

Lithium Attenuates Cannabinoid-Induced Dependence in the Animal Model: Involvement of Phosphorylated ERK1/2 and GSK-3 β Signaling Pathways

Hamid Reza Rahimi¹, Ahmad Reza Dehpour², Shahram Ejtemaei Mehr², Mohammad Sharifzadeh¹,
Mohammad Hossein Ghahremani¹, Ali Razmi³, and Seyed Nasser Ostad¹

¹Department of Toxicology and Pharmacology, Faculty of Pharmacy, Pharmaceutical Nanotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran

²Department of Pharmacology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

³Department of Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran

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Abstract- Cannabis is one of the most banned drugs in the world. Cannabinoid-induced dependence or withdrawal signs are indicated by the result of complex molecular mechanisms including upstream protein kinases (PKs), such as an extracellular signal regulated kinase1/2 (ERK1/2) and downstream glycogen synthase kinase-3 β (GSK-3 β), which lead to neuronal plasticity. In this study, we examined the protective effect of lithium (Li) as a potent ERK1/2 and GSK-3 β modulator to prevent the development of dependence on cannabinoids. For this purpose, rats were treated twice daily with increasing doses of WIN 55,212-2 (WIN, 2-8 mg/kg, intraperitoneally (i.p.), for five consecutive days. AM251 (AM, 2 mg/kg), a cannabinoid antagonist, was injected i.p. to induce manifestations of abstinence in rat dependency on WIN, and the subsequent withdrawal signs were recorded. To evaluate the preventive effect of Li, the rats were pre-treated with Li (10 mg/kg, i.p.) twice daily, 30 minutes before every injection of WIN. SL327, as an ERK1/2 inhibitor, was also injected (SL, 50 mg/kg, i.p.) 30 minutes before the last doses of WIN in separate groups. The p-ERK1/2, total ERK1/2, p-GSK-3 β and total GSK-3 β expressions were determined with Western blot method after 60 minutes, prior to the Li, WIN or AM injections. Li and SL pre-treatment attenuated the global withdrawal signs in regarding their modulation effect on the up-regulation of p-ERK1/2 cascade enhanced by AM injection. Furthermore, the p-GSK-3 β expression was up-regulated with SL and Li pre-treatment against AM injection, without alteration on the total contents of ERK1/2 and GSK-3 β level. Therefore, p-ERK1/2 and p-GSK-3 β pathways are involved in the cannabinoid-induced dependence. However, no crosstalk was indicated between these two pathways. In conclusion, Li neuroprotection with regard to cannabinoid abstinence may occur through the regulation of the p-ERK1/2 cascade in consequence of p-GSK-3 β signaling pathways in rats.

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Introduction

Cannabinoids are the active components of *Cannabis sativa* (marijuana). Chronic consumption can cause milder withdrawal signs in comparison to opioids (1-3). Recent studies have reported the relation of opioids to cannabinoids' systems and, therefore, both systems have similar responses including anti-nociception, hypolocomotion and hypothermia, as well as the development of cannabinoid-induced dependence or withdrawal signs (4). Cannabinoid withdrawal can be

enhanced in animals by administering AM251 or SR141716A (rimonabant) as cannabinoid receptor antagonists to further induce withdrawal signs (5,6). Cannabinoid such as WIN 55,212-2 and tetrahydrocannabinol (THC) were also widely used for induction of cannabinoid dependence in animal models (5,7). However, molecular mechanisms or signaling pathways regarding the development of cannabinoid-induced dependence or withdrawal signs are less clear.

The development of dependence or tolerance to cannabinoids may be mediated by CB1 receptors, a G-

Corresponding Author: S.N. Ostad

Department of Toxicology and Pharmacology, Faculty of Pharmacy, Pharmaceutical Nanotechnology Research Center, Tehran University of Medical Sciences, Tehran, Iran

Tel: +98 21 66959105, Fax: +98 21 66959105, E-mail address: ostadnas@tums.ac.ir

protein-coupled receptor (GPCR) and with the interaction to Gi/o proteins. This causes inhibition of adenylylase (AC) activity, changes in Ca^{2+} and K^{+} currents, activation of protein kinases (PKs), such as extracellular signal-regulated kinases (ERK), as well as focal adhesion kinase (FAK) and phosphatidylinositol-3-kinase (PI3K) (8).

