Structural Features of Carbapenem Compounds for Nephrotoxicity: Effect of C-2 Side Chain

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Several β-lactam antibiotics produce acute nephrotoxicity in both human and laboratory animals[1~3]. Two clinically available carbapenems, imipenem (IPM) and panipenem (PAPM), are also nephrotoxic in laboratory animals when each carbapenem is administered without the combinational drug (*i.e.* cilastatin and betamipron, respectively)[4,5]. The nephrotoxicity of these carbapenem antibiotics is characterized by necrosis of the proximal tubule epithelial cells, similar to the well known nephrotoxic cephalosporin, cephaloridine (CER). Meropenem (MEPM), a new dehydropeptidase-I (DHP-I) stable carbapenem antibiotic without neurotoxicity[6,7], has shown only minimal nephrotoxicity at a very high dose in laboratory animals[8]. This profile could make it the first carbapenem that does not need any combinational drug. The structure of MEPM is different from that of IPM and PAPM by the presence of a 1β-methyl group and the lesser basicity of the amino group in the C-2 side chain. The basicity of MEPM is much lower than that of IPM and PAPM (pKa = 7.4[9], 9.9[10], 10.9[11], respectively), so that the physico-chemical properties of MEPM are different from those of the others.

We have reported that the basicity of the C-2 side chain of carbapenem antibiotics plays an important role in inducing convulsions[12]. We find that the β-lactam ring structure is not responsible for the convulsive activity and that the neurotoxicity is related to only a part of the structure that includes the C-2 side chain, but not the carbapenem skeleton itself[12,13]. Although the difference in nephrotoxicity between these carbapenems is also considered due to the different structural features, especially the physico-chemical properties, there is no report on structure-activity relationships for nephrotoxicity of carbapenem antibiotics. The aim of this report is to elucidate the structural feature involved in nephrotoxicity, in particular the role of the carbapenem skeleton and the basicity of the C-2 side chain.

IPM was purified from imipenem/cilastatin (Banyu Pharmaceutical Co., Ltd.) in our laboratories. Other carbapenem compounds were synthesized in our laboratories according to the reported procedures[14,15]. New Zealand white male rabbits were purchased from Nihon Dobutsu Co., Ltd. (2.0~2.5 kg in weight). Each compound, except for IPM, was dissolved in saline at a concentration of 100 mg/ml. Due to insolubility and instability, IPM was dissolved in 0.3% sodium bicarbonate at a concentration of 50 mg/ml. Each solution was given to the rabbits at a dose of 300 mg/kg (IPM, 150 mg/kg). Serum biochemical parameters including total protein, albumin, albumin/globulin ratio, glucose, total cholesterol, calcium, uric acid, sodium, potassium, alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen, creatinine, and alkaline phosphatase (ALP) were assayed using an auto-analyzer. The kidneys were removed and evaluated histopathologically. The specimens were microscopically observed after hematoxylin-eosin staining or the periodic acid-Schiff's (PAS) reaction. The degree of proximal tubule necrosis was classified as follows; slight change, necrosis in few tubules; mild, multifocal necrosis in some tubules; moderate, multifocal necrosis in many tubules; severe, widespread necrosis. The biochemical analysis and histopathological examination were performed independently.

Table 1 shows serum biochemical indices at 48 hours after administration of IPM, N-acetyl thienamycin, and hydrolyzed IPM (the β-lactam ring opened metabolite of IPM). IPM elevated serum urea nitrogen and creatinine levels significantly but other parameters tested did not change. In agreement with the serum biochemical findings, the obvious proximal tubule necrosis was observed in all kidneys from rabbits treated with IPM. To determine whether basicity in the C-2 side chain has an important role in the nephrotoxicity, we examined N-acetyl thienamycin, which lacks basicity in the C-2 side chain and has almost the same molecular size as IPM. Even at a dose of 300 mg/kg, there were no abnormal changes in either serum biochemical data or histopathological observations. This suggests that the nephrotoxicity of IPM relates to the basic center in the C-2 side chain. Since the nephrotoxic activity of IPM was drastically reduced by cleavage of the β-lactam ring, the β-lactam ring is necessary to induce renal toxicity. This result is quite different from the neurotoxic activity, because the β-lactam ring does not play any role in inducing convulsions[13].

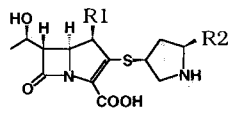
MEPM shows no nephrotoxic findings in either serum biochemical findings or histopathological observations even at a dose of 300 mg/kg (Table 2). To make sure the reduced nephrotoxicity of MEPM is due to its reduced basicity in the C-2 side chain, we synthesized several analogues of MEPM and tested their nephrotoxicity in rabbits. Des-methyl MEPM did not induce nephrotoxicity according to serum biochemistry or histopathology. This result confirms that reduced nephrotoxic activity is not related to the presence of the 1β-methyl group. Then we attempted to increase the basicity in the amino group of the C-2 side chain of MEPM by inserting methylene spacers between the pyrrolidine ring and the electron withdrawing dimethylaminocarbonyl group. Compound A has one methylene spacer connecting a monomethylaminocarbonyl group, and hence, it has the same molecular weight as MEPM. Compound B has two methylene spacers. The pKa values of the amino group

Table 1. Nephrotoxic activity of imipenem, N-acetyl thienamycin, and hydrolyzed imipenem in rabbits.

