Antihyperalgesic/Antinociceptive Effects of Ceftriaxone and Its Synergistic Interactions with Different Analgesics in Inflammatory Pain in Rodents

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ABSTRACT

Background: The β-lactam antibiotic ceftriaxone stimulates glutamate transporter GLT-1 expression and is effective in neuropathic and visceral pain models. This study examined the effects of ceftriaxone and its interactions with different analgesics (ibuprofen, celecoxib, paracetamol, and levetiracetam) in somatic and visceral pain models in rodents.

Methods: The effects of ceftriaxone (intraperitoneally/intraplantarly), analgesics (orally), and their combinations were examined in the carrageenan-induced paw inflammatory hyperalgesia model in rats (n = 6–12) and in the acetic acid-induced writhing test in mice (n = 6–10). The type of interaction between ceftriaxone and analgesics was determined by isobolographic analysis.

Results: Pretreatment with intraperitoneally administered ceftriaxone (10–200 mg/kg per day) for 7 days produced a significant dose-dependent antihyperalgesia in the somatic inflammatory model. Acute administration of ceftriaxone, *via* either intraperitoneal (10–200 mg/kg) or intraplantar (0.05–0.2 mg per paw) routes, produced a significant and dose-dependent but less efficacious antihyperalgesia. In the visceral pain model, significant dose-dependent antinociception of ceftriaxone (25–200 mg/kg per day) was observed only after the 7-day pretreatment. Isobolographic analysis in the inflammatory hyperalgesia model revealed approximately 10-fold reduction of doses of both drugs in all examined combinations. In the visceral nociception model, more than 7- and 17-fold reduction of doses of both drugs was observed in combinations of ceftriaxone with ibuprofen/paracetamol and celecoxib/levetiracetam, respectively.

Conclusions: Ceftriaxone exerts antihyperalgesia/antinociception in both somatic and visceral inflammatory pain. Its efficacy is higher after a 7-day pretreatment than after acute administration. The two-drug combinations of ceftriaxone and the nonsteroidal analgesics/levetiracetam have synergistic interactions in both pain models. These results suggest that ceftriaxone, particularly in combinations with ibuprofen, celecoxib, paracetamol, or levetiracetam, may provide useful approach to the clinical treatment of inflammation-related pain. (**ANESTHESIOLOGY** 2014; 120:737-50)

I N inflammatory somatic and visceral pain, tissue damage or inflammation sensitizes peripheral afferents and dorsal horn neurons.[1] Synaptic input from primary afferents onto second-order neurons in the dorsal horn is excitatory. This excitation is mostly mediated by the release of glutamate, which is the major excitatory neurotransmitter in the central nervous system.[2,3] There are several reports that glutamate elicits hyperalgesia *via* the direct excitation of peripheral afferent fiber terminals.[4,5] Because glutamate is critical for neuroplasticity in nociceptive network in inflammatory pain, there have been considerable efforts to develop therapeutic approaches that suppress glutamatergic activity. There is no enzyme that metabolizes the glutamate in extracellular space, but there is high-affinity, efficient, high-capacity glutamate transporter system within the central and peripheral nervous systems that removes glutamate from the extracellular space. The glutamate transporter GLT-1 is distributed primarily in astrocytes and plays a crucial role in glutamate clearance in the synaptic cleft in the central

> ### What We Already Know about This Topic
> - Ceftriaxone is a third-generation cephalosporin antibiotic capable of increasing the expression of the GLT-1 glutamate transporter. This action may underlie ceftriaxone's analgesic properties.
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> ### What This Article Tells Us That Is New
> - Pretreatment of rodents with ceftriaxone provided antinociceptive effects in inflammatory pain models.
> - By using inflammatory somatic and visceral pain models, synergistic interactions of ceftriaxone and several commonly used analgesics were demonstrated.

nervous system,[6–8] as well as in satellite and Schwann cells in the peripheral nervous system.[9]

A novel strategy to decrease synaptic glutamate by upregulating the glutamate transporter GLT-1 might be effective in mitigating inflammatory pain.[10] Evidence suggests that the β-lactam antibiotic, ceftriaxone, is a potent stimulator of GLT-1 expression[10] and that it can attenuate neuropathic[11,12]

and visceral pain.[13] There are no data about its efficacy in inflammatory somatic pain.

As there is evidence that inflammatory somatic as well as visceral pain processes are associated not only with glutamate but also with prostaglandins and some other released mediators, combination therapy is of value in this context.[1,14–16] Moreover, when combinations of suitable drugs are used, submaximal doses can be applied, with fewer adverse effects and greater efficacy.[17,18] Nonsteroidal antiinflammatory drugs and paracetamol are useful in the management of both somatic and visceral pain. The analgesic effect of nonsteroidal antiinflammatory drugs has traditionally been related to the reduction of prostaglandin synthesis, by inhibition of cyclooxygenases (COX)-1/2. The mechanism of analgesic effect of paracetamol is still a matter of debate. Recent evidence suggests that the reduction of prostaglandin synthesis caused by the inhibition of COX-2 could be the main mechanism of its action.[19,20] Our research group has shown that levetiracetam, a novel antiepileptic drug, induces antihyperalgesia in the somatic inflammatory model of pain *via* adrenergic, opioidergic, 5-hydroxytryptaminergic, γ-aminobutyric acid (GABA)ergic, and adenosine receptors.[21,22] It seems that levetiracetam is favorable for coadministration with other drugs because unlike other antiepileptics, it does not induce and is not a high-affinity substrate for cytochrome P-450 isoforms or glucuronidation enzymes and thus it is devoid of pharmacokinetic (metabolic) interactions with other drugs.[23]

The current study was undertaken to determine the efficacy of ceftriaxone, as well as the types of interactions (additivity, synergism, or antagonism) of ceftriaxone with different analgesics (ibuprofen, celocoxib, paracetamol, and levetiracetam) in somatic and visceral inflammatory pain models in rodents.

Materials and Methods

Animals

All experiments were approved by the Institutional Animal Care and Use Committee of the Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia, and were carried out in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.[24] Experiments were performed on male Wistar rats (weighing 180–220 g) and Swiss Webster mice (weighing 25–30 g) obtained from the Military Medical Academy Breeding Farm, Belgrade, Serbia. The animals were housed under a 12 h light/dark cycle, with food and water available *ad libitum*. For all measurements, the experimenter was blinded to the treatment group.

Drugs and Their Administration

Ceftriaxone (Longaceph; Galenika, Belgrade, Serbia) was dissolved in saline. Ibuprofen (Pharmagen GmbH, Frankfurt, Germany), celecoxib (Celebrex; Pfizer Manufacturing Deutschland GmbH, Illertissen, Germany), paracetamol

(Panadol; GlaxoSmithKline Dungarvan Ltd., Dungarvan, Ireland), and levetiracetam (Keppra; UCB Pharma AG, Bruselles, Belgium) were suspended in distilled water.

Two distinct protocols were used for ceftriaxone administration. In the pretreatment protocol, ceftriaxone was intraperitoneally administered once daily for 7 consecutive days, and the experiments were performed on day 8 (starting 24 h after the last ceftriaxone injection). In the acute treatment protocol, ceftriaxone was administered either *via* intraperitoneal or intraplantar routes only once, at the time of performing experiments. Analgesics were administered by oral gavage and always as an acute treatment. The volumes of the intraperitoneal/oral drugs administration were 2 ml/kg body weight for rats and 10 ml/kg body weight for mice.

Carrageenan λ (Sigma-Aldrich Chemie GmbH, Munich, Germany) was dispersed in saline (1% m/v) and injected intraplantarly. Intraplantar injection of ceftriaxone was either coadministered with the carrageenan into the rat right hind paw or administered alone into the contralateral hind paw. All intraplantar treatments were administered in a final volume of 0.1 ml per paw using a 1 ml syringe with a 26-gauge needle. Diluted acetic acid (0.75%; Zorka Pharma, Šabac, Serbia) was injected intraperitoneally into mice in a volume of 10 ml/kg body weight.

Model of Somatic Inflammatory Hyperalgesia

The difference between the forces (df, expressed in grams) that were applied to the left, healthy, and right, inflamed (carrageenan-injected) hind paws was measured with an analgesimeter (Hugo Sachs Elektronik, March-Hugstetten, Germany) as previously described.[21,22,25–27] The rat was placed with its hind paws on two transducer platforms and gently pushed downward by the investigator's hand so that the force (pressure) was applied simultaneously to both paws until one of the paws exceeded the trigger of 100 g. This pressure represents a mild nociceptive stimulus that is required to detect nociceptive hypersensitivity (hyperalgesia).

