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Characterization of the Analgesic Effects of Paracetamol and Caffeine Combinations in the Pain-induced Functional Impairment Model in the Rat

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Abstract—The analgesic activities of paracetamol (100, 178, 316 and 562 mg kg^{-1}), caffeine (10, 18, 32 and 56 mg kg^{-1}) and combinations of these doses were tested on a pain-induced functional impairment model in the rat. Dysfunction of the right hind limb was induced by an intra-articular injection of 30% uric acid in the knee. Drugs were given orally and the recovery of functionality over time was considered as an expression of analgesia. Paracetamol alone induced a dose-dependent analgesic effect whereas caffeine alone did not show any activity at the assayed doses. Combinations of 316 mg kg^{-1} paracetamol with either 10, 18, 32 or 56 mg kg^{-1} caffeine yielded analgesic effects significantly greater than that of paracetamol alone. The highest potentiation was observed with a paracetamol–caffeine mixture of 316–32 mg kg^{-1}. Caffeine coadministration, however, did not significantly change paracetamol plasma levels. No potentiation was obtained with other combinations. Paracetamol plasma levels and analgesic effect observed with administration of 316 mg kg^{-1} paracetamol alone or 316–32 mg kg^{-1} of paracetamol–caffeine were fitted to the sigmoidal E_{max} model according to the Hill equation. The curves obtained were parallel, but that of the combination was shifted to the left. It is concluded that caffeine is able to potentiate the analgesic effect of paracetamol by a pharmacodynamic mechanism, but this only occurs at certain dose combinations.

Paracetamol is a widely used analgesic agent, but its efficacy is limited in moderate and severe pain (Insel 1990). Therefore, paracetamol has been combined with other drugs, such as caffeine, in order to increase pain relief (Seegers et al 1981; Laska et al 1983). Although oral formulations containing paracetamol and caffeine are extensively used, the rational bases for this combination are not yet clear. There is controversy on whether caffeine is able to increase the analgesic effects of non-steroidal anti-inflammatory drugs in clinical situations (Moertel et al 1974; Laska et al 1983). Conflicting results are probably due to the difficulties in accurately measuring pain in patients and to the use of different protocols (Laska et al 1984).

There are reports on potentiation by caffeine of the analgesic effects of some non-steroidal anti-inflammatory drugs in animal models (Siegers 1973; Vinegar et al 1976; Seegers et al 1981). However, it is questionable whether the noxious stimuli used in these studies are comparable to pathological pain in clinical situations (Pircio et al 1975; Wheeler-Aceto & Cowan 1991). Moreover, since behavioural responses were evaluated, false positive or negative results may occur since learning processes are involved (Winter 1965; Wood 1984).

Pardo & Rodríguez (1966) developed a method of pain-induced functional impairment (PIFI) in dogs which allowed them to quantitatively estimate the effect of analgesic agents and follow their time course. We have recently used a similar procedure, modified for rats, which allowed us to establish a mathematical relationship between paracetamol plasma concentrations and analgesic effect (Granados-Soto et al 1992). In this work, we used the PIFI procedure to investigate the potentiation by caffeine of paracetamol-induced analgesia and to examine whether the mechanism of this synergism is pharmacokinetic or pharmacodynamic.

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Materials and Methods

Animals

Female Wistar rats, 180–220 g, were used. Twelve hours before the initiation of the experiments, food was withheld, but animals had free access to drinking water.

Measurement of analgesic activity

Pain was induced and the analgesic effect of caffeine–paracetamol mixtures was measured using the PIFI procedure as described previously (Granados-Soto et al 1992). Rats received an intra-articular injection of 0·05 mL 30% uric acid suspended in mineral oil in the knee joint of the right hind limb under light anaesthesia with ether. Immediately, an electrode was adhered to each hind paw between the plantar pad. Rats were allowed to recover from anaesthesia and were then placed on a stainless steel cylinder of 30 cm diameter. The cylinder was rotated at 4 rev min^{-1}, forcing the rats to walk. The variable measured in this method was the time of contact between each of the rat's hind paws and the cylinder. When the electrode placed on the animal's paw made contact with the cylinder floor, a circuit was closed and the time that the circuit remained closed was recorded. The cylinder was rotated for 2 min periods, during which time recordings were made, allowing the rats to rest for 30 min between recording periods.

