Synergistic interaction between dexamethasone and tramadol in a murine model of acute visceral pain

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INTRODUCTION

Glucocorticoids are anti-inflammatory drugs used in a wide range of inflammatory diseases. Their mechanism of action involves the interaction with intranuclear glucocorticoid receptors resulting in the modulation of gene transcription. The expression of steroid-induced genes is modulated at the protein level, 2–3 h after the administration of the drug [1]. The genomic anti-inflammatory effects of glucocorticoids are mediated by a reduction of prostaglandin synthesis due to a decrease in the activity of phospholipase A2, and a selective block of COX-2 mRNA expression [2]. Glucocorticoids also inhibit hyperalgesia induced by inflammatory mediators such as tumour necrosis factor-α, interleukin 1-β and interleukin-6 [3].

When administered perioperatively, glucocorticoids have analgesic and antiemetic effects [4,5]. Due to the slow onset of the genomic effects (hours), it has been recommended that glucocorticoids should be administered in the preoperative period [6]. However, a rapid onset of the analgesic and anti-hyperalgesic effects of glucocorticoids (seconds to minutes) has also been demonstrated in human and animal models of nociception, including neuropathic pain [1,3,6,7].

Interactions between opioids and glucocorticoids have been previously reported on a wide range of physiological responses, including cardiovascular [8], gastrointestinal [9], immunological [10], endocrine [11] and behavioural responses [12] including pain, in laboratory investigations. However, the interaction between glucocorticoids and opioids on nociception is controversial, as

Keywords
drug interactions, glucocorticoids, isobolograms, opioid analgesics

ABSTRACT

Tramadol is effective in the management of mild to moderate postoperative pain, but its administration is associated with nausea and vomiting. Patients treated with tramadol, often receive dexamethasone as antiemetic. The aim of our investigation was to assess if the two drugs interact in a murine model of acute visceral pain. Using the acetic acid writhing test in mice, we assessed the antinociceptive effects of tramadol and dexamethasone (a glucocorticoid with antiemetic effect) administrated individually and in a 1 : 1 fixed ratio combination. Tramadol and dexamethasone induced a dose-dependent inhibition of the writhing response when administered individually, with ED₅₀ values of 2.9 [2.09–4.31, 95% confidence limit (CL)] mg/kg, and 0.13 (0.05–0.29, 95% CL) mg/kg, respectively. The ED₅₀ of the combination was 0.13 (0.01–0.29, 95% CL) mg/kg; the isobolographic and interaction index analysis revealed a synergistic interaction. The results suggest that the combination of tramadol and dexamethasone could be beneficial in the management of postoperative pain in humans.
both additivity and antagonism have been reported [13,14].

Pain and postoperative nausea and vomiting are challenging adverse events in the postoperative setting [15]. These events are closely related, as the treatment of postoperative pain with opioids such as tramadol is a major factor predisposing to nausea and vomiting. In the postoperative period, opioids and antiemetics are used concomitantly for pain management and the prevention of nausea and vomiting; tramadol is one of the opioids more frequently used in clinical practise in the management of mild–moderate pain. Clinical studies suggest a decrease in the postoperative analgesic effects of tramadol when administered concomitantly with ondansetron, a 5-HT\textsubscript{3} serotonin receptor antagonist [16,17]. Recently, antagonism between tramadol and two antiemetics (ondansetron and droperidol) has been demonstrated by our group in different animal models of nociception [18]. Thus, due to the antagonism between tramadol and other antiemetics, the co-administration of tramadol and dexamethasone is widely used at present in the postoperative period, and was the main reason to test their possible interaction in an animal model of nociception.

The aim of the present study was to establish a possible interaction between tramadol and dexamethasone on antinociception in an acute visceral pain model [acetic acid writhing test (AAWT)] in mice. We also wanted to assess the role of the rapid non-genomic effects of dexamethasone on antinociception and its role on the dexamethasone–tramadol interaction.

**Materials and Methods**

**Animals**

Female Swiss CD1 mice weighing 25–30 g (Charles River, France) were used in the study. The experiments were performed according to the Ethical Guidelines of the International Association for the Study of Pain [19], and the Ethical Committee for Animal Welfare of the Institution approved the protocols. Mice were housed in plastic cages (five mice per cage) with soft bedding and free access to food and water. They were maintained in a controlled temperature (22 ± 1 °C and 60% relative humidity) and light (12 : 12-h dark : light cycle with light on at 8:00 h) environment, for at least 5 days before the experiments. Behavioural testing was performed during the light phase between 15:00 and 20:00 h in a quiet room maintained at 22–23 °C. Mice were used only once and were killed at the end of the experiment by cervical dislocation.

