Preventive Effect of Betamipron on Nephrotoxicity and Uptake of Carbapenems in Rabbit Renal Cortex

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ABSTRACT—The preventive effect of betamipron (*N*-benzoyl-3-propionic acid: BP) on the renal uptake and nephrotoxicity of carbapenems (panipenem and imipenem) was studied in rabbits. Panipenem, a new carbapenem antibiotic, induced nephrotoxicity at a dose of 200 mg/kg, i.v., but this was less severe than that caused by a single dose of imipenem or cephaloridine. Along with the significant reduction of nephrotoxicity, the uptake of these carbapenems in the renal cortex was remarkably inhibited by simultaneous treatment with BP (200 mg/kg, i.v.). These results suggest that BP reduces the nephrotoxicity of carbapenems through inhibiting the active transport of carbapenems in the renal cortex. Because of the low toxicity of BP (LD₅₀ in the rat, more than 3,000 mg/kg, i.v.), it was concluded that BP might be a good candidate for reducing the nephrotoxicity induced by panipenem or imipenem.

Keywords: Betamipron (*N*-benzoyl-3-propionic acid), Panipenem, Imipenem, Nephrotoxicity (rabbit), Renal uptake (rabbit)

Betamipron (BP) is an amino acid derivative that has benzoyl and carboxyl groups in its structure, and it also has very low toxicity in mammals (LD₅₀ in the rat, more than 3,000 mg/kg, i.v.). We previously found that BP reduced the nephrotoxicity by cephaloridine (CER), a cephalosporin antibiotic, by inhibiting the intracellular accumulation of CER in renal tubules (1).

Panipenem (PAPM) and imipenem (IPM) are both novel carbapenem antibiotics that cause acute renal injuries in some laboratory animals if they are given at doses much above those required clinically. It is considered that the renal injuries induced by large doses of carbapenems are closely related to high intracellular concentration of the antibiotics in renal tubules. The carbapenems are known to be inactivated by dehydropeptidase-I (DHP-I) located on the brush border of the proximal tubular cells.

Thus, in the present paper, the effect of BP, a new organic anion tubular transport inhibitor (2), on the renal intracellular accumulation and nephrotoxicity of the carbapenems was investigated in rabbits.

MATERIALS AND METHODS

Evaluation of nephrotoxicity in rabbits

Male albino rabbits weighing 2.0–3.0 kg were used in this study. Rabbits were given CER (75–200 mg/kg), IPM (100–200 mg/kg), PAPM (50–400 mg/kg) with or without BP (50–800 mg/kg) or cefazolin (CEZ, 200–400 mg/kg); and the urine was collected every day (24 hr from 9:30 to 9:30 on the next day) for measurement of protein and glucose. The intravenous administration of carbapenems and BP were performed simultaneously through the ear vein of the rabbit. The rabbits were decapitated five days after administration of the antibiotics, and their kidneys were immediately excised and weighed. They were stained with hematoxylin-eosin or PAS followed by histopathological examination by optical microscopy. Extent of tubular necrosis was evaluated by the following rating system: very slight, very slightly localized tubular necrosis in the kidney; slight, very slight to less than 20% of tubular necrosis in the kidney; moder-

ate, 20% to less than 50% of tubular necrosis in the kidney; and severe, 50% or more of tubular necrosis in the kidney.

In vivo uptake study of carbapenems in rabbit renal cortex

Rabbits received the carbapenems with or without BP by a single intravenous administration. The rabbits were decapitated 30 min after the administration of the drugs, and their kidneys were excised immediately. Renal cortical samples, extending from the surface of the kidney to just above the red junctional zone between the cortex and medulla, were obtained. Cortical samples were weighed and homogenized in 10% trichloroacetic acid solution, and the concentrations of both carbapenems and BP in the cortex and carotid arterial plasma were measured by an HPLC system with a UV detector.

