
<td>2.76±0.47</td>
<td>0.004</td>
</tr>
<tr>
<td>TDP-43 (pg/mL)</td>
<td>293.00±22.96</td>
<td>312.00±7.50</td>
<td>308.86±22.63</td>
<td>0.166</td>
</tr>
<tr>
<td>DCX (ng/mL)</td>
<td>52.12±3.82</td>
<td>39.28±2.94</td>
<td>40.95±6.47</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2. Intragroup comparison of neurodegeneration biomarkers BDNF and NGF in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Groups comparison</th>
<th>Tukey test P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>BDNF (ng/mL)</td>
<td>0.22±0.03</td>
<td>0.13±0.02</td>
<td>I&amp;2</td>
</tr>
<tr>
<td></td>
<td>0.22±0.03</td>
<td>0.13±0.02</td>
<td>1&amp;3</td>
</tr>
<tr>
<td>NGF (ng/mL)</td>
<td>4.39±1.13</td>
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<td>2&amp;3</td>
</tr>
<tr>
<td></td>
<td>4.39±1.13</td>
<td>3.11±0.44</td>
<td>I&amp;3</td>
</tr>
<tr>
<td></td>
<td>2.76±0.20</td>
<td>3.11±0.44</td>
<td>2&amp;3</td>
</tr>
</tbody>
</table>

APP: Amyloid precursor protein; BDNF: Brain-derived neurotrophic factor; CREB: Cyclic AMP response element binding protein; DCX: Doublecortin; NGF: Nerve growth factor; TDP-43: TAR DNA binding protein 43 (p<0.05 or p<0.01).
Regardless, these antidiabetics have some undesirable profile, and positive effects on gastrointestinal tolerability.

In diabetes, changes occur in the cerebral vessels and cause a decrease in blood fluidity. Subsequently, hypoxia and neuronal damage occur, respectively. This is because it results in lipid accumulation in brain vessels and ultimately various cerebrovascular endothelial dysfunctions.[8] In diabetes, changes occur in the cerebral vessels and damage resistance of neurons. While the majority of neurotrophins are produced by the CNS, some are also produced by peripheral tissues. It is largely found in the hypothalamus, the limbic system, and other parts of the brain such as the hippocampal nucleus.[9] Central effects on brain tissue and effects on energy homeostasis are among the effects of BDNF. Studies have reported that BDNF is related to metabolic effects, especially glucose metabolism and insulin resistance. NGF is a neurotrophin that plays an important role in the normal development of the nervous system, the survival of nerve cells, and their function. It has been suggested that NGF plays an important role in the diagnosis and treatment of important diseases such as neurodegenerative diseases, cancer, pain, retinal diseases, or diabetes as well as other diseases.[10]

The most prescribed drugs in T2DM patients are sulfonylureas. They are highly preferred drugs with a decrease in the mean glycosylated hemoglobin rate, good safety profile, and positive effects on gastrointestinal tolerability. [11] Regardless, these antidiabetics have some undesirable effects. In addition to the antidiabetic effects of sulfonylureas, there may also be beneficial effects to be explored further. Gliclazide, a second-generation sulfonylurea group, has been reported to have a reducing effect on free radicals as well as regulating blood sugar.[11] Reduction of free radicals in circulation may result in amelioration of oxidative stress and perhaps prevention or delay of complications. In a study, it was reported that gliclazides have protective effects from cardiovascular system diseases by having a positive effect on plasma lipid profile and platelet functions.[12] Alp et al.[13] suggested that gliclazide is a substance that protects the brain and nerve tissues against diabetic oxidative stress.

Our study showed that the levels of neurotrophins BDNF and NGF were significantly lower in diabetic rats given and not given gliclazide compared to the healthy control group. Although there was an increase in both BDNF and NGF levels in diabetic rats given gliclazide, this difference was not significant.

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Studies investigating the relationship between BDNF levels and glycemic parameters have shown conflicting results. While some studies have reported an increase in BDNF levels in T2DM patients, others have reported a decrease. [14,15] Increased BDNF levels have been reported in patients with T2DM receiving metformin therapy. In our study, gliclazide was given to diabetic mice, and we observed that the gliclazide level was higher in these mice than in diabetic mice that were not given gliclazide.

Studies have shown that NGF levels decrease significantly in cognitive disorders, exogenous NGF applications provide improvement in cognitive disorders, and NGF levels are significantly lower in diabetic patients.[16-18] The neurotropic and metabotropic potentials of NGF are thought to have an impact on the molecular mechanism and pathogenesis of diabetes, and multidisciplinary studies are being conducted on the NGF-DM interaction.[19] Boyuk et al.[20] reported that BDNF levels were higher in diabetic patients than in the healthy control group.

In our study, BDNF level and, similarly, NGF level were lower in diabetic rats compared to healthy rats. There was an increase in BDNF and NGF levels in rats with diabetes and given gliclazide. Moreover, in our study, BDNF showed positive correlation with NGF, CREB, and c-FOS neurodegeneration biomarkers, while there was negative correlation with APP, TDP-43, and DCX.

