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Protective effect of telmisartan against morphine-induced analgesic tolerance in mice

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ABSTRACT: Morphine tolerance is a serious clinical problem characterized by a decreased analgesic effect resulted from the long-term use of morphine with uncertain etiology and therapeutic interventions are limited. In this study, we aimed to investigate the effect of telmisartan in a mouse model of morphine-induced analgesic tolerance and the underlying mechanisms of its action. Morphine (10 mg/kg) was injected subcutaneously twice daily for five days. Mice were pretreated with Telmisartan (TEL; 5 and 15 mg/kg) orally by gavage 30 min before each morphine injection. L-NAME (10 mg/kg), L-arginine (L-ARG,300 mg/kg) or N-acetylcysteine (NAC, 50 mg/kg) was administered intraperitoneally 45 min prior to morphine. Analgesic efficacy was evaluated by hot-plate test on 1st, 3rd and 5th days, 60 min after morphine injection. Spinal cord samples of mice were used to examine the protein expressions of nNOS and iNOS by western blotting and total GSH content. Repeated morphine administration caused a significant decrease in analgesic efficacy, demonstrating the development of morphine tolerance. High-dose TEL treatment effectively prevented morphine-induced analgesic tolerance. L-ARG abolished the inhibitory effect of TEL on morphine tolerance, while L-NAME and NAC did not alter. GSH level and nNOS expression were decreased, as well as iNOS expression was increased in the spinal cords from morphine-tolerant mice. TEL (15 mg/kg) treatment prevented the decrease in GSH level and the increase in iNOS expression of spinal cords. TEL would be a potential therapeutic candidate in preventing morphine tolerance through its activity on antioxidant systems and, in part, on the nitric oxide pathway.

KEYWORDS: hot-plate test; mice; morphine tolerance; nitric oxide; telmisartan

1. INTRODUCTION

Morphine is a natural alkaloid obtained from poppy seeds, an opioid receptor agonist and used in the treatment of different types of pain with its strong analgesic and sedative effects. Morphine shows its potent analgesic effect by preventing the transmission of pain impulses in the spinal cord and through modulation of the pain perception [1]. Although it is known as one of the most potent analgesic substances, morphine is commonly associated with serious problems such as the development of tolerance to its effects and physical dependence, which limits its clinical use [2]. Morphine tolerance is characterized by a reduced therapeutic response to morphine at therapeutic doses and the need for higher doses to maintain the desired effect of morphine. Moreover, it leads to the occurrence of side effects of opioid analgesics such as nausea, urinary retention and severe constipation, and poor compliance with therapy [3]. Although the precise molecular mechanisms underlying morphine tolerance are still largely unknown, numerous studies have shown that neuroinflammation [2], oxidative stress [4], nitric oxide (NO) pathway, and protein kinase C activation [5] play important roles in opioid tolerance. It has been shown that chronic morphine treatment causes the production of reactive oxygen species (ROS) and inhibits the antioxidant defense system [6, 7]. In a clinical trial, chronic morphine treatment altered the glutathione (GSH)-dependent oxidative balance and tented to vulnerable the patients to oxidative damage [8].

The role of NO on morphine tolerance is controversial. While some studies have shown that the blockage of excessive NO biosynthesis and selective NO synthase (NOS) inhibitors (especially inducible NOS –iNOS- inhibitors) prevent morphine tolerance and dependence [9, 10], other studies have revealed that increased NO level in the induction phase of tolerance delays the formation of opioid tolerance and dependence [11]. Babey et al. suggested that NO precursor, L-arginine (L-ARG), decreased morphine analgesic potency and also accelerated the development of opioid tolerance when coadministered with

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morphine [12]. Ozdemir et al., underlined that L-NAME, a NOS inhibitor, treatment prevented the morphine analgesic tolerance in hot plate test [13].

Telmisartan (TEL), an angiotensin II type 1 (AT1) receptor antagonist, is an antihypertensive agent that is frequently preferred in the treatment of hypertension in clinic. In addition it has antihyperlipidemic, neuroprotective, cardioprotective and antidiabetic activity that are considered to be related with the inhibition of ROS production by TEL [14-16]. In neuronal cells, TEL prevented glucose-induced neuronal damage by increasing antioxidant enzyme activities and GSH levels [15]. Besides its antioxidant effects, TEL is also known to interact with NO pathway, especially by inhibiting iNOS activity [17, 18]. Several studies also emphasized the antiinflammatory effects of TEL in neuronal damage, which is attributed to the inhibition of iNOS activity, thus the production of NO that is a causative factor involved in inflammation and the suppression of proinflammatory cytokines such as tumor necrosis factor-alpha and interleukins [19, 20].

In the light of these studies, we aimed to investigate the protective effect of TEL in the development of morphine-induced analgesic tolerance in mice and to elucidate the role of the antioxidant system and NO pathway in its action.

