Choline and Parecoxib Sodium Induced Pain Inhibition in Rats After Chronic Constriction Injury of the Sciatic Nerve

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Research Article

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Abstract

Purpose

We aimed to examine the pain inhibitory effects of systemic administration of choline and parecoxib sodium in a rat model of neuropathic pain induced by a chronic constriction injury (CCI) of the sciatic nerve, and to determine the changes of high mobility protein-1 (HMGB1) and NF- κ Bp65 expression in the dorsal root ganglion (DRG) after drug treatment.

Methods

Adult rats were randomly divided into 5 groups (N = 8/group). Sham surgery group received an intraperitoneal (i.p.) injection of saline. CCI rats received saline, choline (cho, 6, 12 and 24 mg/kg), parecoxib sodium (pare, 3, 6, 12mg/kg), or a combination of choline and parecoxib sodium with each drug delivered at a sub-effective dose. Mechanical and heat pain thresholds were measured at 30 min after drug treatment at day 3, 5, 7, 10, and 14 after CCI. In a separate study, rats were also divided into 5 groups (N = 6/group) including sham, CCI + saline, CCI + cho-6 mg/kg, CCI + pare-3mg/kg, and CCI + cho-6mg/kg + pare-3mg/kg group. After repeated drug treatment for 7 days, five rats were randomly selected in each group, and the lumbar DRGs (L4-6) were harvested for Western blot study.

Results

Choline significantly attenuated the mechanical and heat hypersensitivities in CCI rats at 12 and 24 mg/kg doses ($P < 0.05$), but was not effective at 6 mg/kg dose. Parecoxib sodium exerted significant pain inhibitory effects at 6 and 12 mg/kg doses ($P < 0.05$), but not at 3 mg/kg dose. Combining a low dose of choline (6 mg/kg) and parecoxib sodium (3 mg/kg) produced significant pain inhibition in CCI rats, and also reduced the expression of HMGB1 and NF- κ Bp65 in L4-6 DRGs.

Conclusion

A combination of choline and parecoxib sodium may effectively relieve neuropathic pain, and the underlying mechanisms may involve an inhibition of HMGB1/TLR4/NF- κ B signaling pathway in DRG neurons.

Introduction

Neuropathic pain is a chronic disease caused by the injury of the nervous system or functional disorders.

^[1] After the nervous system injury, the release of inflammatory factors and mediators will induce inflammatory responses. These changes may increase the excitability of nociceptive sensory neurons

causing peripheral and central sensitization, which are involved in neural plasticity and contribute to the occurrence and maintenance of neuropathic pain.^[2]

Because of its complex underlying mechanisms, the clinical treatment of neuropathic pain remains to be unsatisfactory. Previous studies showed that choline and paracetin sodium had synergistic analgesic effects in an acetic acid-induced pain model. Their interaction may play a role in modulating the NF- κ B signaling pathway.^[3] In this study, we examined the two drugs for the treatment of neuropathic pain, and determined whether they may exert additive or synergistic effect, which remain unknown. We further explored the mechanisms underlying the pain inhibitory drug action.

Materials And Methods

Animals

Adult male Sprague Dawley (SD) rats (6–8 weeks, 180-220g) were provided by the Academy of Military Medical Sciences Experimental Animal Center (SCXK-Jun 2012-0004). All experiment protocols were reviewed and approved by the PLA General Hospital Ethics Committee. Animals were acclimated to the testing environment for 2 days. Animals received sub-cage feeding, and were housed (room temperature 20–25 °C, humidity 60% -70%) with free access to food and water.

Materials

Tartaric acid choline (CAS No. 87-67-2, Shanghai Aladdin Biotechnology Co., Ltd, China), parecoxib sodium (Pfizer Ltd, lot: J20080044, USA), pentobarbital sodium (Beijing Chemical Reagent Company, Beijing, China), 0.9% saline solution (Shijiazhuang four drugs Co., Ltd., Hebei) were stored at 4 °C.

