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Effects of Dexamethasone and Brain-Derived Neurotropic Factor in NT2 Cells

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Abstract

Stress is a major risk factor that can evoke neuropathological changes within the cortico-limbic system in neuropsychiatric, neurodegenerative, and metabolic disorders. Many of these disorders implicate the regulation of glucocorticoids (GCs) and neurotrophins, such as the brain-derived neurotropic factor (BDNF). GCs are steroidal hormones that have anti-inflammatory and immunosuppressive effects. They are widely used to treat allergy, inflammation and autoimmune diseases. GCs' roles and functions in the central nervous system (CNS) is varied and not well understood at this time. BDNF is commonly known to play important roles in the survival, growth-promoting and synaptic plasticity of the CNS. However, it has also been reported that continuous exposure to BDNF results in widespread neuronal death. While several studies have shown functional interactions between BDNF and GCs in neural events, the relationship between these interactions has not been clearly defined. The goal of this study was to determine the effects of dexamethasone (DEX) and BDNF in Ntera-2 (NT2) cells. Our results show a decline in cell viability and proliferation in a time and dose dependent manner when NT2 cells were treated with DEX alone. Treatment with BDNF did not affect NT2 cell viability. Interestingly, when NT2 cells were treated with a combination of DEX and BDNF, there appeared to be greater loss of cell viability and cell proliferation compared to the treatment with DEX alone. This synergistic effect possibly occurred via the co-activation of the BDNF receptor p75 and glucocorticoid receptor common pathways that may be responsible for apoptosis and cellular death.

Keywords: Dexamethasone (DEX); Brain-Derived Neurotropic Factor (BDNF); Ntera-2 (NT2) Cells; Cytotoxicity.

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1. Introduction

In the brain, stress is a major risk factor that can evoke neuropathological changes within the cortico-limbic system through neuropsychiatric, neurodegenerative, and metabolic disorders; many of these pathologies involve irregularities with respect to glucocorticoids (GCs) and neurotrophins [1-4]. In response to stress, the hypothalamic–pituitary–adrenal axis is activated to release the steroidal hormone GC (cortisol is primarily in humans and corticosterone is primarily in rodents) that regulates the stress response [5]. GCs have widespread effects on the body and can be lifesaving therapies when they are used as anti-inflammatory and immunosuppressive drugs. This makes them widely used to treat allergy, inflammation, and autoimmune diseases. However, excess GCs can produce negative outcomes, such as neuronal damage after suffering from stress or brain injury [6]. In the cell, GCs mediate their actions through the GR, a nuclear receptor that is highly expressed in the majority of human tissues and organs and are believed to play an important role in the regulation of stress response [7, 8]. GCs work genomically by binding to an intracellular GR that translocate into the nucleus where it dimerizes and controls gene transcription [9]. GCs can also act through nongenomic pathways independent of any effects on transcription to influence the activity of multiple kinases such as phosphatidylinositol-3-kinase (PI3K), AKT (protein kinase B (PKB)), and mitogen-activated protein kinase (MAPK) [10, 11]. The role and functions of GC in the central nervous system (CNS) are varied and not well understood. While GCs can exhibit neuroprotective, anxiolytic, and anticonvulsive effects, they are also linked to depression, anxiety, and memory changes [6,12]. The beneficial effects of moderate levels of GCs include modulation of hippocampal-dependent cognition and synaptic plasticity [6]. It has been reported that low concentration of GC stimulated axonal transport; critical for neuronal survival and function in Alzheimer's patients [13]. On the other hand, long-term exposure of high GC levels during stress has resulted in neuronal cell death [6]. A variety of neuropsychiatric disorders associated with stress, especially posttraumatic stress disorder, chronic pain, and chronic fatigue syndrome, paradoxically reveal somewhat lower levels of GCs [14]. Thus, the mechanism by which GCs may be exerting their beneficial or detrimental effects is presently not well understood. Brain-derived neurotropic factor (BDNF) is the most abundant neurotrophin in the brain and improves neuronal survival, transmission, synaptic plasticity, and axonal/dendritic growth and branching [15]. However, BDNF can make neurons highly vulnerable to oxygen and glucose deprivation; possibly by enhancing Ca2+ influx and nitric oxide production by the N-methyl-D-aspartate (NMDA) glutamate receptors [16]. BDNF exerts its actions by binding to two cell surface receptors, the high affinity tropomyosin-related kinase receptor B (TrkB), and the low affinity pan-neurotrophin receptor (p75) [17]. The TrkB pathway appears to be responsible for most of the neuronal effects of BDNF, such as cell survival, long-term potentiation (LTP), transcription, synaptic plasticity, and behavioral plasticity [15]. On the other hand, the p75 receptor is a member of the tumor necrosis factor (TNF) receptor family that can influence cell death and modulate the effects of other neurotrophins [18,19]. While studies have shown functional interactions between BDNF and GCs in neural events, the relationship between these interactions has not been clearly defined [20]. GC-BDNF interaction can result in toxic effects as it has been reported that low concentrations of BDNF increase the GR desensitization and vulnerability to stress [21]. On the other hand, recent evidence suggests that high GC levels due to stress decreases expression/function of BDNF *in vivo* [20]. This could result in the development/progression of neurodegenerative pathologies such as Alzheimer's disease that involves depression

