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Neuroprotective effect of cannabidiol on NTF-3 and IGF-1 genes expression

Neda Miandashti[1], Reza Safaralizadeh*[,1,+], Mohammad Ali Hosseinpourfeizi[1] & Majid Mahdavi[1]

[1]Department of Animal Biology, Faculty of Natural sciences, University of Tabriz, 29 Bahman Blvd, Tabriz, Iran, Postal code: 5166616471

E-mail: [+]safaralizadeh@tabrizu.ac.ir

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Cannabidiol is a herbal compound that has been found to be effective in improvement of inflammatory demyelinating diseases and could be useful to increase supportive factors in remyelination. It is derived from *Cannabis sativa* known as Marijuana. The aim of this research was to investigate the changes in the expression of neuroprotective NTF-3 and IGF-1 genes with focus on multiple sclerosis (MS) disease after treating U373-MG glial cells with cannabidiol. In this regard U373-MG astrocyte cells were treated at timescales of 24 h, 48 h, and 72 h with doses of cannabidiol, the total cellular RNA was extracted and converted into cDNA. Eventually, changes in NTF-3 and IGF-1 gene expressions were evaluated by quantitative real time polymerase chain reaction (qRT-PCR).

The results supported the theory that very low doses of the drug are neuroprotective for glial cells and CNS. Probably cannabidiol fulfills it by stimulating the growth and differentiation of oligodendrocyte precursor cells to become mature oligodendrocytes. However, further research work and application of complementary techniques are necessary to ensure trust worthy *in vivo* and *in vitro* results.

Keywords: Cannabidiol, IGF-1, Neuroprotective, Olygodendrocyte, Remyelination, NTF-3

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MS is the prominent cause of disability in young adults (1-3). Although the cause of MS is still unknown and there is no certain treatment for it, it is believed that various changes in different genes and environmental factors play a role in causing this disease. Demyelination in Central Nervous System (CNS) is the main pathological characteristic of MS which is an initial inflammatory process; repetition of inflammation eventually leads to the loss of axons, chronic neurological disabilities and neuropathological defects (2,4,5).

Remyelination– the production of new myelin in the adult CNS - is a primary repair mechanism that occurs in areas of neuronal injury. This improves neurological function and messaging and leads to creating myelin sheath which is often thinner and shorter than non-remyelination axons; however, this process is restricted gradually and will be removed during disease progress (3,6). Generally, it is believed that remyelination occurs via Oligodendrocyte Precursors Cells (OPCs) that are distributed throughout the CNS (1, 3, 4, 6, 7). To this end, OPCs must be dispatched to the demyelinating areas and converted (after differentiation) into mature myelination cells which surround the axon membrane (1,3-6,8). Mature oligodendrocytes move toward axons, make contact with them and cover them in the form of concentric layers of myelin membrane. Ultimately, by compressing the layers around axons, the functional myelin be formed (3) it appears that astrocytes are involved in remyelination as *in vivo* and *in vitro* studies on the processes of demyelinating and remyelinating have demonstrated that activation, proliferation and migration of OPCs into the affected areas occurs by release of chemotropic and neuroprotective factors, as well as expression specific transcription factors such as NKX2 and Olig 2 that are secreted by activated microglia and or astrocytes (1,3,4).

One of these neuroprotective factors is NTF-3 from a family of neurotrophic factors (NTFs) which is considered as a group of protein growth factors (9-11). The neurotrophin family is a family of mediators which are soluble in water and contain nerve growth factor (NGF), Brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4 (NT-4) (10-12). It is proposed that pathogenesis of neurodegenerative disorders, may be the result of

*Corresponding author

changes in the expression of neurotrophic factors and their receptors (10). NT-3 is the third neurotrophic factor that was detected after NGF and BDNF factors in 1990. Studies using EAE and in vitro studies suggest that NT-3 increases myelination of neurons by inducing differentiation of OPCs into myelin-forming cells (9). Another important neuroprotective factor is IGF that is a nutritional support factor of the oligodendrocyte cell lines, and includes IGF-1, IGF-2, and insulin-like growth factor-binding protein (IGF BPS) (6).

