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RESEARCH

Metformin prevents morphine-induced apoptosis in rats with diabetic neuropathy: a possible mechanism for attenuating morphine tolerance

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Abstract

Morphine is a drug of choice for the treatment of severe and chronic pain, but tolerance to the antinociceptive effect limits its use. The development of tolerance to morphine has recently been associated with neuronal apoptosis. In this study, our aim was to investigate the effects of metformin on morphine-induced neuronal apoptosis and antinociceptive tolerance in diabetic rats. Three days of cumulative dosing were administered to establish morphine tolerance in rats. The antinociceptive effects of metformin (50 mg/kg) and test dose of morphine (5 mg/kg) were considered at 30-min intervals by thermal antinociceptive tests. To induce diabetic neuropathy, streptozotocin (STZ, 65 mg/kg) was injected intraperitoneally. ELISA kits were used to measure caspase-3, bax, and bcl-2 levels from dorsal root ganglion (DRG) tissue. Semi-quantitative scoring system was used to evaluate apoptotic cells with the the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) method. The findings suggest that co-administration of metformin with morphine to diabetic rats showed a significant increase in antinociceptive effect compared to morphine alone. The antinociceptive tests indicated that metformin significantly attenuated morphine antinociceptive tolerance in diabetic rats. In addition, metformin decreased the levels of apoptotic proteins caspase 3 and Bax in DRG neurons, while significantly increased the levels of antiapoptotic Bcl-2. Semi-quantitative scoring showed that metformin provided a significant reduction in apoptotic cell counts in diabetic rats. These data revealed that metformin demonstrated antiapoptotic activity in diabetic rat DRG neurons and attenuated morphine tolerance. The antiapoptotic activity of metformin probably plays a significant role in reducing morphine tolerance.

Keywords Metformin · Apoptosis · Morphine tolerance · Diabetic neuropathy · Dorsal root ganglion

Introduction

Neuropathic pain is a serious complication of diabetes and constitutes an important clinical problem due to resistance to treatment and occurs in approximately 50% of patients (Hicks and Selvin, 2019; He et al. 2021). Opioid therapy such as morphine for diabetic neuropathy may be beneficial for a group of people; however, the development of tolerance and physical dependence with long-term use limits its use (Jamison and Mao 2015). Therefore, morphine is an opioid approved as third-line therapy in the management of diabetic neuropathy (Patil et al. 2015). Experimental studies reveal that morphine cannot show an effective antinocicepitive effect in the treatment of painful diabetic neuropathy (Chen and Pan 2002). Increase in blood and brain glucose concentration in diabetes creates significant changes in the endogenous opioid system and the functioning of antinociceptive processes (Forman et al. 1985). These changes occurring in the opioid system with hyperglycemia cause the development of tolerance to the antinociceptive effect of morphine in diabetic animals (Gullapalli et al. 2002).

Spinal and supraspinal administration of μ-opioid receptor (MOR) agonists to animals with diabetic neuropathy resulted in a significant reduction in antinociceptive effect (Kamei et al. 1992). It has been suggested that neuronal and

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non-neuronal factors are responsible for the decrease in the antinociceptive activity of MOR agonists in animals with painful diabetic neuropathy (Mousa et al. 2013; Zychowska et al. 2013). Experimental studies have suggested that the reduction in antinociceptive effect may be related to impaired G-protein responses to MOR or loss of MOR (Mousa et al. 2013). Furthermore, activation of non-neuronal microglia cells in the spinal cord causes a decrease in the antinociceptive effect of morphine in neuropathic pain. Consistent with this, minocycline, a specific microglia inhibitor, increases morphine antinociception in streptozotocin (STZ)-induced diabetic mice (Zychowska et al. 2013). Chronic administration of morphine to mice causes neurotoxic effects by activating apoptotic pathways (Emeterio et al. 2006). Morphine induces apoptosis in the rat dorsal raphe nucleus, suggesting it may be a possible mechanism for morphine antinociceptive tolerance (Charkhpour et al. 2010). In addition, chronic administration of morphine to neonatal rats has been shown to increase neuronal apoptotic cell death (Bajic et al. 2013). Long-term administration of morphine induces upregulation of proapoptotic elements such as Bax and caspase-3 in the spinal cord, while downregulating the antiapoptotic protein Bcl-2 (Mao et al. 2002; Hassanzadeh et al. 2011). However, metformin prevents apoptosis by decreasing Bax and caspase-1 protein levels and increasing Bcl2 protein levels (Kolivand et al. 2017). In addition, chronic morphine administration stimulates glial activity and increases inflammatory cytokines in animals with tolerance to morphine (Raghavendra et al. 2004; Shen et al. 2011).

It has been reported that mammalian target of rapamycin complex 1 (mTORC1) plays an important role in the development of morphine tolerance (Shirooie et al. 2019; Lutz et al. 2015). Furthermore, repeated injections of morphine increase mTORC1 activity in the dorsal horn of the spinal cord, and rapamycin, an mTORC1 inhibitor, blocks these effects (Xu et al. 2014). mTOR is effective in regulating apoptotic cell death, and activation of mTOR inhibits apoptosis regulatory molecules (Faivre et al. 2006). Metformin is used in the clinic to lower blood sugar in patients with type 2 diabetes and has anti-inflammatory, anti-cancer and antioxidant properties (Vazquez-Martin et al. 2009). Furthermore, it has been stated that metformin exerts a potent neuroprotective effect against etoposide-induced apoptotic cellular death in primary cortical neurons of cultured rats (El-Mir et al. 2008). Metformin directly inhibits the mTOR complex 1 signaling pathway by activating the 5′ adenosine monophosphate activated protein kinase (AMPK). Metformin is also able to directly suppress mTOR independently of AMPK (Shirooie et al. 2019). Although the effects of metformin on neuropathic pain are known, its effects on the antinociceptive activity and tolerance of morphine in animals with diabetic neuropathy have not been fully elucidated yet.

In the light of all this information, our aim in this study is to investigate the effects of metformin on morphine-induced apoptosis and antinociceptive tolerance in rats with diabetic neuropathy using biochemical and histochemical methods and thermal antinociceptive tests.

Materials and methods

Animals

Male adult Wistar albino rats weighing 230 to 260 g were used for this study. The animals were obtained from the local Experimental Animal Center (Cumhuriyet University, Sivas, Turkey). All rats were housed under a 12:12 h light/dark (lights on at 7:00 a.m.) schedule at room temperature (22 ± 2 °C) and a humidity of 45–50%. Standard laboratory chow and tap water were available ad libitum. The persons who performed the animal experiments did not know about the previous treatment of the rats. All experiments were carried out between 10.00 and 16.00 h. Before the study, the experimental protocols were approved by the Animal Ethics Committee of Cumhuriyet University (Ethic no: 2019–181). The housing and treatment of the rats followed the guidelines of the "Guide for the Care and Use of Laboratory Rats" (Institute of Laboratory Animal Resources).

Drugs

Metformin (50 mg/kg) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in saline prior to intraperitoneal (i.p.) administration. Morphine HCl (5 mg/kg; Cumhuriyet University Hospital, Sivas, Turkey) was dissolved in sterile 0.9% normal saline and was injected i.p. into rats using 1-ml insulin syringes. Streptozotocin (STZ, Sigma, St. Louis, MO, USA) was dissolved in citrate buffer (pH 4.5) to make a fresh solution and injected intraperitoneally at a dose of 65 mg/kg. The determination of drug doses was based on data obtained from previous similar studies and pre-tests (Zhang et al. 2017; Ozdemir et al. 2011; Zangiabadi et al. 2014].

Induction of morphine tolerance

Morphine tolerance was established in rats with a 3-day cumulative dose administration (Zarrindast et al. 2002). The treatment schedule consisted of twice-daily doses of morphine administered at 30 mg/kg (i.p.) (a.m.) and 45 mg/kg (p.m.) on days 1, 60, and 90 mg/kg on day 2, and 120 mg/kg twice on day 3. The development of tolerance to morphine in rats was evaluated by antinociceptive tests on day 4. Diabetes was induced by STZ (65 mg/kg, i.p.) injection on day 4, and metformin (50 mg/kg, i.p.) and morphine (5 mg/kg) were administered 1 day later. Then, antinociceptive tests

were performed (Fig. 1). Morphine tolerance was defined as a significant decrease in antinociceptive effect when morphine was injected into rats at the test dose (5 mg/kg). First, tail flick (TF) and hot plate (HP) antinociceptive tests were performed for each rat to determine the mean baseline latency time. Then, post-drug latency times for each rat were determined by TF and HP tests at 15, 30, 60, 90, and 120 min after injection of the morphine test dose. Saline group rats were administered the same dose of saline twice a day for 3 days as in the drug groups.

Induction of diabetes

Freshly prepared streptozotocin (STZ, single dose 65 mg/kg) in 0.1-mol/l sodium-citrate buffer (pH 4.5) was injected intraperitoneally in order to induce diabetic neuropathy (Zangiabadi et al. 2014). One day after STZ injection, blood glucose levels were measured by GolDeal Gluco Prober (Aurum Biomedical Tecnology, Hsinchu City, Taiwan). A drop of blood was taken from the tail vein of the animal with a syringe and dripped onto the instrument strip. In the next step, blood glucose levels were read from the device monitor. Rats with a glucose level of ≥ 250 mg/dl were classified as diabetic. After diabetes induction, animals were used for evaluating the effect of metformin on morphine antinociception and tolerance in the diabetic condition.

