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Relationship Between Paracetamol Plasma Levels and its Analgesic Effect in the Rat

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Abstract—The relationship between plasma levels of paracetamol and its analgesic effect was studied in the rat using a model of pain-induced functional impairment (PIFI). Female Wistar rats received an intra-articular injection of 30% uric acid in the knee of the right hind limb, inducing its dysfunction. Animals then received oral paracetamol at doses of 178, 316 or 562 mg kg^{-1} and the recovery of functionality over time was considered as an expression of analgesia. Paracetamol plasma levels were determined by HPLC. Results showed that there is a direct relationship between paracetamol plasma levels and its analgesic effect that follows a sigmoidal model according to the Hill equation. The PIFI model appears to be a useful tool to establish pharmacokinetic/pharmacodynamic relationships for non-narcotic analgesics.

The importance of simultaneous modelling of pharmacokinetics and pharmacodynamics has been emphasized in the last decade, as it allows a prediction of the time course of the intensity of the pharmacological effect in-vivo (Holford & Sheiner 1981; Schwinghammer & Kroboth 1988). This is one of the major goals of clinical pharmacology, but is equally important in animal studies. As it has been stated by Colburn (1987), animal models allow the establishment of appropriate pharmacodynamic measures for use in clinical trials and the early evaluation of metabolites, routes of administration and sample sites.

For certain drugs, for which the pharmacological response can be accurately determined, pharmacokinetic/pharmacodynamic models have been established. In some cases, it has been possible to determine direct relationships between plasma levels and the measured pharmacodynamic response; for example, the antiarrhythmic effect of tocainide (Winkle et al 1976), the vasodilator activity of the calcium entry blockers nifedipine (Kleinbloesem et al 1984) and nitrendipine (Eichelbaum et al 1988), the inhibition of gastric acidity by timoprostil (Wills et al 1985), tiotidine and cimetidine (Kaojarern et al 1981), theophylline-induced bronchodilation (Mitenko & Ogilvie 1973) and the anticoagulant action of warfarin (Nagashima et al 1969). In other cases, there is no direct relationship between the effect and concentrations in plasma although other fluids may show a relationship e.g. the salivary concentration of procainamide and its antiarrhythmic activity (Galeazzi et al 1976).

Information on pharmacokinetic/pharmacodynamic relationships for analgesic agents is scarce. A model relating the analgesic effect of morphine to mathematically derived effector compartments in the rat has been described (Dahlström et al 1978). However, there is practically no pharmacokinetic/pharmacodynamic model for non-steroidal anti-inflammatory drugs. It has been proposed that the effect of paracetamol cannot be defined by pharmacokinetics (Shibasaki et al 1979), probably due to the lack of adequate methods to evaluate accurately the time course of analgesia intensity.

Pardo & Rodríguez (1967) developed a procedure for inducing pain by injecting formalin into the ankle joint of the hind limb of dogs, called pain-induced functional impairment (PIFI). Those authors measured the impairment produced by the irritant as an expression of pain, while the recovery of the use of the limb was considered an estimation of analgesic effect. With this procedure, they were able to follow the time course of the analgesic effect of aspirin (Pardo & Rodríguez 1966). This model closely resembles the clinical situation in which pain occurs before analgesic administration and the noxious stimulus is long lasting. In the present paper we report the use of a similar procedure, modified for rats, to study the relationship between the pharmacokinetics and the pharmacodynamics of paracetamol, one of the most frequently used non-opioid analgesic agents. We were able to develop a mathematical model relating plasma concentrations to the observed pharmacological response.

Materials and Methods

Animals

Female Wistar rats, 180–220 g, were used in this study. Twelve hours before the initiation of experiments, food was withheld, but animals had free access to drinking water. Determination of analgesic activity and of paracetamol plasma concentration was performed in the same animals.

