Research Article

Semi-mechanistic Modeling of the Interaction Between the Central and Peripheral Effects in the Antinociceptive Response to Lumiracoxib in Rats

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Abstract. The model-based approach was undertaken to characterize the interaction between the peripheral and central antinociceptive effects exerted by lumiracoxib. The effects of intraplantar and intrathecal administrations and of fixed ratio combinations of lumiracoxib simultaneously administered by these two routes were evaluated using the formalin test in rats. Pain-related behavior data, quantified as the number of flinches of the injected paw, were analyzed using a population approach with NONMEM 7. The pain response during the first phase of the formalin test, which was insensitive to lumiracoxib, was modeled using a monoexponential decay. The second phase, which was sensitive to lumiracoxib, was described incorporating synthesis and degradation processes of pain mediators that were recruited locally after tissue injury. Upregulation at the local level and in the central nervous system (CNS) was set to be proportional to the predicted levels of pain mediators in the local (injured) compartment. Results suggest a greater role of upregulated COX-2_{Local} in generating the pain response compared to COX-2_{CNS}. Drug effects were described as inhibition of upregulated COX-2. The model adequately described the time course of nociception after formalin injection in the absence or presence of lumiracoxib administered locally and/or spinally. Data suggest that the overall response is the additive outcome of drug effects at the peripheral and central compartments, with predominance of peripheral mechanisms. Application of modeling opens new perspectives for understanding the overall mechanism of action of analgesic drugs.

KEY WORDS: formalin test; interaction model; lumiracoxib; NONMEM; pharmacodynamic modeling.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are being currently used in the treatment of several diseases related with acute and chronic musculoskeletal pain, such as osteoarthritis and rheumatoid arthritis. However, it is well known that their long-term use may cause gastrointestinal ulceration, perforation, and bleeding (1), being the local inhibition of the cyclooxygenase-1 (COX-1) enzyme, which generates cytoprotective prostaglandins in the gastrointestinal tract (2, 3) responsible of the adverse effects. The identification of COX-2 that is upregulated as a consequence of an inflammation process (4) was followed by the development of COX-2 selective inhibitors. Lumiracoxib, the most selective COX-2 inhibitor that has been used in humans (5), has a similar analgesic efficacy, and cardiovascular risk (6), and greater gastrointestinal safety (7) in comparison to NSAIDs that are not selective COX-2 inhibitors.

It has been suggested recently that withdrawal from the market of several coxibs due to their side effects was in part due to the lack of information of the pharmacokinetic/pharmacodynamic (PK/PD) properties and the fact that dose selection was done mainly based on pain relief and less based on understanding of the relationship between the degree of COX inhibition and analgesia (8). The dosing regimens for most drugs, including coxibs, are selected on the sole basis of pharmacokinetic data. Pharmacodynamic is seldom taken into consideration. It should be noted that a rational dosing regimen should consider the rates of appearance and disappearance of the pharmacological effects, both therapeutic and untoward. This requires the understanding of the principal mechanisms involved in the pharmacological response.

We have previously reported a PK/PD model which adequately describes the time course of the antinociceptive

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response of systemic lumiracoxib as a function of its inhibitory effect on COX-2 (9). However, it is well known that the overall antinociceptive response of NSAIDs (10–12), and particularly of lumiracoxib (13), depends on the actions at the site of inflammation as well as at the central nervous system (CNS). However, the relative contribution of peripheral and central COX-dependent mechanisms in the overall antinociceptive response remains elusive (10). In other words, our current knowledge on the mechanisms underlying the antinociceptive action of NSAIDs is still fragmentary and far from being complete.

In order to improve our understanding on the mechanism of analgesic action of lumiracoxib, the objective of the current study was to characterize the *in vivo* contribution of the local and central signaling pathways involved in the antinociceptive effect. The rat formalin model was employed to experimentally induce nociception. The quantitative approach was used to integrate into a single computational PK/PD type model all data generated, isolating system-dependent parameters from drug-related parameters. This approach makes inferences regarding the mechanisms of action and characterizes the absence (additivity) or the presence of interaction (antagonism/synergism) in the analgesic effects exerted between the peripheral and central compartments. The results described in the current work suggest that the overall response is the additive outcome of drug effects at the peripheral and central compartments, with predominance of peripheral mechanisms.

