

Investigating the Effect of Neuroaid on the Expression of BDNF Gene in the Cerebellum of Morphine-Addicted Rats

Saina Rasooli¹, Faryad Sharifian²

¹Department of pharmacy, faculty of pharmacy, Yeditepe University, Istanbul, Turkey

²MSc molecular genetics, Islamic Azad University, North Tehran Branch, Tehran, IR Iran



Abstract – Background: Nowadays, addiction is one of the biggest problems concerning not only physicians but also all people who care about their personal and social health and well-being. Addiction distracts the neural circuits involved in reward system, motivation and memory in the brain, resulting in biologic, physiologic, social and mental consequences. Most of the studies in transcription factors engaged in addiction involved the accumbens nucleus of the brain. In the present study, we investigated the effect of Neuroaid on the expression of morphine-dependent BDNF gene in the rat cerebellum.

Methods: The expression levels of BDNF gene in four main groups checked by Real Time-PCR method after RNA extraction and cDNA synthesis. The comparative threshold cycle ($2^{-\Delta\Delta C_T}$) method and T-test was used to compared expression in treatment and control groups.

Results: Results of BDNF gene analysis showed that Neuroaid increased BDNF gene expression compared to the control group ($P=0.031$). Also, results of morphine-addicted rat show that Neuroaid compensates for reduced BDNF gene expression in morphine-addicted rat ($P=0.037$).

Keywords – Opium, BDNF, expression, Real Time-PCR method, Rat, Neuroid, morphine.

I. INTRODUCTION

Opioids are highly addictive, and opioid abuse has become a national crisis in the United States. Statistics highlight the severity of the epidemic, with the National Institute on Drug Abuse reporting that more than 2 million Americans abuse opioids and that more than 90 Americans die by opioid overdose every day, on average (Hser et al. 2015). Opioid addiction is a chronic mental illness that causes the addicted individuals to experience many relapses and remissions throughout their life, and they suffer from many uncomfortable symptoms, including tolerance development and withdrawal (Juurlink and Dhalla 2012).

Compared to smoking and alcohol consumption, opioid addiction is less common; however, it has imposed a heavy burden on both healthcare systems and the criminal justice system (Leung et al. 2017). Over the last two decades, the opioid epidemic or opioid crisis in the United States has raised public awareness, and effective interventions are urgently needed (Ward et al. 2011). Medications for opioid addiction such as methadone and buprenorphine are used to treat addicted individuals by reducing the intensity of withdrawal and craving symptoms, and naloxone is used to treat opioid overdose or opioid intoxication (Dugosh et al. 2016). Though effective medications for opioid addiction

are available, relapse and remission are still common among addicted individuals. The risk of relapse is heightened due to the craving feeling with terrible withdrawal symptoms, as well as neurobiological changes in brain caused by the repeated abuse of opioids. According to studies, *in vitro* models, the continued use of morphine and its related compounds can cause to producing abnormal proteins, affect the transcription of various genes in different tissues and parts of the body, including the cerebellum, leading to their increase and decrease. Disruption of expression of such genes affecting normal cerebellar activity can have deleterious effects (Krokmyrdal and Andenæs 2015).

The human BDNF gene consists of 11 exons, and its different splicing enables formation of transcripts specific to various tissues and responsive to different stimuli. The conservative structure of BDNF, with 85.9–100 % identity among genes of various vertebrates and humans, determines its physiological function, to a large extent independently of the stage of phylogenetic development (Fritsch et al. 2010). BDNF is a member of the neurotrophins family, which also includes nerve growth factor (NGF), neurotrophin 3 (NT3), and neurotrophin 4 (NT4). A constantly growing body of evidence indicates involvement of BDNF in a wide range of neurophysiological processes (Lubin et al. 2008). This can be explained based on its characteristic pattern of synthesis, which involves several biologically active isoforms that interact with different receptors, thereby controlling numerous signaling pathways (Castrén and Rantamäki 2010). BDNF is present in nearly all brain regions. Its function differs depending on both the stage of brain development as well as the neuronal, glial, or vascular constituents of the brain tissue. The most important functions of BDNF include developmental processes, regulation of neuronal, glial, and synaptogenesis, neuroprotection, and control of short and long-lasting synaptic interactions that influence mechanisms of memory and cognition (Björkholm and Monteggia 2016).

