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Tramadol and caffeine produce synergistic interactions on antinociception measured in a formalin model

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ABSTRACT

Drug combinations have been used in clinical practice for the main purpose of increasing therapeutic effect efficacy. The aim of this study was to determine the antinociceptive effect of tramadol and caffeine administered separately or in combination, as well as their synergistic interaction. The formalin test was used. Nociceptive behavior was evaluated by flinching response of the formalin-treated paw. Rats were divided into five groups and received tramadol alone (4.9–49.6 mg/kg, s.c.), caffeine alone (1–17.8 mg/kg, p.o.), or combinations of tramadol (4.9, 8.8, 15.6 and 20.8 mg/kg, s.c.) and caffeine (1, 3.16 and 10 mg/kg, p.o.). Tramadol showed dose-dependent antinociceptive effect in both phases of the formalin test. Caffeine only presented antinociceptive effect in the second phase and this effect was also dose-dependent. In Phase 1, combinations of tramadol and caffeine showed antinociceptive effect similar to that of tramadol alone. In Phase 2, the dose–response curve shifted to the left with the combination of tramadol and each dose of caffeine. Synergism analysis resulted in synergistic effect in ten combinations and antagonism in two combinations. In conclusion, the synergism observed in the majority of tramadol and caffeine combinations used in this study suggests that this drug combination is useful in the treatment of pain.

1. Introduction

Tramadol is a synthetic opioid analgesic widely used in the treatment of moderate to moderately severe pain (Lewis and Han, 1997). It exhibits good analgesic efficacy and is comparable to morphine in the treatment of postoperative pain (Lewis and Han, 1997). With regard to other types of pain it is less effective than morphine and its potency is similar to that of codeine (Miranda and Pinardi, 1998). The adverse effect profile of tramadol is different from other opioids (Budd and Langford, 1999). All effects of tramadol are due to its complex action mechanism. It has a weak affinity for μ-opioid receptors (Raffa et al., 1992) and inhibits neuronal reuptake of serotonin and norepinephrine (Oliva et al., 2002).

The administration of combinations of analgesics has demonstrated that subeffective doses are able to improve therapeutic effects and at the same time minimize adverse effects. Many analgesic combinations have been studied, such as morphine + metamizole (Hernandez-Delgadillo et al., 2002), ketorolac + tramadol (Lopez-Muñoz et al., 2004) and paracetamol + aspirin (Diener et al., 2005). On the other hand, combinations of analgesics with adjuvants have been analyzed. Clinically, caffeine has been used as an adjuvant for many years, both to eliminate sleepiness induced by antihistamines and to provide better analgesic efficacy.

Combinations of non-steroidal anti-inflammatory drugs (NSAIDS) with caffeine have been analyzed in different animal models; caffeine with ibuprofen, aspirin, ketorolac in pain-induced functional impairment in the rat (PIFIR model) (Medina et al., 2006; López-Muñoz et al., 1996; Castañeda-Hernández et al., 1994), paracetamol + caffeine in hot plate (Engelhardt et al., 1997) and aspirin + caffeine in carrageenan-induced edema in the hind paw test (Vinegar et al., 1976). Misra et al. (1985) reported that the antinociceptive effect of morphine was potentiated by caffeine. Morphine is the only opioid analgesic that has been studied in combination with caffeine.

The mechanisms that participate in the potentiation of antinociceptive effects by caffeine are still not fully understood. Plasmatic level analyses of certain analgesics or caffeine have discarded the possibility that pharmacokinetic mechanisms participate in this potentiation (Engelhardt et al., 1997; Gayawali et al., 1991; Castañeda-Hernández et al., 1994). Synergism can be explained through pharmacodynamic mechanisms.

The aim of this study was to determine the antinociceptive effect of tramadol and caffeine when administered separately or in combination, as well as their synergistic interaction.

