Enhanced Analgesic Effects of Propacetamol and Tramadol Combination in Rats and Mice

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Drug combinations have more potential advantage of greater analgesia than monotherapy. By the combination of analgesics with different mechanism, potency of analgesia can be maximized while the incidence of adverse effects is minimized. This study was aimed to assess a possible interaction in the antinociceptive effects between tramadol (T) and propacetamol (P) when administered in combination against nociceptive effects induced by physical or chemical injury in mice and rats. Three series of experiments were performed. The first was to determine effects of P and T alone or in combination in the acetic acid (AA)-induced writhing test in mice. Combination of T/P (3.9/67.5, 7.8/135, 15.6/271 mg/kg, intraperitoneally (i.p.)) elicited dose-dependent antinociception. The second determined whether the antinociceptive effects of the drugs observed in a test of persistent chemical pain could be seen in a test of acute thermal pain and the back-paw licking response was tested on the hot plate. The back-paw licking latency at different times after drugs obtained with the combination (16/270, 32/540 mg/kg, i.p. T/P) was longer than the respective values obtained with the individual agents. The third was designed to compare the antinociceptive effects between the drugs, either alone or in combination in the rat tail-flicks test. Combination of T/P (5.5/96, 11/192 mg/kg i.p.) both showed effects of higher potency than T and P, respectively. The data obtained confirmed that propacetamol is able to enhance the antinociceptive activity of tramadol.

Key words tramadol; propacetamol; combination; analysis of variance; analgesic effect

Morphine, one of opioid drugs, still has the greatest analgesic effects upon the treatment of severe pain till now. Receptor-binding studies and subsequent cloning confirmed the existence of three main receptor types, , , and . A fourth member of the opioid peptide receptor family, the nociceptor orphanin FQ (N/OFQ) receptor, was cloned as well. The opioid drugs act on the corresponding opioid receptors and produce their antinoiceptive effects. They are fully efficacious on a variety of pains, but show many unwanted side effects and dependence/tolerance problems. Tramadol, a synthetic analogue of opioid drugs, stimulates -opioid receptor weakly a bit. In addition, some of its analgesic effects are produced by inhibiting the uptake of norepinepherine and serotonin. It is rapidly and extensively absorbed by oral as well as parenterally administration and provides clinically significant analgesic effects when acutely administered. However, physical dependence and abuse of tramadol have also been reported by Dayer et al.4 Propacetamol, the diethylamino-acetic ester of acetaminophen, is a prodrug of acetaminophen (paracetamol) that was used in Europe as an IV formulation in the treatment of pain and fever. One gram of propacetamol is hydrolyzed in blood to release 0.5 g of acetaminophen and pharmacologically inactive N,N-diethylglycine. The advantage of propacetamol is that it can be administered parenterally compared with acetaminophen, so it is possible to be used for the patients who can’t take drugs orally. Acetaminophen’s analgesic efficacy has been extensively studied for treatment of low-to-moderate pains in many clinical settings. Warner et al. has reported acetaminophen might be a preferential inhibitor of central cyclooxygenases to peripheral ones, but the inhibition of cyclooxygenase is not sufficient to explain its antinociceptive activity. Smith reported that analgesic effects of acetaminophen might come from the interaction of other multiple neurotransmitters, like those involved in serotonergic, opioidergic, noradrenergic, cholinergic, and nitric oxide synthase systems. In addition, it is very important for acetaminophen to depend its positive action on the serotonergic descending inhibitory pathways in order to produce the analgesic effects.11