Other studies have indicated that CB1 receptors mediated ERK activation through dopaminergic and glutamatergic mechanisms (9-12). Furthermore, SL327, a specific inhibitor of mitogen-activated protein kinase (MAPK) and the upstream kinase of ERK can prevent the development of tolerance to cannabinoid hypolocomotion (13). GSK-3 β is another downstream molecular mechanism related to cannabinoids, which is essential in the induction of drug-induced withdrawal signs and has an important regulator of survival and apoptosis subject in neuronal cells (14,15). GSK-3 β can interact with Bax, a protein of the intrinsic apoptotic cascade, which is involved in mitochondrial pore formation (16). GSK-3 β is also related to JNK activation as apoptotic signaling (17). Therefore, upstream PKs such as the PI3K/Akt and ERK1/2 signaling pathways are capable of rescuing the neurons from induced apoptosis. However, the PI3K/Akt pathway is defined as the main pathway to phosphorylation of GSK-3 β to its in-activation state and to survival route in neurons (18, 19). Therefore, p-ERK1/2 and p-GSK-3 β activation plays an important role in regulating cannabinoid abstinence. Li, as a potent inhibitor of the GSK-3 β pathway and modulator of the ERK1/2 activation (20), inhibits cannabinoid tolerance (21), morphine-induced withdrawal signs and the development of physical dependence (22-24).

Aim of this study was to investigate the effect of Li pre-treatment on cannabinoid-induced dependence or withdrawal signs leading to neuronal plasticity in the animal model. The ERK1/2 and GSK-3 β phosphorylation, as intrinsic molecular pathways related to cannabinoid-induced dependence or withdrawal signs, were studied. Authors also evaluated the crosstalk of p-ERK1/2 and p-GSK-3 β signaling by administering SL327 in cannabinoid-dependent rats.

After this study, the authors can state that Li pre-treatment can attenuate cannabinoid-induced dependence or withdrawal signs through the regulation of p-ERK1/2 and p-GSK-3 β signaling pathways in animals; therefore, it will provide a potent pharmacotherapy target to overcome the cannabinoid-induced disorders.

Materials and Methods

Animals

Adult male Wistar rats, weighing 150-200 g, were obtained from Faculty of Pharmacy, Tehran University of Medical Sciences (TUMS). Animals were housed in groups of six per cage for one week under standard 12 h light/dark cycle at 22-24 °C, and then were treated with approved Ethical Committee protocols for the use and care of laboratory animals of TUMS (25).

Induction of cannabinoid dependence

Rats were administered intraperitoneally (i.p.) twice daily for five consecutive days with increasing doses of WIN (Day 1: 2 and 2 mg/kg; Day 2: 2 and 4 mg/kg; Day 3: 4 and 4 mg/kg; Day 4: 8 and 8 mg/kg; Day 5: 8 mg/kg) following the standard protocol (13, 26).

Expression of withdrawal signs

Two hours after the last dose of WIN (2-8 mg/kg, i.p.); rats were placed in a circular clear plastic observation area for a 15-minute period of habituation. Then the animals were immediately observed for 15 minutes, followed by injections of AM251 (AM, 2 mg/kg, i.p.). The rats were then observed for a 30 minutes. The global withdrawal signs, including body tremor, piloerection, mastication, genital licks, sniffing, paw tremors and wet-dog shakes were checked for in each rat during 30 minutes at 5-minute intervals, and then the level of severity was obtained as the composite score for each animal as indicated with the global withdrawal score ranging from 0% to 100% (4,5,26).

Treatment procedures

To evaluate the relation of p-GSK-3 β and p-ERK1/2 expression in the cannabinoid-induced dependence or withdrawal signs, groups of rats were pre-treated with Li (10 mg/kg, i.p.), 30 minutes before every dose of WIN (2-8 mg/kg, i.p.), and SL (50 mg/kg, i.p.) was administered 30 minutes before the last dose of WIN following the studies (13,26).

Western blotting

Rats were anesthetized with a mixture of ketamine (100 mg/kg, i.p.) and xylazine (25 mg/kg, i.p.), and sacrificed one hour after the end of the experimental period in separate groups. The cerebellum brain section of the rats were immediately removed and put on ice and then stored at -80°C until used. Western blotting was performed as described (27). Briefly, cerebellum tissues were homogenized with a manual homogenizer and then

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were incubated with 200 μ l of lyses buffer, including 10 mmol/l Tris pH= 8, 0.1% SDS, 1% NP40, 0.5 mmol/l DDT, 0.5% Na-deoxycholate, 0.5 mmol/l PMSF and 0.5 μ g/ml leupeptinad, one complete protease inhibitor cocktail tablet per 10 ml of buffer (Roche), at 4°C for 30 minute for the prevention of phospho-protein breaks by protease or phosphatase enzymes (28), and then the products were centrifuged at 14000 rpm in 4°C for 5 minutes and the supernatant was removed. At this point, 20 μ l of loading buffer, including 50 mmol/l Tris pH= 6.8, 2% SDS, 4% 2-mercaptoethanol, 10% glycerol and 0.1% bromophenol, was added to the product and boiled for 10 minutes. The samples were once again centrifuged in 14000 rpm for five minutes; equal amounts of supernatants (20 μ l) were loaded to 10% SDS-PAGE, and then transferred to nitrocellulose membranes prepared from Amersham (GE Healthcare, UK).

