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Isobolographic analysis of the analgesic interactions between ketamine and tramadol

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Abstract

Owing to different mechanisms of analgesia, we hypothesized that the combination of ketamine and tramadol could produce synergistic or additive antinociceptive effects. Swiss albino mice were administered intraperitoneally with ketamine, tramadol, a combination of ketamine and tramadol, or saline, and the resulting antinociceptive effects were tested in the mouse tail-flick and formalin tests. The potencies of the two drugs alone or in combination were obtained by fitting data to the Sigmoid E_{max} equation. Isobolographic analysis was performed to evaluate the interaction. CNS depression was also monitored. Results showed that tramadol exhibited apparent dose-dependent effects in the tail-flick test, and in phase 1 and phase 2 of the formalin test. Ketamine dose-dependently inhibited the phase 2 responses, but failed to modify the phase 1 and tail-flick responses. Combination of tramadol and ketamine produced significant synergistic interactions only in phase 2 of the formalin test ($P < 0.05$). The synergistic combinations also displayed less CNS depression than when an equi-analgesic dose of ketamine was administered alone. We conclude that in the acute thermal or chemical pain model, ketamine is not effective and the net effect of ketamine and tramadol in combination was simply additive after systemic administration. However, the coadministration produced synergistic antinociception in the chemical-induced persistent pain model.

Introduction

Tramadol hydrochloride is a synthetic opioid analgesic agent with dual mechanisms: relatively weak and selective affinity at the μ receptors and activation of descending monoaminergic inhibitory pathways (inhibition of norepinephrine and serotonin reuptake) (Lee et al 1993). Because of its favourable safety record – few opioid-related side-effects such as respiratory depression, constipation, tolerance and dependence – tramadol is widely used for post-operative and gynaecological pain, refractory cancer pain, chronic inflammatory disorders and neuropathic pain (Lewis & Han 1997).

Ketamine is a clinically available general anaesthetic. It has been used in clinical practice for more than 30 years and has been extensively studied because of its non-competitive antagonism of *N*-methyl-D-aspartate (NMDA) receptors (Hirota and Lambert 1996; Kohrs and Durieux 1998). In many animal pain models and in clinical practice, it produces antinociception as an NMDA receptor antagonist (Felsby et al 1995; Davidson & Carlton 1998; Klimscha et al 1998).

Clinically, physicians often combine different analgesics that would provide weak efficacy or severe adverse effects when used unimodally (Kehlet et al 1999). These combination regimens produce synergistic antinociceptive effects, but with less

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incidence or severity of adverse reactions. At present, researchers often concentrate on drug combinations of classic opioids with either NMDA receptor antagonists, α_2-adrenoceptor agonists or local anaesthetics (Dickenson & Sullivan 1993; Dickenson 1997a).

There is considerable data to suggest that addition of NMDA receptor antagonists can potentiate the analgesic effects of μ opioid receptor agonists in-vitro and in-vivo (Bhargava 1997; Nishiyama 2000). Considering the mechanisms of action and the pharmacological characters (efficacy and adverse effects) of tramadol and ketamine, we hypothesized that systemic coadministration of tramadol and ketamine would show a synergistic or additive interaction and reduced side-effects. The present study was designed to investigate the antinociceptive interaction of an intraperitoneal combination of tramadol and ketamine in the tail-flick and formalin tests in mice, and to evaluate the incidence of adverse reactions after single and combined administrations in the central nervous system (CNS) depression test in mice.

Materials and Methods

Animals

Experiments were carried out according to a protocol approved by the Laboratory Animal Centre of the National University of Singapore. Male Swiss albino mice, 25–30 g, were used. In order to reduce the impact of environmental changes and handling during nociceptive responses, mice were acclimatized to their surroundings and trained in the test situation for 3 days before the experiment. All tests were performed during the light cycle. Each dose group contained 8–10 mice and each mouse was used only once.

Drugs

Racemic tramadol hydrochloride was generously provided by Grünenthal (Stolberg, Germany). Racemic ketamine hydrochloride was purchased from Warner Lambert Ltd (Co. Dublin, Ireland). Both drugs were dissolved and diluted with physiological saline, and administered intraperitoneally in a constant volume of 0.1 mL/10 g bodyweight. Control animals received an injection of an equal volume of saline.