as a risk factor [21]. GCs and BDNF interactions also display positive effects. A study in rat hippocampal and cortical neuron cultures reported that GCs can selectively activate TrkB receptor tyrosine kinases independent of increased levels of BDNF resulting in neuroprotection [6]. In addition, binding of BDNF to TrkB promoted the transcriptional activity of a synthetic GR reporter, suggesting a direct effect of BDNF signaling on GR function [22]. In addition, high levels of BDNF can increase the antidepressant effect, which is facilitated by GR-mediated signaling, phosphorylation, and specific gene transcriptions in mouse brains [23]. The present study investigated the effects of dexamethasone (DEX) in a time and dose dependent manner in Ntera-2 (NT2) cells. NT2 cells are human teratocarcinoma cell line which has been derived from a malignant testicular cancer and closely resemble human embryonic neural stem cells [24, 25]. We hypothesized that DEX treatment would be protective in low concentrations but toxic in high concentrations, especially with long-term exposure. Furthermore, as BDNF is known for its regulation of neuronal development, plasticity, function, survival and growth, we examined the effects of BDNF on NT2 cells in a time and dose dependent manner in this study. Given that the relationship between DEX and BDNF is not well understood, our study also examined the combined effects of DEX and BDNF on NT2 cells.

2. Materials and Methods

2.1. Cell Line and Cell Culture

NT2 cells were purchased from American Type Culture Collection® (ATCC®). Cells were seeded and grown according to ATCC® instructions.

2.2. Reagents and Drugs

DEX was purchased from SIGMA-ALDRICH®. BDNF was purchased from GIBCO®. CyQUANT and BrdU ELISA kits were purchased from INVITROGEN® and ABACAM®, respectively. DMEM and 10% fetal bovine serum were purchased from ATCC® and R & D Systems®, respectively. All other reagents were purchased from SIGMA®.

2.3. DEX Treatment

After trypsinization, NT2 cells were seeded at different cell densities of 2 x 10^3/well, 2 x 10^4/well, or 2 x 10^5/well in 96-well microplates. Following 48 h of incubation, NT2 cells were treated with either 10 nM, 100 nM, 1000 nM. 100 μM, 500 μM, or 1000 μM of DEX and then incubated for different time intervals (4 h - 72 h). The cells were washed using 1% phosphate buffered saline (PBS) before measuring the cell viability. Cell viabilities of the treated samples versus the control samples were measured and compared using the CyQUANT assay. Likewise, the cell proliferation amounts of the treated samples versus the control samples were compared but measured via the BrdU ELISA.

2.4. BDNF Treatment

After trypsinization, NT2 cells were seeded at a cell density of 2 x 10^4/well in 96-well microplates. Following

48 h of incubation, NT2 cells were treated with either 100 ng/ml or 1000 ng/ml of BDNF and then incubated for different time intervals (24 h - 72 h). The cells were washed using 1% PBS before measuring the cell viability. Cell viabilities of the treated samples versus the control samples were measured and compared using the CyQUANT assay.

2.5. DEX and BDNF Combined Treatment

After trypsinization, NT2 cells were seeded at a cell density of 2 x 10^4/well in 96-well microplates. Following 48 h of incubation, NT2 cells were either pre-treated with 1000 µM DEX, or 1000 ng/ml BDNF, treated simultaneously with a combination of multiple concentrations of DEX and BDNF, or post-treated with 1000 µM DEX or 1000 ng/ml BDNF. NT2 cells were then incubated for different times after each treatment. Cell viability and proliferation were measured using the CyQUANT and BrdU ELISA assays, respectively.

2.6. CyQUANT Assay

CyQUANT Assay is a cell viability assay based on the analysis of the cellular DNA content via a fluorescent dye binding. This assay uses CyQUANT® GR dye that has proprietary green fluorescence exhibited strongly when bound to cellular nucleic acids. The range of cell viability was measured by comparing cell counts of samples treated with agents of interest combined with CyQUANT® GR dye to control samples that have cells treated with media contained CyQUANT® GR dye only. The cell viability in a 96-well plate was examined by a fluorescence microplate reader (Biotek® - Synergy 2) with excitation at ~ 485 nm and emission detection at ~ 530 nm [26].

2.7. BrdU Cell Proliferation ELISA Assay

Abcam's BrdU Cell Proliferation ELISA (colorimetric) is an *in vitro* assay designed for an accurate detection of DNA synthesis and cell proliferation in 96-well plate. In this assay, bromodeoxyuridine (BrdU), which is a thymidine analog, replaces thymidine that is incorporated as cells enter the S phase. Following partial denaturation of double stranded DNA, BrdU is incorporated into recently synthesized DNA strands of cells that are actively proliferating. BrdU is detected immunochemically allowing the evaluation of the cell populations, which are actively synthesizing DNA. The amount of cell proliferation was measured by comparing cell samples treated with agents of interest combined with BrdU to control samples that have cells treated with media contained BrdU only. The cell proliferation in the 96-well plate was examined by a fluorescence microplate reader (Biotek® - Synergy 2) set at a dual wavelength of 450/550 nm [27].

2.8. Statistical Analysis

The average percentage of cell viability and proliferation amounts data were obtained from fluorescence intensities. The represented data are shown as mean ± standard error of the mean (SEM) from multiple independent experiments. Data were tested using one-way ANOVA (when comparing three or more groups) and Student's t-test (when comparing two groups), where a P-value <0.05 was considered significant.

