Biochemical Pharmacology, Vol. 51, pp. 557–561, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
SSDI 0006-2952(95)02237-6

Cephalosporin and Carbacephem Nephrotoxicity

ROLES OF TUBULAR CELL UPTAKE AND ACYLATING POTENTIAL

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ABSTRACT. Three beta-lactams, desacetylcephaloglycin, ampicillin, and loracarbef, were studied to test a hypothesis derived from retrospective analysis of previously studied cephalosporins: that beta-lactam nephrotoxicity develops in approximate proportion to tubular cell antibiotic concentrations and lactam ring reactivities. Concentrations of each beta-lactam (and inulin) in rabbit renal cortex and serum were measured at the end of 0.5-hr infusions of 100 mg antibiotic/kg body weight and 0.5 to 0.67 hr later. Total cortical AUCs (total areas under the curve of concentration and time in renal cortex) and transported cortical AUCs (total minus inulin-space beta lactam) were calculated from these measurements. Reactivities, determined by the rate constants of lactam-ring opening at pH 10, were taken from the literature. Nephrotoxicity was quantified by grades of proximal tubular cell necrosis and by serum creatinine concentrations 2 days after infusion of 100–1500 mg/kg of the antibiotics. Desacetylcephaloglycin was slightly less nephrotoxic than cephaloglycin; the AUCs, reactivities, and toxicities of these two cephalosporins fit the proposed model, particularly when allowance is made for hepatic and renal deacetylation of cephaloglycin. The very low AUCs, limited reactivity, and absence of nephrotoxicity of ampicillin also fit the model. Loracarbef had a transported AUC less than three times, and reactivity one-thirtieth, those of cefaclor, respectively. Although only at 1500 mg/kg, loracarbef was significantly more nephrotoxic than cefaclor. If the reactivity of loracarbef with its targeted bacterial proteins, which is essentially the same as that of cefaclor, is considered instead of the base hydrolysis rate constant, then loracarbef also fits the model. By the same analysis, the comparatively high *in vitro* stability of other carbacephems, although pharmaceutically convenient, may not limit their nephrotoxicity. BIOCHEM PHARMACOL 51;4:557–561, 1996.

KEY WORDS. beta-lactam; cephalosporin; cephem; kidney; nephrotoxicity; transport

The phenylglycyl beta-lactams, a group of antibiotics with qualities of acid-stability and enteric absorption that favor their oral use [1], have several properties that make them valuable in the study of beta-lactam nephrotoxicity. First, a sensitive fluorimetric assay allows measurement of their concentrations in tissues and biological fluids [2]. Second, although they have a common phenylglycyl R_1 side-group [1], they have broad ranges of tubular cell uptake [3], cephem-ring reactivity [4–9], and nephrotoxic potential [10].

A retrospective analysis of measurements with cephaloridine, ceftazidime, and three phenylglycyl cephalosporins (cephaloglycin, cefaclor, and cephalexin) provided the basis for the hypothesis that nephrotoxicity develops in approximate proportion to the proximal tubular cell uptake and the lactam ring reactivity of each [3]. The present study tests this hypothesis by adding: (1) measurements of renal cortical uptake of the remaining available phenylglycyl beta-lactams [desacetylcephaloglycin, the principal metabolite of cephaloglycin; loracarbef, the 1-carba-1-dethiacephalosporin (carbacephem) analogue of cefaclor; and ampicillin (phenylglycyl penicillin), the penicillin analogue of cephalexin]; (2) calculations of both total areas under the curve of concentration and time in renal cortex (cortical AUCs†) and transported cortical AUCs of all of the beta-lactams studied; and (3) measurements of the comparative nephrotoxicities of all six phenylglycyl beta-lactams.

Cortical AUCs, published rate constants of lactam ring opening (reactivities), and nephrotoxic doses are collectively analyzed from the perspective of a model of renal toxicity through beta-lactam acylation of tubular cell proteins, analogous to their attack on bacterial proteins [3]. The bacterial targets include several membrane-bound enzymes essential for cell wall synthesis [11], while the targets in the tubular cell include the mitochondrial carriers responsible for transport of respiratory substrates into the inner matrix [12–14].

