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Pharmacological interaction between oxcarbazepine and two COX inhibitors in a rat model of inflammatory hyperalgesia

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ABSTRACT

Oxcarbazepine, ibuprofen and etodolac have efficacy in inflammatory pain. The combination of different drugs activates both central and peripheral pain inhibitory pathways to induce additive or synergistic antinociception, and this interaction may allow lower doses of each drug combined and improve the safety profile, with lower side-effects. This study aimed to examine the effects of oxcarbazepine–ibuprofen and oxcarbazepine–etodolac combinations, in a rat model of inflammatory hyperalgesia, and determine the type of interaction between drugs. Rats were intraplantarly injected with carrageenan (0.1 ml, 1%) and the hyperalgesia was assessed by modified paw pressure test. The anti-hyperalgesic effects of oxcarbazepine, ibuprofen and etodolac and oxcarbazepine–ibuprofen and oxcarbazepine–etodolac combinations were examined. Drugs were co-administered in a fixed-dose fractions of the ED_{50} and the type of interaction was determined by isobolographic analysis.

Oxcarbazepine (40–160 mg/kg; p.o.), ibuprofen (10–120 mg/kg; p.o.) and etodolac (5–20 mg/kg; p.o.) produced a significant, dose-dependent anti-hyperalgesia in carrageenan-injected rats. ED_{50} values (mean ± SEM) for oxcarbazepine, ibuprofen and etodolac were 88.17 ± 3.65, 47.07 ± 10.27 and 13.05 ± 1.42 mg/kg, respectively. Oxcarbazepine–ibuprofen and oxcarbazepine–etodolac combinations induced significant and dose-dependent anti-hyperalgesia. Isobolographic analysis revealed that oxcarbazepine exerts a synergistic interaction with ibuprofen, with almost 4-fold reduction of doses of both drugs in combination. In contrast, there was an additive interaction with etodolac.

Synergistic interaction of oxcarbazepine with ibuprofen and its additive interaction with etodolac provide new information about the combination pain treatment and could be explored further in patients with inflammatory pain. Adverse effect analysis of the combinations is necessary to verify possible clinical use of the mixtures.

1. Introduction

Oxcarbazepine, keto analog of carbamazepine, is used principally for treating epilepsy and occasionally for relieving neuropathic pain (Beydoun and Kutluay, 2002). Increasing evidence for antinociceptive effects of oxcarbazepine has been obtained in models of nerve injury (Fox et al., 2003; Kiguchi et al., 2004; Jang et al., 2005; Dogra et al., 2005), as well as somatic and visceral tissue injury followed by inflammation (Kiguchi et al., 2004; Tomić et al., 2004; Shannon et al., 2005; Stepanović-Petrović et al., 2008a). Ibuprofen, a nonselective cyclo-oxygenase-1/2 (COX-1/2)-inhibitor is effective especially against inflammatory pain, which is a consequence of tissue damage. Etodolac, a cyclo-oxygenase-2 (COX-2) selective inhibitor is also effective in inflammatory pain, but recent evidence showed that it alleviates hyperalgesia in neuropathic pain models in rats and mice, too (Suyama et al., 2004; Inoue et al., 2009). All three drugs are without substantive effect in models of acute pain in uninjured animals, but normalize the lowered nociceptive threshold induced by some kind of tissue injury (Kiguchi et al., 2004; Hurley et al., 2002). Tissue injury provokes damage to peripheral nerves and spinal cord changes, causing hypersensitivity and spontaneous firing of afferent nerve endings at the site of injury, as well as in central nervous system (Hurley et al., 2002; Kolosov et al., 2010). Several receptors and neurotransmitters are involved in this hyperexcitability, as well as an increased expression of Na^+- and Ca^{+2}-channels in sensory neurons and spinal cord (Waldmeier et al., 1995; Li et al., 2006; Kolosov et al., 2010). Antinociceptive effect of oxcarbazepine is mediated via Na^+-channels and neurotransmitter receptors, centrally and peripherally (Waldmeier et al., 1995; Ichikawa et al., 2001; Kiguchi et al., 2004; Tomić et al., 2004; Vučković et al., 2006;

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Stepanović-Petrović et al., 2008b). Ibuprofen exerts its anti-hyperalgesic activity by decreasing production of the prostaglandins (PGs) mainly on peripheral, but also on central levels. PGs sensitize nociceptors to inflammatory mediators and enhance firing frequency in nociceptive neurons by phosphorylation of $Na_V1.8$, high-threshold Na^+ channel isoform which is expressed only in nociceptive neurons (Akopian et al., 1996; Momin and McNaughton, 2009). In the spinal cord, PGs develop sensitization partly by activating nonselective cation current in dorsal horn neurons (Baba et al., 2001). It has been suggested that etodolac attenuates mechanical allodynia in a mouse model of neuropathic pain by suppressing the expression of genes for Ca^{+2}-channel $\alpha 2\delta$ subunit in the dorsal root ganglion (Inoue et al., 2009).

A hypothesis assumes that the simultaneous activation of different pain inhibiting pathways may be effective in pain therapy (Horvath and Kekesi, 2006). Thus, the co-administration of drugs that are efficacious in inflammatory pain, and interfere with different systems may be an effective method to its relief. The combination of different drugs activates both central and peripheral pain inhibitory pathways to induce additive or synergistic antinociception, and this interaction may allow lower doses of each drug combined and improve the safety profile, with lower side-effects (Desmeules et al., 2003; Miranda et al., 2009).

The purpose of this study was to determine the type of interaction (additive or synergistic) between oxcarbazepine and different COX inhibitors as ibuprofen (COX 1/2-inhibitor) and etodolac (COX 2-inhibitor). The type of interaction was evaluated by means of the isobolographic analysis using inflammatory paw pressure test in rats.

