

Pharmacokinetic/Pharmacodynamic Modeling of Antipyretic and Anti-Inflammatory Effects of Naproxen in the Rat

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ABSTRACT

Pharmacokinetic/pharmacodynamic modeling was used to characterize the antipyretic and anti-inflammatory effects of naproxen in rats. An indirect response model was used to describe the antipyretic effects of naproxen after short intravenous infusions. The model assumes that basal temperature (T^a) is maintained by the balance of fever mediators given by a constant (zero order) rate of synthesis (K_{syn}), and a first order rate of degradation (K_{out}). After an intraperitoneal injection of lipopolysaccharide, the change in T^a was modeled assuming an increase in fever mediators described as an input rate function [IR(t)] estimated nonparametrically. An inhibitory E_{max} model adequately described the inhibition of IR(t) by naproxen. A more complex model was used to describe the anti-inflammatory

response of oral naproxen in the carrageenin-induced edema model. Before carrageenin injection, physiological conditions are maintained by a balance of inflammation mediators given by K_{syn} and K_{out} (see above). After carrageenin injection, the additional synthesis of mediators is described by IR(t) (see above). Such mediators induced an inflammatory process, which is governed by a first order rate constant (K_{IN}) that can be inhibited by the presence of naproxen in plasma. The sigmoidal E_{max} model also well described the inhibition of K_{IN} by naproxen. Estimates for IC_{50} [concentration of naproxen in plasma eliciting half of maximum inhibition of IR(t) or K_{IN}] were 4.24 and 4.13 $\mu\text{g/ml}$, for the antipyretic and anti-inflammatory effects, respectively.

As other nonsteroidal anti-inflammatory drugs (NSAIDs), naproxen has been extensively used during the past decades in the treatment of rheumatoid arthritis, febrile syndrome, and pain (Davies and Anderson, 1997). Naproxen inhibits both cyclooxygenase 1 (COX-1; constitutive) and cyclooxygenase 2 (COX-2; induced in settings of inflammation) and thereby the synthesis of prostaglandins (PGs) and thromboxanes (Vane, 1971; Ehrlich et al., 1999; Langenbach et al., 1999).

Limited insight on the in vivo pharmacodynamic (pd) properties of NSAIDs, based on plasma/biophase levels and drug effect relationships, have been published. Recently, clinical protocols characterizing the antipyretic effects of ibuprofen in children related to biophase concentration (Kelley et al., 1992; Brown et al., 1998) or to plasma concentration by an indirect response model (Garg and Jusko, 1994; Trocóniz et al., 2000), have been proposed. Preclinical studies are useful

alternatives to investigate different pharmacokinetic/pharmacodynamic (pk/pd) models to get insight into the "in vivo" mechanism of drug action. To our knowledge pk/pd modeling of the antipyretic effects of NSAIDs has not been performed in experimental models. Moreover, only few studies dealing with the time description of the anti-inflammatory effects of these agents (Castañeda-Hernández et al., 1995; Landoni and Lees, 1996; Landoni et al., 1999) have been published.

The injection of a wide range of doses of lipopolysaccharide (lps) into animals induces fever (Kluger, 1991; Yirmiya et al., 1994; Wachulec et al., 1997). It is known that lps-induced fever is driven by enhanced formation of cytokines such as interleukin- 1β , interleukin-6, interferons α and β , and tumor necrosis factor- α . The cytokines increase the synthesis of PGE_2 in circumventricular organs and near to the preoptic hypothalamic area. There PGE_2 , via cyclic AMP increasing, triggers the hypothalamus to elevate body temperature (T^a). NSAIDs suppress this response by inhibiting the synthesis of PGE_2 (Ruwe et al., 1985; Oka et al., 1997; Zhang et al., 1997).

On the other hand, a time-dependent inflammatory reaction is observed following diverse doses of carrageenin in-

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ABBREVIATIONS: NSAID, nonsteroidal anti-inflammatory drugs; COX, cyclooxygenase; PG, prostaglandin; T^a , body temperature; pd, pharmacodynamic; pk, pharmacokinetic; lps, lipopolysaccharide; CL, clearance; AUC, area under the curve.

jected to the paw of rats (Di Rosa et al., 1971; Castañeda-Hernández et al., 1995). In this model, COX-2 levels are elevated with a concomitant increase in prostaglandin production. Although these mediators do not appear to have direct effects on vascular permeability, both PGE₂ and PGI₂ markedly enhance edema formation and leukocyte infiltration by promoting blood flow in the inflamed region. Systemic inhibition of COX leads to a decreased production of PGs at sites of inflammation, and in the spinal cord (Coderre et al., 1990; Seibert et al., 1994). Vane and Botting (1994) suggested that PGs play an important role in promoting the signs and symptoms of inflammation. Nonetheless, as in the case of antipyretic effect of naproxen, the kinetics of its anti-inflammatory action is yet to be characterized.

Therefore, the goal of the current study was to develop a suitable pk/pd model for the antipyretic and anti-inflammatory effects of naproxen. To estimate its pd properties, experimentally induced pyresis or inflammation models were required. Thus, baseline variations within the time span of the experiments, as occurs with the adjuvant-induced alterations, represent an additional complexity, which should be included into the models.

Materials and Methods

This work includes results from the pk/pd modeling of the antipyretic (study I) and anti-inflammatory (study II) effects of naproxen in the rat.

Chemicals

The (*S*)-6-methoxy- α -methyl-2-naphthaleneacetic acid (naproxen) was supplied by Syntex S.A. (Madrid, Spain; study I) and by Syntex S.A. (Mexico City, Mexico; study II). Bayer (Madrid, Spain) and Novartis (Mexico City, Mexico) kindly supplied ibuprofen and sodium diclofenac (used as internal standards), respectively.

Lipopolysaccharide from *Escherichia coli*, serotype 0111:B4 was purchased from Sigma (Barcelona, Spain). Carrageenin was purchased from Sigma (St. Louis, MO). All reagents and solvents used were purchased from commercial sources and were of analytical grade.