Surgical procedures for CCI

The rat CCI model was prepared according to the methods described by Bennett et al.^[4] Rats were deeply anesthetized with 2% pentobarbital sodium (45 mg/kg, i.p.), and secured on the operating table. Skin in the middle of the left limb was opened after disinfection. The left sciatic nerve was exposed at mid-thigh level by blunt dissection through the biceps femoris muscle. Four loosely constrictive ligatures (4 – 0 chrome line, bubble in sterile saline for 30 min 4) were tied around the nerve near the bifurcation site, and at spaces of ~ 1 mm. The nerve ligation was performed slowly which caused a mild fibrillation in calf muscle, but did not block blood circulation. The muscle layer was approximated and the skin was closed with sutures.

Drug treatment

Rats were randomly divided into 5 groups (N = 8/group) including sham: rats received sham surgery and saline injection; CCI + saline: rats received CCI surgery and saline treatment; CCI + choline: CCI rats received choline (6, 12, 24 mg/ kg); CCI + parecoxib: CCI rats received parecoxib (3, 6, 12mg/kg); CCI + choline + parecoxib: CCI rats received both parecoxib and choline, each drug was delivered at a sub-

effective dose. Drugs were given by intraperitoneal (i.p.) injection in the morning (9: 00–10: 00 AM) for 7 days (one injection/day).

Mechanical and thermal pain threshold tests

Rats were tested on day 3, 5, 7, 10, and 14 after surgery. Behavior study was conducted between 8: 00 AM and 5: 00 PM. The room temperature was maintained at 20–24 ° C. The pain threshold was measured at 30 min after each drug injection.

To examine mechanical pain threshold, rats were placed in an empty cage and remained calm. Mechanical stimulation (von Frey filaments) was applied to the dorsal surface of the left hind paw, which was ipsilateral to side of nerve injury. Each von Frey filament was applied perpendicularly to the skin to slowly bend the cilia. There was a 30 sec interval between two tests. If a withdrawal response was evoked, the next stimulus was applied after the rat resettled.

To examine the thermal pain threshold, rats were placed in the plexiglass box which was put on a 6 mm thick glass plate. After 15 minutes of adaptation, rats were stimulated with a light beam applied to the plantar side of the left hind paw. The time (sec) between the starting of heat stimulation and an evoked paw withdrawal response was measured as the thermal withdrawal latency. The cut-off time was set to 20 sec to prevent tissue damage. The light intensity was set to 40% and kept consistent during the experiment. Each rat was measured 3 times at an interval of 5 minutes, and data were averaged for analysis.

Tissue harvest

Rats were divided into 5 groups (N = 6/group) including Sham (S), CCI + saline (M), CCI + cho-6mg/kg (P), CCI + pare-3mg/kg (C), and CCI + cho-6mg/kg + pare-3mg/kg (L). CCI rats received drug treatment for 7 days. After that, L4-6 DRGs were quickly removed and stored in liquid nitrogen. Tissues from five rats which were randomly selected in each group were used in Western blot study to examine the protein levels of HMGB1 and NF- κ Bp65.

Statistical analysis

All statistical analysis was carried out with SPSS22.0 statistical software. Comparisons between groups were made by using single factor analysis of variance (ANOVA), Data are expressed as mean $\pm$ standard error. Statistical significance was set at $P < 0.05$ in all tests.

Results

Dose-dependent pain inhibition produced by choline and parecoxib sodium in CCI rats

Compared to the pre-injury condition, saline-treated CCI rats developed mechanical hypersensitivity on the ipsilateral hind paw, as indicated by a significant decrease of mechanical withdrawal threshold, which

occurred at 3 day after injury and persisted till the end of the study. In contrast, saline-treated sham rats did not show mechanical hypersensitivity. Choline significantly inhibited mechanical hypersensitivity in CCI rats at 12 and 24 mg/kg doses ($P \leq 0.05$), but not at 6 mg/kg dose (Fig. 1A). Parecoxib induced significant anti-allodynic effects at 6 and 12 mg/kg doses ($P \leq 0.05$), but not at 3 mg/kg dose (Fig. 1B).

Saline-treated CCI rats also developed heat hypersensitivity at 3 day post-injury, and persisted till the end of the study. Saline-treated sham rats did not show heat hypersensitivity. In CCI rats, choline reduced heat hypersensitivity at 12 and 24 mg/kg doses ($P \leq 0.05$), but not at 6 mg/kg dose (Fig. 2A). Parecoxib reduced heat hypersensitivity at 6 and 12 mg/kg doses ($P \leq 0.05$, Fig. 2B).

