Neurotoxicity of Panipenem/Betamipron, a New Carbapenem, in Rabbits: Correlation to Concentration in Central Nervous System

Atsushi KURIHARA,* Masafumi HISAOKA,* Naoji MIKUNI,** and Katsuo KAMOSHIDA**

Product Development Laboratories, Sankyo Co., Ltd.,* Hiromachi, Shinagawa-ku, Tokyo, 140, Japan and Institute of Science Technology, Inc.,** Kitashinagawa, Shinagawa-ku, Tokyo, 140, Japan

(Received November 19, 1991)

The neurotoxic potential of panipenem/betamipron (PAPM/BP), a new carbapenem antibiotic, was compared with that of imipenem/cilastatin (IPM/CS). The drug concentration in cerebrospinal fluid (CSF) at the onset of epileptogenic electroencephalographic (EEG)-activity and the drug distribution into the central nervous system (CNS) were evaluated.

Epileptogenic reactions correlated well with drug levels in CSF, but not with drug levels in circulating plasma. The concentration of PAPM in CSF at the onset of epileptogenic EEG-activity was almost twice that of IPM, suggesting that neurotoxic activity of PAPM is about half that of IPM. In addition, in terms of incidence percent for the epileptogenic EEG-activity, PAPM/BP was found to be less toxic than IPM/CS within the dose of 1.0—1.2 g/kg.

Concentrations of PAPM in CSF and brain extracellular fluid after PAPM/BP i.v. infusion were comparable with those of IPM after IPM/CS infusion, indicating the similar characteristics of distribution into the CNS for the two antibiotics.

From these results of pharmacologic effects and drug distributions, it is suggested that the neurotoxicity of PAPM/BP is less than half that of IPM/CS.

Keywords — carbapenem; panipenem (PAPM); betamipron (BP); imipenem (IPM); EEG-activity; cerebrospinal fluid (CSF); brain extracellular fluid; microdialysis; neurotoxicity; pharmacokinetics

Introduction

Panipenem (PAPM) is a new carbapenem developed at Sankyo Co. Ltd. (Tokyo, Japan). It is strongly resistant to beta-lactamase produced by bacteria, and expresses potent antimicrobial activities against various types of G(+) and G(−) microorganisms.1) PAPM induces the nephrotoxicity at very high doses in rabbits. Betamipron (BP) is an analogue of an amino acid, and it has an inhibitory effect on organic anion transport at sites of nephrotoxicity.2) PAPM/BP, a mixture of PAPM and BP in equivalent amounts, was developed for clinical use, due to the reduction of nephrotoxicity by PAPM3) (Fig. 1).

It is well known that many antibiotics have neurotoxicity.4) Epileptogenic reactions by benzylpenicillins and beta-lactam antibiotics have been reported at very high doses or with clinical use in patients suffering renal failure or neuropathic failure.5) In addition, neurotoxic reac-

![Chemical Structures of PAPM and BP](image)

Fig. 1. Chemical Structures of PAPM and BP
tions caused by newer beta-lactam antibiotics belonging to the carbapenem group have also been reported. Imipenem/cilastatin (IPM/CS), the first and only one carbapenem antibiotic on the market at present has been associated with epileptogenic reactions in both animals and man.\(^7\)

The penetration of many drugs into the central nervous system (CNS) is limited by the blood-brain barrier\(^8\) and the blood-cerebrospinal fluid (CSF) barrier,\(^9\) especially for drugs with low lipophilicity. Beta-lactam antibiotics,\(^10\) which are hydrophilic, have been reported to be much more neurotoxic after direct application to the CNS than following intravenous administration, suggesting that penetration into the CNS is key to the neurotoxic reaction.

In the present study, the neurotoxicity of PAPM/BP was compared with that of IPM/CS in view of the correlation between pharmacodynamics and pharmacokinetics in rabbits. That is, drug levels in the CSF at the onset of epileptogenic electroencephalographic (EEG) activity were determined, and the distribution of drugs into the CSF and brain extracellular fluid were evaluated.