2. Methods

2.1. Animals

Experiments were performed on 180–220 g male Wistar rats (Military Academy Breeding Farm, Belgrade, Serbia). The animals were housed in groups of four in home cages (42.5×27×19 cm) and maintained on a 12/12 h light/dark cycle at 22±1 °C. Food and water were freely available, except during the experimental procedure. All experiments were carried out at the same time of the day between 8:00 and 16:00 h to avoid diurnal variation in behavioral tests. All experimental groups consisted of 6 to 8 rats. All experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee and were carried out in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs administration

Oxcarbazepine (TRILEPTAL, Novartis Pharma AD, Basel, Switzerland), ibuprofen (gift from Galenika, Belgrade, Serbia) and etodolac (gift from Alkaloid, Skopje, Macedonia) were suspended in distilled water with the addition of one drop of Tween 80. Drugs/drug combinations were administered to fasted rats orally in a volume of 2 ml/kg. Carrageenan (Sigma-Aldrich Chemie, Germany) was dissolved in saline (1% m/v), 24 h before the experiment and was injected intraplantarly (i.pl.) in a final volume of 0.1 ml/paw, using 1 ml syringe and 24 G (0.55×25 mm) needle.

2.3. Model of inflammatory hyperalgesia

The carrageenan-induced hyperalgesia and the anti-hyperalgesic activity of drugs/drug combinations were determined by a modified "paw pressure" test (Randall and Selitto, 1957; Tomić et al., 2004). The apparatus (Hugo Sachs Elektronik, March-Hugstetten, Germany) was used to evaluate the force exerted by rat hind paws in order to determine right/left differences. The rat was placed with its hind paws on two transducer platforms and pushed slowly and smoothly downwards with investigator hand, so that the force (pressure) is applied simultaneously to both paws. The pressure is applied until one of the paws exceeds the trigger level set at 100 g. At this point, an audible click by the apparatus is heard and the measurement is stopped automatically. The forces applied on the paws are read on the displays, and the difference (d) is calculated as (Tomić et al., 2004):

$$d = \text{force (g) applied on un} - \text{inflamed paw} - \text{force (g) applied on inflamed paw.}$$

Measurements were repeated 3 times at each time point, and the average d for each rat was used for further calculations.

Pre-treatment d was obtained before the induction of inflammation and peroral (p.o.) administration of drugs/drug combinations. Inflammatory hyperalgesia was induced immediately after p.o. drug/drug combination administration by injecting carrageenan (CAR) intraplantarly (i.pl.) into the right hind paw (Morris, 2003). According to data about the time-course of carrageenan hyperalgesia and the time-course of anti-hyperalgesic effect and/or pharmacokinetic properties of examined drugs (Cayen et al., 1981; Khan and Akhter, 2005; Tomić et al., 2010), administering the drugs and carrageenan at the same time was suitable for recording the appearance and increase of effect, its peak and the decrease during time for all examined drugs and drug combinations. Post-treatment d was measured 1, 2, 3, 4, and 5 h after the treatment. Control animals received the same volume of corresponding vehicle (p.o.) instead of test compounds.

Force differences for each rat are expressed as a percent anti-hyperalgesic activity (%AH) and calculated according to the following formula:

$$\%AH = (dc_{av} - dt) / (dc_{av}) \times 100,$$

according to Tomić et al. (2004), where the dc_{av} denotes control group average d and dt denotes d of each rat in the test group. If the dt was greater than dc_{av}, a value of 0% AH was assigned.

The values for %AH were calculated after each measurement of d (1, 2, 3, 4, and 5 h after treatment) to establish the time of peak effect. The log dose–response curves were determined at the time of peak effect. The ED_{50} (the dose that was expected to result in 50 %AH) with 95% confidence limits were estimated from corresponding log dose–response curves (Tallarida and Murray, 1986).

2.4. Isobolographic analysis

The interaction between ibuprofen and etodolac with oxcarbazepine was evaluated by co-administration of fixed proportions of each drug, and performing an isobolographic analysis (Tallarida et al., 1997). At first, an ED_{50} value of each drug has to be obtained from the corresponding log dose–response curves.

In the next step, oxcarbazepine and ibuprofen were co-administered at fixed-dose fractions of the ED_{50} (0.0625 $ED_{50\ \text{OXCARBAZEPINE}}$ + 0.0625 $ED_{50\ \text{IBUPROFEN}}$, 0.125 $ED_{50\ \text{OXCARBAZEPINE}}$ + 0.125 $ED_{50\ \text{IBUPROFEN}}$, 0.25 $ED_{50\ \text{OXCARBAZEPINE}}$ + 0.25 $ED_{50\ \text{IBUPROFEN}}$, 0.5 $ED_{50\ \text{OXCARBAZEPINE}}$ + 0.5 $ED_{50\ \text{IBUPROFEN}}$). For drug mixture, experimental ED_{50} ($ED_{50\ \text{mix}}$) and its associated 95% confidence intervals were determined by linear regression analysis of the log dose–response curve and compared to a theoretical additive ED_{50} ($ED_{50\ \text{add}}$) obtained from the calculation:

$$ED_{50\,\text{add}} = f \times ED_{50\,\text{OXCARBAZEPINE}} + (1-f) \times ED_{50\,\text{IBUPROFEN}},$$

where f denotes a fraction of the corresponding ED_{50} in drug mixture (in our study, $f=0.5$). In this equation, $ED_{50\ \text{add}}$ is the total dose, and the variance of $ED_{50\ \text{add}}$ was calculated as (Tallarida et al., 1997):

$$\text{Var}\,ED_{50\,\text{add}} = f^2 \times \text{Var}\,ED_{50\,\text{OXCARBAZEPINE}} + (1-f)^2 \times \text{Var}\,ED_{50\,\text{IBUPROFEN}}.$$