Importantly, the combination of a low dose choline (6 mg/kg) and parecoxib (3 mg/kg) resulted in a significant inhibition of both mechanical and heat hypersensitivities ($P < 0.05$, Fig. 3A-B).

Changes of HMGB1 and NF- κ Bp65 expression in lumbar DRGs after drug treatment

Compared to the sham group, the expression levels of HMGB1 and NF- κ Bp65 were significantly increased in CCI rats which received saline injection (Fig. 4). Compared to that in CCI rats after saline treatment, the levels of HMGB1 and NF- κ Bp65 were significantly decreased in CCI rats which received combined choline and parecoxib treatment for 7 days ($P < 0.05$). However, the levels of HMGB1 and NF- κ Bp65 were not significantly decreased in CCI rats after receiving single drug treatment.

Discussion

Neuropathic pain is an intractable chronic pain condition. The efficacy of sodium channel inhibitors for treating neuropathic pain has been examined in the past decade.^[5] Because current therapies for neuropathic pain are not suitable for some patients, the search for new pain medications and therapies must continue.^[6]

In this study, we examined pain inhibitory effects of choline and parecoxib sodium in rats with neuropathic pain. We used the CCI model because it has been reported that this model may simulate some characteristics of neuropathic pain in humans, and CCI of the sciatic nerve induced rapid and significant mechanical and heat hypersensitivities in rats.^[7] Similarly, our study showed that CCI rats began to develop pain hypersensitivity at 3 days after injury, and persisted through the study period. The affected hind limb (i.e., ipsilateral to the side of CCI) showed eversion, and were often lifted to avoid touching the ground. The paw withdrawal threshold to mechanical stimulation and paw withdrawal latency to heat stimulation were significantly decreased, as compared to that before the injury.

Parecoxib sodium is a highly selective COX-2 inhibitor. It inhibits the production of PGE2 and nitric oxide and regulates neurotransmission in the spinal cord dorsal horn.^[8] Parecoxib sodium has been reported to reduce the accumulation of tumor necrosis factor- α (TNF- α) and neutrophils in inflammatory exudates (Add reference please). Compared to other commonly used NSAIDs (e.g., diclofenac and nimesulide),

parecoxib sodium induces a quicker pain inhibition in humans.^[9] Studies also showed that parecoxib sodium reduced pain and inflammatory responses in formalin and other inflammatory pain models.^[10] Our study demonstrated that after 7 days of treatment with 6 mg/kg and 12 mg/kg parecoxib sodium, CCI rats showed significant reductions of mechanical and heat hypersensitivities, as compared to pre-drug condition. However, 3 mg/kg dose was not effective. Thus, moderate and high doses of parecoxib sodium reduced neuropathic mechanical and heat hypersensitivities, and these findings are consistent with a previous report.^[11]

As a specific $\alpha 7$ nAChR agonist, choline exerts anti-inflammatory and analgesic effects through activation of cholinergic anti-inflammatory pathway,^[12] which was shown in several pain models and sepsis models.^[13] In acetic acid torsion model, choline induced dose-dependent analgesic effects.^[14] In current study, choline attenuated mechanical and heat hypersensitivity in CCI rats at 12 mg/kg and 24 mg/kg doses, but 6 mg/kg was not effective. Under chronic neuropathic pain condition, the decomposition and synthesis of ACh were reduced in the hypothalamus, causing a low level of cholinergic activity.^[15] Our findings suggest that choline supplement may help to alleviate chronic pain after nerve injury.

In patients with diabetic neuropathy and postherpetic neuralgia, a previous study showed that morphine in combination with a low dose of gabapentin provided a better pain relief than morphine alone.^[16] In current study, co-treatment of CCI rats with a low, sub-effective dose of choline (6 mg/kg) and parecoxib sodium (3 mg/kg) induced a significant pain inhibition, indicating that two drugs may exert an additive or synergistic pain inhibitory effect. Using low doses may limit the dose-limiting adverse effects for each drug .