Basal df was obtained before the induction of inflammation and administration of the drugs. The time points when the drugs were administered were chosen according to data about the time course of carrageenan hyperalgesia[28] and the time course of the antihyperalgesic effects and/or pharmacokinetic properties of the examined drugs.[11,21,26,27,29] Ceftriaxone was administered in both pretreatment and acute treatment protocols. The analgesics were administered only as an acute treatment. In the acute treatment protocols, ceftriaxone was either administered intraperitoneally 1 h after carrageenan or coadministered intraplantarly with carrageenan. To exclude a potential systemic effect of intraplantarly administered ceftriaxone, the highest tested dose of the drug was given contralaterally (into the left hind paw) to a separate group of rats. Ibuprofen and celecoxib were administered at the same time as carrageenan. Paracetamol and levetiracetam were administered 1 h after

carrageenan. Posttreatment df values were measured at six time points during a 300 min period after the induction of inflammation.

Model of Visceral Nociception

The writhing test described previously[26,30] was used. Mice were injected intraperitoneally with acetic acid solution. The number of writhes (N) was counted during a 15 min period, starting 5 min after the administration of the acetic acid. Ceftriaxone was administered in both pretreatment and acute treatment protocols. The analgesics were administered only as an acute treatment. In the acute treatment protocols, ceftriaxone and analgesics were administered 25 and 55 min before the acetic acid administration, respectively.

Calculations in the Hyperalgesia/Nociception Models

To calculate the effective dose that produces 50% of the antihyperalgesic/antinociceptive effect (ED_{50}) of each drug, the df or N values were converted to percentages of antihyperalgesic (%AH) or antinociceptive (%AN) activities, according to the following formulas:

$$\%AH = [(\text{Control group average df} - \text{df of each rat in the test group})/(\text{Control group average df})] \times 100^{25\text{-}27}$$

and

$$\%AN = [(\text{Control group average N} - \text{N of each mouse in the test group})/(\text{Control group average N})] \times 100^{26}$$

ED_{50} values were estimated from corresponding log dose–response curves by linear regression.[31]

Analysis of Interactions between Ceftriaxone and Analgesics

Interactions between ceftriaxone and the analgesics were evaluated by isobolographic analysis at the ED_{50} level of the effect as described previously.[26,27,31] In the experiments in which the drugs were combined, the two drugs were administered at fixed-dose fractions of their respective ED_{50}. Ceftriaxone was applied as a pretreatment and the analgesic as an acute treatment. The experimental ED_{50} for the drug mixture ($ED_{50\ mix}$) was calculated from the corresponding log dose–response curve. When the drug combination produced an $ED_{50\ mix}$ that was significantly lower than the calculated theoretical ED_{50} ($ED_{50\ add}$), it was interpreted as there is a supraadditive (synergistic) interaction between the drugs.[31] In addition, an interaction index was used to describe the magnitude of the interaction.[26,27,32]

Analysis of Duration of the Effect of Drugs/Drug Combinations in a Model of Somatic Inflammatory Hyperalgesia

To compare the duration of the effect of the drug when applied alone with the duration of the effect produced by the same drug when applied in combination, the slopes of the %AH–ΔAUC regression lines were calculated as previously described.[26,27] In brief, the areas under the time–df curves (AUC) were calculated for the control (AUC_C) and the drug/drug combination (AUC_D) groups, and the differences were calculated as ΔAUC = AUC_C – AUC_D.[33] The ΔAUC for each drug/drug combination group is presented as a function of its peak %AH (ΔAUC = slope × %AH + intercept). In this regression line, the slope is the relative measure of the duration of the drug/drug combination effect; the treatment with a significantly greater slope has a longer effect than the treatment with a lesser slope.[26,27,34] A high correlation coefficient of the %AH–ΔAUC regression line indicates that the duration of the effect of the drug/drug combination treatment is dose dependent.[26,27,34]

Rotarod Test

Rotarod performance was assessed to evaluate the effects of ceftriaxone and levetiracetam on motor coordination or sedation.[35] The test was performed using a rotarod apparatus (Treadmill for rats 47700 or Treadmill for mice 7600; Ugo Basile, Milano, Italy), consisting of a rod rotating at a constant speed of 15 rpm.[30,36] The animals were trained to drive the rotarod four times a day for 2 days. Only those animals that could stay on the rod for 180 s on two consecutive trials were used in the experiments. Ceftriaxone was administered in both pretreatment and acute treatment protocols, and levetiracetam was administered only as an acute treatment. In the acute treatment protocols, ceftriaxone and levetiracetam were administered 30 or 60 min before the testing, respectively. The posttreatment latency to remain on the rotating rod (with a 180 s cutoff) was recorded at four time points, during 240 min.

Statistical Analysis

All pharmacological computations were performed using Pharm PCS (Micro-Computer Specialists, Philadelphia, PA) and Pharm Tools Pro (The McCary Group, Schnecksville, PA). The statistical analysis was conducted using SigmaPlot 11 (Systat Software Inc., Richmond, CA). The data were normally distributed (Shapiro–Wilk test) and are presented as the means ± SEM. Time-course data in the inflammatory hyperalgesia model were analyzed using a two-way repeated-measures ANOVA, followed by Tukey honestly significant difference test for between-group comparisons and comparisons at each time point. Two-way ANOVA was used to compare the peak effects of each ceftriaxone dose that was achieved after the pretreatment and the acute treatment. Data from the visceral nociception model were analyzed by one-way ANOVA. Tukey honestly significant difference test was used for *post hoc* comparisons. Data from the rotarod test were analyzed by independent sample Student *t* test. The differences between $ED_{50\ mix}$ and $ED_{50\ add}$ were examined by modified *t* test.[31] The slopes of the %AH–ΔAUC regression lines were compared by the test for parallelism.[33] A *P* value less than 0.05 was considered statistically significant.

Results

Effects of Ceftriaxone on Somatic Inflammatory Hyperalgesia

Seven-day pretreatment with intraperitoneally administered ceftriaxone (10–200 mg/kg per day) produced a dose-dependent reduction of hyperalgesia ($P < 0.001$ by two-way repeated-measures ANOVA; fig. 1A). The maximal effects were observed 120–240 min after the induction of inflammation. The corresponding $ED_{50} \pm SEM$ calculated from the peak effect values was 31.96 ± 12.02 mg/kg per day (table 1).

Acute treatment with intraperitoneally administered ceftriaxone (10–200 mg/kg) also produced a dose-dependent antihyperalgesia ($P < 0.001$ by two-way repeated-measures ANOVA; fig. 1B), which peaked 300 min after the induction of inflammation (240 min after ceftriaxone administration). The peak effect produced with each tested dose of ceftriaxone in the acute treatment was significantly lower than the peak effect of the same dose produced after the 7-day pretreatment ($P < 0.001$ by two-way ANOVA; fig. 1C).

Acute treatment with intraplantar ceftriaxone (0.05–0.2 mg per paw) reduced hyperalgesia in a dose-dependent manner ($P < 0.001$ by two-way repeated-measures ANOVA;

fig. 2A). The effect of ceftriaxone was local because it was not observed after the injection of the highest dose into the contralateral paw ($P > 0.05$ by Tukey honestly significant difference *post hoc* test; fig. 2A). The peak antihyperalgesic effects of the intraplantarly administered ceftriaxone (fig. 2B) occurred 180–300 min after administration.

Effects of Ceftriaxone on Visceral Nociception

Intraperitoneal pretreatment with ceftriaxone (25–200 mg/kg per day) for 7 days produced a dose-dependent antinociception, as observed in the writhing test ($P < 0.001$ by one-way ANOVA; fig. 3A). The corresponding $ED_{50} \pm SEM$ was 69.32 ± 2.76 mg/kg per day (table 2).

Acute treatment with intraperitoneally administered ceftriaxone (25–200 mg/kg) did not produce significant antinociception ($P = 0.089$ by one-way ANOVA; fig. 3B) although a tendency for a reduction of the number of writhes in a dose-dependent manner could be observed. When the effects of each acutely administered dose of ceftriaxone were compared with the effects of the same dose when applied as a 7-day pretreatment, it was significantly less for 50, 100, and 200 mg/kg ($P < 0.001$ by two-way ANOVA; fig. 3C).

