Synergistic Interaction Between Fentanyl and a Tramadol:Paracetamol Combination on the Inhibition of Nociception in Mice

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Abstract. Multimodal analgesic approaches to manage acute and chronic pain are commonly used in humans. Here, we attempted to characterize a synergistic interaction between fentanyl, tramadol, and paracetamol on the inhibition of nociception in a model of visceral pain in mice. The three-drug combined treatment displayed a potent synergistic antinociceptive effect, together with a significant reduction of gastrointestinal transit inhibition. Furthermore, selective μ- and κ-opioid receptor antagonists reversed these synergistic antinociceptive effects, thus suggesting a pivotal role of the opioid system. Overall, this study presents accurate pre-clinical data that might be useful to improve the clinical management of opioid-mediated analgesia.

Keywords: opioid multimodal analgesia, drug–drug interaction, synergy

In clinics, the use of drug–drug combinations in order to manage acute and chronic pain is well established. On the one hand, analgesics are staggered according to pain severity: mild, moderate, and severe pain, and thus patients should progress through this analgesic ladder depending on the pain relief obtained. On the other hand, analgesic adjuvants should also be added in order to reach analgesia using lower doses of the drugs (i.e., opioids) used, thus reducing the incidence of adverse side-effects. However, although there is precise categorization of analgesics within drug–drug combinations, these multimodal analgesic approaches are most of the times based on empirical instead of accurate experimental data. In such a way, tramadol (TRM) and paracetamol (PRC) have been revealed as analgesic drugs that would synergically interact with opioid drugs (1, 2). Furthermore, the combination of TRM and PRC is also a common multimodal analgesic strategy that has been shown to be effective and generally well tolerated in patients with moderate to severe pain (3). Accordingly, in the present study we attempted to characterize the combination of the major opioid fentanyl (FEN) with a tramadol:paracetamol (TRM:PRC) mixture, in order to examine whether the analgesic effects of the mixture would be enhanced together with a FEN-dosage reduction and the consequent diminution of opioid-related adverse effects (i.e., constipation). In addition, we evaluated the role of opioid receptors in such drug–drug interaction, since both TRM and PRC possess diverse and complex mechanisms of action, in which the modulation of the opioid system would be included (4–6).

First of all, we analyzed the antinociceptive effects of the individual drugs. Briefly, male Swiss CD1 mice (20 – 25 g) housed under controlled standard conditions (12-h dark/light cycle, 22°C temperature and 66% humidity) were injected with one or a mixture of the following drugs: FEN (KernPharma, Barcelona, Spain); TRM (Grüenthal, Madrid, Spain); PRC (Bristol-Myers Squibb, Madrid, Spain); naloxone (Sigma-Aldrich, St. Louis, MO, USA); naltrindole and nor-binaltorphimine (nor-BNI) (Tocris Bioscience, Bristol, UK) subcutaneously 30 min before testing. Then, mice were injected intraperitoneally with an acetic acid solution (0.6% v/v, 10 ml/kg) and placed in a clear plexiglass cylinder to observe after 5 min the number of writhes (a single writhe is defined as a contraction of the abdominal wall together with the twisting of the trunk and hind limbs extension) in a 5-min period (6). The Institutional Committee on Animal Use and Care, in accordance with the International Association for the Study of Pain guidelines on ethical standards for investigation in animals, approved the protocol. As previously described, FEN and PRC