After peripheral nerve injury, glial cells were activated and pro-inflammatory cytokines were released in the spinal cord.^[17] HMGB1 is a late inflammatory factor. It can promote the release of pro-inflammatory factors and more HMGB1 from inflammatory cells, thus forming a positive feedback loop and aggravating inflammatory responses.^[18] NF- κ Bp65 is an activated nuclear signal transduction protein, and mediates the production of inflammatory mediators.^[19] Our study showed that the expressions of HMGB1 and NF- κ Bp65 were increased in DRGs after CCI, which may increase the inflammatory responses and pain.

Parecoxib sodium was shown to reduce the expression of HMGB1 in cerebral ischemia model, and hence it plays a neuroprotective role.^[20] Choline-activated $\alpha 7$ nAChR signaling pathway was suggested to be sensitive to control HMGB1 release.^[21] In this study, although there was a trend that parecoxib sodium at 3 mg/kg dose reduced HMGB1 and NF- κ Bp65 levels, the difference was not statistically significant. Similarly, choline at 6 mg/kg dose did not significantly reduced the upregulated HMGB1 and NF- κ Bp65 expression in CCI rats. However, a combination of these drugs significantly reduced the expressions of HMGB1 and NF- κ Bp65, which was consistent with findings in our behavioral study. HMGB1 can bind and activate TLR4 receptors, and induces the production and release of inflammatory factors. NF- κ B, as a major nuclear signal transduction protein that mediates the production of a variety of inflammatory

mediators, is downstream of HMGB1/TLR4 pathway.^[22–23] Therefore, these findings suggest that choline and parecoxib sodium may both inhibit HMGB1/TLR4/NF-κB signaling pathway, and may be used together for the treatment of neuropathic pain which remains a great clinical challenge.

Parecoxib sodium is frequently used in the clinic as an analgesic, but has dose-limiting side effects.^[24] The cholinergic pathway may also be targeted for pain inhibition by inducing an anti-inflammatory effect.^[25] Current findings suggest that combining choline and parecoxib sodium, which have different properties, may improve neuropathic pain inhibition and decrease the side effects of each drug by reducing the required dose. This notion needs to be further tested in future clinical studies.

Conclusion

1. In a rat model of chronic neuropathic pain (CCI), at a certain dose, choline or parecoxib sodium can alleviate mechanical pain and thermal hyperalgesia caused by CCI.
2. The combination of choline and parecoxib sodium in non-analgesic doses can effectively relieve neuropathic pain, and its mechanism may be related to the inhibition of HMGB1/TLR4/NF-κB pathway.

Declarations

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Contributions

Methodology and Original draft preparation: NZ; Statistical analysis and Review: YL; Supervision, Resource, and Review: ZF. All authors read and approved the final manuscript.

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Ethics declarations

This study was approved by the Animal Care and Use Committee of Chinese PLA General Hospital on 16 February 2021 (ethic approval ID: 20210216–001) and performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The study was carried out in compliance with the ARRIVE guidelines.

Consent for publication

Informed consent obtained from all the participants to publish the information/image(s) in an online open-access publication.

Competing interests

We declare that we have no conflicts of interest in this work.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. Nanna B, Rohini K, Troels S. Neuropathic Pain: From Mechanisms to Treatment. *Physiol Rev.* 2021;101(1):259–301.
2. Délia S, János T, Aliz N, László V. Therapeutic Approaches for Peripheral and Central Neuropathic Pain. *Behav Neurol.* 2019;2019:8685954.
3. Morena Z, Valentina L, Davide F, Andrea P, Rita L, Stefano S, Anna C, Pietro G, Stefano M. Ciprofloxacin and levofloxacin attenuate microglia inflammatory response via TLR4/NF-kB pathway. *J Neuroinflam.* 2019;16(1):148.
4. Bennett A, Villa G. Nimesulide: an NSAID that preferentially inhibits COX-2, and has various unique pharmacological activities. *Expert Opin Pharm.* 2000;1:277–286.
5. Nanna B, Rohini K, Troels S. Neuropathic Pain: From Mechanisms to Treatment. *Physiol Rev.* 2021;101(1):259–301.