**Drugs**

Tramadol (Grünenthal, Madrid, Spain) and dexamethasone (Merck, Mollet del Valles, Spain) were used. Their combinations were prepared in pyrogen-free water just before use, and administered subcutaneously (s.c.) at the nape of the neck in a final volume of 10 mL/kg, 30 min before behavioural testing. Evaluation after s.c. administration was selected on the basis of previous reports [20,21].

**Behavioural testing**

The AAWT was performed as previously reported [18]. The intraperitoneal (i.p.) injection of a weak solution of acetic acid induces a nociceptive stereotyped behaviour (writhing) that mimics acute visceral pain. The model has been validated in rats and mice and is widely used to evaluate the antinociceptive effects of opioid and non-opioid analgesics [22,23]. Thirty minutes after the s.c. injection of the drugs (alone or combined), the animals received 10 mL/kg i.p. of a 0.6% acetic acid solution, and were immediately placed in a test box. After 5 min, the number of abdominal constrictions was cumulatively counted over a 10-min period. The inhibitory effects of the drugs on the AAWT are expressed as the percent inhibition of the number of writhings in a drug-treated animal, when compared with the mean number of writhings measured in a group of saline-treated mice (controls), according to:

\[
\%\text{Inhibition} = \left(\frac{\text{No. of writhings saline}}{\text{No. of writhings treated}}\right) \times 100.
\]

**Experiments performed**

Dose–response curves were obtained for each drug individually and combined in a 1 : 1 proportion. The dose that produced a 50% of the maximal effect (ED\textsubscript{50}) of antinociception for each drug or a combination of drugs was calculated as a measure of potency, according to the method of Tallarida [24]. The analysis of the interaction was accomplished using isobolograms and interaction indexes, which are well established as valid methods to assess drug–drug interactions when both drugs show a significant antinociceptive effect when administered individually.

**Construction of isoboles**

Isoboles are graphic representations of equally effective doses of two (or more) agents. The dose of each drug that
produces a given level of response (i.e. 50%) is plotted on the axes of the graph. A diagonal line is drawn to join the isoeffective doses on the axis. Doses of drug combinations producing the same effect are then plotted. Points falling on the diagonal line represent zero interaction (additivity), while those located above and below are antagonistic and synergistic, respectively. Mean and 95% confidence limits (CL) were calculated for all the doses plotted, and points were considered to differ significantly from additivity if their 95% CL values did not overlap. Moreover, for each dose of the combination plotted, the values were compared with the doses that would produce the same level of inhibition, under the assumption that the drugs were merely additive (Student’s t-test). These theoretical additive values were obtained using the following equation:

$$ED_{add} = ED_1/(P_1 + R P_2)$$

where $R$ is the potency ratio of the drugs when tested individually, and $P_1$ and $P_2$ are the proportions of each drug in the combination [24]. The diagonal non-interaction line is described by the equation $da/Da + db/Db = 1$, where $Da$ and $Db$ are the doses of drugs (a and b) producing a given level of effect when administered individually, and $da$, $db$ are the doses of drugs (a and b) producing the same level of effect when given in combination. The solution to the equation is called the interaction index; if it is different than 1, an interaction is present: either synergy (index <1) or antagonism (index >1) [24].

**Statistical analysis**

The results are expressed as percentages of inhibition of writhing. Statistical calculations were performed as described by Tallarida [24]. $ED_{50}$ values (95% CL) were determined by linear regression analysis of dose–response relations based on at least four different doses and 8–10 mice per dose. For comparison between groups we used the Student’s t-test, and a $P < 0.05$ was considered statistically significant.

**RESULTS**

In the AAWT, the s.c. administration of tramadol induced a dose-related inhibition of writhing when administered individually 30 min before testing, with a resulting $ED_{50}$ of 2.9 (2.09–4.31 95% CL) mg/kg. A dose–response curve could also be obtained for s.c. dexamethasone administered 30 min before the AAWT, with an $ED_{50}$ of 0.13 (0.05–0.29 95% CL) mg/kg (Table I). However, when dexamethasone was injected 3 h before testing, no dose–response relationship could be generated. Figure 1 shows the dose–response curves for dexamethasone and tramadol, each one individually, on the AAWT. The slopes of the curves were significantly different ($P < 0.05$, Student’s t-test).

