Dezocine attenuates neuropathic pain by inhibiting ERK1/2 signaling in rats

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Research article

Keywords: Dezocine, neuropathic pain, ERK1/2 signaling

Posted Date: August 8th, 2019

DOI: <https://doi.org/10.21203/rs.2.12507/v1>

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Abstract

Background Neuropathic pain severely impacts patients' life quality. Dezocine can be used for the treatment of pain. The present study intended to explore the effects of dezocine in chronic constriction injury (CCI) induced neuropathic pain as well as the possible responsible molecules in rats. **Methods** There were 3 subgroups, ie, control group, CCI group and dezocine+CCI group. The values of paw withdrawal threshold (PWT) and paw withdrawal latency (PWL) in rats were determined by a dynamic plantar esthesiometer. The ipsilateral lumbar spinal cords in rats were extracted for the detection of protein levels of phosphorylated-mammalian target of rapamycin (p-mTOR) and p-extracellular signal-regulated kinase 1/2 (p-ERK1/2) by western blot analysis; and the mRNA and protein expression levels of interleukin (IL)-6, tumor necrosis factor (TNF)- α , and cyclooxygenase-2 (COX-2) by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and enzyme-linked immunosorbent assay (ELISA), respectively. **Results** In comparison with control group, there were lower values of PWT and PWL in CCI group, which were partially reversed by dezocine. In addition, compared to control group, the expression levels of p-mTOR, p-ERK1/2, IL-6, TNF- α and COX-2 were upregulated by CCI, which were attenuated by dezocine. **Conclusions** In conclusion, the analgesic effect of dezocine on CCI induced neuropathic pain might be correlated with inhibiting of the p-mTOR and p-ERK1/2 signaling pathway.

Background

Neuropathic pain, a syndrome of chronic pain, is resulted from a lesion or dysfunction in the somatosensory system which are brought about by trauma, autoimmune disease, or cancer, *etc* [1, 2]. Neuropathic pain is common in clinical, with the morbidity of approximately 7%- 10% [3]. The life quality of patients with neuropathic pain are as low as those with clinical depression, coronary artery disease, recent myocardial infarction, or diabetes mellitus [4]. Whereas, the treatment of neuropathic pain remains a huge difficulty in clinical.

Opioid analgesics are widely used for the treatment of pain, while there is controversy for them in treating neuropathic pain [5]. Dezocine is projected to be the bridged aminotetralin analog for pentazocine, which was developed in 1970s and acted as a mixed partial agonist/antagonist for opioid receptor, to improve the efficacy of analgesics [6, 7].

Dezocine exhibits less liability of analgesic tolerance as well as physical dependence than opioids [8, 9]. Dezocine is reported to antagonize morphine analgesia in mice acute nociception model [10]. Moreover, dezocine is discovered to have antinociceptive effects on complete Freund's adjuvant-induced inflammatory pain in rats [11]. The present study meant to explore the effects of dezocine on CCI-induced rat neuropathic pain model and the possible molecules that were involved in.

Methods

Animals

Adult (8-week old) male Sprague-Dawley rats which were purchased from Shanghai Experimental Animal Institute (Shanghai, China) were used for the establishment of CCI model of neuropathic pain. Rats were fed in the environment with controlled temperature, humidity, 12-h light/dark cycle, and ad libitum access to food/water. The experiments were approved by the Animal Care and Welfare Committee of Hubei Maternal and Child Health Hospital Affiliated to Huazhong University of Science and Technology and carried out according to the principles of the Committee for Research and Ethical Issues of International Association for the Study of Pain (IASP).

CCI model of neuropathic pain in rats

Rats were randomly divided into 3 groups, ie, control group, CCI group and dezocine (Yangzi River Pharmaceuticals Group, Taizhou, Jiangsu, China) +CCI group.