6. Bouhassira D. Neuropathic pain: Definition, assessment and epidemiology. *Revue Neurol.* 2019;175(1–2):16–25.
7. Wu J, Wang C, Ding H. LncRNA MALAT1 promotes neuropathic pain progression through the miR–154–5p/AQP9 axis in CCI rat models. *Mol Med Rep.* 2020;21(1):291–303.
8. Wu F, Wang W, Duan Y, Guo J, Li G, Ma T. Effect of Parecoxib Sodium on Myocardial Ischemia-Reperfusion Injury Rats. *Med Sci Monit.* 2021;27:e928205.
9. Zhuang Q, Tao L, Lin J, Jin J, Qian W, Bian Y, Li Y, Dong Y, Peng H, Li Y, Fan Y, Wang W, Feng B, Gao N, Sun T, Lin J, Zhang M, Yan S, Shen B, Pei F, Weng X. Postoperative intravenous parecoxib sodium followed by oral celecoxib post total knee arthroplasty in osteoarthritis patients (PIPFORCE): a multicentre, double-blind, randomised, placebo-controlled trial. *BMJ Open* 2020;10(1):e030501.
10. Sun Y, Xu Q, Wu Z, et al. Parecoxib inhibits inflammatory responses in a mouse model of sepsis. *FEBS Open Bio.* 2020.
11. Bian YY, Wang LC, Qian WW, Lin J, Jin J, Peng HM, Weng XS. Role of Parecoxib Sodium in the Multimodal Analgesia after Total Knee Arthroplasty: A Randomized Double-blinded Controlled Trial. *Orthop Surg.* 2018;10(4):321–327.
12. Donald B. Cholinergic modulation of the immune system presents new approaches for treating inflammation. *Pharmacol Therap.* 2017;179:1–16.
13. Gisela A, Clare S, Brendan M, Katarzyna K, Stefania B, Lisa D, Roger L, Palmer T. Synthesis, Pharmacological Characterization, and Structure-Activity Relationships of Noncanonical Selective Agonists for $\alpha 7$ nAChRs. *J Med Chem.* 2019;62(22):10376–10390 doi:10.1021/acs.jmedchem.9b01467
14. Lotte S, Nicole J, Michiel G, Rima O, Marc M, Diederik E. Natural Choline from Egg Yolk Phospholipids Is More Efficiently Absorbed Compared with Choline Bitartrate; Outcomes of A Randomized Trial in Healthy Adults. *Nutrients.* 2019;11(11).
15. Laleh R, Sakineh S, Alizadeh A, Soheila F, Abbas H. Spinal Orexin-2 Receptors are Involved in Modulation of the Lateral Hypothalamic Stimulation-Induced Analgesia. *Neurochem Res.* 2019;44(5):1152–1158.
16. Minarda D, Irene D, Alma R, Adriana M, Francisco J. Anti-nociceptive synergism of morphine and gabapentin in neuropathic pain induced by chronic constriction injury. *Pharmacol Biochem Behav.* 2009;92: 457–464.
17. Zhao Z, Li X, Li Q. Curcumin accelerates the repair of sciatic nerve injury in rats through reducing Schwann cells apoptosis and promoting myelination. *Biomed Pharm.* 2017;3(92):1103–1110.
18. Leon G, Zou J, Crews F. Microglial-derived miRNA let-7 and HMGB1 contribute to ethanol-induced neurotoxicity via TLR7. *J Neuroinflamm.* 2017;14(1):22.
19. Tao Y, Wang M, Wu D, Luo C, Tang H, Chen E. Apolipoprotein A5 alleviates LPS/D-GalN-induced fulminant liver failure in mice by inhibiting TLR4-mediated NF- κ B pathway. *J Trans Med.* 2019;17(1):151

