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Usefulness of the Pain-induced Functional Impairment Model to Relate Plasma Levels of Analgesics to Their Efficacy in Rats

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Abstract

In this work we show that the pain-induced functional impairment model (PIFIR) can be used with cannulated rats as a useful procedure for pharmacokinetic/pharmacodynamic modelling. This model evaluates analgesia by measuring motor impairment of the right limb after intra-articular administration of uric acid. Time of contact with a rotating cylinder is referred to the control limb. We studied the pharmacokinetic and pharmacodynamics of naproxen after six peroral doses to Wistar rats, and we examined the adjuvant action of caffeine with naproxen.

Surgery and blood sampling did not produce any difference on functional impairment either in rats without uric acid or in the dysfunction produced by uric acid. The relation between naproxen plasma concentration and the analgesic effect was obtained with few rats. Caffeine alone did not produce any significant modification in functional impairment but the co-administration significantly increased the effect of naproxen. Plasma levels of naproxen did not change when caffeine was co-administered.

The PIFIR model with blood sampling is a suitable method for pharmacokinetic/pharmacodynamic relationship studies and is specially useful to characterize drug-drug interactions.

Literature on pharmacokinetic/pharmacodynamic relationships for analgesic agents is relatively sparse. This is probably due to the lack of methods for objectively measuring nociception and analgesia in animals and man. There are a few clinical studies that have shown a significant correlation between analgesia and circulating levels of non-opioid drugs (Day et al 1982; Laska et al 1986). In animal models pharmacokinetic/pharmacodynamic modelling of analgesia requires continuous evaluation avoiding behavioural artifacts. Vocalization responses after electrical stimulation (Dahlström et al 1978) and the classical tail flick in rats (Bhargava et al 1991; Gårdmark et al 1993; Oullet & Pollack 1993), have been reported.

The pain-induced functional impairment model (PIFIR (López-Muñoz et al 1993)) is a model for the objective evaluation of pain in rats, permitting the time course of the analgesic effects to be followed in the same animals. This model has been used to relate paracetamol plasma levels to analgesic efficacy (Granados-Soto et al 1992, 1993). However, 234 rats were used to obtain only one concentration–analgesia data set for paracetamol. Plasma samples can be obtained in the same animals using a cannula in the caudal artery. If that cannulation does not interfere with the pain or the analgesic effect in the PIFIR model, it would be adequate for modelling studies of analgesic drugs with fewer animals.

A number of controlled clinical trials have indicated that caffeine can improve analgesic efficacy (Sawynok & Yaksh 1993). It is now known that caffeine induces actions which interfere with nociception at the peripheral and central levels, mainly by interaction with adenosine receptors (Sawynok & Yaksh 1993). In previous studies the caffeine adjuvant efficacy was not clear (Moertel et al 1974; Laska et al 1983), probably due to the difficulties in accurately measuring pain in patients and in the use of different protocols (Laska et al 1984).

These considerations determined the objectives of the present work: to relate plasma concentration and analgesic effect of naproxen; and to examine the caffeine–naproxen interaction with the PIFIR model.

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Materials and Methods

Experimental animals

Experiments were on female Wistar rats (Clr:(WI)BR), 180–220 g, from our own breeding facilities. All experimental procedures followed the recommendations of The Committee for Research and Ethical Issues of the International Association for the Study of Pain (Covino et al 1980) and the guidelines on ethical standards for investigations of experimental pain in animals (Zimmermann 1983). The number of experimental animals was kept to a minimum. Animals were housed six to a large cage, the floor of which was covered with sawdust, and were kept in an animal room at a constant temperature of 22°C, with a 12 h alternating light/dark cycle. Twelve hours before experiments, food was withheld, with free access to water.

Compounds

Apart from anaesthetic (ethyl ether), the drugs used in the

present study were the following: uric acid, naproxen and caffeine, purchased from Sigma Chemical Co. (St Louis, MO, USA). Uric acid was suspended in mineral oil, whereas naproxen and caffeine were suspended in 0·5% carmellose. The doses mentioned in the text refer to the salts of substances.

Cannulation and analgesic activity

Rats were cannulated in the caudal artery with a PE-10 cannula (i.d. 0·28 mm, o.d. 0·61 mm; Clay Adams, Parsippany, NJ, USA) connected to a PE-50 cannula. A cannula was introduced into the caudal artery, and was kept potent with heparin and stoppered with a needle. The antinociceptive effect was determined using the PIFIR model as described previously (López-Muñoz et al 1993). The animals were anaesthetized with ether. Then, 0·5 mL uric acid (30%) suspended in mineral oil was administered by an intra-articular injection into the knee joint of the right hind limb to induce pain. Immediately afterwards, an electrode was attached to each hind-paw between the plantar pads. Rats were allowed to recover from anaesthesia and then were exercised on a cylinder, which was rotated for periods of 2 min. The time of contact between each electrode on the hind limbs of the rat and the cylinder was recorded with a computer. When a zero value for the functionality index (FI) was recorded, naproxen or naproxen–caffeine was administered. Experiments lasted a further 4 h. Analgesia was estimated as the recovery of the FI.