Membranes were then immunoblot with anti-ERK1/2, anti-phospho-ERK1/2, anti-GSK-3 β and anti-phospho-GSK-3 β at 1: 1000 dilution from Cell signaling Technology Company (USA), or anti β -actin at 1: 2000 dilution as the internal control (CTRL), prepared from Santa Cruz Biotech (USA) at 4°C, overnight. For the detection of an antibody reaction, blots were incubated with horse-radish peroxidase (HRP)-conjugated secondary antibody at 1: 10000 dilutions for 60 minutes at room temperature. Detection was carried out using a chemiluminescence Western Blotting Kit (Roche; Mannheim, Germany) on X-ray film (Kodak; Rochester, NY). Immunoreactive bands were analyzed using ImageJ software (NIH; USA), normalized to β -actin, and then expressed as a percent of CTRL to mean

\pm SD of at least three independent experiments.

Statistical analysis

All results were presented as mean \pm SD. The statistical analyses were performed using one-way Analysis of Variance (ANOVA) following the post-hoc test of Tukey. Differences with p-value less than 0.05 were considered statistically significant.

Results

Behavioral studies

Results of this study indicated that acute injection of AM (2 mg/kg, i.p) enhanced significant increases ($P<0.001$) of global withdrawal signs in dependent rats treated with increasing doses of WIN (2-8 mg/kg, i.p) as compared to CTRL (Figure 1). However, pre-treatment with Li can significantly reduce ($P<0.01$) these withdrawal signs as compared to the AM+WIN group. Furthermore, acute pre-treatment of rats with SL (50 mg/kg, i.p.) significantly ameliorated the abstinence signs induced by AM injection, as compared to the AM+WIN group. It was indicated that the potential effect of Li and SL was to prevent the cannabinoid-induced withdrawal in animals, and, therefore, it was suggested that ERK1/2 phosphorylation was required for excitation induced by cannabinoid receptor antagonists in animals. Therefore, its in-activation may be one of the protective intrinsic mechanisms of Li against neuronal plasticity induced by cannabinoid abstinence.

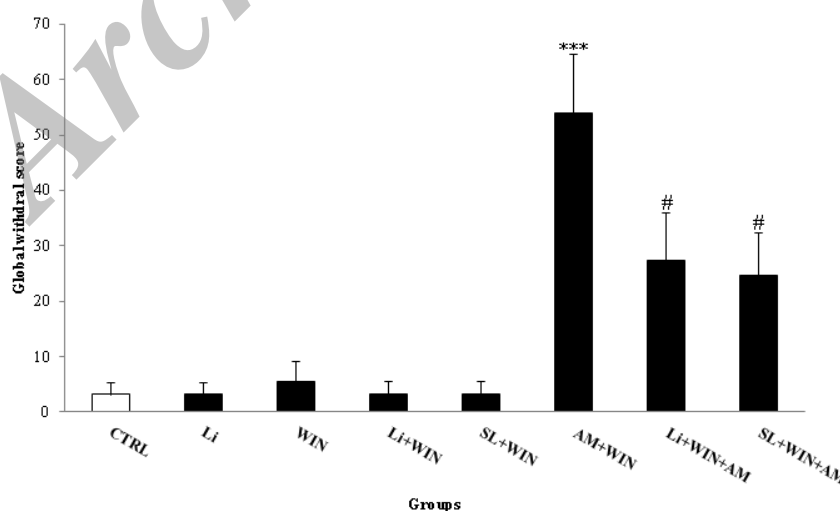


Figure 1. Somatic expression of cannabinoid withdrawal enhanced by acute administration of AM251 (AM) in rats dependent upon WIN 55, 212, 2 (WIN). Rats were treated twice daily with increasing doses of cannabimimetic amino-alkyl-indole, WIN 55, 212, 2 (WIN, 2-8 mg/kg, i.p., n = 6) for five consecutive days, and more than two hours after the last dose of WIN, the cannabinoid antagonist AM was acutely injected (2 mg/kg, i.p., n = 6). All data is presented as mean \pm SD. * $P<0.05$; ** $P<0.001$; *** $P<0.0001$ compared to control (CTRL). # $P<0.05$ compared to AM+WIN group.

Molecular evaluations

To determine the possible molecular mechanisms related to cannabinoid withdrawal signs, the authors removed the cerebellum of the experimental rats after the end of experiment period, and the expressions of p-ERK1/2, total ERK1/2, p-GSK-3 β and total GSK-3 β proteins were analyzed by the Western blotting method (Figure 2A). Treatment of rats with WIN significantly down-regulated the p-ERK1/2 and up-regulated the p-GSK-3 β expressions in the cerebellum ($P=0.041$ and $P<0.01$), respectively, as compared to CTRL (Figure 2 B & C). Furthermore, no significant change was indicated with pre-treatment of Li on the p-ERK1/2 and p-GSK-

3 β expressions in the cerebellum as compared to CTRL (Figure 2 B & C). Furthermore, Li pre-treated to WIN indicates no significant change on the expression of p-ERK1/2; however, Li pre-treated to WIN significantly ($P<0.001$) up-regulated the expression of p-GSK-3 β , as compared to CTRL (Figure 2C). AM injection significantly ($P<0.001$) up-regulated the p-ERK1/2 and down-regulated the p-GSK-3 β expression in cannabinoid dependent rats, as compared to CTRL (Figures 2 B and C). This data indicated that p-ERK1/2 and p-GSK-3 β signaling pathways were important in the cannabinoid-induced dependence or withdrawal signs in rats.