Numerous studies have suggested that the *Cannabis sativa* plant can probably increase the level of the neuroprotective factors in the body and play an important role in the improvement of patients. It is believed that cannabinoids mainly act via two different receptors: (i) the cannabinoid-1 (CB-1) receptor, predominantly expressed on the neurons; and (ii) the cannabinoid-2 (CB-2) receptor, predominantly expressed on cells of the immune system (13). However, CB-2 receptor expression is seen on glial as well as on neuronal cells in several areas of the brain (14).

The aim of this study was to evaluate the therapeutic potential of cannabidiol (non-psychoactive compound of cannabis) in increasing neuroprotective factors, NTF-3 and IGF-1. We hope that the pharmaceutical industry could utilize compounds that can increase levels of neuroprotective factors and play an important role to improve MS patients' condition.

Methodology

Cell culture

In the present study, U373-MG cell lines (purchased from National Cell Bank of Iran (NCBI) at Pasteur Institute) were used. U373-MG is a human glioblastoma astrocytoma that can be grown in adherent cultures. It was maintained while supplemented with 10% heat-inactivated fetal bovine serum (FBS). Cells were kept at 37°C, under a humidified atmosphere of 95% and 5% CO2 in 25 mL flasks.

Dilution cannabidiol

Cannabidiol (solution) was purchased from Sigma Aldrich Company. For each use, it was diluted to the amount desired with RPMI solution.

Cytotoxicity assay (MTT)

After determining the viability of the cells with Trypan blue dye, the researchers used MTT assay to assess toxicity of doses of cannabidiol. In this test, cells in 96-well plates at 6,000 cells with a final volume of 200 µL of 4% FBS at 24 h, 48 h and 72 h with different doses of drug (0 to 50 mM) were examined. Finally, the absorption of each well was read by analyzer device at a wavelength of 540 nm.

Treatment

Cells were seeded in a 6-well plate at a density of 3×10^5 and then treated with cannabidiol at a concentration of 0, 0.5, 1, and 2 µM cannabidiol. The plates were incubated in specified periods of time (24 h, 48 h and 72 h).

RNA isolation, reverse transcription (RT), and real-time PCR

Total RNA was extracted from astrocytes using RNA isolation reagent (TRIzol reagent, Invitrogen). The researchers conducted reverse transcription polymerase chain reaction for the conversion of RNA into cDNA, using RNA-dependent DNA polymerase enzyme (Thermo Scientific, USA). Based on the concentration obtained by Picodrop of RNA samples, equal amounts of RNA from each treated sample were used for cDNA synthesis. The primers were purchased from GENERAY Company. Real-time PCR was performed in the Rotor-Gene Q (Qiagen, Germany) and SYBR Green Universal PCR Master Mix (Amplicon), DNA template (cDNA), water and forward and reverse primers were used in this reaction. The GAPDH gene was selected as an internal control. The PCR protocol for NTF-3 gene consisted of 40 cycles of denaturation at 95°C for 25 s followed by 60°C for 25 s annealing and 72°C for 35 s to allow extension and amplification of the target sequence. The annealing for IGF-1 gene was 57°C for 25 s. primer sequences are shown in table (Table 1).

Statistical Analysis

All experiments, including MTT and qRT-PCR assays were performed in triplicate and the data were statistically analyzed by SPSS PASW statistic 18 and were evaluated by using linear and bivariate regression correlation tests. Linear and bivariate regression correlation tests were used for MTT and qRT-PCR-related data analysis, such as relationship

Table 1 — Primer sequences

5'-TGCTCTTAACACCTGTGTTTCCT -3' F	NTF-3 primers
5'-TTGAGCGAGTCTTCTGGCAA-3' R	
5'-GTGACATTGCTCTCAACATCTCC-3' F	IGF-1 primers
5'-TGGGTTGGAAGACTGCTGATT-3' R	
5'-TTCAACAGCGACACCCACTC-3' F	GAPDH primers
5'-GGTCTCTCTCTTCCTCTTGTGC-3' R	

between IGF-1 gene expression and different doses of cannabidiol.

Results

Results of MTT assay

Two sets of time periods (48 h and 72 h) were compared to a 24 h period indicating the effect of time on cytotoxicity of the cannabidiol on the cells. Column chart and linear graph of the IC50 and percentage of cell viability U373-MG with cannabidiol are shown in Fig. 1 and Fig. 2.

Results of Real Time PCR reaction

NTF3 and IGF-1 gene expression changes, as a result of different doses of cannabidiol, are shown in Fig. 3 & Fig. 4.