2. Materials and methods

2.1. Animals

Male Wistar rats weighing 180–200 g were used in this study. All animals were housed at 25 °C with a 12-h light/dark cycle. Food was

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withheld 12 h before the experiments with water provided ad libitum. Each rat was used only once and was euthanized at the end of the test by cervical dislocation. All experimental procedures were carried out according to protocol approved by the local Animal Ethics Committee and procedures stipulated in the Guidelines on Ethical Standards for Investigations of Experimental Pain in Animals (Zimmermann, 1983) were followed. The number of experimental animals was kept at a minimum.

2.2. Drugs

The following drugs, dissolved in NaCl 0.9% as the vehicle, were used: Tramadol hydrochloride (100 mg/2 ml ampules, Tradol®) was obtained from Laboratories Grünenthal, Mexico, and caffeine was obtained from Sigma Aldrich Co. (St. Louis, MO, USA).

2.3. Formalin test

Antinociception was assessed by the formalin test (Wheeler-Aceto and Cowan, 1991). Rats were placed in a Plexiglas observation chamber for 30 min to allow them to adapt to the environment. They were then removed and subcutaneously injected with 50 μL of diluted formalin (2%). The formalin was administered into the dorsal surface of the right hind paw. Animals were returned to the chambers and nociceptive behavior was observed immediately after formalin injection. Mirrors were placed at the back of the chamber to allow complete view of the injected paw. Nociceptive behavior was quantified as the number of flinches per minute, every 5 min up to 60 min after injection. Time-courses were constructed for the antinociceptive response to individual drugs by graphing the mean number of flinches as a time function.

2.4. Study design

Tramadol and caffeine were administered either individually or in combination by group. First, corresponding dose–effect curves were obtained with different doses of tramadol (4.9, 8.8, 15.6, 20.8, 27.8 and 49.6 mg/kg) and caffeine (1, 3.16, 10 and 17.8 mg/kg) administered separately. Second, different combinations of doses of tramadol (4.9, 8.8, 15.6 and 20.8 mg/kg) with three doses of caffeine (1, 3.16 and 10 mg/kg) were administered to analyze possible synergistic interactions. All control groups received saline solution, alone, to assess the magnitude of nociception produced by formalin 2%. Doses were selected on the basis of previous pilot studies under the authors' experimental conditions (data not shown). Tramadol and caffeine were administered fifteen minutes before formalin injection based on pharmacokinetic studies in which these drugs are found in plasma at 15 min (Liu et al., 2003; Sawynok and Yaksh, 1993). Each dose was administered to 6 animals resulting in a total of 22 doses for the entire study. All experiments were carried out by the same researcher who prepared all solutions and observed all animal behavior.

2.5. Data presentation and statistical analysis

The number of flinches per minute was the nociceptive response scored every 5 min up to 60 min after injection. Drug time courses (TCs) were constructed by graphing the mean number of flinches ± SEM as a time function. The cumulative nociceptive effect was analyzed and determined as area under the curve (AUC) of the TC. AUC was obtained by the trapezoidal rule (Shargel et al., 2005). Per cent of maximum possible effect (%MPE) was regarded as the antinociceptive effect percentage and was calculated from the AUC obtained from the drug group (AUC_D) and from the control group (AUC_C) with the following formula:

$$\%MPE = [(AUC_C - AUC_D) / AUC_C] \times 100.$$

The antinociceptive effect percentage corresponding to the first phase of the assay was determined from 0 to 10 min with regard to formalin administration and it was calculated from 15 to 60 min in the second phase. Percent of maximum possible effect was calculated for individual tramadol and caffeine administration in both phases, whereas it was only calculated for the combination of tramadol and caffeine in Phase 2 due to the fact that the combined effect was not different from that of tramadol, alone (see Results).

Synergism was determined based on the Combination Index method (CI) (Chou, 2006) calculated with the following formula:

$$CI = [(D)_{TRAM} / (Dx)_{TRAM} + (D)_{CAF} / (Dx)_{CAF}],$$

D_{TRAM} and D_{CAF} are the doses that, when combined, produce a % X of the antinociceptive effect.

$(Dx)_{TRAM}$ and $(Dx)_{CAF}$ are the theoretical drug doses that, when administered separately, would produce the same effect percentage as the experimental drug combination.