Though the opioid drugs remain the most effective therapy available for treatment of moderate to severe pains, the problems arising from unwanted side effects on the central nervous system, including respiratory depression and the development of physical dependence persist. Therefore, the combination of analgesic drugs is commonly used for attenuating postoperative pain clinically and overcoming the unwanted side effects caused by a single agent. Drug combinations have the potential advantage of greater analgesia than monotherapy. Researches on the interaction and possible use of analgesic combination in a variety of painful conditions have been published.12–15 Synergistic antihyperalgesic effect of etoricoxib and tramadol combination suggested that the combination might have clinical utility in mechanical hyperalgesia associated with spinal injury.16 Most of the researches on the combination that have positive synergistic interaction clinically, but only few of these have been analyzed in preclinical animal models. The experiments described in this report were conducted for the purpose of determining the analgesic potential of propacetamol and tramadol combination in mice and rats models of nociception. And the effects were compared with those produced by the tramadol alone. Although the effects of tramadol combined with acetaminophen have been reported in Europe, the combination of tramadol and propacetamol is not a pharmaceutical preparation used in the management of moderate pain in humanbeings in China as far as we know, and few of literature relevant to it can be searched. The effects of the
compounds on acute thermal pains were assessed by using the hot-plate and tail-flick tests, and as for more persistent pains, visceral pain was assessed by using the abdominal constriction assay in this research.

MATERIALS AND METHODS

Animals According to the rules of animal experimentation and the Guide for the Care and Use of Laboratory Animals of Shenyang Pharmaceutical University, this protocol was approved by the local Animal Ethics Committee. The subjects were Kunming male mice (female mice were used in the hot plate test) and Sprague-Dawley male rats were obtained from Animal Center of Shenyang Pharmaceutical University, the size of which ranged from 25—30 g and 200—230 g in this research. The animals were group-housed (8 mice per cage) in standard environmental conditions (22 ± 1 °C, humidity 60 ± 5%, 12 h light : 12 h dark cycle) with free access to a standard commercial diet and water ad libitum. After a 7-d adaptation period, all experiments were performed during the light phase. All the animals used as the subjects were used only once except where it is noted.

Drugs Propacetamol hydrochloride, tramadol hydrochloride (Shenyang Huatai Ltd. of Medicine Research, Shenyang, China) were used. They were dissolved in distilled water. Injection of morphine hydrochloride and pethidine (Shenyang 1st Pharmaceutical Ltd., Shenyang, China) were diluted when necessary with 0.9% sterile saline before injecting. 0.9% sterile saline was produced by Shenyang Zhiying Pharmaceutical factory (Shenyang, China). The drugs were administered according to the constant volume of 10 ml/kg of body weight.

Abdominal Constriction Assay Persistent chemical pain was assessed by means of an abdominal constriction assay in mice. In this test, mice were divided into 7 groups and received 0.9% saline (n = 9), 2.0 mg/kg morphine (n = 7), 10.0 mg/kg tramadol (n = 7), 200 mg/kg propacetamol (n = 9) or combination of tramadol (T) and propacetamol (P) (3.9 mg/kg T + 67.5 mg/kg P, 7.8 mg/kg T + 135 mg/kg P, 15.6 mg/kg T + 271 mg/kg P) (n = 8), 10, 10) min prior to subjection to the abdominal constriction, respectively. The mice were injected intraperitoneally with 0.1 ml/10 g body weight in the other area of the irritant 0.6% acetic acid (AA) in distilled water. Mouse was placed in an individual plastic cage (29×18×16 cm) for observation and the number of writhes as compared to control animals in the 20-min period from the time when the animal was placed on the heated surface of the plate. The latency was defined as the time when the animal licked its back paw or jumped off to avoid thermal pain. The mice with latency of 10—30 s by duplicated detection were selected to be used in the formal experiment. Mice were divided into 7 groups, 10 mice each group (n = 10) and received 0.9% saline, tramadol (16.0, 32.0 mg/kg), propacetamol (270, 540 mg/kg) or combination of T and P (16.0 mg/kg T + 270 mg/kg P, 32.0 mg/kg T + 540 mg/kg P) respectively before suffering from the hot plate test. Back-paw licking latency was measured after 0, 15, 30 and 45 min after the test compounds were administered.