Discussion

Recent studies on animal models and human populations using drugs based on cannabinoids derived from cannabis, offer promising results for the treatment of various disorders and diseases such as cancer, neurodegenerative, and chronic inflammatory diseases (15). At least 60 active compounds can be extracted from this plant, all of which are known as cannabinoids and among them cannabidiol has received considerable attention in medicine, recently. This is due to its antioxidant and neuroprotective activity and ability to regulate the immune system as well as its lack of psychoactive effects (3,16-18). Most previous studies have suggested that cannabinoids are modulators of immune responses and have anti-inflammatory roles which are carried out by TH1 response switch to TH2 responses. Thus, by reducing inflammation and secretion of anti-inflammatory cytokines, the disease process can be slowed down (19,20).

In the mid-1980s, studies conducted on mice cells treated with THC (the main composition of the cannabis plant), showed reduced levels of IFN-1 (IFN-β and IFNα) after stimulation with LPS. the first evidence provided basis for the fact that cannabinoids can regulate the production of cytokines (21). Most of the later experiments focused on changes in inflammatory factors after the administration of cannabinoid in vitro and in vivo; these experiments were developed to studies investigating other factors including neuroprotective factors. One of the studies dealing with in vivo

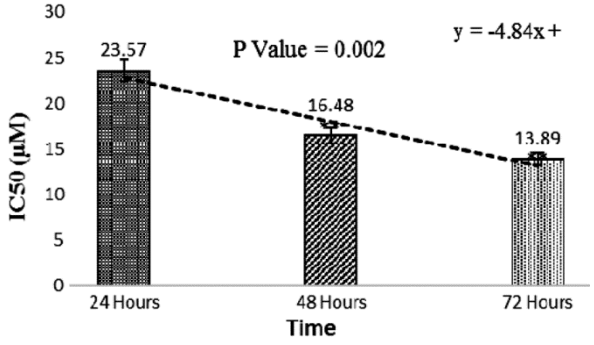

Fig. 1 — IC50 value of CBD (µM) in periods of 24 h, 48 h and 72 h on cell line U373-MG with Pv≤0.01. Two sets of time periods (48 h and 72 h) were compared to a 24 h period indicating the effect of time on cytotoxicity of the cannabidiol on the cells.

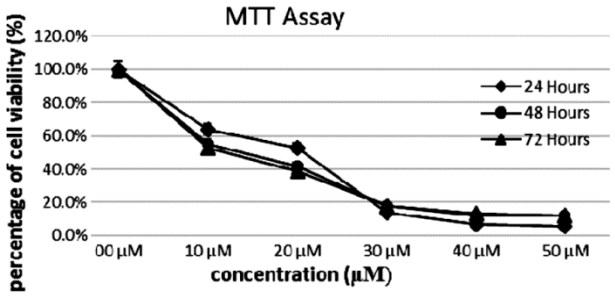

Fig. 2 — Linear graph of the percentage of cell viability U373-MG with cannabidiol in periods of 24 h, 48 h and 72 h (Pv≤0.01).

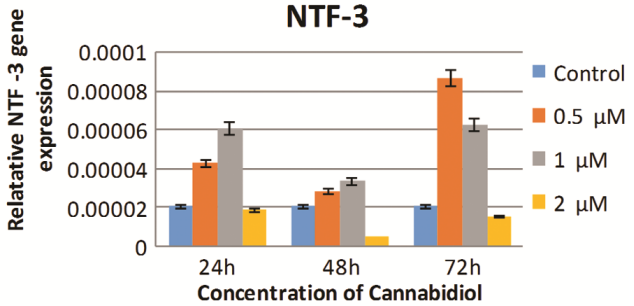

Fig. 3 — Chart of NTF3 gene expression changes as a result of different doses of cannabidiol Compared with the control over periods of 24 h, 48 h and 72 h (Pv≤0.01)

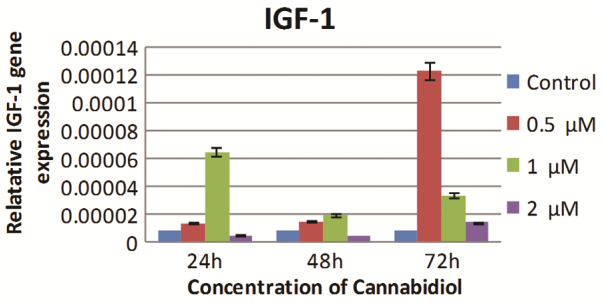

Fig. 4 — Chart of IGF-1 gene expression changes as a result of different doses of cannabidiol compared with the control over a period of time (24 h, (Pv≤0.05); 48 h and 72 h (Pv≤0.01)).

treatments with CBD, found an increase in brain-derived neurotrophic factor, also known as BDNF in peripheral blood cells of mice (22).