This method predicts synergistic interaction when $CI<1$, additive interaction when $CI=1$ and antagonistic interaction when $CI>1$ (Chou, 2010).

Due to the fact that this study design was made with non-constant dose rations, a normalized isobologram was constructed after calculating CI for each of the drugs and was related to the DE_{50} for each of the separately administered drugs. This was graphed on the X $[D_{TRAM}/(DE_{50})_{TRAM}]$ and Y $[D_{CAF}/(DE_{50})_{CAF}]$ axes. The oblique line indicates the theoretical doses that produce an additive effect. If the points obtained from the experimental drug combinations were below the line, the interaction was considered to be synergistic and if they were above the line it was considered to be antagonistic (Chou and Talalay, 1984; Chou, 2008).

All data in the text and figures were expressed as means ± S.E.M for six experimental observations. The antinociceptive effect of each group was measured and the Student *t* test was used for between-group comparisons. An analysis of variance (ANOVA) was used for multiple comparisons, followed by the post-hoc Tukey test. Statistical significance was defined at $P<0.05$.

3. Results

3.1. Antinociceptive effects of tramadol and caffeine

The antinociceptive effect of tramadol was analyzed using the inflammatory pain test in which hind paw flinching, shaking, biting or licking can be observed after formalin administration. In the present study only hind paw flinching was scored due to the fact that biting and licking were not present during the entire observation period, probably because formalin was administered at 2%. The injection of formalin (2%) into the hind paw produced the two characteristic phases. The first phase began immediately after formalin administration and finished approximately 10 min later. The second phase started after 15 min and lasted for 60 min (Fig. 1). Fig. 1 shows the TCs of nociceptive behaviors obtained in animals injected with tramadol and caffeine (20.8 mg/kg and 17.8 mg/kg, respectively). Tramadol reduced the number of flinches per minute with regard to the control group in both phases, whereas caffeine only reduced nociception in Phase 2. The total antinociceptive effect (%MPE) of caffeine was not significantly different with regard to control in this phase ($P>0.05$ with Student *t* test).

Dose–effect curves (DEC) for tramadol and caffeine were constructed. In Phase 1 tramadol presented a dose-dependent effect and its maximum effect was 75.68 ± 3.63% when the animals received 27.8 mg/kg. The DE_{50} value in this phase of the formalin test was 20.137 mg/kg (Fig. 2A). In Phase 1, caffeine did not produce antinociceptive effect with the doses administered. Tramadol and caffeine DEC obtained in Phase 2 (Fig. 2B) showed that the effect of tramadol was also dose-dependent and it achieved its maximum effect with 49.6 mg/kg (97.54 ± 0.73%).The DE_{50}

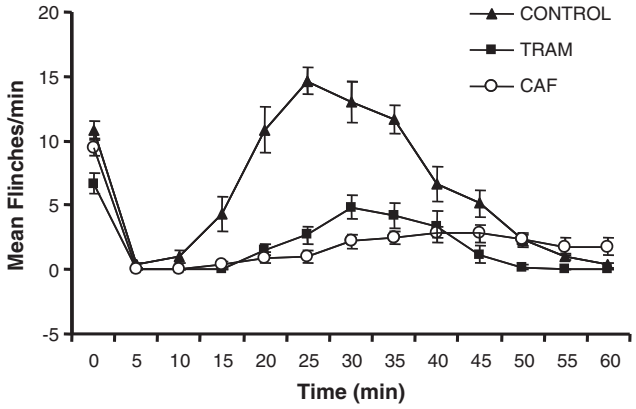

Fig. 1. Time-course of antinociceptive effects of systemic administration of tramadol (TRAM, 20.8 mg/kg, subcutaneous) and caffeine (CAF, 17.8 mg/kg, oral) in phases one and two of 2% formalin test. Control group was treated with vehicle. Data are expressed as mean ± SEM of six rats.