MATERIALS AND METHODS

Except where otherwise noted, reagents were purchased from the Sigma Chemical Co. (St. Louis, MO). All of the cephems were provided by the Eli Lilly Co. (Indianapolis, IN); desace-

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† *Abbreviations:* AUC, area under the curve (of concentration and time); C/S, cortex-to-serum (ratio); and $T_{1/2}$, half-life.

Received 5 July 1995; accepted 4 October 1995.

tylcephaloglycin was specially prepared for these studies through the assistance of Dr. Walter Wright and Mr. Richard Heiney. Ampicillin sodium was obtained from Wyeth Laboratories Inc. (Philadelphia, PA). Each of the antibiotics was dissolved immediately before use in a solution containing 100 mg/mL of antibiotic base, using 1 mEq/mL of sodium bicarbonate for the phenylglycyl cephems and sterile water for ampicillin. Inulin (J. T. Baker Chemical Co., Phillipsburg, NJ) was also dissolved in 0.9% saline at a concentration of 100 mg/mL.

Female New Zealand white rabbits (Nitabell Rabbitry, Hayward, CA) weighing 1.6 to 2.0 kg were allowed free access to food (Standard Rabbit Maintenance Diet, Manna Pro Corp., Fresno, CA) and water until the morning of study. All animals were anesthetized with 45–60 mg/kg of intraperitoneal pentobarbital (Abbott Laboratories, North Chicago, IL) before the antibiotic infusions for *in vivo* studies of cortical uptake or nephrotoxicity.

Renal Cortical Uptake

Loading doses of 30 mg/kg of desacetylcephaloglycin, ampicillin, or loracarbef, and 200 mg of inulin, were administered through an ear vein over 1 min, followed by an additional 70 mg/kg of the beta-lactam and 300 mg of inulin over 0.5 hr. Separate groups of animals were killed by decapitation immediately after the 0.5-hr infusions and 0.5 to 0.67 hour later. Carotid artery blood was collected, and the kidneys were removed immediately. Renal cortex and serum were prepared for measurement of antibiotic and inulin concentrations as previously described for the study of cephaloglycin [2]. Cortical (μg/g wet tissue) and serum (μg/mL) concentrations were determined, and the following were calculated: C/S ratios, cortical and serum $T_{1/2}$s, and cortical AUCs.

The following equations were used to calculate $T_{1/2}$s and cortical AUCs:

$$T_{1/2} = t \cdot \ln 0.5/\ln (C_t/C_0) \quad (1)$$

where t = the time (hr) between measurements, and C_0 = the initial and C_t = the final beta-lactam concentrations in cortex or serum; and

$$\text{Cortical AUC} = (0.25 \cdot C_0) + (C_0 \cdot T_{½_{Ctx}}/\ln 0.5) \quad (2)$$

where C_0 = the 0.5-hr post-infusion cortical concentration and $T_{½_{Ctx}}$ = the cortical half-life.

C/S inulin ratios have been consistently 1.4 ± 0.2 in studies of C/S beta-lactam ratios, reflecting a relatively constant interstitial and filtered fluid pool of solute [3]. In contrast, C/S beta-lactam ratios have ranged from 1.4 to 16 at steady-state, and from 2.5 to 26 at later sampling times, reflecting widely differing contributions of actively transported (mostly intracellular [15]) and non-transported antibiotic. To compare the AUCs without the contributions of non-transported beta-lactam, transport-pool $T_{1/2}$s and transported AUCs were calculated for each antibiotic by multiplying each cortical concentration (C_0 and C_t) by the corresponding fraction:

$$(\text{C/S beta-lactam} - \text{C/S inulin})/\text{C/S beta-lactam}. \quad (3)$$

