Pharmacokinetic-Pharmacodynamic Modeling of the Electroencephalogram Effect of Imipenem in Healthy Rats

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A pharmacokinetic-pharmacodynamic (PK-PD) modeling approach was developed to investigate the epileptogenic activity of imipenem in rats. Initially, animals received an intravenous infusion of imipenem at a rate of 2.65 mg min⁻¹ for 30 min. Blood samples were collected for drug assay, and an electroencephalogram (EEG) was recorded during infusion and postinfusion. A dramatic delay was observed between concentrations of imipenem in serum and the EEG effect; this effect was accompanied by tremors and partial seizures. Indirect-effect models failed to describe these data, which were successfully fitted using an effect compartment model. The relationship between effect and concentration at the effect site was best described by a spline function. The elimination rate constant from the effect compartment was severalfold lower than that from the central compartment. The robustness of the model was then confirmed after administering the imipenem dose over 60 and 90 min. In conclusion, the successful PK-PD modeling of the imipenem EEG effect in rats constitutes a major improvement for better prediction of the epileptogenic risk associated with this antibiotic.

Antibiotics may occasionally induce central nervous system (CNS) side effects, including seizures, in humans. These are observed with fluoroquinolones (5, 39) and β-lactams, in particular carbapenems (33). In this family the leading compound, imipenem, presents a relatively high risk of convulsions (1, 3), especially in patients with renal impairment or with bacterial meningitis (2, 41).

Various experimental approaches have been developed to investigate the convulsion liabilities of antibiotics in vivo, including direct intracerebroventricular injection (15, 40). However, this approach does not take into consideration the CNS diffusion characteristics of these drugs, which may have major impacts on their convulsant activity (11). The proconvulsive activity of antibiotics on pentylenetetrazol has also been investigated (8, 30), but many parameters may contribute to the observed proconvulsant effects, making data interpretation complex and potentially misleading (27).

An in vivo experimental approach to the study of the pharmacokinetic-pharmacodynamic (PK-PD) contributions to the convulsant activity of fluoroquinolones alone (11, 12) or in association with other drugs (13, 25) has recently been developed. This approach is based upon previous studies which showed that for convulsant compounds, such as pentylenetetrazol (31) or theophylline (32), the cerebrospinal fluid (CSF) was part of the biophase, meaning that the convulsant activities of these substances are directly related to their concentrations in CSF (7). However, unlike fluoroquinolones, the concentration of imipenem in CSF is not predictive of its convulsant activity (20).

Electroencephalogram (EEG) recording was then considered as a suitable alternative. This approach had occasionally been used to assess the epileptogenic effects of drugs, including antibiotics (14, 16, 17), and could be considerably improved by coupling quantitative EEG recording with PK-PD modeling analysis, as previously done to investigate the desirable CNS effects of drugs (6, 26).

MATERIALS AND METHODS

Animals. This work was done in accordance with the Principles of Laboratory Animal Care. Male Sprague-Dawley rats (Depres Breeding Laboratories, St. Doulchard, France) with a body weight between 320 and 350 g were housed in the animal breeding facilities of the laboratory (authorization no. 0028). The animals were placed in wire cages in a 12-h light-dark cycle for 1 week to adjust to the new environment and to overcome stress possibly incurred during transit. They had free access to food (AO4; U. A. R., Villemoisson, France) and water.

Surgery. Five days before the experiment, each rat had five cortical EEG electrodes implanted while under anesthesia (1.25 mg of ketamine [Ketalar], at a concentration of 50 mg ml⁻¹; Parke Davis Laboratories, France, and 0.5 mg of xylazine hydrochloride [Rompum]; Bayer Laboratories, France). The electrodes were screwed into little holes drilled into the skull at the following positions, in relation to bregma: 2 mm anterior, 2 mm lateral (F1 and F2); 4 mm posterior, 2 mm lateral (P1 and P2); and 4 mm anterior, 2 mm lateral (reference electrode). The stainless steel electrodes were connected to a miniature plug fixed to the skull with dental cement. One day before the experiment, two permanent polyethylene catheters were implanted while each rat was under anesthesia (thiopental sodium, 60 mg kg of body weight⁻¹; Sanofi Laboratories, France): one in the left femoral vein for drug administration and the other one in the left femoral artery for blood sample collection. Animals were housed individually in plastic cages under a 12-h light-dark cycle for 1 week to adjust to the new environment.
The rats in group I (n = 6, weight [mean ± standard deviation] = 335 ± 15 g) received an intravenous (i.v.) infusion of imipenem-cilastatin at a rate of 2.65 mg of imipenem per min for 30 min, corresponding to a dose of 80 mg of imipenem. The rats in groups II and III received the same dose of imipenem, either at an infusion rate of 1.325 mg min⁻¹ corresponding to a dose of 80 mg of imipenem. The rats in group I (n = 6) received the same dose of imipenem, either at an infusion rate of 1.325 mg min⁻¹ for 60 min (group II, n = 6, weight = 335 ± 15 g) or at an infusion rate of 0.883 mg min⁻¹ for 90 min (group III, n = 6, weight = 335 ± 15 g). Drug infusions started between 9:00 a.m. and 12:00 p.m.