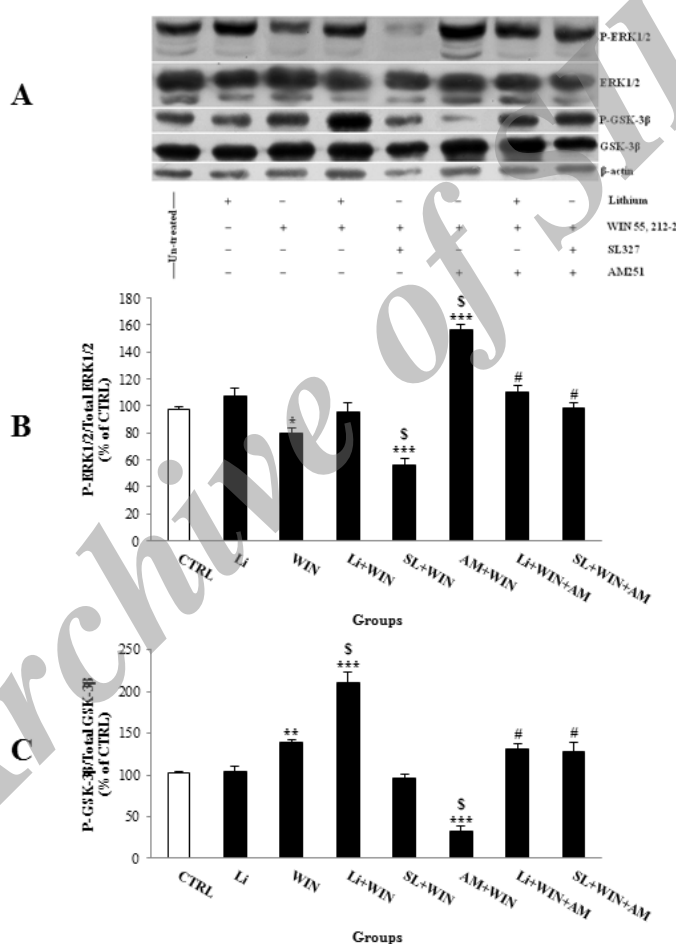


Figure 2. The effect of the cannabinoid receptor antagonist AM251 on WIN 55, 212-2-included changes in phosphorylated-ERK1/2 (p-ERK1/2), phosphorylated-GSK-3 β (p-GSK-3 β) and total amount of ERK1/2 and GSK-3 β in the rat cerebellum. The animals were treated twice daily with increasing doses of the cannabinoid antagonist WIN 55, 212-2 (WIN, 2-8 mg/kg, i.p., n = 6) for five consecutive days, and more than two hours after the last dose of WIN, the cannabinoid antagonist AM was injected (2 mg/kg, i.p., n = 6). Rats were pre-treated with lithium (Li, 10 mg/kg, i.p., n = 6) twice daily, 30 minutes before each dose of WIN. Rats were also pre-treated with an acute dose of SL327 (SL, 50 mg/kg, i.p., n = 6), as a selective ERK1/2 inhibitor, 30 minutes before the last dose of WIN. Control (CTRL) rats received normal saline (NS, 1 ml/kg, i.p., n = 6). (A) Representative Western blots of p-ERK1/2, total ERK1/2, p-GSK-3 β and total GSK-3 β protein expressions; (B & C) the quantitative analysis of p-ERK1/2, total ERK1/2, p-GSK-3 β and total GSK-3 β . All data is presented as mean \pm SD. * $P<0.05$; ** $P<0.001$; *** $P<0.0001$ compared to control (CTRL).^S $P<0.05$ compared to WIN group. # $P<0.05$ compared to AM+WIN group.

Lithium and SL327 regulates p-ERK1/2 and p-GSK-3 β pathways

Pre-treatment of rats with Li (20 mg/kg/day, i.p.) and acute injection of SL (50 mg/kg, i.p.) prevented the significant ($P < 0.001$) up-regulation of the p-ERK1/2, and also significantly ($P < 0.001$) blocked the down-regulation of the p-GSK-3 β expression by AM injection, as compared to the AM+WIN group (Figures 2 B & C). These results showed that no crosstalk was between the p-ERK1/2 and p-GSK-3 β pathway, and therefore more other PKs than ERK1/2 may be mediated the phosphorylation of GSK-3 β pathway. Furthermore, no significant change was in the total amount of the ERK1/2 and GSK-3 β expression in experimental groups, as compared to CTRL (Figure 2A), wherein no breakdown occurred in the ERK1/2 and GSK-3 β proteins. Therefore, Li pre-treatment can attenuate cannabinoid abstinence enhanced by AM through regulation of p-ERK1/2 cascade in consequent of the p-GSK-3 β signaling pathway.