Experimental protocol

In the first experimental series, four groups of six rats were used. Two groups (A and B) had cannulation surgery and the others (A′ and B′) did not. The groups B and B′ received uric acid intra-articularly. The FI was determined for all the groups each 30 min for 6 h. Two hours after the uric acid treatment (time 0) the analgesic was administered and analgesia evaluated. For the groups with surgery, blood samples were taken at 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, and 240 min in the same animals, in volumes of 50, 100 or 200 μL (the total volume of blood taken was 850 μL). The groups A and A′ were used to determine if the blood sampling, by itself, produced any motor impairment. The groups B and B′ were used for determining if the blood sampling changes the dysfunction produced in the PIFIR model.

In the second experimental series, to obtain the curve of the relationship between naproxen plasma concentrations and analgesic effect on cannulated rats, six groups of six rats were used. All rats were cannulated, then received uric acid intra-articularly, and 2 h later, each group received one different dose of naproxen at time 0. Doses of naproxen were: 0·56, 1·00, 1·77, 3·16, 5·62, and 10·0 mg kg^{-1}, orally. The FI value was evaluated at 0, 10, 20, 30, 45, 60, 75, 90, 120, 150, 180, 210 and 240 min, and immediately after each FI determination, the blood samples were taken; the total volume of blood taken was 850 μL. The plasma was obtained by centrifugation and frozen in liquid nitrogen. Plasma was stored at −20°C until assayed for naproxen by HPLC. Naproxen plasma levels were measured according to the method of Kazemifard & Moore (1990), with slight modifications.

In the third experimental series, to examine if caffeine is able to potentiate the analgesic effect of naproxen in the PIFIR model, four groups of six rats were used. Caffeine was administered at doses of 31·6 and 56·2 mg kg^{-1}, orally, combined with the 1·77 mg kg^{-1} dose of naproxen. Caffeine plasma levels were measured according to the method of Kennedy et al (1992). The FI and blood sampling were evaluated at the times listed above. Surgery and uric acid injection was at 0700 h. Naproxen alone, caffeine alone or naproxen–caffeine was given at 0900 h and the time course of analgesia was followed from 0900 to 1300 h.

Data presentation and statistical analysis

Data are expressed as the functionality index (FI). The FI was defined as the contact time of the right limb divided by the contact time of the left leg (expressed as percentage). The curves represent FI (%) vs time (h). All values in the text and figures are given as arithmetic means ± s.e.m. of at least six animals. Two-way analysis of variance was used to compare the groups. $P < 0·05$ was regarded as statistically significant.

Results

Cannulation effect on the dysfunction

Rats were able to walk with the cannula. Surgery and blood sampling did not produce any differences in the FI in rats without uric acid (Fig. 1). Two hours after the injection of uric acid the rats were unable to use the injured leg. Hence, the FI reached zero, and remained there in rats without analgesic despite the surgery. The dysfunction with uric acid was the same in animals with and without blood sampling (Fig. 1).

Relationship between naproxen plasma levels and analgesic effect after naproxen administration

Fig. 2A shows the time course of the analgesic effects of naproxen. Naproxen analgesia was dependent on the dose, reaching an observed maximal effect of 94% in the FI with

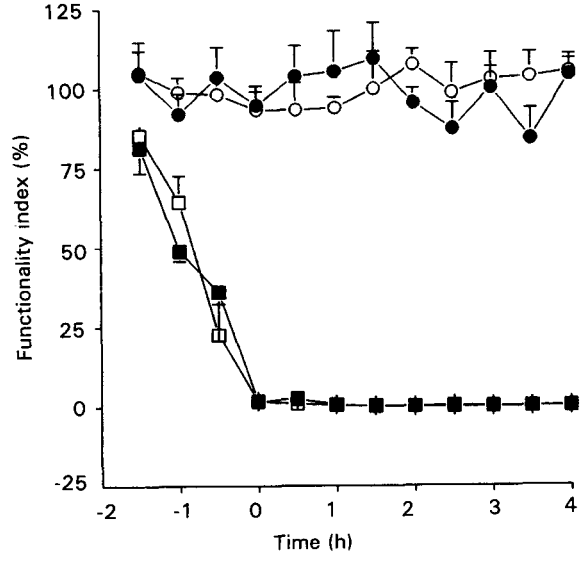

FIG. 1. Comparison of time course of functionality index in rats with or without cannulation surgery (cannula). There were no statistical differences due to the cannulation surgery after two-way analysis of variance for rats either with normal walking or with uric acid. ○ Normal walk, ●, normal walk + cannula, □ uric acid, ■ uric acid + cannula.