After the uric acid injection, rats developed a progressive dysfunction of the injured limb. This was recorded as a diminished time of contact between the right hind paw and the cylinder. Data are expressed as the functionality index, i.e. the time of contact of the injected limb divided by the time of contact of the control left limb, multiplied by 100. After 2 h, the functionality index was zero; the injured limb made no contact with the cylinder. At this time, rats received the analgesic agents suspended in 0·5% carboxymethyl cellulose (4 mL kg^{-1}) by gavage and recordings were carried out during the next 4 h. Recovery of the functionality index was considered as the expression of the analgesic effect.

Analysis of paracetamol in plasma

Plasma concentrations of paracetamol were determined by HPLC (Ameer et al 1981). Briefly, to 0·5 mL plasma was added 50 ng 2-acetamidophenol (internal standard) and the mixture was extracted with 5 mL ethyl acetate. The solvent was then evaporated and the residue redissolved in 0·05 mL methanol. Portions (0·01 mL) were injected into a HPLC system (model 5000, Varian, Palo Alto, CA, USA) equipped with a 150 × 3·9 mm reversed-phase column (Novapak C_{18}, Waters Assoc., Milford, MA, USA) eluted with a mixture of sodium acetate 0·05 M (pH 4·0) with acetonitrile 96·5 : 3·5 at a constant flow of 1 mL min^{-1}. The effluent from the column was recorded by UV detection at 254 nm. Retention times were 4·6 and 10·1 min for paracetamol and the internal standard, respectively.

Determination of the analgesic effect of paracetamol–caffeine mixtures

Rats injured with uric acid received paracetamol, caffeine or mixtures of paracetamol and caffeine at different doses. Twenty-five groups of six animals were used, each group received a different paracetamol, caffeine or paracetamol–caffeine dose. The assayed doses (mg kg^{-1}) were: vehicle control; paracetamol alone 100, 178, 316 and 562; caffeine alone 10, 18, 32 and 56; paracetamol–caffeine 100–10, 100–18, 100–32, 100–56, 178–10, 178–18, 178–32, 178–56, 316–10, 316–18, 316–32, 316–56, 562–10, 562–18, 562–32 and 562–56. Once the functionality index had reached zero (2 h after uric acid injection), each rat received the analgesic agent and the functionality index was measured every 30 min for 4 h. Thus, the same rat was used during the whole observation period and participated in eight determinations of nociception.

Functionality index vs time curves were constructed. The area under the curve (AUC_E) was calculated by the trapezoidal rule and was considered as an expression of the overall analgesic effect during the whole observation period. AUC_E values observed with paracetamol alone and with paracetamol–caffeine combinations were compared by the Dunnett's test (Steel & Torne 1960).

Simultaneous determination of paracetamol plasma concentration and analgesic effect

Paracetamol plasma concentration and analgesic effect were determined simultaneously in rats which received either 316–32 mg kg^{-1} of paracetamol–caffeine or 316 mg kg^{-1} of paracetamol alone. The combination was studied in a group of 104 animals which was divided into 13 subgroups of 8 rats each, whereas paracetamol alone was studied in a group of 78 rats divided into 13 subgroups of 6 animals. Functionality indices were determined at 10, 20, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 min after administration of the analgesic agent, each time corresponding to one subgroup. Thus, each animal participated in only one determination of nociception. Immediately after the functionality index determination, rats were killed by cervical dislocation, blood was extracted by cardiac puncture and plasma was obtained. Plasma samples were frozen in liquid nitrogen and stored at −20°C until assayed for paracetamol by HPLC.