METHODS

Animals

Experiments were performed on adult female Wistar rats (body weight range, 180–220 g) of 6 to 7 weeks of age. Animals were obtained from our own breeding facilities (Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City, Mexico) and had food and water *ad libitum* before experiments. Experiments were in accordance with the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (14) and were approved by the Institutional Animal Care and Use Committee (Centro de Investigacion y de Estudios Avanzados, Mexico, DF, Mexico).

Intrathecal Surgery

Chronic catheterization of the intrathecal subarachnoid space was performed as described by Yaksh and Rudy (1976) under ketamine–xylazine mixture (45–12 mg/kg, i.p.) anesthesia. Briefly, a polyethylene catheter (PE-10, 8.0 cm length) was inserted intrathecally and located at the level of the thoracolumbar junction (11). Animals were allowed to recover from surgery for at least 5 days. Once the experiments were finished, the correct position of the catheter was assessed as it has been described previously (13) and animals were euthanized in a CO₂ chamber afterwards.

Measurement of Antinociceptive Activity

Antinociception was assessed using the formalin test (11, 12, 15). Briefly, 50 μ L of a solution of formalin (1%) was injected subcutaneously into the dorsal surface of the right

hind paw and immediately after nociception was observed. Nociception was expressed as the number of flinches or shakings of the injected paw during 1-min periods every 5 min, up to 60 min after injection (11, 16).

Drug

Lumiracoxib was kindly provided by Novartis Farmaceutica (Mexico City). Lumiracoxib was dissolved in dimethyl sulfoxide 20% and 100% for local peripheral and intrathecal administration, respectively. Subsequent dilutions were performed in isotonic saline.

Study Design

Eighty-six rats were randomized into 14 groups of six animals each as follows: four groups for the local lumiracoxib administration, four groups for the intrathecal route, five groups for the combined route administration, and one for the animals with saline. For local peripheral drug administration, rats received an intraplantar (i.pl.) injection into the dorsal surface of the right hind paw of vehicle or increasing doses (10, 30, 100, and 300 μ g) of lumiracoxib 20 min before formalin injection. For intrathecal (i.th.) administration, rats received an i.th. injection of increasing doses (10, 30, 100, and 300 μ g) of lumiracoxib 10 min before formalin injection into the right paw, and nociceptive behavior was assessed. Doses and times of administration were selected on the bases of pilot studies and previous reports (12, 13). For the combined route administration, rats received an i.pl. injection into the dorsal surface of the right hind paw of increasing doses of lumiracoxib 20 min before formalin injection and an i.th. injection of increasing doses 10 min before (i.pl. + i.th.: 13.52 + 13, 26 + 27, 52 + 54, 104 + 108, and 208 + 216 μ g). These animals received simultaneously, both an i.pl. and i.th. injection of lumiracoxib, to get a dose–response curve in a fixed ratio (17), based on the ED₃₀ of each individual route (12). Figure 1a summarizes schematically the experimental design using in the current work.

The dose–response curves were constructed and the experimental points fitted using least-squares linear regression. ED₃₀ $\pm$ standard error was calculated according to the method described by Grabovsky (18). ED₃₀ was used instead of ED₅₀ values since lumiracoxib was not able to reach more than 50% of antinociception in the formalin model (13). To perform the isobolographic analysis, spinal and peripheral lumiracoxib were administered in combination as fixed ratios of equieffective ED₃₀ for each drug.

Data Analysis

Despite the number of flinches that represents a count variable, it was analyzed as continuous due to the way it was collected. The number of flinches was not recorded continuously during the experiment; therefore, there are time windows with no data available. All data available (number of flinches vs. time) were modeled on the basis of the population approach using the software NONMEM 7 with the first-order conditional estimation method together with the INTERACTION option (Icon development solutions). During the population analysis, the typical model parameters (common to all animals), the

Fig. 1. **a** Schematic representation of the experimental design used in the current study. **b** Time course of formalin-induced pain response after saline and 300 µg local and intrathecal administration of lumiracoxib observed in average. Note that all groups included saline received an injection of formalin which elicited transient nociception

magnitude of their variability, and the residual error associated to the observations are estimated simultaneously. Inter-animal variability and residual error were modeled using exponential and additive models, respectively.

