The Antinociceptive Effects of Anticonvulsants in a Mouse Visceral Pain Model

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BACKGROUND: There is evidence supporting the antinociceptive effects of carbamazepine, oxcarbazepine, gabapentin, and topiramate in various models of neuropathic pain as well as inflammatory somatic pain. Data are lacking on the antinociceptive potential of these drugs against visceral pain. In this study, we examined and compared the effects of carbamazepine, oxcarbazepine, gabapentin, and topiramate in the writhing test as a visceral pain model in the mouse. In addition, the influence of these anticonvulsants on motor performance was examined to compare the tolerability of these anticonvulsants when used against acute visceral pain.

METHODS: The antinociceptive effects of these anticonvulsants were examined in the acetic acid writhing test in mice. The side effect propensity of these drugs was examined using the rotarod test.

RESULTS: Carbamazepine (25–60 mg/kg; p.o.), oxcarbazepine (10–40 mg/kg; p.o.), gabapentin (10–70 mg/kg; p.o.), and topiramate (5–30 mg/kg; p.o.) caused a significant dose-dependent reduction the number of writhes in the writhing test. In the rotarod test, carbamazepine (60–140 mg/kg; p.o.) and oxcarbazepine (120–450 mg/kg; p.o.) significantly reduced the time spent on the rotarod in a dose- and time-dependent manner. Gabapentin (1000–2000 mg/kg; p.o.) and topiramate (400–1500 mg/kg; p.o.) did not produce significant impairment of motor performance at the highest doses used. The therapeutic index (motor impairing dose TD₅₀/writhing ED₅₀) values were topiramate (>148.5) > gabapentin (>60.2) > oxcarbazepine (15.2) > carbamazepine (2.3).

CONCLUSIONS: These results indicate that oxcarbazepine, gabapentin, and topiramate are effective in the writhing model in mice, in a dose range, which is not related to motor impairment; topiramate is the most potent and the most tolerable drug.

Visceral pain (e.g., angina, colic, dyspepsia, pancreatitis, appendicitis, dysmenorrhea, hysterectomy, tumor invasion of viscera), caused by activation of nociceptors in viscera, constitutes a large portion of clinically treated pain.¹⁻³ If an inflammatory process or tissue injury occurs in internal organs, or if non-noxious stimuli are applied in a repeated and/or prolonged fashion locally, the visceral structures may become hypersensitive.¹,⁴ In fact, intense activation of nociceptive primary afferent fibers by visceral tissue injury and inflammation produces central sensitization or hyperexcitability of nociceptive neurons in the spinal cord dorsal horn that contributes to hyperalgesia.¹

Intraperitoneal administration of dilute acetic acid (AA) produces a characteristic writhing response in mouse. This behavior is considered to be evidence of peritoneal visceral pain, since AA directly activates visceral and somatic nociceptors innervating the peritoneum and induces inflammation not only in subdiaphragmatic visceral organs, but also in subcutaneous muscle walls.⁵ There is evidence that polymodal C fibers and Aδ fibers are present in the gut.⁵,⁶ AA causes tissue damage and releases pain-producing substances that activate nociceptors on the sensory nerve fibers.⁷

Carbamazepine, oxcarbazepine, gabapentin, and topiramate are used as antiepileptic drugs and for treating the neuropathic pain.⁸,⁹ There is evidence of the antinociceptive effects of carbamazepine, oxcarbazepine, gabapentin, and topiramate in various models of neuropathic pain.¹⁰⁻¹³ Substantial experimental data have been provided on the antinociceptive effects of carbamazepine, oxcarbazepine, and gabapentin in models of inflammatory somatic pain after systemic,¹¹,¹⁴⁻¹⁷ as well as local peripheral administration.¹⁸⁻²¹ Topiramate did not produce statistically significant analgesic effects in models of inflammatory somatic pain after systemic administration,¹⁵ but there are no data after local peripheral administration.