Analytical methods

Carbapenems and BP were determined by an HPLC (LC-6A; Shimadzu Co., Kyoto) equipped with a variable wavelength UV detector (SPD-6A, Shimadzu Co.). The conditions of HPLC used for assays of PAPM and BP were as follows: for PAPM: column, YMC-PAC A-312 ODS, 15 cm × 6 mm I.D. (YMC Co., Ltd., Kyoto); mobile phase, a mixture of acetonitrile / methanol / ammonium acetate (pH 5) / PIC B7 (1 : 6 : 92 : 1); flow rate, 1.5 ml/min; wavelength 290 nm; column temperature, 40°C; for BP: column, Cosmosil 5C18-P, 15 cm × 4.6 mm I.D. (Nacalai Tesque, Inc., Kyoto); mobile phase, acetonitrile

/ acetic acid / water (10 : 1 : 89); flow rate, 1.0 ml/min; wavelength, 260 nm; column temperature, 40°C. IPM was determined by the method of Norrby et al. (3).

Urinary protein was determined according to the Kingsbury-Clark method, and urinary glucose levels were quantitated by a spectrophotometric glucose oxidase kit (Glucose B-Test; Wako Pure Chemical Industries Ltd., Osaka).

Materials

Imipenem and cilastatin were prepared from commercially available Tienam (Banyu Pharmaceutical Co., Tokyo). Cephaloridine and cefazolin were also purchased from Shionogi & Co. (Keflordin, Osaka) or Sigma Chemical Co. (St. Louis, MO, USA) and Fujisawa Pharmaceutical Industry Co. (Cefamezin, Osaka). *N*-Benzoyl-3-propionic acid (betamipron) was purchased from Tokyo Kasei Kogyo Co. (Tokyo). Panipenem was chemically synthesized at the Chemical Research Laboratories, Sankyo Co., Ltd., Tokyo. The other chemicals used for the experiments were of the highest purity available.

RESULTS

Preventive effect of BP on carbapenem-induced nephrotoxicity in rabbits

The severity of nephrotoxicity induced by PAPM, IPM and CER is shown in Fig. 1. The tubular necrosis induced by PAPM was observed at a dose of 150 mg/kg or more, that by IPM was observed at doses greater than 100

Fig. 1. Intensity of nephrotoxicity induced by carbapenems and cephalosporins in rabbits. Renal tubular necrosis was observed five days after administration of the antibiotics. The extents of tubular necrosis were classified into five levels, and they were given scores of 0, 1, 2, 3 and 4 points. Each point represents the mean value of five rabbits. IPM/cilastatin and PAPM/BP show coadministrations of equal doses of IPM and cilastatin and equal doses of PAPM and BP, respectively. IPM: imipenem, PAPM: panipenem, CER: cephaloridine, CEZ: cefazolin, BP: betamipron.

mg/kg, and that by CER at 75 mg/kg or more, i.v. in rabbits. However, coadministration of equal amounts of BP markedly decreased the degree of PAMP nephrotoxicity as compared to the effect of PAMP alone. Similar results were obtained after coadministration of equal amounts of cilastatin and IPM. CEZ, which is used as the control in the nephrotoxicity test, induced slight renal tubular necrosis at doses greater than 200 mg/kg. A dose of PAMP at 200 mg/kg induced swelling, decoloration, ascites and tubular necrosis of the rabbit kidney (Table 1).

The maximal values of both urinary protein and urinary glucose were observed on the 2nd day after a single

Table 1. Protective effect of betamipron (BP) against the nephrotoxicity induced by panipenem (PAMP) in rabbits

Dose (mg/kg)	Animal No.	Renal histology			
		Swelling	Decolor	Ascites	Tubular necrosis
PAMP 200 alone	1	++	++	—	+++
	2	++	++	—	++
	3	++	+++	++	+++
	4	+	++	—	+++
	5	+	+++	—	+++
PAMP 200 + BP 200	1	—	—	—	—
	2	—	—	—	—
	3	—	—	—	—
	4	—	—	—	—
	5	—	—	—	—

Tubular necrosis was observed together with swelling, decoloration and ascites of the kidney following an intravenous dose of PAMP with or without BP in rabbits. The observations were performed 5 days after the administration. The definitions of the symbols are the follows: — normal, + slight, ++ moderate, +++ severe.

dose of PAMP, and the values slowly decreased thereafter (Fig. 2). These results indicated that renal dysfunction by PAMP (200 mg/kg, i.v.) grew gradually up to the 2nd day after a single administration of the antibiotic, and the function was then restored a few days later. On the other hand, proximal tubular necrosis was observed during two or three days to five days after the administration of PAMP. These renal dysfunctions were, however, reversible in many cases of low dose (200 mg/kg) administration of the antibiotic.