The establishment of the CCI model was conducted according to a previously reported study [12]. Briefly, rats were anesthetized with sodium pentobarbital (40 mg/kg) before surgery. In CCI group, the male SD rats were subjected to partial ligation of the sciatic nerve and intraperitoneal (IP) injection of 2 ml saline/day during the experiments. In dezocine+CCI group, rats were subjected to partial ligation of the sciatic nerve and IP injected with 2 ml dezocine (3 mg/kg/day). As for rats in control group, the nerves were exposed but not ligation, followed by IP injection of 2 ml saline/day.

Measurement of PWL and PWT values

The values of PWL and PWT in rats were determined by a Dynamic Plantar Aesthesiometer (Ugo Basile) at 1, 3, 7, and 14 days after CCI.

The PWL was applied for the evaluation of nociceptive response in thermal hyperalgesia [13]. PWL values were recorded at 1 day before and 1, 3, 7, 14 days after CCI. Rats were first adapted to the new environment for 30 min. Afterwards, rat was put in a transparent plexiglass cage (23×18×13cm) followed by heat radiation, which was 40 mm below the glass floor beneath the left hind paw of each rat. The maximum exposure time to radiation was set at 20 seconds to avoid injury. PWL, the period from the starting of heat to the paw withdrawal, was automatically recorded by a digital timer.

The PWT was applied for the evaluation of nociceptive response in mechanical allodynia by Von Frey filaments according to a previous report [14]. PWT values were recorded at 1 day before and 1, 3, 7, 14 days after CCI. Rats were first adapted to the new environment for 30 min. Afterwards, rat was put in a transparent plexiglass cage with wire mesh floor. Each filament was subjected to the left hind paw of each rat. The corresponding force (g) of filament was recorded when paw withdrawal accompanied by biting, head turning and/or licking.

At day 14, rats were decapitated immediately after they were euthanized by sodium pentobarbital (0.12 g/kg, i.p.). The ipsilateral lumbar 4–6 spinal cord of each rat was obtained for the following experiments.

RT-qPCR

Total RNA of ipsilateral lumbar 4–6 spinal cord was extracted by TRIzol (Invitrogen, California) according to the manufacturer's protocol. RNA was reverse transcript to cDNA by M-MLV Reverse Transcriptase Kit (Invitrogen). RT-qPCR was conducted by SYBR Green PCR Kit (Toyobo, Osaka, Japan) on a CFX96™ real-time PCR system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). GAPDH was reference for TNF- α , IL-1 β , and COX-2. The $2^{-\Delta\Delta C_q}$ method was applied for the quantification of the relative expression levels of mRNA [15].

Western blot

Briefly, whole proteins from ipsilateral lumbar 4–6 spinal cord were extracted by radioimmunoprecipitation assay buffer, followed by separation by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferration onto polyvinylidene difluoride membranes. After blocking by 5% skim milk at room temperature for 1 h, the polyvinylidene difluoride membranes were subjected to incubation by primary antibodies for p-ERK1/2 (#4377, 1:1000, Cell Signaling Technology), ERK1/2 (#4695, 1:1000, Cell Signaling Technology) and GAPDH (60004-1-Ig, 1:1000, Proteintech) at 4 °C overnight, and secondary antibody-goat anti-rabbit peroxidase (HRP, 1:5000; A0208, Beyotime) at room temperature for 2 h, successively. The protein bands were detected by enhanced chemiluminescence kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and the intensity of protein band was quantified by Quantity One Imaging Software (Bio-Rad, California).

ELISA

The protein expression levels of TNF- α , IL-1 β and COX-2 in the lumbar 4–6 spinal cord were determined by ELISA kits (Neobioscience: ERC102a.96; ab100768, Abcam; ab6665, Abcam) according to the manufacturer's instructions.

Statistical analysis

SPSS22.0 (SPSS Inc., Chicago, IL) was applied for the analysis. Data were presented as means $\pm$ SD. One-way analysis of variance followed by a post-hoc Newman Keuls was used for the analysis among 3 groups. $P < 0.05$ was set statistical significance.

Results

Effects of dezocine on rat behavior as a result of neuropathic pain

On account of the induction of thermal hyperalgesia and mechanical allodynia by nerve injury in rats [13, 14], the values of PWL and PWT in rats after CCI and the administration of dezocine were detected.