From these variances, confidence intervals were calculated and resolved according to the ratio of the individual drug in the combination. Supra-additivity is defined as the effect of a drug combination that is

higher and statistically different (ED_{50} significantly lower) than the theoretical calculated equieffect of a drug combination with the same proportions. When the drug combination gives an experimental ED_{50} not statistically different from the theoretical calculated ED_{50}, the combination has an additive effect (Tallarida et al., 1997). Additionally, to describe a magnitude of interaction, an interaction index (γ) was calculated according to the formula (Tallarida, 2002):

$$ED_{50\,\text{OXCARBAZEPINE COMBINED WITH IBUPROFEN}} / ED_{50\,\text{OXCARBAZEPINE GIVEN ALONE}} + ED_{50\,\text{IBUPROFEN COMBINED WITH OXCARBAZEPINE}} / ED_{50\,\text{IBUPROFEN GIVEN ALONE}}.$$

An interaction index is a quantitative marker for the drug combination that indicates the changed potency of the combination. Values near 1 indicate additive interaction; values more than 1 imply an antagonistic interaction and values less than 1 indicate a synergistic interaction (Tallarida, 2002).

The same procedure was followed for examining the oxcarbazepine–etodolac combination, except that in this case we examined different fractions of ED_{50} of each drug (0.125, 0.25, 0.5 and 0.75).

2.5. Analysis of duration of effect of drugs and drug combinations

In order to compare the duration of effect of each drug applied alone with the duration of effect produced by the same drug applied in combination, the data were expressed as the area under the curve (AUC), *e.g.* the area of a series of trapezoids in which height was the paw pressure difference between non-injected and carrageenan-injected paw (d) and the base, the interval (h) between measurements (Tallarida and Murray, 1986). Difference between AUC for control (vehicle-treated) group (AUC_C) and AUC for the each dose of drug/drug combination (AUC_D) is calculated as $\Delta AUC = AUC_C - AUC_D$, and expressed as the function of %AH (achieved at time of peak effect). The slope of the %AH–ΔAUC regression line ($\Delta AUC = a \times \%AH + b$; where "$a$" denotes the slope and "b" the y-intercept) is the relative measure of the duration of drug (or drug combination) effect: the drug treatment with significantly greater slope exerts the effect of longer duration than that with lesser slope (Yaksh et al., 1986). If the slopes of the %AH–ΔAUC regression lines for two drug treatments do not differ significantly, the equal duration of action is assumed. Additionally, high correlation coefficient of %AH–ΔAUC regression line indicates that the duration of the effect of drug/drug combination treatment is dose-dependent (Yaksh et al., 1986).

2.6. Statistical analysis

All computations were done according to Tallarida and Murray (1986), Tallarida et al. (1997) and Tallarida (2000) using computer programs Pharm PCS and Pharm Tools Pro. Differences between corresponding means were verified by using Student's t-test or analysis of variance (One-Way ANOVA with repeated measures), followed by Tukey's HSD test. Test for parallelism was used to compare the slopes of two regression lines (Tallarida and Murray, 1986). The difference between theoretical ED_{50} and experimental ED_{50} was examined by Student's t-test. A p value of less than 0.05 was considered statistically significant.

3. Results

3.1. Effects of oxcarbazepine, ibuprofen and etodolac in the paw pressure test in carrageenan-injected rats

In the paw pressure test in rats, oxcarbazepine (40–160 mg/kg; p.o.), ibuprofen (10–120 mg/kg; p.o.) and etodolac (5–20 mg/kg; p.o.) caused significant dose-dependent reduction of the hyperalgesia induced by carrageenan (Figs. 1A, B, C, 3A, and 4A). The peak effects of oxcarbazepine occurred 2 h after the administration. Maximum anti-hyperalgesic effects of ibuprofen were achieved either 2 h (120 mg/kg; p.o.), or 3 h (10, 40 and 80 mg/kg; p.o.) after administration. Anti-hyperalgesic effects of etodolac peaked 2 h (10 and 20 mg/kg; p.o.) or 3 h (5 and 15 mg/kg; p.o.) after administration. The ED_{50} values for oxcarbazepine and etodolac are calculated at 2 h time point and for ibuprofen at 3 h time point after administration, and all are presented in Table 1.

3.2. Effects of oxcarbazepine–ibuprofen combination in the paw pressure test in carrageenan-injected rats

Oxcarbazepine–ibuprofen drug mixture administered in fixed-dose fractions of the ED_{50} (1/16, 1/8, 1/4 and 1/2) caused significant and dose-dependent reduction of the anti-hyperalgesia induced by carrageenan in a paw pressure test in rats (Figs. 2A and 3A). The peak effect of oxcarbazepine–ibuprofen combination occurred at 2 h ($1/4 + 1/4\ ED_{50}$; p.o.), or 3 h time point after administration ($1/2 + 1/2\ ED_{50}$, $1/8 + 1/8\ ED_{50}$ and $1/16 + 1/16\ ED_{50}$; p.o.) (Fig. 2A, small graph).