Discussion

In this study, Li, as a neuroprotective drug at a lowered dose (20 mg/kg/day, i.p.), and pre-treatment experiments can attenuate the development of cannabinoid dependence or withdrawal signs enhanced by cannabinoid receptor antagonists in the animal model. Two molecular signaling pathways, including p-ERK1/2 and p-GSK-3 β pathways were predominantly related to CB1 receptor, which were highly expressed in the cerebellum brain section (29). Then Li neuroprotective molecular mechanisms, including p-ERK1/2 and p-GSK-3 β modulation, were evaluated in rats. Although cannabinoids were used for a long time for medicinal and recreational goals, new pharmacological effects, including their anti-cancer properties, was discovered recently (30,31). Cannabis disorders affect more adults in the world and number of its users have increased (32,33), but a few cannabis users do seek pharmacotherapy for their disorders.

The protective effect of Li against peripheral neuropathy induced by Vincristine (VCR) with the dose of 20 mg/kg/day i.p. was reported in a previous study in animals (34). These Li neuroprotective effects have occurred at the sub-therapeutic concentrations without association to its received therapeutic plasma concentration (34,35). Studies have reported the amelioration effect of acute and chronic administration of Li on the opioid-induced dependence or withdrawal

signs (36,37). Opioids and other drugs, such as cocaine, have functional interaction with the endocannabinoid system/CB1 receptors to induce similar withdrawal signs and are insensitive to further concern in humans (4,38,39). Furthermore, the inhibitory effect of Li on cannabinoid tolerance was showed *in vitro* (21). However, the attenuating effect of Li on the cannabinoid-induced dependence or withdrawal signs are less clear in the animal model, and little is known about the possible molecular mechanisms or signaling pathways about the protective role of Li has been reported.

Therefore, we indicated that pre-treatment with Li (20 mg/kg/day, i.p.) has a preventive effect on the cannabinoid withdrawal signs enhanced by acute injection of AM (2 mg/kg, i.p.) in cannabinoid dependent rats treated with increasing doses of WIN (2-8 mg/kg, i.p.). Studies have indicated the relation of dopaminergic (dopamine, D2 receptor), glutamatergic (glutamate, GLUT) and GABAergic (Gamma-aminobutyric-acid, GABA) systems on the drug-induced withdrawal signs (36,40,41).

GLUT interacted with the NMDA/NO cascade, which has played an important role in the induction of cannabinoid-induced withdrawal, leading to neuronal plasticity and Ca²⁺/calmodulin clusters (42). Therefore, NMDA/NO inhibition was the other neuroprotective mechanism of Li introduced against excitation in the brain, and it has regulated the signaling pathway with ERK (43). It has also shown that D2 receptors regulated the cyclic adenosine mono phosphate (cAMP)-PKA (protein kinase A) and Ca²⁺ pathway by mediating GPCRs, wherein GPCR can function through a PKB (Akt)-GSK-3 signaling pathway (18) since, the cannabinoid withdrawal was underlying to calmodulin-stimulated AC and PKA activities in the cerebellum (44). Therefore, the cAMP/CREB/PKA mediating pathway is a major documented neuroprotective mechanism of Li against toxicants (45,46). ERK1/2 and GSK-3 β activation have been shown to have a relationship with these systems (18) and. Therefore, they can regulate the expression of behavior alteration in cannabinoid abstinence induced in rats. WIN down-regulated the p-ERK1/2 (activation) cascade and up-regulated the p-GSK-3 β expression (in-activation), in which the antagonist with AM injection was found. It has reported that activation of β -adrenergic receptors by isoproterenol mimics forskolin (FSK) to elicit a cAMP-dependent PKA inhibition of CB1 receptor functions, and it conceded the inhibitory effect of

cannabimimetic aminoalkylindole, WIN 55, 212-2 on the activation/phosphorylation of the ERK1/2 pathway against AM injection (40). Furthermore, the presynaptic inhibitory action of CB1 receptors at corticostriatal synapses could be negatively regulated by a cAMP/PKA-mediated receptor phosphorylation related to glutamatergic transmissions to induce withdrawal sign expressions (40). Therefore, ERK1/2 in-activation and GSK-3 β induced phosphorylation (in-activation) were regulated with the activation of β -adrenergic and in-activation of glutamatergic/dopaminergic receptors, respectively (40). Therefore, both the p-ERK1/2 and p-GSK-3 β were important in the cannabinoid-induced dependence or withdrawal signs.

