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Comparison of *In Vivo* Nephrotoxicity in the Rabbit by a Pyrrolidinyl-Thio Carbapenem CW-270031

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CW-270031 is a novel synthesized carbapenem antibiotic with a broad antimicrobial activity. Carbapenem antibiotics are well known for their nephrotoxicity. In this study, we evaluated the nephrotoxicity potential of this compound in rabbits, which are known for being more sensitive than other animals to renal insult. CW-270031 was administered to NZW male rabbits *via* an ear vein (200 mg/kg, single injection). Blood samples were collected on 2, 3, and 4 days after treatment. Urea nitrogen and creatinine in plasma were quantified. Four days after the treatment, all animals were autopsied and histopathological examinations were performed on their kidneys, revealing that cephaloridine and imipenem were highly nephrotoxic, and cefazolin had mild renal toxicity, whereas CW-270031 as well as meropenem and tienam had no toxicity to the kidney. The present findings suggest that CW-270031 is a potential carbapenem antibiotic with no nephrotoxicity.

Keywords: Carbapenem, CW-270031, nephrotoxicity, rabbits

Since the discovery of thienamycin from *Streptomyces cattleys* [8], carbapenem compounds have provided a new generation of β-lactam antibiotics, which are highly potent against a broad range of bacterial species [2]. Although imipenem could not be marketed owing to its chemical and biological instabilities [9], to overcome these problems, many groups started to develop stable compounds and ways of synthesizing thienamycin because enzymatic production was not effective. Under these circumstances, imipenem [2] containing cilastatin as an inhibitor of renal dehydropeptidase-I (DHP-I) was marketed by Merck and Co. [9]. The Sankyo group marketed panipenem containing betamipron for the alleviation of nephrotoxicity [15].

Carbapenems generally demonstrate nephrotoxicity in most experimental animals. Carbapenem antibiotics have been evaluated for their nephrotoxic potentials in the rabbit [4]. The rabbit has been a popular animal model for nephrotoxicity assessments because of the unique sensibility of this species to the carbapenem compared with that of other laboratory animals.

In order to study a carbapenem compound with broad-spectrum antibacterial activity and stability to renal DHP-I, we synthesized a new pyrrolidinyl-thio carbapenem, known as CW-270031 (Fig. 1) [10], which is a potential injectable carbapenem that has a methyl group at the 1-β position. This report presents the results of the *in vivo* nephrotoxicity of carbapenem in the rabbit.

Materials and Methods

Antibiotics

CW-270031, imipenem, and cilastatin were obtained from Choong Wae Pharmaceutical Co., Ltd. (Hwasung, Korea). Cefazolin was a gift from Dong-A Pharmaceutical Co. Ltd. (Seoul, Korea) and cephaloridine was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Meropenem was obtained from Sumitomo Pharmaceutical Co., Ltd. (Tokyo, Japan).

Animals and Care

New Zealand white male rabbits weighing 2.2±0.2 kg (Samtaco, Osan, Korea) were used in this study. Each group of rabbits was housed singly in cages and metabolic caged groups of five rabbits were individually injected intravenously (i.v.) through the ear vein of the rabbit with 200 mg of each antibiotic per kg of body weight. The antibiotics were dissolved in distilled water and filtered (Millipore filter, 0.22 μm). Animal care was carried out as described