The coadministration of BP significantly reduced these renal dysfunctions. A single dose of BP (100–800 mg/kg) did not induce any nephrotoxicity, including tubular necrosis, in three rabbits (data not shown). A simultaneous dose of BP (200 mg/kg, i.v.) completely prevented the tubular necrosis induced by PAMP (200 mg/kg, i.v.) (Table 1). The values of both urinary protein and urinary glucose remained at normal levels by the coadministration of BP (Fig. 2).

The combination ratio of BP against PAMP was subsequently studied with regard to tubular necrosis in the rabbits. Tubular necrosis was induced by an intravenous dose of 150 mg/kg or more of PAMP (Fig. 3). A quarter-dose combination of BP against PAMP prevented the tubular necrosis induced by less than 200 mg/kg of PAMP. A half-dose combination of BP against PAMP prevented the tubular necrosis induced by PAMP at a dose of less than 250 mg/kg. An equivalent-dose combination of BP against PAMP prevented the tubular necrosis by less than 300 mg/kg of PAMP. These results indicated that the carbapenem-induced nephrotoxicity was produced dose-dependently and that the preventive effect of BP on the carbapenem-induced nephrotoxicity was also dose-dependent.

Fig. 2. Changes in urinary protein and urinary glucose in rabbits following administration of panipenem (PAMP) with or without betamipron (BP). Each point represents the mean value of three rabbits. ●: PAMP (200 mg/kg), ○: PAMP/BP (200 mg/kg : 200 mg/kg), △: control (saline).

Fig. 3. Relationships between intensity of tubular necrosis and dose ratios of panipenem (PAPM) and betamipron (BP). Each value represents the mean of three rabbits. The tubular necrosis was evaluated using the classification of 0, 20, 40, 60, 80 and 100% (tubular necrosis) according to the procedure described in Materials and Methods. 1/0, 1/0.25, 1/0.5 or 1/1 indicates the dose ratio of PAPM against BP.

Inhibitory effect of BP on carbapenem uptake in rabbit renal cortex

The concentration-time profiles of PAPM and BP in the rabbit renal cortex after each intravenous dose of 100 mg/kg are shown in Figs. 4 and 5. Both PAPM and BP were accumulated in the renal cortex immediately after the intravenous administration. The uptake of PAPM into the renal cortex was remarkably inhibited by simultaneous treatment with BP (Fig. 4). However, the uptake of BP was not influenced by the coadministration of PAPM (Fig. 5). The treatment with BP tended to inhibit

Fig. 4. Renal accumulation-time profiles of panipenem (PAPM) in rabbits. The accumulated amounts of PAPM in the renal cortex were examined following intravenous administration of 100 mg/kg of PAPM with (●) or without (○) 100 mg/kg of BP. Each point represents the mean ± S.E. of three rabbits.

Fig. 5. Renal accumulation-time profiles of betamipron (BP) in rabbits. The accumulated amounts of BP in the renal cortex were examined following intravenous administration of 100 mg/kg of BP with (●) or without (○) 100 mg/kg of PAPM. Each point represents the mean ± S.E. of three rabbits.

the uptake of PAPM and IPM into the renal cortex. However, the plasma levels of both PAPM and IPM were not changed by the treatment with BP (Fig. 6).

The correlation between dose and renal accumulation of PAPM and BP were studied. The accumulation of PAPM in the renal cortex was apparently saturated at doses of more than 200 mg/kg, i.v. (Fig. 7), whereas the accumulation of BP increased dose-dependently up to a dose of 800 mg/kg, i.v. (Fig. 8). This finding was consistent with the results on the tubular necrosis shown in Fig. 3. That is, the minimal dose of PAPM that induced approximately 100% tubular necrosis was consistent with

Fig. 6. Renal accumulation and plasma levels of panipenem (PAPM) and imipenem (IPM) in rabbits. The renal accumulation and plasma levels of PAPM and IPM were examined 30 min after intravenous administration of 100 mg/kg of PAPM or IPM with (■) or without (□) 100 mg/kg of BP. Each point represents the mean ± S.E. of three rabbits.