Antinociceptive tests

Standard thermal pain tests TF test (May TF 0703 TF Unit; Commat, Ankara, Turkey) and HP test (May AHP 0603, Hot Plate; Commat) were used to measure antinociception. In the TF test, after the administration of the drugs to the rats, the radiant heat source was directed to the 2.5-cm distal part of rat tail and the tail flick latency (TFL) times were recorded. The radiant heat source was calibrated so that the basal TFL occurs in an average of 2.8 ± 0.5 s in the pain test. Rats with abnormal responses and basal TFL below 2.3 s or greater than 3.3 s were excluded from subsequent antinociceptive tests. The cut-off latency was set as 20 s to prevent tissue damage. Animals still not responding even within 20 s were excluded from the TF test. Responses to the TF test are often associated with central pain mechanisms (Ramabadran et al. 1989: Kanaan et al. 1996).

In the HP test, rats were placed in a hot plate device at a temperature of 53 ± 0.5 °C. The delay at the first sign of the rat's jump or paw licking response to avoid heat was considered an index of the pain threshold. The cut-off time was arranged at 30 s in order to avoid damage to the rat paw in this test. It is reported that the antinociceptive response in the HP test results from a combination of peripheral and central mechanisms (Kanaan et al. 1996).

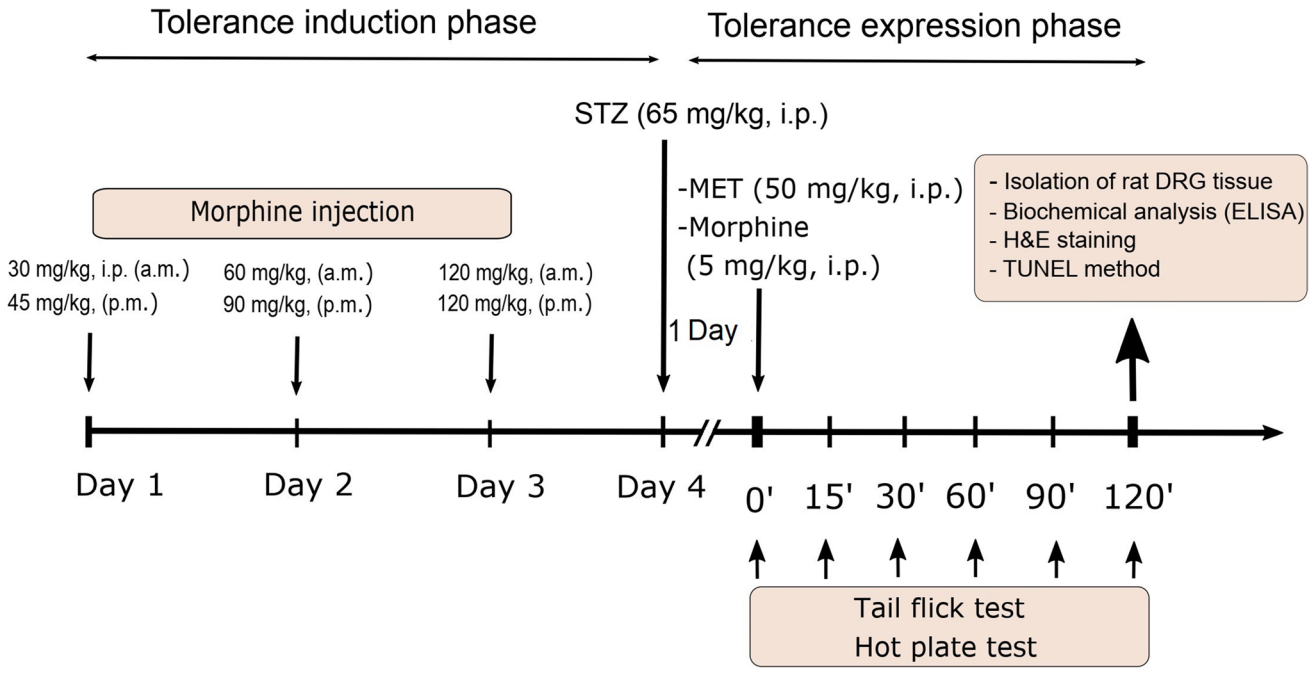

Fig. 1 The timeline shows the experimental protocol. DRG, dorsal root ganglion; MET, metformin; STZ, streptozotocin; i.p., intraperitoneal; H&E, hematoxylin and eosin

Homogenate preparation of the dorsal root ganglia

Wistar-albino rats were euthanized by cervical dislocation, and dorsal root ganglia (DRGs) were aseptically collected from L3 to S4 spinal levels. DRG tissue samples of animals in cold phosphate-buffered saline solution (pH 7.4) were homogenized using a mechanical homogenizer (Analytik Jena speedmill plus, Jena, Germany). It was centrifuged at 4000 rpm for 10 min at a temperature of 4 °C (Avci and Taskiran 2020). After obtaining the supernatants, they were stored at − 80 °C until biochemical analysis. Total protein levels in tissue samples were determined using the Bradford method protein assay kit (Merck KGaA, Darmstadt, Germany) (Kruger 1994).

Measurement of caspase-3, Bax, and Bcl-2 levels

ELISA kits (YL Biont, Shanghai, China) were used to measure caspase-3, Bax, and Bcl-2 levels from DRG tissue samples. The study was performed in accordance with the manufacturer's instructions. In brief, standard and tissue samples were added to the plate and incubated at 37 °C for approximately 60 min. After the washing step was completed, staining solutions were added and incubated for approximately 15 min. The reading was obtained at 450 nm following the addition of the stop solution. Standard curves were drawn to determine the value of the samples. The coefficients of variation within and between the plates were found to be less than 10%.

Detection of apoptotic cells

First, rats were euthanized by ketamine injection and antinociceptive tests 2 h after drug administration for tissue preparation. The explant of DRG neurons was prepared according to the methods described previously (Wang et al. 2016). Briefly, rats were anesthetized and sacrificed. DRGs of the lumbosacral spinal cord at the L3-S4 level were isolated and quickly transferred to a glass petri dish (Corning, USA) containing hanks balanced salt solution at 4 °C in the fixative solution (4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS).

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay was carried out using the in situ cell death detection kit (fluorescein) (Roche Diagnostics GmbH, Mannheim, Germany). It detects DNA fragmentation by labeling the 3′-hydroxyl ends in the double-stranded DNA breaks produced during apoptosis. All procedures were carried out in accordance with the manufacturer's protocol. The DRG tissues were fixed in 10% neutral buffered formalin and washed for total histological examination. Then, it was passed through a series of increasing alcohol (70%, 80%, 90%, and 100%) and triple xylene series and embedded in paraffin. Sagittal and horizontal serial sections of 3–4 µm were taken from tissue samples with a microtome (Leica SM 2000R, Heidelberg, Germany), and they were placed on slides. It was kept in the oven overnight and left for chemical deparaffinization in xylol twice. In order to add water to the tissue, it was passed through a series of decreasing ethyl alcohol, first distilled water, and then PBS 2 times. All tissues were kept in proteinase K solution in a dark and humid environment at 21–37 °C for 30 min. At the end of the period, the tissues were passed through PBS 2 times. About 50 µl of the kit label solution was applied to the negative controls. The enzyme solution was mixed with the label solution and left for incubation. Before the mixture is applied to positive controls, for the detection of DNA breaks, in DNase 1 recombinant solution (3000 U/ml to 3 U/ml in 50 mM Tris–HCl, pH 7.5, 10 mM MgCl2, 1 mg/ml BSA) at 15–25 °C for 10 min was suspended. Afterwards, TUNEL mix solution was applied to the tissues, which were passed through PBS twice, 50 µl on each sample and left to incubation for 60 min in a humid and dark environment at 37 °C. Each tissue sample was washed 3 times with PBS-Triton-X-100. Then, apoptosis was examined semi-quantitatively under fluorescence microscopy (Olympus BX51). Stained sections were photographed with a microscope (Leica DM2500, Nussloch, Germany).

Semi-quantitative scoring system was used to evaluate apoptotic cells with the TUNEL method. Ganglion cells glowing bright green with chromatin aggregation in their nucleus were considered as apoptotic cells. Apoptotic neuron numbers were expressed as percentages. The grades were as follows (Schmeichel et al. 2003): 1, light staining and only affecting ≤ 5% of neurons; 2, staining affecting 5–10% of neurons; 3, staining affecting 10–15% of neurons; 3, staining affecting 15–20% of neurons; 5, staining affecting ≥ 20% of neurons.

For hematoxylin–eosin staining, firstly the tissues obtained from DRG sections were deparaffinized by soaking in xylol for 10 min. Then, the preparations were passed through 95%, 80%, and 70% alcohol, respectively, for 10 min. Tissues were stained with hematoxylin for 5–6 min and washed with water. Washed tissues were immersed in acid alcohol to turn pale blue in a few seconds. Afterwards, the preparations were left in the eosin solution for 3–4 min. To remove excess eosin, the tissues were passed through 70%, 80%, and 95% absolute alcohol, respectively, and were treated with xylol 3 times for 10 min. Preparations extracted from xylol were glued with synthetic resin and left to dry.