Antinociceptive tests

Tail-flick test

The radiant heat tail-flick test was first described by D'Amour & Smith (1941). It was modified slightly and employed to measure the response to a noxious phasic stimulus using a tail-flick analgesicmeter (Apelex, Paris, France). The latency between switching on the light and withdrawal of the tail from the high-intensity beam was recorded. The beam was focused on the dorsal surface of the tail (2.5 cm from the distal ending), which was blackened to aid uniform absorption of the heat. The instrument was calibrated to provide a narrow baseline latency range (3.5–5.5 s), and the baseline latency was the mean of three determinations. A cut-off latency of 12 s was used to minimize tissue damage.

After determining the baseline latency and to establish the time course of antinociceptive effect, two groups of mice were injected intraperitoneally with tramadol (25 mg kg^{-1}) and ketamine (33.6 mg kg^{-1}), respectively. According to the established maximum effect time, the dose–response relationships of intraperitoneal tramadol and ketamine alone were determined with sequentially increasing doses (4.5, 8, 14, 25, 45 mg kg^{-1} and 5, 10, 18.8, 33.6 mg kg^{-1}, respectively; the dose interval was approx. 0.25 log units). To assess the drug interactions between tramadol and ketamine, drug combinations (1 : 1 and 3 : 1, w/w) were intraperitoneally administered based on their peak effect times. The doses of tramadol and ketamine administered in combination in the 1 : 1 group were (mg kg^{-1}): 33.6 : 33.6, 25 : 25, 14 : 14, 8 : 8 and 4.5 : 4.5. The doses in the 3 : 1 group were (mg kg^{-1}): 45 : 15, 25 : 8.3, 14 : 4.7, 8 : 2.7 and 4.5 : 1.5. The latencies of combinations were recorded when both drugs produced simultaneously the maximum analgesic effects.

Formalin test

The formalin test was assessed according to Nishiyama (2000). The mice were acclimatized to the test environment by placing them into a Plexiglas chamber (15 cm × 15 cm × 20 cm) for 45 min before the formalin injection. The mice were slightly anaesthetized with pure diethyl ether (Merck KGaA, Darmstadt, Germany) and were immediately removed from the anaesthesia box. Then, 20 μL 5% formalin was subcutaneously injected into the dorsal surface of the left hind paw using a 100-μL syringe with a 29-gauge needle (Terumo Medical, Elkton, USA). The mice were then individually returned to the chamber for observation of licking/biting of the injected paws. The recording of responses was divided into two phases: phase 1 started immediately following the injection (0–5 min) and phase 2 started between 20 and 45 min after the formalin injection. Pain response was measured by recording the time of spontaneous behaviours for six continuous 5-min periods and the total time was calculated for each phase. A preliminary