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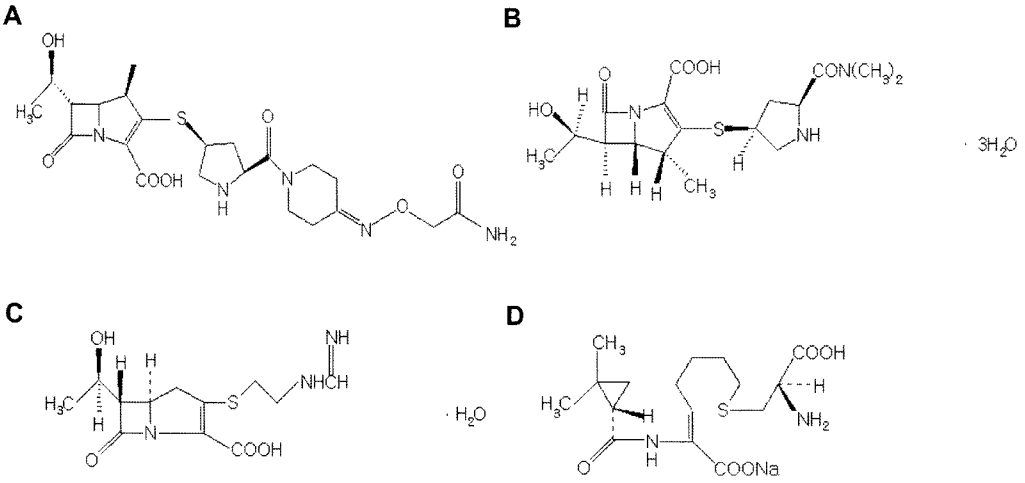

Fig. 1. Chemical structure of (**A**) CW-270031, (**B**) meropenem, (**C**) imipenem, and (**D**) cilastatin.

previously with a slight modification [7]. All procedures were performed in compliance with the *Guiding Principles in the Care and Use of Animals* (National Research Council, 1996) by the Council of American Physiological Society, and the Animal Welfare Committee of Kyungpook National University. The animal received tap water and chow *ad libitum* and were maintained in a room under standard laboratory conditions (23°C±1°C and 50%±5% humidity) with 12 h of dark/light cycle. We strictly adhered to the rules for animal experiments including ethical care under guidance of the Committee.

Blood Chemistry

Rabbits were given antibiotics, and blood was collected every day for the measurement of urea nitrogen and creatinine (96 h before and 48, 72, and 96 h after dosing) [13]. The results were analyzed quantitatively using a commercial kit (Sigma Diagnostic) or an automatic blood chemistry analyzer (Merck Vitalab Selectra II).

Gross Lesions

The rabbits were decapitated four days after the administration of the antibiotics, and their kidneys were immediately excised and weighed. Gross lesions on the kidney and other organs were classified as no significant lesion (-), or mild (+), moderate (++), severe (+++), and extremely severe (++++) lesions.

Histopathological Lesions of the Kidneys

The rabbits were decapitated four days after administration of the antibiotics, and their kidneys were immediately excised and weighed. Both right and left kidneys for all animals were fixed in a neutral buffered 10% formalin. The kidneys were embedded in paraffin wax, and sections were then cut at 4 μm, stained with hematoxylin and eosin, and examined by a light microscope. Histopathological lesions were classified as no significant lesion (-), or mere (±), mild (+), moderate (++), severe (+++), and extremely severe (++++) lesions [14].

Statistical Analyses

All statistical analyses were performed with an unpaired Student's *t*-test for paired differences.

Results

CW-270031 and the related compounds after an ear intravenous administration of a single 200 mg/kg dose in the rabbits are given here. The body weight and kidney weight per specimen treated with imipenem and cephaloridine were significantly decreased compared with the control

Table 1. Body weight and kidney weight changes in each group.

Group	Body weight (kg)		% Change of body weight[a]	Kidney weight (g)	Kidney weight/ 100 g of body weight
	Day 0	Day 4			
Control	2.50±0.23	2.54±0.26	101.6±8.3	8.6±0.6	0.34±0.03
Imipenem	2.34±0.19	2.22±0.28	96.5±7.6*	9.6±0.7	0.44±0.02**
Tienam	2.56±0.28	2.63±0.24	102.7±8.8	10.4±0.4	0.39±0.03
Meropenem	2.34±0.30	2.43±0.33	103.9±9.1	8.8±0.4	0.36±0.04
Cefazolin	2.35±0.25	2.40±0.19	102.1±5.4	7.1±0.6	0.30±0.03
Cephaloridine	2.39±0.17	2.22±0.29	92.9±8.2**	11.1±0.7	0.50±0.02**
CW-270031	2.38±0.21	2.40±0.26	100.8±7.9	8.8±0.6	0.37±0.03

[a]Data represent the relative percentage (%) of body weight on day 4 to body weight on day 0.
*Significantly different from control group at $p<0.05$ ($n=5$).
**Significantly different from control group at $p<0.01$.