Animals

Male Wistar rats, weighing 180 to 270 g were used in studies I and II. Animals were kept under laboratory standard conditions on a 12-h light/dark cycle with light from 8:00 AM to 8:00 PM, in a temperature (22°C)-controlled room, and were acclimatized for a minimum of 2 days before experiments were performed. They were housed in individual cages after the surgical procedures, with free access to water. Food was withheld for 12 h before the start of experiments. The protocols of the studies were approved by the Committee of Animal Experimentation of the University of Navarra and of the Centro de Investigación y Estudios Avanzados del Instituto Politécnico Nacional (Mexico City), respectively.

Surgical Procedure

The day before the experiments, rats were anesthetized i.p. with 100 mg/kg ketamine (Ketolar; Parke-Davis, Madrid, Spain) and 15 mg/kg xylazine (Rompun; Bayer, Mexico City, Mexico) for studies I and II, respectively.

In study I, two polyethylene (0.5 mm i.d., 11-cm-long; 0.3 mm i.d., 21-cm-long; Vygon, Ecouen, France) catheters were implanted in the right femoral artery and in the left jugular vein for blood sampling and drug administration, respectively. Rats in study II were surgically implanted with polyethylene catheters into the caudal artery as described previously (Granados-Soto et al., 1995). All catheters were filled with heparinized saline solution (20 IU/ml) to prevent clotting.

Experimental Protocol

Study I. Figure 1, left, represents the dosing and pk and pd data collection schedules used for study I.

Induction of fever and pd data measurements. Animals ($n = 30$) were randomly divided into five groups. Sterile saline solution (0.5 ml) containing 0.1 mg/kg lps was given i.p. to groups II to V, whereas the group I (baseline) only received 0.5 ml of sterile saline solution i.p. The lps solution was prepared at the beginning of the experiment and was injected at 37°C to animals. T^{re} was monitored in the rectum once every 30 min for 11 h just before and after the injection of the lps with a rectal thermometer (Panlab model 0331; Barcelona, Spain).

Drug administration and pk data collection. Naproxen was administered as an aqueous solution neutralized with NaOH and filled up to the administered volume with a phosphate-buffered solution (Lau-

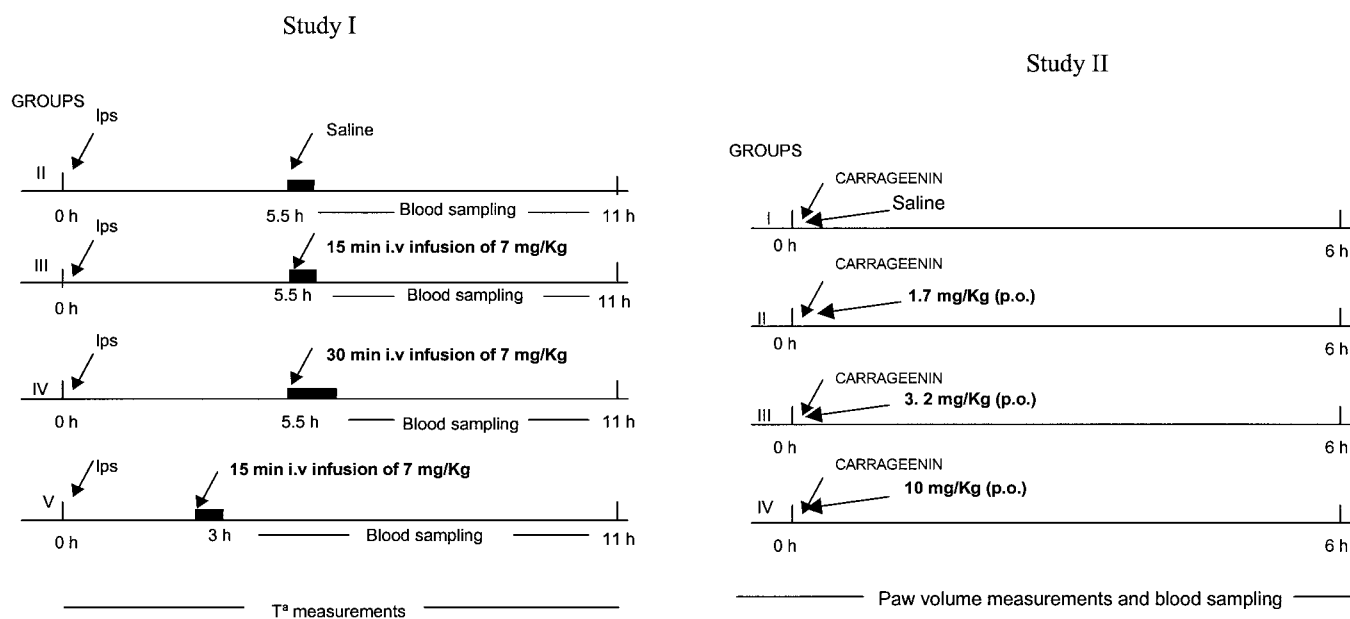


Fig. 1. Experimental design carried out in the current study for the analysis of the antipyretic (left) and anti-inflammatory (right) effects.

roba et al., 1986). The pH of the administered solution was 7.6 and the maximum infused volume was 0.7 ml.

Naproxen was given as different i.v. infusions after lps injection on the basis of the following schedule: group III, a total dose of 7 mg/kg was infused over 15 min, 5.5 h after lps injection; group IV, a total dose of 7 mg/kg was infused over 30 min, 5.5 h after lps injection; group V, a total dose of 7 mg/kg was infused in 15 min, 3 h after lps injection. Group II (control) received in 15 min an i.v. infusion with the same volume of saline that was given to group III.