3. Results

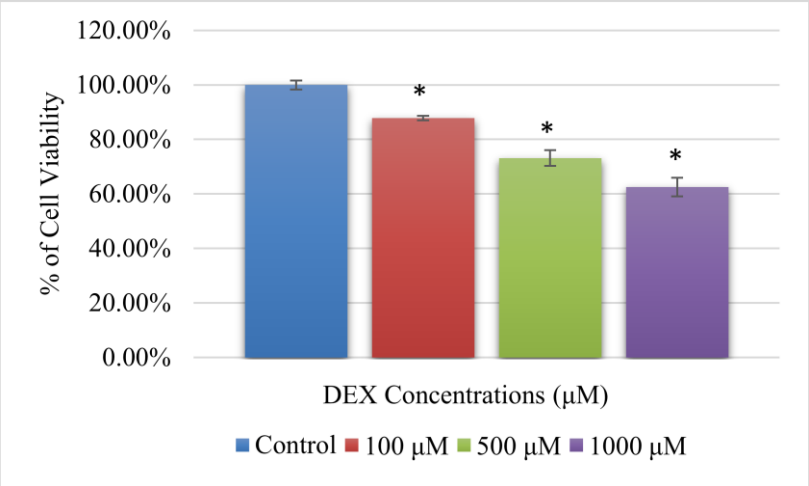
3.1. Time and Dose Dependent Effects of DEX on NT2 Cell Viability

Table 1 summarizes the results which indicated that low concentrations of DEX had no significant effect on NT2 cell viability regardless of the cell density or duration of treatment.

Table 1: Effects of 10 nM, 100 nM, and 1000 nM of DEX on NT2 cell viability. NT2 cells were seeded at different densities for 48 h, then treated with different DEX concentrations at different incubation periods shown above. The treated groups were compared to a control groups (media) at the time of cell viability measurement using the CyQUANT assay. One-way ANOVA and t-test (compared to the control group); $P > 0.05$. In the next set of experiments, NT2 cells were incubated with higher concentrations (100 µM, 500 µM, and 1000 µM) of DEX for 24 h (Figure 1A) and for 48 h (Figure 1B). The results indicated that DEX concentrations of 100 µM, 500 µM, and 1000 µM significantly decreased NT2 cell viability 24 h and 48 h after treatment. Viability of NT2 cells treated with DEX significantly decreased as higher concentrations of DEX were presented in a dose and time dependent manner.

Cell Density	Treatment Duration	DEX Doses	Effect on NT2 Cell Viability
$2x10^3$/well	6 h, 24 h, 48 h, and 72 h	10 nM, 100 nM, and 1000 nM	No significant effect
$2x10^4$/well	6 h, 24 h, 48 h, and 72 h	10 nM, 100 nM, and 1000 nM	No significant effect
$2x10^5$/well	6 h, 24 h, 48 h, and 72 h	10 nM, 100 nM, and 1000 nM	No significant effect

A



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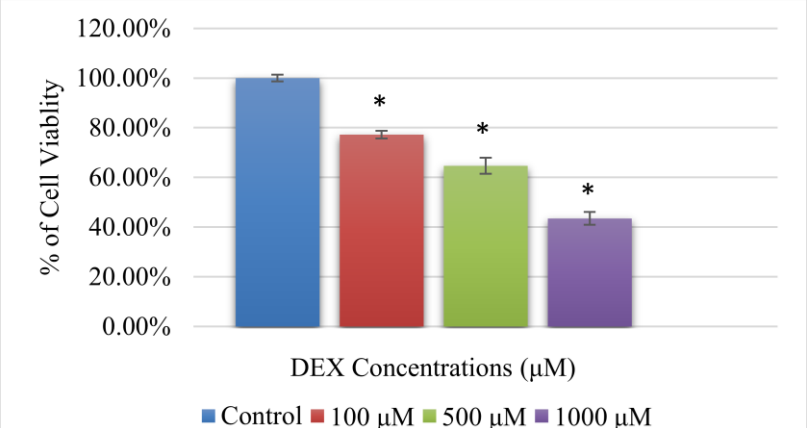


Figure 1: Effects of 24 h and 48 h of incubations with 100 µM, 500 µM, and 1000 µM of DEX on NT2 cell viability. Cells were seeded at $2x10^4$/well cell density in 96-well plate for 48 h then treated by DEX with each concentration shown above, followed by 24 h of incubation (A) and 48 h of incubation (B). The treated groups were compared to a control groups (media) at the time of cell viability measurement using the CyQUANT assay. The experiments were replicated three times with different passages of cell cultures. Data are represented as mean ± SEM and tested using one-way ANOVA and t-test (compared to the control group) (*P < 0.05).

3.2. Time and Dose Dependent Effects of BDNF Effects on NT2 Cell Viability

BDNF was incubated for 24 h (Figure 2A), 48 h (Figure 2B), and 72 h (Figure 2C) in NT2 cells at concentrations of 100 ng/ml and 1000 ng/ml. The results indicated that regardless of the dose or duration of BDNF treatment, BDNF did not have a significant effect on NT2 cell viability.