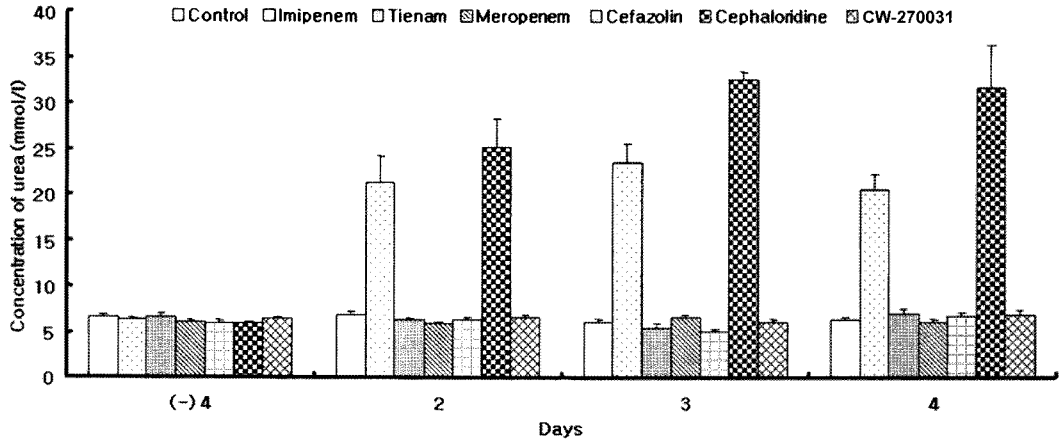

Fig. 2. Concentration of plasma urea nitrogen before and after drug administration.
All compounds were dissolved in saline and were administrated *via* the ear vein on day 0 at a concentration of 200 mg/kg body weight. Each set of data represents mean±standard deviation of 5 animals. *Significantly different from vehicle ($p<0.01$).

group's weight per specimen ($p<0.05$, $p<0.01$). There were no important changes in body weight and kidney weight/100 g body weight indices for those animals dosed with tienam, meropenem, cefazolin, and CW-270031 (Table 1). The concentrations of urea nitrogen and blood creatinine were significantly increased on the 2nd, 3rd, and 4th days after imipenem and cephaloridine ($p<0.01$), and the maximum values gradually increased on the 3rd day after an administration. They were four to five times higher than for the control group. However, there were no important changes in blood chemistry indices for those animals dosed with tienam, meropenem, cefazolin, and CW-270031 (Figs. 2 and 3). The gross lesions of CW-270031 and the reference compounds are summarized in Table 2. Test compounds were given by an ear intravenous administration of a single 200 mg/kg dose in the rabbits. The rabbits were decapitated four days after the administration of the antibiotics, and their kidneys were immediately excised and weighed. Imipenem-treated animals demonstrated severe lesions with renal cortex decoloration, cortical necrosis, and moderate renal swelling. The cefazolin-treated animals showed mild lesions with renal cortex decoloration, cortical necrosis, and moderate atrophy. There were observed in cephaloridine-treated groups extremely severe lesions with renal cortex decoloration, cortical necrosis, and severe renal swelling. No gross lesions were seen in the kidney and other organs (liver, lung, spleen, heart and digestive system) of the animals who received saline, tienam, meropenem, or CW-270031. The histopathological lesions of the kidneys are summarized in Table 3. Moderate tubular dilatation, severe tubular necrosis, and severe protein cast of the kidney were observed in imipenem-treated groups. Mild tubular dilatation and mild tubular necrosis were observed after the administration of cefazolin. A single dose of cephaloridine