Li is a putative inhibitor of the GSK-3 β intrinsic pathway in which it can inhibit the GSK-3 β activity by inducing its phosphorylation (19). It has been reported that GSK-3 β phosphorylation is regulated mainly with the PI3K/Akt kinase pathway, and it was important to the actions of the dopaminergic system (18); however, the knowledge of the mediating effect of the other upstream PKs, such as ERK1/2 to induce cannabinoid-induced dependence or withdrawal signs is limited. Studies have demonstrated the important role of the upstream RAS/ERK1/2 cascade phosphorylation (activation) in the cannabinoid-induced tolerance; however, its crosstalk to the downstream pathway to induce cannabinoid abstinence has not yet cleared. However, it has indicated the crosstalk of NO and ERK1/2 signaling pathways to mediate opioid withdrawal signs in rats (47). The down-stream GSK-3 β pathway can regulate the special gene expressions in nuclear neuronal cells that are important to induce dependence or withdrawal signs. To say that there is crosstalk and sequence relations of p-ERK1/2 to p-GSK-3 β signaling pathway, it must down- or up-regulate p-ERK1/2 expressions consequently and respectively, with down- or up-regulation of the p-GSK-3 β expression. Current study showed that Li and SL pre-treatment reduced the up-regulation of p-ERK1/2 and up-regulated the p-GSK-3 β expression against acute administration of rats with AM injection. The authors indicated that when p-ERK1/2 was down-regulated with WIN or SL and Li pre-treatment (SL+WIN or Li+WIN) in experimental groups, however, the p-GSK-3 β expression was up-regulated in WIN or Li+WIN groups, and no significant change was indicated between the SL+WIN group as compared to CTRL. Furthermore, when the p-ERK1/2 level was up-regulated in the AM+WIN group, however, no significant increase was indicated in the expression of p-GSK-3 β in the AM+WIN group, compared to

CTRL. Therefore, no crosstalk occurred between these two pathways. This means that Li pre-treatment attenuates cannabinoid abstinence through the regulations of p-ERK1/2 cascade in consequence of p-GSK-3 β pathway. Indeed, the PI3K/Akt pathway has a major up-stream kinase to regulate the GSK-3 β phosphorylation. However, both the p-ERK1/2 and p-GSK-3 β pathways were inconsequentially playing a central role in the development of cannabinoid dependence and induction of its withdrawal signs. Present results will provide a therapeutic molecular target to reduce the neuronal plasticity and suggests that Li neuroprotective mechanisms prevent the development of cannabinoid-induced dependence or withdrawal signs. Therefore, regulating the effect of Li on CB1 receptors and p-ERK1/2 and p-GSK-3 pathways is a potential strategy for preventing clinical manifestations, including pain, drug dependence, epilepsy, anxiety, depression, Parkinson's and Huntington's diseases, amyotrophic lateral sclerosis, stroke, cancer, glaucoma, autoimmune uveitis, osteoporosis, sepsis, renal, hepatic, intestinal and cardiovascular disorders induced by cannabinoids (48).

In conclusion, present results suggested the potential of Li pre-treatment for reducing cannabis disorders. Furthermore, the regulation of ERK1/2 and GSK-3 β phosphorylation was an important mechanism to neuroprotective mechanism of Li on the development of cannabinoid-induced dependence or withdrawal signs in rats. However, no crosstalk between these two pathways is indicated. Therefore, a clinical trial study to include the Li as a maintenance therapy to prevent cannabinoid-induced disorders is recommended, and it may be the next step in this type of study in the future.

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References

1. Perkonig A, Lieb R, Höfler M, et al. Patterns of cannabis use, abuse and dependence over time: incidence, progression and stability in a sample of 1228 adolescents. *Addiction* 1999;94(11):1663-78.
2. Crowley TJ, Macdonald MJ, Whitmore EA, et al. Cannabis dependence, withdrawal, and reinforcing effects among adolescents with conduct symptoms and substance use disorders. *Drug Alcohol Depend* 1998;50(1):27-37.