The isobologram was constructed by connecting the ED_{50} of the oxcarbazepine plotted on the abscissa with the ED_{50} of ibuprofen plotted on the ordinate to obtain the additivity line (Fig. 3B). Thus, the ED_{50} of oxcarbazepine (88.17 mg/kg) is shown at point (88.17, 0) and that of ibuprofen (47.07 mg/kg) is plotted at (0, 47.07); these points are connected by a solid theoretical line for additivity. For oxcarbazepine–ibuprofen combination, the $ED_{50\ mix}$ and the 95% confidence limits (CL) of the total mixture is calculated by linear regression of the log dose–response curve (Fig. 3A) and resolved into its component parts according to the dosing ratio. This point is plotted in the isobologram (Fig. 3B). In this study, the components were co-administered at fixed-dose fractions of the ED_{50}:

$$1/2\,ED_{50\ \text{OXCARBAZEPINE}} + 1/2\,ED_{50\ \text{IBUPROFEN}} = 44.09\ \text{mg/kg} + 23.54\ \text{mg/kg},$$

$$1/4\,ED_{50\ \text{OXCARBAZEPINE}} + 1/4\,ED_{50\ \text{IBUPROFEN}} = 22.04\ \text{mg/kg} + 11.67\ \text{mg/kg},$$

$$1/8\,ED_{50\ \text{OXCARBAZEPINE}} + 1/8\,ED_{50\ \text{IBUPROFEN}} = 11.02\ \text{mg/kg} + 5.88\ \text{mg/kg}\ \text{ and}$$

$$1/16 ED_{50\ \text{OXCARBAZEPINE}} + 1/16 ED_{50\ \text{IBUPROFEN}} = 5.51\ \text{mg/kg} + 2.94\ \text{mg/kg}.$$

Thus, fixed drug–dose ratio based on mass quantity for oxcarbazepine and ibuprofen was 1.9:1. The total $ED_{50\ mix}$ for oxcarbazepine–ibuprofen combination is 35.71 mg/kg (Table 1), representing 23.28 mg/kg oxcarbazepine plus 12.43 mg/kg ibuprofen. This point is plotted as (23.28, 12.43) on the oxcarbazepine–ibuprofen isobologram (Fig. 3B); likewise, the CL for the total dose is also resolved into the two components. According to the formula for $ED_{50\ add}$ presented in Methods section, theoretical additive ED_{50} for this combination is $ED_{50\ add} = 0.5 \times 88.17 + 0.5 \times 47.08 = 67.63$ mg/kg (Table 1) (representing 44.09 mg/kg oxcarbazepine plus 23.54 mg/kg ibuprofen). The t-test applied to the potency ratio between the $ED_{50\ mix}$ and the $ED_{50\ add}$ reveals a significant difference; thus, this combination presents a synergistic interaction. A graphic illustration on the isobologram (Fig. 3B) shows that the CL of these two points does not overlap. Additionally, interaction index was less than one (Table 1), confirming the synergistic interaction for oxcarbazepine–ibuprofen combination.

The duration of the effects of oxcarbazepine, ibuprofen and their combination were expressed as the slopes of the %AH–ΔAUC regression line (see Methods). The slope of %AH–ΔAUC regression line for oxcarbazepine was significantly greater than the slopes for ibuprofen and oxcarbazepine–ibuprofen combination ($p<0.05$, test for parallelism) (Table 2), suggesting a shorter duration of the effect of ibuprofen and the drug combination compared with duration of effect of oxcarbazepine. The high correlation coefficients of the %AH–ΔAUC line for all three treatments indicated that the duration of the effect was dose-dependent (Table 2).

