Synergistic Analgesic Effect of Choline and Parecoxib Sodium and Its Mechanism: Postprint

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Abstract

Objective: To investigate the interaction between choline and parecoxib sodium in analgesia and to conduct a preliminary study on its underlying mechanism.

Methods: Acetic acid writhing model: 150 male Kunming mice were randomly divided into 4 groups: (1) Control group (S) (n=10): tail vein injection of 0.9% normal saline at 0.2 mL/20 g; (2) Choline group (C) (n=50): five dose levels of 3, 6, 12, 24, and 48 mg/kg were established in 50 mice; (3) Parecoxib sodium group (P) (n=50): five dose levels of 1.5, 3, 6, 12, and 24 mg/kg were established in 50 mice; (4) Combination group (C+P) (n=40): four dose levels corresponding to 1/2, 1/4, 1/8, and 1/16 of the ED50 of both drugs were established in 40 mice to determine the ED50 of the combination. All drugs were administered via tail vein before model establishment. Choline was administered 2 hours before modeling, and parecoxib sodium 30 minutes before modeling. The effects of normal saline control group (S), ED50 choline group (C), ED50 parecoxib sodium group (P), and 1/2 ED50 choline and parecoxib sodium group [1/2(C+P)] on blood cytokines and inflammatory mediators in acetic acid writhing model mice were investigated. Drug pretreatment regimens were identical to those described above, and orbital blood samples were collected at 10 minutes after intraperitoneal administration of acetic acid. Serum levels of IL-1, TNF- , PGE2, NF-kB, and I-kB were measured using ELISA kits.

Results: (1) In the acetic acid writhing model, the ED50 values obtained after separate tail vein administration were 8.64 mg/kg for choline and 6.33 mg/kg for parecoxib sodium; when used in combination, the ED50 values were 2.13 mg/kg for choline and 1.56 mg/kg for parecoxib sodium. (2) In the isobologram, the measured ED50 for the combination fell below the theoretical value, with $P<0.05$ for t-test between the two points. The combination index $CI<0.9$. (3) Compared with group S, IL-1 and TNF- levels were decreased in groups C, P, and 1/2(C+P) ($P<0.05$), with the reduction being more pronounced in

group 1/2(C+P) than in groups C and P administered separately (P<0.05). PGE2 levels in groups P and 1/2(C+P) were decreased compared with the control group (P<0.05), and group 1/2(C+P) showed a greater decrease in PGE2 content compared with group C (P<0.05). Compared with group S, NF-kB content was decreased in groups C, P, and 1/2(C+P) (P<0.05), while I-kB content showed statistically significant decreases in groups 1/2(C+P) and C, with NF-kB and I-kB showing more pronounced reductions in group 1/2(C+P) than in group P (P<0.05).

Conclusion: Choline and parecoxib sodium exhibit synergistic analgesic effects, and their interaction may be related to NF-kB expression in vivo.

Full Text

Synergistic Analgesic Effect of Choline and Parecoxib Sodium in Mice and Its Mechanism

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Abstract

Objective: To investigate the synergistic analgesic effect of choline and parecoxib sodium and study its mechanism.

Methods: In male Kunming mice with acetic acid-induced writhing, the ED50 of choline and parecoxib sodium (administered via the tail vein at 2 h and 30 min before modeling, respectively) and their combined use were determined. In saline (control) group, ED50 choline (C) group, ED50 parecoxib sodium (P) group, and 1/2ED50 choline and parecoxib sodium (1/2[C+P]) group, blood samples were collected from the eyeball 10 min after intraperitoneal administration of acetic acid to detect the levels of IL-1, TNF- , PGE2, NF-kB, and I-kB using ELISA kits.

Results: In the acetic acid-induced writhing model, the ED50 of choline and parecoxib sodium was 8.64 and 6.33 mg/kg, and when combined, their ED50 was 2.13 and 1.56 mg/kg, respectively. The isobolograms of parecoxib sodium and choline showed that the measured ED50 of the two drugs combined was below the theoretical ED50 value (P<0.05) with a combination index (CI) of <0.9. Compared with the control group, C group, P group, and 1/2 (C+P) group all showed significantly lowered IL-1 and TNF- levels (P<0.05), especially in 1/2 (C+P) group (P<0.05). PGE2 level was significantly lower in P group and 1/2

(C+P) group compared with the control group (P<0.05). NF- B and I- B levels were significantly lowered in C, P, and 1/2 (C+P) groups (P<0.05), and the reduction was the most obvious in 1/2 (C+P) group (P<0.05).