Inflammatory hyperalgesia model
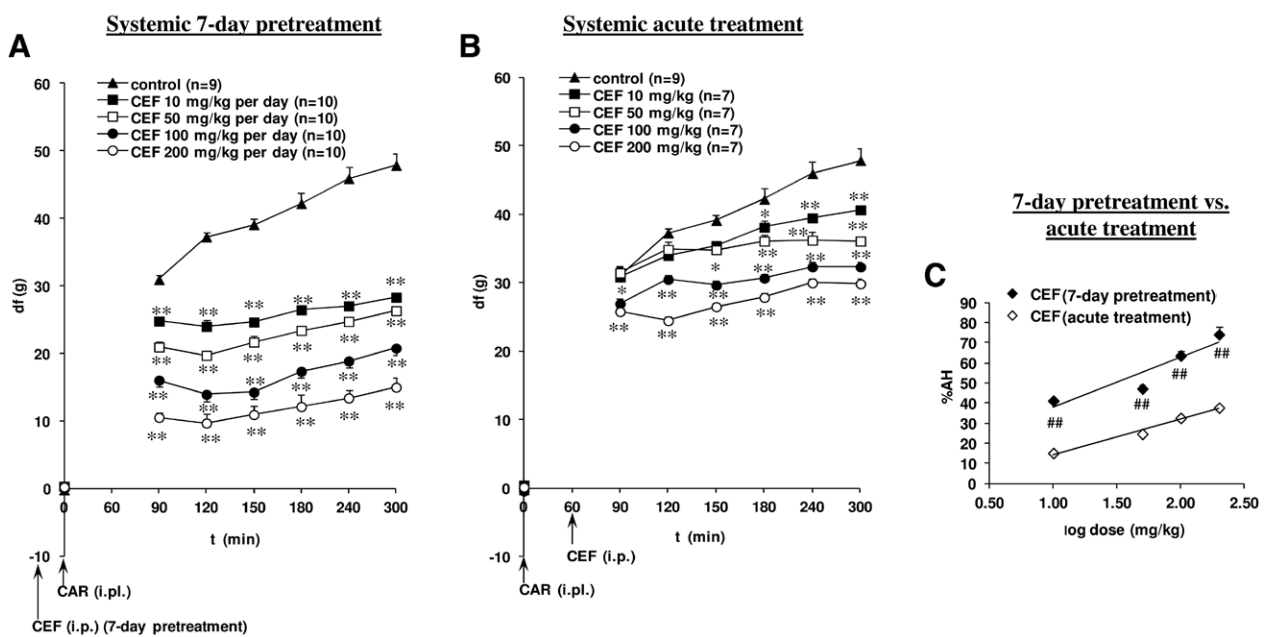
Fig. 1. Time course of the systemic antihyperalgesic effects of CEF after 7-day pretreatment (*A*) and after an acute treatment (*B*). Basal df (plotted at vertical axis) was obtained before drug administration and the induction of inflammation by an i.pl. injection of CAR (denoted by *arrows*). Each *point* represents the mean ± SEM. *$P < 0.05$, **$P < 0.01$ compared with control group at each time point (Tukey honestly significant difference *post hoc* test after two-way repeated-measures ANOVA). (*C*) Log dose–response curves for CEF antihyperalgesia at the time of peak effects, after 7-day pretreatment, and after an acute treatment. ##$P < 0.01$ compared with the effect of the same dose after an acute treatment (Tukey honestly significant difference *post hoc* test after two-way ANOVA). %AH = percentage of antihyperalgesic activity; CAR = carrageenan; CEF = ceftriaxone; df = paw pressure difference between noninjected and CAR-injected rat hind paw; i.p. = intraperitoneal; i.pl. = intraplantar.

Table 1. Parameters of Isobolographic Analysis for Ceftriaxone–Analgesics Combinations in a Model of Inflammatory Hyperalgesia

Drug/Drug Combination		ED_{50}* ± SEM (95% Confidence Limits)	
Ceftriaxone		31.96 ± 12.02 (6.32–161.61) mg/kg per day	
Ibuprofen		30.78 ± 1.08 (26.45–35.82) mg/kg	
Celecoxib		7.76 ± 0.46 (6.06–10.01) mg/kg	
Paracetamol		94.93 ± 4.41 (77.70–115.98) mg/kg	
Levetiracetam		28.52 ± 5.04 (13.80–59.48) mg/kg	

|	Mass Quantity Drug Ratio	$ED_{50\,add}$‡	$ED_{50\,mix}$§	γ†
Ceftriaxone + ibuprofen	1.04:1	31.37 ± 4.64 (21.35–44.62)	6.42 ± 0.45 (4.75–8.68)‖	0.20
Ceftriaxone + celecoxib	4.12:1	19.86 ± 3.07 (12.60–28.72)	3.83 ± 0.70 (1.75–8.42)‖	0.19
Ceftriaxone + paracetamol	1:2.97	63.44 ± 9.95 (39.10–89.24)	12.10 ± 0.89 (8.81–16.62)‖	0.19
Ceftriaxone + levetiracetam	1.12:1	30.24 ± 5.24 (19.32–45.83)	5.93 ± 0.77 (3.39–10.38)‖	0.20

* ED_{50} is the effective dose that produces 50% antihyperalgesic activity. † Interaction index, $\gamma = ED_{50\,CEFTRIAXONE\,COMBINED\,WITH\,ANALGESIC}/ED_{50\,CEFTRIAXONE\,GIVEN\,ALONE}$ + $ED_{50\,ANALGESIC\,COMBINED\,WITH\,CEFTRIAXONE}/ED_{50\,ANALGESIC\,GIVEN\,ALONE}$. Values near 1 indicate additive interaction, values >1 imply an antagonistic interaction, and values <1 indicate a synergistic interaction.[32] ‡ $ED_{50\,add}$ is the theoretical additive ED_{50} for drug mixture. § $ED_{50\,mix}$ is the experimental ED_{50} for drug mixture. ‖ $P < 0.05$ between $ED_{50\,add}$ and $ED_{50\,mix}$ (t test) indicates a synergistic interaction.[31]

Inflammatory hyperalgesia model

Local acute treatment
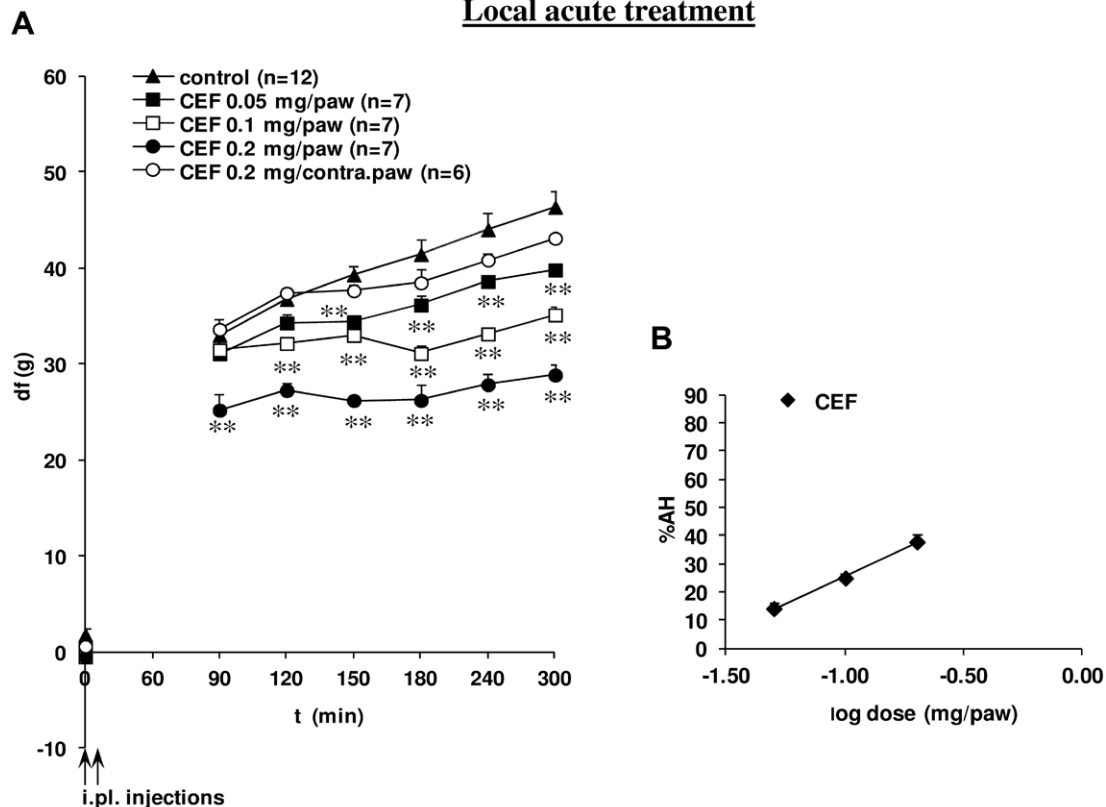
Fig. 2. Time course of the local peripheral antihyperalgesic effects of CEF (*A*). Basal df (plotted at vertical axis) was obtained before CEF and CAR i.pl. administration (denoted by *arrows*). Each *point* represents the mean ± SEM. **$P < 0.01$ compared with control group at each time point (Tukey honestly significant difference *post hoc* test after two-way repeated-measures ANOVA). (*B*) Log dose–response curve for CEF local peripheral antihyperalgesia at the time of peak effects. %AH = percentage of anti-hyperalgesic activity; CAR = carrageenan; CEF = ceftriaxone; contra. = contralaterally; df = paw pressure difference between noninjected and CAR-injected rat hind paw; i.pl. = intraplantar.

