Asian Journal of Psychiatry 46 (2019) 70–73

Contents lists available at ScienceDirect

Asian Journal of Psychiatry

journal homepage: www.elsevier.com/locate/ajp

Minocycline induces the expression of intra-accumbal glutamate transporter-1 in the morphine-dependent rats

Reza Arezoomandan[a,c], Abbas Aliaghaei[b], Fariba Khodagholi[c], Abbas Haghparast[c,*]

[a] Addiction Department, School of Behavioral Sciences and Mental Health (Tehran Institute of Psychiatry), Iran University of Medical Sciences, Tehran, Iran
[b] Neuroscience Lab, Biology and Anatomical Sciences Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[c] Neuroscience Research Center, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Keywords:
Minocycline
Glutamate transporter 1
Nucleus accumbens
Morphine dependency
Immunohistochemistry
Rat

ABSTRACT

Glial glutamate transporters (GLT-1) is responsible for glutamate homeostasis. GLT-1 expression and glutamate uptake can be affected by addictive drugs and can be used as a target in addiction pharmacotherapy. It has been shown that minocycline, an antibiotic with anti-inflammatory, and neuroprotective properties, can upregulate the expression of GLT-1. In the present study, in morphine-dependent rats, the effect of minocycline on expression of GLT-1 in nucleus accumbens was investigated by immunohistochemistry. The expression of GLT-1 significantly increased in minocycline treated animals. In line with other studies, our findings showed that restoring GLT-1 expression with minocycline might be considered as a potential target for correcting pre-clinical and clinical manifestations of drug addiction.

A critical aspect of drug addiction is alterations in neuronal structure, biochemistry and function in reward-related brain areas (Chou and Narasimhan, 2005). The main area is nucleus accumbens (NAc), which by receiving dopaminergic and glutamatergic projections from ventral tegmental area and medial prefrontal cortex (mPFC) plays a critical role in reward-related phenomena (Chou and Narasimhan, 2005). Many studies show the essential role of excitatory amino acid, particularly glutamate, in the development of morphine dependence and induction of morphine reinstatement (Gass and Olive, 2008; Quintero, 2013a; Marquez et al., 2017). It has been shown that glutamatergic *N*-methyl-D-aspartate (NMDA) antagonists such as memantine (Do Couto et al., 2005) metabotropic receptors antagonists (Moussawi and Kalivas, 2010) and AMPA receptor antagonist (Rasmussen et al., 1996) modified the rewarding effects of morphine. Furthermore, in vivo microdialysis studies suggests that after injection of addictive drugs like heroin, cocaine or nicotine there is an increase in the extracellular levels of glutamate in the NAc (Quintero, 2013b).

Sodium-dependent glutamate transports of glia and neurons including glutamate transporter-1 (GLT-1), glutamate aspartate transporter (GLAST), excitatory amino acid transporter (EAAT) and excitatory amino-acid carrier-1 (EAAC1) play a fundamental role in the regulation of glutamate in the mammalian brain. GLT-1 and GLAST are mainly expressed in astrocytes, whereas EAAC1 and EAAT4 are mainly in neurons (J Roberts-Wolfe and W Kalivas, 2015). GLT-1 responsible for the majority of glutamate reuptake and plays an important role in maintaining low extracellular glutamate in the brain as well in the NAc (Knackstedt et al., 2009). Many studies have shown the involvement of GLT-1 in morphine reward. Expression of GLT-1 mRNA was significantly decreased in the striatum and thalamus of morphine-dependent rats and significantly increased in withdrawal phase (Ozawa et al., 2001). Also, self-administration of heroin significantly decreased GLT-1 protein and glutamate uptake in NA-core (Shen et al., 2014). Several lines of evidence have shown that addictive drugs can reduce GLT-1 and glutamate uptake, and described the agents which restored these effects as addiction pharmacotherapy (J Roberts-Wolfe and W Kalivas, 2015).