Measurement of analgesic activity

Pain intensity and the analgesic effect of paracetamol was measured using a pain-induced functional impairment (PIFI) procedure similar to that reported by Pardo & Rodriguez (1966, 1967), modified for rats. Animals received an intra-articular injection of 0·05 mL of 30% uric acid

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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 a speed of 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 (Fig. 1) i.e. the injured limb made no contact with the cylinder. At this time, rats received an oral paracetamol dose suspended in 0·5% carboxymethyl cellulose (4 mg kg^{-1}) 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 samples was added 50 ng 2-acetamidophenol (internal standard) and the mixture was extracted with 5 mL of ethyl acetate. The solvent was then evaporated and the residue redissolved in 0·05 mL of methanol. Portions (0·01 mL) were injected into an HPLC system (model 5000, Varian, Palo Alto, CA, USA) equipped with a 150 × 3·9 mm reversed-phase column (Novapak C_{18}, Waters Associates 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.

Study design

Three groups of 78 rats were used in this study. Animals in the first group received an oral dose of 178 mg kg^{-1} paracetamol, those in the second group 316 mg kg^{-1} paracetamol, and those in the third group 562 mg kg^{-1} paracetamol. Groups were divided into 13 subgroups of 6 rats, to determine functionality indices at 0, 10, 20, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 min after dosing. 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 by centrifugation and frozen in liquid nitrogen. Plasma was stored at −20°C until assayed for paracetamol by HPLC.

Three additional groups of control rats were studied. Animals from one of these groups were not injected with uric acid, but received the paracetamol doses, in order to determine if the analgesic agent, by itself, produced any motor impairment. Another group was injected with uric acid but received only vehicle, not paracetamol, in order to establish if there was any spontaneous recovery of leg functionality. The third group was not injected with uric acid and received no drug. Functionality index was determined at the times listed above.

Since it has been reported that paracetamol disposition shows diurnal variation (Bélanger et al 1987), all experiments were performed to the same schedule. Uric acid was injected at 0700 h, paracetamol was given at 0900 h and the time course of analgesia was followed from 0900 to 1300 h.

Analysis of results

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

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

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 induces an effect equivalent to 50% of the maximal effect and h is the Hill coefficient.

Results

The measurement of nociception and of analgesic effect using the PIFI model in the rat is shown in Fig. 1. Control rats which were not injected with uric acid nor received any analgesic agent walked normally on the rotating cylinder, both hind limbs exhibited similar contact times with the cylinder floor resulting in a functionality index of 100%. Uric acid injection produced a progressive dysfunction of the right hind limb resulting in a reduction of the functionality index. Values reached zero, 2 h after injection, and this time was considered as time 0 and the analgesic agent was

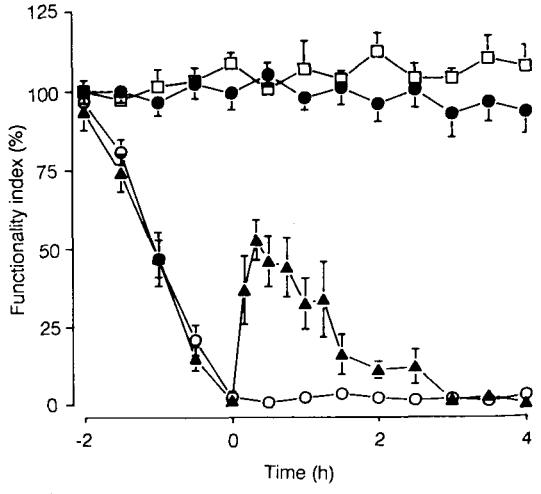

FIG. 1. Time course of functionality index in rats. ● Control rats. ○ Rats injected with uric acid in the right hind knee at time −2 h and with vehicle at time 0. ▲ Rats injected with uric acid at time −2 h and given oral paracetamol (316 mg kg^{-1}) at time 0. □ Rats given oral paracetamol (316 mg kg^{-1}) at time 0. Data are expressed as mean ± s.e.m. of six determinations.