Visceral nociception model
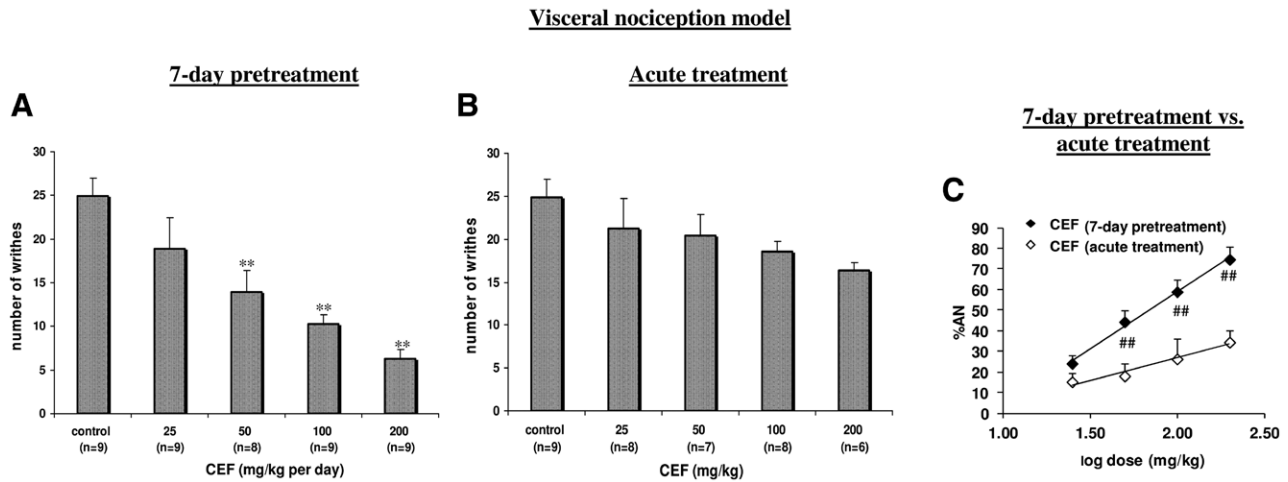
Fig. 3. Antinociceptive effects of CEF after 7-day pretreatment (*A*) and after an acute treatment (*B*). Each column represents the mean ± SEM number of writhes induced by acetic acid (i.p.). **$P < 0.01$ compared with control group (Tukey honestly significant difference *post hoc* test after one-way ANOVA). (*C*) Log dose–response curves for CEF antinociception after 7-day pretreatment and after an acute treatment. ##$P < 0.01$ compared with the effect of the same dose after an acute treatment (Tukey honestly significant difference *post hoc* test after two-way ANOVA). %AN = percentage of antinociceptive activity; CEF = ceftriaxone; i.p. = intraperitoneal.

Interactions between Ceftriaxone and Analgesics in a Model of Somatic Inflammatory Hyperalgesia

Orally administered ibuprofen (12.5–100 mg/kg), celecoxib (3.75–30 mg/kg), paracetamol (50–200 mg/kg), and levetiracetam (10–200 mg/kg) caused dose-dependent antihyperalgesia ($P < 0.001$ by two-way repeated-measures ANOVA, not shown). Their ED_{50} values were calculated from the corresponding log dose–response curves (fig. 4) and are summarized in table 1.

Two-drug combinations of intraperitoneally administered ceftriaxone (7-day pretreatment) with oral analgesics also caused a dose-dependent reduction of hyperalgesia ($P < 0.001$ by two-way repeated-measures ANOVA; fig. 5). For all of the examined combinations, the $ED_{50 \; mix}$ was significantly lower than the $ED_{50 \; add}$ ($P < 0.05$ by *t* test)

and the interaction index was less than 1 which indicates a synergistic interaction (fig. 6 and table 1). According to the interaction index values, at all of the examined combinations, there was almost the same degree of potentiation with approximately 10-fold reduction of doses of both drugs when the drugs were applied in combination compared with the doses that produced the same level of effect after individual administration.
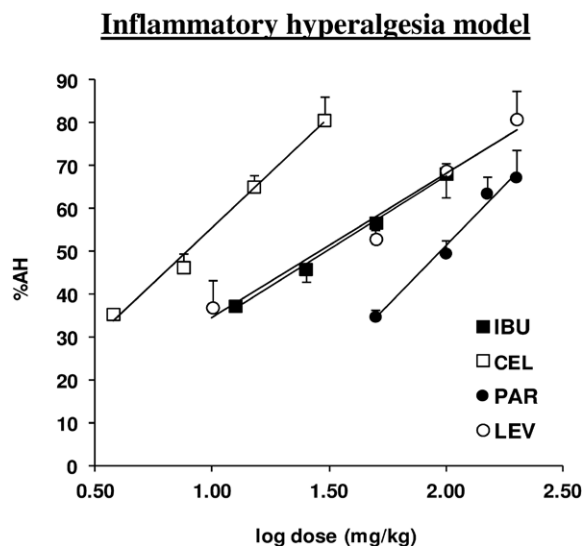
Analysis of the Duration of the Effects of Ceftriaxone, Analgesics, and Ceftriaxone–Analgesic Combinations in a Model of Somatic Inflammatory Hyperalgesia

Slope of the %AH–ΔAUC regression line for ceftriaxone (7-day pretreatment) was greater than the slope for each analgesic ($P < 0.05$ by test for parallelism; table 3). This

Table 2. Parameters of Isobolographic Analysis for Ceftriaxone–Analgesics Combinations in a Model of Visceral Nociception

Drug/Drug Combination	ED_{50}* ± SEM (Confidence Limits)		
Ceftriaxone	69.32 ± 2.76 (58.39–82.31) mg/kg per day		
Ibuprofen	38.11 ± 3.77 (24.89–58.36) mg/kg		
Celecoxib	9.13 ± 1.13 (5.35–15.58) mg/kg		
Paracetamol	48.43 ± 14.48 (13.36–175.58) mg/kg		
Levetiracetam	6.03 ± 1.62 (1.90–19.13) mg/kg		

	Mass Quantity Drug Ratio	$ED_{50 \; add}$‡	$ED_{50 \; mix}$§	γ†
Ceftriaxone + ibuprofen	1.82:1	53.65 ± 2.33 (48.24–59.68)	14.99 ± 1.59 (9.50–23.67)‖	0.28
Ceftriaxone + celecoxib	7.59:1	39.26 ± 2.07 (34.51–44.67)	4.56 ± 0.62 (1.76–9.69)‖	0.12
Ceftriaxone + paracetamol	1.43:1	58.88 ± 7.46 (41.37–78.56)	16.13 ± 0.82 (15.07–18.65)‖	0.27
Ceftriaxone + levetiracetam	11.50:1	37.68 ± 4.28 (27.79–49.07)	4.38 ± 0.60 (1.69–9.33)‖	0.12

* ED_{50} is the effective dose that produces 50% antinociceptive activity. † Interaction index, $\gamma = ED_{50 \; \text{CEFTRIAXONE COMBINED WITH ANALGESIC}} / ED_{50 \; \text{CEFTRIAXONE GIVEN ALONE}} + ED_{50 \; \text{ANALGESIC COMBINED WITH CEFTRIAXONE}} / ED_{50 \; \text{ANALGESIC GIVEN ALONE}}$. Values near 1 indicate additive interaction, values >1 imply an antagonistic interaction, and values <1 indicate a synergistic interaction.[32] ‡ $ED_{50 \; add}$ is the theoretical additive ED_{50} for drug mixture. § $ED_{50 \; mix}$ is the experimental ED_{50} for drug mixture. ‖ $P < 0.05$ between $ED_{50 \; add}$ and $ED_{50 \; mix}$ (*t* test) indicates a synergistic interaction.[31]

Inflammatory hyperalgesia model

Fig. 4. Log dose–response curves for antihyperalgesia induced by IBU, CEL, PAR, and LEV in a model of somatic inflammatory hyperalgesia in rats at the time of peak effects. Each *point* represents the mean ± SEM obtained in 6-9 animals. %AH = percentage of antihyperalgesic activity; CEL = celecoxib; IBU = ibuprofen; LEV = levetiracetam; PAR = paracetamol.

result indicates that the effects produced by individually administered ceftriaxone lasted longer than the effects produced by individually administered analgesics. The slopes for all two-drug combinations of ceftriaxone were not different from the slope for ceftriaxone *per se* ($P > 0.05$ by test for parallelism; table 3). This indicates that the duration of the effects of ceftriaxone was unchanged when it was applied alone and when it was applied with analgesics. However, the slopes for ceftriaxone–ibuprofen and ceftriaxone–celecoxib combinations were greater than the slopes for the corresponding analgesics when applied individually ($P < 0.05$ by test for parallelism; table 3). This indicates that the actions of ibuprofen and celecoxib were longer when combined with ceftriaxone. The high correlation coefficients of the %AH–ΔAUC line for all of the treatments indicate that the durations of the effects were dose dependent (table 3).