Data analysis

To calculate the percentage of maximum possible effect (% MPE), the hot plate test and tail flick test latencies were

converted to percent antinociceptive effect. The equation is as follows:

$$\%\ MPE = [(test\ latency - baseline)/(cut\ off - baseline)] \times 100.$$

Statistical analysis

After the antinociceptive latency times were determined, the means of MPEs in all group rats were calculated. The obtained data were expressed as means ± S.E.M. Normal distribution was assessed in accordance with the Shapiro–Wilk's test. A paired *t*-test statistical analysis was used for the means of the two groups. Data from more than two groups were analyzed by two-way ANOVA and multiple comparisons determined by Tukey test using SPSS computer program (version 22.0 for windows Chicago, IL, USA). In all groups, $p < 0.05$ was considered statistically significant.

Results

Body weight and blood glucose levels in diabetic rats

Before thermal antinociceptive tests, blood glucose levels and body weights of rats were measured before and after STZ administration. The mean body weight of the diabetic rats ($n = 8$) was significantly reduced compared to before STZ injection ($p < 0.01$; Table 1). Furthermore, blood glucose levels (396.66 ± 2.31) were significantly increased after STZ injection into rats ($p < 0.001$). The mean blood glucose level met the criteria for diabetic rats (≥ 250 mg/dl).

Antinociceptive effects of morphine on painful diabetic neuropathy

Antinociceptive test results showed that the maximal antinociceptive effect was observed at 60 min after the administration of morphine (5 mg/kg) in antinociceptive tests of all group rats (Fig. 2A and B). The antinociceptive effect (% MPE) of morphine was significantly higher in both tail flick ($F_{3,20} = 16.85$) and hot plate ($F_{3,20} = 20.23$) tests compared to the saline group ($p < 0.01$). On the contrary, the antinociceptive effect of morphine in the diabetic group significantly decreased compared to the morphine group rats ($p < 0.05$).

Table 1 Body weight and blood glucose levels of STZ-induced diabetic rats

Body weight (g)		Blood glucose (mg/dL)	
Before STZ injection	End of experiment	Before STZ injection	End of experiment
246.16 ± 1.54	218.73 ± 2.47**	129.10 ± 1.83	396.66 ± 2.31***

The data are presented as the mean ± SEM

**$p < 0.01$ and ***$p < 0.001$ compared to before STZ injection (paired *t*-test)

Effects of metformin on morphine antinociception in diabetic rats

Co-administration of metformin (50 mg/kg) with morphine (Fig. 3A and B) showed a significant increase in antinociceptive effect compared to that injected with morphine alone in diabetic rats ($p < 0.05$; $F_{4,25} = 17.62$, and $F_{4,25} = 18.51$, respectively). However, maximal antinociceptive activity was observed in normal rats in which metformin was combined with morphine in antinociceptive tests ($p < 0.001$). Furthermore, these data demonstrated that metformin alone has a significant antinociception in both tail flick and hot plate tests compared to the saline group rats ($p < 0.01$).

Effects of metformin on morphine tolerance

Administration of metformin to morphine tolerant rats significantly attenuated morphine tolerance (increased % MPE) in both the tail-flick ($p < 0.05$; Fig. 4A) and hot-plate test assays ($p < 0.05$; Fig. 4B), as compared to the morphine tolerant rats ($F_{4,25} = 21.3$ for TF test; $F_{4,25} = 23.2$ for HP test). Similarly, administration of metformin to diabetic rats significantly reduced morphine tolerance ($p < 0.05$). In this test, the maximum antinociceptive activity was obtained at 30 min of measurements.

Effect of metformin on caspase 3, Bax and Bcl-2 levels

Caspase 3 levels were significantly increased in the dorsal root ganglia of morphine injected diabetic rats compared to the saline group ($p < 0.01$; Fig. 5A). However, administration of metformin to rats in this group caused a significant decrease in caspase 3 levels ($p < 0.05$). In addition, administration of metformin to morphine-tolerant rats significantly reduced the level of caspase 3 in ganglion tissue ($p < 0.01$). The data showed that the Bax protein level was higher in rats with painful neuropathy than in the saline group ($p < 0.01$; Fig. 5B). Similarly, injection of metformin into morphine-tolerant rats significantly decreased Bax protein levels ($p < 0.01$).

There was a significant decrease in antiapoptotic Bcl-2 protein level in diabetic rats compared to the saline group rats ($p < 0.01$; Fig. 5C). Administration of metformin to morphine-tolerant rats resulted in a significant reduction in Bcl-2 levels ($p < 0.01$).

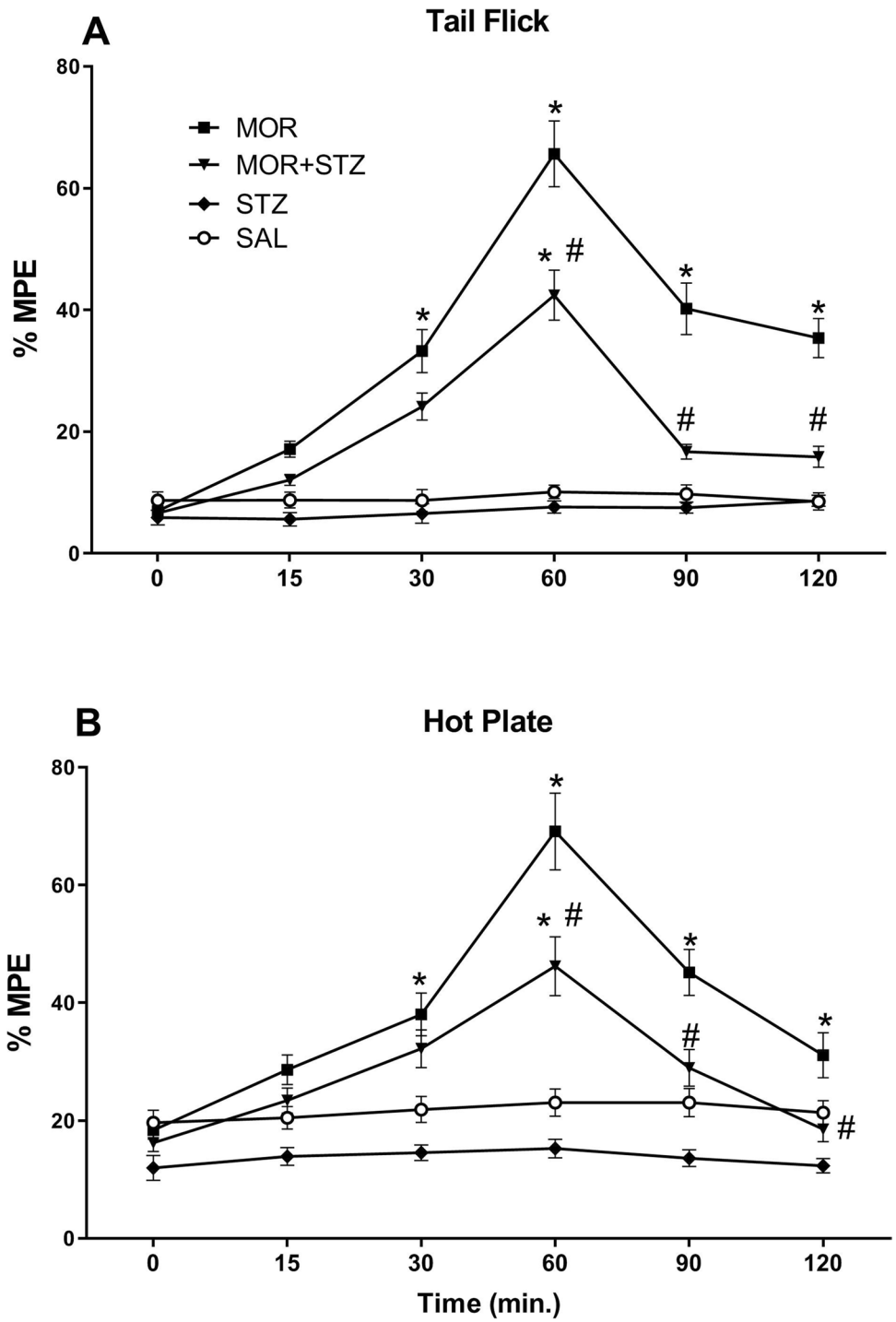

Fig. 2 Antinociceptive effects of morphine on painful diabetic neuropathy. The antinociceptive effects of morphine are demonstrated in (**A**) tail-flick test and (**B**) hot plate test. Each point represents the mean ± SEM of % MPE for 8 rats. $^{*}p < 0.01$ vs. saline group and $^{\#}p < 0.05$ vs. morphine group (two-way ANOVA followed by Tukey HSD post hoc test). MOR, morphine; STZ, streptozotocin; SAL, saline

Effect of metformin on morphine-induced apoptosis

Hematoxylin–eosin (H&E) staining shows that ganglia are located linearly along the axons and surrounded by a single layer of satellite cells surrounding the ganglia in the saline, morphine and metformin groups (Fig. 6A). There were significant improvements in ganglion cell morphology, a decrease in satellite cell count, and an improvement in the structural integrity of the connective tissue in the metformin-administered groups. TUNEL staining revealed important information regarding apoptotic cell numbers in DRG neurons. A semi-quantitative scoring system was used to evaluate apoptotic cells. The percentage of apoptotic cells in the diabetic group was significantly higher than in the saline group ($p < 0.01$; Fig. 6B). Moreover,