2. RESULTS

2.1. Effect of TEL treatment on the development of morphine-induced analgesic tolerance

Repeated morphine administration lead to a significant decrease in the analgesic effect of morphine on the 3rd and 5th days, indicating the development of morphine tolerance. TEL treatment at 5 mg/kg could not prevent the decrease in analgesic effect when compared to the morphine-tolerant group, whereas high dose TEL (15 mg/kg) treatment significantly increased the analgesic response of morphine when compared to the morphine-tolerant group (Figure 1, $p<0.05$).

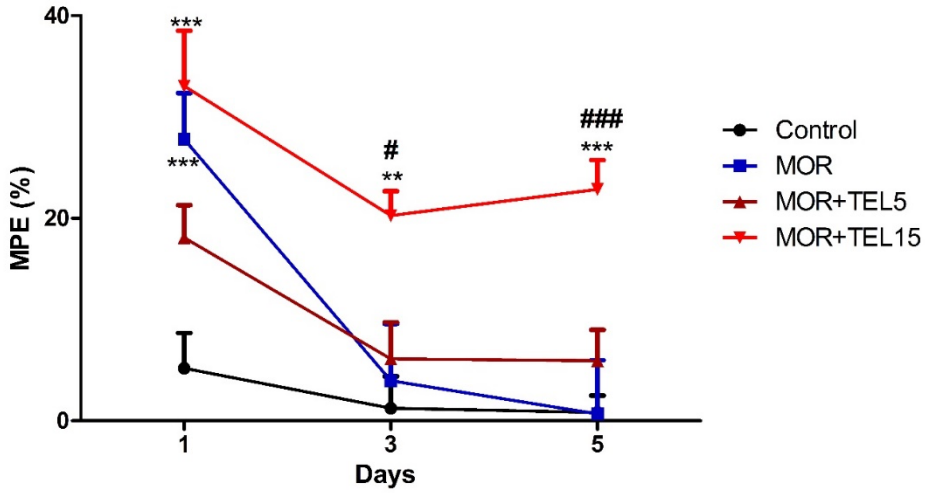

Figure 1. Effects of TEL treatment on the development of morphine-induced analgesic tolerance in mice. Data were expressed as mean±standard error (n=6-8). ***p<0.001, **p<0.01 significantly different from the control, ### p<0.001, # p<0.05 significantly different from the MOR. MOR, morphine (10 mg/kg). TEL, telmisartan.

2.2. Role of NO pathway and antioxidant system on the effect of TEL treatment on the morphine-induced analgesic tolerance

As shown in Figure 2, high dose of TEL (15 mg/kg) prevented the development of tolerance to morphine analgesia, administration of L-ARG antagonized the therapeutic effect of TEL on the morphine-induced analgesic tolerance at day 3 and 5 ($p<0.05$). Co-treatment with L-NAME or NAC did not change the effect of TEL on the morphine-induced analgesic tolerance (Figure 2).