**Materials and Methods**

**Reagents** — PAPM and BP were synthesized at Sankyo Co. Ltd. (Tokyo, Japan). IPM/CS (Tienam\(^\text{®}\) ) was purchased from Banyu Pharmaceutical Co., Ltd. (Tokyo, Japan). Chloramphenicol base was purchased from Sigma Chemical Co. (St. Louis, U.S.A.). 3-(N-Morpholino)propanesulfonic acid (MOPS) was purchased from Wako Pure Chemical Industry (Osaka, Japan). All other reagents and solvents were commercial products of analytical and high performance liquid chromatography (HPLC) grade.

**Animals** — Male Japanese-White rabbits weighing 2.5—3.5 kg (Shiraishi Laboratory Animal, Tokyo, Japan) were used throughout this study.

**Measurement of Epileptogenic EEG-Activity** — Rabbits received implantation of silver-ball-type electrodes in the left prefrontal area and parietal association area of the brain, and also a stainless electrode in the hippocampus area. Implantation was carried out more than a week before the experiment. Electroencephalographic (EEG) activity was measured and recorded with an EEG recorder (EEG 3118, Nihon Koden, Tokyo, Japan). Drug solutions of PAPM/BP and IPM/CS (30/30 mg/ml of saline) were infused into an ear vein at rates of 11 and 20 mg/min/kg until the onset of epileptogenic EEG-activity. CSF and plasma samples were taken at the onset of epileptogenic EEG-activity, and assayed for PAPM and IPM. Epileptogenic EEG-activity was defined as the continuation of at least three spikes during a period of 20 s. CSF samples were obtained by cisternal puncture with a 25-gauge needle after exsanguination from the carotid artery under light anesthesia with pentobarbital. All CSF samples were free from visible blood contamination. Blood samples were heparinized to obtain plasma samples.

**CSF Distribution** — Sterile saline solutions containing PAPM/BP and IPM/CS (30/30 mg/ml) were administered to rabbits by 30 min-infusion into an ear vein at doses of 160/160, 320/320, and 640/640 mg/kg. CSF and blood samples were taken just after 30 min-infusions. CSF samples were obtained as described above.

**Microdialysis Method** — The technique of microdialysis\(^11\) was applied to assess the drug distribution into extracellular fluid of the rabbit brain. A dialysis probe (o.d. 0.5 mm, 4 mm membrane length, Mw cut off 20000 Da, Carnegie Medicin, Stockholm, Sweden) was implanted into the hippocampus area of the rabbit brain (5.0 mm posterior to bregma, 5.0 mm lateral to the midline, 5.0 mm below dura) under anesthesia about 4 h before the experiments. The microdialysis probe was perfused with Ringer’s solution (147 mM NaCl, 2.3 mM CaCl\(_2\), and 4 mM KCl) at a rate of 2 \(\mu\)l/min. After the animals recovered from anesthesia, drug solutions of PAPM/BP and IPM/CS (30/30 mg/ml of saline) were infused into an ear vein for 30 min at a total dose of 200/200 mg/kg. Outflow solutions from the microdialysis probe were collected during and after the drug infusion, and assayed for drug concentration. Drug recovery
for each probe, which was checked in vitro using Ringer's solution containing a known amount of drug, was 15.0 ± 0.4% and 19.0 ± 0.2% for PAPM and IPM, respectively (mean ± S.E., n = 3). The concentration in brain extracellular fluid was calculated by dividing the concentration in microdialysis perfusates by the in vitro recovery.

**Determination of PAPM and IPM** — Concentrations of PAPM and IPM were determined by HPLC as developed by Hisaoka et al.\textsuperscript{12} with minor modifications. Plasma and CSF samples were mixed with equal volumes of 1 M MOPS just after the sampling for storage until the assay. Stabilized plasma and CSF samples were added to equal volumes of internal standard solution (10 μg/ml of chloramphenicol base in 1 M MOPS), and the mixed solutions were applied to an HPLC system (LC-6A, Shimadzu, Tokyo, Japan). Microdialysis samples were injected into HPLC without any pretreatment, immediately after sampling. HPLC conditions for each compound were as follows. An HPLC column of Partisphere SCX (7.5 mm × 125 mm, Whatman, Inc., Clifton, U.S.A.) with a guard column of Partisphere CX was used. Mobile phases used were 0.04 M CH\textsubscript{3}COONH\textsubscript{4} (pH 4.8)—CH\textsubscript{3}CN (96:4) for PAPM and 0.04 M CH\textsubscript{3}COONH\textsubscript{4} (pH 4.8) for IPM. PAPM and IPM were detected with UV absorption at lengths of 300 and 313 nm, respectively.