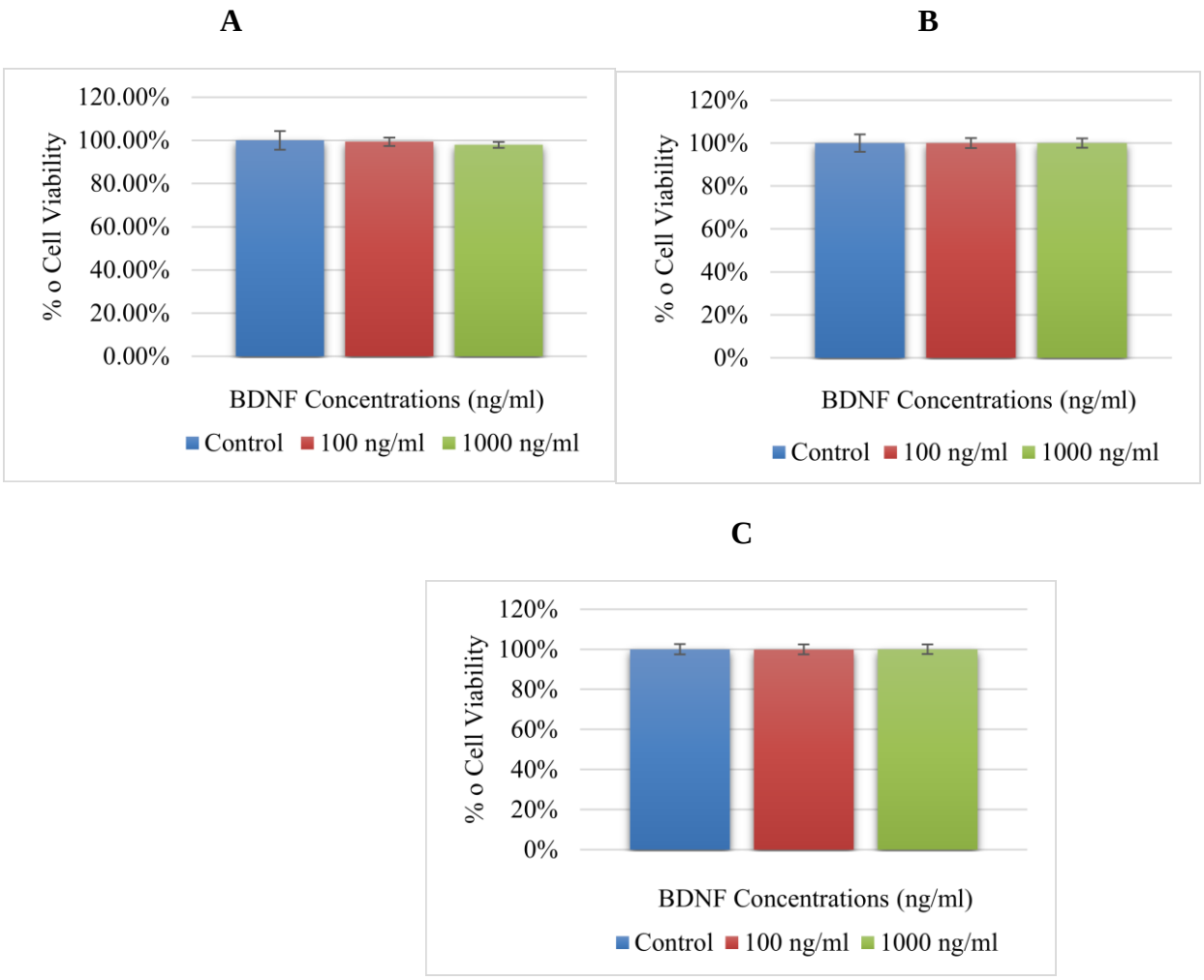


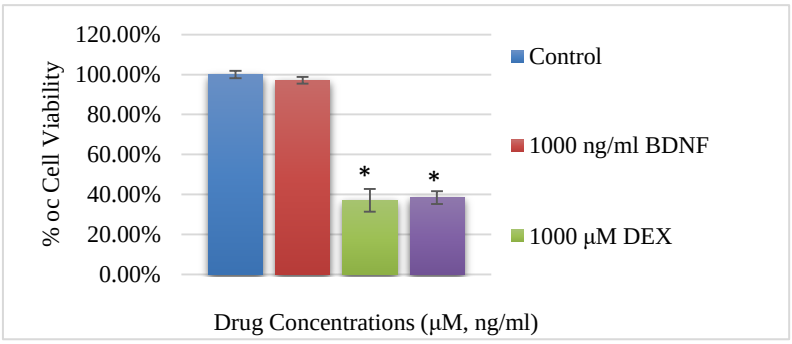
Figure 2: Effects of 24 h, 48 h, and 72 h of incubations with 100 ng/ml and 1000 ng/ml of BDNF on NT2 cell viability. Cells were seeded at $2x10^4$/well cell density in 96-well plate for 48 h then treated by BDNF with each concentration shown above, followed by 24 h (A), 48 h (B), and 72 h (C) of incubation. The treated groups were compared to a control groups (media) at the time of cell viability measurement using the CyQUANT assay. The experiments were replicated three times with different passages of cell cultures. Data are represented as mean ± SEM and tested using one-way ANOVA and t-test (compared to the control group) ($P > 0.05$).

3.3. Effect of DEX and BDNF on NT2 Cell Viability

The effect of DEX and BDNF on NT2 cell viability was examined in three ways. First, we tested the effects of 1000 ng/ml BDNF pretreatment followed by 1000 µM DEX treatment on NT2 cell viability (Figure 3A). The result indicated that BDNF pretreatment had no significant effect on cell viability followed by the addition of DEX. Second, we tested the effects of 1000 µM DEX pretreatment followed by 1000 ng/ml BDNF treatment on NT2 cell viability (Figure 3B). The result indicated a significant decrease in NT2 cell viability. Third, the effects

of 1000 µM DEX and 1000 ng/ml BDNF simultaneous treatment on NT2 cell viability was studied (Figure 3C). The result also indicated a significant decrease in cell viability compared to the effect of each drug administered alone.