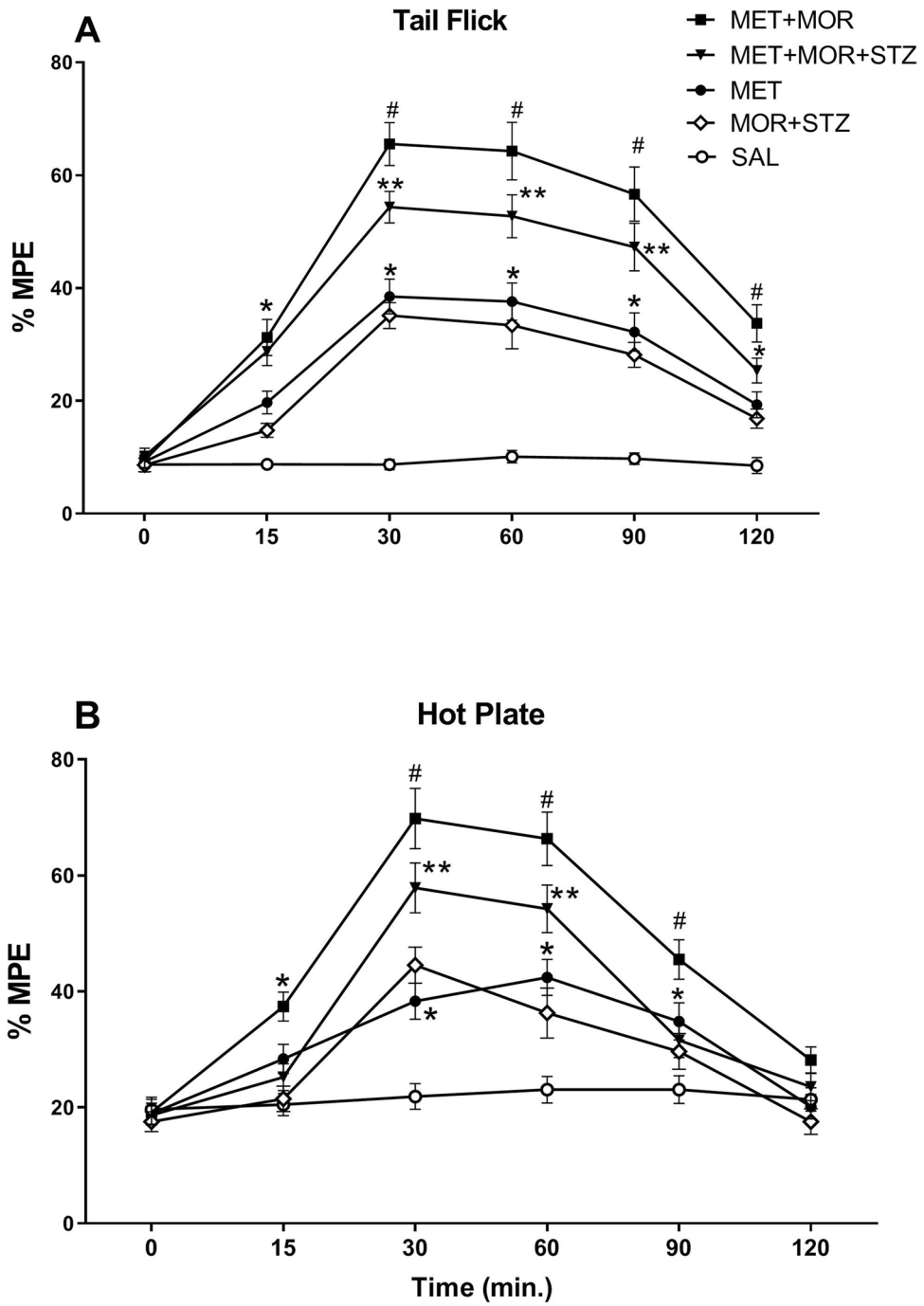

Fig. 3 Effects of metformin on morphine analgesia in diabetic rats. The effects of metformin are demonstrated in (**A**) tail-flick test and (**B**) hot plate test. Each point represents the mean ± SEM of % MPE for 8 rats. $^{\#}p < 0.001$ vs. saline-treated group, $^{*}p < 0.01$ vs. saline-treated group, and $^{**}p < 0.05$ vs. morphine + STZ group (two-way ANOVA followed by Tukey HSD post hoc test). MET, metformin; STZ, streptozotocin; MOR, morphine; SAL, saline

administration of metformin to diabetic rats significantly reduced the number of apoptotic cells ($p < 0.05$; $n = 6$).

Hematoxylin–eosin staining ganglion cell count was decreased in morphine-tolerant rats, and there was a significant increase in satellite cells. In addition, disruptions in the structure of the connective tissue between the ganglion and satellite cells were detected (Fig. 7A). Similarly, the percentage of apoptotic cells showed a significant increase in the morphine tolerant group compared to the saline ($p < 0.01$; Fig. 7B). However, injection of metformin into morphine-tolerant rats resulted in a significant reduction in the number of apoptotic cells ($p < 0.05$).

Discussion

In this study, we demonstrated that the intraperitoneal administration of metformin provided a significant decrease in morphine antinociceptive tolerance. Co-administration of metformin with morphine increased the antinociceptive

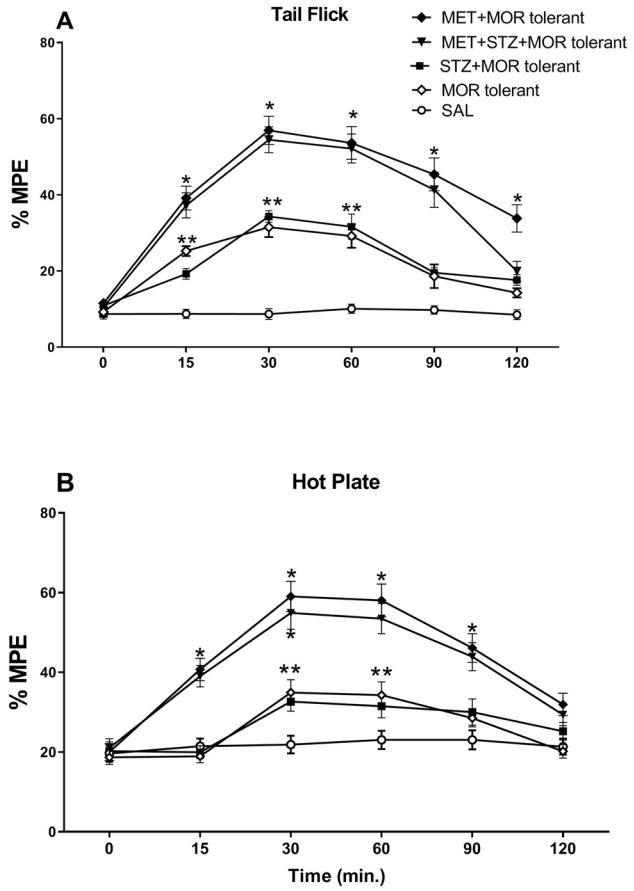

Fig. 4 Effects of metformin on morphine tolerance. The effects of metformin are demonstrated in (**A**) tail-flick test and (**B**) hot plate test. Each point represents the mean ± SEM of % MPE for 7–8 rats. $^{**}p < 0.01$ vs. saline group, $^{*}p < 0.05$ vs. MET + STZ + MOR-tolerant group (two-way ANOVA followed by Tukey HSD post hoc test). MET, metformin; STZ, streptozotocin; MOR, morphine; SAL, saline

activity of morphine in diabetic rats. The data suggested that metformin reduced the levels of the apoptotic proteins caspase 3 and Bax in DRG neurons, while increasing the levels of the antiapoptotic Bcl-2. The apoptosis inhibitory activity of metformin probably plays a significant role in the reduction in morphine tolerance.

The antinociceptive effects of opioids in the management of neuropathic pain have been a topic of current debate in clinical practice and opioid research (Balogh et al. 2019). Opioids are an important drug in acute neuropathic pain states and provide rapid pain relief (Dowell et al. 2016). It has been reported that the antinociceptive activity of morphine is reduced in diabetic animals following systemic administration of μ-opioid receptor (MOR) agonists such as morphine (Courteix et al. 1998; Nielsen et al. 2007). Impaired opioid antinociception occurs as a result of a decrease in the number of opioid receptors (Zhang et al. 1998). Similarly, opioid receptor density is markedly reduced in spinal cord tissues of animals with diabetes (Shaqura et al. 2013). Our results are in agreement with others suggesting that the antinociceptive effect of morphine decreases in diabetic rats. In addition, co-administration of morphine and metformin in morphine-tolerant rats potentiated the antinociceptive effect in diabetic rats. This increase may be attributable to some extent to the antinociceptive effect of metformin, but the reduction in apoptosis may be a more important factor in the reduction in morphine tolerance. Also, according to another view, metformin reduces diabetic neuropathy by controlling blood sugar level and thus causes an increase in MOR concentration in diabetic rats. The decrease in MOR density may be effective in reducing the antinociceptive effect of morphine in diabetic rats (Kou et al. 2016). High doses of morphine are required to reduce neuropathic pain, but this high dose of morphine causes tolerance (Hervera et al. 2012).