**Pharmacokinetic Analysis** — The half life was calculated from a log-linear, least-squares fit of the terminal linear portion of concentration-time curves. The area under the blood concentration–time curve (AUC) was obtained by trapezoidal rule and determined from time zero to infinity. The area from final sampling time to infinity was calculated as the value of concentration at final sampling time divided by 2.303 and by the slope of the terminal log-linear portion. The analysis for drug distribution into CSF in EEG experiments was performed with the hybrid model described by Sato et al.,\textsuperscript{13} with minor modification (Fig. 2). Since CSF volume is so small compared with plasma volume, the drug amount in CSF can be neglected in the calculation of drug concentrations in plasma. Mass balance equations of drug in CSF and plasma can be expressed as follows:

\[
dV_{\text{CSF}} \cdot C_{\text{CSF}} / dt = k \cdot C_p - K \cdot C_{\text{CSF}} - F \cdot C_{\text{CSF}} \tag{1}
\]

\[
dV_d \cdot C_p / dt = I_0 - K_c \cdot V_d \cdot C_p \tag{2}
\]

where \( V_{\text{CSF}} \) and \( V_d \) are the volumes of CSF and central compartment, respectively; \( C_{\text{CSF}} \) and \( C_p \) are the concentrations of drug in CSF and plasma, respectively; \( K \) is CSF transport clearance; \( F \) is CSF bulk flow; \( I_0 \) is i.v. infusion rate; and \( K_c \) represents the elimination rate constant from the central compartment. From Eq. (1) and (2), the following equations can be derived.

\[
C_{\text{CSF}} = (K \cdot I_0 / V_d \cdot V_{\text{CSF}})(V_{\text{CSF}} / ((K + F) \cdot K_c))
+ V_{\text{CSF}} \cdot e^{-(K + F)/(K + F) \cdot (K + F)/(V_{\text{CSF}} - K_c)} - e^{-K_c \cdot t} / (K_c \cdot (K + F) / V_{\text{CSF}} - K_c)) \tag{3}
\]

\[
C_p = (I_0 / V_d K_c) \cdot (1 - e^{-K_c \cdot t}) \tag{4}
\]

The values of \( K_c \) and \( V_d \) were determined from the plasma concentration–time profile in the microdialysis experiment, which values were used in fitting the observed CSF concentrations to Eq. (3) with a non-linear least squares method.\textsuperscript{14}

**Results**

**Drug Levels in CSF at the onset of EEG-Activity**

As for incidences of epileptogenic reactions (Table I), no abnormality on EEG-activity or behavior was induced in several rabbits tested, even at total doses of 1.0—1.2 g/kg. PAPM/BP was found to be less toxic than IPM/CS in terms of incidence percent for epileptogenic EEG-activity.
TABLE I. Inductive Incidences of Epileptogenic EEG-Activity during Infusions of PAPM/BP and IPM/CS in Rabbits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Infusion rate (mg/min/kg)</th>
<th>Infusion time (min)</th>
<th>Total dose (mg/kg)</th>
<th>Incidence Numbers</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAPM/BP</td>
<td>11/11</td>
<td>100</td>
<td>1100/1100</td>
<td>6/10</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>20/20</td>
<td>60</td>
<td>1200/1200</td>
<td>5/6</td>
<td>83</td>
</tr>
<tr>
<td>IPM/CS</td>
<td>11/11</td>
<td>90</td>
<td>990/990</td>
<td>6/7</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>20/20</td>
<td>60</td>
<td>1200/1200</td>
<td>6/6</td>
<td>100</td>
</tr>
</tbody>
</table>

TABLE II. Concentrations of PAPM and IPM in Plasma and CSF at Onset of Epileptogenic EEG-Activity during Infusions of PAPM/BP and IPM/CS in Rabbits