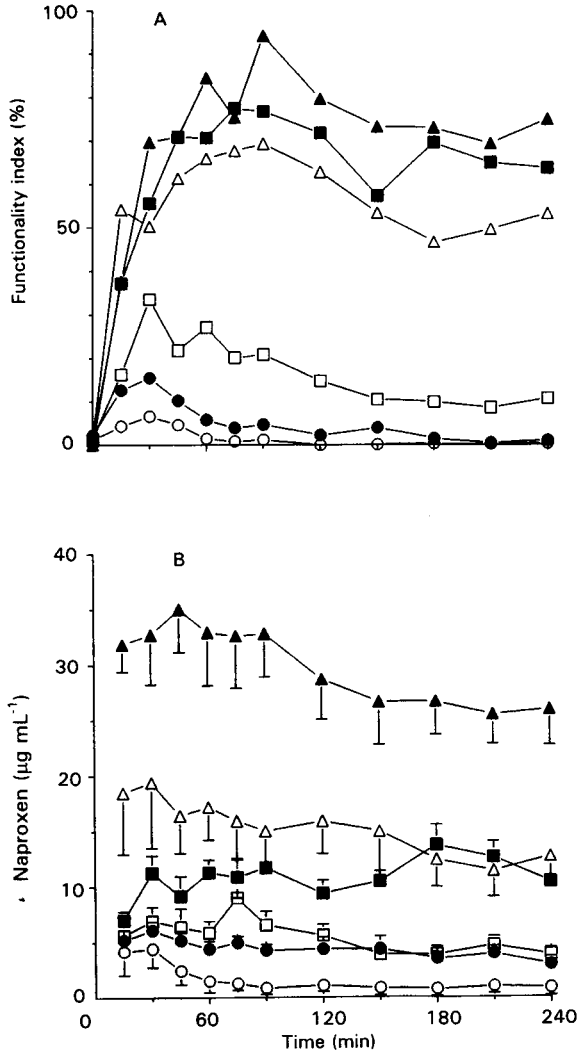

FIG. 2. A. Time course of naproxen analgesic effect in rats submitted to pain-induced functional impairment after several doses of naproxen given orally. B. Plasma levels of naproxen in the same rats. Data are presented as means of at least six rats. The s.e.m. values are not shown for the sake of clarity. Both pharmacokinetics and pharmacodynamics were dependent on the dose. ○ 0·56, ● 1·00, □ 1·77, ■ 3·16, △ 5·62 and ▲ 10·00 mg kg^{-1}.

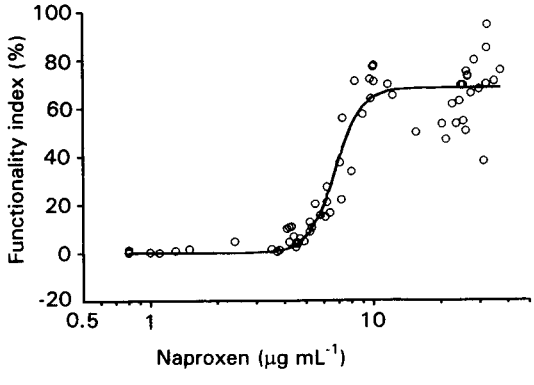

FIG. 3. Concentration–response curve in rats which received naproxen orally. Each point corresponds to the mean of at least six rats. Fitting is shown for illustrative purposes only.