Drug dosing and blood sampling. The rats in group I (n = 6, weight [mean ± standard deviation] = 335 ± 15 g) received an intravenous (i.v.) infusion of imipenem-cilastatin at a rate of 2.65 mg of imipenem per min for 30 min, corresponding to a dose of 80 mg of imipenem. The rats in groups II and III received the same dose of imipenem, either at an infusion rate of 1.325 mg min⁻¹ for 60 min (group II, n = 6, weight = 335 ± 15 g) or at an infusion rate of 0.883 mg min⁻¹ for 90 min (group III, n = 6, weight = 335 ± 15 g). Drug infusions started between 9:00 a.m. and 12:00 p.m.

The femoral vein cannula was connected to a motor-driven syringe pump (Program 2; Vial Inc., France) containing the imipenem solution. Arterial blood samples (300 μl) were collected in dry tubes immediately before and at the following times after infusions had started: 15, 30, 40, 50, 60, 75, 90, 105, and 120 min (group I); 30, 60, 70, 80, 90, 105, 120, 135, 150, and 180 min (group II); or 30, 60, 90, 100, 110, 120, 135, 150, 165, and 180 min (group III). Blood samples were immediately centrifuged to collect serum. Blood was replaced by an equal volume of 0.9% NaCl solution. Before storage, samples were diluted (1:1, vol/vol) with a stabilizer (0.5 M HEPES buffer, pH 6.8; ethylene glycol; h.p.l.c.-grade water [1:0.5:0.5, vol/vol/vol]) and kept frozen at 80°C until analysis.

Drug analysis. A previously described high-performance liquid chromatography assay (4) was used with minor modifications for imipenem determination. Proteins from serum samples were precipitated by the addition of methanol (1:2, vol/vol), the mixture was centrifuged, and the supernatant was injected. Separation was performed with a Nucleosil C8 (5 μm) 250 by 0.4 mm [inner diameter] column. The mobile phase consisted of 0.2 M aqueous borate buffer, pH 7.2, containing 15% (vol/vol) methanol, and the flow rate was 1 ml min⁻¹. The retention time of imipenem was equal to 5.5 min. The chromatographic system consisted of a model L 6000 Merck-Hitachi pump and a Waters 717 plus refrigerated autosampler connected to a Waters 484 UV absorbance detector (λ = 313 nm). Chromatographic data were recorded and processed using a Waters 746 integrator. The limit of quantitation of imipenem was 0.5 μg ml⁻¹ in serum. Intraday coefficients of variation calculated at two concentrations were equal to or less than 10%. Corresponding interday coefficients were equal to or less than 13%.

Modeling procedures. A one-compartment open model with zero-order input (R₀) was used to characterize the serum concentration-versus-time profiles of imipenem during and after infusion:

\[ C = \frac{R_0}{k_{e1}} \left( 1 - e^{-k_{e1}T} \right), \text{ for } T \leq T_{d} \]  

\[ C = \frac{R_0}{k_{e1}} \left( 1 - e^{-k_{e1}T_d} \right) e^{-k_{e1}(T-T_d)}, \text{ for } T > T_{d} \]  

where C is the concentration of imipenem in serum at time T, Tₕ is the duration of infusion, kₑ₁ is the elimination rate constant, and V is the apparent volume of distribution.

The pharmacokinetic parameters were then fixed, and the pharmacodynamic models were regressed to the EEG data for each individual rat, using the nonlinear least-squares program WinNonlin (version 1.1; SCI Software, Cary, N.C.). An indirect-response model (9) and an effect compartment model (35)

\[ V \text{ (ml/kg) } 467.4 \pm 45.7 \]

\[ k_{e1} \text{ (min⁻¹) } 0.007 \pm 0.000 \]

\[ T_{d} \text{ (min) } 46.5 \pm 47.0 \]

\[ C_{d} \text{ (μg/ml) } 177.8 \pm 37.0 \]

\[ 14.5 \pm 1.7 \]

\[ 14.5 \pm 1.7 \]