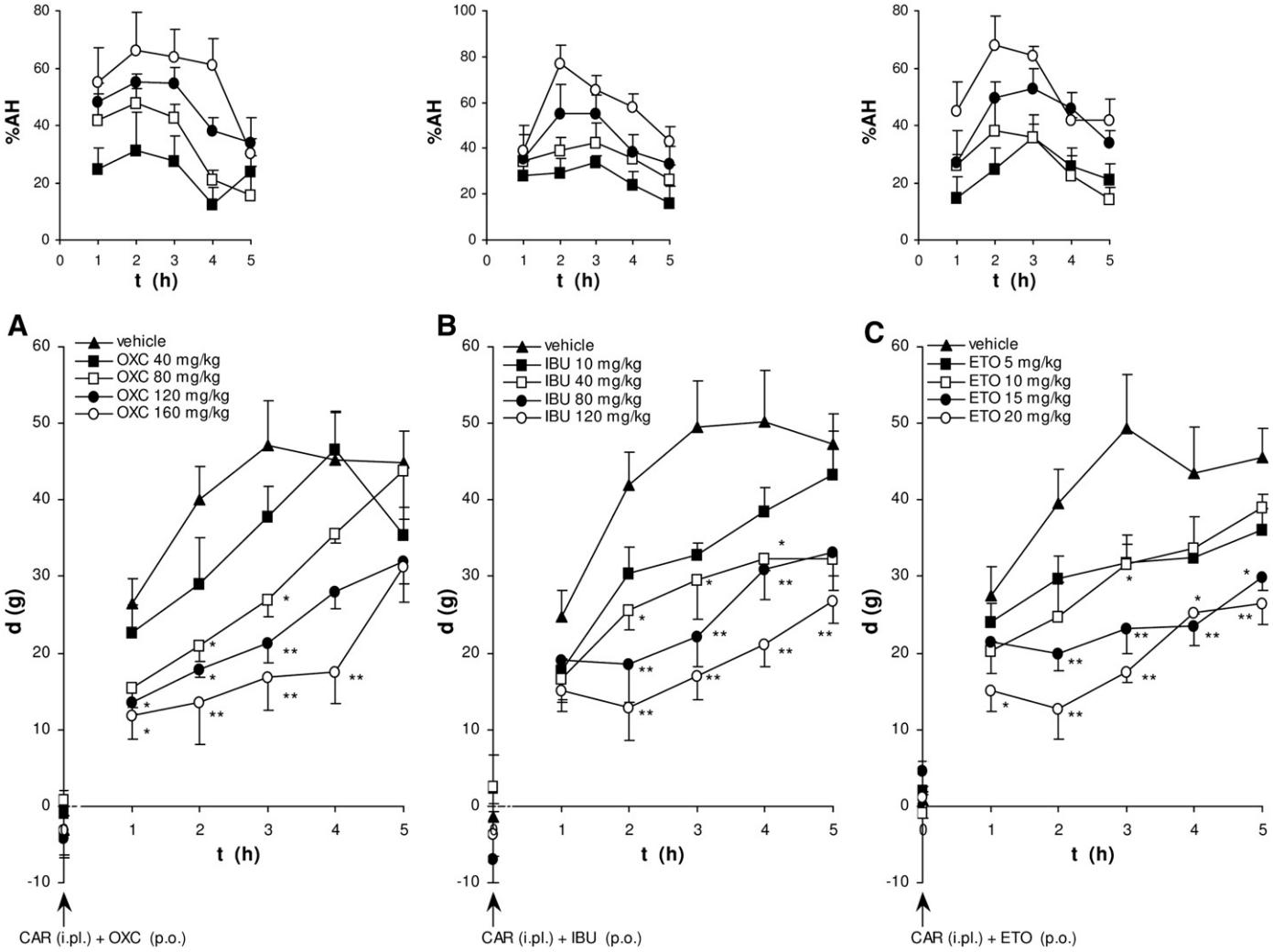

Fig. 1. Time course of the anti-hyperalgesic effects of oxcarbazepine (OXC) (A), ibuprofen (IBU) (B), and etodolac (ETO) (C) expressed as paw pressure differences in g (*d*) between non-injected and carrageenan (CAR) injected rat hind paw (bigger graphs) or as percent of anti-hyperalgesic activity (%AH) (smaller graphs). Pre-treatment *d* (plotted at vertical axis) was obtained before induction of inflammation and administration of drugs. Intraplantar (i.pl.) CAR injection was given immediately after peroral (p.o.) drug (OXC or IBU or ETO) administration (denoted by arrows). Each point represents the mean ± SEM of paw pressure differences (*d*) (bigger graphs) or %AH (smaller graphs) obtained in 6–8 animals. Statistical significance (*$p<0.05$, **$p<0.01$; One-Way ANOVA with repeated measures followed by Tukey's HSD) was determined by comparison with the curve for vehicle.

Table 1
ED_{50} ± SEM values (mg/kg) with 95% confidence limits and interaction index (γ) obtained at time of peak effect after p.o. administration of oxcarbazepine, ibuprofen, etodolac and oxcarbazepine–ibuprofen and oxcarbazepine–etodolac combination in carrageenan-injected rats.

Drugs/drug combination	ED_{50}[a] ± SEM (confidence limits)		γ[b]
Oxcarbazepine	88.17 ± 3.65 (73.64–105.57)		
Ibuprofen	47.07 ± 10.27 (18.46–120.02)		
Etodolac	13.05 ± 1.42 (8.16–20.87)		
	$ED_{50\ add}$[c]	$ED_{50\ mix}$[d]	
Oxcarbazepine + ibuprofen (total dose)	67.63 ± 5.44 (51.36–83.80)	35.71 ± 2.90* (25.16–50.70)	0.53
Oxcarbazepine + etodolac (total dose)	50.54 ± 2.35 (45.09–56.65)	39.08 ± 5.90 (20.41–74.82)	0.77

[a] ED_{50} = Effective dose required to produce 50% anti-hyperalgesic activity.
[b] $\gamma = ED_{50\ \text{OXCARBAZEPINE COMBINED WITH IBUPROFEN/ETODOLAC}} / ED_{50\ \text{OXCARBAZEPNIE GIVEN ALONE}} + ED_{50\ \text{IBUPROFEN/ETODOLAC COMBINED WITH OXCARBAZEPINE}} / ED_{50\ \text{IBUPROFEN/ETODOLAC GIVEN ALONE}}$. Values near 1 indicate additive interaction, values more than 1 imply an antagonistic interaction and values less than 1 indicate a synergistic interaction (Tallarida, 2002).
[c] $ED_{50\ add}$ = Theoretical additive ED_{50} for drug mixture.
[d] $ED_{50\ mix}$ = Experimental ED_{50} for drug mixture.
* $p<0.05$ between $ED_{50\ add}$ and $ED_{50\ mix}$ (*t*-test), indicates a synergistic interaction (Tallarida et al., 1997).