Compound (Dose)	Urea nitrogen (mg/dl)	Creatinine (mg/dl)	No. of rabbits with proximal tubular necrosis #			
			−	±	+	++
Control	16±5	1.0±0.2	3	0	0	0
Imipenem (150 mg/kg)	68±6**	2.7±0.2**	0	0	3	0
N-Acetyl thienamycin (300 mg/kg)	16±3	1.1±0.1	3	0	0	0
Hydrolyzed imipenem (300 mg/kg)	16±2	1.3±0.1	3	0	0	0

Each value represents mean ± s.d. (n=3).
** : $p<0.01$, significantly different from the control (saline treated group) by one-way variance analysis
# −, not observed; ±, slight; +, mild; ++, moderate.

Table 2. Nephrotoxic activity of meropenem and related compounds in rabbits.



Compound (No. of rabbits)	Structure R1	Structure R2	Urea nitrogen (mg/dl)	Creatinine (mg/dl)	No. of rabbits with proximal tubular necrosis #			
					−	±	+	++
Meropenem (4)	CH_3	$CON(CH_3)_2$	19±3	1.2±0.3	4	0	0	0
Des-methyl meropenem (4)	H	$CON(CH_3)_2$	12±2	0.9±0.1	4	0	0	0
Compound A (3)	CH_3	$CH_2CONHCH_3$	86±19**	5.2±1.9**	0	0	2	1
Compound B (3)§	CH_3	$CH_2CH_2CONHCH_3$	91±11**	3.4±0.2**	0	0	2	0
Compound C (3)	CH_3	$CONH_2$	14±3	0.9±0.1	3	0	0	0
Control (3)			16±5	1.0±0.2	3	0	0	0

Dose: 300 mg/kg.
Each value represents mean ± s.d.
** : $p<0.01$ Significantly different from the control (saline treated group) by one-way variance analysis.
# −, not observed; ±, slight; +, mild; ++, moderate.
§:one of three animals died immediately after administration.

of the C-2 side chain of compound A, B, and MEPM were calculated at 8.9, 10.0, and 7.2, respectively[16]. When these compounds were administered to rabbits, both compounds significantly elevated serum urea nitrogen and creatinine levels, and induced obvious tubular necrosis. One animal in the group treated with compound B died immediately, probably due to other toxicity such as central nervous system toxicity. On the other hand, compound C, has an aminocarbonyl group as R2 (shown in Table 2), so that its basicity in the C-2 side chain is low like MEPM (pKa = 7.2)[16], and it is less bulky at the end of the side chain than MEPM. This compound did not affect the serum biochemistry or histopathology. These results clearly indicate that the basicity of the C-2 side chain in this series of carbapenems is important in evoking nephrotoxicity.

Although the mechanism of nephrotoxicity of β-lactam antibiotics has not been fully elucidated, the accumulation of the antibiotics in the proximal tubule cell and the reactivity with the target molecule are considered to be important[17]. Of the two structural features responsible for the nephrotoxicity of the carbapenem compounds, the importance of the basicity of the C-2 side chain and the necessity of the β-lactam ring seem to relate to the distribution and the reactivity, respectively. CER is actively transported into the proximal tubule cell by the organic anion transport system, and is accumulated probably because the cationic charge in the C-3 side chain restricts its movement from cell-to-tubular fluid[3]. Both IPM and PAPM are also accumulated intracellularly by this transporter, and the nephrotoxicity of these three antibiotics is reduced by the inhibition of this transport system[4,5]. Therefore, it is likely that the basicity is important in the cellular accumulation to cause nephrotoxicity.

It is suggested that nephrotoxic cephalosporins have an active leaving group in their C-3 side chain and can cause mitochondrial injury by acylating and inactivating the mitochondrial transporters[17]. Although IPM does not have any leaving group, it is reported that IPM also causes similar mitochondrial toxicity[18,19]. Our observation is consistent with the hypothesis that the β-lactam ring of carbapenems is essential to acylate the target protein that leads to nephrotoxicity. The carbapenem skeleton itself has much higher reactivity due to strained chemical structure than cephalosporin skeleton. That is considered to be one reason why carbapenems show nephrotoxicity without an active leaving group which is important for the cephalosporin nephrotoxicity. Since it is reported that CER's zwitter ion region is also implicate to the toxicity for mitochondrial toxicity[20], the basicity may also be important for interacting with the target. We are now investigating whether the basicity is important for accumulation in the tubule cells or interaction with the target such as mitochondria or both of them, using these series of carbapenem compounds.

In conclusion, our observations clearly indicate that the β-lactam ring and the basicity of the C-2 side chain play an important role in the nephrotoxicity of carbapenem antibiotics. This information as well as the structural features responsible for neurotoxicity will be helpful to design next generational carbapenem antibiotics with low side-effects.