\[ 15.5 \pm 4.4 \]

\[ 14.4 \pm 3.2 \]

\[ 14.4 \pm 3.2 \]

\[ 244.1 \pm 43.4 \]

\[ 244.1 \pm 43.4 \]

\[ 277.4 \pm 20.3 \]

\[ 277.4 \pm 20.3 \]

\[ 11.7 \pm 1.7 \]

\[ 11.7 \pm 1.7 \]

\[ 457.4 \pm 46.5 \]

\[ 457.4 \pm 46.5 \]

\[ 272.9 \pm 47.0 \]

\[ 272.9 \pm 47.0 \]

\[ 0.010 \pm 0.010 \]

\[ 0.010 \pm 0.010 \]

\[ 13.1 \pm 2.2 \]

\[ 13.1 \pm 2.2 \]

\[ 0.012 \pm 0.001 \]

\[ 0.012 \pm 0.001 \]

\[ 13.1 \pm 2.7 \]

\[ 13.1 \pm 2.7 \]

\[ 177.8 \pm 37.0 \]

\[ 177.8 \pm 37.0 \]
were applied for analysis of the PK-PD relationship, with uniform weights according to the constant variance. The baseline value, \( P_0 \), was estimated by averaging the 10th min of the EEG record.

The first approach was to correlate the EEG effect with imipenem concentration using an indirect-response model based on the mechanism of action of the drug. Because it has been proposed that the convulsant activity of imipenem was mainly related to inhibition of the GABA system (8), an indirect-response model with inhibition of the response, frequently referred to as model II by its authors (23), was selected. The general form of the corresponding inhibition function is as follows:

\[
I = \frac{1 - \frac{I_{\text{max}} \cdot C}{IC_{50} + C}}
\]

where \( I_{\text{max}} \) represents the maximum inhibition capacity, \( IC_{50} \) represents the imipenem concentration producing 50% of the maximum drug-induced inhibition, and \( \gamma \) is the sigmoidicity factor. However, when the PD effect tends toward infinity without reaching a maximum, as was the case with the total power of the EEG signal of imipenem, \( I_{\text{max}} \) must be given a value of one (34). Therefore, a simplified form of equation 3 was used as the inhibition function:

\[
I = 1 - \frac{C}{IC_{50} + C}
\]

FIG. 3. Measured total power of the EEG signal versus imipenem concentrations in serum (A) and at the effect site (B), for the same rat as in Fig. 1. The solid line represents the best fit of the data according to the spline function model (equation 8).

The second approach used to describe the time course of the effect was to include a hypothetical effect compartment in the PK-PD model, allowing minimization of the hysteresis observed in the serum concentration-EEG effect relationship, according to the following equations:

\[
C_e = \frac{k_{0e} \cdot R_0}{k_{e1} \cdot V(k_{0e} - k_{e1})} \left(1 - e^{-k_{e0} T}\right) + \frac{R_0}{V(k - k_{e0})} \left(1 - e^{-k_{e0} T}\right), \text{ for } T \leq T_{\text{inf}}
\]

\[
C_e = \frac{k_{0e} \cdot R_0}{k_{e1} \cdot V(k_{0e} - k_{e1})} \left(1 - e^{-k_{e0} T_{\text{inf}}}\right) e^{-k_{e0} (T - T_{\text{inf}})} + \frac{R_0}{V(k_{e1} - k_{0e})} \left(1 - e^{-k_{e0} T_{\text{inf}}}\right) e^{-k_{e0} (T - T_{\text{inf}})},
\]

for \( T > T_{\text{inf}} \)

In these equations, \( C_e \) is the drug concentration in the effect compartment at time \( T \), and \( k_{e0} \) is the rate constant for elimination of the drug from the effect compartment. The other parameters are the PK parameters previously defined and estimated for being used as constants for the PK-PD modeling (35).

The profile of the EEG effect was described using a spline function derived from the Hill equation (10):

\[
P = P_0 + B \cdot C^\gamma
\]

In this equation, \( P \) is the total power (EEG effect) corresponding to \( C \), \( P_0 \) is the baseline effect value, \( B \) is the combined parameter \( E_{\text{max}}/EC_{50} \), and \( \gamma \) is a factor determining the steepness of the curve.

The goodness-of-fit of each model was assessed by visual inspection and analysis of the residuals, and coefficient of variation percentage associated with parameter estimates (22).