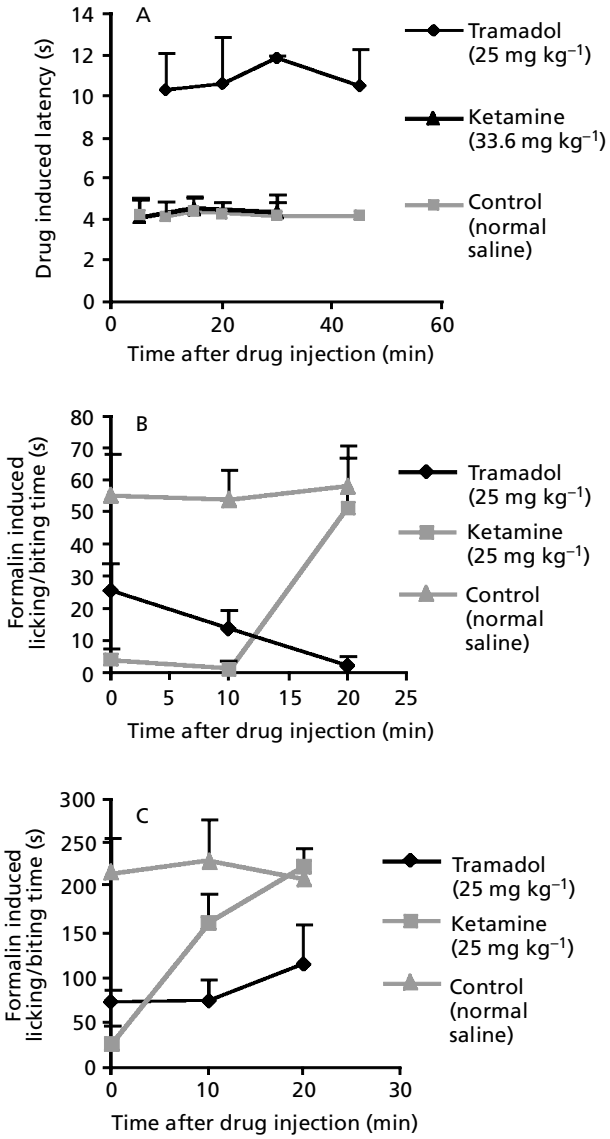

Figure 1 Time courses of drug-induced responses in the tail-flick test (A), and phase 1 (B) and phase 2 (C) of the formalin test after separate intraperitoneal administrations of tramadol and ketamine. Data are presented as mean±s.d., n = 8–10 mice for each dose.

test established that the slight anaesthesia had no effect on licking/biting behaviour during the two phases.

In order to determine the peak time for tramadol to inhibit nociceptive behaviour, three groups of mice were administered with 25 mg kg^{-1} tramadol and then injected with 20 μL 5% formalin at three different time intervals (0, 10 and 20 min, respectively). The same procedures were repeated to establish the time course of ketamine antinociception.

Based on their respective peak times, the dose–response relationships of tramadol and ketamine alone were determined with sequentially increasing doses (phase 1: 4.5, 8, 14, 25 mg kg^{-1} and 8, 14, 25 mg kg^{-1}, i.p.; phase 2: 8, 14, 25, 45 mg kg^{-1} and 4.5, 8, 14, 25 mg kg^{-1}, i.p.). There were 8–10 mice in each dose group. For evaluating the interactive effects during phase 1, tramadol and ketamine were given at ratios of 1:1 and 3:1. The same strategy was adopted for phase 2 to assess these interactions, but three dose ratios (3:1, 1:1 and 1:3) were used.

CNS depression test

To achieve a sensitive numeric index, CNS depression was evaluated by using the scale proposed by Shimoyama et al (1997). This scale consists of six graded contents as follows: 0 = normal; 1 = cannot stand on hind limbs (slight ataxia); 2 = cannot negotiate 60° inclined mesh (marked ataxia); 3 = loss of righting reflex; 4 = immobility (reaction to pain present, determined by paw pinch); and 5 = no reaction to pain (anaesthesia). Thus, the normal baseline CNS depression score was 0 and the complete damage score was 5.

CNS depression was assessed at 5, 10 and 20 min after injection of 33.6 mg kg^{-1} ketamine and 45 mg kg^{-1} tramadol, respectively. These doses were the maximum doses used in this study. An additional 25 mg kg^{-1} ketamine was then administered for this test. The antinociceptive effects of tramadol (45 mg kg^{-1}) and ketamine (25 mg kg^{-1}) were at approximately 90% of the maximum possible effect (MPE) in phase 2 of the formalin test. In the drug combination group that showed synergism, the equi-analgesic (near 90% MPE) combination dose was given to determine the depressant effects.

Data and statistical analysis

To construct dose–response curves, all response (latency and licking/biting time) data were converted to the percentage of MPE for each single drug and combination regimen.

For the tail-flick test, this was computed by the following equation:

$$\%\,\text{MPE} = ((\text{Drug-induced latency} - \text{baseline latency})/(12 - \text{baseline latency})) \times 100$$

For the formalin test, the conversion was realized by the following equation:

$$\%\,\text{MPE} = ((\text{Pre-treatment response} - \text{post-treatment response})/\text{pre-treatment response})) \times 100$$

Table 1 ED50 (mg kg^{-1}) and 95% CI of tramadol, ketamine and their combination in the tail-flick and formalin tests.