Fig. 7. Accumulation capacity of panipenem (PAPM) in rabbit renal cortex. The accumulated amounts of PAPM were examined 30 min after intravenous administration of PAPM with (○: PAPM/BP, 1:0.5; △: PAPM/BP, 1:1) or without (●) BP as a function of different PAPM doses and combination ratios. Each point represents the mean $\pm$ S.E. of three rabbits.

the minimal dose of PAPM that showed saturated accumulation in the renal cortex. Therefore, the intravenous dose of 200 mg/kg PAPM was concluded to be sufficient to produce severe tubular necrosis in rabbits. The accumulated amount of PAPM in the renal cortex was also reduced significantly by the treatment with BP dose-dependently.

DISCUSSION

Carbapenems generally have very low toxicity in most experimental animals. However, some species of experimental animals show acute renal dysfunction if given these drugs at a dose much above that required clinically. Histological changes induced by large doses of PAPM and IPM (4) were similar in their location, morphologic appearance and rate of occurrence to those induced by CER in rabbits and monkeys. The degree of renal damage by the carbapenems (4) and CER (5, 6) and the dose necessary to produce renal damages vary among animal species. Rabbits were the most sensitive to the nephrotoxicity of carbapenems.

The results in the single-dosing study of the carbapenems and CER in rabbits showed that the doses of CER, IPM and PAPM necessary to produce the proximal tubular necrosis were less than 75 mg/kg, 100 mg/kg and 150 mg/kg, respectively (Fig. 1). Urinary levels of protein and glucose rapidly increased until two days after a single intravenous dose of 200 mg/kg PAPM, and they slowly decreased thereafter (Fig. 2). These results indicate that

Fig. 8. Accumulation capacity of betamipron (BP) in rabbit renal cortex. The accumulated amounts of BP were examined 30 min after intravenous administration. Each point represents the mean $\pm$ S.E. of three rabbits.

the renal damage induced by high doses of PAPM is acute renal dysfunction, although it was reversible in many cases.

The renal cortical accumulation of PAPM was saturated at doses of antibiotic greater than 200 mg/kg, i.v. (Fig. 7). This result suggests that the intracellular accumulation capacity of PAPM was relatively small, but the saturated concentration of the antibiotic in the tubular cells was sufficient to induce renal injury, including proximal tubular necrosis. In contrast, the accumulated amount of BP increased dose-dependently up to at least the intravenous dose of more than 800 mg/kg (Fig. 8). This result suggests that BP has relatively low affinity to a protein like a carrier protein or BP transported into the renal cortex is immediately excreted. In fact, it was shown that BP strongly inhibited the renal secretion of CER in our previous study (1). In addition, it was shown that the treatment with BP significantly inhibited the uptake of PAPM and IPM into the renal cortex without changing their plasma levels (Fig. 6). In a previous paper (2), we suggested the following reason for this: The treatment with BP significantly inhibited the uptake of the carbapenems into the renal cortex, but scarcely altered their pharmacokinetics. This is considered to be the reason why the secreted amounts of carbapenems were not due to their protein binding and also did not influence their distribution volumes after treatment of BP (2).

BP also inhibited the renal cortical accumulation of IPM. It has been reported that IPM-induced nephrotoxicity is reduced by the coadministration of cilastatin (3). BP does not exhibit inhibitory activity against DHP-I (7), which exists in tubular cells and inactivates the carbap-

enems by breaking the β -lactam bond, whereas cilastatin has high inhibitory activity against DHP-I. These results suggest that BP may be able to prevent the nephrotoxicity induced by carbapenems. It can be recognized that BP is a novel organic anion transport inhibitor in renal tubules and is very useful for reducing the carbapenem-induced nephrotoxicity because of its efficacy and safety. BP is expected to be a novel candidate for reducing the nephrotoxicity induced by β -lactam antibiotics, including carbapenems and cephalosporins.

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