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References

1) ATKINSON, R. M.; J. P. CURRIE, B. DAVIS, D. A. H. PRATT, H. M. SHARPE & E. G. TOMICH: Acute toxicity of cephaloridine, an antibiotic derived from cephalosporin C. Toxicol. Appl. Pharmacol. 8: 398~406, 1966
2) FOORD, R. D.: Cephaloridine, cephalothin and the kidney. J. Antimicrobial. Chemother. 1 (Suppl.): 119~133, 1975
3) TUNE, B. M.: The nephrotoxicity of β-lactam antibiotics. *In* Toxicology of the Kidney. Second edition. *Ed.*, J. B. HOOK and R. S. GOLDSTEIN, pp. 257~281, Raven Press Ltd., New York, 1993
4) BIRNBAUM, J.; F. M. KAHAN, H. KROPP & J. S. MACDONALD: Carbapenems, A new class of beta-lactam antibiotics: Discovery and development of imipenem/cilastatin. Am. J. Med. 78 (suppl. 6A): 3~21, 1985
5) NAGANUMA, H.; H. TOKIWA, Y. HIROUCHI, Y. KAWAHARA, J. FUKUSHIGE, M. FUKAMI, K. HORITA, S. MURAMATSU, H. TAKAHAGI, K. INUI, Y. TANIGAWARA, M. YASUHARA, R. HORI & S. KUWAHARA: Nephroprotective effect and its mechanism of betamipron (1): Relationships of renal transport. Chemotherapy (Tokyo) 39 (S-3): 166~177, 1991
6) OHNO, Y.; A. HIROSE, R. TSUJI, T. KATO, M. NAKAMURA, J. R. EDWARD & J. B. PATEL: Behavioral and electroencephalographic studies on the central action of a novel carbapenem: Meropenem. Chemotherapy (Tokyo) 40 (S-1): 175~181, 1992
7) PATEL, J. B. & R. E. GILES: Meropenem: evidence of lack of proconvulsive tendency in mice. J. Antimicrobial. Chemother. 24 (Suppl. A): 307~309, 1989
8) TOPHAM, J. C.; L. B. MURGATROYD, D. V. JONES, U. R. P. GOONETILLEKE & J. WRIGHT: Safety evaluation of meropenem in animals: studies on the kidney. J. Antimicrob. Chemother. 24 (Suppl. A): 287~306, 1989
9) TAKEUCHI, Y.; M. SUNAGAWA, Y. ISOBE, Y. HAMAZUME & T. NOGUCHI: Stability of a 1β-methylcarbapenem antibiotic, meropenem (SM-7338) in aqueous solution. Chem. Pharm. Bull. 43: 689~692, 1995
10) SUMITH, G. B & E. F. SCHOENEWALDT; Stability of N-formimidoylthienamycin in aqueous solution. J. Pharm. Sci. 70: 272~276, 1981
11) CARBENIN: Product brochure. Sankyo Co., Ltd.
12) SUNAGAWA, M.; H. MATSUMURA & M. FUKASAWA: Structure-activity relationships of carbapenem and penem compounds for the convulsive property. J. Antibiotics 45: 1983~1985, 1992
13) SUNAGAWA, M.; H. MATSUMURA, Y. SUMITA & H. NOUDA: Structural features resulting in convulsive activity of carbapenem compounds: Effect of C-2 side chain. J. Antibiotics 48: 408~416, 1995

14) SUNAGAWA, M.; H. MATSUMURA, T. INOUE, M. FUKASAWA & M. KATO: A novel carbapenem antibiotic, SM-7338: Structure-activity relationships. J. Antibiotics 43: 519~532, 1990
15) SUNAGAWA, M.; H. MATSUMURA, T. INOUE, M. FUKASAWA & M. KATO: A new series of carbapenem antibiotics with 5'-substituted pyrrolidinylthio group at C-2 position. J. Antibiotics 44: 459~462, 1991
16) TAFT, R. W. Jr.: "Steric effects in organic chemistry", M. S. NEWMAN, *Ed.*, Chap. 13, JOHN WILEY & Sons, New York, 1956
17) TUNE, B. M.: Renal tubular transport and nephrotoxicity of beta-lactam antibiotics: Structure-activity relationships. Miner Electrolyte Metab. 20: 221~231, 1994
18) TUNE, B. M.; D. FRAVERT & C.-Y. HSU: Thienamycin nephrotoxicity: Mitochondrial injury and oxidative effects of imipenem in the rabbit kidney. Biochem. Pharmacol. 38: 3779~3783, 1989
19) TUNE, B. M. & C.-Y. HSU: The renal mitochondrial toxicity of beta-lactam antibiotics: In vitro effects of cephaloglycin and imipenem. J. Am. Soc. Nephrol. 1: 815~821, 1990
20) TUNE, B. M. & C.-Y. HSU: Toxicity of cephaloridine to carnitine transport and fatty acid metabolism in rabbit renal cortical mitochondria: Structure-activity relationships. J. Pharmacol. Exp. Ther. 270: 873~880, 1994