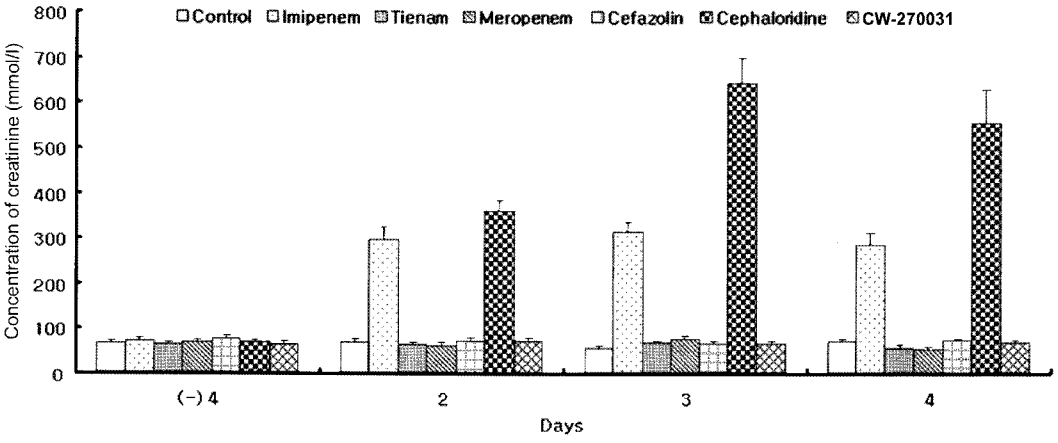

Fig. 3. Concentration of plasma creatinine before and after drug administration.
All compounds were dissolved in saline and were administrated *via* the ear vein on day 0 at a concentration of 200 mg/kg body weight. Each set of data represents mean±standard deviation of 5 animals. *Significantly different from vehicle ($p<0.01$).

Table 2. Gross lesions of the kidney and other organs.

Group	Kidney				Other organs
	Decoloration of surface	Cortical necrosis	Swelling	Atrophy	
Control	-	-	-	-	-
Imipenem	+++	+++	++	-	-
Tienam	-	-	-	-	-
Meropenem	-	-	-	-	-
Cefazolin	+	+	-	++	-
Cephaloridine	++++	++++	+++	-	-
CW-270031	-	-	-	-	-

Lesions were classified as no (-), mere (+), moderate (++), severe (+++), or extremely severe (++++).

did induce nephrotoxicity, including mild tubular dilatation, extremely severe tubular necrosis, severe protein cast, and moderate calcium disposition [3]. A single dose of CW-270031 induced only mild tubular dilatation, which was observed on the 4th day after the treatment (Fig. 4).

Discussion

It is well documented that the antibiotic is a self-defense, bioactive agent, which can be obtained by microbial sources and chemically synthesized [11, 12, 16, 17]. Carbapenems, a new generation antibiotic, generally produce nephrotoxicity in most experimental animals. Carbapenem antibiotics have been evaluated for their nephrotoxic potentials *in vivo* in the rabbit. The result for the single-dosing study of the imipenem and cephaloridine in rabbits was to produce acute proximal tubular necrosis [16]. However, there was no nephrotoxicity after co-administration of the dehydropeptidase-I (DHP-I) inhibitor cilastatin [1, 9, 14]. The carbapenems are known to be inactivated by DHP-I located on the border of the proximal tubular cells [1]. It is well known that imipenem induces nephrotoxicity, whereas meropenem is very stable against the rabbit's DHP-I enzyme. Meropenem could directly resist metabolism by renal DHP-I without additives like cilastatin. Therefore, when meropenem was administrated without the DHP-I inhibitor, it did not induce nephrotoxicity. CW-270031 is a