Model Selection

Selection between different models was performed mainly by (1) exploring visually the agreement between observations and typical/individual model predictions, (2) taking into account the precision of the model parameter estimates (based on the standard error given by NONMEM), and (3) using as additional criteria to guide during model selection, the log-likelihood ratio test and the Akaike information criteria (AIC) (19) for nested and non-nested models, respectively. For nested models, a decrease in the minimum value of the objective function provided by NONMEM and approximately equal to $-2 \times \log(\text{likelihood})$ ($-2LL$) of 6.63 or 10.83 points for an added parameter was regarded as a significant model improvement corresponding to p values of 0.01 and 0.001, respectively. For non-nested models, the model corresponding to a lower AIC value calculated as $AIC = -2LL + 2 \times N_p$, where N_p is the number of parameter in the model, was selected.

Model Evaluation

Model performance was evaluated through visual and numerical predictive checks (VPC and NPC, respectively) (9). Five hundred studies of the same design characteristics as the original studies were simulated. To develop the VPC, at each time of measurement, the 2.5, 50, and 97.5th percentiles were calculated in every simulated study. Then, the 95% prediction intervals from the resultant 50th percentiles, and the 50th

percentiles of the 2.5 and 97.5th resultant percentiles were computed, and represented over time together with the raw data. NPC, for each simulated study and dose level, the maximum and total number of flinches were recorded in each animal and the median value was calculated. The 2.5th, 50th, and 97.5th percentiles of the median values across the 500 simulated studies were then computed and compared with the median raw data.

Model Development

Model development was based on the observed number of flinches. During model development, it has been assumed that the number of flinches were directly proportional with the (unobserved) amount of COX-2 which was also assumed to be directly proportional with the (unobserved) amount of pain mediators denoted by MED that were not identified in the current study. The main components of the selected model are described in detail below. Nevertheless during the model building process, different variants of the final model were also explored.

1. Model for the Formalin-Induced Pain

Figure 1b shows the formalin-induced pain response after saline and 300 µg local and intrathecal administration of lumiracoxib observed in average. Note that all groups included saline received an injection of formalin which elicited transient nociception. Nociception induced by formalin showed a clear biphasic time profile. The first phase was characterized by a very early peak of nociception followed by a rapid decay. The time profile of the first pain phase, PN_1 (insensitive to lumiracoxib),

was modeled according with the following expression (Eq. 1):

$$\frac{dPN_1}{dt} = -K_{PN1} \times PN_1 \quad (1)$$

where dPN_1/dt reflects the rate of change of the pain mediators responsible of the first nociceptive phase, and K_{PN1} is the first-order rate constant of degradation of those pain mediators. At the time of formalin injection (initial condition) $PN_1 = PN_{1,0}$, a parameter to be estimated during the modeling process together with K_{PN1} .

The second phase appeared with a certain delay (15 min) with respect to the formalin insult and showed a slower increase and decay of the pain response compared to PN_1 . An early activation of both COX-2 mRNA and protein, after i.pl. injection of formalin, has been reported (20, 21) followed by the synthesis of pain mediators (MED). Such synthesis of early pain precursors was described using the analytical solution of the transit compartments model trying to mimic the synthesis delay of COX2 and other pain mediators; however in the end, the outcome variable is the number of flinches. In the absence of COX2 observations and other pain mediators, it is not possible to propose a more mechanistic/physiological model. The solution of this model has been previously applied to describe drug absorption process (22) and has the following expression (Eq. 2):

$$MED = MED_0 \times \frac{(K_{TR} \times t)}{NC!} \times e^{-K_{TR} \times t} \quad (2)$$

where MED_0 was arbitrarily set to 1. NC is the number of transit pain mediator compartments, and K_{TR} the first-order rate constant of transfer. At time of formalin injection, MED is equal to 0. This equation describes the shape of the second phase of the nociceptive effect as a consequence of the formalin injection consisting of an increase followed by a decrease in the number of flinches. Later, two new model parameters (θ_{COX-2_L} and θ_{COX-2_CNS}) will be used as scaling factors allowing the fitting of the model to the number of flinches.