Neuroaid is a Traditional Chinese Medicine which has been shown to stimulate growth of brain cells and connections in animals. Neuroaid may improve blood flow in the brain and functional recovery after stroke in patients (Heurteaux et al. 2013).

We investigated the effect of Neuroaid on the expression of morphine-dependent BDNF gene in the rat cerebellum.

II. MATERIAL AND METHODS

2.1 Animal samples

In this study, 20 Wistar rats with a mean weight of 200–220 g were used. These animals were obtained from the Faculty of Pharmacology of the University of Tehran and kept in transparent plastic cages for animal storage. The animals were kept in a 12-hour light-dark physical environment from 8 am to 6 pm (temperatures varying from 21 to 25° C) without any noise or noise pollution.

2.2 Injection and grouping of samples

The rats were divided into four main groups: control, morphine, Neuroid, and morphine with Neuroid. Intravenous injection of Neuroaid as the main drugs into mice at a concentration of 2.5 mg per kg and all crocin injections were administered from day 1 of the experiment to day 15 (one day in between). Then, the cerebellum of each control rat and morphine-treated rat is extracted using a multi-atlas segmentation propagation framework.

2.3 RNA extraction and cDNA synthesis

Total RNA was isolated from each tissue by using RiboEx (GeneAll, Korea) according to the manufacturer's specifications. The concentration of total RNA in the final eluate was determined by spectrophotometry and the absorbance 260/280 ratio was controlled between 1.8 and 2.0. The synthesis of cDNA (240 ng of total RNA per 20 µL reaction mixture) was performed using the Prime Script RT reagent kit (Perfect Real Time) RR037A (Takara, Japan) according to the manufacturer's specifications. The obtained cDNAs were stored in -80°C until use.

2.4 Real-time quantitative PCR

Real-time PCR was performed using an StepOnePlus™ Real-Time PCR Systems (ABI Applied Bio-systems, Thermo Fisher Scientific, USA) in a 15-µl reaction containing 7.5-µl of RealQ Plus 2x Master Mix Green High ROX™ (Ampliqon, Denmark), 2-µl of cDNA, 4.5-µl of H₂O and 1-µl of mixed forward and reverse primers (10 Pmol/µl concentration). Real-time PCR amplifications were done as follows: PCR amplification was set to an initial 95°C for 10 min and then for BDNF gene, a total of 35 cycles, 95°C for 15 seconds and 58°C for 1 min (step and hold). All samples were analyzed in duplicate and B-actin was used as an internal control. The primers used for real-time PCR are listed in Table 1.

Table 1. Primer sequences of BDNF gene and B-actin.

Target genes		Sequences (5' → 3')	Product length
BDNF	Forward	TGACCCCACATCTCCTGCAAT	128bp
	Reverse	GTGGACGTAGGCAGCAATTATC	
B-actin	Forward	CATCAAGAAGGTGGTGAAGCA	146bp
	Reverse	GCGTCAAAGGTGGAGGAGTG	

2.5 Statistical Analysis

The comparative threshold cycle ($2^{-\Delta\Delta CT}$) method was used to estimate gene expression and T-test was used to compare expression of BDNF in control and addicted group. All tests, was performed using the GenX software and P value <0.05 was considered statistically significant.

III. RESULTS

3.1 Effect of morphine on BDNF gene expression

Results of the BDNF gene analysis showed that morphine decreased BDNF gene expression compared to the control group ($P=0.02$) (Fig. 1).