The robustness of the compartment effect model was assessed by comparing the parameters characteristic of the PK-PD model (\( B, \gamma \), and \( k_{e0} \)) between groups. The derived parameters, such as the maximum intensity of the EEG signal (\( P_{\text{max}} \)), the corresponding time of occurrence (\( TP_{\text{max}} \)), and the period of time elapsed between the end of infusion and the effect peak (\( \Delta T \)) observed after infusing 80 mg of imipenem at a rate of 1.325 mg min\(^{-1}\) (group II) or 0.833 mg...
RESULTS

Following a 30-min infusion of imipenem at a rate of 2.65 mg min⁻¹, corresponding to a dose of 80 mg (group I), the decay of imipenem concentration in serum with time was monoeponential and rapid after infusion was stopped. Limited interindividual variability was observed. PK parameters are presented in Table 1. A dramatic temporal delay between concentration in serum and EEG effect was observed in every individual rat. Only isolated spikes appeared during imipenem infusion, with limited effects on the total power of the EEG signal. Then the frequency and amplitude of the spikes increased dramatically, leading to a relatively sudden increase of the total power, occurring in most animals between one and two half-lives postinfusion. This was accompanied by behavioral troubles, including tremors and partial seizures. The EEG signal reached a maximum at three elimination half-lives after the total power sharply increased, and it was only ca-

tration in the effect compartment, which could be adequately fitted with a spline function (Fig. 3B). Overall, the effect compartment model provided a good fitting of the relationship between imipenem concentration in serum and EEG effect, as illustrated in Fig. 4, with corresponding parameters accurately estimated (Table 2). The non-statistically significant differences between the parameter values estimated for the three groups (Table 2) and between the observed and predicted values of the derived parameters (Table 3) attest to the robustness of the compartment effect model in various experimental settings. Note that the pharmacokinetic parameters of imipenem were also unaffected by the change in infusion rate (Table 1).

DISCUSSION

Quantitative EEG recording constitutes an interesting experimental approach offering a sensitive, objective, and continuous measure of the neurotoxic effects of imipenem in rats. Furthermore, since variations in the total power of the EEG signal were related to behavioral modifications and occurrence of tremor and partial seizures, changes in EEG can be considered as an appropriate surrogate pharmacodynamic endpoint for the investigation of the epileptogenic activity of imipenem in rats. Compared to EEG recording used in previously published studies (14, 16), the integrated PK-PD modeling approach used here constituted a major improvement. Because imipenem is supposed to interact with GABA_A receptors (8, 16), an indirect-response model with inhibition of the factors controlling drug response (that is, inhibition of GABA_A binding to its receptor sites) was initially selected but was unable to capture the lag time followed by a sudden increase in the EEG effect (Fig. 2).

The ability of the effect compartment model to describe imipenem data suggests that this delay is due to the limited and slow CNS diffusion of imipenem (19, 22, 28), which is also responsible for the important differences observed between the elimination rate constants from the central and effect compart-

### Table 2. PD parameters characteristic of imipenem infused i.v. to rats at a dose of 80 mg at various rates, derived from the compartment effect model

<table>
<thead>
<tr>
<th>Group</th>
<th>Infusion rate (mg min⁻¹)</th>
<th>Infusion duration (min)</th>
<th>P₀⁺ (mV²)</th>
<th>B⁺</th>
<th>n⁺</th>
<th>ke⁺ (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (n = 6)</td>
<td>2.65</td>
<td>30</td>
<td>0.21 ± 0.07</td>
<td>0.0264 ± 0.0147</td>
<td>7.1 ± 6.6</td>
<td>0.0054 ± 0.0031</td>
</tr>
<tr>
<td>II (n = 6)</td>
<td>1.325</td>
<td>60</td>
<td>0.16 ± 0.07</td>
<td>0.0236 ± 0.0199</td>
<td>4.5 ± 4.4</td>
<td>0.0099 ± 0.0075</td>
</tr>
<tr>
<td>III (n = 6)</td>
<td>0.883</td>
<td>90</td>
<td>0.18 ± 0.07</td>
<td>0.0155 ± 0.0091</td>
<td>5.1 ± 4.1</td>
<td>0.0128 ± 0.0083</td>
</tr>
</tbody>
</table>

* a, non-statistically significant differences between groups based on one-way ANOVA.