The time course of COX-2 in the compartment where formalin was injected ($COX-2_{Local}$) was set to be proportional to the concentration of pain mediators in that compartment (MED). Local tissue damage also generates a widespread induction of COX-2 expression in the spinal cord neurons and in other regions of the CNS (23). In the current analysis, it has been also assumed that the level of COX-2 in CNS ($COX-2_{CNS}$) was proportional to MED. Equations 3 and 4 described the relationships for $COX-2_{Local}$ and $COX-2_{CNS}$, respectively.

$$COX-2_{Local} = \theta_{COX-2_L} \times MED \quad (3)$$

$$COX-2_{CNS} = \theta_{COX-2_CNS} \times MED \quad (4)$$

where θ_{COX-2_L} and θ_{COX-2_CNS} are scaling factors allowing fitting of the model to the number of flinches, and within the current model, they represent the proportionality parameters between $COX-2_{Local}$, $COX-2_{CNS}$, and MED, respectively. θ_{COX-2_L} and θ_{COX-2_CNS} can be estimated precisely due to the fact that during the experiment lumiracoxib was injected locally, intrathecally, and both locally and intrathecally to

different groups of animals. At time of formalin administration, $COX-2_{Local}$ and $COX-2_{CNS}$ are equal to 0.

The pain response corresponding to the second phase (PN_2) is the sum of $COX-2_{Local}$ plus $COX-2_{CNS}$.

$$PN_2 = COX-2_{Local} + COX-2_{CNS} \quad (5)$$

Finally the complete response profile (PAIN) integrating PN_1 and PN_2 is given by Eq. 6:

$$PAIN = PN_1 + PN_2 \quad (6)$$

2. Model for the Antinociceptive Effects of Lumiracoxib

The unobserved time profiles of lumiracoxib levels (unit less) in the local and intrathecal compartments ($LUMX_{Local}$ and $LUMX_{CNS}$, respectively) were modeled assuming a monoexponential decay represented by the following two expressions:

$$\frac{dLUMX_{Local}}{dt} = -K_{D_Local} \times LUMX_{Local} \quad (7)$$

$$\frac{dLUMX_{CNS}}{dt} = -K_{D_CNS} \times LUMX_{CNS} \quad (8)$$

K_{D_Local} and K_{D_CNS} are the first-order rate constant of elimination of lumiracoxib from the local and CNS compartments, respectively. At times at which lumiracoxib was injected, the initial conditions of both compartments were equal to the administered dose.

Antinociceptive effects of lumiracoxib administered locally (E_{Local}) or intrathecally (E_{CNS}) were described as follows:

$$E_{Local} = \frac{1}{1 + LUMX_{Local}} \quad (9)$$

$$E_{CNS} = \frac{1}{1 + LUMX_{CNS}} \quad (10)$$

Finally, PN_2 in Eq. 5 has the form of:

$$PN_2 = COX-2_{Local} \times E_{Local} + COX-2_{CNS} \times E_{CNS} \quad (11)$$

To summarize, when $LUMX_{Local}$ and $LUMX_{CNS}$ are greater, E_{Local} and E_{CNS} become lower as well as PN_2 (Eq. 11), describing finally the net effect of lumiracoxib, reflected as a decrease in the number of flinches. Figure 2 shows the schematic representation of the selected model based on Eqs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11.

3. Characterization of the Type of Interaction Between the Local and CNS Antinociceptive Effects of Lumiracoxib

The type of interaction was evaluated creating the isobolograms associated to a level of effect corresponding to the 20% change in the (1) maximum number of flinches, N_{MAX} , and (2) cumulative number of flinches, N_{TOT} , reached during the second pain phase between lumiracoxib and saline administration. We simulated all possible dose-route (local/intrathecally) combinations from 0 to 300 μ g in increments of 50 μ g for both routes. The

Fig. 2. Schematic representation of the selected population model. MED, unobserved pain mediators, $LUMX_{Local}$, $LUMX_{CNS}$, and unobserved levels of lumiracoxib in the local and intrathecal compartments, respectively. PN1, PN2, pain responses corresponding the first and second phases observed after local injection of formalin, respectively. The rest of the terms are defined in the text

non-simulated intermediate doses were calculated by means of a bilinear interpolation process. We calculated from these data all possible dose–route combinations that achieved 20% effect for both descriptors, N_{MAX} and N_{TOT} .