Treatment	Tail-flick test		Phase 1 of formalin test		Phase 2 of formalin test		Total fraction value
	ED50 (mean±s.e.)	95% CI	ED50 (mean±s.e.)	95% CI	ED50 (mean±s.e.)	95% CI	
Tramadol	22.4±3.1	16.2–28.7	12.4±2.0	8.2–16.6	20.9±2.4	16.0–25.8	–
Ketamine	–	–	–	–	12.9±2.5	7.8–18.0	–
Experimental value (1:1)	49.2±7.0	34.9–63.4	16.9±1.3	14.1–19.6	6.8±0.9**	4.9–8.8	0.4
Theoretical value (1:1)	44.8±12.4	19.9–69.8	24.8±8.2	8.0–41.6	16.0±1.8	12.4–19.6	1
Experimental value (3:1)	29.4±4.6	20.1–38.8	16.6±1.0	14.5–18.8	12.0±1.7*	8.6–15.5	0.7
Theoretical value (3:1)	29.9±5.5	18.8–41.0	16.5±3.7	9.1–24.0	18.1±1.8	14.5–21.7	1
Experimental value (1:3)	–	–	–	–	16.4±0.8	14.8–18.1	1.2
Theoretical value (1:3)	–	–	–	–	14.3±2.1	10.1–18.5	1

*$P < 0.05$, significant difference between experiment value and theoretical value (t-test); **$P < 0.001$, significant difference between experiment value and theoretical value (t-test).

The effective dose resulting in a 50% MPE was defined as the median effective dose (ED50). The ED50s and 95% confidence intervals (CI) of drugs and combinations were calculated by fitting the data to the Sigmoid E_{max} dose–effect equation (GraphPad Prism, San Diego, USA). For combination of tramadol and ketamine, the total dose of combined drugs was used for data fitting.

For drugs and combinations that were ineffective and could not reach ED50 and 95% CI, the data were compared with baseline using a one-way analysis of variance followed by Tukey's test for between-group comparison. To analyse the CNS depression scores, the depression score at the different time-points for each mouse was cumulated as its total score. The analysis of significant differences between these scores was achieved using Kruskal–Wallis's analysis of variance by ranks followed by Dunns's test for between-group analysis. A value of $P < 0.05$ was considered to be statistically significant.

Test for an additive or synergistic interaction between drugs was performed by isobolographic analysis when at least one of the drugs produced dose-dependent antinociception when administered alone. The theoretical additive ED50 and 95% CI were calculated for the combination of drugs based on the individual ED50 and the fixed dose ratio (Tallarida et al 1989; Tallarida 1992). Drug interactions may be suggested by constructing an isobologram. The ED50s of the two drugs are respectively plotted on the x and y axes. The straight line connecting these two points is the theoretical additive line. If the experimental derived isobole (a point representing x, y coordinates for ED50) is plotted significantly below the theoretically additive isobole, the interactive effect is identified to be synergistic. For the statistical estimation of the difference between the experimentally derived potency and the theoretical additive counterpart, a t-test was used based on known ED50s and standard errors.

Additionally, the method of total fractions was used when both drugs had apparent potencies (ED50s) when injected alone. The total fraction values were calculated using the following equation:

Total fraction value =
(ED50 dose of Drug 1 in combination/ED50 dose of Drug 1 injected alone)+(ED50 dose of Drug 2 in combination/ ED50 dose of Drug 2 injected alone)

A value near 1 indicates an additive interaction and a value less than 1 indicates synergistic interaction between the coadministered agents (Tallarida et al 1989).

Results

Tail-flick test

Administration of tramadol and ketamine alone

Tramadol (25 mg kg^{-1}) alone produced apparent antinociceptive effects at all observation points and the mean peak effects were observed at 30 min after drug injection. Ketamine (33.6 mg kg^{-1}) did not induce antinociception at any time point (Figure 1), and the drug-induced latencies did not significantly differ from the baseline latency ($P > 0.05$). From the time–response curves, the starting points for recording the latency were set at 30 and 15 min after the respective administration