Minocycline is a tetracycline antibiotic with powerful anti-inflammatory and neuroprotective properties, thought to be a result of its ability to attenuate glial activation (Garrido-Mesa et al., 2013). Recently, growing evidence has described the ability of minocycline to interfere with the rewarding effects of morphine (Zhang et al., 2012; Arezoomandan and Haghparast, 2015). Studies have proved that minocycline can upregulate the expression of GLT-1 and also ameliorate the down-regulation of GLT-1 in neuropathic rats (Fujio et al., 2005). In our previous study, we showed daily intra-NAc injection of minocycline during the extinction period attenuated the maintenance and also, attenuated the priming-induced reinstatement (Arezoomandan and

Abbreviations: GLT-1, glial glutamate transporters; NAc, nucleus accumbens; mPFC, medial prefrontal cortex; NMDA, *N*-methyl-D-aspartate; CPP, condition place preference

* Corresponding author at: Neuroscience Research Center, Shahid Beheshti University of Medical Sciences, P.O. Box 19615-1178, Tehran, Iran.
E-mail address: Haghparast@sbmu.ac.ir (A. Haghparast).

https://doi.org/10.1016/j.ajp.2019.10.007
Received 27 July 2019; Received in revised form 6 October 2019; Accepted 7 October 2019

Haghparast, 2015). After behavioral studies, we investigated the effect of minocycline on the expression of GLT-1 in NAc using immunohistochemistry.

In the acquisition phase, condition place preference (CPP) was induced by administration of morphine (5 mg/kg for 3 days, s.c.). During extinction phase, the animals were given daily bilateral injections of either minocycline (1, 5 and 10 µg/ 0.5 µl/NAc) or saline (0.5 µl/NAc). The animals were tested for conditioning score 60 min after each injection. To induce reinstatement, a priming dose of morphine (1 mg/kg) was given 1 day after the final extinction day. After behavioral studies, the brains were removed and the levels of GLT-1 were measured using immunohistochemistry. Under deep anesthesia, the rats were perfused through the heart with 0.1 M PBS followed by 4% paraformaldehyde in PBS (pH 7.4). The brains were removed immediately and post-fixed in 4% paraformaldehyde for 48 h and then prepared for immunohistochemical analysis and calculation of the number of GLT-1-positive cells density in the NAc area. Five µm thick Paraffin-embedded slides were cut and mounted on the lam. The sections were processed according to the immunohistochemistry technique incubated with primary antibody for GLT1 (diluted 1: 100) overnight at 40 °C. All prepared sections were then incubated overnight with the avidin-biotin complex substrate and treated with 0.05% 3,3-diaminobenzidine tetrahydrochloride and 0.03% hydrogen peroxide in 0.05 ml of Tris buffer (pH 7.6). At the end of the immunohistochemical reaction, the numerical density of the GLT-1 cells was determined using the optical dissector method. Statistical analysis was performed using one-way ANOVA- test. P-values less than 0.05 considered significant. All experiments were performed in accordance with the guide for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences.

The results showed daily bilateral injections of minocycline (0.5 µl/NAc) increased the expression of GLT-1 in the NAc (Fig. 1). This difference was not significant in the group which received 1 µg minocycline ($p = 0.944$), but minocycline 5 and 10 µg significantly increased the expression of GLT-1 in comparison with the control group [$F(3, 28) = 8.762$, $P < 0.0001$] (Fig. 2).