Conclusion: Choline and parecoxib sodium has a synergistic analgesic effect, and their interactions may involve the in vivo expression of NF- B.

Key words: choline; parecoxib sodium; ED50; nuclear factor- B

Introduction

Pain is a complex sensory experience generated by noxious stimuli and transmitted to the brain through the nervous system [1], which seriously affects human physical and mental health. Postoperative pain is the most common type of acute pain that concerns clinical anesthesiologists [2]. Parecoxib sodium has become an ideal non-steroidal anti-inflammatory analgesic drug for postoperative analgesia in various clinical departments, but it still has certain side effects, and increased doses can cause a series of adverse reactions in the gastrointestinal system [3-4]. Combined analgesic therapy is currently a widely used method in clinical practice, which achieves optimal efficacy by combining analgesic drugs with different mechanisms of action to utilize their additive or synergistic effects [5]. The cholinergic anti-inflammatory pathway (CAP) is a novel inflammatory regulatory mechanism in which anti-inflammatory signals, via released acetylcholine binding to 7 nicotinic acetylcholine receptors (7nAChR) expressed on immune cells such as macrophages, inhibit the release of pro-inflammatory cytokines to exert anti-inflammatory and analgesic effects [6]. Choline is a specific agonist of 7nAChR that can inhibit the release of various pro-inflammatory cytokines by activating 7 receptors, thereby exerting analgesic effects in different inflammatory pain models. Studies have found that the cholinergic system may interact with the arachidonic acid metabolic pathway [7], but no research has yet investigated the interaction between these two agents. This experiment combined parecoxib sodium with choline in an acetic acid writhing model to determine whether the two drugs have synergistic analgesic effects and to explore their mechanism.

Materials and Methods

1.1 Experimental Animals

Male Kunming mice, aged 6-8 weeks and weighing 18-22 g, were provided by the Laboratory Animal Center of the Academy of Military Medical Science (Animal Certificate No.: SCXK-Jun 2002-001). The study was approved by the Ethics Committee of the General Hospital of PLA. The animals were acclimated for 2 days and housed in separate cages at room temperature 20-25°C with humidity 60%-70%, fresh air, good ventilation, and free access to food and water.

1.2 Materials

Choline bitartrate (Shanghai Aladdin Bio-Chem Technology Co., Ltd., CAS No.: 87-67-2), parecoxib sodium for injection (specification: 40 mg, Pfizer Ltd, USA, batch No.: J20080044), acetic acid (Sinopharm Chemical Reagent Co., Ltd., batch No.: 20150616), and 0.9% saline injection (Shijiazhuang No. 4 Pharmaceutical Co., Ltd., Hebei) were used. Drugs were stored at 4°C.

1.3 Acetic Acid Writhing Model

The acetic acid writhing model was established by injecting 0.6% acetic acid solution into the mouse peritoneal cavity at 0.2 mL/10 g to stimulate the visceral and parietal peritoneum, inducing deep, extensive, and prolonged inflammatory pain. This caused behavioral responses including abdominal concavity, trunk and hind limb extension, and elevated hips, collectively called writhing responses. The number of writhing episodes within 20 min after injection was used as a quantitative pain indicator. Mice were randomly divided into 4 groups: (1) Control group (S) (n=10): tail vein injection of 0.9% saline 0.2 mL/20 g; (2) Choline group (C) (n=50): 50 mice received 5 doses of 3, 6, 12, 24, and 48 mg/kg; (3) Parecoxib sodium group (P) (n=50): 50 mice received 5 doses of 1.5, 3, 6, 12, and 24 mg/kg; (4) Combination group (C+P) (n=40): 40 mice received 4 doses at 1/2, 1/4, 1/8, and 1/16 of the ED50 of both drugs to calculate the ED50 of the combination. All drugs were administered via tail vein before modeling. Choline was given 2 h before modeling, and parecoxib sodium was given 30 min before modeling.

1.4 ELISA Detection of Serum IL-1, TNF- , PGE2, NF-kB, and I-kB

Forty mice were randomly divided into 4 groups: saline control group (S), ED50 choline group (C), ED50 parecoxib sodium group (P), and 1/2ED50 choline and parecoxib sodium group [1/2(C+P)]. Drug pretreatment was the same as above. Ten minutes after intraperitoneal administration of acetic acid, eyeball blood collection was immediately performed, with consistent timing from acetic acid administration to blood collection within and between groups. The collected blood was centrifuged at 3000 r/min for 10 min, the upper serum was carefully aspirated and stored in 1.5 mL EP tubes at -80°C. Within one month, ELISA kits were used to detect IL-1, TNF- , PGE2, NF-kB, and I-kB levels.

