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Differential Expression of BDNF and BIM in Streptozotocin-induced Diabetic Rat Retina After Fluoxetine Injection

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Abstract. *Background: Chronic diabetic retinopathy (DR) is a diabetic complication that causes blindness. Brain-derived neurotrophic factor (BDNF) expression is induced by fluoxetine. We observed the effects of fluoxetine on a streptozotocin (STZ)-induced diabetic rat model in this study. Materials and Methods: Rats were divided into three groups: Control, diabetic (65 mg/kg STZ injection), and diabetic with fluoxetine injection (20 mg/kg/week, six times). Western blotting was performed using anti-BDNF and anti-hexa-ribonucleotide-binding protein-3. Expression of BCL2 apoptosis regulator-like protein 11 (BIM) was analysed using a reverse transcription-polymerase chain reaction. Results: BDNF levels were significantly higher in the diabetic group treated with fluoxetine than in the untreated diabetic group. BIM expression was higher in the diabetic group than in the control group. BIM gene expression was lower in the fluoxetine-treated diabetic group than in the untreated diabetic group. Conclusion: Fluoxetine had an anti-apoptotic effect with up-regulation of BDNF expression in the retina of rats with STZ-induced diabetes.*

Diabetic retinopathy (DR) is a complication of diabetes that causes blindness (1). Important mechanisms of blindness are macular edema and alteration of neovascularisation (2, 3). Vascular endothelial growth factor (VEGF) is the most effective mediator of DR progression (4). Anti-VEGF effects are associated with the regression of retinal neovascularisation (5) and anti-VEGF treatment is the most effective approach for DR therapy. However, some patients show poor response to this treatment (6). Thus, comprehension of the pathogenesis of DR is critical. Although DR is traditionally known as a vascular disease, some studies have shown that neuronal pathological changes occur in the retina, including physiological and structural alterations (7, 8).

Brain-derived neurotrophic factor (BDNF) is a neuroprotective growth factor that affects neuronal cell proliferation and survival (9). BDNF binds to tropomyosin receptor kinase B (TRKB), which leads to nerve cell survival, repair, and development through the neurotrophic signalling pathway (10). BDNF has also been observed in retinal neuronal cells. BDNF is expressed in retinal ganglion cells (RGCs) and was found to play an important role in their survival in rats with streptozotocin (STZ)-induced diabetes (11). Cusato *et al*. reported that BDNF prevented cell death in the inner nuclear layer of the retina (12). Uzel *et al*. showed that BDNF is a good marker for the early diagnosis of DR (13).

In a previous study, BDNF expression was induced by fluoxetine, a selective inhibitor of serotonin reuptake, to treat major depression (14). In this study, we analysed the expression of BDNF and BCL2 apoptosis regulator-like protein 11 (BIM) after fluoxetine injection in the STZ-induced diabetes rat model to investigate the effect of fluoxetine on BDNF signalling.

Materials and Methods

Rat model of DR. All animal studies were approved by the Chosun University Institutional Animal Care and Use Committee (approval number: CIACUC2019-A0049). Male Sprague-Dawley rats (5-6 weeks old) were supplied by a certified breeder (Damul Laboratory Animals, Republic of Korea). The animal experimental design is illustrated through the schematic diagram shown in Figure 1. The

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Key Words: BDNF, BIM, diabetic, fluoxetine, retina.

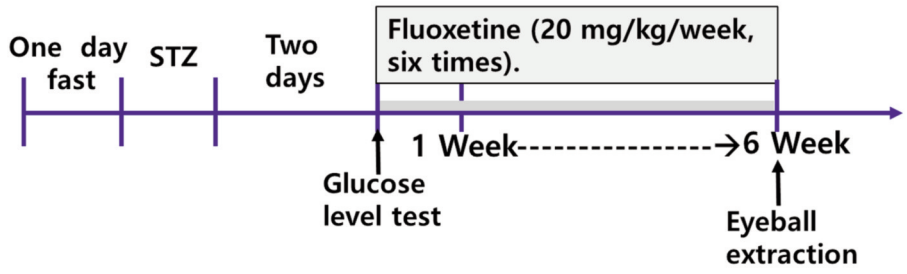

Figure 1. *Timetable for the experimental procedures. Blood glucose levels were measured using the tail vein. A high glucose level (>250 mg/dl) was defined as diabetes mellitus (DM).*