It is well known that the glutamatergic system's activity at the level of the NAc plays a critical role in relapse after drug extinction and the release of glutamate from the PFC to the NAc increases during reinstatement phase (Quintero, 2013b). Glutamate induces plastic changes in the circuitry from the PFC to the NAc which are essential for dependency and reinstatement (Quintero, 2013b). It is accepted that glial cells are involved in drug dependency (Miguel-Hidalgo, 2009). The glia cells, via expression of GLT-1, play a critical role in maintaining physiological levels of glutamate and also the behaviors of addiction (Conti and Weinberg, 1999). Activating of GLT-1 attenuated the rewarding effects of morphine and methamphetamine (Nakagawa et al., 2005). In addition, GLT-1 inhibitor potentiated the rewarding effect induced by morphine and methamphetamine (Sekiya et al., 2004). Furthermore, the other studies showed that the expression of GLT-1 decreased in the brain reward area of morphine dependence rats (Nakagawa and Satoh, 2004). These findings suggested that glutamate transporters, such as GLT-1, play inhibitory roles in drug-induced reward (Fujio et al., 2005). Several brain circuits and pathophysiological mechanisms are involved in addiction, integrative pharmacology may be the way to move forward for finding new pharmacological treatments (Collis, 2006). Over the past decade, many studies reported some promising pharmacological agents such as minocycline (Garrido-Mesa et al., 2013), N-acetylcysteine (Sangobowale et al., 2018) and galantamine (Carroll et al., 2019) for treatment of several symptoms associated with drug abuse. The growing body of evidence shows the

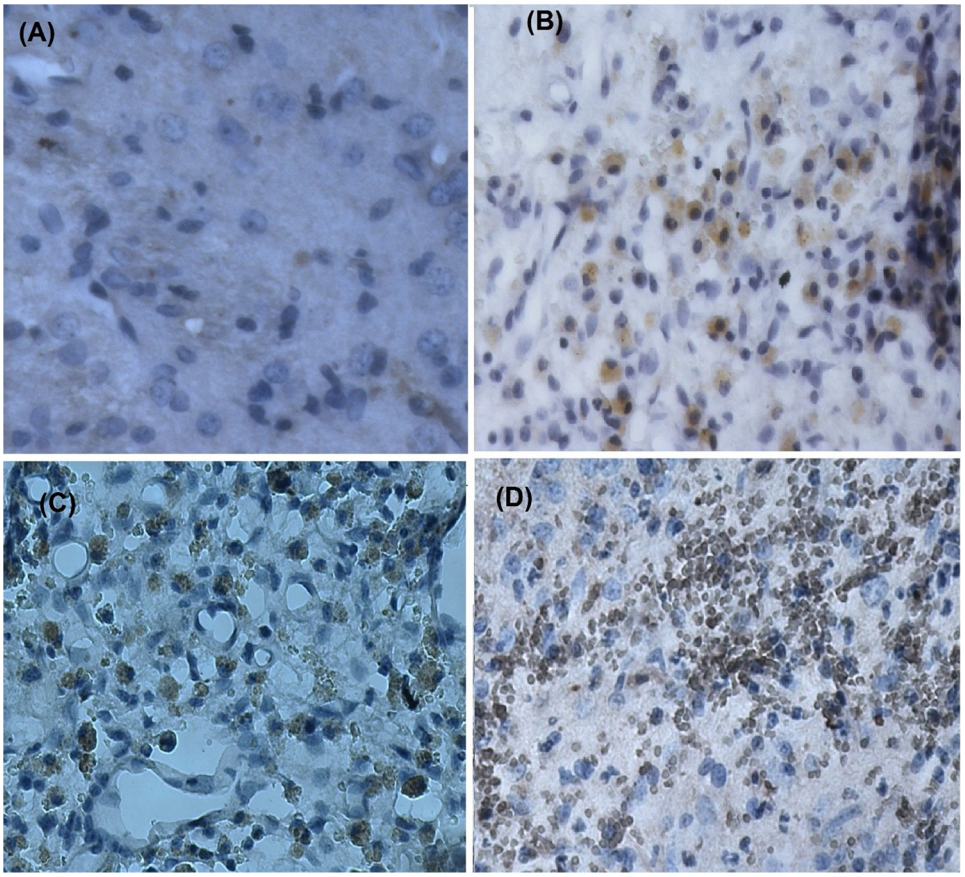

Fig. 1. GLT-1 immunoreactivity in nucleus accumbens after behavioral study and in reinstatement phase. (A) Control group, (B) 1 µg minocycline, (C) 5 µg minocycline, (D) 10 µg minocycline.