As exhibited in Figure 1 and 2, compared with control group, the values of PWL and PWT were significantly declined in CCI group, which were remarkably attenuated by dezocine in dezocine+CCI group, in consistent with a previous report [8]. Thereafter, we planned to explore the potential molecules that might be responsible for the above changes.

Effects of dezocine on p-mTOR as a result of neuropathic pain

mTOR signaling pathway, crucial for the persistence of pain states [16], was activated in CCI induced neuropathic pain [17, 18]. We next verified whether mTOR was involved in the attenuation of CCI induced neuropathic pain which was realized by dezocine.

Compared with control group, significantly higher p-mTOR levels were found in CCI group, which were reduced by dezocine in dezocine+CCI group (Figure 3A and B). Whereas, the total mTOR levels were not obviously changed among the 3 groups (Figure 3A and C).

Effects of dezocine on p-ERK1/2 as a result of neuropathic pain

Since the supraspinal mechanism of mTOR and ERK1/2 in the persistence of neuropathic pain was reported [19]. ERK1/2, whose activation modulated noxious stimuli induced peripheral/central sensitization [20], regulated neuronal activity in neuropathic pain [21, 22]. We next verified whether ERK1/2 was involved in the attenuation of CCI induced neuropathic pain which was realized by dezocine.

Compared with control group, p-ERK1/2 levels were significantly upregulated in CCI group, which were remarkably reduced by dezocine in dezocine+CCI group (Figure 4A and B), while there were not obviously changes for total ERK1/2 levels among the 3 groups (Figure 4A and C).

Effects of dezocine on TNF- α and IL-1 β as a result of neuropathic pain

Proinflammatory cytokines were upregulated in the spinal cord after nerve injury [23]. TNF- α and IL-1 β were downstream targets for ERK1/2 signaling pathway in regulating neuropathic pain [24]. We next verified whether TNF- α and IL-1 β were involved in the attenuation of CCI induced neuropathic pain which was realized by dezocine.

Compared with control group, the mRNA levels of TNF- α and IL-1 β were significantly upregulated in CCI group, which were dramatically reduced by dezocine in dezocine+CCI group (Figure 5A and B). The protein levels of TNF- α and IL-1 β showed the similar trends with those of mRNA (Figure 5C and D).

Effects of dezocine on COX-2 expression

TNF- α and IL-1 β preceded the release of COX-2 (hyperalgesic mediator), which sensitized nociception [25]. Herein, we detected the expression profile of COX-2 accordingly to verify whether COX-2 was involved in the attenuation of CCI induced neuropathic pain which was realized by dezocine.

Compared with control group, evidently induced mRNA level of COX-2 was found in CCI group, which was markedly inhibited by dezocine in dezocine+CCI group (Figure 6A). The protein level of COX-2 showed the similar trend with that of mRNA (Figure 6B).

Discussion

The management of pain remains a huge challenge for patients, specifically for whom suffer from chronic pain. Dezocine is first discovered by Pfizer for the treatment of perioperative pain [26]. Later reports demonstrate that dezocine exhibits anti-nociception effect in a rat neuropathic pain model [8], as well as antihypersensitivity in neuropathy by activating μ -opioid receptor in the spinal cord [27]. With the molecules that might be involved in the process remaining to be explored.

In the current study, we first verified the establishment of rat neuropathic pain model with the lower values of PWL and PWT in CCI group than in control group. And the effects of dezocine in neuropathic pain was proved by the higher PWL and PWT values in dezocine+CCI group than in CCI group. The findings suggested that dezocine successfully rescued CCI-induced neuropathic pain in rats, which was in consistent with the previous reported study [8]. Thereafter, we explored the potential molecules that might be responsible for the above changes.

The mTOR signaling pathway, activated in CCI-induced neuropathic pain [17, 18], is involved in the transmission and modulation of pain [16, 28, 29]. Moreover, the blockage of mTOR signaling pathway alleviates neuropathic pain [30], and there is a supraspinal mechanism of mTOR and ERK1/2 in the development and persistence of neuropathic pain [19], with the latter regulating neuronal activity in neuropathic pain [20, 21] and pain sensitization [31]. The findings exhibited that there were higher p-mTOR and p-ERK1/2 levels in CCI group than in control group, which were lowered by dezocine in dezocine+CCI group, indicating that dezocine might attenuate neuropathic pain by inactivating mTOR signaling pathway and ERK1/2 signaling pathway in rats.