The most abundant type of glial cells is astrocyte that has Cannabinoid receptors. The functional role of astrocytes in the pathogenesis of neurodegenerative and neuroinflammatory diseases has been demonstrated in the CNS (23). Binding of cannabidiol ligand to CB1 and CB2 receptors causes the activation of routes that lead to the regulation of immune responses, inflammation, and secretion of neuroprotective factors (21,24). NTF3 is a member of the neurotrophin family of growth factors. In this study, it was shown that low concentrations of cannabidiol can enhance its secretion. Increased secretion of this factor from astrocytes affects the oligodendrocyte cells and stimulates the growth and differentiation of oligodendrocyte progenitor cells to form mature oligodendrocytes.

IGFs are neuroprotective trophic factors for cells of the oligodendrocyte lineage which are expressed at high levels in neuron-rich areas of the brain (6). Previous studies based on the injection of IGF-1 into animals with EAE, have reported a decrease in the numbers and area of demyelinated lesions and an increase in the number of axons containing regenerating myelin segments as well as an enhancement in myelin gene expression. These changes have been shown to be associated with rapid clinical and pathological recovery in the treated animals (6,25). This factor is effective in improving multiple sclerosis patients' condition, possibly through increased expression of transcription factors. These factors are involved in stimulating the growth and differentiation of oligodendrocyte precursor cells into mature olygodendrocytes that make myelin, and are playing a neuroprotective role in increasing remyelination in nervous system.

In this study, 0.5 μM of cannabidiol had the greatest effect on the expression of NTF-3 and IGF-1 genes in 72 h ($Pv \leq 0.01$). This indicated the influence of time factor on the effect of this drug. However, the best effectiveness in 24 and 48 h treatments with cannabidiol was observed at a dose of 1 μM ($Pv \leq 0.01$ and $Pv \leq 0.05$ respectively).

Moreover, 2 μM of cannabidiol, approximately in all of 3 treatment periods, resulted in a decreasing trend in the expression of mentioned genes. This trend was significant for NTF-3 only at 72 h period ($Pv \leq 0.01$). This decreasing trend in the last treatment period can be due to the stimulation of apoptosis factors which consequently leads to morphology change and ultimately cell death.

Conclusion

Scientific research on the effects of cannabinoids and especially one of them that called cannabidiol (non-psychoactive combination) continues to develop and to find the therapeutic potential and neuroprotective properties of such compounds for the treatment of neurodegenerative and neuroinflammatory diseases such as multiple sclerosis. In this study, data showed significantly ($Pv \leq 0.05$) increased expression of NTF3 and IGF-1 genes after treatment of U373-MG cells with cannabidiol. Doses of 0.5 and 1 μM of this drug offered results supporting its neuroprotective effects; however, further studies with complementary techniques are necessary to ensure obtaining of trustworthy *in vivo* and *in vitro* results.