Interactions between Ceftriaxone and Analgesics in a Visceral Nociception Model

Oral ibuprofen (12.5–100 mg/kg), celecoxib (3.75–30 mg/kg), paracetamol (5–150 mg/kg), and levetiracetam (1–25 mg/kg) caused a dose-dependent reduction of writhes induced by acetic acid ($P < 0.001$ by one-way ANOVA, not shown). The corresponding log dose–response curves are shown in fig. 7, and the ED_{50} values are summarized in table 2.

Two-drug combinations of intraperitoneally administered ceftriaxone (7-day pretreatment) with oral analgesics also reduced nociception in a dose-dependent manner ($P < 0.001$ by one-way ANOVA; fig. 8). Isobolographic analysis revealed synergistic interactions for all of the examined combinations (table 2 and fig. 9). Interaction index values

indicated a different degree of potentiation with the following rank: ceftriaxone–celecoxib = ceftriaxone–levetiracetam > ceftriaxone–ibuprofen = ceftriaxone–paracetamol. A more than 17-fold reduction of doses of both drugs in the ceftriaxone–celecoxib and ceftriaxone–levetiracetam combinations and a more than seven-fold reduction of doses in the ceftriaxone–ibuprofen and ceftriaxone–paracetamol combinations are observed when compared with the corresponding doses after individual administration.

Effects of Ceftriaxone and Levetiracetam in a Rotarod Test

Intraperitoneally administered ceftriaxone either after 7-day pretreatment (200 mg/kg per day) or an acute treatment (200 mg/kg) did not influence the rotarod performance in both rats and mice ($P > 0.05$ by Student t test, not shown). Oral levetiracetam (200 mg/kg) was also without significant effect in both species ($P > 0.05$ by Student t test, not shown).

Discussion

Antihyperalgesic/Antinociceptive Effects of Ceftriaxone in Somatic and Visceral Inflammatory Pain Models

The current study shows that the administration of ceftriaxone for 7 days caused a significant and dose-dependent reduction of carrageenan-induced mechanical hyperalgesia in rats and acetic acid-induced nociception in mice. Our results widen the findings by Lin *et al.*,[13,37] Hu *et al.*,[11] and Yang *et al.*[38] who observed that ceftriaxone, in preventive 7-day administration, is effective not only in chronic neuropathic and visceral pain but also in somatic inflammatory pain. The animal model of carrageenan-induced hyperalgesia, as a model of somatic pain, mimics clinical inflammatory conditions.[39] Our study shows for the first time that the acute treatment with ceftriaxone in the same dose range produces a significant and dose-dependent antihyperalgesic effect which is significantly lower than that produced by 7-day treatments with all doses. As ceftriaxone did not produce a significant impairment of motor performance in the rotarod test in rats and mice at the highest used doses, it would appear that the antihyperalgesic/antinociceptive effects of ceftriaxone were not due to motor impairment or sedation. The current study also demonstrates a modest efficacy of ceftriaxone as a local peripheral antihyperalgesic drug in the carrageenan-induced pain inflammatory model. There is no literature data regarding ceftriaxone's local peripheral antinociceptive efficacy. The local nature of this action was verified by the absence of effect of ceftriaxone after it was injected into the contralateral hind paw. A significant local effect of ceftriaxone (0.05–0.2 mg per paw) was obtained with much lower doses (up to 40 times lower) than with the lowest effective systemic dose (10 mg/kg). This finding suggests that when ceftriaxone is given systemically (either as an acute dose or a 7-day pretreatment), it achieves effective concentrations at the periphery. It also points to the contribution of a peripheral antihyperalgesic effect to the net effect of systemically administered ceftriaxone.

Inflammatory hyperalgesia model
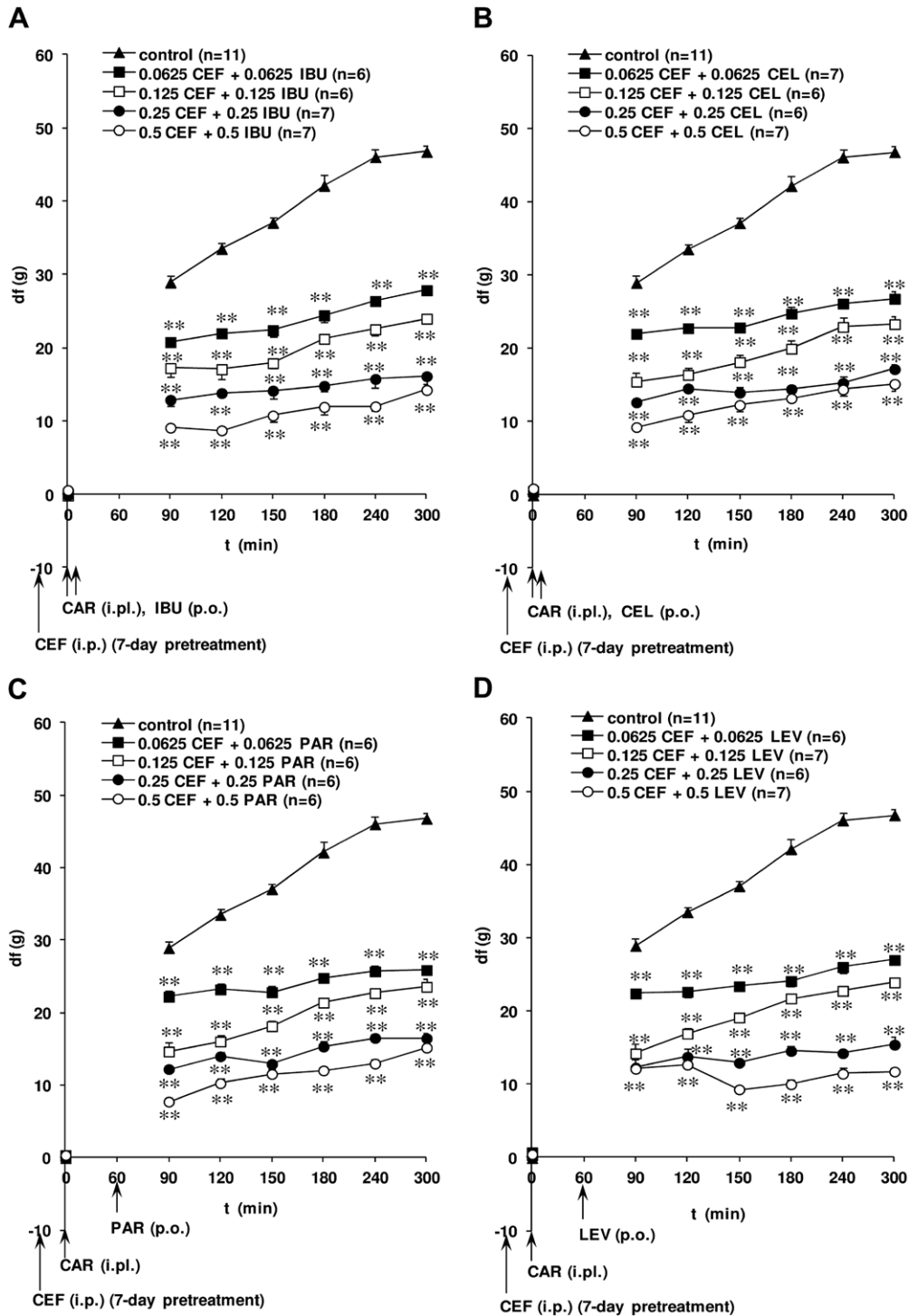
Fig. 5. Time course of the antihyperalgesic effects of CEF + IBU (*A*), CEF + CEL (*B*), CEF + PAR (*C*), and CEF + LEV (*D*) combinations. Basal df (plotted at vertical axis) was obtained before drugs administration and the induction of inflammation by an i.pl. injection of CAR. CEF was administered i.p. once daily for 7 days before induction of inflammation. IBU and CEL were administered p.o. immediately after CAR, whereas LEV and PAR were administered p.o. 60 min after CAR (denoted by *arrows*). Drugs were administered at fixed-dose fractions of their respective ED_{50} (1/16 = 0.0625, 1/8 = 0.125, 1/4 = 0.25, and 1/2 = 0.5). Each *point* represents the mean ± SEM. **$P < 0.01$ compared with control group at each time point (Tukey honestly significant difference *post hoc* test after two-way repeated-measures ANOVA). CAR = carrageenan; CEF = ceftriaxone; CEL = celecoxib; df = paw pressure difference between noninjected and CAR-injected rat hind paw; ED_{50} = effective dose that produces 50% of the antihyperalgesic effect; IBU = ibuprofen; i.p. = intraperitoneal; i.pl. = intraplantar; LEV = levetiracetam; PAR = paracetamol; p.o. = oral.