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Gabapentin markedly reduced visceral nociception, but data are lacking on the antinociceptive potential of carbamazepine, oxcarbazepine, and topiramate against visceral pain. An additional issue relates to defining the therapeutic ratio of these drugs. Simply put, using the preclinical model we can predict not only the antinociceptive activity, but also the efficacy relative to the propensity to produce side effects. In the present study, we examined the dose-dependent effect on motor function using the rotarod test.

METHODS

Animals

Experiments were performed on 25–30 g male Swiss Webster mice (Military Farm, Belgrade, Serbia) and were approved by the Institutional Animal Care and Use Committee. The animals were housed in groups of 20 in home cages (42.5 × 27 × 19 cm) under standard conditions: temperature of 22°C ± 1°C, and a 12/12 h light/dark cycle. Food and water were freely available, except during the experimental procedure. Before experimental manipulation, the animals were given at least 3 days to adapt to the laboratory. All experiments were performed at the same time of the day between 8:00 and 12:00 h to avoid diurnal variation in behavioral tests. All experimental groups consisted of 8–12 mice. Each animal was used only once and was killed with diethyl-ether immediately after the experiment.

Writhing Test

This test was selected as a model of acute peritoneal nociceptive pain, which could be a model of clinically relevant intestinal pain in humans. Mice were injected i.p. with 10 mL/kg of 0.75% AA and the number of writhes was counted during a 15 min period starting 5 min after administration of AA solution. A writh was defined as a contraction of the abdominal muscles accompanied by an elongation of the body and extension of the hindlimbs. The drugs (or the vehicle in the control group) were administered p.o. (in a volume of 10 mL/kg), 60 min before the AA injection.

The data are expressed quantally as the number of animals in which the antinociception was observed versus total number of animals receiving the same treatment. For antinociception, the following criteria was used: an antinociceptive effect was said to have occurred if the observed number of writhes was equal to or less than half of the average number of writhes in the control group. The ED50 (the dose that was antinociceptive in 50% of animals tested) with 95% confidence limits was calculated from a corresponding quantal dose–response curve (Litchfield & Wilcoxon II procedure) according to Tallarida and Murray, by using the following criteria: an animal was said to be motorically impaired if it did not remain on the rotarod for the entire 60 s.

Drugs Administration

Oxcarbazepine (Trileptal®, Novartis Pharma AD, Basel, Switzerland), carbamazepine (Tegretol®, Novartis Pharma AD), gabapentin (Neurontin®, Pfizer H.C.P. Corporation, NY), and topiramate (Topamax®, Janssen-Cilag AG, Baar, Switzerland) were suspended in distilled water and sonicated for 15 min for proper distribution. The drugs were administered by oral gavage (p.o.) in a final volume of 10 mL/kg.

Statistical Analysis

All computations were done according to the methods of Tallarida and Murray. Differences in observed parameter between corresponding groups of animals were verified by using Kruskal–Wallis test (nonparametric analysis of variance), followed by Mann–Whitney U-test. The Litchfield & Wilcoxon II procedure was used to calculate ED50 and TD50 values from corresponding quantal dose–response curves. Additionally, when data for a second quantal dose–response curve was entered, the same procedure calculated the potency ratio [with confidence limits (CL)] for corresponding curves. In that way, relative potencies for carbamazepine, oxcarbazepine, gabapentin, and topiramate were calculated for antinociceptive as well as toxic effects. Relative potency estimates were considered statistically significantly different when 95% CL did not overlap 1.0. Also, the potency ratios between TD50 and ED50, denoted as the TI (therapeutic index), for each drug were determined. If the 95% confidence interval for a TI fails to include 1.0, then TD50 and ED50 are significantly different.