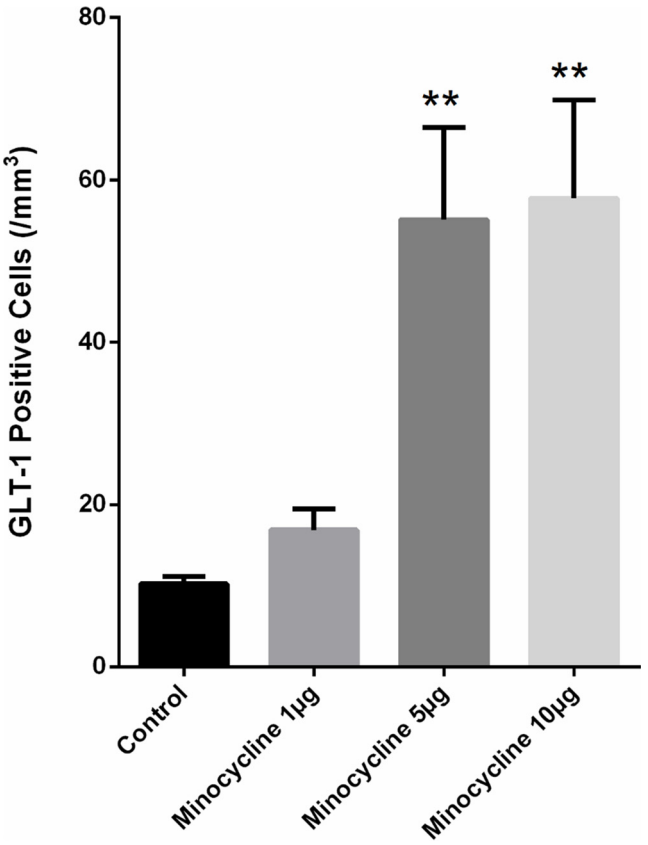

Fig. 2. Daily bilateral injections of minocycline (0.5 µl/NAc) during extinction phase increased the expression of GLT-1 in nucleus accumbens. ** $P < 0.01$ compared with control group.

potential role of minocycline alone or in combination with N-acetylcysteine for improvement of cognition (Abdel Baki et al., 2010; Sangobowale et al., 2018) and treatment of psychiatric disorders (Koola, 2019) and correcting pre-clinical and clinical manifestations of drug addiction (Zhang et al., 2012; Arezoomandan and Haghparast, 2015). In our previous studies, we showed that that daily intra-NAc administration of minocycline attenuated the maintenance and reinstatement of methamphetamine and morphine-induced CPP (Arezoomandan and Haghparast, 2015; Attarzadeh-Yazdi et al., 2014). Some studies have shown that minocycline can upregulate the expression of GLT-1 also, attenuated the down-regulation of GLT-1 (Maier et al., 2007). The present study showed that intra-NAc administration of minocycline could restore the reduction of GLT-1 expression induced by morphine. In line with other studies, our findings showed that minocycline alone or in combination with other drugs can consider as a potential therapeutic target for correcting morphine-induced behavioral changes, and at least part of these effects is mediated by GLT-1 activity.

Role of funding source

Funding for this study was provided by the grant (No. 92-261-A) from Vice-Chancellor for Research & Technology of Shahid Beheshti University of Medical Sciences, Tehran, Iran. The Vice-Chancellor for Research & Technology of Shahid Beheshti University of Medical Sciences had no further role in the design of the study; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

Reza Arezoomandan and Abbas Aliaghaei: Performed the experiments, analyzed the data and wrote the manuscript. Fariba Khodagholi and Abbas Haghparast: Designed the study, interpretation of results and wrote the manuscript. All authors approved the fnal submission.