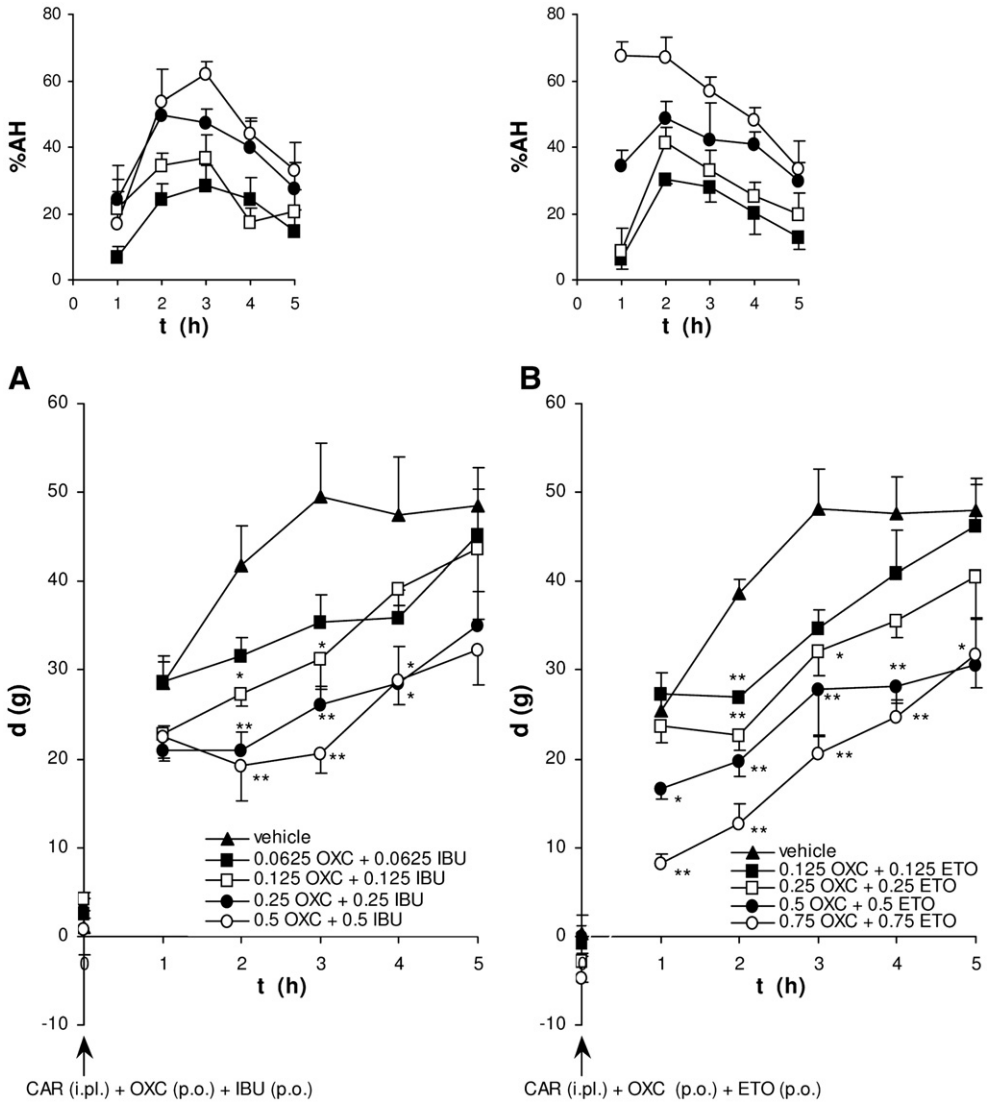

Fig. 2. Time course of the anti-hyperalgesic effect of oxcarbazepine–ibuprofen (OXC + IBU) (A), and oxcarbazepine–etodolac (OXC + ETO) (B) combination, expressed as paw pressure differences in g (*d*) between non-injected and carrageenan (CAR) injected rat hind paw (bigger graphs) or as percent of anti-hyperalgesic activity (%AH) (smaller graphs). Pre-treatment *d* (plotted at vertical axis) was obtained before induction of inflammation and administration of drug combinations. Intraplantar (i.pl.) CAR injection was given immediately after peroral (p.o.) drug combination administration (denoted by arrows). Each point represents the mean ± SEM of paw pressure differences (*d*) (bigger graphs) or % AH (smaller graphs) obtained in 6–8 animals. Statistical significance (*$p<0.05$, **$p<0.01$; One-Way ANOVA with repeated measures followed by Tukey's HSD test) was determined by comparison with the curve for vehicle.

3.3. Effects of oxcarbazepine–etodolac combination in the paw pressure test in carrageenan-injected rats

Oxcarbazepine–etodolac drug mixture administered in fixed-dose fractions of the ED_{50} (3/4, 1/2, 1/4 and 1/8) caused significant and dose-dependent reduction of the hyperalgesia induced by carrageenan in a paw pressure test in rats (Figs. 2B and 4A). The peak effect of oxcarbazepine–etodolac combination occurred at 1 h (3/4 + 3/4 ED_{50}; p.o.), or 2 h after administration (1/2 + 1/2 ED_{50}, 1/4 + 1/4 ED_{50} and 1/8 + 1/8 ED_{50}; p.o.) (Fig. 2B, small graph).

The isobolographic analysis for mixture of oxcarbazepine with etodolac was performed as described in details for the mixture with ibuprofen. Fixed drug–dose ratio based on mass quantity for oxcarbazepine and etodolac was 6.76:1. The total $ED_{50\ mix}$ for oxcarbazepine–etodolac combination is 39.08 mg/kg (Table 1), representing 34.04 mg/kg oxcarbazepine plus 5.04 mg/kg etodolac. The theoretical additive ED_{50} for this combination is 50.54 mg/kg (Table 1), representing 44.02 mg/kg oxcarbazepine plus 6.52 mg/kg etodolac. Despite the interaction index value less than one (Table 1), the *t*-test applied to the potency ratio between the $ED_{50\ mix}$ and the $ED_{50\ add}$ has not revealed a significant difference, indicating an additive interaction, as shown in the corresponding isobologram (Fig. 4B).

The duration of the effects of oxcarbazepine, etodolac and their combination were also expressed as the slopes of the %AH–ΔAUC regression line (see Methods). There is significant difference between the slopes for oxcarbazepine and etodolac ($p<0.05$, test for parallelism) but no difference between the slopes for oxcarbazepine and oxcarbazepine–etodolac combination ($p>0.05$, test for parallelism) (Table 2), indicating a shorter duration of effects of etodolac and longer duration of the effects produced by oxcarbazepine alone and in combination with etodolac. A relatively high correlation coefficients of the %AH–ΔAUC lines for both treatments indicated that the duration of the effect was dose-dependent (Table 2).