Arterial blood samples ($n = 13$) of 100 to 200 μl were withdrawn during and after the drug or saline infusions. Sampling was carried out to define the plasma drug concentrations versus time profiles (groups III to V), or to explore the influence of blood sampling on rectal T^{ra} (group II). Blood samples were transferred to heparinized tubes and were immediately centrifuged to separate the plasma, which was frozen and kept at -20°C until analysis was performed. The same volume of extracted blood was reconstituted with physiological saline solution.

Drug analysis. Briefly, plasma samples (50–100 μl) were spiked with 50 μl of methanol containing the internal standard (ibuprofen) at a concentration of 200 $\mu\text{g/ml}$ followed by acidification with 0.3 ml of 2 N HCl. After the double addition of 2 ml of *tert*-butylmethylether, samples were shaken on a vortex mixer for 1 min and centrifuged for 5 min at 3500 rpm. The organic phase was transferred to a 10-ml tube and evaporated to dryness at vacuum in a vortex evaporator at 40°C . The dried residues were reconstituted with 500 μl of mobile phase and 100 μl were injected into the chromatographic system. This consisted of a Hewlett Packard high performance liquid chromatograph HP 1100 equipped with a quaternary pump, an autosampler, and a diode-array detector set at 229 nm.

Separations were achieved on a reversed phase Kromasil 100 C18 column (250×4 mm i.d., 5- μm particle size) provided by Tecknokrroma (Barcelona, Spain). The mobile phase was acetonitrile in 0.01 M phosphate buffer adjusted to pH 1.5 (60:40 v/v). The mobile phase was delivered at a constant flow rate of 1 ml/min and the chromatograph was carried out at 40°C . The signal showed linearity over the range 0.05 to 75 $\mu\text{g/ml}$ with intra- and interday coefficients of variation of the assay of 3.36 and 3.86%, respectively.

Study II. Figure 1, right, depicts the dosing and pk and pd data collection schedules used for study II.

Induction of paw inflammation and pd data collection. Animals ($n = 24$) were randomly allocated into four groups. To induce inflammation all animals were injected s.c. into the right hind paw with 0.1 ml of a 1% carrageenin suspension in 0.9% saline. Induced inflammation was measured by plethysmography as it has been described before (Winter et al., 1962). Paw swelling was determined once just before and during the following 6 h to carrageenin and drug administration.

Drug administration and pk data collection. Animals were treated orally with naproxen 1.7 mg/kg (group II), 3.2 mg/kg (group III), and 10 mg/kg (group IV), suspended in 0.5% carboxymethyl cellulose solution. The drug was given in a volume equal to 4 ml/kg and was simultaneously administered with the injection of carrageenin. Group I (control) received only carrageenin and blood samples were taken to study the influence of sampling on the time course of paw swelling.

Arterial blood samples ($n = 7$) of 200 μl were withdrawn at selected times for 6 h. Plasma was obtained by centrifugation, frozen, and kept at -20°C until analysis. The same volume of withdrawn blood was replaced with sterile saline.

Drug analysis. Measurements of naproxen in plasma were carried out using the method previously described by Borgå and Borgå (1997) with some modifications. Briefly, plasma samples (10 μl) were spiked with 20 μl of methanol containing the internal standard (diclofenac) at a concentration of 50 $\mu\text{g/ml}$. This mixture was diluted to 500 μl with phosphate buffer (pH = 7) and stirred until homogenization. Then, 20 μl was injected into the chromatograph.

The chromatographic system consisted of a Waters 510 pump

(Waters Associates, Milford, MA), a Rheodyne 7725i injector with a 20- μl loop (Cotati, CA), and a Waters 484 variable wavelength detector set up at 231 nm. Separations were achieved on a Symmetry C18 150×3.9 -mm (i.d.) column, 4- μm particle size (Waters Associates) using a mixture of 1 mM phosphoric acid and acetonitrile (62:38 v/v) as the mobile phase. The mobile phase was delivered at a constant flow rate of 0.8 ml/min and chromatography was carried out at room temperature. The signal showed linearity over the range 0.1 to 50 $\mu\text{g/ml}$. The intra- and interday coefficients of variation of the assay were 3.14 and 4.94%, respectively.

Data Analysis

The time course of the drug concentration and antipyretic/anti-inflammatory effect were analyzed with a population approach using the first order method implemented in NONMEM (version V) software (Beal and Sheiner, 1992). This approach makes it possible to simultaneously fit data from all individuals, describing both mean population tendencies and individual profiles, and provides estimates for the interindividual variability and residual (intraindividual) error. Data for study I and II were analyzed separately.

Interanimal variability was fitted using exponential models. Residual variability was modeled by using a constant-coefficient variation model for plasma drug concentrations, and an additive model for effect measurements.

Model selection was based on the exploratory analysis of goodness-of-fit plots performed with Xpose package (Jonsson and Karlsson, 1999), the estimates of the parameters, and their confidence intervals. The minimum value of the objective function provided by NONMEM was also used as a criterion for model selection. The difference in the objective function between two hierarchical models is approximately chi square distributed; $p < 0.05$ was used as the level of significance. Results from data analysis are presented as estimated values and their relative standard error. Estimates of the interanimal and residual variability are expressed as coefficients of variation (%).

Pharmacokinetic Models. Naproxen disposition properties were characterized by compartmental models. In the case of study II, different absorption models were tested: first order, zero order, and Michaelis-Menten absorption models; the presence of a lag time was also explored.

Pharmacodynamic Models. Figure 2 shows the models fitted to the data for studies I and II.

Study I: antipyretic effect. After injection of lps control group showed a time-varying response, which was modeled using the following model:

$$\frac{dR}{dt} = K_{\text{syn}} + \text{IR}(t) - K_{\text{out}} \times R \quad (1)$$

where dR/dt represents the rate of change in response (T^{ra}); K_{syn} is the zero order rate constant of response formation and K_{out} is the first order rate constant of response degradation. $\text{IR}(t)$, the input rate function representing the increase in the formation of fever mediators accounting for the temporal increase in response, was modeled nonparametrically using a linear spline. A linear spline is characterized by a number of real numbers called breakpoints and a number of polynomials, which simply join at the breakpoints (DeBoor, 1978). In this study the breakpoints were located at the times of observations. The number and location of the breakpoints were selected as a part of the model-building process. The heights of the spline at each of the breakpoints were parameters to be also estimated during the model-building process together with the rest of the pharmacodynamic parameters, and were constrained to be non-negative.