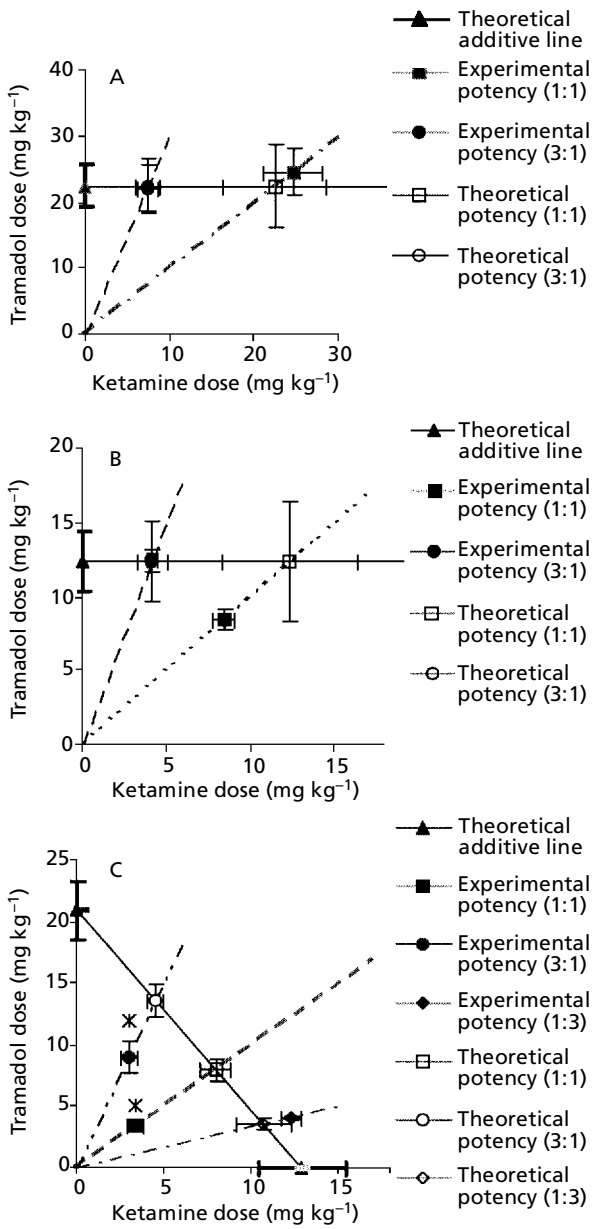

Figure 2 Isobolograms for the antinociceptive interaction of intraperitoneal coadministration of tramadol and ketamine at different fixed dose ratios (1:1, 3:1 or 1:3, as shown in graph) in the tail-flick test (A), and phase 1 (B) and phase 2 (C) of the formalin test. The straight solid line is the theoretical additive line, and the point shown on this line is the theoretical additive ED50 point. The dashed lines denote the three fixed dose ratios. The standard error of the theoretical additive and experimental derived potencies (ED50) is resolved into the ketamine (horizontal) and tramadol (vertical) components and show on the graphs. In the tail-flick test, the experimental point is above (1:1) or almost overlaps (3:1) with the additive line. The difference between experimental and theoretical points was not significant ($P > 0.05$), indicating an additive antinociceptive effect at both ratios. In phase 1 of the formalin test, the experimental point for combinations is below (1:1) or almost (3:1) overlaps with the additive line. The difference between experimental and theoretical points was not significant ($P > 0.05$), indicating an additive antinociceptive effect at both ratios. In phase 2 of the formalin test, the experimental point for combinations is below (1:1 and 3:1) or above (1:3) the additive line. The differences between experimental and theoretical points were significant at the ratios of 1:1 and 3:1 ($P < 0.05$), indicating synergistic effect, whereas they were not significant at the ratio of 1:3 ($P > 0.05$), indicating an additive effect.

of tramadol and ketamine. With the sequentially increasing doses, a significant dose-dependent response curve could be established for tramadol, but not for ketamine. Thus, over the dose range used in this test, ketamine could not evoke antinociceptive effects in the tail-flick test.

Coadministration of tramadol and ketamine

At two fixed dose ratios of tramadol to ketamine (1:1 and 3:1, w/w), a series of combinations produced dose-dependent effects. ED50s and 95% CIs for tramadol and combinations are summarized in Table 1. Experimentally derived ED50s and CIs for combinations plotted in the isobologram were located above (1:1) or almost overlapped (3:1) with the theoretically additive line (Figure 2). Statistical analysis revealed that the differences between experimental potencies and theoretical potencies were not significant ($P > 0.05$).