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induced a dose-dependent inhibition of writhes with similar efficacy (100%) and the following $ED_{50}$’s: FEN = 0.021 ± 0.003, PRC = 109.3 ± 2.5 mg/kg (6). Similarly, TRM also dose-dependently inhibited acetic acid–induced nociception (TRM = 3.18 ± 0.27 mg/kg). Once the individual $ED_{50}$ values were obtained, TRM and PRC were combined, based on previous studies (7, 8), in a fixed ratio (1:8) combination (TRM:PRC). The interaction between the different drugs was analyzed using the previously described procedure (6) based on the method developed by Tallarida et al. (9). Thus, the mixture induced dose-dependent antinociceptive effects, with similar efficacy (100%), and the dose–response curve was shifted to the left with respect to the theoretical additive dose–response curve ($P < 0.05$, Student’s $t$-test). Synergism was also assessed by means of isobolographic analysis (Fig. 1A), obtaining an interaction index of 0.40 ± 0.08. Next, the double combination TRM:PRC was treated as a single drug and mixed with FEN in a fixed ratio (1:1) combination based on $ED_{50}$ fractions. Figure 1B shows the isobologram when adding FEN to the mixture TRM:PRC (1:8). The interaction index (0.37 ± 0.06) was significantly lower than 1 ($P < 0.05$, Student’s $t$-test), thus clearly demonstrating that these drugs displayed a potent synergistic effect on the inhibition of acetic acid–mediated nociception. Thereafter, we evaluated the effects of selective opioid antagonists on the antinociceptive effect of the triple combination. Interestingly, this antinociceptive effect was completely antagonized when treating with naloxone [both at non-selective (1 mg/kg) and $\mu$-opioid selective (0.1 mg/kg) doses (10), s.c. administration immediately before the analgesic drugs]. Furthermore, the selective $\kappa$-opioid receptor antagonist nor-BNI partially blocked while the $\delta$-opioid receptor antagonist naltrindole did not affect the combined treatment–mediated antinociception (Fig. 2A). Finally, we also evaluated the effects of the combined treatment on gastrointestinal transit (GIT) inhibition, a common side effect of opioid therapy. GIT inhibition was measured by orally-administering to 18-h fasted animals a charcoal meal (0.25 ml of a suspension of 10% vegetable charcoal in 5% gum acacia, Sigma-Aldrich) and calculating the percent of the distance traveled by the charcoal relative to the total length of the intestine (11). Interestingly, when comparing the effects on GIT inhibition of the mixture TRM:PRC plus FEN with that of FEN alone, the dose–response curve of the former was parallel and shifted rightward ($P < 0.05$, Student’s $t$-test) (Fig. 2B), thus indicating that the combined treatment diminished FEN-mediated GIT inhibition.

Overall, our results show that a synergistic interaction occurred between the three drugs in the relief of acetic acid–mediated nociception in mice and that this multimodal approach enabled a decrease in FEN dosage and reduced the appearance of adverse effects, namely GIT inhibition. First, after testing the antinociceptive effects of the individual drugs, we evaluated the effects of the combination of TRM plus PRC. This multimodal approach has been widely validated in clinics, for instance, in the treatment of postsurgical-associated pain (3), while it has not been fully studied in animal models. Interestingly, our results are in accordance with that obtained recently in a streptozotocin-induced diabetic rat model, in which a TRM:PRC mixture induced supra-additive

![Fig. 1](image-url). Isobolographic representation of the antinociceptive effects of the combination of tramadol plus paracetamol (A) and a mixture of tramadol:paracetamol (TRM:PRC) in a fixed 1:8 ratio plus fentanyl (FEN) (B), at the $ED_{50}$ level of effect, in the writhing test. The filled square in the line joining the x- and y-axes corresponds to the theoretical $ED_{50}$ with 95% CL; the open square below the isobole corresponds to the experimental $ED_{50}$ with 95% CL. There were significant differences between the experimental and the theoretical values ($P < 0.05$, Student’s $t$-test).
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anti-hyperalgesic effects (12), while other authors did not find a synergic effect of the TRM:PRC mixture in rats with neuropathic spinal cord injury pain (13). Needless to say, it would seem likely that the controversies could be explained either by the nociceptive stimuli used or by the proportion of the drugs in the mixture. Accordingly, the rationale behind the use of the TRM:PRC (1:8) proportion was based on two evidences. First, it was previously shown that only ratios over 1:5.7 were expected to be supra-additive in relieving visceral-induced nociception in mice (7); and second, ratios of approximately 1:8 have been developed by pharmaceutical companies for combination therapy in clinics (8).