<table>
<thead>
<tr>
<th>Drug</th>
<th>Infusion rate (mg/min/kg)</th>
<th>Rabbit numbers</th>
<th>Onset time (min)</th>
<th>Total dose (mg/kg)</th>
<th>Concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CSF</td>
</tr>
<tr>
<td>PAPM</td>
<td>11/11</td>
<td>6</td>
<td>73.9 ± 2.6</td>
<td>821 ± 41</td>
<td>1571 ± 204</td>
</tr>
<tr>
<td></td>
<td>20/20</td>
<td>5</td>
<td>44.7 ± 5.0</td>
<td>894 ± 99</td>
<td>2119 ± 243</td>
</tr>
<tr>
<td>IPM</td>
<td>11/11</td>
<td>6</td>
<td>39.4 ± 4.6</td>
<td>436 ± 51</td>
<td>1234 ± 121</td>
</tr>
<tr>
<td></td>
<td>20/20</td>
<td>6</td>
<td>23.8 ± 3.1</td>
<td>475 ± 61</td>
<td>1741 ± 171</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± S.E.

As for rabbits which showed no epileptogenic EEG-activity, the drug concentrations in CSF at the end of infusion were almost twice those for rabbits with epileptogenic EEG-activity (data not shown). Therefore, we used concentration data only from rabbits with epileptogenic EEG-activity, assuming that the other rabbits were extraordinarily resistant to such neurotoxicity. Drug concentrations at the onset of epileptogenic EEG-activity (spiked waves) and total doses until the onset time are shown in Table II and Fig. 3. The total dose of PAPM/BP was twice that of IPM/CS at any infusion rate. Plasma concentrations of PAPM and IPM at the onset of

Fig. 3. Concentrations of PAPM (●) and IPM (○) in Plasma (A) and CSF (B) at Onset of Epileptogenic EEG-Activity during Infusions of PAPM/BP and IPM/CS in Rabbits

Two infusion rates of 11/11 mg/min/kg (L) and 20/20 mg/min/kg (H) were adopted for drug administrations. Solid lines were simulated by hybrid model for CSF distribution (see text).

Results are expressed as mean ± S.E. (n = 5—6).
epileptogenic EEG-activity depended on the drug infusion rate. The drug concentrations in plasma at the onset time were higher at the high infusion rate than at the low infusion rate. CSF concentrations at the onset time, however, were almost constant at both infusion rates, suggesting that epileptogenic EEG-activity is related with the drug level in CSF. CSF concentrations at the onset time for PAPM were almost twice those for IPM. From these results, the neurotoxic activity of PAPM is suggested to be half that of IPM.

Pharmacokinetic analysis was carried out using a hybrid model of drug distribution into CSF on these drug concentration data. The values of $V_d$ and $K_e$ calculated from the data of plasma concentration time profiles in the microdialysis experiment are as follows:

PAPM: $V_d = 232$ ml/kg, $K_e = 1.56$ l/h  
IPM: $V_d = 187$ ml/kg, $K_e = 1.87$ l/h

Plasma concentration-time profiles at two infusion rates were simulated substituting these values into Eq. (4) (Fig. 3 (A), solid lines). Observed plasma concentrations at the onset of epileptogenic EEG-activity dispersed on the predicted lines for each of PAPM and IPM. Then, these values of $V_d$, $K_e$ and an $F$ value of 0.202 ml/h/kg from the literature\(^{15}\) were used to calculate parameters of $V_{CSF}$ and $K$, by fitting each observed CSF concentration data to Eq. (3) with a non-linear least-squares method. Simulated lines were well fitted to the observed CSF concentrations for each of PAPM and IPM (Fig. 3 (B)). Obtained parameters were as follows:

PAPM: $V_{CSF} = 0.244$ ml/kg,  
$K = 0.0030$ ml/h/kg  
IPM: $V_{CSF} = 0.213$ ml/kg, $K = 0.0028$ ml/h/kg

Both parameters of $K$ and $V_{CSF}$ for PAPM were almost the same as those for IPM, suggesting similar distribution into the CSF for two antibiotics.