Previous studies indicate that the mechanisms of morphine tolerance are complex and involve many factors such as receptors, ion channels, and neural networks (Xu et al. 2014; Ozdemir et al. 2011; Altun et al. 2015). Moreover, recent evidence indicates that neuronal apoptosis and inflammation play a crucial role in the development of morphine tolerance (Charkhpour et al. 2010; Lutz et al. 2015). The antidiabetic drug metformin exerts anti-inflammatory and neuroprotective effects on neurons of the central and peripheral nervous system and reducing hyperalgesia and neuropathic pain in damaged nerves (Pan et al. 2016; Afshari et al. 2018). In addition, metformin plays an important role in autophagy and locomotor recovery processes after spinal cord injury through mTOR inhibition (Zhang et al. 2017). Numerous studies indicate that the mTOR plays an important role in the development of tolerance to morphine. Activation of the mTOR pathway inhibits opioid-induced antinociception and accelerates the development of tolerance to opioids (Shirooie et al. 2019). In addition, repeated long-term injections of morphine increase thermal latency and mTORC1 activity in the dorsal horn of the spinal cord. Co-injection of rapamycin (an mTORC1 inhibitor) with morphine inhibits these effects (Xu et al. 2014). In this study, inhibition of the MTOR pathway by metformin probably played an important role in reducing morphine tolerance.

Current studies indicate that activation of non-neuronal cells such as microglia plays an important role in the development of neuropathic pain and that these cells are activated in conditions of diabetes in the spinal cord (Daulhac et al. 2006; Mika et al. 2009). Activated microglia in the spinal cord cause neuropathic pain generation through the release of proinflammatory cytokines, which are common mediators of allodynia and hyperalgesia (Mika et al. 2009; Watkins et al. 2007). In addition, available evidence indicates that chronic morphine treatment induces glial activity in the spinal cord and increases proinflammatory cytokines in

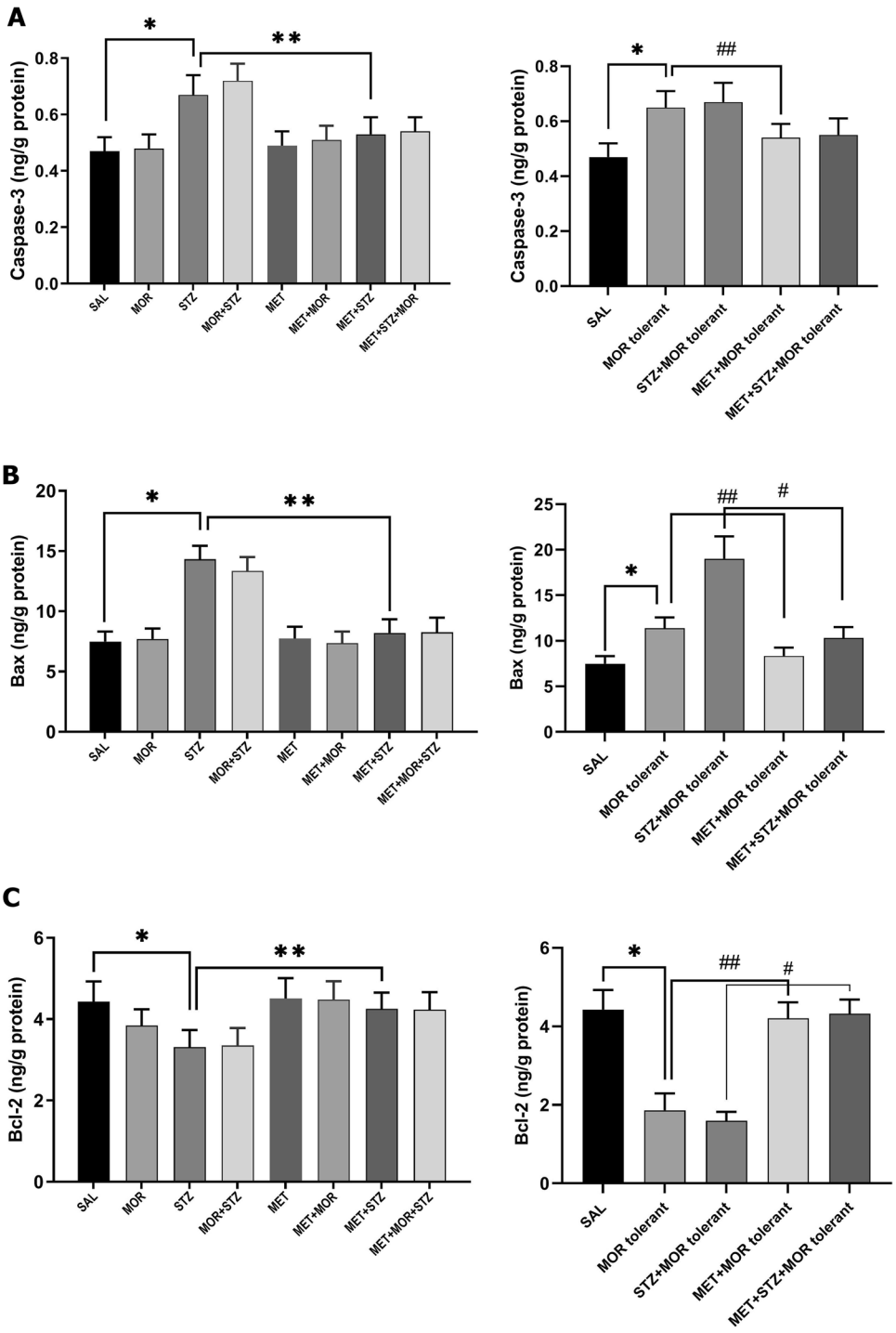

Fig. 5 The effect of metformin on caspase 3 (**A**), Bax (**B**), and Bcl-2 (**C**) levels. Each point represents the mean ± SEM, $n = 6$. $^{*}p < 0.05$ vs. STZ group, $^{**}p < 0.01$ vs. saline group, $^{\#}p < 0.05$ vs. MOR-tolerant group, and $^{\#\#}p < 0.01$ vs. STZ + MOR-tolerant group. MET, metformin; STZ, streptozotocin; MOR, morphine; SAL, saline

morphine-tolerant animals (Shen et al. 2011). Recent reports demonstrate that activation of the p38 MAPK pathway in dorsal horn microglia is responsible for diabetes-induced hyperalgesia (Daulhac et al 2006). Metformin increases AMPK phosphorylation and significantly reduces opioid tolerance by preventing morphine-induced microglial activation. Moreover, metformin significantly reduces the upregulation of TNF-α mRNA and IL-6 level induced by morphine, which plays an important role in the attenuation of morphine tolerance (Pan et al. 2016).

Classical evidences suggest that opioid-induced antinociception and tolerance are a function of the central nervous system. However, recent studies have revealed the critical role of nociceptive DRG neurons in expressing antinociception and inducing opioid tolerance (Muchhala et al. 2021). Knockout of μ-opioid receptors in DRG neurons expressing