Subsequently, our aim consisted of performing a multimodal analgesic paradigm, in which the three drugs were combined. It is noteworthy that we recently demonstrated that FEN and PRC interact in a synergic manner on the inhibition of acetic acid–induced nociception in mice (6), and similarly FEN and TRM have been shown to induce synergic antinociceptive effects in the writhing test (14), validating the clinical strategy of using opioid–opioid combinations in the management of pain, usually in patients in which opioid tolerance appears (2). On the basis of these results, we expected supra-additive antinociceptive effects when combining the three drugs, and effectively FEN:TRM:PRC displayed a potent synergistic interaction on the inhibition of acetic acid–mediated nociception. On the other hand, regarding the mechanism of action of such drug–drug interaction, our results point out the opioid system as one of the responsible systems. Interestingly, naloxone completely reversed the antinociceptive effects of the three-drug combined treatment, while the selective δ-opioid receptor antagonist naltrindole did not affect them and the selective κ-opioid-receptor antagonist nor-BNI partially blocked these effects. As previously described, PRC as well as the atypical opioid agonist TRM have been suggested to induce their antinociceptive effects, along with other mechanisms, via opioid receptors (4, 5). It would then seem likely that the antinociceptive effects of the distinct drugs may converge at opioid receptors, either by a direct activation of the receptors or sensitization of their intracellular signaling pathways or by an increase in the release of endogenous opioid peptides; accordingly, opioid receptors (mainly the μ- but also the κ-subtype) would play a central role integrating the antinociceptive effects of the present drug–drug combination. Interestingly, the role of opioid receptors on the effects of the three-drug combined treatment was also observed when examining GIT inhibition. Thus, the prominent function of opioid receptors was further demonstrated by the parallelism of the obtained dose–response curves that suggested the same mechanism of action in both experimental conditions (14). On the other hand, while an anti-transit effect of PRC has not been reported, it is well-described that not only FEN but also TRM, as an atypical opioid ago-

Fig. 2. Reversion of the antinociceptive effects, and GIT inhibition, of the triple combination. A) Effects of opioid receptor antagonists: naloxone (0.1 mg/kg), naltrindole (3 mg/kg), and nor-binaltorphimine (nor-BNI) (10 mg/kg) on the inhibition of acetic acid–mediated nociception (% inhibition), assessed by the determined effective dose (ED80) of the individual drugs (FEN = 0.037 mg/kg) and of a mixture containing the three drugs (TRM:PRC plus FEN; TRM = 0.25 mg/kg, PRC = 56.74 mg/kg, FEN = 0.011 mg/kg). In control animals, saline was injected instead of the antagonist. Each column represents mean values of at least eight mice, and vertical bars indicate S.E.M. * indicates statistically significant differences (P < 0.05, Student’s t-test) between saline and antagonist-treated mice for each treatment (FEN, TRM:PRC + FEN). B) Dose–response regression lines for the administration of fentanyl (FEN, squares) and the combination of a fixed (1:8) tramadol:paracetamol dose plus fentanyl (TRM:PRC + FEN, triangles; TRM = 0.14 mg/kg, PRC = 31.26 mg/kg, FEN = 0.01 – 0.1 mg/kg) on the inhibition of gastrointestinal transit (% GIT inhibition). Each point represents mean values of at least eight mice, and vertical bars indicate S.E.M.
nist, induce constipation (15). Therefore, it has to be also considered that the diminution of the anti-transit effect would be mostly mediated by the reduction of the opioid drugs in the mixture, which in fact was one of the aims of the present drug–drug combination, reducing undesirable but increasing antinociceptive effects.

In conclusion, our study shows that the combination of FEN, TRM, and PRC leads to synergistic antinociceptive effects and that \( \mu \) - and \( \kappa \)-opioid receptors play a central role in such drug–drug interaction. Furthermore, this multimodal analgesic approach permits reducing possible undesired secondary opioid-associated effects. We have therefore characterized a common multimodal analgesic strategy used in clinics, validating this approach for the management of pain in which opioid drugs are used.

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References