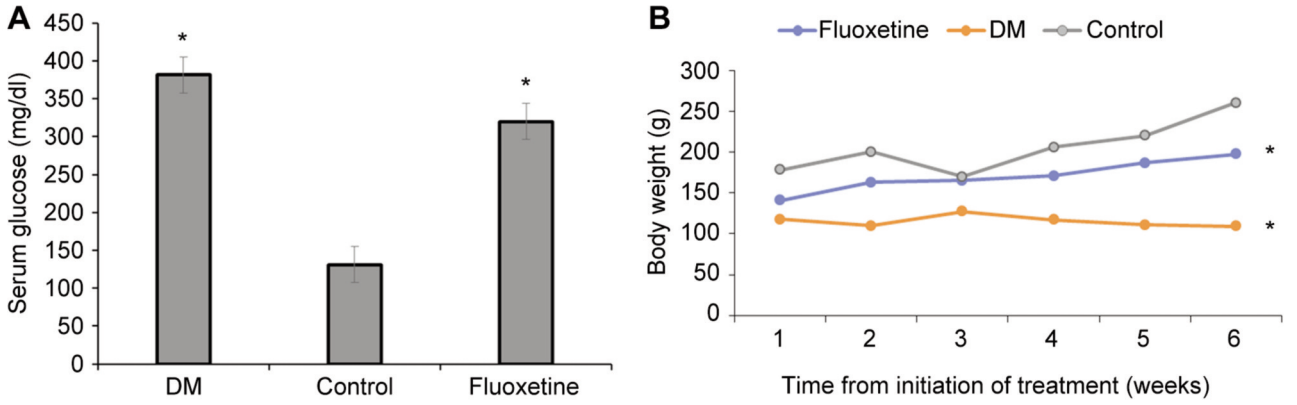

Figure 2. *Analysis of serum glucose (A) and body weight (B) in rats of control, diabetes mellitus (DM) and fluoxetine-treated DM groups. The data are expressed as the mean±standard error of the mean. *Significantly different from the control at p<0.05.*

rats were divided into three groups: Control (n=7), diabetic (n=7), and diabetic with fluoxetine injection (n=9, 20 mg/kg/week, six times). After one day of fasting, STZ (65 mg/kg, in 10 mM citrate buffer, pH 4.5; Sigma-Aldrich, St. Louis, MO, USA) was injected intraperitoneally into the rats in the diabetic groups. Two days later, blood glucose levels were measured using the tail vein. A high glucose level (>250 mg/dl) was defined as diabetes.

Western blot analysis. After 6 weeks, all Sprague-Dawley rats (n=23) were anaesthetised using sevoflurane inhalation (1.0-2.0%, end-tidal concentration) and the eyeballs were extracted under anaesthesia. Retinas were isolated from each eye. Retinal tissues were lysed using 0.1% Triton X-100 extraction buffer. Protein quantification in tissue lysates was performed using a bicinchoninic acid protein assay. After protein quantification, sodium dodecyl sulphate gel electrophoresis was performed using equal volumes of protein. Proteins were transferred to nitrocellulose membranes (GE Healthcare, Piscataway, NJ, USA). The membrane was then washed with primary antibodies against β-actin (1:1,000; Santa Cruz, CA, USA), and rabbit anti-BDNF (1:1000; Abcam, Cambridge, UK).

Reverse transcription-quantitative polymerase chain reaction (RT-PCR) analysis. The expression levels of BCL2-interacting mediator of cell death (*Bim*) were analysed using qPCR. Total RNA was extracted from the retinas of rats using Triazol. The synthesis of cDNA was performed using ReverTra Ace® qPCR RT Kit (Toyobo Corporation, Osaka, Japan). The glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*) gene was used as an internal control. The following primers were used: *Gapdh* forward: 5'-CCATCAACGACCCCTTCATT-3', and reverse: 5'-CACGACATACTCAGCACCAGC-3' (Gene ID: AF106860.2); *Bim* forward: 5'-AGATACGGATCGCACAGGAG-3', and reverse: 5'-ACC AGA CGG AAG ATG AAT CG-3' (Gene ID: NM_171988.2). The reaction mixture was as follows: One cycle for 1 min at 95˚C, 45 cycles of 5 s at 95˚C, and 5 s at 58˚C. Data were analysed using the $2^{-\Delta\Delta Cq}$ method (15).