Nephrotoxicity

For comparisons of *in vivo* nephrotoxicity, 100 mg/kg of cephaloglycin or desacetylcephaloglycin, or 1000–1500 mg/kg cefaclor, loracarbef, ampicillin, or cephalexin was injected intravenously over 4–6 min. Ceftazidime toxicity was also evaluated using a 1500 mg/kg dosage, because *in vitro* observations showing mild cytotoxicity [16, 17] pose a possible challenge to the lack of ceftazidime nephrotoxicity found in lower-dose animal and human studies [18–20]. Two days after infusion of the antibiotics [21], the rabbits were anesthetized and decapitated. Carotid artery blood was collected. Full transverse sections of kidney were fixed in Bouin's solution, transferred after 1 hr to 10% formaldehyde, then subsequently embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

Histopathologic interpretation was done with randomly assorted slides marked only with a code number. Degrees of tubular necrosis were quantified as follows: mild, < 10% of tubules necrotic; moderate, 10 to < 25% necrotic; severe, ≥ 25% necrotic [2]. Creatinine concentrations were measured in carotid arterial serum using a Beckman Creatinine Autoanalyzer (Beckman Instruments Inc., Mountain View, CA).

Analytical

Measurements of antibiotic and creatinine concentrations are presented as means ± SEM. The significance of differences of serum creatinine concentrations between groups was determined by ANOVA. Results of histopathologic scoring are presented as numbers of animals in each category and are analyzed by the exact test. Differences in both cases were judged to be significant where $P < 0.05$.

RESULTS

Cortical Uptake

Post-infusion and declining concentrations of desacetylcephaloglycin, ampicillin, and loracarbef in renal cortex and serum are shown in Table 1, with the corresponding C/S ratios. Initial and subsequent cortical and serum concentrations, and C/S ratios, varied widely between the three beta-lactams, reflecting differences of secretory uptake and elimination (mostly through glomerular filtration and tubular secretion [15, 22, 23]) as great as those found in comparable studies with other cephalosporins [3].

Nephrotoxicity

Cephaloglycin was significantly more toxic than desacetylcephaloglycin after equal dosage (Table 2), both by comparison of tubular necrosis (normal-to-moderate vs severe, $P = 0.03$) and serum creatinine concentrations ($P < 0.05$). Lor-

TABLE 1. Concentrative uptake of beta-lactam antibiotics in rabbit renal cortex

	Cortical concentrations*	Serum concentrations*	Cortex/Serum ratios
Desacetylcephaloglycin			
Steady-state	993 ± 73	171 ± 22	6.2 ± 0.8
0.67 hr	301 ± 19	25 ± 2	12.7 ± 1.5
Half-life (hr)†	*0.39*	*0.24*	
Ampicillin			
Steady-state	359 ± 69	139 ± 20	2.5 ± 0.2
0.5 hr	144 ± 22	17 ± 3	8.9 ± 0.7
Half-life (hr)†	*0.38*	*0.17*	
Loracarbef			
Steady-state	1751 ± 239	211 ± 35	8.7 ± 0.9
0.5 hr	338 ± 46	26 ± 5	14.7 ± 11
Half-life (hr)†	*0.21*	*0.17*	

* Concentrations (μg/g tissue in cortex, μg/mL in serum) after i.v. infusion of 100 mg/kg over 0.5 hr, and at the indicated times after the end of infusion. Values are means ± SEM, N = 6.

† Cortical and serum $T_{1/2}$s = sampling interval · ln 0.5/ln (final/initial concentrations).

acarbef was considerably less toxic than both desacetylcephaloglycin and cephaloglycin, but more toxic at 1500 mg/kg than cefaclor (normal-to-mild vs moderate-to-severe necrosis, $P < 0.001$; serum creatinine concentrations, $P < 0.001$).

DISCUSSION

The nephrotoxic beta-lactam antibiotics produce a selective proximal tubular necrosis, starting within 1–5 hr and fully developed by 24 hr after parenteral administration of a single toxic dose [2, 21, 24–26]. Every nephrotoxic beta-lactam studied undergoes secretory transport, with concentrative uptake by the proximal tubular cell [10, 27]. Toxicity can be prevented by inhibition of organic anion secretion [26–30], and augmented by manipulations that increase tubular cell concentrations [31, 32].