Formalin test

Administration of tramadol and ketamine alone

In phase 1, 25 mg kg^{-1} tramadol produced apparent time-dependent analgesic effects (Figure 1) and the peak effect of inhibiting the licking/biting behaviour was seen when the injection interval between tramadol and formalin was 20 min. A dose of 25 mg kg^{-1} ketamine could induce apparent behavioural changes at 0 and 10 min after ketamine administration ($P < 0.05$). However, the results of the CNS function test showed that at these two points, mice licking/biting behaviours were affected by ketamine's inhibition of the CNS function. At 20 min after the injection of ketamine, the animal behaviour induced by formalin did not significantly differ from that of control animals ($P > 0.05$). Therefore, tramadol and ketamine were simultaneously administered 20 min before the injection of 5% formalin to achieve the maximal effects in this phase. With sequentially increasing doses, a dose-dependent response curve was constructed for tramadol, but not for ketamine. Similar to the tail-flick test, ketamine could not evoke antinociceptive reactions in phase 1 of the formalin test.

In phase 2, both tramadol (25 mg kg^{-1}) and ketamine (25 mg kg^{-1}) produced time-dependent antinociception (Figure 1). Both peak effects could be observed when the

time interval between the injection of the drugs and formalin was 0 min. Thus, formalin should be injected immediately after administration of the two drugs. Two dose-dependent response curves could be constructed for tramadol and ketamine.

Coadministration of tramadol and ketamine

In phase 1, two fixed dose ratios (1 : 1 and 3 : 1) were used to evaluate the interactive effects of the drugs. ED50s were plotted on the isobologram and compared with the theoretical additive ED50s (Figure 2). From the graph, it was found that the experimental point of 1 : 1 was below the theoretical point, whereas the experimental point of 3 : 1 almost overlapped with its theoretical counterpart. There was no significant difference between the experimental and theoretical values for all ratios ($P > 0.05$). The experimental and theoretical ED50s and 95% CIs in phase 1 are pooled in Table 1.

In phase 2, three ratios of tramadol and ketamine (3 : 1, 1 : 1 and 1 : 3) were used. The interactions were analysed by constructing the isobologram and using the *t*-test (Figure 2). In this phase, the 3 : 1 and 1 : 1 combinations produced synergistic antinociceptive effects ($P < 0.05$), but the 1 : 3 combination did not ($P > 0.05$). These results were consistent with those of the total fraction method (Table 1).

CNS depression test

According to the CNS depression scoring system, all mice used in the present study showed a CNS depression score of 0 (i.e. no depression before treatment). Tramadol at a dose of 45 mg kg^{-1} did not induce CNS depression throughout the observation period. CNS depression scores were consistently 0 for 20 min after injection of tramadol (Table 2). For ketamine at doses of 33.6 and 25 mg kg^{-1}, depressant behaviour was clearly observed at first and then disappeared at 20 min after injection. The total scores were significantly different from the control group ($P < 0.01$). At 20 min after the injection of ketamine, the depression scores were not different from that of the control group ($P > 0.05$), but some mice in the 33.6 mg kg^{-1} dose group displayed some depression that did not affect tail-flick behaviour, but did affect licking/biting behaviour. Ketamine at 25 mg kg^{-1} did not produce any CNS depression at 20 min and the behaviours were not disturbed.

In the synergistic combination groups (1 : 1 and 3 : 1 in phase 2), two combination doses of tramadol and ketamine, 16 mg kg^{-1} (8 : 8) and 24 mg kg^{-1} (18 : 6), displayed significantly less CNS depression than when an equi-analgesic dose (25 mg kg^{-1}) of ketamine was administered alone ($P < 0.01$). Furthermore, there was no significant difference between the total scores of these two combinations and that of equi-analgesic tramadol (45 mg kg^{-1}) or the control group ($P > 0.05$).