To appropriately interpret the results of the isobolograms for each type of effects explored, i.e., N_{MAX} and N_{TOT} , a dose–response curve was established for lumiracoxib injected locally or intrathecally. The rationale behind this exercise relies on the fact that, only when equally effective doses have the same ratio over the range of effects being studied, the straight line plotted in the isobologram (called isobole) represents additive (non-interaction) effects (18). Therefore, the real isoboles corresponding to N_{MAX} and N_{TOT} obtained simply as the sum of the simulated effects obtained from each route of administration alone were also calculated and compared with those obtained from the pain model developed.

4. Analysis of the Impact of the Route of Administration on the Antinociceptive Response

Depending on the administration route used, same doses of lumiracoxib exert different N_{MAX} and N_{TOT} . We simulated all possible dose–route (local/intrathecally) combinations from 0 to 150 μg in increments of 50 μg for both routes and calculated N_{MAX} and N_{TOT} . The non-simulated intermediate doses were calculated by means of a bilinear interpolation process. For every dose/route combination, we also calculated N_{MAX} and N_{TOT} as if the same drug amount was given alone using solely one route (local or intrathecal). The difference between the combined *vs.* unique administration was symbolized through color maps.

RESULTS

During the data analysis process, different models were explored. For example, a nonlinear function model was initially

assayed to describe the time course of the second pain phase, in a manner analogous to the modeling of the pain response induced by injection of carrageenan in rats (9). This model was discarded as a poor fit was obtained. For the case of upregulated $COX-2_{Local}$ and $COX-2_{CNS}$, delayed time courses compared with MED were also tested, but it was found that predicted COX-2 and MED profiles were undistinguishable. Nonlinear E_{MAX} -type relationships between COX-2 and MED were evaluated but data description did not improve significantly ($p > 0.05$). Scaling the predicted lumiracoxib concentrations in Eqs. 9 and 10 introducing two IC_{50} parameters was found to be not significant for both $LUMX_{Local}$ and $LUMX_{CNS}$ ($p > 0.05$).

Table I lists the parameter estimates obtained for the model selected. Estimates of the parameters were obtained with good precision. Data showed moderate inter-animal variability on NC, $PN_{1,0}$, and $\theta_{COX-2-L}$. Variability was also tested in the rest of the parameters but resulted as not significant ($p < 0.05$). Figure 3 shows the response *vs.* time profiles for animals chosen at random within each dose (single and combination) group and route of administration, and where it can be observed that the two phases of the pain response profiles are described, in general, very well by the selected model.

The simulation-based procedures undertaken in the current analysis to internally evaluate the performance of the model (visual and numerical predictive checks) also indicated that the model is supported by the data available. In Fig. 4, all data gathered are represented together with the results obtained from 500 simulated datasets. The mean tendency of the data and their dispersion are well captured by the model for each experimental condition [dose (single/combination) and route]. Similarly, Table II, where the results from the numerical predictive check are summarized, supports the selection of the final model. For the two response descriptors, N_{MAX} and N_{TOT} , the 50th percentiles

Table I. Model Parameter Estimates Corresponding to the Selected Model

Parameter	Estimates (CV%)	IAV (CV%)
K_{D_Local} (min^{-1})	0.129 (5.34)	—
K_{D_CNS} (min^{-1})	0.073 (22.16)	—
θ_{COX-2_L} (flinches)	94.0 (7.79)	15.87 (42.85)
θ_{COX-2_CNS} (flinches)	28.5 (24.91)	—
$PN_{1,0}$ (flinches)	18.7 (3.14)	22.4 (20.51)
K_{PN1} (min^{-1})	0.279 (6.37)	—
NC (n)	6.5 (4.92)	11.09 (21.13)
K_{TR} (min^{-1})	0.233 (4.20)	—
Residual error (flinches)	2.93 (19.11)	—

Parameters are listed as estimated with the coefficient of variation (CV%) in parenthesis. Model parameters are defined in the text IAV inter-animal variability

calculated from the raw data fall (for most of the cases) within the 2.5–97.5th percentile range obtained from the model-based simulations and are close to the simulated 50th percentiles.