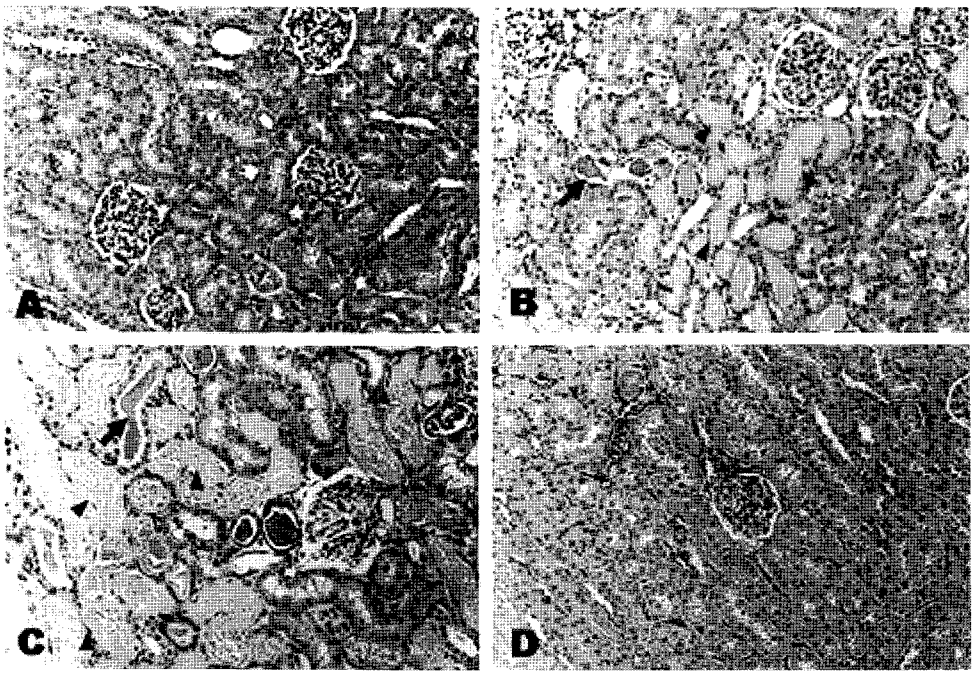

Fig. 4. Microphotographs of the cortex of rabbit kidneys injected by i.v. with (**A**) saline, (**B**) imipenem, 200 mg/kg, (**C**) cephaloridine, 200 mg/kg, or (**D**) CW-270031, 200 mg/kg.

A. No morphological changes were found. **B**. Cortical tubules showed severe necrosis (arrow head), and protein casts were contained in the lumen of the tubules (arrow). **C**. Photograph shows massive necrosis of the cortical tubules (arrow heads) and the formation of intratubular protein cast (arrow). **D**. There were no morphological changes. Hematoxylin and eosine stain, ×200.

Table 3. Histopathological lesions of the kidney.

Group	Microscopical lesions			
	Tubular dilatation	Tubular necrosis	Protein cast	Calcium disposition
Control	±	-	-	-
Imipenem	++	+++	+++	-
Tienam	-	-	-	-
Meropenem	±	-	-	-
Cefazolin	+	+	-	-
Cephaloridine	+	++++	+++	++
CW-270031	+	-	-	-

Lesions were classified as no (-), mere (±), mild (+), moderate (++), severe (+++), or extremely severe (++++).

β-lactam antibiotic that has a methyl group at the 1-β position in its structure [10], and it also has been very stable against DHP-I in the mouse, rabbit, and dog. Thus, in the present study, the effect of CW-270031 on a single dosing of nephrotoxicity was investigated in rabbits. The results of these tests were revealed in the single-dosing study of carbapenem in the rabbits. The imipenem- and cephaloridine-treated group's body weights significantly decreased compared with the control group's ($p<0.05$, $p<0.01$), and other treatments groups' kidney weights per specimen were significantly increased ($p<0.05$, $p<0.01$). However, there were no important changes in body weight and kidney weight/100 g body weight indices for those animals dosed with tienam, meropenem, cefazolin, and CW-270031.

This study shows that tienam, meropenem, cefazolin, and CW-270031 have no nephrotoxicity to rabbits. On the other hand, imipenem and cephaloridine caused renal damage, which were included as a positive control. Collectively, the present study reveals that CW-270031 can be a potential candidate for alleviating nephrotoxicity.

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