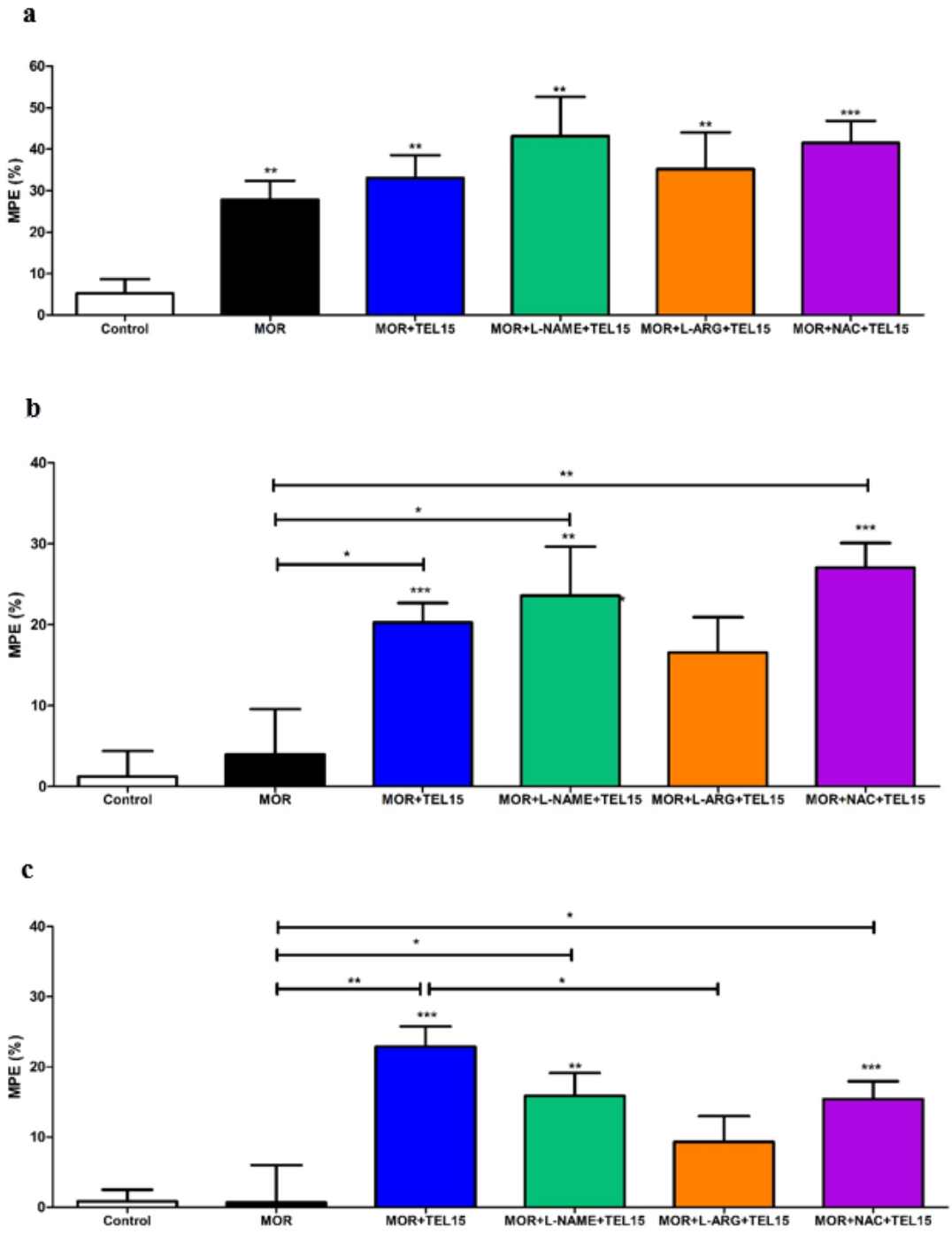

Figure 2. Effects of L-NAME (10 mg/kg), L-ARG (300 mg/kg) or NAC (50mg/kg) co-treatment on TEL-induced prevention of the development of analgesic tolerance in mice on (a) day 1, (b) day 3, (c) day 5. Data were expressed as mean±standard error (n=6-8). $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$ significantly different from control and as indicated. L-ARG, L-arginine. L-NAME, L-N (G)-nitroarginine methyl ester. MOR, morphine (10 mg/kg) . NAC, N-acetylcysteine. TEL, telmisartan.

2.3. Effect of TEL and co-treatment with L-ARG, L-NAME or NAC on morphine-induced alteration of spinal cord GSH level

Chronic morphine treatment caused a significant reduction in GSH level of spinal cords from morphine-tolerant mice compared with control ($p<0.001$) and TEL (15 mg/kg) augmented spinal GSH content compared to morphine-tolerant group. Moreover, co-treatment with L-NAME and NAC caused a marked increment in GSH level compared to morphine-tolerant mice ($p<0.05$). L-ARG administration did not change the effect of TEL on morphine-induced alterations of spinal cord GSH level (Figure 3).

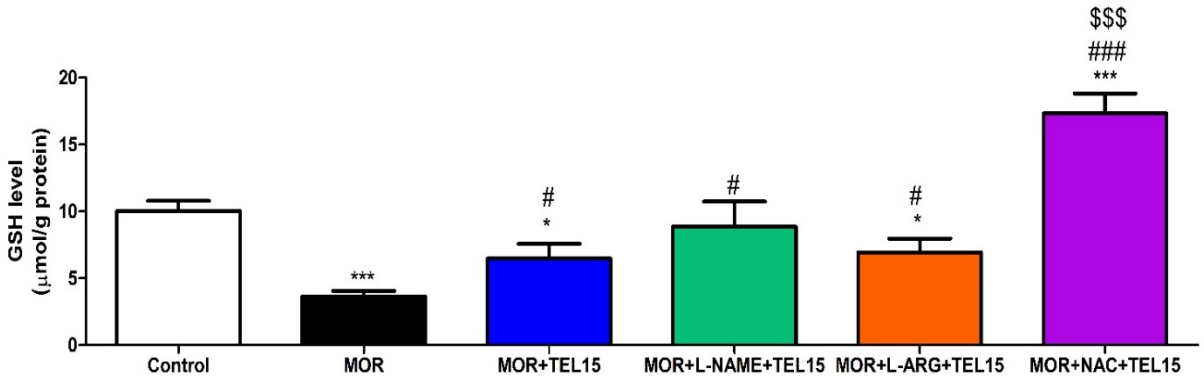

Figure 3. Effects L-NAME (10 mg/kg), L-ARG (300 mg/kg) or NAC (50mg/kg) co-treatment with TEL on total GSH level in mice spinal cord. Data were expressed as mean±standard error (n=6-8). ***p<0.001, *p<0.05 significantly different from the control, ### p<0.001, # p<0.05 significantly different from the MOR, $$$ p<0.001 significantly diffferent from the MOR+TEL15. L-ARG, L-arginine. L-NAME, L-N (G)-nitroarginine methyl ester. MOR, morphine (10 mg/kg). NAC, N-acetylcysteine. TEL, telmisartan.

2.4. Effect of TEL on morphine-induced alteration of spinal cord nNOS protein expression

As shown in Figure 4, chronic morphine treatment caused a significant decline in spinal cord nNOS protein expression compared to the control ($p<0.05$) and TEL treatment was not able to prevent the reduction of nNOS protein expression in spinal cord compared to the morphine-tolerant group (Figure 4, $p<0.001$).