Figure 5 explores the kinetics and dynamics of the underlying processes integrated in the selected model. Figure 5a shows the contribution of the PN_1 and PN_2 variables to the overall formalin-induced pain response. The profile corresponding to PN_1 is the result of assuming an exponential decay governed by the parameter K_{PN1} estimated in 0.279 min^{-1} , with an initial pain load of 18.7 flinches. The time course of pain mediators (MED in Eq. 2) after injection of saline is represented in panel B, superimposed with the

kinetics of the upregulated COX-2 in the local and CNS compartments. The degree of COX-2 upregulation is greater in the local compartment in comparison to the CNS compartment, the estimates being: $\theta_{COX-2_L}=94$ flinches and $\theta_{COX-2_CNS}=28.5$ flinches (Table I). The model predicted lumiracoxib kinetic profiles in the local and CNS compartments are shown in Fig. 5c. These profiles are controlled by parameters K_{D_Local} and K_{D_CNS} with estimated values of 0.129 and 0.073 min^{-1} , respectively. The relationship between the inhibitory effects of lumiracoxib and its predicted levels in both compartments are presented in Fig. 5d together with maximum predicted concentrations for each administered dose. The kinetic and dynamic profiles shown in Fig. 5b–d conditioned the profiles shown in Fig. 5e, where the time course of upregulated COX-2 in the local and CNS compartments are shown after saline and single dose administration of lumiracoxib.

In the model selected in the current analysis, the mechanism of action of lumiracoxib administered i.pl. or i.th. is the inhibition of upregulated COX-2_{Local} and COX-2_{CNS} resulting from pain mediators synthesis induced by the formalin injection. Data did not support any interaction between upregulated COX-2_{Local} and COX-2_{CNS}, and therefore lumiracoxib exerted independent effects and consequently an additive (non-interactive) effect was expected. However, when isobolograms were constructed for the 20% change from baseline in N_{MAX} and N_{TOT} , a synergistic interaction is suggested (upper panels in Fig. 6). Lower panels in Fig. 6 represent the dose–response relationship for the N_{MAX} and N_{TOT} effects when lumiracoxib was administered i.pl. or i.th.

Fig. 3. Individual observations (circles) and individual model predicted (solid lines) pain response vs. time profiles. Each panel represents a single individual chosen at random from each dose group

Fig. 4. Results from the visual predictive check. Circles raw data; solid lines median of the raw data. Dashed lines represent the 50th percentile of the 2.5 and 97.5th simulated percentiles. The gray area shows the 95% prediction intervals from the simulated 50th percentiles

It can be observed that the dose–response relationships were not parallel, which makes the interpretation of the isobolograms meaningless. In fact the real isoboles obtained simply as the sum of the simulated effects from each route of administration alone were almost superimposed to those resulted from the selected model (Fig. 6, upper panels).

Figure 7 compares N_{MAX} (left panel) and N_{TOT} (right panel) when lumiracoxib is administered at different dose/route combinations and when the same amount of drug dose is given using solely a unique route [local (Fig. 7a) or intrathecal (Fig. 7b)]. The difference between combination vs. single administration is symbolized by color maps. When

Table II. Results from the Numerical Predictive Check

Dose level (μg)	N_{MAX} (flinches)		N_{TOT} (flinches)	
	Raw	50th (2.5–97.5th percentiles)	Raw	50th (2.5–97.5th percentiles)
Local				
0	23.83	19.01 (14.72–25.08)	112.83	103.25 (81.69–131.61)
10	20.5	18.64 (14.35–24.16)	109.33	100.52 (80.17–129.38)
30	18.67	18.08 (13.24–21.12)	99.33	97.41 (78.37–124.57)
100	18.5	16.15 (12.12–21.97)	87.17	89.80 (70.91–114.72)
300	15	14.08 (11.36–17.73)	70.33	76.54 (60.61–97.90)
Intrathecal				
10	20.33	17.24 (13.29–22.76)	99.5	94.78 (72.88–121.77)
30	19.5	16.15 (12.12–21.97)	92.33	89.32 (67.42–119.81)
100	19	15.22 (11.27–21.07)	88	84.08 (63.32–112.29)
300	16.88	14.83 (10.67–20.61)	75.75	81.06 (59.88–111.03)
Combined routes^a				
26.52 (13+13.52)	19	16.57 (12.71–21.76)	86.75	90.64 (69.44–118.59)
53 (26+27)	17.88	15.49 (11.87–20.72)	79.63	84.90 (65.15–111.78)
106 (52+54)	16.13	14.37 (10.93–19.17)	76.63	78.52 (59.20–104.69)
212 (104+108)	16.38	12.93 (9.58–17.40)	73.63	69.95 (52.01–95.55)
424 (208+216)	17.75	11.24 (8.32–15.20)	69.13	60.68 (43.49–83.00)