Drug effect (DRUG) was incorporated to the previous equation as follows:

$$\frac{dR}{dt} = K_{\text{syn}} + \text{IR}(t) \times (1 - \text{DRUG}) - K_{\text{out}} \times R \quad (2)$$

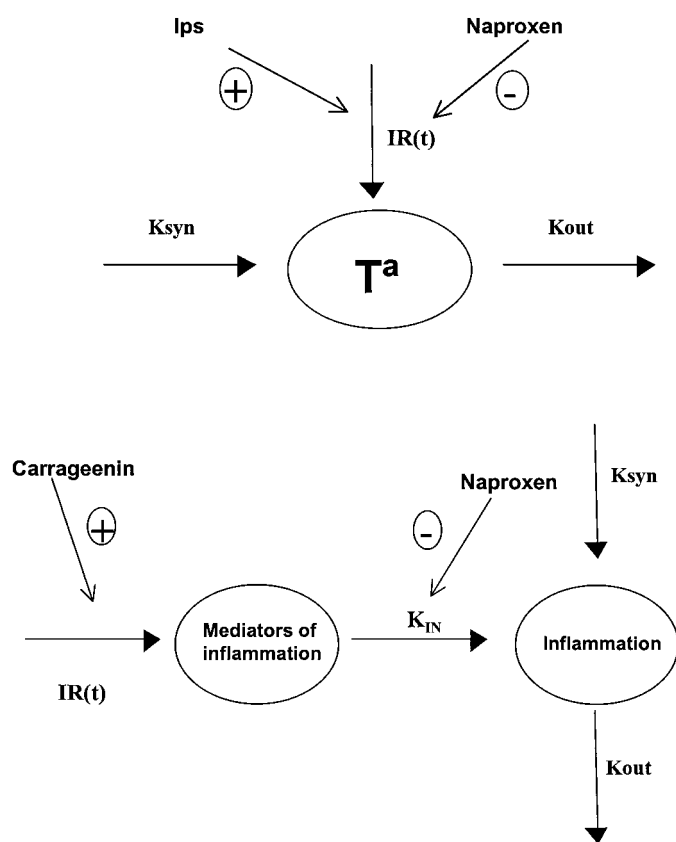


Fig. 2. Schematic representation of the pharmacodynamic model used to describe the data for study I (top) and study II (bottom). $IR(t)$, input rate function of fever or inflammation mediators; K_{IN} , first order rate constant of release of inflammation mediators into the inflammation compartment; K_{syn} , zero order rate constant of formation of basal fever or inflammatory response; K_{out} , first order rate constant of degradation of fever or inflammatory response.

This model assumes that naproxen exerts its action by the inhibition of the lps-induced synthesis of fever mediators. Different models were tested for DRUG: linear, E_{max} , and sigmoidal E_{max} models.

Study II: anti-inflammatory effect. Although previous equations were suitable for describing T^a data when applied to inflammation data, the model predictions obtained were not satisfactory. Thus, a more complex model was built to account for the delayed increase in inflammation in naproxen-treated groups in comparison with control. This model assumes that 1) carrageenin injection elicits a transient formation of inflammatory mediators (M), which is described by the input rate function $IR(t)$ (see above); 2) the mediator-induced inflammatory response is governed by a first order rate constant (K_{IN}), which can be inhibited by naproxen in plasma; and 3) in absence of carrageenin and/or drug in the body, a certain degree of inflammation (baseline level) is maintained by the balance between the production (represented by the zero order rate constant, K_{syn}) and the degradation (represented by the first order rate constant, K_{out}) of inflammatory response. The model is represented by the following set of differential equations:

$$\frac{dM}{dt} = IR(t) - K_{IN} \times (1 - DRUG) \times M \quad (3)$$

$$\frac{dR}{dt} = K_{IN} \times (1 - DRUG) \times M + K_{syn} - K_{out} \times R \quad (4)$$

where dM/dt represents the rate of change of inflammatory mediators, dR/dt is the rate of change of inflammatory response, and

DRUG is the effect of naproxen on the system. Different models were tested for DRUG: linear, E_{max} , and sigmoidal E_{max} models.

Statistical Analysis. Results from studies I and II after injection of lps or carrageenin are shown as mean data with their corresponding standard deviations. Within each study, comparisons of the observed responses between the different groups were made by one-way ANOVA followed by a Tukey's posteriori test. Statistical significance was set at $p < 0.05$.

Results

Study I

Pharmacokinetics. A three-compartment model was used to describe the kinetics of naproxen in plasma. Estimates of the typical pk parameters and their values of inter-animal variability are listed in Table 1. Mean observed and typical model-predicted plasma concentration versus time profiles are shown in Fig. 3. Mean observed plasma concentrations at the time the infusions were stopped were 46.22 ± 2.11 , 55.45 ± 6.39 , and 52.85 ± 2.72 $\mu\text{g/ml}$ for the 30- and 15-min infusions, respectively.

Pharmacodynamics. Figure 4 shows the mean observed T^a versus time profiles for all groups injected with lps in study I. Baseline group showed a constant basal body T^a over a 12-h period with a mean \pm S.D. value of $37.2 \pm 0.06^\circ\text{C}$. Basal T^a recorded at the time of lps injection did not differ statistically among groups I to V ($p > 0.05$). In addition, at the times T^a were recorded between lps injection and the start of the drug infusions no statistical differences in T^a ($p > 0.05$) were found among groups II to V. A mean maximal increase in body T^a of $38.27 \pm 0.15^\circ\text{C}$ located at 5.3 ± 0.4 h after lps injection was found for group II; T^a then returned gradually to baseline; 11 h after lps injection mean observed T^a was $37.92 \pm 0.33^\circ\text{C}$. Mean times to baseline were 8 ± 1.5 , 11 ± 1 , and 4.5 ± 1.6 h for groups III, IV, and V, respectively. The onset of the antipyretic effects was fast in the three groups. However, T^a returned to baseline with a 2- to 3-h delay with respect to time to peak plasma concentrations, indicating that observed effects and plasma drug concentrations could not be related directly.