4. Discussion

In this examination we used isobolographic analysis to characterize the nature of the interaction of oxcarbazepine with ibuprofen or

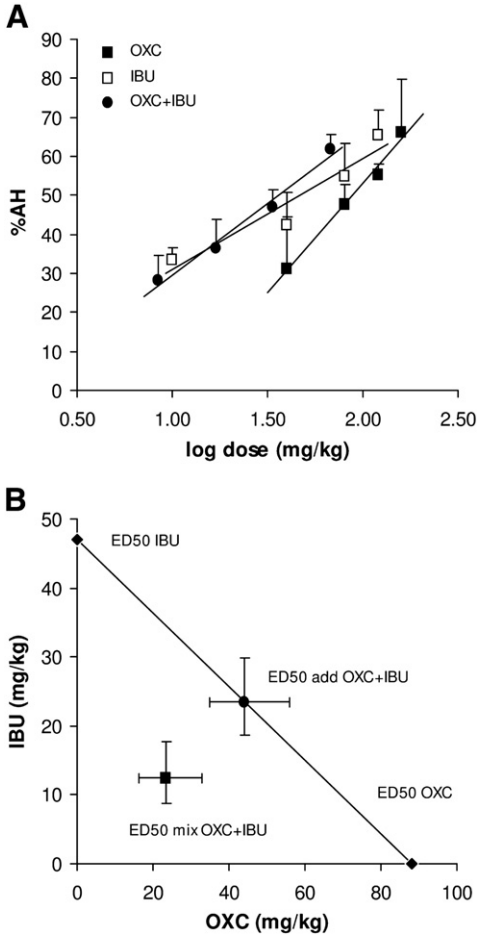

Fig. 3. A) Log dose–response curves for oxcarbazepine (OXC), ibuprofen (IBU) and oxcarbazepine–ibuprofen combination (OXC + IBU) for anti-hyperalgesia at the time of peak effects in paw pressure test in carrageenan-injected rats. Data are expressed as a percent anti-hyperalgesic activity (%AH). Each point represents the mean ± SEM of %AH obtained in 6–8 animals. B) Isobologram for the oxcarbazepine (OXC)–ibuprofen (IBU) combination in a paw pressure test in carrageenan-injected rats. The ED_{50} values for each drug (obtained 2 h after administration of OXC, or 3 h after administration of IBU) 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 $ED_{50\ add}$ (theoretical additive ED_{50}). There is a significant difference ($p<0.05$; *t*-test) between the $ED_{50\ add}$ and the $ED_{50\ mix}$ (experimental ED_{50} for drug mixture), indicating a synergistic drug interaction for combination tested.

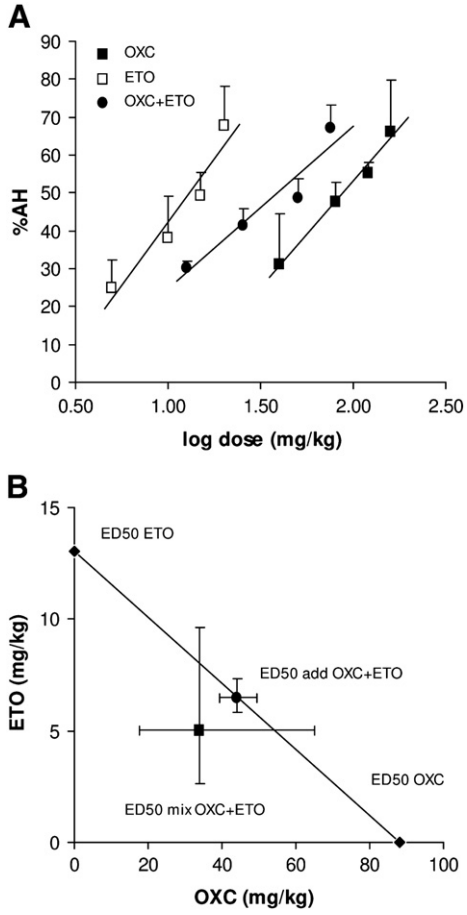

Fig. 4. A) Log dose–response curves for oxcarbazepine (OXC), etodolac (ETO) and oxcarbazepine–etodolac combination (OXC + ETO) for anti-hyperalgesia at the time of peak effects in paw pressure test in carrageenan-injected rats. Data are expressed as a percent anti-hyperalgesic activity (%AH). Each point represents the mean ± SEM of %AH obtained in 6–8 animals. B) Isobologram for the oxcarbazepine (OXC)–etodolac (ETO) combination in a paw pressure test in carrageenan-injected rats. The ED_{50} values for each drug (obtained 2 h after administration) 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 $ED_{50\ add}$ (theoretical additive ED_{50}). There was no significant difference ($p>0.05$; *t*-test) between the $ED_{50\ add}$ and the $ED_{50\ mix}$ (experimental ED_{50} for drug mixture), indicating an additive drug interaction for combination tested.

etodolac in an animal model of inflammatory pain. Our results revealed that the anticonvulsant oxcarbazepine exerts: synergistic interaction with ibuprofen and additive interaction with etodolac in reducing carrageenan-induced mechanical hyperalgesia in rats. An understanding of the sites and mechanisms that mediate the effect of these drugs alone is a useful preface to any discussion of the mechanism of their synergistic or additive interaction.

Table 2
Slopes ± SEM and correlation coefficients (*r*) of the %AH–ΔAUC regression lines, for the p.o. oxcarbazepine, ibuprofen, etodolac, and oxcarbazepine–ibuprofen and oxcarbazepine–etodolac combinations in carrageenan-injected rats.

Drug/drug combination	Slope[a] ± SEM	*r*
Oxcarbazepine	2.84 ± 0.15	0.99
Ibuprofen	1.75 ± 0.18*	0.99
Etodolac	1.82 ± 0.23*	0.98
Oxcarbazepine + ibuprofen	1.73 ± 0.32*	0.97
Oxcarbazepine + etodolac	2.12 ± 0.27	0.98

[a] Slope of the %AH–ΔAUC regression lines (see Methods) is a relative measure for the duration of the effect of drug/drug combination (Yaksh et al., 1986).
* $p<0.05$; comparing to the slope for OXC, test for parallelism.