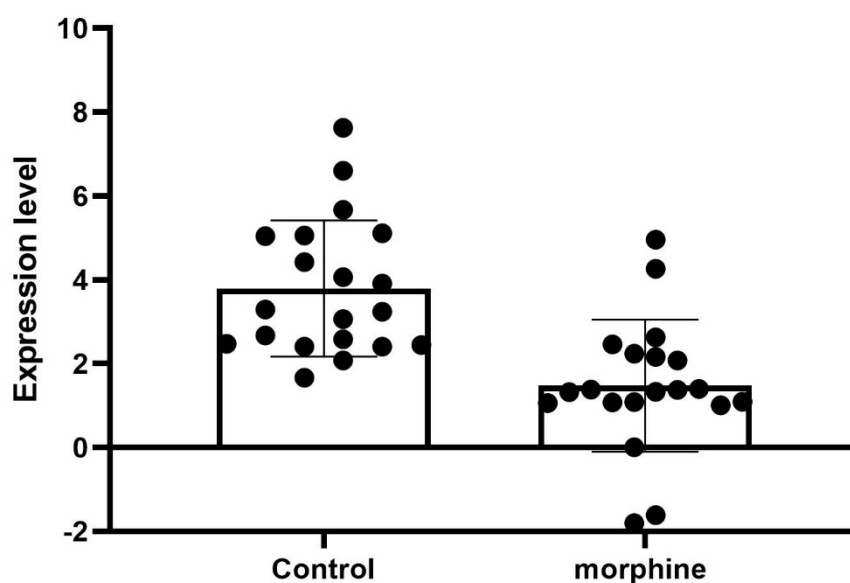


Figure 1. BDNF gene expression in control compared to morphine groups.

3.2 Effect of Neuroaid on BDNF gene expression

Results of the BDNF gene analysis showed that Neuroaid increased BDNF gene expression compared to the control group ($P=0.031$) (Fig. 2).

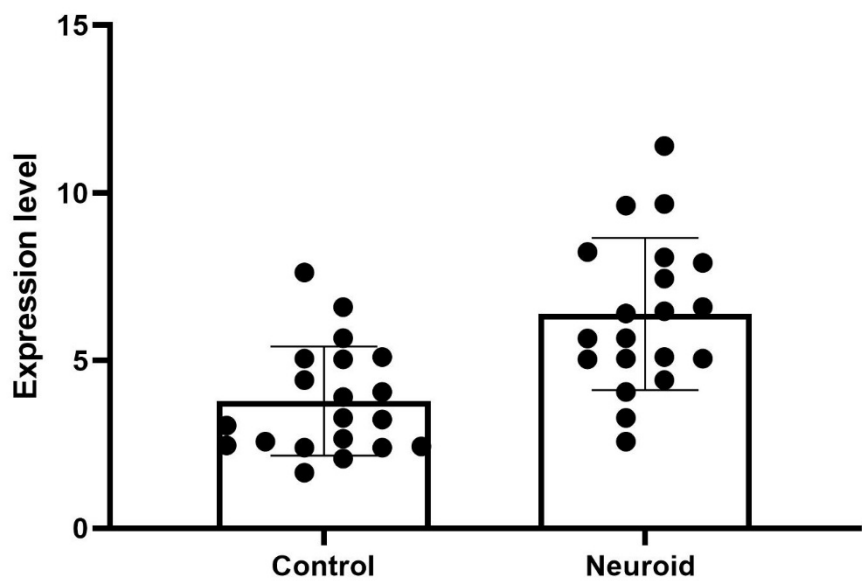


Figure 2. BDNF gene expression in control compared to Neuroaid groups.

3.3 Effect of Neuroaid on BDNF gene expression in morphine-addicted rat

Results of morphine-addicted rats show that Neuroaid compensates for reduced BDNF gene expression in morphine-addicted rats ($P=0.037$) (Fig. 3).