**Distribution of PAPM and IPM into CNS**

Drug concentrations in CSF just after 30 min-infusions of PAPM/BP or IPM/CS at the dose of 640/640 mg/kg were $5.0 \pm 0.8$ and $4.8 \pm 0.8$ μg/ml (mean ± S.E., $n = 3$) for PAPM and IPM, respectively. Plasma concentrations determined at the same time were $1609 \pm 122$ and $2307 \pm 182$ μg/ml (mean ± S.E., $n = 3$) for PAPM and IPM, respectively. PAPM concentrations in both CSF and plasma were linearly related with dose at a range of 160/160—640/640 mg/kg. As for IPM,
Table III. Pharmacokinetic Parameters for Brain Extracellular Distribution

<table>
<thead>
<tr>
<th>Drug</th>
<th>Brain extracellular fluid (B)</th>
<th>Plasma (P)</th>
<th>B/P Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( AUC ) (( \mu g \cdot h/\text{ml} ))</td>
<td>( t_{1/2} ) (h)</td>
<td>( C_{\text{max}} ) (( \mu g/\text{ml} ))</td>
</tr>
<tr>
<td>PAPM</td>
<td>43.8 ± 12.0</td>
<td>0.86 ± 0.12</td>
<td>29.9 ± 7.8</td>
</tr>
<tr>
<td>IPM</td>
<td>57.2 ± 3.5</td>
<td>0.69 ± 0.03</td>
<td>38.9 ± 3.8</td>
</tr>
</tbody>
</table>

PAPM/BP (200/200 mg/kg) and IPM/CS (200/200 mg/kg) were administered to rabbits by 30 min i.v. infusion. Results were expressed as mean ± S.E. (PAPM: n = 3, IPM: n = 4).

the same lineairities were observed for CSF and plasma concentration at the same dose range, suggesting linear pharmacokinetics within plasma concentration range of about 500 to 2000 \( \mu g/\text{ml} \) for both antibiotics.

Drug concentrations in the brain extracellular fluid determined by microdialysis method are shown in Fig. 4 with plasma concentrations. Concentrations of PAPM and IPM in plasma and also in brain extracellular fluid did not significantly differ from each other. Pharmacokinetic parameters for brain extracellular distribution are listed in Table III. The values of \( AUC \) and half life for PAPM concentrations in brain extracellular fluid were comparable with those for IPM concentrations. \( AUC \) ratio of brain extracellular fluid to plasma for PAPM was not significantly different from that for IPM. For both PAPM and IPM, the half life of drug concentrations in brain extracellular fluid was about twice that in plasma.

**Discussions**

Neurotoxic reactions caused by antibiotics generally seem to be influenced by the distribution of drugs into the CNS.\(^{16}\) It is, however, not obvious which sites in the brain have correlation to such reactions. The inhibition of agonist binding to GABA-benzodiazepine receptor in the brain has been thought to be one of the mechanisms for inducing convulsions.\(^{17}\) It is reported that binding sites of the receptor are exposed to extracellular fluids of the brain.\(^{18}\) Therefore, it is of importance to evaluate drug levels in brain extracellular fluid rather than those in total brain or circulating plasma in order to investigate the neurotoxicity of drugs. In the present study, CSF levels instead of brain extracellular fluid levels were determined at the onset of epileptogenic EEG-activity. And the distribution of drugs into CSF and brain extracellular fluid were investigated. In general, drug concentration in CSF is closely related with that in CNS, especially that in brain extracellular fluid. If diffusion of drugs in the CNS is not a rate-limiting step, concentrations in CSF correspond to those in the brain extracellular fluid.\(^{19}\)

Drug concentrations in CSF, but not in plasma, were related with the onset of epileptogenic EEG-activity for both PAPM and IPM. Therefore, the potency of neurotoxic reaction was evaluated by comparing the drug concentration in CSF at the onset time. CSF concentrations of PAPM at the onset of epileptogenic EEG-activity were about twice those of IPM, suggesting that the neurotoxic potential of PAPM is about half that of IPM.