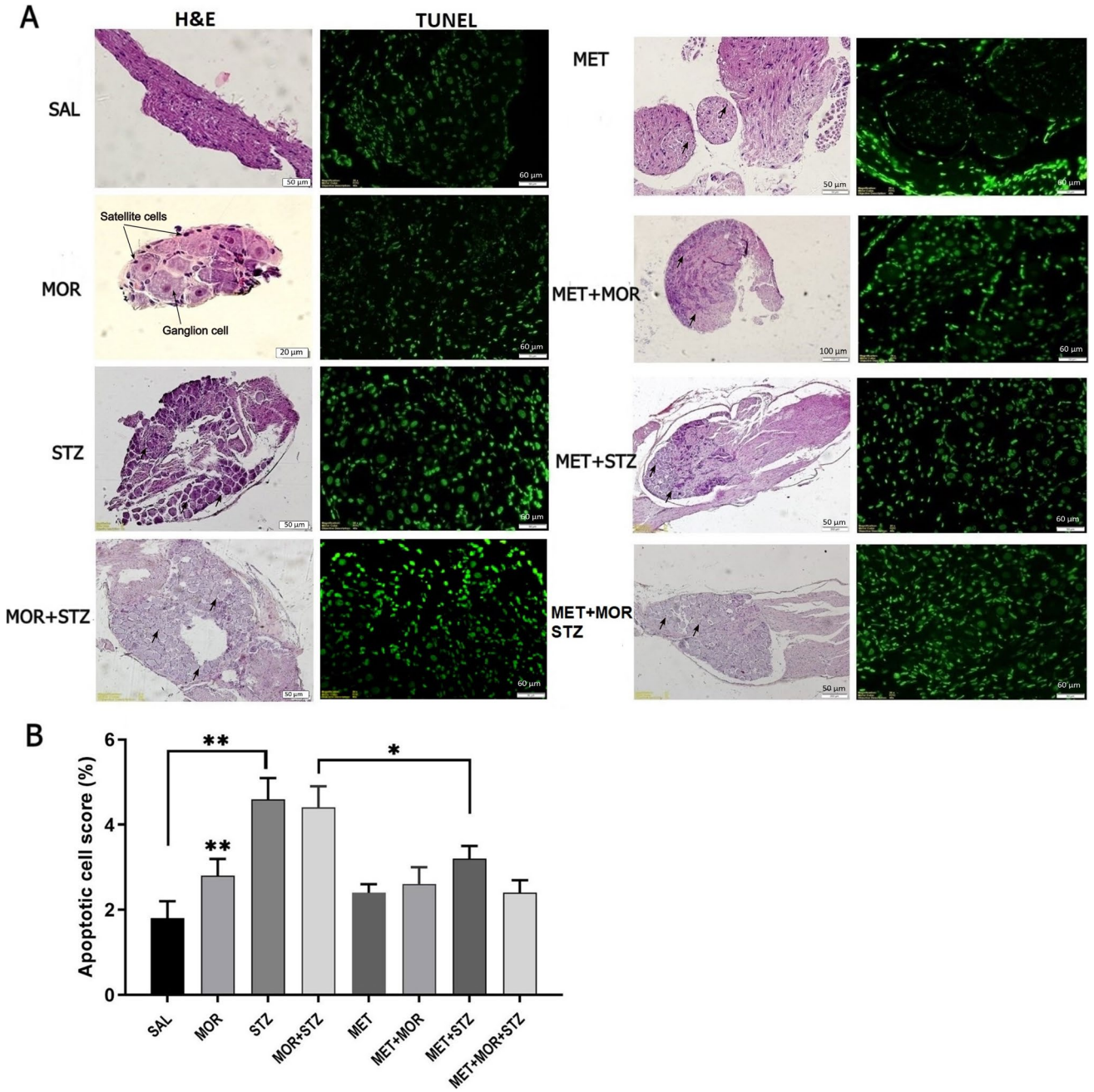

Fig. 6 Effect of metformin on cell morphology and morphine-induced apoptosis in diabetic rats. Tissue sections were prepared from rat dorsal root ganglia. H&E staining in tissue sections was used to evaluate cell morphology. H&E-stained cell nuclei and tissue elements are seen in blue and cytoplasmic elements in light pink. Slides were analyzed with a light microscope (×100 objective). Apoptosis was tested with cell death detection kit (fluorescein). Apoptotic cells scattered throughout the tissue section were prominently stained (fluorescent) with TUNEL (×100 objective). (**A**) shows hematoxylin–eosin and TUNEL staining in DRG tissue, (**B**) shows percantage of apoptotic cells by semiquantitative scoring system. $n=6$. $^{**}p<0.01$ vs. saline-treated group, $^{*}p<0.05$ vs. MOR + STZ group. MET, metformin; STZ, streptozotocin; MOR, morphine; SAL, saline

transient receptor potential vanilloid-1 (TRPV1) or ablation of DRG neurons expressing TRPV1 channel reduces opioid tolerance (Chen et al. 2007; Corder et al. 2017). Additionally, selective deletion of μ-opioid receptors from Nav1.8-containing neurons in DRG causes a decrease in the antinociceptive activity of morphine in inflammatory pain (Weibel et al. 2013). However, elimination of μ-opioid receptors from DRG neurons blocks the acute supraspinal and spinal antinociceptive activities of opioids (Sun et al. 2020; Boronat et al. 2001). β-Arrestin-2 is a critical mediator

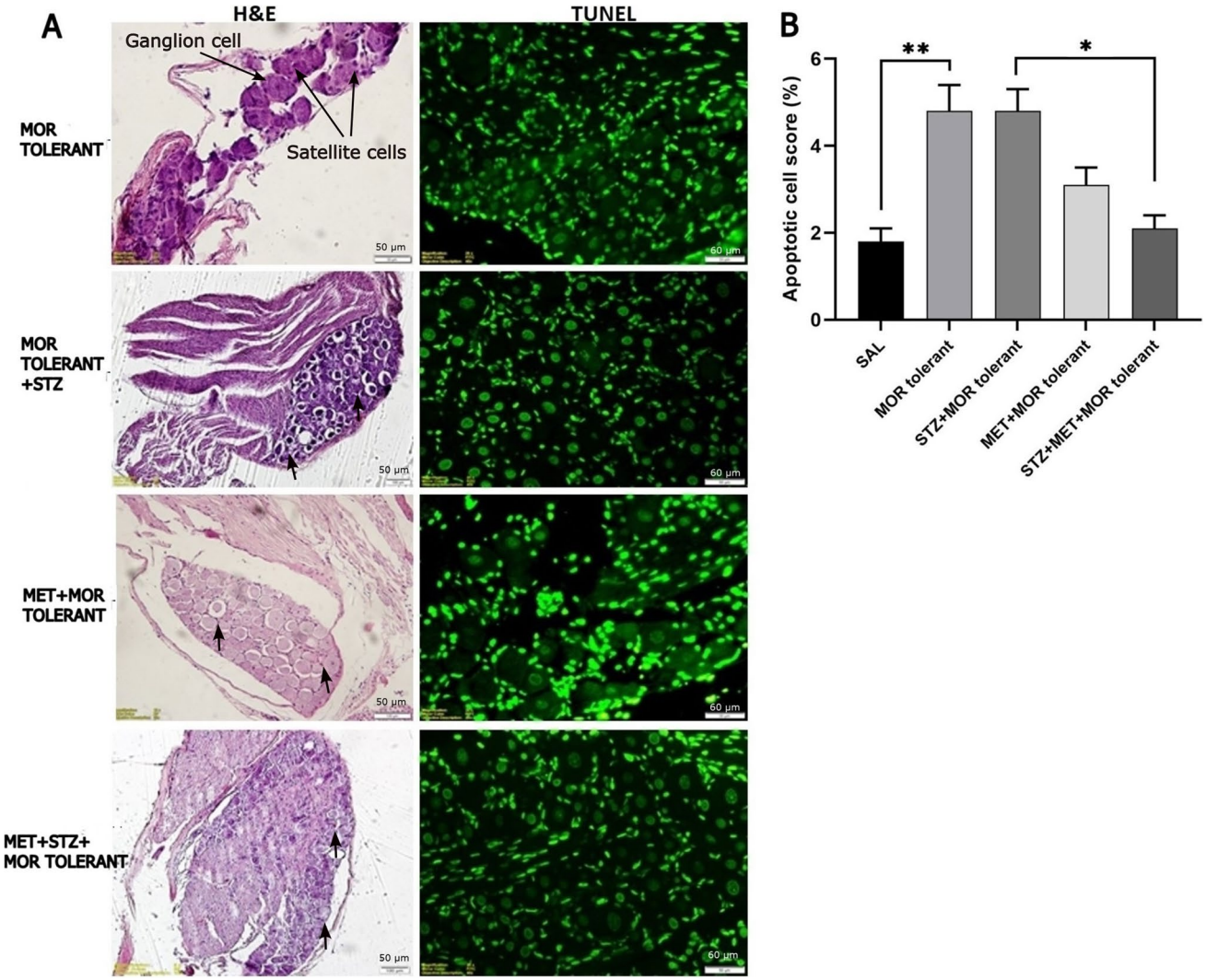

Fig. 7 Effect of metformin on cell morphology and morphine-induced apoptosis in morphine-tolerant rats. Tissue sections were prepared from rat dorsal root ganglia. H&E staining in tissue sections was used to evaluate cell morphology. H&E-stained cell nuclei and tissue elements are seen in blue and cytoplasmic elements in light pink. Slides were analyzed with a light microscope (×100 objective). Apoptosis was tested with cell death detection kit (fluorescein). Apoptotic cells scattered throughout the tissue section were prominently stained (fluorescent) with TUNEL (×100 objective). (**A**) shows hematoxylin–eosin and TUNEL staining in DRG tissue (×100), (**B**) shows percantage of apoptotic cells by semiquantitative scoring system. $n=6$. $^{**}p<0.01$ vs. saline-treated group, $^{*}p<0.05$ vs. STZ + MOR-tolerant group

in the development of acute tolerance to opioids in DRG nociceptive neurons. Therefore, it can be understood that DRG neurons have an important role in the development of anticiceptive tolerance to opioids.

Different anti-apoptotic mediators reduce the antinociceptive tolerance of opioids by preventing apoptosis in the central nervous system. However, chronic morphine administration in rats causes significant changes in Bax and Bcl-2 proteins and induces apoptosis (Hassanzadeh et al. 2011; Boronat et al. 2001). It has been shown that donepezil, an anti-apoptotic drug, attenuates opioid tolerance by increasing anti-apoptotic Bcl-2 protein mRNA levels, while decreasing proapoptotic Bax gene expression (Shafie et al. 2015). Biochemical data in this study indicated that metformin decreased apoptosis by increasing the antiapoptotic Bcl-2 levels, while decreasing the levels of caspase 3 and Bax, which are apoptotic proteins in DRG neurons. Mitochondrial permeability transition pore (PTP) or Bcl-2 family proteins play an important role in the regulation of the intrinsic apoptotic cascade. Under stress conditions, PTP opens, and the outer mitochondrial membrane is locally ruptured, and apoptogenic proteins, apoptosis-inducing factor, and cytochrome c are released into the cytosol (Kroemer and Reed 2000). Metformin inhibits PTP

opening and subsequent cell death in different endothelial cell types exposed to high glucose levels (Detaille et al. 2005). In one study, administration of minocycline (a second-generation tetracycline) to rats reduced apoptotic cell numbers in TUNEL staining of the spinal cord and attenuated development of tolerance to morphine (Hassanzadeh et al. 2011). Similarly, TUNEL staining data of our study demonstrated that metformin reduced apoptotic cell numbers in DRG neurons of diabetic rats. The apoptotic score protection of metformin in morphine-administered rats is an important factor in increasing the antinociceptive effect. This study demonstrated important results in terms of the effects of metformin on morphine antinociceptive activity and tolerance in animals with diabetic neuropathy. Further studies should be planned to fully elucidate the mechanism of action of metformin on morphine tolerance in animals with diabetic neuropathy.