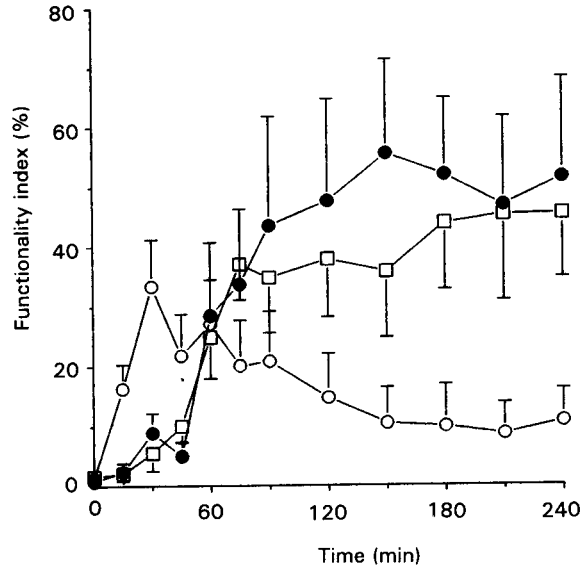

FIG. 4. Synergism between naproxen and caffeine for the analgesic effect. Both caffeine doses produced a similar interaction with naproxen. ○ Naproxen 1·77 mg kg^{-1}, ● naproxen + caffeine 31·6 mg kg^{-1}, □ naproxen + caffeine 56·2 mg kg^{-1}.

the dose of 10 mg kg^{-1}. The doses 3·16, 5·62 and 10 mg kg^{-1} induced a good recovery of the FI, keeping the FI above 50% for most of the time. The analgesia diminished more rapidly with doses of 0·56 and 1·00 mg kg^{-1}. Maximal recovery of functionality with all doses was between 30 and 90 min after the administration.

Fig. 2B shows the time course of naproxen plasma concentration. Plasma levels of naproxen reached peak values in about 15 min after oral administration. Naproxen elimination had a half-life of about 4 h, but it changed with the doses. Because of the short duration of these experiments (4 h) and the variability due to the absorption, it was not appropriate to construct a complete estimate of the pharmacokinetic parameters.

The relation between naproxen plasma concentration and the analgesic effect is shown in Fig. 3. The relationship was obtained with data from six doses of naproxen. The calculated E_{max} was 68%, EC50 was 6·8 μg mL^{-1} and the slope factor was 7·2.

Analgesic effects of naproxen–caffeine mixtures

Oral administration of naproxen alone resulted in an increase of FI, which reached a peak and then decayed slowly. Caffeine alone did not produce any significant modification in FI. Caffeine co-administration significantly increased the effect of naproxen (Fig. 4).

Plasma levels of naproxen did not change when caffeine was co-administered. Nevertheless, the analgesic effect of the drug was greatly increased. Average plasma levels of caffeine were about 20 and 40 ng mL^{-1} for the doses of 31·6 and 56·2 mg kg^{-1}, respectively.

Discussion

The purpose of the present work was to show that the PIFIR model with blood sampling is a useful method for pharma-

cokinetic/pharmacodynamic relationship studies and to examine if caffeine is able to potentiate the analgesic effect of naproxen in the PIFIR model.

The PIFIR model with blood sampling is an objective method to evaluate the pharmacokinetic/pharmacodynamic relationship of analgesics. This model can yield relevant information because of its resemblance to the clinical situation. Since it produces chronic pain, stable for at least 4 h, it is closer to the clinical pain in contrast with models of acute pain, such as electrical stimulation or tail flick. Ethical issues were improved with this approach compared with the PIFIR model in as much as a small number of animals is required.

The variability of the absorption did not allow a good fit for the pharmacokinetics. For this reason, the plasma levels and the analgesic effects of naproxen were averaged to plot the concentration–response curve, instead of using the individual data. With intravenous administration better pharmacokinetic/pharmacodynamic relationships would be feasible and fewer doses would need to be tested.

The pharmacokinetic/pharmacodynamic relationship of naproxen is well-correlated at low doses (0·56, 1·00 and 1·77 mg kg^{-1}) but the variability is greater at higher doses (5·62 and 10 mg kg^{-1}). We suggest that the maximal effect is reached at a dose of 3·16 mg kg^{-1}. It is possible that lower doses reach an equilibrium between the plasma and the effect compartment more rapidly than at the higher doses, although no hysteresis was observed. Although it appears that an intravenous administration can improve significantly the fitting exercises, in this work we have attempted to show the possibilities of the model.

The naproxen–caffeine interaction is similar to other non-steroidal anti-inflammatory drug-coadjuvant interactions. This particular combination was tested for the first time in this study. Our data clearly demonstrate the pharmacodynamic nature of this interaction since the levels of naproxen were not changed by the co-administration with caffeine. To our knowledge, there are no studies characterizing these interactions.

A criticism of this study could be the use of females, since the oestrous cycle has been shown to influence the clearance of caffeine (Brugerolle 1992).

Day et al (1982) have reported a relationship of naproxen serum levels with analgesic efficacy in patients, but the pain-relief score was subjective and the naproxen levels were measured at limited times.

In conclusion, the PIFIR model with blood sampling is a suitable method for studying pharmacokinetic/pharmacodynamic relationships and is specially useful to characterize drug-drug interactions.

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