Statistical analysis. Differences between the control, diabetic, and injection groups were evaluated using Kruskal-Wallis one-way analysis of variance. To examine the mean difference among groups, the Mann-Whitney test with Bonferroni adjustment was performed. All data were analysed using the Statistical Package for Social Sciences, Information Analysis Systems (IBM, Armonk, NY, USA). All data are expressed as the mean±standard error of the mean. *p*-Values of less than 0.05 were considered statistically significant.

Results

Bodyweights were checked weekly after fluoxetine administration. The bodyweights of rats in the diabetic group were significantly lower than those of rats in the control group (Figure 2). The bodyweights of rats in the fluoxetine-

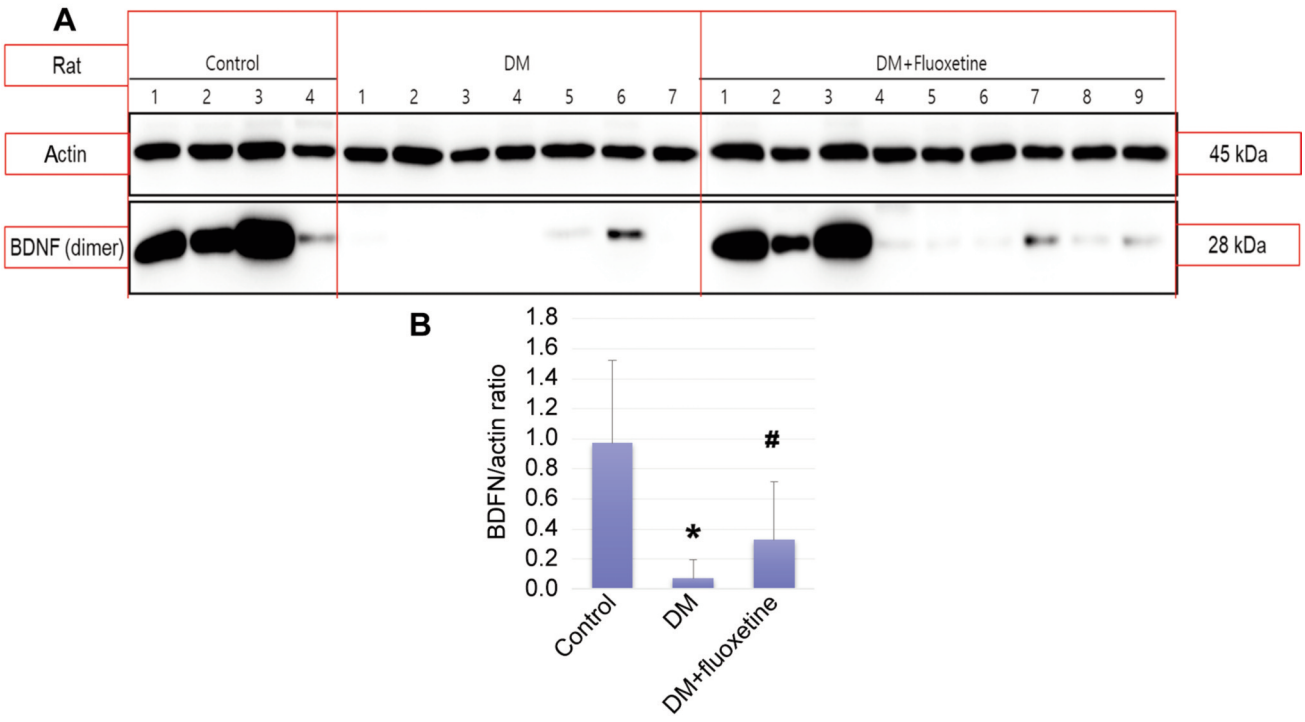

Figure 3. *Representative photographs (A) and quantification (B) of brain-derived neurotrophic factor (BDNF) expression on western blot in rats of control, diabetic (DM), and fluoxetine-treated DM groups. A: BDNF levels were significantly increased in the fluoxetine-treated group compared to that in the diabetic group. B: The results were expressed as a ratio compared to actin. The data are expressed as mean and standard error values. Significantly different at p<0.05 compared with *control and #DM.*

treated diabetic group were significantly higher than those of rats in the diabetic group but was not significantly different from that of the control group. The mean blood glucose levels of the diabetic and the fluoxetine-treated diabetic groups were significantly higher than that of the control group (Figure 2).

