Investigating the Effect of Crocin on the Expression of TRKB Gene in the Cerebellum of Morphine-Addicted Rats

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Abstract

Background: Addiction to opium and other drugs is a problem that is increasingly widespread across the world. 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. Therefore, in this study, we investigated the effect of crocin on the expression of morphine-dependent TRKB gene in the rat cerebellum.

Methods: The expression levels of TRKB gene in four main groups checked by Real Time-PCR method after RNA extraction and cDNA synthesis. The comparative threshold cycle (2^-△△CT) method and T-test was used to compare expression in treatment and control groups.

Results: Results of TrkB gene analysis showed that crocin increased TrkB gene expression compared to the control group (P > 0.05). Also, results of morphine-addicted rat show that crocin compensates for reduced TrkB gene expression in morphine-addicted rat (P <0.01).

Keyword – Opium, TRKB, Expression, Real Time-PCR Method, Rat, Crocin, Morphine.

I. INTRODUCTION

Addiction to opium and other drugs is a problem that is increasingly widespread across the world. One of the major effects of morphine abuse and its related compounds in the development of drug dependence that can have severe and even irreversible effects on the consumer (Brindley 2019). In the year 2012, 259 million prescription opioid medications were prescribed, much higher than needed, and in the year 2015, 276000 teenagers were given opioid medications because of pain due to surgery, and fifty percent of them were addicted to these drugs (Fallahzadeh et al. 2017).

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 (Moossavi et al. 2018).

The human TrkB gene is located on chromosome 9 and at chromosomal position 9q21.33. The Trk family contains several receptors including TrkA, TrkB, and TrkC that play important roles in the cellular effects of neurotrophins (Winkel et al. 2018). In general, tyrosine kinase receptors of this family are involved in the development and maturation of the central and peripheral nervous system through regulation of neuronal survival, proliferation, migration, differentiation, formation and synaptic plasticity (Harward et al. 2016).

Crocin is a carotenoid chemical compound that is found in the flower’s crocus and gardenia. Crocin is the chemical primarily responsible for the color of saffron (Sun et al. 2014). Chemically, crocin is the diester formed from the disaccharide gentiobiose and the dicarboxylic acid crocetin.
Crocin is an antioxidant, and neural protective agent (Sapanidou et al. 2015). The antioxidant behavior of crocin is related to the sugar moiety in the crocin molecule which has a vital role in its chemical reactivity. It has also been shown to have an antiproliferative action against cancer cells in vitro (Ghaeni et al. 2014). Limited evidence suggests possible antidepressant properties of crocin in mice and humans. Crocin increases the efficiency of antibiotics and reduces blood cholesterol levels and atherosclerosis severity (Jam et al. 2017). We investigated the effect of crocin on the expression of morphine-dependent TRKB gene in the rat cerebellum.

II. MATERIAL AND METHODS

2.1 Animal samples

In this study, 20 Wistar rats with a mean weight of 210-230 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, crocin, and morphine with crocin. Intravenous injection of crocin 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), 1-μl of cDNA, 5.5-μl of H2O and 1-μl of mixed forward and reverse primers (6 Pmol/μl concentration). Real-time PCR amplifications were done as follows: PCR amplification was set to an initial 95°C for 15 min and then for TRKB gene, a total of 40 cycles, 95°C for 15 seconds and 60°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 TRKB genes and B-actin.

<table>
<thead>
<tr>
<th>Target genes</th>
<th>Sequences (5’→3’)</th>
<th>Product length</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRKB</td>
<td>Forward GCATGACAGCGAAAGGTCTAC</td>
<td>148bp</td>
</tr>
<tr>
<td></td>
<td>Reverse GGGTCTTTATCCGCAACAGG</td>
<td></td>
</tr>
<tr>
<td>B-actin</td>
<td>Forward CATCAAGAAGGTGGTGAAGCA</td>
<td>146bp</td>
</tr>
<tr>
<td></td>
<td>Reverse GCGTCAAAGGTGGAGGAGTG</td>
<td></td>
</tr>
</tbody>
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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 TRKB in control and addicted group. All tests, was performed using the GenX software and \( P \) value <0.05 was considered statistically significant.

III. 3. RESULTS

3.1 Effect of crocin on TrkB gene expression in morphine-addicted rat

Results of the TrkB gene analysis showed that crocin increased TrkB gene expression compared to the control group (\( P > 0.05 \)). Also, results of morphine-addicted rats show that crocin compensates for reduced TrkB gene expression in morphine-addicted rats (\( P < 0.01 \)) (Fig. 1).
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IV. DISCUSSION

The main objective of this study was to investigate the effects of morphine on the brain's rat as well as the effects of crocin in preventing these effects (Fischer et al. 2014). Many studies indicate morphine consumption and alteration in gene expression. Morphine addiction impairs the brain's neural circuitry on reward, motivation, and memory, and disruption of the brain system causes biological, physiological, social, and psychological effects (Kelly et al. 2015). Many studies indicate the negative effects of morphine on the expression of genes. Most studies show different changes in TRKB gene expression in the presence of morphine (Kubica et al. 2016). Homira Hetami and Mojgan Rasti, demonstrated a decrease in TRKB gene expression in the presence of morphine (Rasti et al. 2015). In the present study, we evaluated the effect of morphine on the cerebellum by increasing doses of morphine and evaluated the expression of the TrkB gene in four groups. According to the results, the expression of the TrkB gene in the cerebellar segment decreased. Crocin is a pharmacologically active ingredient of Crocus sativus (saffron) used in traditional Chinese medicine. The results of the use of crocin in this study on morphine-addicted rats showed that this drug can compensate for the decrease of expression of the TrkB gene in morphine-addicted rats.

V. CONCLUSIONS

In this paper, we investigated the effect of crocin on the expression of morphine-dependent TRKB, gene in the rat cerebellum. According to the results, the expression of the TrkB gene in the cerebellar segment decreased. Also, the Results of TrkB gene analysis showed that crocin increased TrkB gene expression compared to the control group (P>0.05).

REFERENCES


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