J Infect Chemother 22 (2016) 346–349

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Note

Pharmacokinetics and brain penetration of carbapenems in mice

Kazuaki Matsumoto [a, *], Yuji Kurihara [a, b], Yuko Kuroda [a], Seiji Hori [c], Junko Kizu [a]

[a] Division of Practical Pharmacy, Keio University Faculty of Pharmacy, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan
[b] Molecular Medical Bioscience Laboratory, Yokohama City University Graduate School of Medical Life Science, 1-7-29 Suehiro-cho, Turumi-ku, Yokoyama 230-0045, Japan
[c] Department of Infectious Diseases and Infection Control, Jikei University School of Medicine, 3-25-8 Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan

ARTICLE INFO

Article history:
Received 13 July 2015
Received in revised form
12 November 2015
Accepted 24 November 2015
Available online 22 January 2016

Keywords:
Carbapenems
Pharmacokinetics
Brain penetration

ABSTRACT

An adverse effect associated with the administration of carbapenems is central nervous system (CNS) toxicity, with higher brain concentrations of carbapenems being linked to an increased risk of seizures. However, the pharmacokinetics and brain penetration of carbapenems have not yet been examined. Thus, the aim of this *in vivo* investigation was to determine the pharmacokinetics and brain penetration of carbapenems in mice.

Blood samples and brain tissue samples were obtained 10, 20, 30, 60, and 120 min after the subcutaneous administration of carbapenems (91 mg/kg). We obtained the following values for the pharmacokinetic parameters of carbapenems in mice: 1.20–1.71 L/h/kg for CL_{total}/F, 1.41–2.03 h^{-1} for K_e, 0.34–0.51 h for $T_{1/2}$, 0.66–0.95 L/kg for V_{ss}/F, 0.49–0.73 h for MRT, 83.46–110.58 μg/mL for $C_{max,\ plasma}$, and 0.28–0.83 μg/g for $C_{max,\ brain\ tissue}$. The $AUC_{0-\infty}$ of the carbapenems tested in plasma were in the following order: doripenem > meropenem > biapenem > imipenem, and in brain tissue were: imipenem > doripenem > meropenem > biapenem. The degrees of brain tissue penetration, defined as the $AUC_{0-\infty,\ brain\ tissue}/fAUC_{0-\infty,\ plasma}$ ratio, were 0.016 for imipenem, 0.004 for meropenem, 0.002 for biapenem, and 0.008 for doripenem.

The results of the present study demonstrated that, of the carbapenems examined, imipenem penetrated brain tissue to the greatest extent.

Carbapenem antibiotics possess a broad antimicrobial spectrum and are commonly used to treat complicated and serious bacterial infections. Although carbapenems are tolerated well by most patients, an important adverse effect associated with their administration is central nervous system (CNS) toxicity [1]. Early clinical trials reported a relationship between the use of imipenem and development of seizures [1], which remains a concern, particularly at high doses of carbapenems [2]. A previous study investigated the risk of seizures with the use of carbapenems and reported rates as high as 6%, especially when dosing was not carefully adjusted with respect to renal function [3]. Therefore, higher brain concentrations of carbapenems have been linked to an increased risk of seizures. The penetration of drugs into brain tissue may vary according to the kind of carbapenems administered. A meta-analysis previously revealed that the odds ratios for the risk of seizures from imipenem, meropenem, and doripenem relative to other antibiotics were 3.50 (95% confidence interval (CI) 2.23, 5.49), 1.04 (95% CI 0.61, 1.77), and 0.44 (95% CI 0.13, 1.53), respectively [4]. Furthermore, among the carbapenems examined, meropenem and biapenem induced weaker convulsive activity than imipenem in animals [5,6]. Nevertheless, the pharmacokinetics and brain penetration of carbapenems have not yet been examined. Therefore, the aim of this *in vivo* investigation was to determine the pharmacokinetics and brain penetration of carbapenems in mice.

This study was reviewed and approved by the Animal Experimentation Committee of Kyoritsu University of Pharmacy (#42), and was performed in compliance with its Animal Experimental Guidelines. Male ddY mice (5-week-old) were supplied by Sankyo Labo Service Co., Ltd. (Tokyo, Japan), kept under a 12 h/12 h light–dark cycle for a week with free access to food and water, and were used in experiments at the age of 6 weeks old.