Inflammatory hyperalgesia model
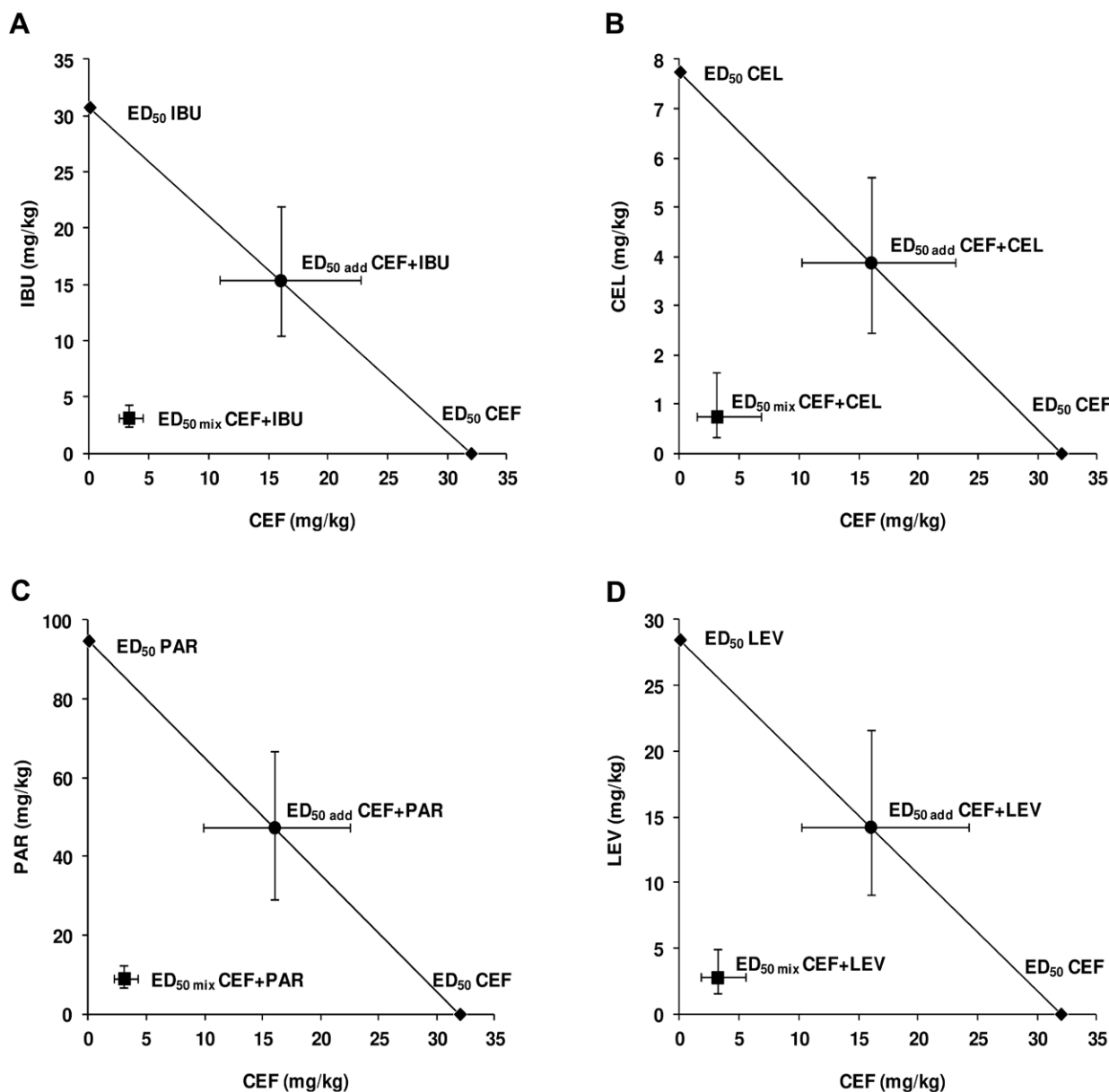
Fig. 6. Isobolograms for CEF + IBU (*A*), CEF + CEL (*B*), CEF + PAR (*C*), and CEF + LEV (*D*) combinations in the model of somatic inflammatory hyperalgesia in rats. The ED_{50} values for each drug (obtained at the time of peak effects) are plotted at the axes. The *straight line* connecting the each ED_{50} value is the theoretical additive line, and the *point* in this line is the $ED_{50\ add}$. There is a significant difference ($P < 0.05$; *t* test) between the $ED_{50\ add}$ and the $ED_{50\ mix}$ in each isobologram indicating a synergistic drug interaction for all of the examined combinations. CEL = celecoxib; CEF = ceftriaxone; ED_{50} = effective dose that produces 50% of the antihyperalgesic effect; $ED_{50\ add}$ = theoretical additive ED_{50}; $ED_{50\ mix}$ = experimental ED_{50} for drug mixture; IBU = ibuprofen; LEV = levetiracetam; PAR = paracetamol.

Peripheral inflammation induced by carrageenan causes glutamate release from primary sensory afferents and excitatory interneurons in the dorsal horn of the spinal cord, producing inflammatory hyperalgesia through the activation of peripheral and spinal glutamate receptors.[1,40–42] Ceftriaxone can selectively upregulate the expression of glutamate transporter GLT-1 and accordingly reduce intrasynaptic glutamate.[10]

GLT-1 is considered to be the glutamate transporter in the central[8,43] and peripheral[9] nervous systems. Because ceftriaxone produced analgesic activity in the paw pressure test after systemic and local peripheral application, it could be suggested that the reduction of intrasynaptic glutamate in the spinal cord and peripheral sensory terminals would contribute to the antihyperalgesic effect of ceftriaxone in somatic inflammatory pain.

Table 3. Parameters of the Duration of Action of Ceftriaxone, Analgesics, and Their Combinations in a Model of Inflammatory Hyperalgesia

Drug/Drug Combination	Slope* ± SEM	Correlation Coefficient†
Ceftriaxone	1.62 ± 0.36	1
Ibuprofen	1.04 ± 0.16‡	0.98
Celecoxib	1.21 ± 0.22‡	1
Paracetamol	1.04 ± 0.13‡	0.98
Levetiracetam	1.23 ± 0.16‡	1
Ceftriaxone + ibuprofen	1.63 ± 0.08§	1
Ceftriaxone + celecoxib	1.74 ± 0.13§	0.99
Ceftriaxone + paracetamol	1.50 ± 0.30	1
Ceftriaxone + levetiracetam	1.41 ± 0.19	1

* Slope of the %AH–ΔAUC regression line is the relative measure of the duration of the drug/drug combination effect.[26,27,34] † Correlation coefficient of the %AH–ΔAUC regression line. ‡ $P < 0.05$; comparing with the slope for ceftriaxone, test for parallelism. § $P < 0.05$; comparing with the slope for analgesic alone, test for parallelism.

%AH = percentage of antihyperalgesic activity; ΔAUC = difference between area under the time–df curve for control and each dose of drug/drug combination groups; df = paw pressure difference between noninjected and carrageenan-injected rat hind paw.