Lactam-ring reactivity, an important element in the antibacterial action of the beta-lactams [33], has also been identified as a probable contributor to their nephrotoxicity [3, 34]. Although nephrotoxicity does not occur in the absence of secretory transport, some beta-lactams that undergo significant cellular uptake, like cephalexin, are not nephrotoxic [3]. Cephaloglycin and cephalexin have almost the same cortical concentrations and AUCs after 100 mg/kg infusions [3], but have at least a 30-fold difference in nephrotoxic dosage ([2] and Table 2).

The renal cortical AUCs and lactam-ring reactivities of several previously studied cephalosporins [3] are presented in Table 3, together with the same values for the beta-lactams added in the current study. The data for cortical concentrations differ from the previous calculations by including both total AUCs and transported AUCs (derived by subtracting from each C/S beta-lactam ratio the corresponding C/S inulin ratio, which reflects filtered and peritubular antibiotic). The validity of this correction is supported by the reduction of the transported cortical AUC of cephaloridine, approximately 100% of which is intracellular [35, 36], from 6177 mg · hr/g to zero by prior administration of probenecid [29, 37, 38]. Reactivities, determined by the rate constants of lactam-ring opening at pH 10, were taken from the literature [4–9]. Nephrotoxicity was quantified by scoring proximal tubular cell necrosis and by serum creatinine concentrations 2 days after single-dose intravenous infusions of 75–1500 mg/kg of the beta-lactams ([2] and Table 2).

It has been noted previously [3] that cephaloridine, although meeting the criteria for nephrotoxicity through tubular

TABLE 2. Nephrotoxicity of several beta-lactam antibiotics in the rabbit

		Proximal tubular necrosis†				
	β-Lactam dose*	None	Mild	Moderate	Severe	Serum creatinine‡
Desacetylcephaloglycin	100	0	3	3	0	1.08 ± 0.12
Cephaloglycin	100	0	0	3	5	3.33 ± 0.70
Ampicillin	1500	5	0	0	0	0.84 ± 0.09
Cephalexin	1500	5	0	0	0	0.68 ± 0.09
Loracarbef	1000	3	1	0	0	0.83 ± 0.05
	1500	0	0	1	3	4.80 ± 1.70
Cefaclor	1500	3	2	0	0	0.92 ± 0.05
Ceftazidime	1500	5	0	0	0	0.76 ± 0.07

* Single intravenous dose (mg/kg) 48 hr before killing.

† Tubular necrosis scores: mild, 10%; moderate, 10 to < 25%, severe, ≥ 25% of proximal tubules necrotic.

‡ Serum creatinine concentrations (mg/dL) at 48 hr; means ± SEM; normal: 0.79 ± 0.03 (SEM), N = 24.

TABLE 3. Cortical concentrations, reactivity, and nephrotoxicity of beta-lactam antibiotics

	AUCs*			
	Total	Transported	Reactivity† ($\times 10^{-2}$/hr)	ND-100‡ (mg/kg)
Cephaloridine	6069	6177	68	150§
Desacetylcephaloglycin	803	678	30–60‖	100
Cephaloglycin	1287	1064	60	75§
Loracarbef	970	835	3.2	<1500
Cefaclor	459	293	94	>1500
Ampicillin	286	339	19	⩾1500
Cephalexin	1129	980	6.9	⩾1500
Ceftazidime	497	0	130	⩾1500

* AUCs = cortical areas-under-the-curve of concentration and time (mg · hr/g). Transported AUCs were adjusted to delete the vascular, interstitial, and filtered cephem by multiplying cortical concentrations by the ratio (C/S cephem minus C/S inulin)/(C/S cephem) at each sampling time.

† Published rate constants for β-lactam ring opening at pH 10 [4, 6, 7, 9].

‡ The lowest dose producing some degree of tubular necrosis in 100% of rabbits (>1500: some toxicity demonstrated, but not in all animals; ⩾ 1500: no toxicity demonstrated).

§ Reference 2.