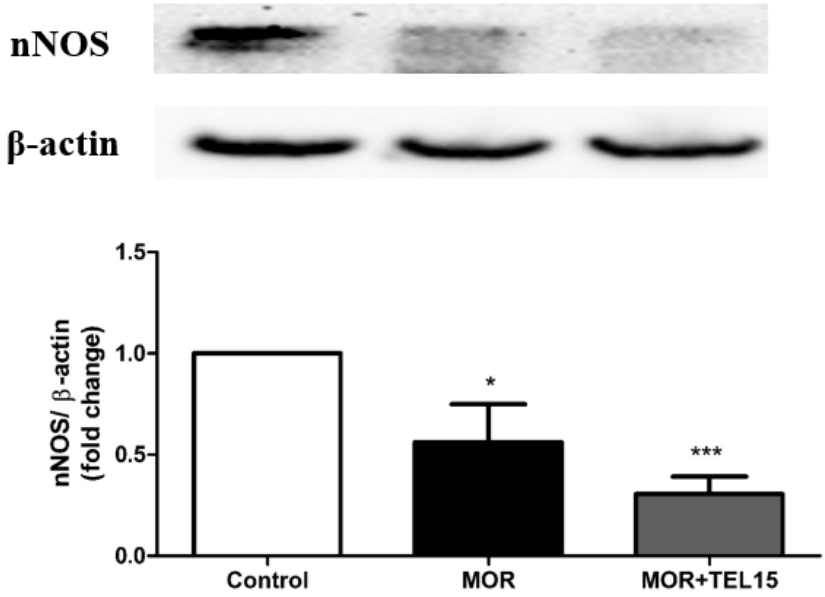

Figure 4. Effects of TEL (15 mg/kg) treatment on nNOS protein expression levels of spinal cords from morphine-treated mice. Upper panel, representative image showing the nNOS protein expressions. Data were expressed as mean±standard error (n=5-6). ***p<0.001, *p<0.05 significantly different from the control. MOR, morphine (10 mg/kg). TEL, telmisartan.

2.5. Effect of TEL on morphine-induced alteration of spinal cord iNOS protein expression

As shown in Figure 5, chronic morphine treatment significantly increased iNOS protein expression in the spinal cord compared to the control ($p<0.05$) and in TEL-treated group, iNOS protein expression was similar compared to control (Figure 5).

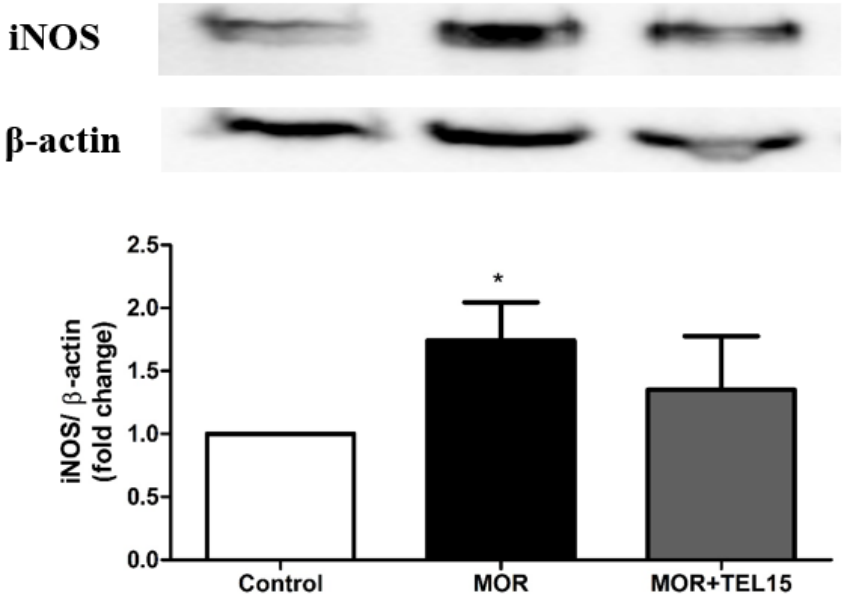

Figure 5. Effects of TEL (15 mg/kg) treatment on iNOS protein expression levels of spinal cords from morphine-treated mice. Upper panel, representative image showing the iNOS protein expression. Data were expressed as mean±standard error (n=5-6). *p<0.05 significantly different from the control. MOR, morphine (10 mg/kg). TEL, telmisartan.

3. DISCUSSION

Morphine and other opioid agents are the most effective drugs that are frequently preferred in the treatment of chronic pain due to their high potency. However, the decrease in the analgesic effect called tolerance that develops as a result of long-term use of morphine, limits its clinical use. In case of opioid tolerance, higher doses of drugs are required to achieve the effect at the therapeutic level. This problem exacerbates many opioid-related side effects like physical dependence and respiratory depression, which may be life-threatening [1, 3]. Therefore, there is an urgent need to develop potent and safe adjuvant agents to prevent opioid tolerance.