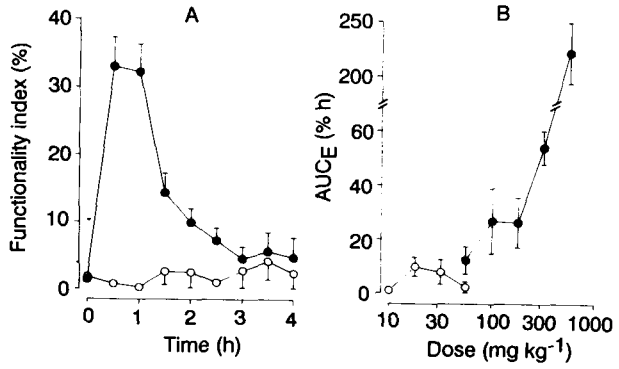

FIG. 1. A. Time course of functionality index recovery in rats submitted to pain-induced functional impairment by intra-articular injection of 30% uric acid in the right hind limb which received 316 mg kg^{-1} paracetamol (●) or 32 mg kg^{-1} caffeine (○). B. Overall analgesic effect in 4 h, expressed as the area under the functionality index vs time curve (AUC_E), of several oral doses of paracetamol (●) or caffeine (○). Data are presented as mean ± s.e.m. of six determinations.

Paracetamol plasma concentration vs time curves were constructed and peak plasma levels (C_{max}) and time to reach the peak (t_{max}) were determined directly from these plots. The area under the plasma level vs time curve (AUC) was

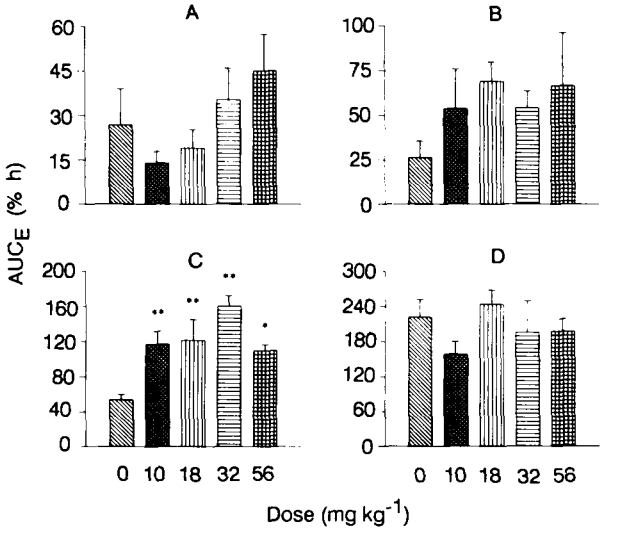

FIG. 2. Overall analgesic effect in 4 h, expressed as the area under the functionality index vs time curve (AUC_E), of combinations of either 100 (A), 178 (B), 316 (C) or 562 (D) mg kg^{-1} paracetamol with several caffeine doses. Animals were submitted to pain-induced functional impairment by intra-articular injection of 30% uric acid in the right hind knee. Data are expressed as mean ± s.e.m. of six determinations. Significant differences from paracetamol alone are denoted by: $*P < 0.05$, $**P < 0.01$, Dunnett's test.