Imipenem and cilastatin were purchased from USP (Rockville MD, USA) and Wako Pure Chemical Industies, Ltd. (Osaka, Japan), respectively. Meropenem, biapenem, and doripenem were a gift

* Corresponding author. Tel./fax: +81 3 5400 2656.
E-mail address: matsumoto-kz@pha.keio.ac.jp (K. Matsumoto).

http://dx.doi.org/10.1016/j.jiac.2015.11.010
1341-321X/

from Sumitomo Dainippon Pharma Co., Ltd. (Osaka, Japan), Meiji Seika Pharma Co., Ltd. (Tokyo, Japan), and Shionogi Co., Ltd. (Osaka, Japan). Other reagents used in this study were of analytical grade.

Single-dose plasma pharmacokinetic studies were performed after the subcutaneous administration of 9.1 mg/mL of the different carbapenems and cilastatin dissolved in saline, because imipenem/cilastatin could dissolve in saline up to 9.1 mg/mL. Cilastatin was administered to avoid the degradation of carbapenems. Blood samples and brain tissue samples, after decapitation under ether anesthesia, were obtained 10, 20, 30, 60, and 120 min after the subcutaneous administration of carbapenems (91 mg/kg) (four animals per time point). The blood samples collected at each sampling time point were centrifuged (3000 rpm, 10 min) immediately after collection, and the obtained plasma was stored frozen at −80 °C for later analyses. After the brain tissue sample was washed with saline and weighed, the sample was added at 2 times its volume weight to the mobile phase. The mixture was homogenized using a polytron homogenizer to prepare a brain tissue suspension, which was then centrifuged (13,000 rpm, 10 min). The obtained supernatant was stored frozen at −80 °C for later analyses.

Carbapenem concentrations in plasma and brain tissue were measured using high-performance liquid chromatography (HPLC) [7]. Ninety microliters of the mobile phase was added to a 10-μL aliquot of a plasma sample. The whole volume was then pipetted into a centrifugal filter (10,000 NMWL Filter Unit; Merck Millipore, Darmstadt, Germany) and centrifuged (14,000 rpm, 20 min), after which a 20-μL aliquot of the filtrate was injected into the HPLC equipment. A 140-μL aliquot of the supernatant obtained from brain tissue was pipetted into a centrifugal filter device and centrifuged (14,000 rpm, 30 min), after which a 20-μL aliquot of the filtrate was injected into the HPLC equipment.

HPLC employed a Luna 5um C18(2) 100 Å column (250 × 4.6 mm; Phenomenex Ltd, Torrance, USA) and detected ultraviolet absorbance at a wavelength of 300 nm. The mobile phase consisted of 0.1 M phosphate buffer (pH 7.8):methanol = 92:8 for imipenem and biapenem, and 78:22 for meropenem and doripenem. The quantification limits were 0.02 μg/mL for imipenem and biapenem and 0.04 μg/mL for meropenem and doripenem. The intra- and inter-day accuracy (as absolute values of the relative errors of the means) and precision (as coefficient of variation values) were within 10%.

A non-compartmental pharmacokinetic analysis was conducted to estimate the rate and extent of the penetration of carbapenems from the systemic circulation into brain tissue. C_{max} was defined as the observed maximum concentration of carbapenems, and T_{max} was the time to C_{max}. The area under the drug concentration−time curve from 0 to infinity ($AUC_{0-\infty}$) and mean residence time (MRT) were calculated based on the trapezoidal rule (Microsoft Excel 2010). Using plasma concentration data, total clearance (CL_{total}/F) was estimated as dose/$AUC_{0-\infty}$, and the volume of distribution at a steady state (V_{ss}/F) was calculated as CL_{total}/F*MRT. The elimination half-life ($T_{1/2}$) was estimated by dividing 0.693 by the elimination rate constant K_e (= $CL_{total}/F/V_{ss}$). Since protein binding rates of imipenem, meropenem, biapenem and doripenem in mouse serum were previously reported to be 2.5, 18.9, 3.8 and 25.2%, respectively [8,9], the free $AUC_{0-\infty}$ for plasma were calculated: $fAUC_{0-\infty,\ plasma}$ = (1 − protein binding rates of carbapenems)*$AUC_{0-\infty,\ plasma}$.

Data were subjected to an analysis of variance using SPSS version 22 for Windows (IBM Japan Co., Ltd., Tokyo, Japan). Data were analyzed by Tukey's HSD tests. Differences of $P < 0.05$ were considered significant.