Discussion

The present study has yielded the following findings: (1) intraperitoneal tramadol, at doses that do not cause CNS depression, exerted dose-dependent antinociceptive effects in the mouse tail-flick test and during both phases of the mouse formalin test, but ketamine only induced analgesia in phase 2 of the formalin test; (2) coadministration of tramadol and ketamine, at the fixed dose ratios tested, produced synergistic analgesic effects.

The mouse tail-flick test is a classic acute thermal pain

Table 2 CNS depression scores after intraperitoneal injection of tramadol, ketamine and their combination.

Drug	Dose (mg kg^{-1})	CNS depression score[a]			
		5 min	10 min	20 min	Total
Saline	–	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Tramadol	45	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Ketamine	33.6	2 (2, 2)*	1 (1, 1)*	0 (0, 0)	3 (3, 3.5)*
Ketamine	25	1 (1, 2)*	0.5 (1, 1)	0 (0, 0)	2 (2, 3)*
Combination (1 : 1)	8 : 8	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)
Combination (3 : 1)	18 : 6	0 (0, 0)	0 (0, 0)	0 (0, 0)	0 (0, 0)

[a]Values are median (25th, 75th percentile); *$P < 0.01$ versus saline group (Kruskal–Wallis's analysis of variance by ranks followed by Dunns's test for between-group analysis).

model and determines a spinal nociceptive reflex (Danneman 1997). Noxious stimuli (high-intensity light beam) cause direct activation of the peripheral nociceptive afferent terminal and release of amino acids (glutamate and aspartate) from both small and large fibres. Therefore, the non-NMDA receptors, especially AMPA receptors, are activated to mediate the phasic nociceptive responses. However, because the thermal noxious stimulus is not prolonged or repeated, post-synaptic NMDA receptors are not activated and the channels are not opened. The early phase of the formalin test appears to reflect the nocifensive response to the direct chemical stimulation. The pre- and post-synaptic mechanisms are similar to those of the tail-flick test (Dickenson 1994, 1997b; Kohrs & Durieux 1998).

Phase 2 of the formalin test has been widely established as one of persistent pain models (Danneman 1997; Dickenson 1997b). In this phase, formalin induces the release of excitatory amino acids and neuropeptides (SP, neurokinin A and CGRP) (Skilling et al 1988; Dickenson 1994, 1997b), which may extend depolarization that causes removal of the voltage-dependent Mg^{2+} blockade, then activates NMDA receptors and opens the ion channels, leading to an increase of intracellular Ca^{2+} concentration. Further, the influx of Ca^{2+} activates protein kinase C- and nitric oxide-mediated positive feedback loops that make NMDA receptors progressively sensitized and greatly enhances the level of excitability of the neurons, which then leads to prominent and long-lasting wind-up, central sensitization and spontaneous discharge (Dickenson 1994, 1997b; Mao 1999; Mayer et al 1999; McNally 1999). Additionally, the enhancement of intracellular Ca^{2+} initiates production of the NO-activated poly (ADP ribose) synthetase that causes the loss of function of spinal cord inhibitory interneurons (Mayer et al 1999). Thus, both NMDA receptor-mediated central sensitization and possible disinhibition may contribute to central hyperalgesia in phase 2 of the formalin test.

Tramadol only displays a modest affinity for μ opioid receptors and weaker affinity for δ and κ opioid receptors (Lee et al 1993). As an agonist, systemic tramadol inhibits the release of peptides (e.g. SP and CGRP) from spinal terminals of primary afferent nociceptors (McNally 1999). These direct pre-synaptic actions reduce the responsiveness of post-synaptic nociceptive neurons (McNally 1999) and directly disrupt the ascending transmission of nociceptive information from the spinal cord. Supraspinal activation of the monoaminergic inhibitory neuronal system has also been considered as an important source of tramadol's antinociception (Lee et al 1993). Because of its dual mechanisms of action, tramadol showed antinociception not only in the tail-flick test, but also in phase 1 of the formalin test. Because the peripheral stimulation continues in phase 2 of the formalin test, wind-up, peripheral and central sensitization may subtract the inhibitory activity of opioids, which leads to reduced opioid sensitivity (Dickenson 1997a, b). In the present study, the ED50 of tramadol in phase 2 was significantly greater than that in phase 1, possibly indicating that the effects of tramadol were reduced by NMDA receptor-mediated hyperalgesia.