Our study has many limiting points. One of these limitations is the lack of histopathological examination of the nervous system. Since it is a rat model study, it has a limited number of samples.

### Table 3. Correlation of BDNF and NGF with other neurodegeneration biomarkers.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BDNF (ng/ml) R.P</th>
<th>NGF (ng/ml) R.P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CREB (ng/L)</td>
<td>0.642, 0.002</td>
<td>0.74, &lt;0.000</td>
</tr>
<tr>
<td>c-FOS (ng/ml)</td>
<td>0.740, &lt;0.001</td>
<td>0.653, 0.001</td>
</tr>
<tr>
<td>APP (ng/ml)</td>
<td>-0.552, 0.009</td>
<td>-0.474, 0.030</td>
</tr>
<tr>
<td>B-SECRETASE1 (ng/ml)</td>
<td>-0.419, 0.058</td>
<td>-0.483, 0.027</td>
</tr>
<tr>
<td>TDP-43 (pg/mL)</td>
<td>-0.171, 0.459</td>
<td>-0.380, 0.089</td>
</tr>
<tr>
<td>DCX (ng/mL)</td>
<td>0.504, 0.020</td>
<td>0.580, 0.580</td>
</tr>
</tbody>
</table>

APP: Amyloid precursor protein; BDNF: Brain-derived neurotrophic factor; CREB: Cyclic AMP response element binding protein; DCX: Doublecortin; NGF: Nerve growth factor; TDP-43: TAR DNA binding protein 43 (p<0.05 or p<0.01).
Conclusion
As a result, serum BDNF and NGF levels were significantly higher in non-diabetic healthy control group rats than in diabetic rats. While low serum levels of BDNF and NGF neurotrophins, which increase in neurodegeneration, were observed in diabetic rats, this level was observed to be higher in diabetic rats given gliclazide. Although there are many studies on the effects of treatments on the nervous system as well as diabetic complexity, there is still not enough information on this subject. Detailed studies are needed to better understand the positive and negative effects of gliclazides, which are sulfonylurea antidiabetic drugs. We hope that our study will contribute to this issue.

Ethics Committee Approval
This study approved by the Dicle University, Faculty of Medicine Clinical Research Ethics Committee (Date: 17.04.2023, Decision No: E-35582840-020-482040).

Informed Consent
Retrospective study.

Peer-review
Externally peer-reviewed.

Authorship Contributions

Conflict of Interest
None declared.

REFERENCES
Amaç: Bu çalışmada, ikinci kuşak bir sülfonilüre grubu olan gliklazidlerin nörodejenerasyon biyobelirteçleri olarak kabul edilen BDNF ve NGF plazma düzeyleri üzerindeki etkileri incelenecektir. Çalışmamızı tasarlarken, gliazidlerin olumlu noronal etkileri olabileceğini varsaydık. Gliklazidin olası olumlu etkileri çalışmamızda değerlendirildi.

Gereç ve Yöntem: Çalışmada 21 adet erişkin erkek Wistar-Albino sıçan kullanıldı. Öneriler doğrultusunda ELISA kiti ile analiz edilerek serum BDNF ve NGF düzeyleri belirlendi.

Bulgular: Glikazid verilen diabetik sıçanlarda ve ilaç verilmeyen diyabetik sıçanlarda BDNF düzeyi sağlıklı kontrol grubuna göre anlamlı olarak düşüktü (srasıyla, p=0.017, p<0.001). Glikazid verilen diyabetli sıçanların BDNF düzeyi, diabetli ve ilaç verilmeyen sıçanlara göre artış olmasına rağmen bu fark anlamlı değildi (p=0.107). Benzer şekilde, glikazid verilen sıçanlarda (p=0.009) ve glikazid verilmeyen diyabetik sıçanlarda (p=0.001) NGF seviyeleri sağlıklı kontrol grubuna göre anlamlı derecede düştü. Diyabetik gruplar kendi aralarında karşılaştırıldığında glikazid grubunda NGF düzeyi artmış olmakla birlikte bu fark istatistiksel olarak anlamlı değildi (p=0.638). CREB (p<0.001), c-FOS (p<0.001) ve DCX (p<0.001) düzeyleri diyabetik sıçanlarda daha düşük idi.

Sonuç: Sonuç olarak, diyabetik olmayan sağlıklı kontrol grubu sıçanlarda serum BDNF ve NGF düzeyleri diyabetik sıçanlara göre anlamlı olarak yükseldi. Nörodejenerasyonda artan BDNF ve NGF nörotrofinlerinin serum düzeyleri diyabetik sıçanlarda düşük görülürken gliklazid verilen diyabetik sıçanlarda bu düzeyin daha yüksek olduğu gözlemdi.

Anahtar Sözcükler: Beyin kaynaklı nörotrofik faktör (BDNF); gliklazid; nörodejenerasyon; sinir Büyüme faktörü (NGF).