‖ No published rate constant by desacetylcephaloglycin; the constant for desacetylcephalothin is between 50 and 100% of that of cephalothin, more likely in the lower range [5, 7].

cell uptake and acylating potential, is one-half to two-thirds as toxic as cephaloglycin, while its relative AUCs and reactivity would better fit with 5–7 times greater toxicity. This discrepancy has been attributed to the fixed zwitterionic charge of cephaloridine, unique among the nephrotoxic beta-lactams, which may hinder its access to the targeted mitochondrial anionic substrate carriers [3, 10]. In support of this hypothesis, compared with cephaloglycin and imipenem, cephaloridine has limited early (1 hr) *in vivo* toxicity to the mitochondrial carriers of pyruvate and the short-chain fatty anions [39].

The absence of toxicity of ceftazidime, the most reactive of these beta-lactams, is attributed to its very low cortical AUCs, which are no greater than can be accounted for by extracellular antibiotic (Table 3). The lack of nephrotoxicity of ampicillin can be explained by both its very low AUCs and limited reactivity.

The AUCs, reactivities, and toxicities of desacetylcephaloglycin and cephaloglycin fit the proposed model, particularly when allowance is made for the hepatic and renal metabolism of cephaloglycin [40, 41]. Approximately 50% of cephaloglycin is metabolized to desacetylcephaloglycin, which reacts equally in the fluorimetric assay used in these studies. Therefore, the AUCs of cephaloglycin represent approximately equal fractions of the parent compound and its less toxic metabolite, suggesting that, were it not deacetylated, cephaloglycin would be even more toxic.

Loracarbef has a transported AUC less than three times, and reactivity one-thirtieth, the AUC and reactivity of cefaclor. However, loracarbef is the more nephrotoxic of the two cephems (Table 2). The unusual antibacterial properties of the carbacephems may offer an explanation for this contradiction of the model of toxicity proportional to uptake and reactivity determined as the base-hydrolysis rate constant. Each cephem in nine matched pairs of carbacephems and their more reactive cephalosporin analogues (including loracarbef and cefaclor as one such pair) has nearly the same antibiotic efficacy against several bacterial species. Because of the identical C-3 and C-7 substituents, the cephems in each pair should have very similar beta-lactamase resistances, diffusion through Gram-negative outer membrane porins, and affinities and off-rates for their penicillin-binding proteins [8].

Therefore, Blaszczak and associates [8] have concluded that the carbacephems have more reactive interactions with their targeted bacterial proteins than would be predicted from the *in vitro* base-hydrolysis assay of reactivity. If, as suggested, the lactam ring of the carbacephems is more sensitive to attack by target-protein nucleophiles than to the hydroxyl ion, then the model of nephrotoxicity in proportion to uptake and acylating potential is supported by the relative toxicities of loracarbef and cefaclor.

Comparison of the cortical AUCs and reactivities of desacetylcephaloglycin and cefaclor (Table 3) suggests that cefaclor should be more nephrotoxic than found in the present study. This analysis might be dismissed because of the uncertainties raised regarding the utility of the base-hydrolysis rate constant as a predictor of protein-acylating reactivity. However, unlike the carbacephem and cephalosporin pairs, identically C-7-substituted cephalosporins with different C-3 substituents, including cefaclor and cephaloglycin, have antibacterial potencies that correlate reasonably well with their relative base hydrolysis rates [6, 8].

A more likely explanation of the limited nephrotoxicity of cefaclor per mg · hr/g cortical AUC is the minimal intracellular sequestration caused by its efficient movement from cell-to-luminal fluid. In support of this interpretation, cefaclor is unique among the transported beta-lactams studied in having C/S ratios that do not rise as serum concentrations decline [3, 32]. Also consistent with this analysis, tubular necrosis was seen in 5/6 kidneys subjected to 2 hr of ureteral obstruction after 800 mg/kg of intravenous cefaclor, compared to 1/6 contralateral control kidneys [32]. The same procedure had significantly less effect on the nephrotoxicity of cephaloglycin, and therefore probably of desacetylcephaloglycin, and no effect on the toxicity of cephaloridine, which undergoes more intracellular sequestration than any other cephalosporin.

This work was sponsored by a grant from the National Institutes of Health (DK 33814).

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