Informed consent

Not applicable

Research involving human and animal participants

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All experiments were performed in accordance with the guide for the care and use of laboratory animals (National Institutes of Health Publication No. 80-23, revised 1996) and were approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences.

All authors (Reza Arezoomandan, Abbas Aliaghaei, Fariba Khodagholi, Abbas Haghparast) declare that they have no confict of interest.

Declaration of Competing Interest

All authors (Reza Arezoomandan, Abbas Aliaghaei, Fariba Khodagholi, Abbas Haghparast) declare that they have no confict of interest.

Acknowledgment

This study was carried out as part of a Ph.D. student thesis project in Shahid Beheshti University of Medical Sciences. This work was supported by a grant (no. 92-261-A) from Neuroscience Research Center of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

References

Abdel Baki, S.G., Schwab, B., Haber, M., Fenton, A.A., Bergold, P.J., 2010. Minocycline synergizes with N-acetylcysteine and improves cognition and memory following traumatic brain injury in rats. PLoS One 5, e12490.
Arezoomandan, R., Haghparast, A., 2015. Administration of the glial cell modulator, minocycline, in the nucleus accumbens attenuated the maintenance and reinstatement of morphine-seeking behavior. Can. J. Physiol. Pharmacol. 94, 257–264.
Attarzadeh-Yazdi, G., Arezoomandan, R., Haghparast, A., 2014. Minocycline, an antibiotic with inhibitory effect on microglial activation, attenuates the maintenance and reinstatement of methamphetamine-seeking behavior in rat. Prog. Neuropsychopharmacol. Biol. Psychiatry 53, 142–148.
Carroll, K.M., Devito, E.E., Yip, S.W., Nich, C., Sofuoglu, M., 2019. Double-blind placebo-controlled trial of galantamine for methadone-maintained individuals with cocaine use disorder: secondary analysis of effects on illicit opioid use. Am. J. Addict. 28, 238–245.
Chou, I.-H., Narasimhan, K., 2005. Neurobiology of addiction. Nat. Neurosci. 8, 1427.
Collis, M.C., 2006. Integrative pharmacology and drug discovery–is the tide finally turning? Nat. Rev. Drug Discov. 5, 377–379.
Conti, F., Weinberg, R.J., 1999. Shaping excitation at glutamatergic synapses. Trends Neurosci. 22, 451–458.
Do Couto, B.R., Aguilar, M., Manzanedo, C., Rodriguez-Arias, M., Minarro, J., 2005. NMDA glutamate but not dopamine antagonists blocks drug-induced reinstatement of morphine place preference. Brain Res. Bull. 64, 493–503.
Fujio, M., Nakagawa, T., Sekiya, Y., Ozawa, T., Suzuki, Y., Minami, M., Satoh, M., Kaneko, S., 2005. Gene transfer of GLT-1, a glutamate transporter, into the nucleus accumbens shell attenuates methamphetamine-and morphine-induced conditioned place preference in rats. Eur. J. Neurosci. 22, 2744–2754.
Garrido-Mesa, N., Zarzuelo, A., Galvez, J., 2013. Minocycline: far beyond an antibiotic. Br. J. Pharmacol. 169, 337–352.
Gass, J.T., Olive, M.F., 2008. Glutamatergic substrates of drug addiction and alcoholism. Biochem. Pharmacol. 75, 218–265.
J Roberts-Wolfe, D., W Kalivas, P., 2015. Glutamate transporter GLT-1 as a therapeutic target for substance use disorders. CNS Neurol. Disord. Drug Targets 14, 745–756.
Knackstedt, L.A., Larowe, S., Mardikian, P., Malcolm, R., Upadhyaya, H., Hedden, S., Markou, A., Kalivas, P.W., 2009. The role of cystine-glutamate exchange in nicotine dependence in rats and humans. Biol. Psychiatry 65, 841–845.
Koola, M.M., 2019. Antipsychotic-minocycline-acetylcysteine combination for positive,