Several studies have confirmed that ketamine, as an antagonist of NMDA receptors, can produce pronounced antinociceptive effects, particularly in persistent pain models (Millan & Seguin 1994; Chaplan et al 1997; Davidson & Carlton 1998). In clinical studies, ketamine was also effective for the management of post-operative pain after major abdominal surgery, post-herpetic neuralgia and chronic neuropathic pain (Bhattacharya et al 1994; Eide et al 1995; Felsby et al 1995). In the present study, it was found that ketamine produced antinociception in phase 2 of the formalin test, but not in the tail-flick test or phase 1 of the formalin test. Thus, our results are consistent with most of the recent research findings, although some studies reported antinociceptive effects in acute pain tests as well (Baumeister & Advokat 1991; Crisp et al 1991). This discrepancy may have resulted from the different routes of drug administration or differences in the administered doses.

When these two drugs were combined, the antinociceptive synergy was observed in phase 2 of the formalin test. Generally, there are two acceptable explanations for synergistic effects. Synergy can occur when two drugs with distinct mechanisms are concurrently administered. The common effects of several receptors on interacting systems could thus provide a premise for synergistic interactions. At separate anatomic sites (pre- and post-), different antinociceptive effects that may act independently and also combine to inhibit spinal nociceptive processing may result in synergy (Yaksh & Malmberg 1994; Przesmycki et al 1997). Tramadol acts pre-synaptically on primary afferents and reduces the release of neurotransmitters or inhibits interneurons early in nociceptive pathways. Ketamine blocks the post-synaptic NMDA receptors and inhibits inflammation- or tissue-damage-induced central hyperalgesia that may be less sensitive to opioids (Dickenson & Sullivan 1993). Considering the activation of descending inhibitory pathways of tramadol, co-administration of tramadol with ketamine improves analgesia by targeting different neuron systems.

Another proposed mechanism of synergy involves pharmacodynamic interactions between the receptors affected by the two drugs (Yaksh & Malmberg 1994). In the spinal cord dorsal horn, the distribution of opioid receptors and NMDA receptors is very close, suggesting an intimate functional relationship between these two classes of receptors (Mao 1999). There is more evidence suggesting that NMDA receptor binding can be affected by opioids and vice versa (Dumont & Lemaire 1994; Bhargava & Kumar 1997). Thus, it is possible that synergistic antinociception between tramadol and ketamine is derived from the their mutual effects on both receptors.

At present, the most frequent side-effects of ketamine are psychotomimetic reactions (e.g. hallucinations, bad dreams) and altered short-term memory, which are normally induced by high-dose ketamine anaesthesia (Kohrs & Durieux 1998; Schmid et al 1999). Tramadol also causes some adverse effects, including dizziness or vertigo, nausea, vomiting, dry mouth and headache (Lewis & Han 1997). Those side-effects could affect the long-term application of ketamine and tramadol for chronic and refractory pain.

In the present study, however, combination of ketamine and tramadol induced not only synergistic antinociceptive effect, but also decreased side-effects by lowering the dose of each drug. Thus, it is reasonable to postulate that this type of combination should be effective for some clinically painful states, particularly for conditions that are refractory to the conventional treatments. The study of the combinational effects of tramadol and other NMDA receptor antagonists within specific neuropathic pain models is warranted.

Conclusions

To our knowledge, this study is the first report of the use of an isobolographic method to determine the interactive analgesic effects of intraperitoneal tramadol and ketamine in the tail-flick and formalin tests in mice. The combination was significantly more potent than predicted by adding the relative contributions of each drug in phase 2 of the formalin test, but only additive in phase 1 of the formalin test and in the tail-flick test. Moreover, the synergistic combinations displayed less CNS depression than when an equi-potent dose of ketamine was administered alone. Synergistic interactions between tramadol and ketamine were shown to be dose-, ratio- and model-dependent. The combinations may be useful in the management of chronic or refractory pain, when pain cannot be controlled by conventional treatments, such as opioids, NSAIDs and adjuvant analgesics, and/or as a result of their side-effects.

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