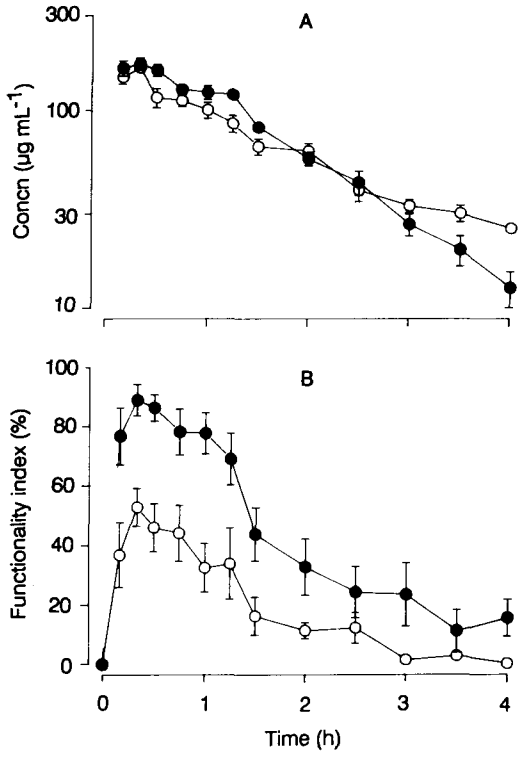

FIG. 3. Time course of paracetamol plasma concentrations (A) and analgesic effect, measured as functionality index recovery (B), in rats submitted to pain-induced functional impairment by intra-articular injection of 30% uric acid in the right hind knee. Animals received 316 mg kg^{-1} paracetamol alone, n = 6 (○), or 316–32 mg kg^{-1} paracetamol–caffeine, n = 8 (●). Data are expressed as mean ± s.e.m.

estimated by the trapezoidal rule. Functionality index vs time plots were also constructed and the maximal observed effect (E_{max}^{obs}), the time to reach the maximal observed effect (t_{Emax}) and the area under the effect vs time curve (AUC_E) were determined. Values of these parameters observed with paracetamol alone and with paracetamol–caffeine were compared by the Student's *t*-test.

Paracetamol plasma concentrations and functionality indices at given times were related using the sigmoidal E_{max} model as described previously (Granados-Soto et al 1992). Fitting was performed by nonlinear regression using the PCNONLIN program according to the Hill equation (Holford & Sheiner 1981):

$$E = \frac{E_{max} \cdot C_p^h}{EC50^h + C_p^h}$$

where E is the observed effect, E_{max} is the maximal effect that can be attained, C_p is the plasma concentration, EC50 is the plasma concentration that induced an effect equivalent to 50% of the maximal effect and h is the Hill coefficient. Hill coefficient and EC50 values obtained by nonlinear regression were compared as described by Tallarida & Murray (1981).

Results

Analgesic effects of paracetamol–caffeine mixtures

Oral administration of paracetamol alone resulted in an increase of functionality index, which reached a peak and then decayed gradually. Caffeine alone did not produce any significant modification in functionality (Fig. 1A). The overall analgesic response during the 4 h observation period, expressed as AUC_E, indicated that paracetamol produced a dose-dependent effect whereas caffeine was inactive at the assayed doses (Fig. 1B).

Table 1. Pharmacokinetic and pharmacodynamic parameters of paracetamol after administration of 316 mg kg^{-1} paracetamol alone (n = 6) or 316–32 mg kg^{-1} paracetamol–caffeine (n = 8) to rats.

Parameter	Paracetamol	Paracetamol–caffeine
C_{max} (μg mL^{-1})	169 ± 7	171 ± 12
t_{max} (min)	20 ± 2·4	20 ± 3·0
AUC (μg h mL^{-1})	253 ± 7	275 ± 11
E_{max}^{obs} (%)	65 ± 3·4	97 ± 2·4*
t_{Emax} (min)	20 ± 2·4	25 ± 4·8
AUC_E (% h)	71·6 ± 9·6	166·3 ± 14·0*

Data are presented as mean ± s.e.m. C_{max} is the maximal plasma concentration, t_{max} is the time to reach C_{max}, AUC is the area under the plasma concentration against time curve, E_{max}^{obs} is the maximal observed analgesic effect expressed as functionality index, t_{Emax} is the time to reach E_{max}^{obs} and AUC_E is the area of the functionality index against time curve. * Statistically significantly different from paracetamol alone, $P < 0.001$, Student's *t*-test.

Fig. 2 depicts the values of AUC_E observed with the assayed paracetamol–caffeine combinations. Caffeine coadministration increased the effect of paracetamol in some, but not in all cases. Association of either 10, 18, 32 or 56 mg kg^{-1} caffeine to 316 mg kg^{-1} paracetamol produced analgesic effects significantly greater than the one observed with paracetamol alone. The highest potentiation was obtained with 316–32 mg kg^{-1} paracetamol–caffeine combination which yielded an AUC_E about 3 times greater than that obtained with 316 mg kg^{-1} paracetamol. However, with all the other assayed paracetamol–caffeine mixtures, the AUC_E values were not significantly different from those of paracetamol alone. This was interpreted as an absence of potentiation.