RESULTS

The Effects of Anticonvulsants in the Writhing Test in Mice

In the writhing test in mice, carbamazepine (25–60 mg/kg; p.o.), oxcarbazepine (10–40 mg/kg; p.o.), gabapentin (10–70 mg/kg; p.o.), and topiramate (5–30 mg/kg; p.o.) were administered p.o. in a final volume of 10 mL/kg. The TD50 (the dose that induces motor impairment in 50% of animals tested) with 95% confidence limits was calculated from a corresponding quantal dose–response curve (Litchfield & Wilcoxon II procedure) according to Tallarida and Murray, by using the following criteria: an animal was said to be motorically impaired if it did not remain on the rotarod for the entire 60 s.
mg/kg; p.o.) caused a dose-dependent reduction of the number of writhes, compared with the control group (Figs. 1 and 3A). The anticonvulsant effect was significant at all doses tested, except for the lowest dose of each anticonvulsant (Fig. 1). The ED50 values for anticonvulsants antinociception are shown in Table 1. The rank of potency ratios calculated versus carbamazepine as the least potent drug in producing antinociception was topiramate (about four times more potent than carbamazepine; \( P < 0.05 \)) > oxcarbazepine (about 2.5 times more potent than carbamazepine; \( P > 0.05 \)) > gabapentin (slightly more potent than carbamazepine; \( P > 0.05 \)) >

![Figure 1. Antinociceptive effect of carbamazepine (CBZ) (A), oxcarbazepine (OXC) (B), gabapentin (GBP) (C), and topiramate (TOP) (D) expressed as number of writhes induced by i.p. injection of acetic acid in mice. Drugs were administered p.o. 60 min prior the acetic acid injection. Each column represents the mean ± SEM of number of writhes obtained in 8–12 animals. Statistical significance (*\( P < 0.05 \), **\( P < 0.01 \); Kruskal–Wallis test followed by Mann–Whitney U-test) was determined by comparison with the curve for vehicle.](image)

### Table 1. ED50, TD50 Values, and the Corresponding Potency Ratios and Therapeutic Indices (TI), with 95% Confidence Limits (95% CL), for Oxcarbazepine (OXC), Carbamazepine (CBZ), Gabapentin (GBP), and Topiramate (TOP) in Inducing Antinociception and Motor Impairment in Mice

<table>
<thead>
<tr>
<th>Substance</th>
<th>Antinociceptiona</th>
<th>Motor impairmentb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED50 mg/kg (95% CL)</td>
<td>Potency ratio vs CBZ</td>
</tr>
<tr>
<td>CBZ</td>
<td>38.9 (31.2–48.2)</td>
<td>1</td>
</tr>
<tr>
<td>OXC</td>
<td>15.8 (11.1–22.3)</td>
<td>2.5 (0.2–27.0)</td>
</tr>
<tr>
<td>GBP</td>
<td>33.22 (18.7–58.9)</td>
<td>1.2 (0.1–13.4)</td>
</tr>
<tr>
<td>TOP</td>
<td>10.1 (6.0–16.6)</td>
<td>3.9 (2.2–6.7)</td>
</tr>
</tbody>
</table>

*a Antinociception was said to have occurred if the observed number of writhes was equal to or less than half the average number of writhes in the control group.

*b Motor impairment was detected in the animal if it did not remain on the rotarod for 60 s.

*c Therapeutic index (TI) is calculated as TD50/ED50 potency ratio for each drug.

*d Approximate values of TD50, potency ratio and therapeutic index for GBP and TOP are given.

* \( P < 0.05 \), Litchfield & Wilcoxon II test. Relative potency estimates were considered statistically significant when 95% CL did not overlap 1.0. If 95% CL for a TI fails to include 1.0, then TD50 and ED50 are significantly different.

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carbamazepine (Table 1). Topiramate is the most potent anticonvulsant we tested, and it is significantly more potent than carbamazepine (potency of topiramate relative to carbamazepine is shown in Table 1) and gabapentin (potency of topiramate relative to gabapentin = 3.3, 95% CL = 1.5–7.1; not shown).