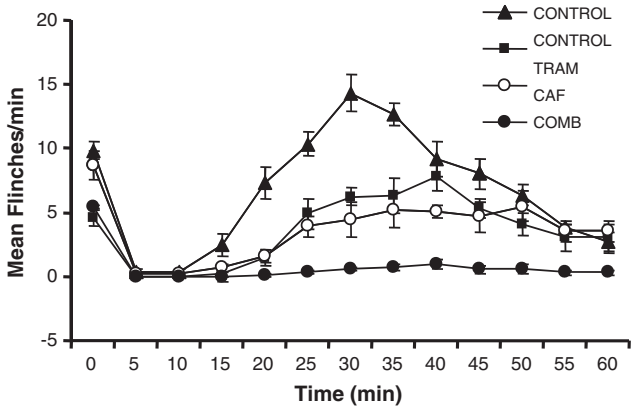

Fig. 3. Time-course of antinociceptive effects of systemic administration of tramadol (TRAM, 15.6 mg/kg, subcutaneous), caffeine (CAF, 3.16 mg/kg, oral) and the combination of them at the same doses, in phases one and two of 2% formalin test. Control group was treated with vehicle. Data are expressed as mean ± SEM of six rats.

value in this phase of the formalin test was 19.226 mg/kg. Likewise, the effect of caffeine was dose-dependent at the doses tested. The maximum effect observed for caffeine was 63.61 ± 8.21% with a dose of 17.8 mg/kg. The DE_{50} value in this phase of the formalin test was 8.328 mg/kg. None of the assayed treatments produced significant motor activity alteration as was demonstrated by the animals' normal mobility inside the observation chamber.

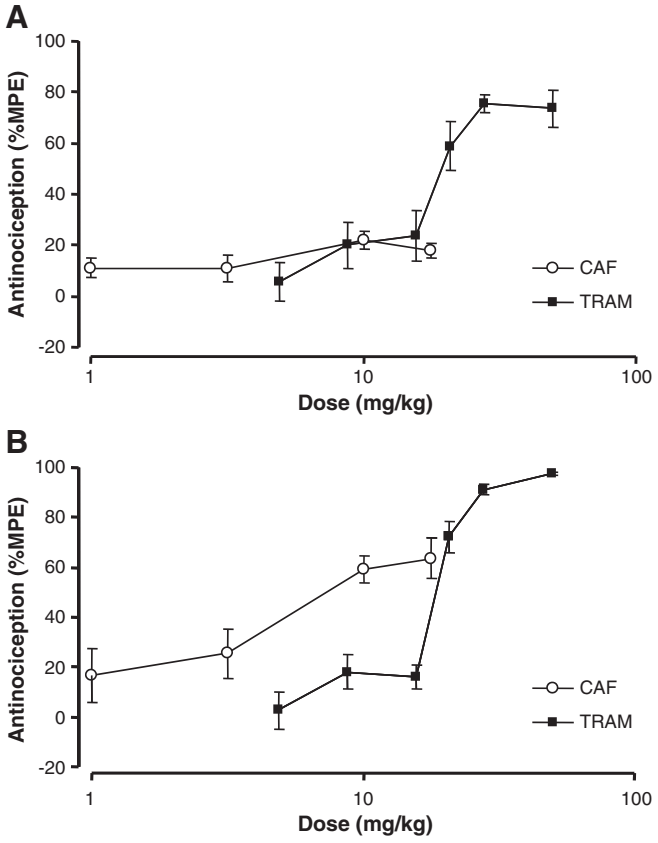

Fig. 2. Dose–effect curves of antinociceptive effects of subcutaneous tramadol (TRAM) and oral caffeine (CAF) in the 2% formalin test. Doses of tramadol were 4.9, 8.8, 15.6, 20.8, 27.8 and 49.6 mg/kg. Doses of caffeine were 1, 3.16, 10 and 17.8 mg/kg. Antinociception was expressed as per cent of maximum possible effect (%MPE) in the first phase (A) and in the second phase (B). Each point corresponds to the mean ± SEM of six rats.