1.5 Statistical Analysis

SPSS 22.0 statistical software was used for analysis. Measurement data were expressed as mean ± standard error, and inter-group comparisons were performed using one-way ANOVA. $P<0.05$ was considered statistically significant. ED50 and 95% confidence intervals were obtained by logistic regression. Theoretical additive ED50 and measured ED50 were compared using t-test.

Results

2.1 Analgesic Effects and Drug Interaction

In the acetic acid writhing model, the ED50 and 95% confidence intervals for choline and parecoxib sodium administered via tail vein are shown in Table 1 . In the isobolographic analysis [8] (Figure 1 [Figure 1: see original paper]), the measured ED50 for the drug combination was significantly different from the theoretical additive ED50 (P<0.05). Using CalcuSyn 2.0 software and the multiple drug effect equation derived by Zheng et al. [9], the combination index CI<0.9 (Table 2), demonstrating synergistic analgesic effects.

Table 1 ED50 values and 95% confidence limits (CL) for the antinociceptive effect of choline and parecoxib sodium

Drug	ED50 (CL) (mg/kg)
Choline	8.64 (7.99-9.29)
Parecoxib sodium	6.33 (6.03-6.63)
Choline+Parecoxib	2.13/1.56 (1.93/1.46-2.33/1.66)

Table 2 Combination index (CI) values of the combination of choline and parecoxib sodium

Drug	ED50	ED75	ED90
Choline+Parecoxib	0.11342	0.25679	0.58139

Note: CI<0.9 indicates synergism; 0.9-1.1 additive effect; >1.1 antagonism.

Figure 1 [Figure 1: see original paper] Isobolograms for the intravenous administration of the combination of choline and parecoxib sodium. A, C: ED50 of parecoxib sodium and choline, respectively; B: Measured ED50 of combined use of parecoxib sodium and choline; D: Theoretical ED50 of parecoxib sodium and choline combined. B vs D, P<0.05.

2.2 Serum Levels of IL-1, TNF- , PGE2, NF-kB, and I-kB

In the acetic acid writhing model, serum levels of IL-1, TNF- , PGE2, NF-kB, and I-kB after intravenous administration of choline and/or parecoxib sodium are shown in Figure 2 [Figure 2: see original paper]. Compared with group S, IL-1 and TNF- levels were significantly decreased in groups C, P, and 1/2(C+P) (P<0.05, P<0.01), with more pronounced reduction in group 1/2(C+P) than in groups C or P alone (P<0.05). PGE2 content was significantly lower in group P and group 1/2(C+P) compared with the control group (P<0.05), and group 1/2(C+P) showed greater PGE2 reduction than group C (P<0.05). NF-kB content was significantly decreased in groups C, P, and 1/2(C+P) compared

with group S ($P<0.05$, $P<0.01$), while I-kB content was significantly reduced in group 1/2(C+P) and group C. NF-kB and I-kB were more significantly decreased in group 1/2(C+P) than in group P ($P<0.05$). No significant differences were found among other groups ($P>0.05$).

Figure 2 [Figure 2: see original paper] Effects of co-administration with choline and parecoxib sodium on the production of pro-inflammatory mediators in the acetic acid writhing model. *$P<0.05$ vs S group; #$P<0.05$ vs 1/2(C+P) group.

Discussion

Parecoxib sodium is often combined with other analgesic drugs for postoperative pain treatment due to its high selectivity and fewer adverse reactions. This not only improves analgesic efficacy but also significantly reduces the dosage of narcotic analgesics, thereby decreasing adverse drug reactions [10-11]. Studies have shown that parecoxib sodium and tramadol have synergistic analgesic effects in the second phase of the orofacial formalin test [12]. Other research has demonstrated that choline and aspirin have synergistic anti-inflammatory effects, with choline enhancing the anti-inflammatory effect of low-dose aspirin in several inflammatory swelling models. Activation of 7 nicotinic receptors is involved in their interaction, though the specific mechanism remains unclear [13-14]. Our results show that combined use of choline and parecoxib sodium produces synergistic analgesic effects while reducing the dosage of both drugs, thus decreasing side effects. As a selective COX-2 inhibitor, parecoxib sodium has more potent analgesic effects and fewer side effects than aspirin and is widely used in multimodal postoperative analgesia [15]. Choline, as a specific agonist of the 7 receptor, is similar to nicotine but without physical dependence, making it safer and more reliable than nicotine [16-17].