Although molecular mechanisms that mediates the development of morphine tolerance and novel preventive strategies are still not fully elucidated, neuroinflammation, free radical production and the NO pathway have been found to play important roles in opioid tolerance [2, 4]. In this study, we aimed to determine the effects and possible mechanisms of action of TEL, an antihypertensive agent known to be associated with NO pathway and antioxidant systems, on morphine tolerance within the scope of drug repositioning strategies.

We observed that chronic subcutaneous injection of morphine (10 mg/kg) for 5 consecutive days significantly decreased maximal analgesic action and generated analgesic tolerance in mice and TEL treatment with high dose (15 mg/kg) markedly prevented the decline in morphine analgesia. A recent study showed that adjuvant treatment with angiotensin receptor antagonists enhanced the analgesic effect of submaximal dose morphine in a neuropathic pain model but acute TEL treatment alone did not show analgesic activity [21]. However, little is known about the their effects and possible underlying mechanisms of angiotensin receptor antagonists on morphine-induced analgesic tolerance.

GSH acts as an antioxidant mediator by scavenging free radicals and inhibiting lipid peroxidation, and also mediates non-enzymatic detoxification of hydroxyl radicals [22]. In opioid tolerance, excitotoxicity develops as a result of increased synaptic glutamate concentrations and a decrease in neuronal antioxidant defenses, which is associated with decreased intracellular cysteine levels and decreased total GSH [23]. Morever, chronic morphine administration exaggerated production of free radicals and produced a progressive decline in intracellular GSH levels in rat brain [24]. According to the our findings, the total GSH level of spinal cords decreased with consecutive morphine administration in morphine-tolerant group and TEL treatment prevented this decrease significantly. Moreover, inhibition of NO production and activation of antioxidant system potentiated the therapeutic effect of TEL on GSH levels. Consistent with our findings, Eslami et al., also found that TEL increased the total GSH level in a glucose-induced oxidative damage in neuronal cell culture [15]. In neuronal cells, it was demonstrated that extracellular cysteine is an rate-limiting

amino acid for GSH synthesis. NAC, a potent antioxidant, is a precursor of L-cysteine and it can be easily penetrate the neurons and act as a cysteine reservoir for neuronal GSH synthesis [25, 26]. Therefore the excessive increment GSH level with the administration of NAC+TEL may be due to cysteine reservoir action of NAC. In this regard, it can be assumed that chronic morphine treatment increase the oxidative damage and also depletes the antioxidant reserve in neuronal cells while TEL may be an effective agent in preventing oxidative damage and supporting the antioxidant defence system via increasing the GSH level.

Growing evidence indicates that the overproduction of NO plays an important role in the development of opioid-analgesic tolerance and dependence [27-29]. It was suggested that chronic morphine treatment enhances NO production that increase cGMP levels and alter phosphorylations of some key proteins implicated in analgesic tolerance. Therefore, the agents that inhibit NO pathway might be promising therapeutic target for opioid-induced tolerance and dependence [30]. To investigate the roles of nitric oxide synthase (NOS) isoforms in the development of morphine tolerance and possible role in TEL therapeutic effect, we evaluated nNOS and iNOS expression levels in spinal cord homogenates by western blot. Our findings showed that nNOS expression decreased and iNOS expression increased in the spinal cords of morphine-tolerant mice. We found that the decrease in nNOS expression was not prevented by TEL treatment and iNOS expression was found to be similar to the control. Similar with our findings, Khan et al. reported that while morphine administration increased iNOS expression, it did not change the nNOS expression level and thalidomide, which was found to be effective on morphine analgesic tolerance, prevented the increase in iNOS expression of morphine-treated cells, and also did not change nNOS expression in neuroblastoma cell culture [31]. Abdel-Zaher et al. also showed that iNOS mRNA expression significantly increased in brain homogenates of morphine-tolerant mice, however, but nNOS mRNA expression did not change [10].

4. CONCLUSION

Taken together, our results provide strong evidence that TEL has significant beneficial effects on morphine-induced analgesic tolerance clinically encountered in the treatment of chronic pain by partially modulating the NO pathway and oxidative stress.

5. MATERIALS AND METHODS

5.1.Chemicals

The following agents were used in experiments; morphine HCl (Galen Pharmaceutical Industry Inc., Türkiye), L-N (G)-nitroarginine methyl ester (Sigma-Aldrich, USA), L-arginine (Sigma-Aldrich, USA), N-acetylcysteine (Haver Pharma, Türkiye), telmisartan (NeuTec Pharma, Türkiye), anti-nNOS antibody (Cell Sigaling Technology, USA), anti-iNOS antibody (Cell Sigaling Technology, USA).

5.2. Animals

Adult 48 male Balb/C mice (20-35 g, 6-8 weeks old) were used in the study. The mice were housed in cages with access to free food and water in the temperature and humidity-controlled (22 ± 1 °C) rooms. The experimental protocol was approved by Institutional Animal Care and Ethics Committee (Approval number: 2021/22).