Relationship between paracetamol plasma levels and analgesic effect after paracetamol–caffeine administration

Since maximal potentiation of the analgesic effect of paracetamol in the PIFI model was observed with coadministration of paracetamol–caffeine 316–32 mg kg^{-1}, this combination was chosen for the simultaneous determination of paracetamol plasma levels and functionality index and compared with 316 mg kg^{-1} paracetamol alone. No comparison was carried out with caffeine alone, as this drug did not show any significant analgesic effect.

Fig. 3 depicts paracetamol plasma levels and functionality indices observed after administration of either the paracetamol–caffeine combination or paracetamol alone. Relevant pharmacokinetic and pharmacodynamic parameters are given in Table 1. Paracetamol plasma concentration vs time curves were similar after administration of either the para-aminophenol derivative alone or with caffeine. There were no significant differences in C_{max}, t_{max} nor in AUC values. Caffeine coadministration did not elevate paracetamol plasma levels, although it did increase the analgesic effect. Both E_{max}^{obs} and AUC_E values were higher with the combination than with paracetamol alone. Caffeine association did not produce any significant change in t_{Emax}.

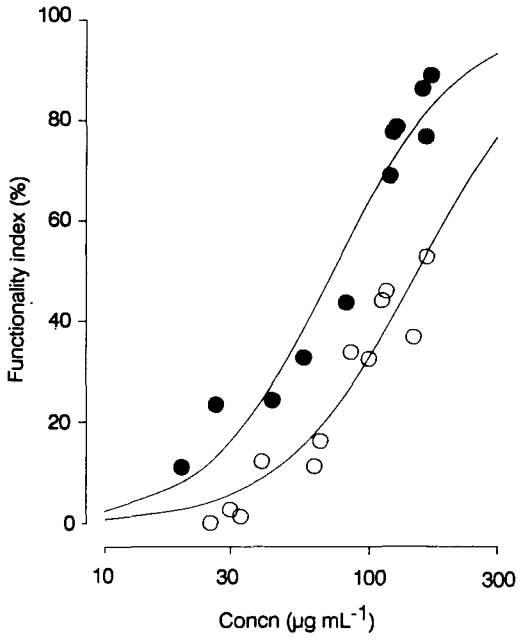

FIG. 4. Relationship between mean paracetamol plasma concentrations and analgesic effect in rats which received either 316 mg kg^{-1} paracetamol alone (○) or 316–32 mg kg^{-1} paracetamol–caffeine (●). Each point corresponds to the mean of at least six rats. Traces correspond to the curves fitted to the experimental data by the Hill equation by nonlinear regression.

Paracetamol plasma concentrations after administration of either 316–32 mg kg^{-1} paracetamol–caffeine or 316 mg kg^{-1} paracetamol alone were correlated with the analgesic effect, expressed as functionality index, by the Hill equation. The maximal effect (E_{max}) was considered as 100%, this assumes that paracetamol is able to induce a complete pain relief in this model (Granados-Soto et al 1992). Nonlinear regression of the data obtained with the combination yielded a sigmoidal curve which was in agreement with the experimental results (Fig. 4). Similarly, the data obtained with paracetamol alone also yielded a sigmoidal curve, but which was shifted to the right compared with the one of the combination.

Table 2 shows the parameters (±s.e.m.) obtained by the nonlinear regression routine of the Hill equations, corresponding to the sigmoidal curves correlating paracetamol plasma levels to functionality indices after administration of either 316 mg kg^{-1} paracetamol alone or 316–32 mg kg^{-1} paracetamol–caffeine. There was no significant difference between the Hill coefficients; therefore both curves can be considered as parallel. However, the EC50 value obtained with the combination was significantly lower than that of paracetamol alone, indicating that caffeine increased the analgesic potency of paracetamol plasma concentrations.

Table 2. Parameters of the Hill equations, obtained by nonlinear regression, relating paracetamol plasma concentration and analgesic effect after administration of 316 mg kg^{-1} paracetamol alone or 316–32 mg kg^{-1} paracetamol–caffeine.