cognitive, and negative symptoms of schizophrenia. Asian J. Psychiatr. 40, 100–102.
Maier, K., Merkler, D., Gerber, J., Taheri, N., Kuhnert, A.V., Williams, S.K., Neusch, C., Bähr, M., Diem, R., 2007. Multiple neuroprotective mechanisms of minocycline in autoimmune CNS inflammation. Neurobiol. Dis. 25, 514–525.
Marquez, J., Campos-Sandoval, J.A., Penalver, A., Mates, J.M., Segura, J.A., Blanco, E., Alonso, F.J., De Fonseca, F.R., 2017. Glutamate and brain glutaminases in drug addiction. Neurochem. Res. 42, 846–857.
Miguel-Hidalgo, J.J., 2009. The role of glial cells in drug abuse. Curr. Drug Abuse Rev. 2, 76–82.
Moussawi, K., Kalivas, P.W., 2010. Group II metabotropic glutamate receptors (mGlu2/3) in drug addiction. Eur. J. Pharmacol. 639, 115–122.
Nakagawa, T., Fujio, M., Ozawa, T., Minami, M., Satoh, M., 2005. Effect of MS-153, a glutamate transporter activator, on the conditioned rewarding effects of morphine, methamphetamine and cocaine in mice. Behav. Brain Res. 156, 233–239.
Nakagawa, T., Satoh, M., 2004. Involvement of glial glutamate transporters in morphine dependence. Ann. N. Y. Acad. Sci. 1025, 383–388.
Ozawa, T., Nakagawa, T., Shige, K., Minami, M., Satoh, M., 2001. Changes in the expression of glial glutamate transporters in the rat brain accompanied with morphine dependence and naloxone-precipitated withdrawal. Brain Res. 905, 254–258.
Quintero, G.C., 2013a. Role of nucleus accumbens glutamatergic plasticity in drug addiction. Neuropsychiatr. Dis. Treat. 9, 1499.
Quintero, G.C., 2013b. Role of nucleus accumbens glutamatergic plasticity in drug addiction. Neuropsychiatr. Dis. Treat. 9, 1499–1512.
Rasmussen, K., Kendrick, W.T., Kogan, J.H., Aghajanian, G.K., 1996. A selective AMPA antagonist, LY293558, suppresses morphine withdrawal-induced activation of locus coeruleus neurons and behavioral signs of morphine withdrawal. Neuropsychopharmacology 15, 497–505.
Sangobowale, M.A., Grin'kina, N.M., Whitney, K., Nikulina, E., St Laurent-Ariot, K., Ho, J.S., Bayzan, N., Bergold, P.J., 2018. Minocycline plus N-Acetylcysteine reduce behavioral deficits and improve histology with a clinically useful time window. J. Neurotrauma.
Sekiya, Y., Nakagawa, T., Ozawa, T., Minami, M., Satoh, M., 2004. Facilitation of morphine withdrawal symptoms and morphine-induced conditioned place preference by a glutamate transporter inhibitor DL-threo-β-benzyloxyaspartate in rats. Eur. J. Pharmacol. 485, 201–210.
Shen, H.-W., Scofield, M.D., Boger, H., Hensley, M., Kalivas, P.W., 2014. Synaptic glutamate spillover due to impaired glutamate uptake mediates heroin relapse. J. Neurosci. 34, 5649–5657.
Zhang, X.Q., Cui, Y., Cui, Y., Chen, Y., Na, X.D., Chen, F.Y., Wei, X.H., Li, Y.Y., Liu, X.G., Xin, W.J., 2012. Activation of p38 signaling in the microglia in the nucleus accumbens contributes to the acquisition and maintenance of morphine-induced conditioned place preference. Brain Behav. Immun. 26, 318–325.