The dose–response curve of the tramadol–dexamethasone 1 : 1 combination showed an $ED_{50}$ of 0.13 (0.01–0.29 95% CL) mg/kg, and the isobolographic and interaction index analysis (Figure 2; Table I) demonstrated a synergy with an interaction index of 0.088. The interaction was synergistic at 20, 50 and 80% level of effect ($ED_{20}$, $ED_{50}$ and $ED_{80}$). The comparison of the experimentally obtained tramadol–dexamethasone dose–response curve with a theoretical additive dose–response curve of the combination was statistically significant (Student’s t-test, $P < 0.01$), demonstrating the presence of synergy.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>$ED_{50}$ (mg/kg)</th>
<th>95% CL</th>
<th>Interaction index (type of interaction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tramadol</td>
<td>2.9</td>
<td>2.09–4.31</td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.13</td>
<td>0.05–0.29</td>
<td></td>
</tr>
<tr>
<td>Tramadol–dexametha-</td>
<td>0.13</td>
<td>0.01–0.29</td>
<td>0.088* (synergy)</td>
</tr>
<tr>
<td>sone 1 : 1 combin-</td>
<td></td>
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</tbody>
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*Synergy represents statistically significant deviation from additivity: $P < 0.01$ (Student’s t-test).
DISCUSSION

The first finding of the present work is the antinociceptive effect induced by dexamethasone in the AAWT, as the effects of tramadol in different animal models of nociception (rats and mice) are well documented in the literature [18,20]. When comparing the ED₅₀ values of the two drugs, we could observe that in our experimental conditions, the antinociceptive potency of dexamethasone was approximately 20 times higher than tramadol.

The analgesic effects of dexamethasone have been reported in humans after oral, orthopaedic and laparoscopic surgery [5,6,25]. The fact that dexamethasone did not induce antinociception when administered 3 h before behavioural testing, suggests a non-genomic mechanism, as the pain model used in our study does not induce a clear inflammatory reaction [26]. It may be noted that prior works also failed to demonstrate an analgesic effect of intravenous dexamethasone when administered 4 h before the AAWT in rats [14]. Similarly, dexamethasone did not induce analgesia when administered 12 h before testing in a clinical model of acute inflammation [27]. The non-genomic glucocorticoid effects could be mediated by at least three mechanisms, mainly physicochemical interactions with cellular membranes (non-specific effects), the activation of membrane-bound-glucocorticoid receptors (mGCR) and cytosolic-glucocorticoid receptors (cGCR). High concentrations of glucocorticoid may alter the characteristics of plasma and mitochondrial membranes leading to an inhibition of calcium and sodium entry and ATP production; these events could participate in the immunosuppressive effects of glucocorticoids [28–30]. The mGCR receptors have been described in neuronal membranes [31], lymphoma cells [32–34] and human mononuclear cells [29,35]. Although there is no evidence for specific signalling pathways associated with these receptors, their activation may explain the rapid dexamethasone-induced adenylate cyclase/protein kinase A-dependent inhibition of chloride ion secretion during inflammation [36,37]. The cGCR is a multi-protein complex, that when activated by glucocorticoids induces not only classical genomic, but also non-genomic effects, by rapidly controlling intracellular signalling through other components of the multiprotein complex. The binding of dexamethasone to cGCR induces not only the release of the receptor protein (that binds to glucocorticoid responsive DNA elements: genomic effects), but also Src from the multi-protein complex. It has been hypothesized that among other effects, Src could inhibit arachidonic acid release in a rapid manner (glucocorticoid receptor-dependent but transcription-independent mechanism) [38].

It is likely that a non-genomic mechanism is involved in the rapid antinociceptive effects of dexamethasone reported in our study, although a precise signalling pathway responsible for this effect has not yet been identified.

The antinociceptive synergism obtained with the combination of dexamethasone and tramadol does not support previous studies reporting a dose-dependent inhibition of morphine analgesia by dexamethasone [14,39]. A reasonable argumentation of the discrepancy could be the atypical mechanism of action of tramadol, characterized by a very low μ-opioid receptor affinity in comparison with morphine and the activation of descending inhibitory pathways (increasing serotonin and noradrenaline levels in the central nervous system) [40].

It has been suggested that synergy between two or more analgesic drugs occurs by the simultaneous activation of complementary pathways of antinociception as the activation of a common mechanism would produce additive effects [41]. Thus, the main antinociceptive effect of tramadol, a central acting analgesic, is due to the opioid and monoaminergic mechanisms [40], while the precise mechanisms implicated in the immediate analgesic effects of dexamethasone remain unknown. The presence of synergy between them is clearly demonstrated from the isobolographic analysis of the interaction, and by the low interaction index obtained.
In conclusion, the results of our study demonstrate a synergistic interaction between tramadol and dexamethasone in a model of acute visceral pain in mice, when both drugs are administered 30 min prior to nociceptive testing. Tramadol is an effective analgesic widely used in the management of mild to moderate postoperative pain in humans, but its administration is usually associated with postoperative nausea and vomiting. To prevent these effects, antiemetics such as ondansetron or dexamethasone are used. As recent reports show an antagonistic interaction with ondansetron for analgesia, we have tested the interaction between tramadol and dexamethasone. Our experiments suggest that the tramadol–dexamethasone combination could be useful in the management of postoperative pain in man. Moreover, further human studies should be performed in order to be able to recommend this drug combination in the perioperative period.

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REFERENCES