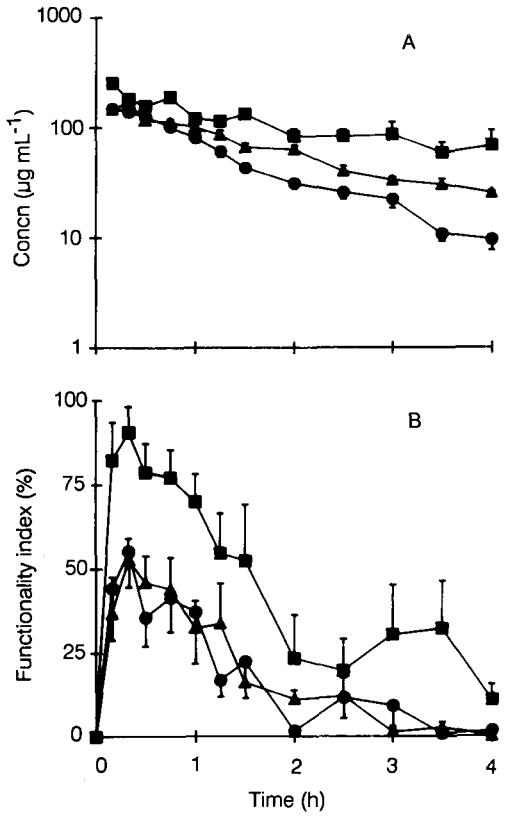

FIG. 2. Time course of paracetamol plasma concentrations (A) and analgesic effect, measured as functionality index recovery (B), in rats submitted to pain-induced functional impairment by intra-articular injection of 30% uric acid in the right hind knee. Animals received 178 (●), 316 (▲) or 562 mg kg^{-1} (■) of paracetamol orally. Data are presented as mean ± s.e.m. of six determinations.

immediately given. Paracetamol administration resulted in a significant analgesic effect, observed as a recovery of the functionality index. When no analgesic agent was given, the functionality index remained at 0 and no spontaneous recovery occurred during the 4 h observation. Rats which were not injected with uric acid, but received paracetamol, exhibited functionality index values not significantly different from control animals, i.e. 100%.

After drug administration, paracetamol plasma concentrations rose quickly reaching a maximum concentration between 10 and 20 min. Plasma concentration decayed with a half-life of 0·8, 1·6 and 1·7 h for the doses of 178, 316 and 562 mg kg^{-1}, respectively (Fig. 2A). Relevant pharmacokinetic parameters are shown in Table 1. Oral paracetamol induced a significant analgesic effect in rats injected with uric acid in the right hind knee, as judged by the recovery of the functionality index. The peak analgesic effect, E_{max}^{obs}, was highest with the 562 mg kg^{-1} dose, but there was no significant difference between the E_{max}^{obs} values observed with the two lower doses. Therefore the effect is not strictly dose-related. Maximum analgesic effect was observed between 10 and 20 min after drug administration (Fig. 2B).

At equivalent plasma concentrations of paracetamol, similar analgesic effects were produced, regardless of the administered dose. This suggests that the analgesic effect of

Table 1. Pharmacokinetic and pharmacodynamic parameters after administration of paracetamol to rats.

	Dose (mg kg^{-1})		
Parameter	178	316	562
C_{max} (μg mL^{-1})	147·5 ± 12·4	169·2 ± 7·3	254·3 ± 18·6
t_{max} (h)	0·28 ± 0·06	0·33 ± 0·04	0·22 ± 0·04
AUC (μg h mL^{-1})	191·7 ± 7·3	253·5 ± 6·6	388·2 ± 21·7
E_{max}^{obs} (%)	55·2 ± 10·7	52·7 ± 6·4	90·5 ± 7·7
t_{Emax} (h)	0·35 ± 0·09	0·33 ± 0·04	0·25 ± 0·04

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 and t_{Emax} is the time to reach E_{max}^{obs}.

paracetamol is directly related to plasma levels. The relationship between plasma levels and the functionality index was established by the sigmoidal E_{max} model according to the Hill equation. When the effect (E, in % of functionality index) was plotted against the observed plasma levels (C_p, in μg mL^{-1}) (Fig. 3), the Hill equation that best fitted the experimental data was:

$$E = \frac{100 \cdot C_p^{2.13}}{124.6^{2.13} + C_p^{2.13}}$$

EC50 and Hill coefficient values were determined by iteration with the PCNONLIN program, being (± s.e. given by the nonlinear regression routine) 124·6 ± 5·5 and 2·13 ± 0·26 μg mL^{-1}, respectively. E_{max} was established as 100%, anticipating that a complete recovery of functionality induced by the drug is possible.