Conclusion

In summary, our study results indicated that the administration of metformin with morphine increased the antinociceptive efficiency of morphine and attenuated antinociceptive tolerance in thermal antinociceptive tests. In addition, metformin prevented morphine-induced apoptosis in the dorsal root ganglia of diabetic rats by increasing antiapoptotic Bcl-2 levels and decreasing proapoptotic caspase 3 and Bax levels. Furthermore, metformin resulted in a significant reduction in the number of apoptotic cells in DRG neurons. Further studies will elucidate possible mechanisms for the modulatory role of metformin on the effects of morphine antinociception in diabetic rats.

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Author contribution All the authors contributed to the study conception and design. OA and EO conceived and designed the study, performed the statistical analysis, writing, and review. AST performed the behavioral tests and biochemical analysis. ZDSI performed the immunohistochemical analyses and wrote the histological comments. SG did the conceptualization, supervision, and formal analysis. The authors declare that all the data were generated in-house and that no paper mill was used.

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Data Availability We confirm that all the data and materials are authentic and available.

Declarations

Ethical approval Experiment protocols were approved by the Cumhuriyet University National Animal Ethics Committee (Ethics no: 2019–181).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Afshari K, Dehdashtian A, Haddadi N-S, Haj-Mirzaian A, Iranmehr A, Ebrahimi MA et al (2018) Anti-inflammatory effects of metformin improve the neuropathic pain and locomotor activity in spinal cord injured rats: introduction of an alternative therapy. Spinal Cord 56:1032–1041

Altun A, Ozdemir E, Yildirim K, Gursoy S, Durmus N, Bagcivan I (2015) The effects of endocannabinoid receptor agonist anandamide and antagonist rimonabant on opioid analgesia and tolerance in rats. Gen Physiol Biophys 34:433–440

Avci O, Taskiran AS (2020) Anakinra, an interleukin-1 receptor antagonist, increases the morphine analgesic effect and decreases morphine tolerance development by modulating oxidative stress and endoplasmic reticulum stress in rats. Turkish J Med Sci 50:2048–2058

Bajic D, Commons KG, Soriano SG (2013) Morphine-enhanced apoptosis in selective brain regions of neonatal rats. Int J Dev Neurosci 31:258–266

Balogh M, Zádor F, Zádori ZS, Shaqura M, Király K, Mohammadzadeh A et al (2019) Efficacy-based perspective to overcome reduced opioid analgesia of advanced painful diabetic neuropathy in rats. Front Pharmacol 10:347

Boronat MA, Garcia-Fuster MJ, Garcia-Sevilla JA (2001) Chronic morphine induces upregulation of the pro-apoptotic Fas receptor and down-regulation of the anti-apoptotic Bcl-2 oncoprotein in rat brain. Br J Pharmacol 134:1263–1270

Charkhpour M, Nayebi AR, Doustar Y, Hassanzadeh K (2010) 8-OH-DPAT prevents morphine-induced apoptosis in rat dorsal raphe nucleus: a possible mechanism for attenuating morphine tolerance. Anesth Analg 111:1316–1321

Chen SR, Pan HL (2002) Hypersensitivity of spinothalamic tract neurons associated with diabetic neuropathic pain in rats. J Neurophysiol 87:2726–2733

Chen S-R, Prunean A, Pan H-M, Welker KL, Pan H-L (2007) Resistance to morphine analgesic tolerance in rats with deleted transient receptor potential vanilloid type 1-expressing sensory neurons. Neuroscience 145:676–685

Corder G, Tawfik VL, Wang D, Sypek EI, Low SA, Dickinson JR et al (2017) Loss of μ opioid receptor signaling in nociceptors, but not microglia, abrogates morphine tolerance without disrupting analgesia. Nat Med 23:164–173

Courteix C, Bourget P, Caussade F, Bardin M, Coudore F, Fialip J et al (1998) Is the reduced efficacy of morphine in diabetic rats caused by alterations of opiate receptors or of morphine pharmacokinetics? J Pharmacol Exp Ther 285:63–70

Daulhac L, Mallet C, Courteix C, Etienne M, Duroux E, Privat AM et al (2006) Diabetes-induced mechanical hyperalgesia involves spinal mitogen-activated protein kinase activation in neurons and microglia via N-methyl-D-aspartate-dependent mechanisms. Mol Pharmacol 70:1246–1254

Detaille D, Guigas B, Chauvin C, Batandier C, Fontaine E, Wiernsperger N et al (2005) Metformin prevents high glucose-induced endothelial cell death through a mitochondrial permeability transition-dependent process. Diabetes 4:2179–2187

Dowell D, Haegerich TM, Chou R (2016) CDC guideline for prescribing opioids for chronic pain-United States. JAMA 315:1624–1645

El-Mir MY, Detaille D, R-Villanueva G, Delgado-Esteban M, Guigas B, Attia S et al (2008) Neuroprotective role of antidiabetic drug metformin against apoptotic cell death in primary cortical neurons. J Mol Neurosci 34:77–87

Emeterio EP, Tramullas M, Hurle MA (2006) Modulation of apoptosis in the mouse brain after morphine treatments and morphine withdrawal. J Neurosci Res 83:1352–1361

Faivre S, Kroemer G, Raymond E (2006) Current development of mTOR inhibitors as anticancer agents. Nat Rev 5:671–678

Forman LJ, Marquis DE, Stevens R, Adler R, Vasilenko P (1985) Diabetes induced by STZ results in a decrease in immunoreactive beta-endorphin levels in the pituitary and hypothalamus of female rats. Diabetes 34:1104–1107

Gullapalli S, Gurumoorthy K, Kaul CL, Ramarao P (2002) Role of L-type Ca2+ channels in attenuated morphine antinociception in streptozotocin-diabetic rats. Eur J Pharmacol 435:187–194

Hassanzadeh K, Habibi-Asl B, Farajnia S, Roshangar L (2011) Minocycline prevents morphine-induced apoptosis in rat cerebral cortex and lumbar spinal cord: a possible mechanism for attenuating morphine tolerance. Neurotox Res 19:649–659

He J, Wang HB, Huang JJ, Zhang L, Li DL, He WY et al (2021) Diabetic neuropathic pain induced by streptozotocin alters the expression profile of non-coding RNAs in the spinal cord of mice as determined by sequencing analysis. Exp Ther Med 22(1):775

Hervera A, Leánez S, Pol O (2012) The inhibition of the nitric oxide-cGMP-PKG-JNK signaling pathway avoids the development of tolerance to the local antiallodynic effects produced by morphine during neuropathic pain. Eur J Pharmacol 685:42–51

Hicks CW, Selvin E (2019) Epidemiology of peripheral neuropathy and lower extremity disease in diabetes. Curr Diab Rep 19(10):86

Jamison RN, Mao J (2015) Opioid analgesics. Mayo Clin Proc 90(7):957–968

Kamei J, Ohhashi Y, Aoki T, Kawasima N, Kasuya Y (1992) Streptozotocin-induced diabetes selectively alters the potency of analgesia produced by mu-opioid agonists, but not by delta- and kappa-opioid agonists. Brain Res 571:199–203

Kanaan SA, Saade NE, Haddad JJ, Abdelnoor AM, Atweh SF, Jabbur SJ, Garabedian BS (1996) Endotoxin-induced local inflammation and hyperalgesia in rats, a new model for inflammatory pain. Pain 66:373–379

Kolivand S, Motevaseli E, Cheki M, Mahmoudzadeh A, Shirazi A, Fait V (2017) The anti-apoptotic mechanism of metformin against apoptosis induced by ionizing radiation in human peripheral blood mononuclear cells. Klin Onkol 30(5):372–379

Kou Z-Z, Wan F-P, Bai Y, Li C-Y, Hu J-C, Zhang G-T et al (2016) Decreased endomorphin-2 and μ-opioid receptor in the spinal cord are associated with painful diabetic neuropathy. Front Mol Neurosci 9:80

Kroemer G, Reed JC (2000) Mitochondrial control of cell death. Nat Med 6:513–519

Kruger NJ (1994) The Bradford method for protein quantitation. In: Protoc P (ed) Handbook. New Jersey, Humana Press, pp 15–22

Lutz BM, Nia S, Xiong M, Tao YX, Bekker A (2015) mTOR, a new potential target for chronic pain and opioid-induced tolerance and hyperalgesia. Mol Pain 11(1):32

Mao J, Sung B, Ji RR, Lim G (2002) Neuronal apoptosis associated with morphine tolerance: evidence for an opioid-induced neurotoxic mechanism. J Neurosci 22:7650–7661

Mika J, Osikowicz M, Rojewska E, Korostyñski M, Wawrzczak-Bargiela A, Przewlocki R, Przewlocka B (2009) Differential activation of spinal microglial and astroglial cells in a mouse model of peripheral neuropathic pain. Eur J Pharmacol 623:65–72

Mousa SA, Shaqura M, Khalefa BI, Zöllner C, Schaad L, Schneider J et al (2013) Rab7 silencing prevents μ-opioid receptor lysosomal targeting and rescues opioid responsiveness to strengthen diabetic neuro-pathic pain therapy. Diabetes 62:1308–1319

Muchhala KH, Jacob JC, Dewey WL, Akbarali HI (2021) Role of β-arrestin-2 in short- and long-term opioid tolerance in the dorsal root ganglia. Eur J Pharmacol 899:174007

Nielsen CK, Ross FB, Lotfipour S, Saini KS, Edwards SR, Smith MT (2007) Oxycodone and morphine have distinctly different pharmacological profiles: radioligand binding and behavioural studies in two rat models of neuropathic pain. Pain 132:289–300