A



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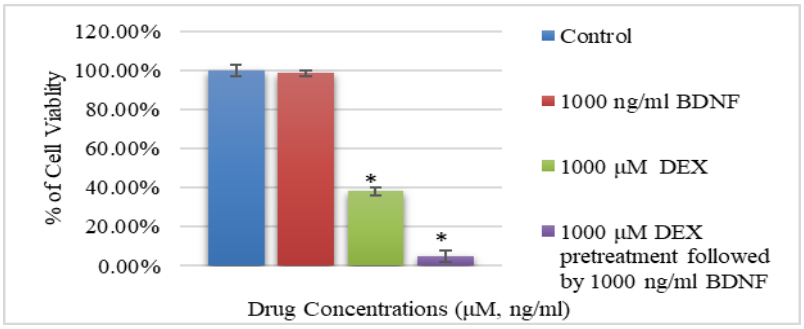

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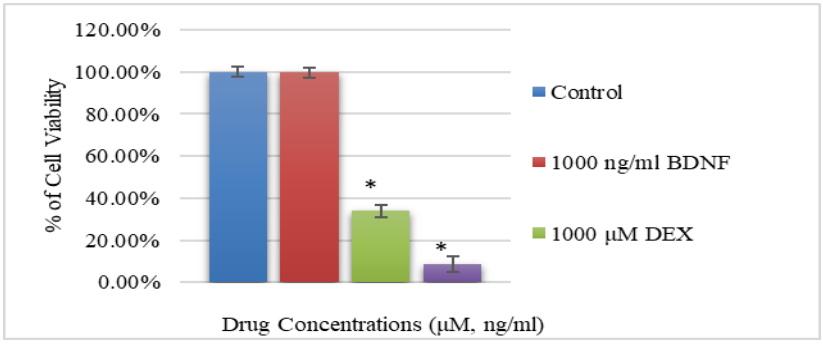

Figure 3: DEX and BDNF combined effects on NT2 cell viability. NT2 cells were seeded at a cell density of 2 x 10^4/well in 96-well microplates. Following 48 h of incubation, NT2 cells were either pre-treated with 1000 ng/ml BDNF (A), or 1000 µM DEX (B), or treated simultaneously with a combination of 1000 µM DEX and 1000 ng/ml BDNF (C). NT2 cells were also treated with 1000 ng/ml BDNF alone and 1000 µM DEX alone for 48 h in two separate groups in all experiments. All treated groups were compared to a control groups (media) at the time of cell viability measurement using the CyQUANT assay. The experiments were replicated three times with different passages of cell cultures. Data are represented as mean ± SEM and tested using one-way ANOVA

and t-test (compared to the control group) (*P < 0.05).

3.4. Effect of DEX on NT2 Cell Proliferation

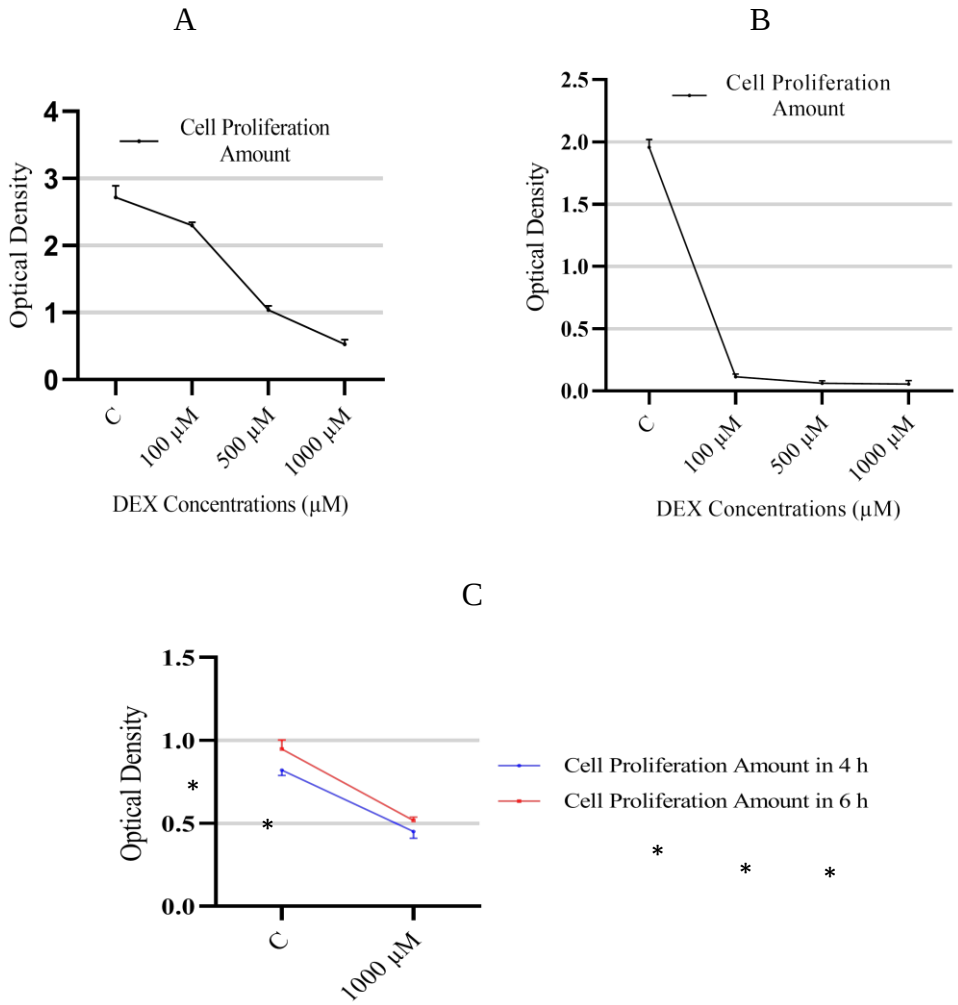

Figure 4: Effect of DEX on NT2 cell proliferation. NT2 cells were seeded at $2x10^4$/well cell density in 96-well plate for 48 h then treated by DEX with each concentration shown above for different times of incubation with BrdU. **A**) Effect of multiple DEX concentrations after incubation for 24 h on NT2 cell proliferation. **B**) NT2 cell proliferation amounts after 24 h of the removal of multiple DEX concentrations. **C**) Effect of 1000 µM DEX incubation for 4 h and 6 h on NT2 cell proliferation. All the treated groups were compared to control groups with BrdU only at the time of cell proliferation. The cell proliferations were measured using BrdU ELISA assay. All experiments were replicated three times with different passages of cell cultures. Data are represented as mean ± SEM and tested using one-way ANOVA and t-test (compared to the control group) (*P < 0.05).