^a Combined route = (local + intrathecal)

N_{MAX} , maximum number of flinches; N_{TOT} , cumulative number of flinches

Fig. 5. a. Contribution of the PN1 and PN2 to the overall pain response profile b. Model predicted kinetics of pain mediators (MED) and upregulated COX-2 in the local and intrathecal compartments c. Model predicted kinetic profiles of lumiracoxib in the local and intrathecal compartments d. Dynamic profile showing the model predicted upregulated COX-2 inhibitory effect vs. predicted levels of lumiracoxib. Symbols represent the maximum predicted levels achieved at each dose level and administration route e. Model predicted time course of arbitrary levels of upregulated COX-2 in the local and intrathecal compartment after injection of different doses of lumiracoxib

we compared combined administration with the use of the local route (Fig. 7a), the antinociceptive effect was always greater, although the bigger differences were not found for the higher local intrathecal doses. This finding resulted more evident for N_{MAX} (left panel). Similarly when comparing the intrathecal route with the combined version, the analgesic effect was also bigger when the lumiracoxib was given in combination (Fig. 7b). However, as shown in the left panel, there are some combinations that elicited smaller effects. In particular, intrathecal doses below 25 μ g combined with local doses tended to be less effective than the unique intrathecal administration of the same drug amount.

DISCUSSION AND CONCLUSIONS

The concept of self-synergism is used to describe the synergism observed following the administration of even a single agent by different routes (17). Recent preclinical studies have showed that spinal plus supraspinal administration of acetaminophen (24) and intraplantar and intraperitoneal administration of tramadol (25) produced antinociception greater than that expected by simply the sum of individual doses.

The formalin test is a widely used model of persistent pain. While the use of the formalin test in pain research is

Fig. 6. **a.** Dose–response relationship established for the maximum **b.** Dose–response for the cumulative number of flinches **c.** Isobologram for the 20% effect in N_{MAX} **d.** Isobologram for the 20% effect in N_{TOT}

Fig. 7. Comparison among administration routes **a.** Combined routes vs. local route **b.** Combined routes vs. intrathecal route. Zero values stand for same achieved effect when the lumiracoxib dose is given using a unique route or combining both routes (colors near green–blue). Positive values stand for bigger effects when the both routes are used (blue–fuchsia) instead of a unique one. Negative values correspond to smaller effects when both routes are used (yellow–red). N_{MAX} in left panel; N_{TOT} in right panel

high, information as to the mechanisms underlying the nocifensive behaviors produced by formalin are not fully understood. It has been suggested that phase I behaviors mainly result from acute activation of nociceptors by formalin, while phase II behaviors are driven in part by the central sensitization of spinal cord circuits secondary to the barrage of input that occurs during phase I.

Understanding of the actual participation of the peripheral and central processes involved in the antinociceptive effects of lumiracoxib, and the way they interact, may allow mechanism-based strategies for development and assessment of new dosing regimens. To the best of our knowledge, the current evaluation represents the first attempt to quantitatively characterize the entire time course of the pain response induced by formalin injection and drug antinociceptive effects. In addition, the quantitative approach undertaken in this work allowed characterization of the contribution to the analgesic effects of local and CNS signaling pathways, which in the case of lumiracoxib, the results revealed additivity (non-interaction).

The selected model has been extensively evaluated using visual and numerical predictive checks, and the results obtained for the evaluation exercise indicated that the model as well as model-derived parameters were supported by the data available and consequently by the experimental design originally planned. The model comprises two main parts, i.e., inflammation progression and drug effect components. The time course of the nociceptive response in absence of any drug showed a marked change over time once formalin was injected. The temporal- and experimentally induced alterations in the basal response in the field of inflammation and analgesia have been modeled in the past (9, 18, 26, 27). After injection of formalin, the nociceptive response showed a complex profile. Since data suggested that the first and more acute phase was insensitive to drug effects, it was decided to model it totally empirically using a monoexponential decay without any attempt of linking it with a biological process. On the other hand, the second and lasting phase was described semi-mechanistically incorporating synthesis and degradation processes of pain mediators that were recruited locally after tissue injury. In our case, and contrary to what was reported by Giraudel *et al.* (28) and Vázquez-Bahena *et al.* (9), for carrageenan-induced inflammation, the use of their proposed non-linear functions did not provide a good description of the data. This suggests that the mechanisms by which pain mediators are recruited and degraded differ between injection of carrageenan and formalin, as it can be anticipated simply by visual inspection of the saline control groups.