Animal model of carrageenan-induced hyperalgesia showed mainly clinical inflammatory conditions (Suyama et al., 2004). The tissue inflammation induced by local peripheral carrageenan injection is associated with local release of pro-inflammatory substances, including prostaglandins (PGs) particularly E series (Dray, 1995; Morris, 2003) resulting in the activation and sensitization of peripheral nociceptive afferents by decreasing the threshold for activation of Na^+ current (Akopian et al., 1996; Momin and McNaughton, 2009). Also, carrageenan paw injection stimulates the release of PGs within the spinal cord that contributes to the development of central sensitization, by activation of nonselective cation current (Yaksh et al., 2001; Baba et al., 2001). Thus, anti-hyperalgesic effects of ibuprofen, in inflammatory pain model, could be explained by its effectiveness, peripherally and centrally, in inhibiting both cyclo-oxygenase enzymes COX-1 and COX-2 (McCormack, 1994; Dirig et al., 1998; Yaksh et al., 2001).

Etodolac, a selective COX-2 inhibitor, was found to have analgesic effects in inflammatory and neuropathic pain models (Inoue et al., 1991a,b, 1994, 2009). The authors suggested that etodolac attenuates neuropathic pain by suppressing the expression of genes for Ca^{+2}-channel α2δ subunit in the dorsal root ganglion (Inoue et al., 2009). Besides strong anti-inflammatory and anti-hyperalgesic effects,

etodolac has low ulcerogenic activity and a good gastric safety due to its selectivity for COX-2 isoenzyme.

Several mechanisms contribute to antinociception by oxcarbazepine in inflammatory pain model in rats, including central and peripheral Na^+ channels as well as some neurotransmitter systems (Kiguchi et al., 2004; Tomić et al., 2004, 2006, 2007; Vučković et al., 2006; Stepanović-Petrović et al., 2008b).

An understanding of the sites and mechanisms that mediate the effects of these drugs guides us to consider the characteristics of their interaction. It has been speculated that supra-additive interactions could be ascribed to the activation of complementary pathways of antinociception (Yoon and Yaksh, 1999; Miranda et al., 2002). As just described, the (predominantly peripheral) sites at which ibuprofen produces anti-hyperalgesia and the (central and peripheral) sites at which oxcarbazepine acts appear to be complementary. Further, the mechanisms by which these two classes of compounds act to suppress pain transmission differ (inhibition of COX-1/2 *versus* inhibition of voltage dependent Na^+ channels and neurotransmitters receptors). Ibuprofen through inhibition the production of PGs, suppresses activation of Na^+-channels in nociceptors and nonselective cation channels in spinal cord. Oxcarbazepine may synergize with these effects through inhibition neuronal Na^+-channels at peripheral and central levels.

The type of interaction between two drugs may also be explained by altering the kinetics of each other (Malmberg and Yaksh, 1992). The pharmacokinetic interaction between ibuprofen and oxcarbazepine was not the point of our interest, so it could not be excluded. Nevertheless, we remarked that the effects of ibuprofen and oxcarbazepine applied alone and in combination peaked at the same time and the duration of the effect of the combination was not longer than the duration of the effects of each component applied alone. Actually, it was equal as the duration of the effect of ibuprofen applied alone and even shorter than the duration of effect of oxcarbazepine applied alone. A prolongation of the effects of drug combination is likely to be expected in the case of pharmacokinetic interaction which would result in potentiation of pharmacologic effects. Therefore, a pharmacokinetic interaction between ibuprofen and oxcarbazepine seems less possible. So, it seems that there is a synergistic pharmacodynamic interaction between ibuprofen and oxcarbazepine, since their fundamentally different central and peripheral mechanisms jointly contribute to the anti-hyperalgesia.

The interaction between systemic oxcarbazepine and etodolac in this model of inflammatory pain does not differ from additivity. It has been suggested that additive interaction could be ascribed to the activation of a common mechanism of the drugs (Yoon and Yaksh, 1999; Miranda et al., 2002). But, oxcarbazepine and etodolac belong to distinct classes of drugs and appear to interact with different endogenous pain regulatory systems. Several factors may govern the nature of drug interaction in an animal pain model, one of them being stimulus intensity. Kissin et al. (1993) showed that the nature of interaction between morphine and barbiturate depends on stimulus intensity: at low intensities, there is a synergistic interaction, while at higher intensities the interaction is additive or less. Whether lower concentrations of carrageenan would reveal synergy between oxcarbazepine and etodolac in a paw pressure test remains to be explored.

An additive interaction may be insufficient for the beneficial outcome of an analgesic drug combination, especially if the interactions for adverse effects are not known. In our study, we were unable to evaluate the benefits of a combination of oxcarbazepine and etodolac in terms of side effects. This is important because the combination may accompany a more frequent incidence of side effects than that produced by each drug alone (Ulugöl et al., 2006). But, there is no data about interactions between oxcarbazepine and nonsteroidal anti-inflammatory drugs (NSAIDs) when they are used in combination. Moreover, etodolac appears to be relatively well tolerated NSAID, with significant high safety profile for the gastrointestinal tract. So, there is no real opportunity for more frequent incidence of side effects accompanied with oxcarbazepine/etodolac combination.

In conclusion, the data of the present study shows that oxcarbazepine in combination with ibuprofen produces a synergistic anti-hyperalgesic activity. Our results might improve the treatment of inflammatory pain, especially because with low doses of the components side effects are not likely to appear. Additionally, oxcarbazepine produces additive interaction with etodolac in inflammatory pain model, which also may have utility in pain pharmacotherapy. Adverse effect analysis of the combinations is needed to validate the possible clinical use of mixtures of these drugs in pain management.

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