5.3. Induction of morphine tolerance and treatment schedule

To induce morphine tolerance; morphine (10 mg/kg) was administered subcutaneously (s.c.) twice a day (at 9:00 a.m. and 4:00 p.m.) for five consecutive days [32]. L-N (G)-nitroarginine methyl ester (L-NAME; non-selective NOS inhibitor, 10 mg/kg) , L-arginine (NO prekursor, 300 mg/kg) and N-acetylcysteine (NAC, antioxidant, 50 mg/kg) were administered intraperitoneally (i.p) 45 minutes before the morphine administration, and TEL (5 and 15 mg/kg) was administered to the animals by oral gavage (p.o) 30 minutes before the morphine administration for five days. In the control group, physiological saline was administered instead of morphine in the same protocol. 0.5% carboxymethylcellulose (CMC) was used as vehicle for TEL.

5.4. Hot-plate test

In the hot plate test, mice were placed on a heater set at 55±0.1 °C and the latency until mice showed first behavioral reaction (hind paw licking or jumping) was recorded. 1st, 3rd and 5th days of experiment, each mouse was placed on a hot plate and baseline latency was measured. Then, the test dose of morphine (10 mg/kg; s.c) was administered to mice and after 60 minutes of morphine administration, latency was recorded three times with an interval of 10 min. The average of three measurements for each mouse was recorded as the latency value for the relevant experimental day. A 30-s cut-off was used to avoid tissue damage [9]. Maximal possible effect (%MPE) was calculated with the following equation for the latency-withdrawal response for each mice:

%MPE = [postdrug latency (s) − baseline latency (s)] / [cut-off value (s) − baseline latency (s)] × 100

On the 5th day following the hot-plate test, the mice were sacrificed by cervical dislocation under anesthesia, the spinal cord tissues were quickly removed, frozen in liquid nitrogen and stored at -80°C for western blot and biochemical analysis.

5.5. GSH content

Spinal cords were homogenized with cold phosphate-buffered saline (pH = 7.4) and the homogenates were centrifuged at 10000 rpm at +4°C. The GSH content were measured in the spinal cord homogenates by using commercial colorimetric kit (Cayman Chemical-703,002, Ann Arbor, MI, USA) following the manufacturer's guidelines and were expressed as µmol/g protein [33].

5.6. Western-blot analysis

Each frozen spinal cords were sonicated respectively in 10 volume of ice-cold fresh TNTE lysis buffer [50 mM Tris-HCl (pH: 7.4), 150 mM NaCl, %1 Triton-X 100, 1 mM EDTA (pH:7.0), 5 mM $Na_4P_2O_7$, protease inhibitor cocktail, %10 Glycerol, 2 mM sodium orthovanadate, 20 mM NaF, 1 mM PMSF] and centrifuged as described previously (23). Total protein content was determined by bicinchoninic acid (BCA) assay kit (Thermo Scientific, 23225, USA). The samples (30 µg/lane) were loaded into 10% acrylamide gel and after electrophoresis, proteins were transferred to polyvinylidene difluoride membranes. Membranes were blocked with 5% skimmed nonfat milk in 0.1% Tween-20 Tris-buffered saline for 90 min and then incubated with anti-nNOS (1:1000), anti-iNOS (1:1000), and anti-β actin antibody (1:3000) overnight at 4° C. Following the primary antibody incubation, membranes were incubated with horseradish peroxidase linked secondary antibody, and protein bands were visualized using enhanced chemiluminescence kit (ECL kit, Laboratories Inc. Hercules, CA, USA). Densitometric analysis of protein bands were performed using Image J software (NIH, USA) and data normalized to relative β-actin [34].

5.7. Statistical analysis

Data were expressed as the mean ± standart error. Statistical analysis was performed using GraphPad Prism (Version 5.01; Graphpad Software, San Diego, CA, USA). The differences between groups were compared with student's t test or two-way ANOVA, followed by Bonferroni multiple comparison test and $p<0.05$ was considered statistically significant.

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REFERENCES

[1] Skrabalova J, Drastichova Z, Novotny J. Morphine as a potential oxidative stress-causing agent. Mini Rev Org Chem. 2013;10(4):367-372. http://doi.org/10.2174/1570193X113106660031

[2] Mansouri MT, Naghizadeh B, Ghorbanzadeh B, Alboghobeish S, Amirgholami N, Houshmand G, Cauli O. oxide Venlafaxine prevents morphine antinociceptive tolerance: The role of neuroinflammation and the l-arginine-nitric pathway. Exp Neurol. 2018;303:134-141. http://doi.org/10.1016/j.expneurol.2018.02.009

[3] de Guglielmo G, Kallupi M, Scuppa G, Stopponi S, Demopulos G, Gaitanaris G, Ciccocioppo R. Analgesic tolerance to morphine is regulated by PPARγ. Br J Pharmacol. 2014;171(23):5407-5416. http://doi.org/10.1111/bph.12851