Both peripheral and central mechanisms contribute to visceral hypersensitivity in the writing test in mice. It seems that visceral hyperalgesia is due to lowering the threshold of "high threshold" receptors and activation of previously unresponsive receptors, and subsequent neuroplastic changes in terms of increased release of the excitatory glutamate, principally in the spinal cord.[44] Therefore, the antinociceptive

Visceral nociception model

Fig. 7. Log dose–response curves for antinociception induced by IBU, CEL, PAR, and LEV in a visceral nociception model in mice. Each *point* represents the mean ± SEM obtained in 6–10 animals. %AN = percentage of antinociceptive activity; CEL = celecoxib; IBU = ibuprofen; LEV = levetiracetam; PAR = paracetamol.

effect of ceftriaxone in the writing test could be explained by enhanced glutamate uptake primarily in the spinal cord.

Moreover, it has been shown that the hyperalgesic/nociceptive activity of carrageenan/acetic acid in the paw pressure/writing test is due to the release of proinflammatory cytokines, tumor necrosis factor-α, and interleukins from immune and glial cells.[45,46] Amin *et al.*[17] observed that a 1-week treatment with ceftriaxone significantly attenuates these cytokines in the spinal cord. Several previous studies have described the synergistic interaction of inflammatory cytokines and glutamate in neuronal sensitization.[17,46] Therefore, ceftriaxone might produce antihyperalgesic/antinociceptive effects after 7 days of systemic administration at least by decreasing cytokine levels. This could also explain the observed antihyperalgesia after acute systemic administration of ceftriaxone. However, the synergistic interactions of inflammatory cytokines and glutamate and the observation that ceftriaxone reduces intrasynaptic glutamate through increased transcription of the GLT-1 gene[10] (hours and days are needed for its full effect) could explain the significantly lower antihyperalgesia/antinociception after an acute systemic administration of ceftriaxone than that after its systemic administration for 7 days in both models.

There is evidence that both somatic and visceral components, as well as inflammation, contribute to many kinds of postoperative pain.[47,48] The available data suggest that ceftriaxone could also work as an adjunctive treatment in certain types of postoperative pain because ceftriaxone is often given for perioperative prophylaxis (acute administration) and treatment (7-day administration) of postoperative infections. Unless the metabolism of ceftriaxone differs in rodents and humans, the lowest and one above the lowest dose of intraperitoneally administered ceftriaxone that produced significant antihyperalgesic effects after both acute and 7-day administration as well as the lowest dose that produced a significant antinociceptive effect after 7 day administration are comparable with the doses used in surgical prophylaxis (1–2 g/day, intramuscular/intravenous) and in severe infections (2–4 g/day, intramuscular/intravenous) in humans.

Effects of Two-drug Combinations of Ceftriaxone and Different Analgesics in Somatic and Visceral Inflammatory Pain

Our results reveal that ceftriaxone exerts a synergistic interaction with ibuprofen, celecoxib, paracetamol, or levetiracetam in reducing carrageenan-induced mechanical hyperalgesia in rats and acetic acid-induced writhing in mice. Synergistic pharmacodynamic interactions could be ascribed to the activation of different complementary pathways of the observed actions.[49] The inflammation and injury induced by carrageenan and acetic acid are associated not only with release of glutamate and cytokines (tumor necrosis factor-α and interleukins) but also with local release of proinflammatory prostaglandins, particularly E series, resulting in activation and sensitization
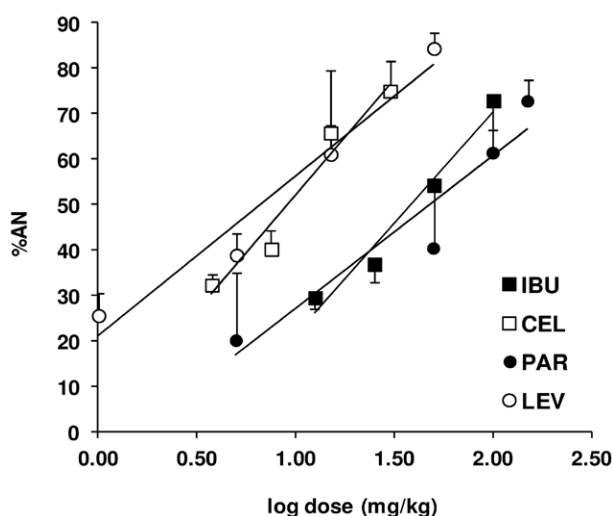
of somatic/visceral peripheral nociceptive afferents.[1,9,14,28,40] The hyperactivity and sensitization of afferent fibers stimulate the release of glutamate, cytokines, and prostaglandins in the spinal cord, contributing to the central sensitization in dorsal horn neurons.[1,50–52] Ibuprofen, a nonselective COX-1/2 inhibitor, and celecoxib, a selective COX-2 inhibitor, exert their antihyperalgesic/antinociceptive actions by blocking prostaglandin synthesis at peripheral and spinal sites.[51,53–55] A satisfactory mechanism of action of paracetamol still remains to be established. The analgesic effect of paracetamol could be ascribed to its inhibitory action on peripheral and central

COX-2,[19] as well to its activation of descending opioid and serotonergic pathways.[56,57] Our research group has shown in the somatic inflammatory pain model that levetiracetam produces an antihyperalgesic effect. This effect is at least in part mediated by central γ-aminobutyric acid type A receptors, peripheral adenosine, and both central and peripheral opioidergic, 5-hydroxytryptaminergic, and α_2-adrenergic receptors.[21,22] Although we have shown for the first time that levetiracetam is effective in the visceral pain model, our results do not clarify the mechanism of the antinociceptive effect of levetiracetam in this model. However, it could be suggested