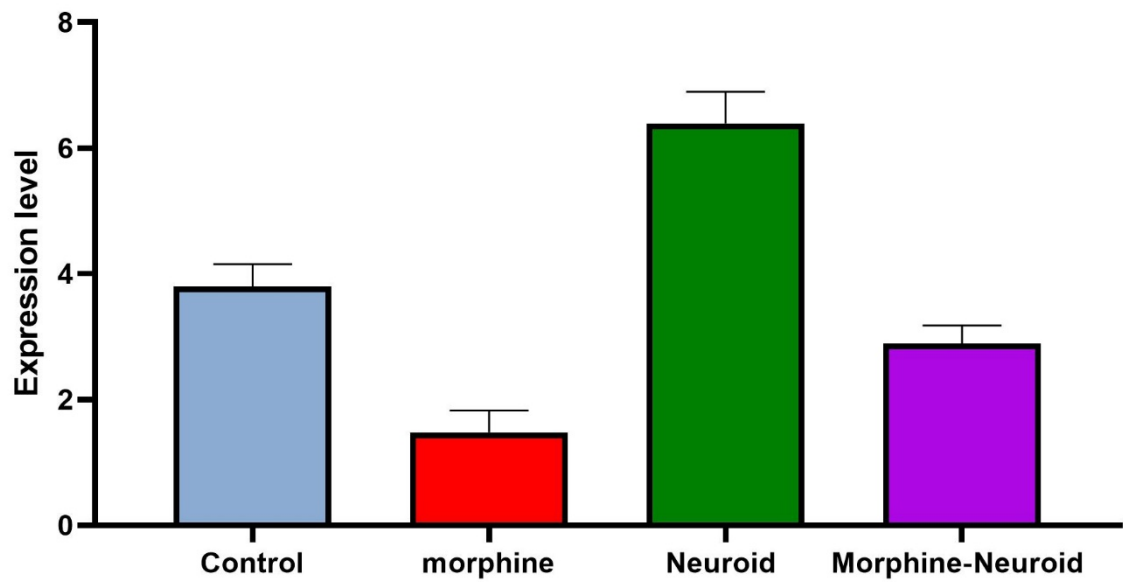


Figure 3. BDNF gene expression in all Groups.

IV. DISCUSSION

Nowadays, addiction is one of the biggest problems concerning not only physicians but also all people who care about their personal and social health and well-being. Addiction is a disease in which the patient constantly repeats a behavior which has bad effects. Addiction distracts the neural circuits involved in reward system, motivation and memory in the brain, resulting in biologic, physiologic, social and mental consequences. In fact, addiction can be divided into physical and mental types. Cerebral reward system includes dopamine-creating neurons in ventral tegmental areas of the mid-brain which send signals to various parts of the frontal areas of the brain involving the accumbens nucleus (one of the nuclei of the limbic system of the brain that plays a role in regulating pain), prefrontal cortex, amygdala (mass below the cortex often known to affect the brain's emotional reactions) and the cerebellum (Ghandehari et al. 2011).

If a person is constantly exposed to too much use or abuse of a drug, its long-term conditions and changes in gene expression regulation will cause distraction in cerebral system. Therefore, drug abuse will cause low and progressive changes in synopsis and in neurons signaling the nucleus.

Morphine, a highly addictive drug, is a strong type of opioid which comes from opium and is considered opium's most important effective combination. Its mechanism involves affecting the central nervous system that reduces pain (Zhang et al. 2017).

Most of the studies in transcription factors engaged in addiction involved the accumbens nucleus of the brain. Rezai et al, examined BDNF and CREB expression by real-time-PCR method. They also used ELISA analysis for assessing the serum BDNF level. The data indicated that morphine treatment could cause a significant decrease in BDNF and CREB gene expression (Rezai et al. 2018).

In the present study, the expression level of BDNF, CRF1 and TRKB was measured in healthy control and addicted rats. We showed the expression level of all three selected genes was reduced in rats which addicted to morphine. Then treatment with crocin increased the expression of these genes. In the present study, we evaluated the effect of morphine on the cerebellum by increasing doses of morphine and evaluated the expression of the BDNF gene in four groups. According to the results, the expression of the BDNF gene in the cerebellar segment decreased. The results of the use of Neuroaid in this study on morphine-addicted rats showed that this drug can

compensate for the decrease of expression of the BDNF gene in morphine-addicted rats.

V. CONCLUSIONS

In this paper, we investigated the effect of Neuroaid on the expression of morphine-dependent BDNF gene in the rat cerebellum. According to the results, the expression of the BDNF gene in the cerebellar segment decreased. Also, the Results of BDNF gene analysis showed that Neuroaid increased BDNF gene expression compared to the control group ($P=0.037$), and, this drug can compensate for the decrease of expression of the BDNF gene in morphine-addicted rats.

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