Pharmacokinetic/Pharmacodynamic Modeling Results. Figure 4 shows the typical model-predicted time course of T^a in all groups on the basis of the model described in Fig. 2 (top) and by eqs. 1 and 2. It can be observed that model predictions for groups III and IV are almost superimposable; the fact that plasma drug concentrations for both groups at early times after the end of the infusion were 9 to

TABLE 1

Pharmacokinetic parameter estimates of naproxen given in 15- or 30-min i.v. infusions of 7 mg/kg to rats

Estimates of interanimal (IAV) variability are expressed as coefficients of variation (%). Precision of the estimates is expressed as relative standard error in parentheses. Relative standard error is standard error divided by the parameter estimate.

Parameter	Estimate	IAV
V_1 (ml/kg)	45.2 (0.097)	20 (0.45)
CL (ml/h/kg)	32 (0.039)	16 (0.37)
V_2 (ml/kg)	73.6 (0.09)	N.E.
V_3 (ml/kg)	60.8 (0.088)	32 (0.73)
CLD ₁ (ml/h/kg)	980 (0.15)	N.E.
CLD ₂ (ml/h/kg)	108.8 (0.24)	N.E.

V_1 , initial volume of distribution; CL, total plasma clearance; V_2 and V_3 , shallow and deep compartment volumes of distribution, respectively; CLD₁ and CLD₂, inter-compartmental clearances between plasma and shallow and between plasma and deep compartment, respectively; N.E., not estimated in the model.

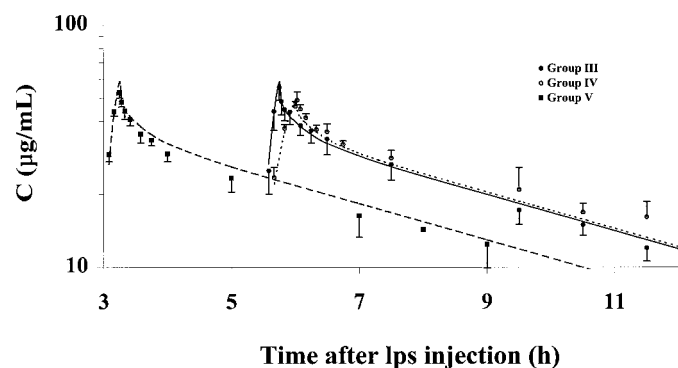


Fig. 3. Time course of plasma concentrations of naproxen in study I. Symbols represent mean observed data, lines represent typical model predictions, and vertical lines represent standard deviations.

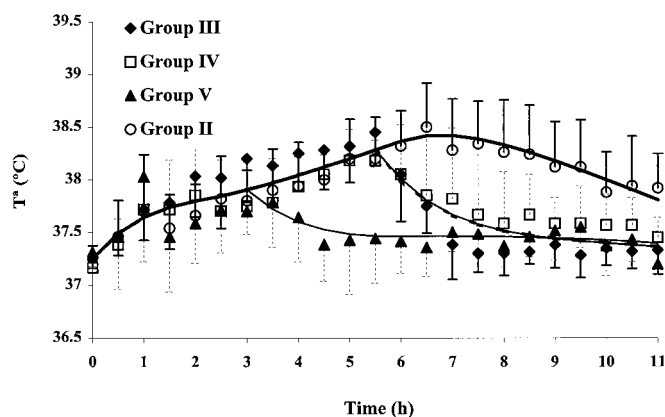


Fig. 4. Time course of rectal T^a after lps injection and naproxen administration (symbols, mean observations; lines, typical model predictions). Vertical lines represent standard deviations.

11 times higher than the estimated value of IC_{50} , together with the high interindividual variability, could explain this issue. Figure 5 shows in solid line the selected shape for the linear spline representing the input rate of fever mediators responsible for the temporal increase in body T^a . The breakpoints were selected at times 0, 2, 6, and 11 h after lps injection. It can be observed that $IR(t)$ has a value different from zero at $t = 0$. This result should be interpreted as an almost instantaneous increase in the synthesis of fever mediators after the lps injection. The effect of naproxen plasma concentrations on the inhibition of $IR(t)$ was described by an inhibitory E_{max} model. Table 2 lists the estimates of the

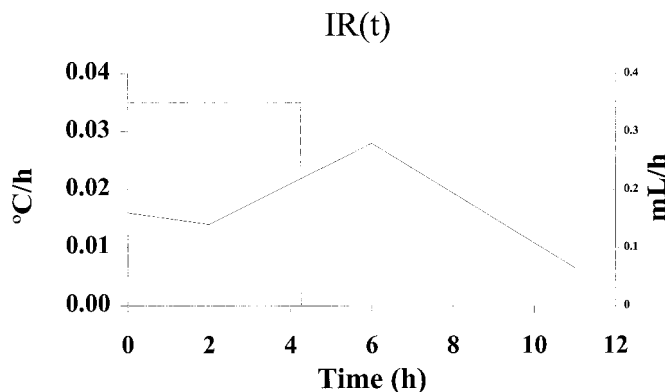


Fig. 5. Profiles of the selected input rate functions of fever (solid line) and inflammation (dashed line) mediators.

TABLE 2

Pharmacodynamic results obtained from the pharmacokinetic/pharmacodynamic modeling of the antipyretic effect of naproxen in the rat

Estimates of interanimal (IAV) variability are expressed as coefficients of variation (%). Precision of the estimates is expressed as relative standard error in parentheses. Relative standard error is standard error divided by the parameter estimate.