The concentrations of carbapenems observed in plasma were shown in Fig. 1. The concentration of doripenem was significantly higher than that of imipenem at 10 min ($P < 0.05$). Furthermore, the concentration of meropenem was significantly higher than those of imipenem and biapenem at 60 and 120 min ($P < 0.05$). Plasma pharmacokinetics parameters were summarized in Table 1, and the following values were obtained: 1.20–1.71 L/h/kg for CL_{total}/F, 1.41–2.03 h^{-1} for K_e, 0.34–0.51 h for $T_{1/2}$, 0.66–0.95 L/kg for V_{ss}/F, 0.49–0.73 h for MRT, and 83.46–110.58 μg/mL for C_{max}. T_{max} was 10 min. The $AUC_{0-\infty}$ of the carbapenems tested in plasma were as follows: doripenem > meropenem > biapenem > imipenem (Table 1). The observed concentrations of carbapenems in brain tissue were shown in Fig. 2. The concentration of imipenem was significantly higher than those of meropenem and biapenem at 10 and 20 min ($P < 0.05$), while the concentration of doripenem was significantly higher than that of biapenem at 20 min ($P < 0.05$). The concentrations of imipenem and doripenem were significantly higher than those of meropenem and biapenem at 30 min ($P < 0.05$). The concentration of imipenem was significantly higher than those of the other carbapenems at 60 min ($P < 0.05$). The concentration of doripenem was significantly higher than that of biapenem at 60 min ($P < 0.05$). The ranges of C_{max} and T_{max} were 0.28–0.83 μg/g and 10–20 min, respectively. The $AUC_{0-\infty}$ of carbapenems in brain tissue were in the following order: imipenem > doripenem > meropenem > biapenem (Table 1).

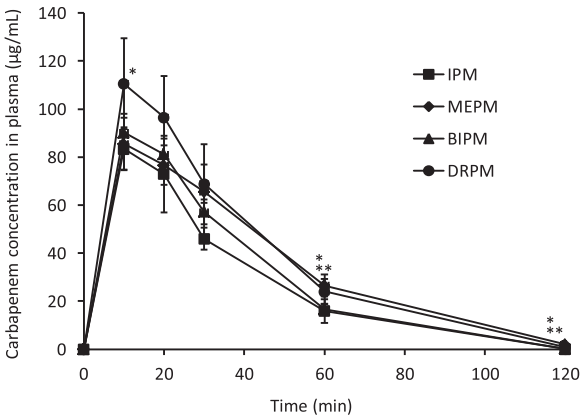

Fig. 1. Observed plasma concentrations of carbapenems in mice after a single subcutaneous administration (91 mg/kg). Data are the average values of 4 experiments (±S.D.). *: $P < 0.05$ significantly different from imipenem, **: $P < 0.05$ significantly different from biapenem.

Furthermore, $fAUC_{0-\infty,\ plasma}$ for imipenem, meropenem, biapenem and doripenem were 52.00, 57.19, 58.02 and 56.92 μg*h/mL, respectively. The degrees of brain tissue penetration, defined as the $AUC_{0-\infty,\ brain\ tissue}/fAUC_{0-\infty,\ plasma}$ ratio, were 0.016 for imipenem, 0.004 for meropenem, 0.002 for biapenem, and 0.008 for doripenem. And $fC_{max,\ plasma}$ for imipenem, meropenem, biapenem and doripenem were 81.37, 69.34, 86.80 and 82.71 μg/mL, respectively. The $C_{max,\ brain\ tissue}/fC_{max,\ plasma}$ ratio, were 0.010 for imipenem, 0.006 for meropenem, 0.003 for biapenem, and 0.007 for doripenem.

We herein showed the detailed pharmacokinetic parameters and penetration into brain tissue of each carbapenem, and demonstrated that, among the carbapenems tested, imipenem had the highest $AUC_{0-\infty,\ brain\ tissue}/fAUC_{0-\infty,\ plasma}$ ratio.

Previous studies on the mouse pharmacokinetics of carbapenems demonstrated that the $AUC_{24\ h}$ of imipenem was 60.6 μg*h/mL at a subcutaneous dose of 128 mg/kg/day [10], the $AUC_{0-\infty}$ of meropenem and biapenem were 61.0 and 77.6 μg*h/mL, respectively, at an intraperitoneal dose of 100 mg/kg/day [11], and the $AUC_{0-\infty}$ of doripenem was 75.3 μg*h/mL at an intravenous dose of 100 mg/kg/day [8]. As shown in Table 1, our results were consistent with these findings. However, detailed pharmacokinetic parameters of carbapenems were not shown in the previous studies, and