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- Kouri EM, Pope HG Jr, Lukas SE. Changes in aggressive behavior during withdrawal from long-term marijuana use. *Psychopharmacology (Berl)* 1999;143(3):302-8.
- Valverde O, Maldonado R, Valjent E, et al. Cannabinoid withdrawal syndrome is reduced in pre-proenkephalin knock-out mice. *J Neurosci* 2000;20(24):9284-9.
- Castañé A, Maldonado R, Valverde O. Role of different brain structures in the behavioural expression of WIN 55,212-2 withdrawal in mice. *Br J Pharmacol* 2004;142(8):1309-17.
- Pan B, Zhong P, Sun D, et al. Extracellular signal-regulated kinase signaling in the ventral tegmental area mediates cocaine-induced synaptic plasticity and rewarding effects. *J Neurosci* 2011;31(31):11244-55.
- Hutcheson DM, Tzavara ET, Smadja C, Valjent E et al. Behavioural and biochemical evidence for signs of abstinence in mice chronically treated with delta-9-tetrahydrocannabinol. *Br J Pharmacol* 1998;125(7):1567-77.
- Howlett AC. The cannabinoid receptors. *Prostaglandins Other Lipid Mediat* 2002;68-69:619-31.
- Valjent E, Pagès C, Rogard M, et al. Delta 9-tetrahydrocannabinol-induced MAPK/ERK and Elk-1 activation in vivo depend on dopaminergic transmission. *Eur J Neurosci* 2001;14(2):342-52.
- Valjent E, Pagès C, Hervé D, et al. Addictive and non-addictive drugs induce distinct and specific patterns of ERK activation in mouse brain. *Eur J Neurosci* 2004;19(7):1826-36.
- Barbara JG, Auclair N, Roisin MP, et al. Direct and indirect interactions between cannabinoid CB1 receptor and group II metabotropic glutamate receptor signaling in layer V pyramidal neurons from the rat prefrontal cortex. *Eur J Neurosci* 2003;17(5):981-90.
- Derkinderen P, Valjent E, Toutant M, et al. Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. *J Neurosci* 2003;23(6):2371-82.
- Rubino T, Forlani G, Viganò D, et al. Ras/ERK signalling in cannabinoid tolerance: from behaviour to cellular aspects. *J Neurochem* 2005;93(4):984-91.
- Wei YM, Li SX, Shi HS, et al. Protracted cocaine withdrawal produces circadian rhythmic alterations of phosphorylated GSK-3 β in reward-related brain areas in rats. *Behav Brain Res* 2011;218(1):228-33.
- Kim S, Lee KY, Koh SH, et al. Role of the phosphatidylinositol 3-kinase and extracellular signal-regulated kinase pathways in the neuroprotective effects of cilnidipine against hypoxia in a primary culture of cortical neurons. *NeurochemInt* 2012;61(7):1172-82.
- Linseman DA, Butts BD, Precht TA, Phelps RA, et al. Glycogen synthase kinase-3 β phosphorylates Bax and promotes its mitochondrial localization during neuronal apoptosis. *J Neurosci* 2004;24(44):9993-10002.
- Hongisto V, Smeds N, Brecht S, et al. Lithium blocks the c-Jun stress response and protects neurons via its action on glycogen synthase kinase 3. *Mol Cell Biol* 2003;23(17):6027-36.
- Beaulieu JM, Gainetdinov RR, Caron MG. The Akt-GSK-3 signaling cascade in the actions of dopamine. *Trends Pharmacol Sci* 2007;28(4):166-72.
- Chuang DM, Wang Z, Chiu CT. GSK-3 as a Target for Lithium-Induced Neuroprotection Against Excitotoxicity in Neuronal Cultures and Animal Models of Ischemic Stroke. *Front Mol Neurosci* 2011;4(1):15.
- Pardo R, Andreolotti AG, Ramos B, et al. Opposed effects of lithium on the MEK-ERK pathway in neural cells: inhibition in astrocytes and stimulation in neurons by GSK3 independent mechanisms. *J Neurochem* 2003;87(2):417-26.
- Rezania F, Mehr SE, Kheirkhah M, et al. The effect of lithium chloride on WIN 55,212-2-induced tolerance in isolated guinea pig ileum. *Eur J Pharmacol* 2010;627(1-3):265-8.
- Dehpour AR, Farsam H, Azizabadi-Farahani M. Inhibition of the morphine withdrawal syndrome and the development of physical dependence by lithium in mice. *Neuropharmacology* 1995;34(1):115-21.
- Dehpour AR, Sadr SS, Nouroddini M, et al. Comparison of simultaneous administration of lithium with L-NAME or L-arginine on morphine withdrawal syndrome in mice. *Hum Psychopharmacol* 2000;15(2):87-93.
- Dehpour AR, Sadr SS, Azizi MR, et al. Lithium inhibits the development of physical dependence to clonidine in mice. *Pharmacol Toxicol* 2002;90(2):89-93.
- Ghasemi M, Dehpour AR. Ethical considerations in animal studies. *J Med Ethics Hist Med* 2009;2(1):12.
- Moranta D, Esteban S, García-Sevilla JA. Acute, chronic and withdrawal effects of the cannabinoid receptor agonist WIN55212-2 on the sequential activation of MAPK/Raf-MEK-ERK signaling in the rat cerebral frontal cortex: short-term regulation by intrinsic and extrinsic pathways. *J Neurosci Res* 2007;85(3):656-67.
- Hosseini-Sharifabad A, Ghahremani MH, Sabzevari O, et al. Effects of protein kinase A and G inhibitors on hippocampal cholinergic markers expressions in rolipram- and sildenafil-induced spatial memory improvement. *Pharmacol Biochem Behav* 2012;101(3):311-9.
- Rahimi HR, Hasanli E, Jamalifar H.A mini review on new pharmacological and toxicological considerations of protease inhibitors' application in cancer prevention and biological research. *Asian J Cell Biol* 2012;7(1):1-12.
- Sim-Selley LJ. Regulation of cannabinoid CB1 receptors