Parameter	Estimate	IAV
$P_{(0)}$ ($^{\circ}C/h$)	0.016 (0.20)	N.E.
$P_{(2)}$ ($^{\circ}C/h$)	0.014 (0.14)	N.E.
$P_{(6)}$ ($^{\circ}C/h$)	0.028 (0.13)	N.E.
$P_{(11)}$ ($^{\circ}C/h$)	0.0065 (0.31)	N.E.
K_{out} (1/h)	0.798 (0.13)	39 (0.42)
E_0 ($^{\circ}C$)	37.3 (0.006)	0.7 (0.24)
IC_{50} ($\mu g/ml$)	4.24 (0.22)	327 (0.42)

$P_{(0)}$, $P_{(2)}$, $P_{(6)}$, and $P_{(11)}$, heights of the linear spline located at the 0-, 2-, 6-, and 11-h breakpoints, respectively. $P_{(0)}$, $P_{(2)}$, $P_{(6)}$, and $P_{(11)}$ determine the shape of $IR(t)$ (see Fig. 5); K_{out} , first order rate constant of degradation of T^a ; E_0 , baseline T^a ; IC_{50} , naproxen plasma concentration eliciting half of maximum $IR(t)$ inhibition; N.E., not estimated in the model.

parameters of the linear spline and pd parameters and their estimates of interanimal variability; all parameters were estimated with an adequate precision. During the model building process E_{max} was estimated close to 1; for that reason, its value was fixed (Table 2). At times before lps injection $dT/dt = 0 = K_{syn} - K_{out} \cdot E_0$, where E_0 is the basal T^a ; then $K_{syn} = K_{out} \cdot E_0$. The typical value of K_{syn} computed using the estimates of K_{out} and E_0 was $29.76^{\circ}C/h$.

Study II

Pharmacokinetics. A one-compartment model was enough to describe the disposition of naproxen in plasma when the drug was given orally. The typical values of the estimates (and their associated interanimal variability) for plasma clearance (CL) and apparent volume of distribution (V), which in the case of the three-compartment model is the sum of the initial volume of distribution, and shallow and deep compartment volumes of distribution (V_1 , V_2 , and V_3 , respectively; Table 1), were very similar between the two studies. Table 3 lists the typical pk parameters and their values of interanimal variability for naproxen obtained from study II; the mean observed and typical model-predicted plasma naproxen concentration versus time profiles are shown in Fig. 6. Relative bioavailability for the group receiving the lowest dose was fixed to 1, and bioavailability for groups receiving 3.2 and 10 mg/kg were estimated with a value significantly lower than 1 ($p < 0.05$); a 25% reduction of bioavailability was found in the highest dose group. Mean maximum plasma concentrations of naproxen were observed 20 min after drug administration in all groups and with

TABLE 3

Pharmacokinetic parameter estimates of naproxen given orally at doses of 1.7, 3.2, and 10 mg/kg to rats

Estimates of interanimal (IAV) variability are expressed as coefficients of variation (%). Precision of the estimates is expressed as relative standard error in parentheses. Relative standard error is standard error divided by the parameter estimate.

Parameter	Estimate	IAV
V (ml/kg)	164 (0.16)	16 (0.34)
CL (ml/h/kg)	33.6 (0.21)	20 (0.44)
K_A (1/h)	14.82 (0.08)	50 (0.48)
F (1.7 mg/kg)	1 FIX	N.E.
F (3.2 mg/kg)	0.8 (0.9)	N.E.
F (10 mg/kg)	0.77 (0.1)	N.E.

V , volume of distribution; CL, total plasma clearance; K_A , first order rate constant of absorption; F , bioavailability; N.E., not estimated in the model; FIX, fixed parameter.

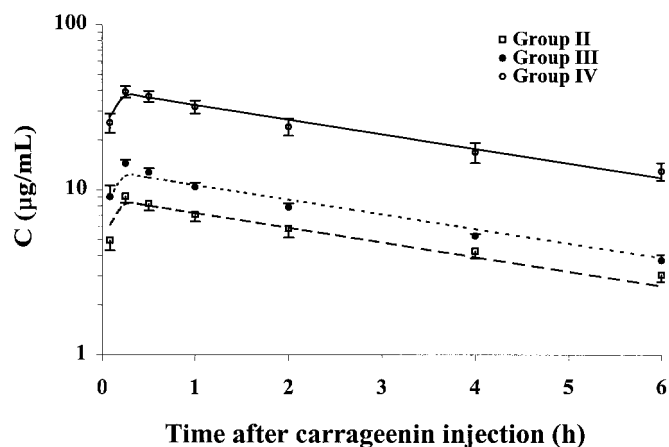


Fig. 6. Time course of plasma concentrations of naproxen in study II. Symbols represent mean observed data, lines represent typical model predictions, and vertical lines represent standard deviations.

values of 9.1 ± 0.8 , 14.4 ± 0.7 , and 39.3 ± 3.1 $\mu\text{g/ml}$ for the 1.7-, 3.2-, and 10-mg/kg dose groups, respectively.

Pharmacodynamics. Figure 7 shows the mean observed paw swelling versus time profiles. Mean basal swelling values did not differ significantly between groups ($p > 0.05$). A maximum paw swelling of 46.55 ± 2 , 48.7 ± 2.5 , 44.5 ± 2.3 , and 40.5 ± 2.4 ml was found for groups I-IV, respectively. These maximums were observed at 3.3 ± 0.8 , 4.8 ± 0.26 , 5.5 ± 0.55 , and 5.9 ± 0.2 h, after the injection of carrageenin, respectively. No statistical differences were found between groups at the end of the experiment ($p > 0.05$). In groups III and IV paw swelling remained at basal levels for 2 ± 0.89 and 3 ± 0.52 h after the start of the experiment, respectively.