Rat Tail-Flicks Test For the tail-flick test, an automated tail-flick test device (analgesia meter SW-200, Chengdu Technology & Market Corp., Ltd., Chengdu, Sichuan, China) was used. Tail rats (distal 1/3 rd) were painted with black ink so as to absorb more heat energy and an adjustable heat source was placed directly under the tail. The animal flicks its tail away from the source of heat on feeling pain. Tail-flick latencies required for the rat to remove its tail were determined in seconds as an index of noxious threshold. The longest time was 30 s laid down for fear of damaging tissues, i.e. maximum exposure time was limited to 30 s. This index was referred to pain threshold. Pain threshold elongation shows the analgesic effect of drug. The rats with latency of 3 to 20 s by duplicated detection were selected to be the subjects in the formal experiment. All rats were divided into 9 groups and received 0.9% saline (n = 9), 5.0 mg/kg pethidine (n = 7), 7.3 and 11.0 mg/kg tramadol (n = 8), 149 and 192 mg/kg propacetamol (n = 8) or combination of T and P (2.8 mg/kg T + 48 mg/kg P, 5.5 mg/kg T + 96 mg/kg P, 11.0 mg/kg T + 192 mg/kg P) (n = 9) respectively previous to subjection to the test mentioned above. The antinociceptive activity was assessed 0, 15, 30, 45, 60, and 75 min after intraperitoneal (i.p.) drug administration.

Statistics Results from each group were calculated as mean ± standard error of mean (S.E.M.). Statistical evaluation was done using analysis of variance (ANOVA) with post-hoc multiple comparison between groups followed by Fisher’s protected least significant difference test to analyze and compare the data, which were analyzed with SPSS 11.5.

RESULTS

Antinociceptive Effect (Reduction in Writhes) of the Drugs on the Writhings Response Administration of morphine, used as reference drug, or tramadol produced an antinociceptive effect and there was significant reduction in the number of writhes as compared to control animals in the algesiometric assay (Table 1). The algesiometric response was also evident by administration of propacetamol. The three

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Number of writhes/20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% Saline</td>
<td>—</td>
<td>9</td>
<td>22.2 ± 5.8</td>
</tr>
<tr>
<td>Morphine</td>
<td>2.0</td>
<td>7</td>
<td>0 ± 0.0 (a)</td>
</tr>
<tr>
<td>Tramadol</td>
<td>10.0</td>
<td>7</td>
<td>0.3 ± 0.3 (a)</td>
</tr>
<tr>
<td>Propacetamol</td>
<td>200</td>
<td>9</td>
<td>11.8 ± 3.8 (a)</td>
</tr>
<tr>
<td>Combination of T &amp; P</td>
<td>15.6 T + 271 P</td>
<td>10</td>
<td>0.0 ± 0.0 (a)</td>
</tr>
<tr>
<td></td>
<td>7.8 T + 135 P</td>
<td>10</td>
<td>4.4 ± 1.8 (a)</td>
</tr>
<tr>
<td></td>
<td>3.9 T + 67.5 P</td>
<td>8</td>
<td>11.8 ± 4.6 (a)</td>
</tr>
</tbody>
</table>

Data were shown as the mean ± S.E.M. a) p < 0.05 and aa) p < 0.01 are the significance levels compared with 0.9% saline group. b) p > 0.05 is not significant compared with 10.0 mg/kg tramadol-treated mice. c) p < 0.05 is significant compared with 200 mg/kg propacetamol-treated mice.
combinations of tramadol and propacetamol elicited dose-dependent antinociception, as compared with 0.9% saline administered (Table 1). Propacetamol used in dose of 200 mg/kg i.p. elicited antinociception with 11.8±3.8 number of writhes during 20 min. Co-injection of 7.8 mg/kg tramadol and 135 mg/kg propacetamol reduced significantly (p<0.05) the number of writhing responses by 63% compared to the effect of propacetamol alone (200 mg/kg) (Table 1). There is not a significant difference between the combination (7.8 T+135 P) and the 10.0 mg/kg tramadol group (Table 1).