Western blot analysis revealed that the mean BDNF level was significantly increased in the fluoxetine-treated group compared to that in the diabetic group (Figures 3 and 4). The gene-expression level of *Bim* in the diabetic group was higher than that in the control group. However, its gene expression was lower in the fluoxetine-treated group than in the diabetic group (Figure 4).

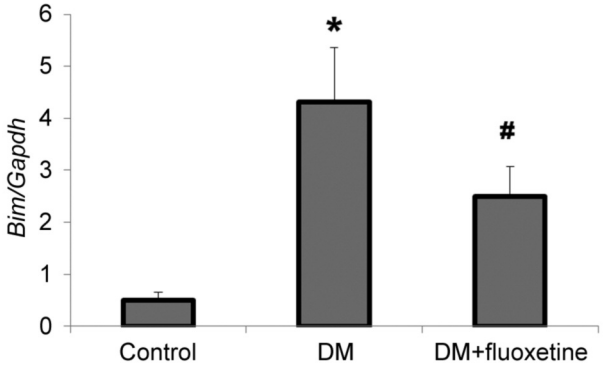

Figure 4. *The mRNA expression of BCL2 apoptosis regulator-like protein 11 (Bim) in retinas of normal, diabetes mellitus (DM) and fluoxetine-treated DM rats. The amounts of BIM mRNA expression were normalized to that of the internal control, glyceraldehyde 3-phosphate dehydrogenase. The data are expressed as mean and standard error values. Significantly different at: p<0.05 compared with *control and #DM.*

Discussion

STZ is an antibiotic that has a side-effect related to pancreatic β-cell destruction (16). This characteristic allows STZ to be widely used to produce an animal diabetes model (17-19). We defined the diabetic group with tail vein glucose levels following a previous study (20, 21).

BDNF expression levels in the diabetic group were lower than those in the control group. Some studies have shown that both protein and mRNA levels of BDNF were lower in patients with diabetes (22, 23). In a previous study, BDNF levels in patients with non-proliferative DR were reduced in serum and aqueous humour (24). This finding was similar to that of another study on BDNF levels in diabetic patients with retinopathy (25). This suggests that the reduction in

serum BDNF levels is related to the development of DR (26). Currently, DR is considered a neurovascular disease (27). The early progression of diabetes compromises retinal ganglion and glial cells (28). One study showed that BDNF suppressed apoptosis of RGCs in a glaucoma model (29). In diabetic conditions, BDNF causes phosphorylation of TRKB and stimulates the activation of extracellular-regulated kinase to play a neuroprotective role in the retina (30).

In our study, fluoxetine induced BDNF expression in rats with diabetes. Fluoxetine is a small molecule that mimics neurotrophic signalling (31). One study showed that fluoxetine has a protective effect on retinal epithelial cells (32). Other studies have shown that fluoxetine promotes the recovery of synaptic proteins in the visual cortex (33, 34). Several studies have shown that fluoxetine improves BDNF expression and concentration (35-37). Some studies have shown that BDNF expression induced by fluoxetine is associated with BDNF-TRKB signalling (38, 39).

We found that *Bim* expression was lower in the diabetic group than in the control group. Expression was restored in the diabetic group with fluoxetine injection. BIM is a well-known mediator of cell death and apoptosis, particularly in neuronal cells (40, 41). In the inner retina, where RGCs exist, proapoptotic molecules such as BIM and active caspase-8 are increased in patients with diabetes (42). Under high glucose conditions, BIM promotes the apoptosis of pericytes in the retina (43). After injection of fluoxetine in the diabetic group, BDNF expression was induced, and *Bim* expression was reduced. Some studies have shown that BDNF has a neuroprotective effect by regulating BIM expression (44, 45). Li *et al*. reported that the down-regulation of BIM was related to activation of TRKB in neuroblastoma cells (46). Regarding fluoxetine-regulated BDNF with TRKB signalling (47, 48), fluoxetine injection was associated with the down-regulation of BIM by TRKB signalling.

In conclusion, fluoxetine appeared to have an anti-apoptotic effect with up-regulation of BDNF expression in retina of rats with STZ-induced diabetes.

Conflicts of Interest

The Authors declare no competing interests in regard to this study.

Authors' Contributions

YHJ and YYC designed the study. RJC and HKS participated in surgical procedures. RJC and HKS analysed the data. HIH performed western analyses. All Authors approved the final article.

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