The Effects of Anticonvulsants in the Rotarod Test in Mice

In the rotarod test in mice, carbamazepine (60–140 mg/kg; p.o.) and oxcarbazepine (120–450 mg/kg; p.o.) caused a dose-dependent reduction of time spent on the rotarod, compared with the control group, but the reduction was significant only at higher doses (80, 100, 140 mg/kg and 300, 450 mg/kg for carbamazepine and oxcarbazepine, respectively) (Figs. 2A, B and 3B). The observed reduction of time spent on the rotarod from these anticonvulsants was also time-dependent; the peak effects occurred 30–60 min after p.o. administration, and the toxic effects were sustained up to 120 min, for carbamazepine and oxcarbazepine, respectively. The TD50 values for carbamazepine- and oxcarbazepine-induced motor impairment are shown in Table 1.

Gabapentin (1000 and 2000 mg/kg; p.o.) and topiramate (400–1500 mg/kg; p.o.) did not produce significant toxic effects on motor performance in mice (Figs. 2C, D and 3B). Higher doses of gabapentin and topiramate were not tested because of difficulties in administration (suspensions were too thick and the accuracy of the administered dose would be questionable). For these reasons, TD50 values, potency ratios, and therapeutic indices for gabapentin and topiramate could not be determined exactly, and statistical analysis could not be performed for these two drugs (Table 1).

The rank of potency ratios calculated versus carbamazepine as the most potent drug in inducing motor impairment was: gabapentin (more than 21 times less potent than carbamazepine; P not determined) < topiramate (more than 16 times less potent than carbamazepine; P not determined) < oxcarbazepine (about 2.6 times less potent than carbamazepine; P > 0.05) < carbamazepine (Table 1).

The corresponding therapeutic indices (calculated as TD50/ED50; TD50 for motor impairment and ED50 for antinociception) are also shown in Table 1. The greater the TI value is, the greater is observed drug
tolerability. The rank of anticonvulsants tested according to TI value was: topiramate (>148.5) > gabapentin (>60.2) > oxcarbazepine (15.2) > carbamazepine (2.3) (Table 1). There was no statistical difference between doses of carbamazepine that produce antinociception and motor impairment. In contrast, a significant difference between the antinociceptive and toxic doses of oxcarbazepine, i.e., high TI indicates that oxcarbazepine is a more tolerable antinociceptive drug than carbamazepine. Also, the present results suggest that gabapentin and topiramate are much less potent than carbamazepine and oxcarbazepine in producing motor impairment, and that they have much greater TIs, i.e., drug tolerability than both carbamazepine and oxcarbazepine (Table 1).

DISCUSSION

In the writhing test, AA activates peripheral nociceptors on the sensory nerve fibers by releasing proinflammatory substances. Visceral hyperalgesia is believed to arise as a consequence of a lowering in threshold of “high threshold” receptors and activation of previously unresponsive receptors (peripheral sensitization), and subsequent neuroplastic changes in the central nervous system, in terms of increased central neuronal activity and excitability (central sensitization), which amplify the effects of pain-related stimuli coming from the affected visceral organs.1

The present study showed that carbamazepine, oxcarbazepine, gabapentin, and topiramate caused a significant and dose-dependent reduction of the nociception induced by AA in a writhing test in mice. This is the first report on the antinociceptive effect of carbamazepine, oxcarbazepine, and topiramate in a visceral pain model.

Carbamazepine- and oxcarbazepine-induced antinociception in the writhing test is consistent with the ability of these anticonvulsants, in a paw pressure test, to reduce somatic nociception caused by proinflammatory agents.11,14,18–20,29 Although our results do not
clarify the mechanism of the antinociceptive effects of these two drugs in the visceral pain model, they may suggest that the inflammatory nature of nociception in writhing and paw pressure tests leading to a facilitated state may be similar. It has been shown that activation of central adenosine (A1) and adrenergic (α2) receptors produced antinociception in a writhing test in mice.30,31 On the other hand, our research group found evidence that the antihyperalgesic effects of carbamazepine and oxcarbazepine are mediated by activation of central and peripheral adenosine (A1) and adrenergic (α2) receptors in a paw inflammatory hyperalgesia in rats.14,16,18–20 Therefore, it could be suggested that the peripheral and/or central activation of adrenergic and purinergic systems by carbamazepine and oxcarbazepine could contribute to their antinociceptive effects in the writhing test.