Comparison of the Antinociceptive Effects (Prolonged Latency) Among the Single Agents and the Combinations on the Hot Plate The combination (32.0 mg/kg T+540 mg/kg P) depicted in Fig. 1 represents a co-treated one that produced the potentiation of the antinociceptive effect, likewise, the back-paw licking latency in 15, 30, and 45 min after administration of combination was longer than that obtained from the single agents. Propacetamol alone did not show any antinociceptive activity in this assay after administration. Both the treatment (tramadol: 32.0 mg/kg, i.p. and the combination: 32.0 mg/kg T+540 mg/kg P, i.p.) showed the analgesic effect, i.e. there is a prolonged back-paw licking latency, but they displayed different efficacy (Fig. 1). Tramadol (32.0 mg/kg) produced a maximal analgesic effect at 15 min following administration. There was a rapid action but the effect sustained a short while when it was compared with the combination group (Fig. 1). In contrast, the combination was found to be long-lasting action (statistically significant effect at 3 tested points of 15, 30, and 45 min, respectively after administration of drugs) with maximal analgesic effect at 30 min following treatment. In addition, the analgesic effect of the combination was more effective than that of 32.0 mg/kg tramadol alone and there is a significance between the two groups at the tested point of 30 min (bb p<0.01, Fig. 1).

Antinociceptive Effect (Prolonged Latency) of the Drugs in the Tail-Flicks Test in Rats Propacetamol added to tramadol increased the analgesic effect of tramadol (Fig. 2), although 192 mg/kg propacetamol alone did not show any analgesic response. The tail flick latency in the combination (11.0 T+192 P) showed a significant increase (p<0.05 and bb p<0.01, significantly different from tramadol 11.0 mg/kg group at 30, 60 and 75 min, respectively). Maximum analgesia was found as early as 30 min after administration of the combination.

We compared the effects among the single agents and the combinations, especially at the equianalgesic doses for people in Fig. 3, which revealed that tramadol alone, at the dose of 7.3 mg/kg (the equianalgesic dose of 50 mg for people in clinical use), yielded evident antinociceptive effects within 75 min after administration but accompanied a shallow dose response curve. And propacetamol, at dose of 149 mg/kg (the equianalgesic dose of 1000 mg for people), showed no evident antinociceptive effect. The combination of 5.5 T+96 P,
being equal to 37.5 mg and 650 mg for people, showed high potency (Fig. 3). Tramadol at dose of 7.3 mg/kg (i.p.) induced antinociception with a pain threshold of 17.00 ± 3.99 S at 30 min. Co-injection of propacetamol and tramadol (5.5 T + 96 P) increased the antinociceptive effect of tramadol, which reached significant levels from 30 (24.28 ± 2.61 S) to 75 (24.75 ± 3.01 S) min (Fig. 3). The antinociceptive effect of 5 mg/kg pethidine which was used as a reference drug was of short duration, lasting only for 45 min (Fig. 3). In contrast, significant effect (Fig. 3) could still be observed at 75 min after the administration of the combination of tramadol and propacetamol (5.5 T + 96 P).

DISCUSSION

The main aim for developing combination analgesics is to gain efficacy and reduce dose and toxicity. It has been reported that the administration of the combinations of opioids and nonsteroidal anti-inflammatory drugs (NSAIDs) can increase therapeutic effects and lead to the use of lower doses of opioids.28 This study also proved a positive potentiation between tramadol and propacetamol in the doses tested when compared to the per se effects. If propacetamol is used together with tramadol clinically, we will minimize doses of tramadol and then enhance the analgesic effect.

Tramadol, propacetamol and the three combinations all produced significantly antinociceptive effects in mice against persistent visceral irritation—the abdominal constriction (writhing) assay. It demonstrates that, under the conditions, persistent visceral irritation—the abdominal constriction produced significantly antinociceptive effects in mice against tramadol/flurbiprofen34) or tramadol/acetaminophen35,36) had synergistic antinociceptive effects, although the study design and experimental model were different.