Visceral nociception model
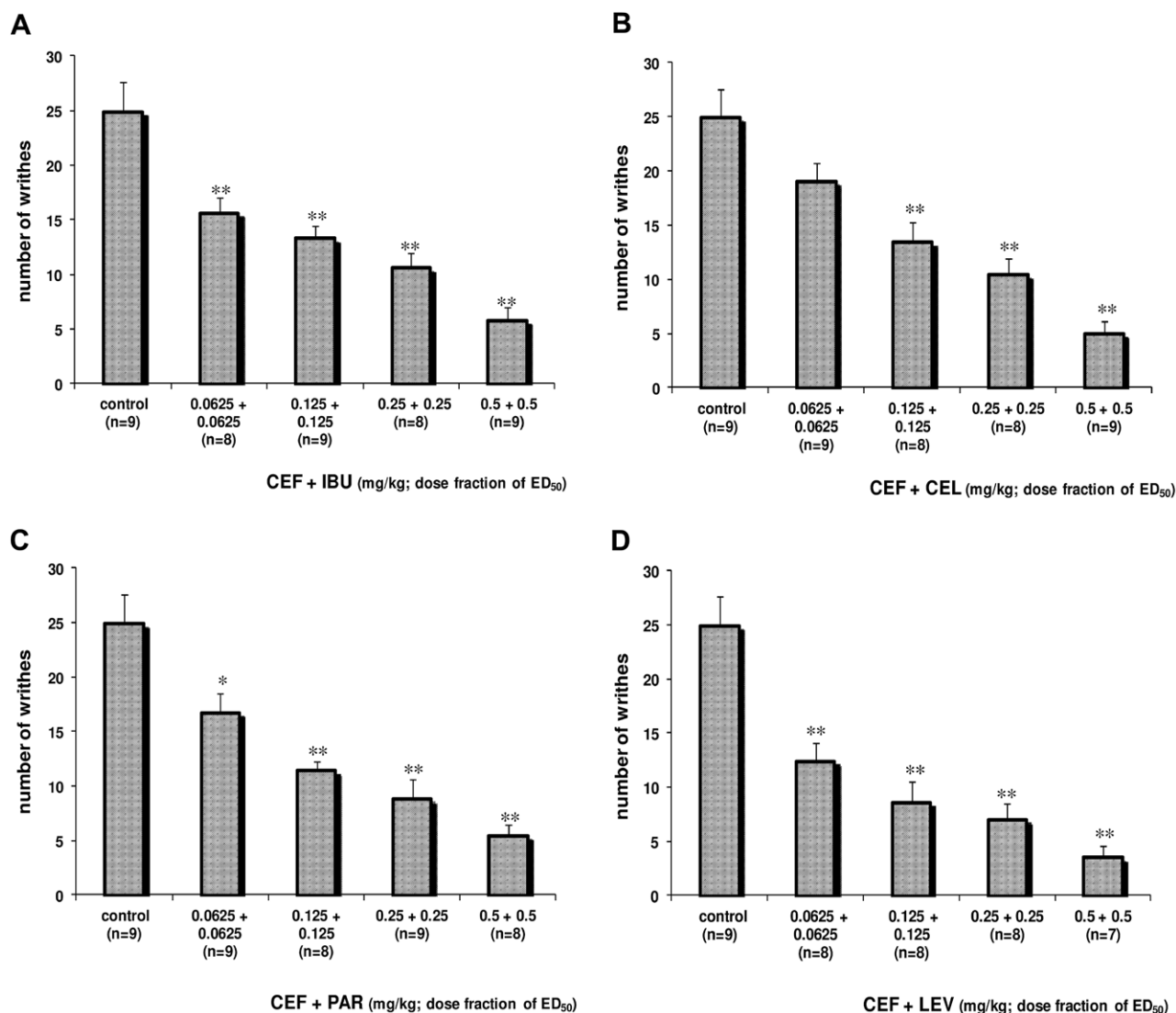
Fig. 8. Antinociceptive effects of CEF + IBU (*A*), CEF + CEL (*B*), CEF + PAR (*C*), and CEF + LEV (*D*) combinations. Analgesics were administered p.o. 55 min before the acetic acid (i.p.). CEF was administered i.p. as 7-day pretreatment. Drugs were administered at fixed-dose fractions of their respective ED_{50} (1/16 = 0.0625, 1/8 = 0.125, 1/4 = 0.25, and 1/2 = 0.5). Each column represents the mean ± SEM number of writhes. *$P < 0.05$, **$P < 0.01$ compared with control group (Tukey honestly significant difference *post hoc* test after one-way ANOVA). CEF = ceftriaxone; CEL = celecoxib; ED_{50} = effective dose that produces 50% of the antinociceptive effect; IBU = ibuprofen; i.p. = intraperitoneal; LEV = levetiracetam; PAR = paracetamol; p.o. = oral.
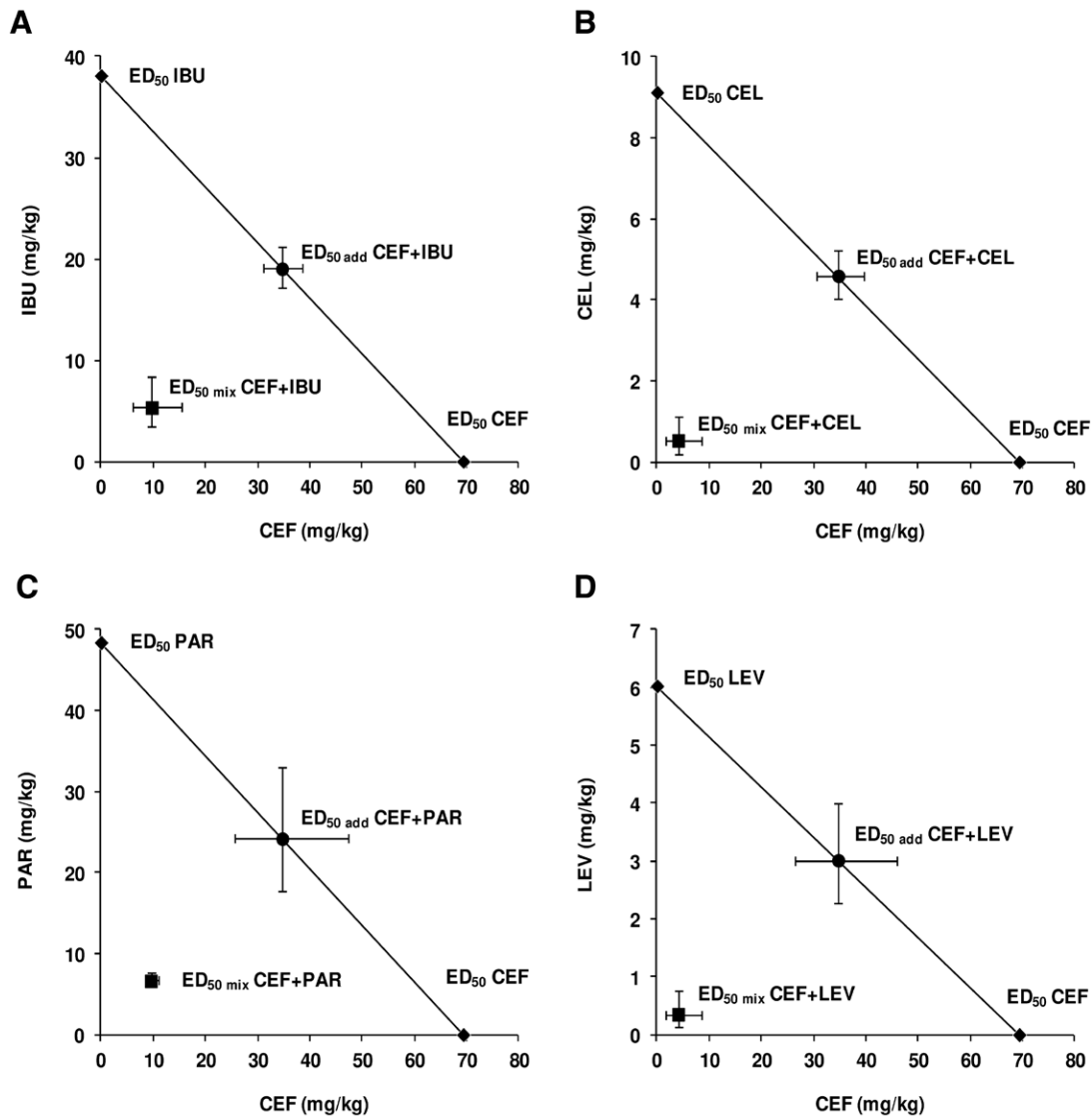
Fig. 9. Isobolograms for CEF + IBU (*A*), CEF + CEL (*B*), CEF + PAR (*C*), and CEF + LEV (*D*) combinations in the visceral nociception model in mice. The ED_{50} values for each drug are plotted at the axes. The *straight line* connecting the each ED_{50} value is the theoretical additive line, and the *point* in this line is the $ED_{50\ add}$. There is a significant difference ($P < 0.05$; *t* test) between the $ED_{50\ add}$ and the $ED_{50\ mix}$ in each isobologram indicating a synergistic drug interaction for all of the examined combinations. CEL = celecoxib; CEF = ceftriaxone; ED_{50} = effective dose that produces 50% of the antinociceptive effect; $ED_{50\ add}$ = theoretical additive ED_{50}; $ED_{50\ mix}$ = experimental ED_{50} for drug mixture; IBU = ibuprofen; LEV = levetiracetam; PAR = paracetamol.

that the inflammatory nature of nociception in writhing test and carrageenan-induced hyperalgesia in paw pressure test leading to a facilitated state may be similar. Enhanced glutamate uptake and attenuated cytokine levels, at least at spinal sites, contribute to the antihyperalgesia/antinociception by ceftriaxone in inflammatory pain models in rodents.[11,13,37,38] Therefore, synergistic interactions between ceftriaxone and ibuprofen, celecoxib, paracetamol, or levetiracetam could be explained by the involvement of multiple different targets and sites in their antihyperalgesic/antinociceptive effects in these inflammatory pain models.

The pharmacokinetic interactions between ceftriaxone and the examined analgesics were not within the scope of

our work. However, they cannot be excluded in the combinations of ceftriaxone with ibuprofen and celecoxib that displayed prolonged actions. A prolongation of the effect of drug combination is likely to be expected in the case of pharmacokinetic interaction which would result in potentiation of pharmacological effects. Thus, it seems that there could be more than one possible mechanism which could explain the synergism observed between ceftriaxone and ibuprofen/ celecoxib. This prolongation of the drug's effects, in addition to their potentiation, could be beneficial in the potential clinical use of these combinations. A pharmacokinetic interaction between ceftriaxone and paracetamol/levetiracetam appears to be less likely (unchanged duration of effect).

The occurrence of side effects with all of these combinations is less likely because the doses of the individual components are markedly lower. Also, because the components have different side effects, the addition/potentiation of their individual effects is not expected.

In conclusion, the major findings of our study are (1) ceftriaxone exerts antihyperalgesia/antinociception in both somatic and visceral inflammatory pain, with higher efficacy after 7-day than after acute administration; (2) ceftriaxone exerts synergistic interactions with different analgesics (ibuprofen, celecoxib, paracetamol, levetiracetam) and is superior to monotherapy for both somatic and visceral inflammatory pain. Our results suggest that ceftriaxone, particularly in combination with certain analgesics, may provide a useful approach to the clinical treatment of inflammation-related pain.

Acknowledgments

Competing Interests

The authors declare no competing interests.

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