### Table 3. Comparisons between simulated parameters characteristic of the EEG effect derived from PK-PD effect compartment modeling conducted in rats from group I and fitted parameters in rats from groups II and III

<table>
<thead>
<tr>
<th>Group</th>
<th>Pmax⁺ (mV²)</th>
<th>Tpmax⁺ (min)</th>
<th>ΔT⁺ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Simulated</td>
<td>Fitted</td>
<td>Simulated</td>
</tr>
<tr>
<td>I (n = 6)</td>
<td>3.6 ± 1.2</td>
<td>4.2 ± 1.4</td>
<td>64.5 ± 7.6</td>
</tr>
<tr>
<td>II (n = 6)</td>
<td>3.8 ± 1.7</td>
<td>3.8 ± 1.7</td>
<td>85.5 ± 7.6</td>
</tr>
<tr>
<td>III (n = 6)</td>
<td>2.0 ± 1.4</td>
<td>2.0 ± 1.4</td>
<td>110.0 ± 7.3</td>
</tr>
</tbody>
</table>

* Values are presented as means ± standard deviations. *, non-statistically significant differences between simulated and fitted values within groups based on the unpaired Student t test.
ments (Tables 1 and 2). Under similar experimental conditions, no hysteresis was found for midazolam, a drug with extensive and rapid CNS diffusion (26), and only a limited, if any, temporal delay between concentrations and effects with values of $k_{e0}$ and $k_{c0}$ close to each other was observed with synthetic opioids (6).

Although the effect compartment provided satisfactory data fitting in one particular situation (80 mg infused over 30 min), it was important to assess its robustness under various experimental conditions. It was initially decided to change the dose as previously done to discriminate between the effect compartment and indirect-effect models (9, 38). However, preliminary experiments showed that no EEG effect was observed when the dose was reduced by 50% and animals died before the end of the experiment when it was increased by 50%. These results are consistent with the sudden and rapid increase of the EEG effect illustrated in Fig. 3A and with the high value of the sigmoidicity factor $n$ in equation 8 (Table 2). However, this precluded dose modification as a way to assess the robustness of the effect compartment model. Keeping the dose constant but changing the duration of infusion by adjusting the infusion rate was therefore considered. Simulations showed that the effect compartment model predicts that the peak of effect should decrease as the duration of infusion increases.

Results obtained following 60- and 90-min infusions, in particular the decrease of $P_{\text{max}}$ as infusion duration increases (Table 3 and Fig. 5A) as well as the lack of statistically significant differences between parameter estimates in the various experimental settings (Table 2), attest to the robustness of the compartment effect model. However, a close look at the data suggests that, although not statistically different, the $k_{e0}$ value had a tendency to increase together with the duration of infusion, indicating that the effect compartment model may not capture all of the complexities of the system. Among these, an active metabolite accumulating with time could contribute to the observed EEG effect. Several authors have suggested that the convulsant activity of imipenem was related to the accumulation of an open lactam metabolite (18, 37), but other results suggest that the parent compound itself would be responsible for the neurotoxicity (29, 36). However, this metabolite is not detected in serum and cannot be included in a PK-PD model. Therefore, the effect compartment model may not be perfect, but at least it was satisfactory in predicting the EEG effect of imipenem in a relatively wide range of experimental settings (Table 3). It provides a new and unique way to approach this complex nonlinear concentration-effect relationship. As an example, it was confirmed experimentally (Table 3) that keeping the dose constant but increasing the duration of infusion (and therefore reducing the input rate) allowed a reduction of the epileptogenic effect. As another consequence of this change in the dosing regimen, $C_{\text{max}}$ decreases (Table 1) but the area under the concentration-time curve (AUC) does not, since dose and clearance are kept constant. One could argue that the CNS toxicity of imipenem is essentially related to high concentrations in serum and that apart from the temporal delay, peak concentrations in serum would be predictive of the epileptogenic risk. Simulations conducted with the compartment effect model show that this would not be the case, as infusing imipenem at various combined input rates and durations in order to keep the $C_{\text{max}}$ constant (but not the AUC) would lead to a completely different EEG effect as well. This is illustrated in Fig. 5B, which shows that a slight change in the dosing regimen (for example, infusing imipenem at a rate of 2.50 mg min$^{-1}$ for 35 min instead of 2.65 mg min$^{-1}$ for 30 min), which has no effect on $C_{\text{max}}$ should result in a dramatic increase of the EEG effect. This confirms that the $C_{\text{max}}$ and AUC alone are not good predictors of the epileptogenic risk associated with imipenem. Interestingly, the model also predicts that a reduction in imipenem clearance, as encountered in patients with renal insufficiency, should lead to a much more than proportional increase in the epileptogenic effect. This is consistent with the current knowledge gained from clinical practice, that imipenem CNS toxicity is essentially observed in patients with renal impairment (2) and dosage adjustment.
based upon renal function may not be adequate to prevent seizures (24).

In conclusion, the PK-PD model validated in this study represents a major improvement in the comprehension of the epileptogenic activity of imipenem in rats.

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REFERENCES