Tramadol, which is a racemic mixture with (−)-tramadol preferentially inhibits noradrenaline (NA) uptake,7 whereas (+)-tramadol inhibits 5-HT uptake, enhances 5-HT release, and binds to µ-opioid receptors.37) In addition, a marked antinociceptive synergy exists between the two enantiomers.38 It is one of the analgesics acting on the CNS evidently, the action of which was produced by opioid and non-opioid modes. Opioids primarily prevent hyperalgesia through their action on spinal and supraspinal level but they also reduce hyperalgesia in periphery.39) NSAIDs can produce analgesia as well as enhance the analgesic action of opioids in several regions of CNS and periphery. Many mechanisms (serotoninergic, nitric oxide-guanosine 5′-cyclic dichloromethane (cGMP) pathway) are thought to govern this interaction, but the precise mechanism was still not clear. As described above, propacetamol is a prodrug of acetaminophen. Therefore, the effect of propacetamol on the tramadol-induced antinociception is mediated by the same mechanism as that of acetaminophen. Acetaminophen is a commonly used non-opioid drug. In spite of the numerous investigations, the mechanism of acetaminophen action is still poorly defined and has not been satisfactorily explained. Unlike NSAIDs, acetaminophen administered at therapeutic doses has little or no antiinflammatory and antiplatelet activity as well as does not share the typical side effect profile with NSAIDs, such as damage of gastrointestinal tract, induction of “aspirin asthma,” etc. The results obtained in previous study suggested that both cyclooxygenase and nitric oxide synthase (NOS) systems were involved in the antinociception produced by orally administered acetaminophen.40 Among various mechanisms of its action, it was suggested that endogenous
opioidergic system might be involved in antinociceptive activity of acetaminophen.\textsuperscript{43}

The development of new pain strategies involves combining analgesics that target many pain pathways to deliver greater analgesia at reduced and tolerable doses of single drugs.\textsuperscript{42,43} Co-administration of two or more drugs may result in additional (sum of the effects produced by each agent), sub-additional (little effect produced by each drug alone), or supra-additional i.e. synergistic (greater effect than the sum of the ones produced by each drug) interaction. In terms of the combination in this paper, there is apparently a supra-additional effect. The mechanisms underlying this potentiation may associate with (1) the central opioidergic system, (2) the serotonin system, and (3) a net interaction of various effects. With respect to the potential site, it is inferred that the synergism of propacetamol and tramadol occurred is probably in connection with a small part on the spinal site but has much more relation with the supraspinal one. It is reported that the potential effect of acetaminophen is partially at spinal cord.\textsuperscript{44} However, the drug did not bind to opioid receptors,\textsuperscript{45} the region for its potentiation was mostly involved in the pathway from the supraspinal to the spinal levels.\textsuperscript{45,46} In contrast, opioid and nonopioid mechanisms of action of tramadol are thought to act synergistically on descending inhibitory pathways in the CNS, including those mediated by the raphe nuclei, periaqueductal grey, locus coeruleus and reticulospinal projection nervous system, which results in the modulation of secondary neurons in the spinal cord.\textsuperscript{47} A study also supported the supraspinal interaction of serotonin and the opioid system in the regulation of analgesic action of tramadol.\textsuperscript{48} Therefore, we think that the analgesic activity of the combination occurred probably on spinal as well as supraspinal levels, but mainly at the high central one. It is obvious, however, that the nature of the molecular mechanisms involved in the relationship among the potentiation described above remains to be elucidated.

In summary, the data obtained in this study indicated that the combination of atypical opioid, tramadol and atypical NSAIDs, propacetamol had more potent antinociceptive effects than those of tramadol and propacetamol respectively in mouse and rat models with acute and persistent pain. It suggests that it is possible to increase the antinociceptive effects and decrease the undesirable side effects of tramadol by co-administrating propacetamol. Thus, the findings of the combination reveal that it has potential for development as one of the new strategies of analgesics.

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REFERENCES