3.2. Interaction between tramadol and caffeine in the formalin test

Synergistic interaction between tramadol and caffeine was carried out on different animal groups. Tramadol was administered at doses of 4.9, 8.8, 15.6 and 20.8 mg/kg in combination with 1, 3.16 or 10 mg/kg of caffeine and the TCs were constructed from the results. In Phase 1 the drug combination did not present a significantly different antinociceptive effect from that of tramadol alone. Fig. 3 shows the TC of one of the combinations (TRAM 15.6 mg/kg + CAF 3.16 mg/kg). The combination TC is clearly seen to be equal to that of TRAM. Similar results were obtained with all combinations therefore synergism was analyzed only in Phase 2.

DECs obtained in Phase 2 of the formalin test are shown in Fig. 4. When drug combination DECs were compared with tramadol DEC, the former shifted to the left with different caffeine doses, showing that tramadol + caffeine combinations produced a greater antinociceptive effect than tramadol alone. Table 1 shows the DE_{50} for the separately administered drugs, and tramadol DE_{50} in the drug combinations is reduced, reflecting an increase in potency.

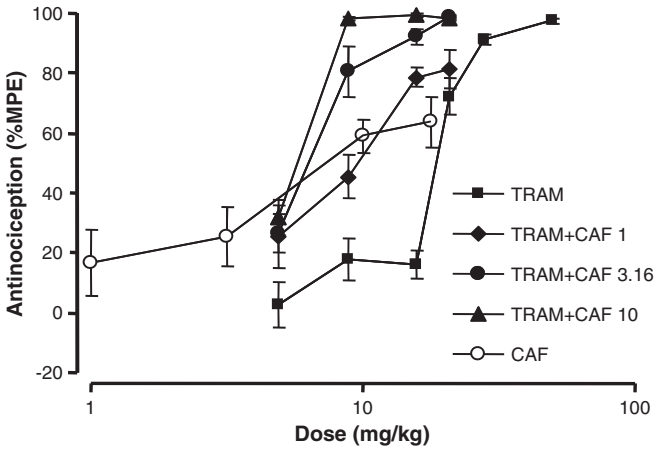

Fig. 4. Dose–effect curves of antinociceptive effects produced by tramadol alone (TRAM), caffeine alone (CAF) and combinations of tramadol with different doses of caffeine (TRAM + CAF). Doses of tramadol were 4.9, 8.8, 15.6, 20.8, 27.8 and 49.6 mg/kg. Doses of caffeine were 1, 3.16, 10 and 17.8 mg/kg. Tramadol was administered at doses of 4.9, 8.8, 15.6 and 20.8 mg/kg in combination with 1, 3.16 or 10 mg/kg of caffeine. Antinociception was expressed as percent of maximum possible effect (%MPE) in the second phase. Each point corresponds to the mean ± SEM of six rats.

Table 1
Interaction between TRAM and CAF in the formalin test, analyzed with the combination index method.

TRAM (mg/kg)	CAF (mg/kg)	%MPE	ED_{50} (mg/kg)	CI	Type of interaction
4.9		2.60 ± 7.6			
8.8		17.97 ± 6.9			
15.6		16.06 ± 4.6			
20.8		72.14 ± 6.2			
27.8		91.30 ± 1.9			
49.6		97.50 ± 0.7	19.226		
	1	16.71 ± 10.9			
	3.16	25.35 ± 9.8			
	10	59.18 ± 5.6			
	17.8	63.61 ± 8.2	8.328		
4.9	1	25.38 ± 10.4		0.746	++[b]
8.8	1	45.51 ± 7.3		0.619	+++[c]
15.6	1	78.72 ± 3.3		0.696	+++
20.8	1	81.28 ± 6.2	8.686	0.895	+[a]
4.9	3.16	26.67 ± 6.6		1.601	−−−[e]
8.8	3.16	80.65 ± 8.4		0.439	+++
15.6	3.16	92.28 ± 2.7		0.585	+++
20.8	3.16	98.88 ± 0.4	6.435	0.568	+++
4.9	10	31.79 ± 5.6		3.337	−−−−[f]
8.8	10	98.30 ± 0.7		0.263	++++[d]
15.6	10	99.30 ± 0.4		0.399	+++
20.8	10	98.30 ± 0.8	5.373	0.611	+++

Chou and Talalay (1984) described the type of interaction in relation to the ranges of CI.
[a] Slight synergism.
[b] Moderate synergism.
[c] Synergism.
[d] Strong synergism.
[e] Antagonism.
[f] Strong antagonism.