[4] Fatemi I, Amirteimoury M, Shamsizadeh A, Kaeidi A. The effect of metformin on morphine analgesic tolerance and dependence in rats. Res Pharm Sci. 2018;13(4):316-323. http://doi.org/10.4103/1735-5362.235158

[5] Xin W, Chun W, Ling L, Wei W. Role of melatonin in the prevention of morphine-induced hyperalgesia and spinal glial activation in rats: protein kinase C pathway involved. Int J Neurosci. 2012;122(3):154-163. http://doi.org/10.3109/00207454.2011.635828

[6] Zhang YT, Zheng QS, Pan J, Zheng RL. Oxidative damage of biomolecules in mouse liver induced by morphine and protected by antioxidants. Basic Clin Pharmacol Toxicol. 2004;95(2):53-58. http://doi.org/10.1111/j.1742-7843.2004.950202.x

[7] Payabvash S, Beheshtian A, Salmasi AH, Kiumehr S, Ghahremani MH, Tavangar SM, Sabzevari O, Dehpour AR. Chronic morphine treatment induces oxidant and apoptotic damage in the mice liver. Life Sci. 2006;79(10):972-980. http://doi.org/10.1016/j.lfs.2006.05.008

[8] Goudas LC, Langlade A, Serrie A, Matson W, Milbury P, Thurel C, Sandouk P, Carr DB. Acute decreases in cerebrospinal fluid glutathione levels after intracerebroventricular morphine for cancer pain. Anesth Analg. 1999;89(5):1209-1215. http://doi.org/10.1213/00000539-199911000-00023

[9] Abdel-Zaher AO, Hamdy MM, Aly SA, Abdel-Hady RH, Abdel-Rahman S. Attenuation of morphine tolerance and dependence by aminoguanidine in mice. Eur J Pharmacol. 2006;540(1-3):60-66. http://doi.org/10.1016/j.ejphar.2006.03.059

[10] Abdel-Zaher AO, Mostafa MG, Farghaly HS, Hamdy MM, Abdel-Hady RH. Role of oxidative stress and inducible nitric oxide synthase in morphine-induced tolerance and dependence in mice. Effect of alpha-lipoic acid. Behav Brain Res. 2013;247:17-26. http://doi.org/10.1016/j.bbr.2013.02.034

[11] Dambisya YM, Lee TL. Role of nitric oxide in the induction and expression of morphine tolerance and dependence in mice. Br J Pharmacol.1996;117(5):914-918. http://doi.org/10.1111/j.1476-5381.1996.tb15280.x

[12] Babey AM, Kolesnikov Y, Cheng J, Inturrisi CE, Trifilletti RR, Pasternak GW. Nitric oxide and opioid tolerance. Neuropharmacology.1994; 33(11):1463-1470. http://doi.org/10.1016/0028-3908(94)90050-7

[13] Ozdemir E, Bagcivan I, Durmus N, Altun A, Gursoy S. The nitric oxide-cGMP signaling pathway plays a significant role in tolerance to the analgesic effect of morphine. Can J Physiol Pharmacol. 2011; 89(2):89-95. http://doi.org/10.1139/y10-109

[14] Yin SN, Liu M, Jing DQ, Mu YM, Lu JM, Pan CY. Telmisartan increases lipoprotein lipase expression via peroxisome proliferator-activated receptor-alpha in HepG2 cells. Endocr Res. 2014;39(2):66-72. http://doi.org/10.3109/07435800.2013.828741

[15] Eslami H, Sharifi AM, Rahimi H, Rahati M. Protective effect of telmisartan against oxidative damage induced by high glucose in neuronal PC12 cell. Neurosci Lett. 2014;558:31-36. http://doi.org/10.1016/j.neulet.2013.10.057

[16] Yoo SM, Choi SH, Jung MD, Lim SC, Baek SH. Short-term use of telmisartan attenuates oxidation and improves Prdx2 expression more than antioxidant β-blockers in the cardiovascular systems of spontaneously hypertensive rats. Hypertens Res. 2015;38(2):106-115. http://doi.org/10.1038/hr.2014.151

[17] Yuen CY, Wong WT, Tian XY, Wong SL, Lau CW, Yu J, Tomlinson B, Yao X, Huang Y. Telmisartan inhibits vasoconstriction via PPARγ-dependent expression and activation of endothelial nitric oxide synthase. Cardiovasc Res. 2011;90(1):122-129. http://doi.org/10.1093/cvr/cvq392

[18] Kumtepe Y, Odabasoglu F, Karaca M, Polat B, Halici MB, Keles ON, Altunkaynak Z, Gocer F. Protective effects of telmisartan on ischemia/reperfusion injury of rat ovary: biochemical and histopathologic evaluation. Fertil Steril. 2010;93(4):1299-1307. http://doi.org/10.1016/j.fertnstert.2008.12.016

[19] Xu Y, Xu Y, Wang Y, Wang Y, He L, Jiang Z, Huang Z, Liao H, Li J, Saavedra JM, Zhang L, Pang T. Telmisartan prevention of LPS-induced microglia activation involves M2 microglia polarization via CaMKKβ-dependent AMPK activation. Brain Behav Immun. 2015;50:298-313. http://doi.org/10.1016/j.bbi.2015.07.015