- in the central nervous system by chronic cannabinoids. *Crit Rev Neurobiol* 2003;15(2):91-119.
30. Pourkhalili N, Ghahremani MH, Farsandaj N, et al. Evaluation of anti-invasion effect of cannabinoids on human hepatocarcinoma cells. *Toxicol Mech Methods* 2013;23(2):120-6.
 31. Farsandaj N, Ghahremani MH, Ostad SN. Role of cannabinoid and vanilloid receptors in invasion of human breast carcinoma cells. *J Environ Pathol Toxicol Oncol* 2012;31(4):377-87.
 32. Swift W, Hall W, Teesson M. Cannabis use and dependence among Australian adults: results from the National Survey of Mental Health and Wellbeing. *Addiction* 2001;96(5):737-48.
 33. Copeland J, Swift W. Cannabis use disorder: epidemiology and management. *Int Rev Psychiatry* 2009;21(2):96-103.
 34. Alimoradi H, Pourmohammadi N, Mehr SE, et al. Effects of lithium on peripheral neuropathy induced by vincristine in rats. *Acta Med Iran* 2012;50(6):373-9.
 35. Pourmohammadi N, Alimoradi H, Mehr SE, et al. Lithium attenuates peripheral neuropathy induced by paclitaxel in rats. *Basic Clin Pharmacol Toxicol* 2012;110(3):231-7.
 36. Sharifzadeh M, Rezaei H, Ghamsari MR. Interactive Effects of Acute and Chronic Lithium with Dopamine Receptor Antagonists on Naloxone-Induced Jumping in Morphine-Dependent Mice. *Iran J Pharm Res* 2003;2(4): 217-223.
 37. Kiyani A, Javadi-Paydar M, Mohammadkhani H, et al. Lithium chloride disrupts consolidation of morphine-induced conditioned place preference in male mice: the nitric oxide/cyclic GMP signaling pathway. *Behav Brain Res* 2011;219(2):240-7.
 38. De Vries TJ, Shaham Y, Homberg JR, et al. A cannabinoid mechanism in relapse to cocaine seeking. *Nat Med* 2001;7(10):1151-4.
 39. Karimi S, Azizi P, Shamsizadeh A, et al. Role of intra-accumbal cannabinoid CB1 receptors in the potentiation, acquisition and expression of morphine-induced conditioned place preference. *Behav Brain Res* 2013;247:125-31.
 40. Huang CC, Chen YL, Lo SW, et al. Activation of cAMP-dependent protein kinase suppresses the presynaptic cannabinoid inhibition of glutamatergic transmission at corticostriatal synapses. *Mol Pharmacol* 2002;61(3):578-85.
 41. Filip M, Zaniowska M, Frankowska M, et al. The importance of the adenosine A(2A) receptor-dopamine D(2) receptor interaction in drug addiction. *Curr Med Chem* 2012;19(3):317-55.
 42. Ghasemi M, Dehpour AR. The NMDA receptor/nitric oxide pathway: a target for the therapeutic and toxic effects of lithium. *Trends Pharmacol Sci* 2011;32(7):420-34.
 43. Cale JM, Bird IM. Inhibition of MEK/ERK1/2 signalling alters endothelial nitric oxide synthase activity in an agonist-dependent manner. *Biochem J* 2006;398(2):279-88.
 44. Tzavara ET, Valjent E, Firmo C, et al. Cannabinoid withdrawal is dependent upon PKA activation in the cerebellum. *Eur J Neurosci* 2000;12(3):1038-46.
 45. Hammonds MD, Shim SS, Feng P, Calabrese JR. Effects of subchronic lithium treatment on levels of BDNF, Bcl-2 and phospho-CREB in the rat hippocampus. *Basic Clin Pharmacol Toxicol* 2007;100(5):356-9.
 46. Zhuang LL, Jin R, Zhu LH, Xu HG, Li Y, Gao S, Liu JY, Zhou GP. Promoter Characterization and Role of cAMP/PKA/CREB in the Basal Transcription of the Mouse ORMDL3 Gene. *PLoS One* 2013;8(4):e60630.
 47. Cao JL, Liu HL, Wang JK, Zeng YM. Cross talk between nitric oxide and ERK1/2 signaling pathway in the spinal cord mediates naloxone-precipitated withdrawal in morphine-dependent rats. *Neuropharmacology* 2006;51(2):315-26.
 48. Pertwee RG. Targeting the endocannabinoid system with cannabinoid receptor agonists: pharmacological strategies and therapeutic possibilities. *Philos Trans R Soc Lond B Biol Sci* 2012;367(1607):3353-63.