Carrying out the synergism analysis according to the Combination Index method (Chou, 2006) for combinations related to non-constant doses, a normalized isobologram was constructed and is shown in Fig. 5. The oblique line was graphed in relation to the DE_{50} of each of the separately administered drugs and represents the theoretical doses that present additive effect. Because the experiments were carried out with non-constant dose fractions only the doses used could be represented in the isobologram. In the graph, ten of these doses were under the line, indicating a synergistic interaction between tramadol and caffeine. Two other doses were above the line, indicating antagonistic interaction. One dose could not be represented because of the size of the axes. However the datum indicating antagonism is shown in Table 1. Both isobologram and CI were used for determining synergistic, additive or antagonistic interaction. In the present study 10 of the 12 combinations resulted in a CI < 1, indicating synergism. Data are summarized in Table 1. As part of this method, the level of synergism that the combination of 2 drugs presented in relation to the CI value was classified. The lowest CI value represented the highest synergism level. Table 1 shows the level of synergism each of the combinations presented. The combination of 8.8 mg/kg of TRAM + 10 mg/kg of CAF with a CI = 0.26 had a stronger synergism while the combination of 4.9 mg/kg of TRAM + 10 mg/kg of CAF with a CI = 3.34 had a stronger antagonism.

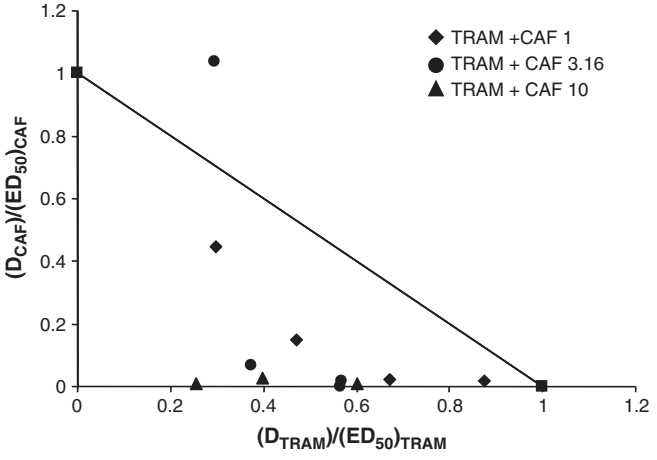

Fig. 5. Normalized ED_{50}-isobologram of non-constant dose rations showing interaction between tramadol (TRAM) and caffeine (CAF). The oblique line between the X and Y axes indicates the theoretical doses that produce an additive effect. The points below the line represented synergistic interactions. The point above the line was considered to be antagonistic.

Another evaluated parameter was the Dose Reduction Index (DRI) which indicates the number of times the dose of each drug is reduced in a synergistic combination. The combination that presented a stronger synergism (8.8 mg/kg of TRAM + 10 mg/kg of CAF) had an antinociceptive effect of 98.3% (%MPE). Theoretically, when administered separately, TRAM dose would have to be 34.54 mg/kg to present a 98.3% antinociceptive effect while CAF dose would have to be 1185.3 mg/kg to reach the same percentage effect. The drug doses that, when combined, presented that same effect level, were much lower. DRI for TRAM was 3.93; that is to say, the dose was reduced to nearly 4 times less. DRI for CAF was 118.53. DRI values for all synergistic combinations are shown in Table 2.