Our results are in agreement with the findings of Feng et al.,22 which provided evidence that gabapentin has an antinociceptive effect on rat writhing responses to peritoneal injection of AA. The authors demonstrated that the antinociceptive effect of gabapentin correlates with the suppression of AA-evoked release of excitatory amino acids, glutamate, and aspartate, in the spinal cord.

Topiramate has no influence on inflammatory pain,15 but has analgesic properties in experimental neuropathy.13,32 Our results show that topiramate has an antinociceptive effect in a visceral pain model. Topiramate has several pharmacological properties that may contribute to its anticonvulsant activity and antinociceptive effect in neuropathic pain, which are: modulating voltage-gated sodium ion channels, enhancing γ-aminobutyric acid inhibition, blocking excitatory glutamate neurotransmission, modulating voltage-gated calcium ion channels, etc.9,13,33 The mechanism(s) underlying the antinociceptive effect of topiramate against visceral pain remain to be elucidated. A relatively low dose of topiramate (10 mg/kg) produced significant antinociception, whereas other studies have used 20–50 mg/kg for neuropathic pain.13,32,34 Moreover, in other studies, topiramate was administered via the i.p. route, but in our experiments we used the p.o. route. Extrapolating to the clinical setting, lower doses are associated with fewer side effects and may be a useful option for the treatment of visceral pain,13 given the correlation between the ED50 values obtained in rats using the writhing test and analgesic doses in humans.24,35

As oxcarbazepine, gabapentin, and topiramate did not alter rotarod performance in the antinociceptive doses used, it is possible that the antinociceptive behavior was not due to motor impairment or sedation. In fact, the present study demonstrates that carbamazepine is the most potent drug for inducing motor impairment and that gabapentin is the least. More importantly, oxcarbazepine, gabapentin, and topiramate have significant antinociceptive effects in the absence of motor impairing actions. The exact TI values for gabapentin and topiramate were not calculated, given the absence of motor impairment evaluation at doses larger than 2000 and 1500 mg/kg for gabapentin and topiramate, respectively, and was expressed as a TI more than 60.2 and 148.5, respectively. Our findings are in agreement with the study by Benes et al.,36 showing that the protective index (TD50/ED50) for oxcarbazepine is more than that for carbamazepine, and with the work of Tutka et al.,37 showing that the protective index value for gabapentin was impossible to calculate because motor impairment cannot be evaluated at doses higher than 1000 mg/kg and expressed as more than 29, regarding their anticonvulsant activity. On the other hand, Luszczki et al.38 succeeded in estimating the TD50 for topiramate in the rotarod test in mice. But, similar to our results, the TD50 for topiramate was still larger in magnitude than that for carbamazepine and oxcarbazepine. In that study, topiramate was administered i.p., but in the present experiments, the drug was administered p.o., so greater doses are necessary to cause acute neurotoxicity than that administered i.p. In our study, oxcarbazepine, gabapentin, and topiramate demonstrated good separation between antinociceptive efficacy in a visceral pain model, and motor impairment induction. Topiramate has shown the comparative advantage among the examined drugs as the most potent antinociceptive drug and, furthermore, as the drug with the best therapeutic tolerability in the model of visceral pain.

These observations suggest that oxcarbazepine, gabapentin, and topiramate are effective in the writhing model in mice, in a dose range that is not related to motor impairment, and that topiramate is the most potent and the most tolerable of these drugs. Carbamazepine, oxcarbazepine, and topiramate efficacy in a visceral pain model was shown for the first time to possess efficacy in this model and, since the writhing test may be predictive, these data may reflect human utility.

REFERENCES

7. Ulugöl A, Özyiğit F, Yesilyurt Ö, Dogrul A. The additive antinociceptive interaction between WIN 55,212-2, a cannabinoi