[20] Torika N, Asraf K, Danon A, Apte RN, Fleisher-Berkovich S. Telmisartan modulates glial activation: In vitro and ın vivo studies. PLoS One. 2016;11(5):e0155823. http://doi.org/10.1371/journal.pone.0155823

[21] Karádi DÁ, Galambos AR, Lakatos PP, Apenberg J, Abbood SK, Balogh M, Király K, Riba P, Essmat N, Szűcs E, Benyhe S, Varga ZV, Szökő É, Tábi T, Al-Khrasani M. Telmisartan ıs a promising agent for managing neuropathic pain and delaying opioid analgesic tolerance in rats. Int J Mol Sci. 2023;24(9):7970. http://doi.org/10.3390/ijms24097970

[22] Motaghinejad M, Karimian SM, Motaghinejad O, Shabab B, Asadighaleni M, Fatima S. The effect of various morphine weaning regimens on the sequelae of opioid tolerance involving physical dependency, anxiety and hippocampus cell neurodegeneration in rats. Fundam Clin Pharmacol. 2015;29(3):299-309. http://doi.org/10.1111/fcp.12121

[23] Salvemini D, Neumann WL. Peroxynitrite: a strategic linchpin of opioid analgesic tolerance. Trends Pharmacol Sci. 2009;30(4):194-202. http://doi.org/10.1016/j.tips.2008.12.005
[24] Zeng XS, Geng WS, Wang ZQ, Jia JJ. Morphine addiction and oxidative stress: The potential effects of thioredoxin-1. Front Pharmacol. 2020;11:82. http://doi.org/10.3389/fphar.2020.00082
[25] Trivedi M, Shah J, Hodgson N, Byun HM, Deth R. Morphine induces redox-based changes in global DNA methylation and retrotransposon transcription by inhibition of excitatory amino acid transporter type 3-mediated cysteine uptake. Mol Pharmacol. 2014;85(5):747-757. http://doi.org/10.1124/mol.114.091728
[26] Tardiolo G, Bramanti P, Mazzon E. Overview on the effects of N-acetylcysteine in neurodegenerative diseases. Molecules. 2018;23(12):3305. http://doi.org/10.3390/molecules23123305
[27] Elliott K, Minami N, Kolesnikov YA, Pasternak GW, Inturrisi CE. The NMDA receptor antagonists, LY274614 and MK-801, and the nitric oxide synthase inhibitor, NG-nitro-L-arginine, attenuate analgesic tolerance to the mu-opioid morphine but not to kappa opioids. Pain. 1994;56(1):69-75. http://doi.org/10.1016/0304-3959(94)90151-1
[28] Majeed NH, Przewłocka B, Machelska H, Przewłocki R. Inhibition of nitric oxide synthase attenuates the development of morphine tolerance and dependence in mice. Neuropharmacology. 1994;33(2):189-192. http://doi.org/10.1016/0028-3908(94)90006-x
[29] Heinzen EL, Pollack GM. The development of morphine antinociceptive tolerance in nitric oxide synthase-deficient mice. Biochem Pharmacol. 2004;67(4):735-741. http://doi.org/10.1016/j.bcp.2003.08.046
[30] Naidu PS, Singh A, Joshi D, Kulkarni SK. Possible mechanisms of action in quercetin reversal of morphine tolerance and dependence. Addict Biol. 2003;8(3):327-336. http://doi.org/10.1080/1355621031000160224 8
[31] Khan MI, Ostadhadi S, Mumtaz F, Momeny M, Moghaddaskho F, Hassanipour M, Ejtemaei-Mehr S, Dehpour AR. Thalidomide attenuates the development and expression of antinociceptive tolerance to μ-opioid agonist morphine through l-arginine-iNOS and nitric oxide pathway. Biomed Pharmacother. 2017;85:493-502. http://doi.org/10.1016/j.biopha.2016.11.056
[32] Lipták N, Dochnal R, Csabafi K, Szakács J, Szabó G. Obestatin prevents analgesic tolerance to morphine and reverses the effects of mild morphine withdrawal in mice. Regul Pept. 2013;186:77-82. http://doi.org/10.1016/j.regpep.2013.07.006
[33] Engin S, Barut EN, Yaşar YK, Soysal AÇ, Arıcı T, Kerimoğlu G, Kadıoğlu M, Sezen SF. Trimetazidine attenuates cyclophosphamide-induced cystitis by inhibiting TLR4-mediated NFκB signaling in mice. Life Sci. 2022;301:120590. http://doi.org/10.1016/j.lfs.2022.120590
[34] Barut B, Yalçın CÖ, Altun Y, Akkaya D, Barut EN, Baş H, Bıyıklıoğlu Z. Evaluation of photodynamic therapy effects of novel zinc (II) phthalocyanine through a possible interaction with toll-like receptor signaling pathway. Appl Organomet Chem. 2023;37(4): e7039. https://doi.org/10.1002/aoc.7039