Analgesic agent	E_{max} (%)	h	EC50 (μg mL^{-1})
Paracetamol	100	1·74±0·28	152·1±12·33
Paracetamol–caffeine	100	1·85±0·23	73·5±5·27*

Data are presented as the parameter value±s.e.m. yielded by the nonlinear regression routine. E_{max} is the maximal analgesic effect that can be attained expressed in functionality index, h is the Hill coefficient and EC50 is the plasma concentration which induces an effect equivalent to 50% of E_{max}. * Significantly different from paracetamol alone, $P<0·001$, Student's t-test.

Discussion

The purpose of the present work was to determine whether caffeine is able to potentiate the analgesic effect of paracetamol in the pain-induced functional impairment (PIFI) model, an experimental procedure in which occurrence of both pain and analgesia, is similar to clinical situations (Pardo & Rodríguez 1966; Granados-Soto et al 1992). Furthermore, we also examined if this potentiation is due to either a pharmacodynamic or a pharmacokinetic mechanism.

We assayed several paracetamol–caffeine combinations and found that, in some cases, the analgesic effect was greater than that observed with paracetamol alone. Our data are in agreement with clinical observations by Laska et al (1983) that caffeine is indeed able to increase paracetamol-induced analgesia. However, this potentiation only occurs if adequate dose combinations are used. These results may explain contradictory reports on caffeine potentiation of non-narcotic analgesics due to the use of different protocols. Maximal potentiation of paracetamol analgesic effect by caffeine on the PIFI model was observed with a combination of 316–32 mg kg^{-1} paracetamol–caffeine.

There is controversy on whether caffeine alone is able to exert any analgesic activity in experimental models. Vinegar et al (1976) did not observe any analgesia, whereas Siegers (1973) reported a significant pain relief with this drug. Our results are consistent with those of Vinegar et al (1976), as in the PIFI model, caffeine alone failed to show any analgesic activity. Under our conditions, caffeine coadministration did not modify paracetamol plasma level vs time curves; C_{max}, t_{max} and AUC observed with 316 mg kg^{-1} paracetamol alone did not significantly differ from those of 316–32 mg kg^{-1} paracetamol–caffeine. These results are consistent with the reports of Vinegar et al (1976) and Collins et al (1979) who also found that caffeine does not affect plasma levels of non-steroidal anti-inflammatory drugs, but disagree with those of Siegers (1973) and Seegers et al (1980).

Although association of 32 mg kg^{-1} caffeine to a 316 mg kg^{-1} dose of paracetamol did not modify the plasma levels of the p-aminophenol derivative, it did increase the analgesic effect in a statistically significant manner. These data indicate that the observed potentiation was not due to a pharmacokinetic mechanism.

Recovery of functionality index after oral administration of 316 mg kg^{-1} paracetamol alone was well correlated with drug plasma levels according to the pharmacokinetic/pharmacodynamic sigmoidal E_{max} model (Holford & Sheiner 1981). The parameters of the Hill equation which described this relationship were not significantly different from those previously reported using several paracetamol doses (Granados-Soto et al 1992). Analgesic effect and paracetamol plasma levels observed after administration of a 316–32

mg kg^{-1} paracetamol–caffeine combination were also well fitted by the same pharmacokinetic/pharmacodynamic model. However, the curve was shifted to the left in a parallel manner compared to that obtained with the dose of the para-aminophenol derivative alone. The EC50 of paracetamol plasma concentrations was significantly lower in the presence of caffeine; this strongly suggests that the methylxanthine increased the potency of the para-aminophenol derivative by a purely pharmacodynamic mechanism of action.

Paracetamol, as other non-steroidal anti-inflammatory drugs, exerts its analgesic action by inhibition of prostaglandin synthesis in the periphery as well as by an effect on the central nervous system (Ferreira et al 1978). It is known that caffeine does not alter the inhibition of prostaglandin synthesis by non-steroidal anti-inflammatory drugs (Vinegar et al 1976), but there is evidence that methylxanthines affect nociception at the central level (Paalzow & Paalzow 1973). It then appears probable that the mechanism of the potentiation of paracetamol-induced analgesia by caffeine is by an action on the central nervous system.

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