4. Discussion

In the present study, synergism of tramadol + caffeine was studied because tramadol is a widely used analgesic in clinical practice due to both its efficacy in relieving moderately severe pain and its lack of adverse effects in relation to other opioids, such as morphine. However, in chronic treatment at therapeutic doses, tramadol may present adverse effects. Experimentally, the racemic mixture of tramadol has been administered to rodents and the dose of 150 mg/kg p.o. has been reported to produce motor dysfunction in 20% of the animals studied (Raffa et al., 1993). In the present study the highest dose administered was 49.6 mg/kg. Animal mobility was normal at this dose as well as at all the combined doses. In this study subeffective doses were used in order to analyze whether or not caffeine was able to improve the effect of tramadol. Tramadol was administered subcutaneously based on pharmacokinetic studies carried out on humans. Oral tramadol undergoes extensive first-pass metabolism (Lewis and Han, 1997), reaching a 68–75% bioavailability with a single dose. However its bioavailability is 80–100% with intramuscular dose (Dayer et al, 1997). On the other hand, oral administration is the most common way to give caffeine and thus was administered orally. Both drugs were administered 15 min before formalin, based on pharmacokinetic studies for tramadol that reported efficacious plasmatic concentrations of tramadol as well as the M1 active metabolite after 15 min of oral administration (10 mg/kg) in rats (Liu et al., 2003). Plasmatic concentrations have also been found

Table 2
Dose Reduction Index calculated to TRAM and CAF in the synergistic interaction.

%MPE	Experimental doses (mg/kg)		Calculated doses (mg/kg)		DRI TRAM	DRI CAF
	TRAM	CAF	TRAM	CAF		
25.38 ± 10.4	4.9	1	16.5	2.2	3.36	2.23
45.51 ± 7.3	8.8	1	18.7	6.7	2.13	6.68
78.72 ± 3.3	15.6	1	23.2	41.2	1.49	41.19
81.28 ± 6.2	20.8	1	23.8	50.1	1.14	50.09
80.65 ± 8.4	8.8	3.16	23.6	47.7	2.68	15.08
92.28 ± 2.7	15.6	3.16	27.5	172.7	1.76	54.64
98.88 ± 0.5	20.8	3.16	36.7	1988.0	1.77	629.12
98.30 ± 0.7	8.8	10	34.5	1185.3	3.93	118.53
99.30 ± 0.4	15.6	10	39.3	3549.0	2.52	354.90
98.30 ± 0.8	20.8	10	34.5	1185.3	1.66	118.53

15 min after oral administration in humans (Roux and Coetze, 2000). Regarding efficacy, Oliva et al. (2002) administered tramadol 15 min before formalin in mice and observed antinociceptive effect. Chen et al. (2002) found that adequate time for tramadol administration to mice treated with formalin at 5% was 20 min before. In regard to caffeine it has been reported that oral doses are completely absorbed in humans as well as in animals. In humans the effects present in very short periods of time (5 min) while plasmatic concentrations are observed in times ranging from 15 to 120 min, with plasmatic peaks at 30–60 min (Sawynok and Yaksh, 1993). In the authors' laboratory caffeine was administered 15 and 30 min before formalin and there was no statistically significant difference in the antinociceptive effect (data not shown).

It has been reported in the formalin test that opioid analgesics show their effect in two phases (Ortiz and Castañeda-Hernandez, 2008). The results of the present study concur with those reports. Tramadol was observed to have a dose-dependent effect in both phases. Efficacy was greater in the second phase because maximum percentage of antinociceptive effect was 97.54 ± 0.73%, whereas in the first phase the highest antinociceptive effect was 75.68 ± 3.63%. This opioid analgesic has been used in humans for mild to moderately severe pain management. However, some cases of intraoperative pain require high doses, increasing the risk of respiratory depression (Radbruch et al., 1996).

On the other hand, in some experimental tests such as the PIFIR model (Medina et al., 2006; Díaz-Reval et al., 2001) and Randall Selitto (Fernández-Dueñas et al., 2008), caffeine has not shown an antinociceptive effect. Nevertheless in neuropathic pain (Wu et al., 2006), hot plate (Malec and Michalska, 1988) and tail immersion (Person et al., 1985) models it has presented an antinociceptive effect. Caffeine has only been effective in relieving headache in humans. In the formalin model, Sawynok and Reid (1996) reported that caffeine only showed antinociceptive effect in the second phase at doses of 12.5–50 mg/kg. In the present study, caffeine did not show antinociceptive effect in the first phase, concurring with results of the researchers just mentioned. However, in the second phase, caffeine showed a dose-dependent effect at doses of 1–17.8 mg/kg. These doses are lower than those used by Sawynok and Reid (1996) but in the present study formalin was administered at a lower concentration (2%).