Local and CNS upregulated COX-2 has been set to be proportional to the predicted levels of pain mediators in the local (injured) compartment. The possibility of delayed predicted COX-2 profiles locally and/or in CNS was also assessed but was not supported by the available data. Model estimates of $\theta_{\text{COX-2-L}}$ and $\theta_{\text{COX-2-CNS}}$ suggested a greater role of upregulated COX-2_{Local} in generating the pain response with regard to COX-2_{CNS}. One important finding of the current work is that, through the inclusion of dose groups where lumiracoxib was injected intrathecally, and together with the quantitative modeling approach, the contribution of upregulated COX-2 in the CNS to the overall pain sensation can be characterized and quantified (20, 21).

The way drug effects were integrated in the model followed the known pharmacodynamic properties of selective COX-2 inhibitors and was characterized as an inhibition (decrease) in the model predicted levels of upregulated COX-2. The same approach was used by Vázquez-Bahena and coworkers (9) when the pharmacokinetics and pharmacodynamics of lumiracoxib were studied in rats submitted to carrageenan-induced inflammation. In the present study, drug levels were not determined. Therefore, it was assumed that lumiracoxib disappears exponentially from the compartments where it was injected by distribution to the central or to other compartments and that there is neither redistribution back to the compartment of administration (i.e., local or spinal) nor distribution from the spinal to the local compartment or vice versa.

$K_{\text{D_Local}}$ and $K_{\text{D_CNS}}$ were included in the model as if they were pharmacokinetic-related parameters. Nonetheless, it is difficult to separate them from the pharmacodynamic properties. Assuming a similar pharmacological behavior of lumiracoxib in the local and intrathecal compartments, the present model estimates of those parameters suggest a longer residence of lumiracoxib in the intrathecal compared to the local compartment.

Increasing the mechanistic understanding of the current study would require a challenging design considering experimental measurements of at least COX-2 and lumiracoxib in both compartments. Nevertheless, the model-based approach here described allows making plausible mechanistic interpretation of the data. In this model, lumiracoxib exerts its antinociceptive effects in the local and CNS compartment using the same mechanism of action on independent pathways (i.e., inhibition of COX-2 upregulated by the presence of pain mediators in the local compartment). Therefore, an additive effect can be expected. However when isobolograms were constructed, synergism was indicated. Only when the model was used to simulate N_{MAX} and N_{TOT} effects in a range of doses greater than the experimentally used, and the dose-response relationships were constructed, it was discovered that synergism had to be questioned. Finally, results obtained by simply adding the N_{MAX} and N_{TOT} effects from the local and intrathecal administration served as an additional support of the additive effects of the two routes of administration.

Although the interaction between both routes of administration is additive, it is important to highlight that the intensity of drug effects depends on the route of administration, as well as the dose and combination, and the response descriptor under evaluation. Here we used color maps to visualize the contribution of these factors for both effect descriptors (N_{MAX} and N_{TOT}).

The results here presented demonstrate that it is possible to model the contributions of central and peripheral mechanisms to the antinociceptive effect of lumiracoxib as inhibition of upregulated COX-2 in these two compartments. Therefore, the model complies with the present knowledge on the pharmacology of lumiracoxib and on the physiopathology of the formalin assay. This opens the possibility of modeling the contribution of central and peripheral mechanisms in the overall antinociceptive effect of analgesic agents. Its use may avoid failures, as it has been the case of those coxibs which have been withdrawn

from the market (8). The number of flinches, despite of being count data, was analyzed as a continuous variable. Another proper alternative would have been monitored continuously the response over the experimental period and once grouped, for example in 1, 2, or 5 min periods, use the discrete distributions (e.g., Poisson, negative binomial, etc. (29)) as the framework to incorporate drug effects and the progression of induced pain.

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Conflict of Interests None

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