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References

1 Keough MB, Yong VW. Remyelination therapy for multiple sclerosis. Neurotherapeutics. 2013; 10(1): 44-54.
2 Katona S, Kaminski E, Sanders H, Zajicek J. Cannabinoid influence on cytokine profile in multiple sclerosis. Clinical & Experimental Immunology. 2005; 140(3): 580-5.
3 Fernández-Ruiz J, Sagredo O, Pazos MR, García C, Pertwee R, Mechoulam R, et al. Cannabidiol for neurodegenerative disorders: important new clinical applications for this phytocannabinoid? British journal of clinical pharmacology. 2013; 75 (2): 323-33.
4 Watzlawik J, Warrington AE, Rodriguez M. Importance of oligodendrocyte protection, BBB breakdown and inflammation for remyelination. Expert review of neurotherapeutics. 2010; 10 (3): 441-57.
5 Aharoni R, Herschkovitz A, Eilam R, Blumberg-Hazan M, Sela M, Bruck W, et al. Demyelination arrest and remyelination induced by glatiramer acetate treatment of experimental autoimmune encephalomyelitis. Proceedings of the National Academy of Sciences. 2008; 105 (32): 11358-63.
6 Zhang J, Kramer EG, Asp L, Dutta DJ, Navrazhina K, Pham T, et al. Promoting myelin repair and return of function in multiple sclerosis. FEBS letters. 2011; 585 (23): 3813-20.
7 John GR. Investigation of Astrocyte–Oligodendrocyte Interactions in Human Cultures. Astrocytes: Methods and Protocols. 2012: 401-14.
8 Wilson HC, Onischke C, Raine CS. Human oligodendrocyte precursor cells in vitro: phenotypic analysis and differential response to growth factors. Glia. 2003; 44 (2): 153-65.
9 Razavi S, Nazem G, Mardani M, Esfandiari E, Salehi H, Esfahani SHZ. Neurotrophic factors and their effects in the treatment of multiple sclerosis. Advanced biomedical research. 2015; 4.
10 Larpthaveesarp A, Ferriero DM, Gonzalez FF. Growth factors for the treatment of ischemic brain injury

(growth factor treatment). Brain sciences. 2015; 5 (2): 165-77.

11 Weissmiller AM, Wu C. Current advances in using neurotrophic factors to treat neurodegenerative disorders. Translational neurodegeneration. 2012; 1(1):1.

12 Maisonpierre PC, Belluscio L, Friedman B, Alderson RF, Wiegand SJ, Furth ME, et al. NT-3, BDNF, and NGF in the developing rat nervous system: parallel as well as reciprocal patterns of expression. Neuron. 1990; 5(4): 501-9.

13 Svíženská I, Dubový P, Šulcová A. Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures—a short review. Pharmacology Biochemistry and Behavior. 2008; 90(4):501-11.

14 Gong J-P, Onaivi ES, Ishiguro H, Liu Q-R, Tagliaferro PA, Brusco A, et al. Cannabinoid CB2 receptors: immunohisto chemical localization in rat brain. Brain research. 2006; 1071(1):10-23.

15 Bindukumar B, Mahajan S, Reynolds J, Hu Z, Sykes D, Aalinkeel R, et al. Genomic and proteomic analysis of the effects of cannabinoids on normal human astrocytes. Brain research. 2008; 1191: 1-11.

16 Hilliard A, Stott C, Wright S, Guy G, Pryce G, Al-Izki S, et al. Evaluation of the effects of Sativex (THC BDS: CBD BDS) on inhibition of spasticity in a chronic relapsing experimental allergic autoimmune encephalomyelitis: a model of multiple sclerosis. ISRN neurology. 2012; 2012.

17 Drysdale AJ, Platt B. [General Articles] Cannabinoids: Mechanisms and Therapeutic Applications in the CNS. Current medicinal chemistry. 2003; 10(24): 2719-32.

18 Huestis M. Pharmacokinetics and metabolism of the plant cannabinoids, Δ 9-tetrahydrocannibinol, cannabidiol and cannabinol. Cannabinoids: Springer; 2005. p. 657-90.

19 Romagnani S. Type 1 T helper and type 2 T helper cells: functions, regulation and role in protection and disease. International Journal of Clinical and Laboratory Research. 1992; 21(2-4): 152-8.

20 Prete GD. Human Th1 and Th2 lymphocytes: their role in the pathophysiology of atopy. Allergy. 1992;47(5): 450-5.

21 Klein TW. Cannabinoid-based drugs as anti-inflammatory therapeutics. Nature Reviews Immunology. 2005; 5(5): 400-11.

22 D'Souza DC, Pittman B, Perry E, Simen A. Preliminary evidence of cannabinoid effects on brain-derived neurotrophic factor (BDNF) levels in humans. Psychopharmacology. 2009; 202(4): 569-78.

23 Jean-Gilles L, Gran B, Constantinescu CS. Interaction between cytokines, cannabinoids and the nervous system. Immunobiology. 2010; 215(8): 606-10.

24 Skaper SD, Di Marzo V. Endocannabinoids in nervous system health and disease: the big picture in a nutshell. Phil Trans R Soc B. 2012; 367(1607): 3193-200.

25 Weiner HL, Hafler DA. Immunotherapy of multiple sclerosis. Annals of neurology. 1988; 23(3): 211-22.