Discussion

Although it has been argued that the major challenge in pharmacokinetic/pharmacodynamic modelling is to develop pharmacokinetic models allowing the determination of drug concentrations at the site of action (Colburn 1987), for

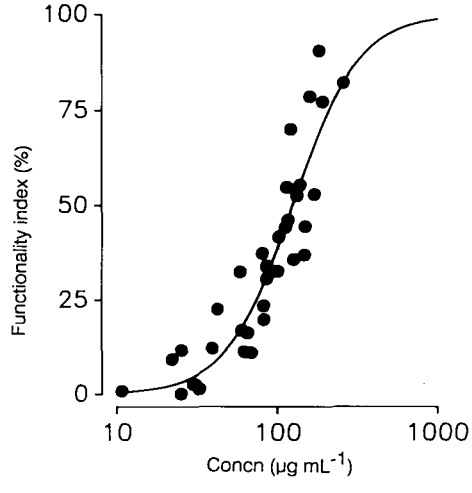

FIG. 3. Relationship between paracetamol plasma concentrations and analgesic effect in rats which received paracetamol orally. Each point corresponds to the mean of 6 rats. The trace corresponds to the curve fitted to the experimental data by the Hill equation, by nonlinear regression.

analgesic drugs the main problem has been the lack of adequate measurements of nocicepetion and analgesia in animals and man. There are few clinical studies that demonstrate a significant correlation between analgesia and circulating levels of non-opioid drugs, mainly due to the difficulty in finding an adequate index for pain relief (Laska et al 1986).

Pardo & Rodríguez (1966) demonstrated that a pain-induced functional impairment (PIFI) procedure in dogs allowed them to follow accurately the time course of the analgesic effect of aspirin. We have confirmed the usefulness of the PIFI model in the rat, by following the time course of the analgesic effect produced by oral paracetamol. Under our experimental conditions, paracetamol reversed the hind limb dysfunction induced by the intra-articular injection of uric acid. We were able to relate this effect to paracetamol plasma concentrations using the Hill equation. According to the best fit obtained by nonlinear regression, the E_{max} that can be obtained with this drug is the theoretical maximum (i.e. functionality index of 100%). This appears highly probable, as a functionality index of 90% was attained with 562·4 mg kg^{-1}. Although a higher dose can theoretically produce a complete recovery, we did not test this as it is well known that very high paracetamol doses produce severe toxic effects (Price & Jollow 1982).

Hence, the PIFI model was found to be adequate in following the analgesic effect of paracetamol. However, uric acid injection induces not only pain, but also inflammation. In patients, painful stimuli are very frequently accompanied by inflammatory processes. Thus, this method does not allow discrimination between pure pain relief and an anti-inflammatory effect of the assayed drug.

Previous information concerning the pharmacokinetic/pharmacodynamic relationship for paracetamol is scarce. Shibasaki et al (1979) were unable to correlate paracetamol circulating levels with the observed analgesic effect in mice, and even suggested that this could not be explained pharmacokinetically. However, the findings of those authors can be attributed to the use of an inadequate experimental method to evaluate analgesia. Pain was induced by applying pressure to the tail and analgesia was measured as the reduction of the pressure threshold that provoked a behavioural response (the mouse biting the compressing device) at several times. Under such conditions, animals learn quickly; after several tail compressions, the response is not only induced by pain but also by a conditioned behaviour (Winter 1965).

In the present study, the use of the PIFI model allowed us to follow the time course of paracetamol-induced analgesia. With this technique for producing pain, there is no possibility of interference by a learning process (Pardo & Rodríguez 1966). Moreover, since there is no spontaneous recovery of the functionality index, it appears that the increase in the value of this parameter depends only on the analgesic or anti-inflammatory effects of the administered drug. Since we were able to relate the recovery of the functionality index (an expression of the analgesic effect) to its plasma concentrations according to the sigmoidal E_{max} model (Holford & Sheiner 1981), our results strongly suggest that the effect of paracetamol can be explained by its pharmacokinetic properties.

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