Since DEX works genomically and could affect cell cycle regulatory genes [28], the effect of DEX on NT2 cell cycle and proliferation was measured using the BrdU ELISA assay to detect the newly synthesized DNA in actively proliferating cells. The result indicated in Figure 4A that DEX decreased NT2 cell proliferation in a dose dependent manner following incubation for 24 hours. Both 500 µM and 1000 µM of DEX were able to

reduce cell proliferation by more than 50%. Given that DEX did not completely abolish cell viability in Figure 1A and B, we also measured NT2 cell recovery and proliferation 24 h after DEX removal (Figure 4B). This experiment was designed to determine whether DEX has a reversible effect on NT2 cell proliferation. The result showed that even after DEX removal, the surviving cells were unable to proliferate again, indicating a potential DEX-induced irreversible effect. When DEX was incubated in NT2 cells at shorter time points (4 h and 6 h) (Figure 4C), the results indicated that 1000 µM of DEX significantly decreased NT2 cell proliferation at both time points.

3.5. Effect of DEX and BDNF in Combination on NT2 Cell Proliferation

Given that DEX and BDNF together led to a greater loss in cell viability, cell proliferation was measured to determine if this combination affected the cell cycle regulation (Figure 5). Results suggested a modest decrease in NT2 cell proliferation by BDNF-DEX combined treatment compared to the treatment with DEX alone.

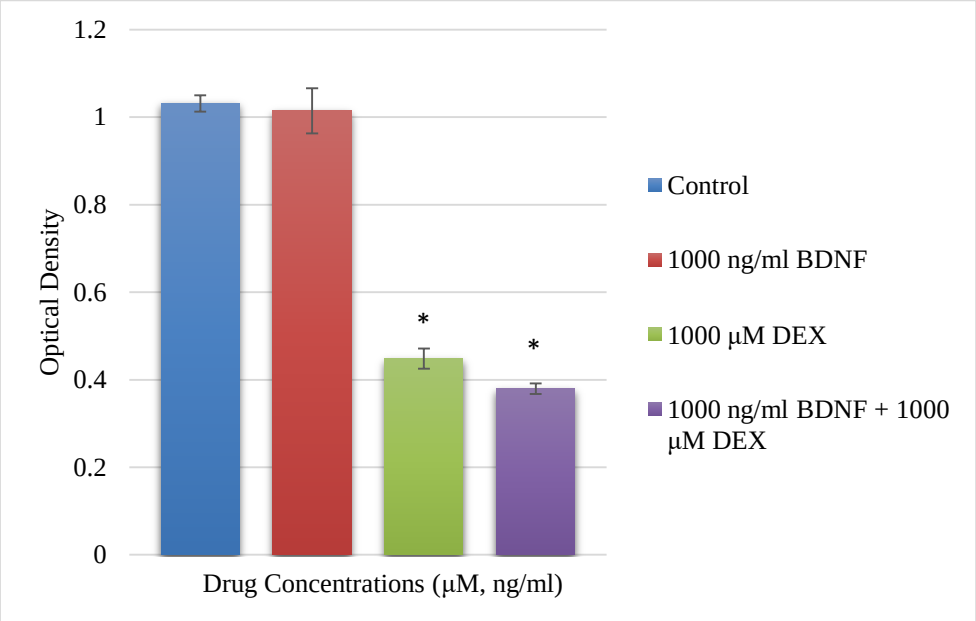

Figure 5: Effects of 1000 µM DEX and 1000 ng/ml BDNF simultaneous treatment on NT2 cell proliferation. Cells were seeded at $2x10^4$/well cell density in 96-well plate for 48 h then treated by 1000 µM of DEX alone, 1000 ng/ml of BDNF alone, and a combination of both. All groups were incubated for 6 h of incubation with BrdU. The treated groups were compared to a control group contained cells with BrdU only at the time of cell proliferation measurement using BrdU ELISA assay. The experiment was replicated three times with different passages of cell cultures. Data are represented as mean ± SEM and tested using one-way ANOVA and t-test (treatment groups compared to the control group and 1000 µM of DEX group compared to 1000 ng/ml of BDNF group) (*$P < 0.05$).