Pharmacokinetic/Pharmacodynamic Modeling Results. Figure 7 shows the typical model predicted time course of the inflammation response using the model described by eqs. 3 and 4 and Fig. 2 (bottom). In this case the shape of the selected linear spline was similar to the profile of a constant input rate function that could be characterized by K_0 (zero order rate constant) and T_{syn} (duration of the input rate function). Figure 5 shows in dashed line the profile of the input rate function of inflammation mediators. The effect of naproxen was described by an inhibitory sigmoidal E_{max} model. The estimates of the pd parameters and their estimates of interindividual variability are listed in Table 4. E_{max} was also fixed to 1, since in the case of groups III and IV,

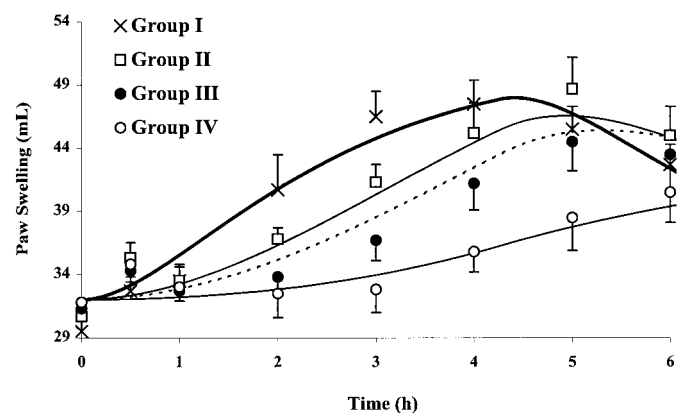


Fig. 7. Time course of inflammation after carrageenin injection and naproxen administration (symbols, mean observations; lines, typical model predictions). Vertical lines represent standard deviations.

TABLE 4

Pharmacodynamic results obtained from the pharmacokinetic/pharmacodynamic modeling of the anti-inflammatory effect of naproxen in the rat

Estimates of interanimal (IAV) variability are expressed as coefficients of variation (%). Precision of the estimates is expressed as relative standard error in parentheses. Relative standard error is standard error divided by the parameter estimate.

Parameter	Estimate	IAV
K_{out} (1/h)	0.6 (0.45)	47 (0.35)
K_{IN} (1/h)	1.05 (0.4)	N.E.
K_0 (ml/h)	0.35 (0.4)	N.E.
T_{syn} (h)	4.26 (0.07)	N.E.
E_0 (ml)	32 (0.01)	36 (0.53)
IC_{50} ($\mu\text{g/ml}$)	4.13 (0.27)	7 (0.31)
γ	1.5 (0.11)	N.E.

K_{out} , first order rate constant of degradation of inflammatory response; K_{IN} , first order rate constant of release of inflammation mediators into the inflammation compartment; K_0 , zero order rate constant rate of synthesis of inflammation mediators; T_{syn} , duration of inflammation mediator synthesis; E_0 , baseline paw swelling; IC_{50} , naproxen plasma concentration eliciting half of maximum K_{IN} inhibition; γ , slope parameter; N.E., not estimated in the model.

plasma drug concentrations were able to block completely the onset of inflammatory response for 2 and 3 h, respectively. K_{syn} , computed as in study I, had a value of 19.2 ml/h.

Discussion

Study I

Pharmacokinetics. The estimates of V_1 (45.2 ml/kg) and CL (32 ml/h/kg) obtained from the fit of i.v. data were in agreement with those published previously in rats (Lauroba et al., 1986; Castañeda-Hernández et al., 1995), and also in humans where the estimates of CL and V reported by Davies and Skjodt (2000) were 42 ml/h/kg and 100 ml/kg, respectively. The fact that a three-compartmental model was selected, when, in general, most authors have used the two-compartmental model to describe the kinetics of naproxen in plasma, could be related to the i.v. infusion design used here and the extensive sampling.

Pharmacodynamics. To describe the time course of the antipyretic effects of naproxen, estimating at the same time reliable pd parameters, we induced fever experimentally by i.p. injection of lps (0.1 mg/kg) from *E. coli* (Wachulec et al., 1997). Several authors have used the same design to study the physiological factors influencing fever response (Cao et al., 1997; Matsumura et al., 1998; Molina-Holgado et al., 1998). Our results were very similar to those obtained by Wachulec et al. (1997), using rats of the same strain, sex, and age, and receiving the same dose of lps; the authors found a maximum mean increase of 38.6°C located 6 h after lps injection. In our study those values were 38.3°C and 5.5 h, respectively. At the time the experiment ended, 11 h after lps injection, mean observed T^{a} was 37.92°C , a reduction of almost 40% on the maximum achieved.

On the basis of observed response versus time profiles, a suitable model should take into consideration the following issues: 1) no circadian variation in T^{a} ; 2) the transient increase in T^{a} , after lps injection, is elicited by an increase in the synthesis of PGE_2 ; 3) naproxen before lps injection does not elicit any change in T^{a} ; 4) in adequate concentrations, naproxen can inhibit the transient increase in T^{a} ; and 5) there is no evidence of a rebound effect.

In our proposed model, the equation $dT/dt = K_{\text{syn}} - K_{\text{out}} \cdot T^{\text{a}}$ predicted a time invariant baseline T^{a} . The estimate of K_{out} (0.798 1/h) was similar to the 0.89 and 1.17 1/h estimates

given by Garg and Jusko (1994) and Trocóniz et al. (2000), respectively, during the pk/pd modeling of the antipyretic effects of racemic ibuprofen in children. Since there was a lack of information regarding the time course of the increasing formation of PGE₂, that profile was estimated by the model using a linear spline constrained to be non-negative. Figure 5 shows that in fact the value of the input rate function, called IR(t), is not constant and once it reaches a peak at 6 h after lps injection it declines linearly. At the end of the experiment (11 h after lps injection) the value of the input rate function was significantly different from zero ($p < 0.05$). Splines have been used for exploratory analysis and modeling purposes (Gries et al., 1999; Fattinger et al., 2000) when there was not an a priori, physiological basis on which to choose a specific form; in our case a time invariant or a periodic input rate constant of synthesis of PGE₂. Allowing IR(t) to be non-negative and independent of K_{syn} , it was possible to account for points 3 and 5 (see above). The most likely mechanism of action of the NSAIDs, the inhibition of the synthesis of PGE₂ (Zhang et al., 1997), is represented by an E_{max} model decreasing IR(t). The estimate of IC₅₀ (4.24 μg/ml) is close to the IC₅₀ values of 10.1 and 6.18 μg/ml given by Garg and Jusko (1994) and Trocóniz et al. (2000), respectively.