20. Hugo R, Carla G, Elena L, Han T, Arsalan Y, Irina M, Dina M, Maria N, Ana B, David J, Lorenzo D. (E)-3-Furan-2-yl-N-p-tolyl-acrylamide and its Derivative DM489 Decrease Neuropathic Pain in Mice Predominantly by $\alpha 7$ Nicotinic Acetylcholine Receptor Potentiation. *ACS Cheml Neurosci*. 2020;11(21):3603–3614
21. Liu J, Chen Y, Wei H, Chang R, Yu D, Wang X, Gong X, Zhang M. Activation of alpha7 acetylcholine receptors reduces neuropathic pain by decreasing dynorphin A release from microglia. *Brain Res*. 2019;1715:57–65
22. Kimberly G, Tissiana G, Aubin M, Samantha PM, Rodolfo D, Ricardo F, Rajesh K. The role of cyclin-dependent kinase 5 in neuropathic pain. *Pain*, 2020 12;161(12):2674–2689
23. Chen X, Wu S, Chen C, Xie B, Fang Z, Hu W, Chen J, Fu H, He H. Omega-3 polyunsaturated fatty acid supplementation attenuates microglial-induced inflammation by inhibiting the HMGB1/TLR4/NF- κ B pathway following experimental traumatic brain injury. *J neuroinflam*. 2017 07 24;14(1):143 bo-controlled trial. *BMJ open*, 2020 01 09;10(1):e030501
24. Jonas J, Benito Y, Simone H; The Cholinergic Anti-Inflammatory Pathway as a Conceptual Framework to Treat Inflammation-Mediated Renal Injury. *Kidney Blood Pressure Res*. 2019;44(4):435–448
25. Wang W, Xu H, Lin X, Molnar M, Ren H. The role of the cholinergic anti-inflammatory pathway in septic cardiomyopathy. *Int. Immunopharmacol*. 2021 Jan;90:107160

Figures

Figure 1

Changes in paw withdrawal threshold to mechanical stimulation after drug treatment in CCI rats. (A) Mechanical withdrawal threshold of the ipsilateral hind paw was measured at 30 min after intraperitoneal (i.p.) injection of saline or choline (6, 12, 24 mg/kg) in rats on day 3, 5, 7, 10 and 14 after chronic constriction injury (CCI). Rats in sham group only received saline injection. (B) Changes in mechanical withdrawal threshold after injection of saline or parecoxib sodium (3, 6, 12 mg/kg, i.p.) in each group.

N=6/group. Data are expressed as the mean $\pm$ SEM, cho: choline; par: parecoxib sodium. * $P<0.05$ versus saline-treated CCI group, # $P<0.05$ versus pre-injury (day 0) and saline-treated sham group.

Figure 2

Changes in thermal withdrawal latency to noxious heat stimulation after drug treatment in CCI rats. (A) Thermal withdrawal latency of the ipsilateral hind paw was measured at 30 min after intraperitoneal (i.p.) injection of saline or choline (6, 12, 24 mg/kg) on day 3, 5, 7, 10 and 14 after chronic constriction injury (CCI). Rats in sham group only received saline injection. (B) Changes in thermal withdrawal latency after injection of saline or parecoxib sodium (3, 6, 12 mg/kg, i.p.) in each group. N=6/group. Data are expressed as the mean $\pm$ SEM, cho: choline; par: parecoxib sodium. * $P<0.05$ versus saline-treated CCI group, # $P<0.05$ versus pre-injury (day 0) and saline-treated sham group.

Figure 3

Changes in mechanical withdrawal threshold and thermal withdrawal latency after individual or combined drug treatment in CCI rats. (A) Paw withdrawal threshold of the ipsilateral hind paw was measured at 30 min after intraperitoneal (i.p.) injection of saline, choline (6 mg/kg), parecoxib sodium (3 mg/kg) or a combination of choline (6 mg/kg) and parecoxib sodium (3 mg/kg) on day 3, 5, 7, 10 and 14 after chronic constriction injury (CCI). Rats in sham group only received saline injection. (B) Changes of thermal withdrawal latency in each group after drug treatment. N=6/group. Data are expressed as the mean $\pm$ SEM, cho: choline; par: parecoxib sodium. * $P < 0.05$ versus saline-treated CCI group, # $P < 0.05$ versus pre-injury (day 0) and saline-treated sham group.

Figure 4

Changes of NF-κB and HMGB1 expression in lumbar dorsal root ganglions (DRGs) in rats after chronic constriction injury (CCI) of the sciatic nerve and drug treatment. Representative immunoblots and quantification of HMGB1 and NF-κBp65 protein levels in lumbar DRGs (L4-6) of CCI rats at day 7 after drug treatment. N=5/group. Data are expressed as the mean $\pm$ SEM. S: sham, M: CCI+saline, P:

CCI+parecoxib-3mg/kg, C: CCI+choline-6mg/kg, L: CCI+parecoxib-3mg/kg+choline-6mg/kg. * $P < 0.05$, # $P < 0.05$ versus the indicated group.

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