4. Discussion

4.1. Effects of DEX on NT2 cells

NT2 cells, a human teratocarcinoma cell line has been proven to be a useful *in vitro* neuronal model [29], and was used in the present study to investigate the effects of DEX and BDNF. To our knowledge, this is the first study that has investigated the effect of DEX and BDNF on NT2 cells. Our results indicate that low concentrations (10 nM, 100 nM, and 1000 nM) of DEX did not significantly affect NT2 cell viability regardless of the cell density ($2x10^3$/well - $2x10^5$/well) and the duration of treatment (6 h – 72 h). We suggest that these concentrations of DEX do not trigger any viability change in NT2 cell line. However, as higher concentrations (100 μM, 500 μM, and 1000 μM) of DEX were applied, DEX significantly decreased NT2 cell viability after 24 h and 48 h of treatment in a dose and time dependent manner (Figures 1A and B). Given that DEX regulates gene expression [28], we investigated the effects of DEX on NT2 cell cycle and proliferation using the BrdU ELISA assay, to detect newly synthesized DNA in actively proliferating cells. DEX at concentrations of 500 μM and 1000 μM for 24 h significantly inhibited NT2 cell proliferation in a dose dependent manner (Figure 4A). Given that DEX did not completely abolish cell viability, we measured NT2 cell proliferation 24 h after DEX removal. The results showed that a small percentage of surviving cells were unable to proliferate even when they were no longer under the influence of DEX (Figure 4B), suggesting a potential irreversible effect on the cell cycle. In addition, when shorter incubation times were used to determine the point at which DEX exerts its effects, we observed that incubation with 1000 μM of DEX for 4 h and 6 h significantly decreased cell proliferation by more than 40% (Figure 4C). Several studies have shown different neuronal effects of GCs on cell viability, proliferation, and survival, depending on cell type, duration of treatment, and doses. For example, Freddy and his team showed that after trophic support deprivation (where cell death occurred within 72 h without B27, an optimized serum-free supplement used for growth and long-term viability of neurons) in hippocampal and cortical neurons, treatment with 1μM DEX rescued more than 30% of these neurons. The study also showed that a treatment of 1μM corticosterone, a GR agonist, had a similar survival effect as DEX. However, treatment with mifepristone (a GR inhibitor), abolished the trophic effects of DEX. In contrast, treatment with 10 μM spironolactone (a mineralocorticoid receptor antagonist) did not show a significant effect. These findings suggested a beneficial effect of DEX in neuronal survival through the GR pathway [6]. However, another study reported that 1-200 μM/ml of DEX for 48 h reduced the growth of rodent and murine glioma tumor cells in a concentration-dependent manner. DEX at low concentrations inhibited glioma cell proliferation, and at higher concentrations, induced cell death. Moreover, DEX did not affect cell viability of primary rodent neurons and primary human astrocytes. This led the researchers to indicate a DEX-selective action on neuron and glioma cell growth in a drug and cell dependent manner [30]. These reports (along with our results) further illustrate the inconsistency of GC effect and suggest that DEX may be regulating specific genes related to cell proliferation, apoptosis and/or necrosis, based on the type of cells, dose, time and other experimental conditions. Accumulating evidence indicates that GCs may lead to cell apoptosis by various mechanisms depending on the availability and responsiveness of the apoptotic machinery in the cell type [31]. For example, primary pericytes cultured from the CNS rat micro vessels showed DEX-induced apoptosis, which was antagonized by the GR antagonist RU486 [32]. DEX also caused apoptosis in cultured bovine trabecular meshwork cells [33], cultured human corneal epithelial cells [34], and rat dentate gyrus neurons [35]. A study suggested that GC-induced apoptosis is initiated by and dependent on the GR that regulates gene expression. In particular, the GR can activate apoptosis via regulating elements of the extrinsic or intrinsic pathways, or both, that involve the activation of caspase 8 and Bcl-2 family members [31]. Additional reports confirmed an up-regulation of pro-

apoptotic modulators like CD95 and caspase-3 activation [36], or down-regulation of anti-apoptotic regulators, such as Bcl-2 [37] and Bcl-XL when activated by DEX [38]. The mechanism by which DEX induces cell death or interferes with cell proliferation is not understood at the present time. While the above studies referred to DEX-induced toxicity by apoptosis, others have indicated that apoptosis was not involved. Kawamura's team investigated the cell growth effect of DEX in human glioblastoma tumor cells such as KNS42, T98G, and A172 cell lines. Their results indicated that DEX inhibited A172 cell growth at all doses (0.1 μM to 100 μM) but caused a dose-dependent increase in cell growth in KNS42 and T98G cells at concentrations of 0.1 μM - 10 μM. Moreover, KNS42, T98G cell counts decreased sharply at 100 μM suggested that this inhibition was not due to DEX-induced apoptosis (since no DNA fragmentation was observed). Rather, it was due to a necrotic effect resulting from cell membrane changes [39]. Another study mentioned that p53, a tumor suppressor protein that regulates the cell cycle and acts differentially depending on the cell type, was found to be involved in the DEX inhibition of cell growth. The study showed that upon exposure to 1 μM of DEX, a functional cross-talk between GR and p53 in HT-22 cell line potentiated the growth arrest properties of p53 in G_1 phase of the cell cycle [28]. Thus, based on our results and reports from other research, it appears that DEX may be mediating its action through GR genomic effects to induce cell proliferation, cell cycle arrest, or apoptosis, and these effects may be dependent on various experimental conditions such as cell type, dose and treatment time.

4.2. Effects of BDNF on NT2 cells

BDNF is the most abundant neurotrophin in the brain and improves neuronal survival, transmission, synaptic plasticity, and axonal/dendritic growth and branching [15]. It can exert its actions by binding to two identified cell surface receptors: TrkB and p75 [17]. TrkB mediates most of the neuroprotective effects of BDNF [15] while p75 influences cell death and modulates the effects of other neurotrophins [19]. While studies have shown functional interactions between BDNF and GCs in neural events, the relationship between these interactions has not been clearly defined [20] Our results show that the 100 ng/ml and 1000 ng/ml of BDNF treatment alone in NT2 cells for 6-72 h did not affect cell viability nor did it exert any toxicity (Figures 2 and 5). This effect is consistent with other studies that reported a lack of an affect. For example, BDNF treatment with 20-100 ng/ml for 5 days did not change cell viability in mouse primary hippocampal neurons [40]. Additionally, a 48-h treatment with 20 ng/mL BDNF did not change cell proliferation in neural stem/progenitor cells [41], nor did 100 ng/ml BDNF affect the growth rate of undifferentiated NB1643 cells [42]. The literature also reports conflicting results when BDNF does exert an effect on cell proliferation; albeit positive or negative. For example, Kawamura's team showed an increased in the total number of trophectoderm cells when treated with 10 ng/ml of BDNF for 72 h; an effect mediated through the TrkB receptor and PI3K signaling pathway [43]. Another study showed an increase in proliferation in primary microglial cells upon BDNF (20 ng/mL) administration for 6 h [44], although the mechanism was not clearly defined. In contrast to these studies that showed an increased proliferation by BDNF, other studies have reported the opposite effect of BDNF. A study of rat cortical cell cultures exposed to several concentrations (10, 30, or 100 ng/ml) of BDNF for up to 48 h reported wide spread neuronal death. This result was explained as BDNF's ability to make neurons highly vulnerable to oxygen and glucose deprivation by enhancing Ca^{2+} influx and nitric oxide production via NMDA glutamate receptors [16]. Another study reported a significant decrease in rat dopaminergic neurons by 500 ng/mL BDNF exposure for 48 h [45]. While the mechanisms for these effects are not clear at this time, it is