In evaluating the neurotoxic reaction of drugs, both pharmacokinetics and pharmacodynamics, namely drug distribution into the CNS and pharmacologic effects, should be taken into account. The present study indicated that the distribution characteristics into CSF and also into brain extracellular fluid are approximately same for PAPM and IPM. Therefore, the difference between doses of two drugs which caused epileptogenic EEG-activity can be attributed to the difference in pharmacodynamics rather than pharmacokinetics.

Observed concentrations in the brain extracellular fluid were considerably higher than those in CSF for both PAPM and IPM. This may be due to the results of a better penetration over the blood-brain barrier than blood-CSF barrier or of active transport of drugs out of the CSF space.
or both, as suggested for benzylpenicillin by Schliamsar et al.\textsuperscript{20} However, further elaborate investigation would be necessary to make clear this point including the problem of in vivo drug recovery of microdialysis probe as described below.

We evaluated the drug distribution into the brain extracellular fluid of rabbits with the technique of microdialysis. This technique was recently developed in the field of neuropharmacology,\textsuperscript{21} and more recently introduced to pharmacokinetic studies.\textsuperscript{22} Using microdialysis, substances in the extracellular space of tissue can be continuously sampled from an individual animal. It must be considered, in the application of the microdialysis technique to pharmacokinetic studies, that the probe may disturb the tissue integrity, especially when applied to the brain, which has a blood-brain barrier. It is, however, reported that the damage of brain tissue caused by probe implantation can recover within 30 min after the operation.\textsuperscript{23} In addition, drug concentrations in the brain extracellular fluid measured in this study were much lower than those in plasma. Moreover, half lives of drug concentrations were different by two-fold between in brain extracellular fluid and in plasma. Therefore, tissue damage might not be serious enough to change the distribution kinetics. It must also be considered that the recovery of drugs from the probe can be different between in vivo and in vitro states.\textsuperscript{24,25} From the preliminary experiment of in vitro probe recovery (data not shown), time lag caused by adsorption to the probe membrane was found to be negligible for this infusion experiment. We calculated the drug concentration in the brain extracellular fluid by dividing the concentration in the brain dialysate by the in vitro recovery, assuming the same probe recovery between in vivo and in vitro states. It is suggested that in vivo diffusion of a substance in the extracellular fluid is influenced by anatomical factors such as tortuosity of the brain cells and the extracellular fluid volume.\textsuperscript{26} It can be, however, possible to compare relatively the drug distribution into the brain extracellular fluid for structurally related drugs. There seems to be no practical method to correct the difference between in vivo recovery and in vitro recovery at the present time.

We focused on PAPM and IPM, not on BP and CS, in order to evaluate the neurotoxicity of PAPM/BP and IPM/CS. It is known that BP (unpublished data) or CS\textsuperscript{27} has much less neurotoxicity than PAPM or IPM. PAPM\textsuperscript{28} and IPM\textsuperscript{29} are known to be converted to open-lactam metabolites, which were not investigated in this study. IPM, however, is thought to be more neurotoxic than its open-lactam metabolite.\textsuperscript{5} Therefore, the open-lactam metabolite of PAPM may similarly be less toxic than PAPM.

In application to man, both PAPM/BP and IPM/CS exert their efficacies at a dose of 500 mg/body by 30 min-drip infusion.\textsuperscript{3,30} Plasma concentrations and pharmacokinetic parameters of PAPM for a 500 mg dose in man are almost the same as those of IPM as follows.\textsuperscript{31,32}

\[
\text{PAPM: } AUC = 41.2 \text{ } \mu g/\text{ml}, \quad C_{\text{max}} = 37.2 \text{ } \mu g/\text{ml}, \text{ half life } = 0.85 \text{ h}
\]

\[
\text{IPM: } AUC = 41.4 \text{ } \mu g/\text{ml}, \quad C_{\text{max}} = 40.1 \text{ } \mu g/\text{ml}, \text{ half life } = 0.97 \text{ h}
\]

And also, we have clarified that drug concentrations in CSF were almost the same between PAPM and IPM on phase III clinical studies. From those results, PAPM/BP is expected to be a less neurotoxic carbapenem than IPM/CS for clinical use.

References

5) S. E. Schliamsar, O. Cars, and S. R. Norrby: Neurotoxicity of beta-lactam antibiotics: predisposing factors and