Noxious stimuli phosphorylate ERK1/2, thus controlling gene transcription and protein expression [31]. TNF- α and IL-1 β , the downstream targets for ERK1/2 signaling pathway in neuropathic pain [24], stimulate nociceptors directly [32] and precede the release of COX-2 that sensitizes nociception [25]. The

findings exhibited that there were higher TNF- α , IL-1 β and COX-2 levels in CCI group than in control group, which were lowered by dezocine in dezocine+CCI group, indicating that dezocine might attenuate neuropathic pain by inhibiting the release of proinflammatory cytokines in rats.

Conclusions

The current study indicated that dezocine attenuated CCI-induced dysregulation of PWT and PWL values, which might be realized by inhibition of p-mTOR/p-ERK1/2 pathway and the following release of IL-6, TNF- α and COX-2 in the spinal cord of rat neuropathic pain model.

List Of Abbreviations

Chronic constriction injury (CCI), paw withdrawal threshold (PWT), paw withdrawal latency (PWL), phosphorylated-mammalian target of rapamycin (mTOR), p-extracellular signal-regulated kinase 1/2 (p-ERK1/2), interleukin (IL)-6, tumor necrosis factor (TNF)- α , cyclooxygenase-2 (COX-2), reverse transcription-quantitative polymerase chain reaction (RT-qPCR), enzyme-linked immunosorbent assay (ELISA), and intraperitoneal (IP).

Declarations

Acknowledgements

We are appreciated for the support of Hubei Maternal and Child Health Hospital Affiliated to Huazhong University of Science and Technology and Union Hospital Affiliated to Huazhong University of Science and Technology in the present study.

Funding

None.

Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate

The experiments were approved by the Animal Care and Welfare Committee of Hubei Maternal and Child Health Hospital Affiliated to Huazhong University of Science and Technology.

Consent for publication

Not applicable.

Availability of data and material

They are available from the corresponding author on reasonable request.

Authors' contributions

NX conceived the study, analyzed the data and prepared the manuscript. CS, SS and LY performed the experiments and analyzed the data. All authors read and approved the final manuscript.

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Figures

Figure 1

Dezocine attenuates CCI induced decrease of PWL. With the postoperative time going on, the PWL values decreased in CCI group compared to those in control group, which in dezocine+CCI group were significantly increased compared to those in CCI group.

Figure 2

Dezocine attenuates CCI induced decrease of PWT. With the postoperative time going on, the PWT values decreased in CCI group compared to those in control group, which in dezocine+CCI group were significantly increased compared to those in CCI group.

Figure 3

Dezocine attenuates CCI induced increase of p-mTOR. p-mTOR levels were increased in CCI group compared with control group, which in dezocine+CCI group were significantly decreased by dezocine (A and B). No significant changes happened to the total mTOR levels (A and C).

Figure 4

Dezocine attenuates CCI induced increase of p-ERK1/2. p-ERK1/2 levels were increased in CCI group compared with control group, which in dezocine+CCI group were significantly decreased by dezocine (A and B). No significant changes happened to the total ERK1/2 levels (A and C).

Figure 5

Dezocine attenuates CCI induced increase of TNF-α and IL-1β. mRNA levels of TNF-α and IL-1β were increased in CCI group compared with control group, which in dezocine+CCI group were significantly decreased by dezocine (A and B). Meanwhile, the protein levels of TNF-α and IL-1β showed the similar trends with those of mRNA (C and D).

Figure 6

Dezocine attenuates CCI induced increase of COX-2. mRNA level of COX-2 was increased in CCI group compared with control group, which in dezocine+CCI group was significantly decreased by dezocine (A). The protein level of COX-2 showed the similar trend with that of mRNA (B).

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