When combinations of tramadol + caffeine were administered, the effect of tramadol was not significantly modified by co-administration of caffeine in the first phase. In other words, caffeine was not able to improve the antinociceptive effect of tramadol. Because of these results no synergism analysis was able to be made. In some experimental models such as PIFIR (Medina, et al, 2006), caffeine is unable to show antinociceptive effect. However, when it is co-administered with NSAIDS, the effect of these analgesics improves significantly. In clinical practice, the effect of analgesics is different in each patient because the stimulus causing the pain is not the same, resulting in different pain mechanisms. Concerning the formalin test, it has been reported that response in the first phase corresponds to a pronounced increase in neural activity lasting a short time, while in the second phase C fiber activation gives rise to spinal release of excitatory amino acids as well as several peptides such as substance P (Malmberg and Yaksh, 1992). Most likely caffeine did not present antinociceptive effect or improve the effect of tramadol in Phase 1 due to the different mechanisms involved in the two phases.

To analyze synergism in Phase 2, tramadol was administered at doses of 4.9, 8.8 and 15. 6 mg/kg and did not produce an effect above 20%. Only one dose presented an effect of 72.14 ± 6.19% (20.8 mg/kg). Caffeine was used at doses of 1 and 3.16 mg/kg, which showed an effect below 30%, and at 10 mg/kg, which presented an effect of 59.18 ± 5.56%. The majority of these combinations presented good effects. Synergism analysis showed that CI<1 in ten combinations which means that they produced synergism and the other two produced antagonism because CI>1 (Chou, 2010). These effects are useful in clinical practice because the goal is to increase the efficacy of therapeutic effect. Different studies have found that caffeine potentiates the effect of various NSAIDS. However, regarding opioids, there is only one study (Misra et al., 1985) showing that caffeine potentiates the effect of morphine. The present study showed that caffeine was capable of producing synergism with an atypical opioid. This is important because this drug is used in moderately severe pain management. As part of the synergism analysis, DRI determination shows that the doses of TRAM combined with CAF can be reduced by factors of 1 to 4. The doses used in the present study cannot be extrapolated to those administered to patients. However, the analysis does give an idea as to what degree dose can be reduced and still be efficacious while at the same time possibly reducing adverse side effects that can present, especially in long-term treatments.

This study did not analyze the action mechanisms that lead to a synergistic effect. Several researchers have suggested that caffeine activates pharmacodynamic mechanisms that improve the antinociceptive effect of NSAIDS. This hypothesis is based on studies that have demonstrated that pharmacokinetic parameters of some NSAIDS are unchanged when administered together with caffeine (Castañeda-Hernandez et al., 1994; Gayawali et al., 1991; Flores-Acevedo et al., 1995). At therapeutic doses, caffeine works mainly as an antagonist of adenosine receptors (Nehlig et al., 1992). Sawynok and Reid (1996) reported that caffeine is able to activate noradrenergic and serotoninergic systems in antinociception evaluated in a formalin model.

On the other hand, tramadol is administered as racemate and its metabolites are (+)-O-desmethyl tramadol ((+)-M1) and (−)-O-desmethyl tramadol ((−)-M1). It has been reported that (+) enantiomers are mainly associated with μ-opioid receptor activity. (+)-M1 has greater affinity than (+)-tramadol, whereas (−) enantiomers are reuptake inhibitors of noradrenaline and (+)-tramadol is a 5-HT reuptake inhibitor (Raffa et al., 1993; Driessen and Reiman, 1992). These data show that tramadol activates endogenous analgesic mechanisms. When tramadol and caffeine are administered, each one acts by different mechanisms, but their combination can in some way interact with the serotoninergic and opioid pathways of the endogenous analgesic pathway, consequently producing an increase in antinociceptive effect. The result of this increment is a synergistic effect. In conclusion, the antinociceptive effect produced by tramadol and caffeine shows synergism when both drugs are administered in combination. These results suggest that reduced tramadol dose combined with caffeine could be useful in moderate to moderately severe pain management.

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