possible that low TrkB receptor expression in NT2 cells may be responsible for the lack of BDNF effect on NT2 cell viability in the present study [46].

4.3. Effects of DEX and BDNF on NT2 cells

Studies have shown functional interactions between BDNF and GCs in neural events. However, the relationship between these interactions has not been clearly defined. While GCs can regulate translation, procession, and secretion of BDNF [47], BDNF can affect GR phosphorylation and signaling [48]. These interactions can result in either neuroprotection or neurodegeneration. Mice with genetic manipulation of the GR expression can alter BDNF mRNA and protein levels. GR+/- mice that had 50% reduction of GR exhibited a significant downregulation of BDNF in the hippocampus and showed a stress-induced depression-like behavioral predisposition. In contrast, transgenic mice overexpressing GR increased BDNF levels [49]. Another study showed that DEX administration did not alter BDNF levels in rat hippocampal and cortical neuron cultures. However, DEX did activate TrkB receptor phosphorylation by a GR genomic action, which resulted in neuroprotective effects [6]. BDNF can also interact with GR to provide neuronal growth and differentiation, which was shown in primary rat cortical neurons by simultaneous treatment with DEX. Binding of BDNF to TrkB promoted the transcriptional activity of a synthetic GR reporter, which suggested a direct effect of BDNF on GR function [22]. Although these previous studies indicate a beneficial effect of BDNF-DEX interaction, it has also been shown that BDNF mRNA expression was reduced by exposure to GCs in primary hippocampal neuron cultures and in neuron-like BZ cell line. These actions are modulated via the GR and linked to the adverse effects of GCs [50]. Additionally, GCs can reduce synaptic plasticity by inhibiting TrkB signaling pathways such as MAPK/ERK in rat cortical neurons [51]. Contrary to expectations, the results of the present study indicated that the combination of DEX and BDNF treatment led to a greater loss of cell viability and cell proliferation in NT2 cells when compared to treatment with DEX alone. It is possible that DEX treatment alters TrkB receptor signaling and therefore attenuates the protective effects of BDNF in NT2 cells. Besides the TrkB signaling pathway, BDNF is reported to exert effects via other mechanisms. For example, BDNF causes neural death by NADPH oxidase-mediated oxidative stress [16]. This effect may be synergistic with DEX, which has been shown to increase cellular oxidative stress [52]. In addition, BDNF exerts cellular apoptosis via a second BDNF receptor p75 [53]. While Trk receptors are known to inhibit p75 apoptotic pathways [54], weakly expressed TrkB receptors compared to p75 receptors [46] in NT2 cells may result in apoptosis with BDNF treatment [53, 55]. It is possible that BDNF by itself does not decrease cell viability and proliferation, but this effect is initiated when DEX is added to activate pathways involving the BDNF p75 receptor. Given that p75-induced apoptosis can be activated through jun kinase, ceramide, and NF-κB cascade (54, 56-59], it is likely that DEX treatment in NT2 cells activates ceramide, Nf-kB, and jun kinase [60-62], leading to cellular death [54, 60, 63]. Thus, the results of the present study suggest that treatment with DEX and BDNF together led to cellular death in NT2 cells through some common apoptotic and oxidative stress pathways. Future studies are warranted to uncover these mechanisms.

5. Limitations of the Study

While several studies have shown functional interactions between BDNF and GCs in neural events, the

relationship between these interactions has not been clearly defined. To the best of our knowledge, the effects of DEX on NT2 cells, and the effects of BDNF with or without DEX in NT2 cells has not been studied previously, thus precluding comparisons in previous literature. While this study looked at NT2 cells specifically, other cell lines were not considered.

6. Conclusion

A growing body of evidence shows that GCs, BDNF, or both evoke neurological changes in the CNS. Studies have shown functional interactions between BDNF and GCs, but without a clear and consistent relationship. The goal of this study was to determine the role (beneficial or detrimental) of DEX, BDNF, or a combination of both in NT2 cells. Our study showed no significant effect of low concentrations of DEX at the cell densities and time of incubation that were used. In contrast, higher DEX concentrations significantly decreased cell viability and proliferation in a dose and time dependent manner, suggesting a DEX differential effect on gene expression and molecular synthesis related to cell proliferation, apoptosis and/or necrosis, depending on the cell type, dose and treatment time. BDNF treatment alone did not protect or alter cell viability/proliferation nor did it exert a neuroprotective effect against high concentrations of DEX. The lack of a protective effect by BDNF may be due to the activation of the p75 receptor. While future studies are warranted to uncover these mechanisms, it appears that DEX and BDNF share a common pathway leading to apoptosis and oxidative stress in NT2 cells.

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