This experiment confirmed the synergistic effect of the two drugs in the acetic acid writhing pain model. ED50 values for individual and combined drugs were obtained through dose-response curves and logistic regression. Isobolographic analysis was then used to determine their interaction, which was further verified by the combination index [18]. This method has been widely applied in studies of drug interactions and has high reliability [19]. Berenbaum [20] proposed that when two or more drugs are used in combination, synergistic effects can be produced if they act on the same or different key points in a common pathway; otherwise, no synergistic effect will occur. In this study, dose-response curves and logistic regression yielded an ED50 of 8.64 mg/kg for choline and 6.33 mg/kg for parecoxib sodium. When combined, the ED50 was 2.13 mg/kg for choline and 1.56 mg/kg for parecoxib sodium. The ED50 values and 95% confidence intervals for the analgesic effects of choline and parecoxib sodium were plotted in an isobologram, showing that the measured point B (2.13, 1.56) fell significantly below the theoretical additive line, with 95% confidence intervals not overlapping the theoretical additive point (4.36, 3.13). T-tests on X and Y axes showed $P<0.05$, indicating statistical significance and demonstrating synergistic effects near equieffective doses (Figure 1). Additionally, the combination index values

for choline and parecoxib sodium were 0.11342 at ED50, 0.25679 at ED75, and 0.58139 at ED90. All CI values were less than 0.9, where CI<0.9 indicates synergism, 0.9-1.1 additive effect, and >1.1 antagonism. Lower CI values indicate stronger synergism, thus verifying the synergistic effect of choline and parecoxib sodium.

To further explore the mechanism of synergism, this study measured pro-inflammatory mediators IL-1 and TNF- in blood. We found that combination of 1/2ED50 choline and parecoxib sodium better inhibited IL-1 and TNF- production than either drug alone, suggesting a common mechanism for combined anti-inflammatory analgesia. PGE2 content was significantly reduced in the parecoxib sodium alone group compared with the control group, suggesting that parecoxib sodium mainly exerts anti-inflammatory and analgesic effects by inhibiting prostaglandin production. In the choline alone group, PGE2 content showed almost no change compared with the control group but was significantly different from the combination group, which showed marked reduction. This suggests that choline does not inhibit PGE2 production during anti-inflammatory processes and may even increase it. In studies on atherosclerosis pathogenesis, cigarette smoke (mainly nicotine) can enhance histamine- and LPS-induced cyclooxygenase-2 (COX-2) expression in endothelial cells [21]. Other studies have also proven that nicotine can upregulate COX-2 expression and PGE2 production. Additionally, we measured blood NF-kB and I-kB levels and found that both individual and combined applications of choline and parecoxib sodium reduced blood NF-kB content, with the combination group showing better efficacy than single-drug groups. This suggests that the common anti-inflammatory target may be NF-kB. Studies on ketamine-induced ulcerative cystitis in rats showed enhanced NF-kB translocation and COX-2 expression, with promoter deletion analysis indicating that NF-kB is a necessary transcription factor for COX-2 gene activation. Regulation of COX-2 expression through the NF-kB pathway can modulate inflammatory signals. Other studies have proven that COX-2 expression is mediated by NF-kB activation [22], and NF-kB binding sites have been identified in the promoter region of the COX-2 gene [23]. The anti-inflammatory mechanism of 7nAChR mainly involves activation of the NF-kB pathway, preventing phosphorylation of inhibitor of NF-kB (I-kB), thereby inhibiting NF-kB transcriptional activity and subsequent pro-inflammatory mediator production to achieve anti-inflammatory effects [24-25]. NF-kB is an early nuclear transcription factor that regulates various stages of early immune and inflammatory responses. Therefore, we speculate that choline and parecoxib sodium may act together on NF-kB and exert anti-inflammatory and analgesic effects through the NF-kB pathway.

In summary, both choline and parecoxib sodium have certain effects on inflammatory pain. This study combined choline with parecoxib sodium in an inflammatory pain model and found synergistic analgesic effects, which reduces the dosage of parecoxib sodium and thus decreases its side effects. The drug interaction may occur through the NF-kB pathway, though the specific mechanism requires further investigation.

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