Study II

Pharmacokinetics. When the area under plasma drug concentrations corrected by mean dose versus time curves (AUC_{dose}) was computed, the value of AUC_{dose} obtained from the 1.7-mg/kg oral dose was comparable with the value of AUC_{dose} from study I (29 and 30 mg · h/ml, respectively), but AUC_{dose} from the 3.2- and 10-mg/kg oral dose groups tended to be lower (23 and 22 mg · h/ml, respectively). On the basis of these preliminary results we assumed complete F for the lowest oral dose group and allowed the estimation of F for the higher oral dose groups. Previous studies have also shown that naproxen has a high but not complete F (Lauroba et al., 1986). The reason for a decrease in F when the dose is increased is not clear but it could be the case that there is a limited time for the drug to be absorbed in the gut, and that time limitation could cause the fraction of dose absorbed to be lower for the highest doses.

Pharmacodynamics. The carrageenin-induced edema model has long been used to evaluate the anti-inflammatory activity of NSAIDs (Seibert et al., 1994; Smith et al., 1998). The time profile of the paw swelling found for the baseline group in the current study was very similar to the one published by Castañeda-Hernández et al. (1995), using rats of the same strain, sex, and age, receiving the same dose of carrageenin. They found a maximum paw swelling increase of 38% located after 4 h after carrageenin injection, whereas we observed corresponding values of 46.2% and 4 h, respectively. By the time the experiment was stopped, average paw volume had been declined to 34.3% in our study and 35% in Castañeda-Hernández et al. (1995) study. On the other hand, Davies and Skjodt (2000) indicated that plasma naproxen concentrations of 50 μg/ml or doses between 3.5 and 22 mg/kg were needed to achieve an adequate anti-inflammatory response in humans, which are values very close to the ones reported in the current study.

To estimate pd parameters of naproxen “in vivo” we proposed several models to fit the experimental data. Previous

studies had shown that COX-2 expression was experimentally induced in the footpad by carrageenin and that the elicited inflammation could be blocked by the administration of a selective COX-2 inhibitor (Seibert et al., 1994). These results support the hypothesis that COX-2-derived PGE₂ plays a crucial role in the initiation and maintenance of inflammatory response. On the basis of these considerations, a first attempt was to propose a model similar to the one used during study I to describe antipyresis. However, the only group that could be adequately described was the control group. The reason for the lack of fit of a simple indirect response model is due to the fact that the effect of naproxen seems to be more related to a delay in the onset of the inflammatory response rather than to a decrease in the extent of inflammation. Indeed, it appears that naproxen acted at the level of blocking the pass from the mediator compartment to the inflammatory response compartment, i.e., acting on the release more than synthesis of mediators.

The model describing the time course of inflammatory response in the control group has a similar structure to the one published recently by Gozzi et al. (1999) to account for the transient increase in plasma concentration of lps-induced tumor necrosis factor- α in the mouse. However, when this model was used to fit our data no satisfactory predictions were obtained. Thus, we adapted the model in such a manner that the drug acts through the inhibition of K_{IN} instead of through the inhibition of K_0 as in Gozzi's study. Our pk/pd model assumes that inflammation mediators increase their production for a certain time after carrageenin injection, and that the increase in the inflammatory response depends on the amount of such mediators. Indeed, Fitzgerald et al. (1981) described a first order rate for the release of a prostaglandin in humans, whereas the period of the observed increase in COX-2 mRNA in vivo, as well as the proinflammatory PG formation, reported by Seibert et al. (1994) and Smith et al. (1998) highly correlates with the duration of inflammatory mediator synthesis (T_{syn}) estimated by the model. It should be noted that the shape of IR(t) selected in the inflammation study differs from the shape obtained during the analysis of the antipyretic effects (Fig. 5), however, the estimates of IC₅₀ for the antipyretic and anti-inflammatory effects, 4.24 and 4.13 μg/ml, respectively, are very similar, and resemble the value for in vitro inhibition of COX-1/COX-2 for the NSAID (2.2/1.3 μg/ml; Mitchell et al., 1993).

It is important to underline that our model was unable to describe the first peak in paw swelling observed at 0 to 15 min. However, the edema produced by this period might be the result of local bradykinin production insensitive to the action of anti-inflammatory NSAIDs as described Seibert et al. (1994). Therefore, this lack of predictability of the model might be considered irrelevant to characterize the pd of naproxen.

In summary, different pk/pd models were proposed to describe the antipyretic and anti-inflammatory effects of naproxen in the rat, but can also be generalized to a class of models dealing with complex interactions between time-variant disease-induced response and drug effects. Antipyresis could be described using a standard indirect response model, but anti-inflammatory response required a more complex model, including an extra compartment dealing with the time course of the inflammatory mediators. These differences may be related to the level of interaction of naproxen with

cyclooxygenase, i.e., if PGE₂ produced in brain is the main factor responsible of lps-induced fever, the inhibition of its biosynthesis would be the limiting step for the antipyretic activity of naproxen. On the other hand, inflammation appears to be a more complex process where edema formation involves vascular events, local and recruited cell activation, production of mediators, and finally synthesis of proinflammatory prostanoids. Thus, there may be more than one target in that cascade of events, which explain the anti-inflammatory effect of naproxen.

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