Ozdemir E, Bagcivan I, Durmus N, Altun A, Gursoy S (2011) The nitric oxide–cGMP signaling pathway plays a significant role in tolerance to the analgesic effect of morphine. Can J Physiol Pharmacol 89:89–95

Pan Y, Sun X, Jiang L, Hu L, Kong H, Han Y et al (2016) Metformin reduces morphine tolerance by inhibiting microglial-mediated neuroinflammation. J Neuroinflammation 13:294

Patil PR, Wolfe J, Said Q, Thomas J, Martin BC (2015) Opioid use in the management of diabetic peripheral neuropathy (DPN) in a large commercially insured population. Clin Pain 31(5):414–424

Raghavendra V, Tanga FY, Deleo JA (2004) Attenuation of morphine tolerance, withdrawal-induced hyperalgesia, and associated spinal inflammatory immune responses by propentofylline in rats. Neuropsychopharmacology 29:327–334

Ramabadran K, Bansinath M, Turndorf H, Puig MM (1989) The hyperalgesic effect of naloxone is attenuated in streptozotocin-diabetic mice. Psychopharmacology (Berl) 97:169–174

Schmeichel AM, Schmelzer JD, Low PA (2003) Oxidative injury and apoptosis of dorsal root ganglion neurons in chronic experimental diabetic neuropathy. Diabetes 52:165–171

Shafie A, Moradi F, Izadpanah E, Mokarizadeh A, Moloud MR, Nikzaban M et al (2015) Neuroprotection of donepezil against morphine-induced apoptosisis mediated through Toll-like receptors. Eur J Pharmacol 764:292–297

Shaqura M, Khalefa BI, Shakibaei M, Winkler J, Al-Khrasani M, Fürst S et al (2013) Reduced number, G protein coupling, and antinociceptive efficacy of spinal mu-opioid receptors in diabetic rats are reversed by nerve growth factor. J Pain 14:720–730

Shen CH, Tsai RY, Shih MS, Lin SL, Tai YH, Chien CC, Wong CS (2011) Etanercept restores the antinociceptive effect of morphine and suppresses spinal neuroinflammation in morphine-tolerant rats. Anesth Analg 112:454–459

Shirooie S, Sahebgharani M, Esmaeili J, Dehpour AR (2019) In vitro evaluation of effects of metformin on morphine and methadone tolerance through mammalian target of rapamycin signaling pathway. J Cell Physiol 234:3058–3066

Sun J, Chen SR, Pan HL (2020) μ-Opioid receptors in primary sensory neurons are involved in supraspinal opioid analgesia. Brain Res 1729:146623

Vazquez-Martin A, Oliveras-Ferraros C, Menendez JA (2009) The antidiabetic drug metformin suppresses HER2 (erbB-2) oncoprotein overexpression via inhibition of the mTOR effector p70S6K1 in human breast carcinoma cells. Cell Cycle 8:88–96

Wang Y, Ni H, Zhang W, Wang X, Zhang H (2016) Downregulation of miR-210 protected bupivacaine-induced neurotoxicity in dorsal root ganglion. Exp Brain Res 234:1057–1065

Watkins LR, Hutchinson MR, Ledeboer A, Wieseler-Frank J, Milligan ED, Maier SF, Norman Cousins Lecture (2007) Glia as the "bad guys": implications for improving clinical pain control and the clinical utility of opioids. Brain Behav Immun. 21:131–46

Weibel R, Reiss D, Karchewski L, Gardon O, Matifas A, Filliol D et al (2013) Mu opioid receptors on primary afferent Nav1.8 neurons

contribute to opiate-induced analgesia: insight from conditional knockout mice. PloS One. 8(9):e74706

Xu JT, Zhao JY, Zhao X, Ligons D, Tiwari V, Atianjoh FE, Lee CY et al (2014) Opioid receptor–triggered spinal mTORC1 activation contributes to morphine tolerance and hyperalgesia. J Clin Invest 124:592–603

Zangiabadi N, Mohtashami H, Hojatipour M, Jafari M, Asadi-Shekaari M, Shabani M (2014) The effect of angipars on diabetic neuropathy in STZ-induced diabetic male rats: a study on behavioral, electrophysiological, sciatic histological and ultrastructural indices. Sci World J 4:1–8

Zarrindast M-R, Dinkoub Z, Homayoun H, Bakhtiarian A, Khavandgar S (2002) Dopamine receptor mechanisms and morphine tolerance in mice. J Psychopharmacol 16:261–266

Zhang D, Xuan J, Zheng BB, Zhou YL, Lin Y, Wu YS et al (2017) Metformin improves functional recovery after spinal cord injury via autophagy flux stimulation. Mol Neurobiol 54:3327–3341

Zhang X, Bao L, Shi T-J, Ju G, Elde R, Hökfelt T (1998) Downregulation of mu-opioid receptors in rat and monkey dorsal root ganglion neurons and spinal cord after peripheral axotomy. Neuroscience 82:223–240

Zychowska M, Rojewska E, Kreiner G, Nalepa I, Przewlocka B, Mika J (2013) Minocycline influences the anti-inflammatory interleukins and enhances the effectiveness of morphine under mice diabetic neuropathy. J Neuroimmunol 262:35–45

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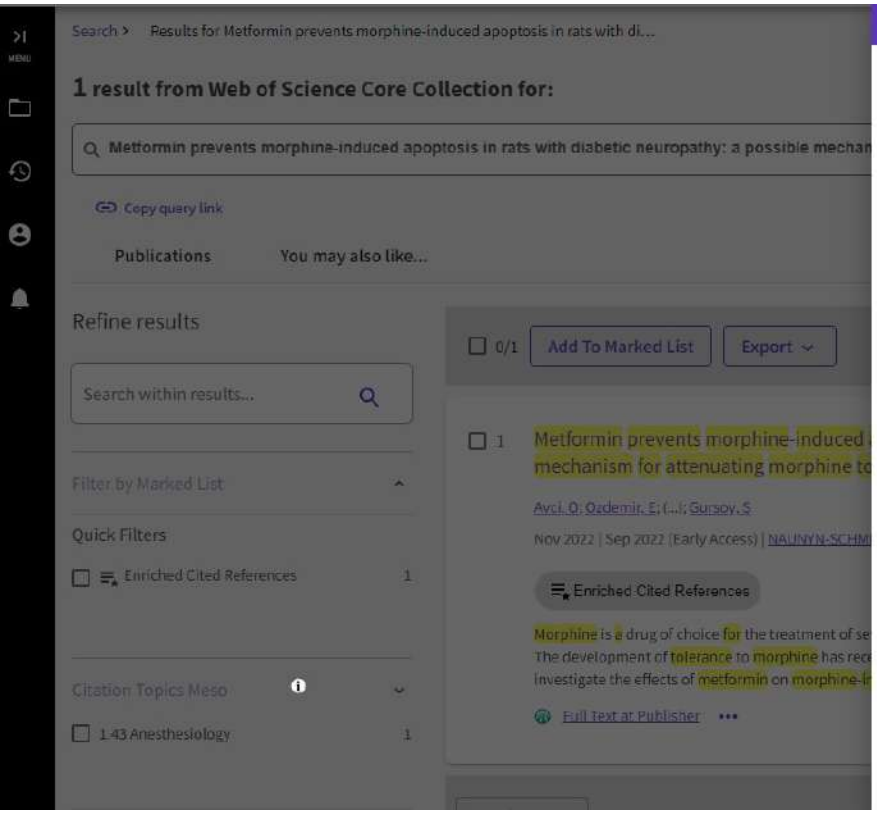
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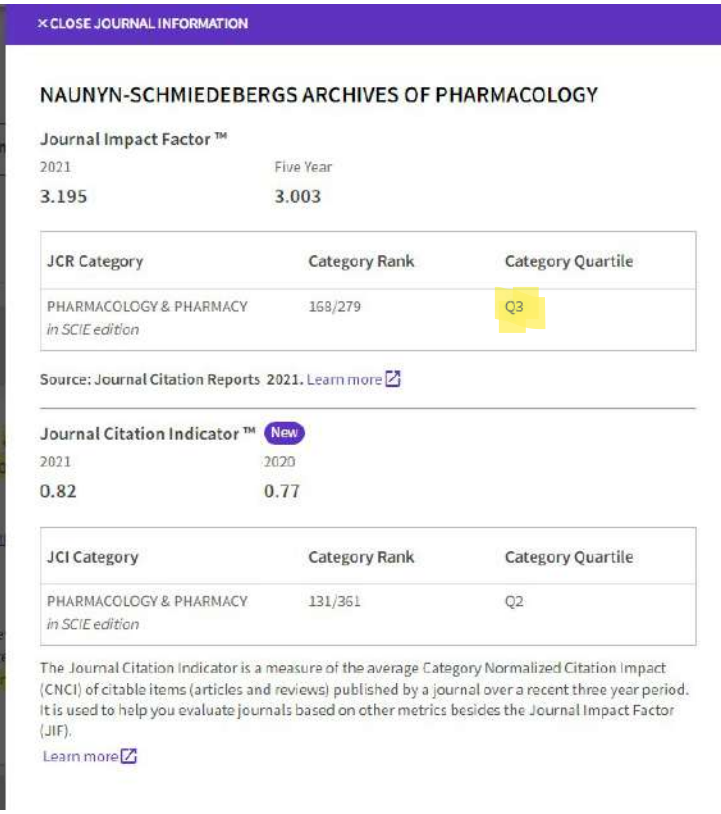
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