Biochemical Pharmacology, Vol. 38, No. 21, pp. 3779–3783, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

THIENAMYCIN NEPHROTOXICITY

MITOCHONDRIAL INJURY AND OXIDATIVE EFFECTS OF IMIPENEM IN THE RABBIT KIDNEY*

BRUCE M. TUNE,† DORIS FRAVERT and CHIEH-YIN HSU
Laboratory of Renal Pharmacology, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA, U.S.A.

(*Received* 25 *November* 1988; *accepted* 20 *March* 1989)

Abstract—The nephrotoxic cephalosoprins cephaloridine and cephaloglycin both produce mitochondrial respiratory toxicity in renal cortex. Recent work has provided evidence that this respiratory toxicity is caused by acylation and inactivation of mitochondrial anionic substrate transporters. While cephaloridine also causes significant lipid peroxidative injury in cortical mitochondria and microsomes, cephaloglycin causes little or no oxidative damage under identical conditions. The recently released thienamycin antibiotic, imipenem, like the toxic cephalosporins, produces acute proximal tubular necrosis which can be prevented completely by prior administration of probenecid. The ability of imipenem to block mitochondrial substrate uptake and respiration and produce oxidative changes has not been examined. We therefore evaluated the effects of imipenem in rabbit renal cortex on the following: (1) mitochondrial function [respiration with and uptake of succinate, and uptake of ADP]; and (2) evidence of oxidative change [depletion of reduced glutathione (GSH), production of oxidized glutathione (GSSG), and production of lipid peroxidative injury, as reflected in microsomal conjugated dienes (CDs)]. The mitochondrial effects of 300 mg/kg body wt of imipenem, given i.v. 1 and 2 hr before killing the animals, were comparable to those of the nephrotoxic cephalosporins. There was significant reduction of respiration with, and unidirectional uptake of, succinate at both times, while mitochondrial ADP transport was comparatively unaffected. Imipenem also depleted GSH and increased GSSG and CDs at 1 hr. These effects, however, were considerably smaller than those of a comparably nephrotoxic dose of cephaloridine, and this evidence of oxidative stress had resolved by 2 hr. We conclude that imipenem and the nephrotoxic cephalosporins have similar effects on mitochondrial substrate uptake and respiration, but differ significantly in their production of oxidative injury.

Acute renal failure is a common complication of Gram-negative bacterial infections. Although endotoxic shock is commonly blamed for the renal injury, the antibiotics used to treat these infections can play an important contributory role. Among the beta-lactam antibiotics, several of the cephalosporins [1], and the new thienamycin imipenem [2], can produce acute proximal tubular necrosis and acute renal failure in laboratory animals when given in large single doses. This injury, as studied most thoroughly with the cephalosporins cephaloridine and cephaloglycin, may occur at therapeutic doses under conditions of risk, such as renal ischemia [3], endotoxemia [4], and combined administration with aminoglycosides [5].

The present studies were undertaken to examine the ability of imipenem to produce two recognized components of cephalosporin toxicity: (1) mitochondrial injury, which is significant with both cephaloridine [6, 7] and cephaloglycin [8, 9], and (2) oxidative damage, which is a major effect of cephaloridine [10] and a minor one of cephaloglycin [7]. The following components of mitochondrial function were studied: respiration with, and the net uptake and efflux of, succinate, and the net uptake of ADP. The following components of oxidative change were examined: depletion of reduced glutathione (GSH), production of oxidized glutathione (GSSG), and production of lipid peroxidative injury, as reflected by conjugated dienes (CDs) in cortical microsomes.

MATERIALS AND METHODS

Except where otherwise indicated, reagents were purchased from Sigma Chemical Co. (St Louis, MO). The following isotopes were used: [2,3-^{14}C]succinic acid (42 mCi/mmol) and [U-^{14}C]sucrose (560 mCi/mmol) obtained from the Amersham Corp. (Arlington Heights, IL), [2,8-^{3}H]adenosine 5′-diphosphate (22.5 Ci/mmol) from New England Nuclear (Boston, MA), and [^{3}H]water (2.3×10^{6} dpm/ml) from E.I. du Pont de Nemours & Co. (North Billerica, MA).

Female New Zealand white rabbits weighing 1.6 to 2.0 kg (Nitabell Rabbitry, Hayward, CA) were allowed free access to food (Wayne 15% Rabbit Ration, Allied Mills, Chicago, IL) and water until the morning of study. Imipenem (supplied by the courtesy of Dr. Helmut Kropp, Merck Sharp & Dohme Research Laboratories, Rahway, NJ) was dissolved in a 10 mM sodium phosphate buffer (pH 7.0) at a concentration of 20 mg/ml of antibiotic base.

* Supported by grants from the National Institutes of Health (DK 33814) and the American Heart Association (890665) with funds contributed by the Northern California Heart Association.

† Correspondent: Bruce M. Tune, M.D., Division of Pediatric Nephrology, Room S-348, Standford University School of Medicine, Stanford, CA 94305.

All studies involved the production of nephrotoxicity *in vivo* in tissues obtained from animals 1 or 2 hr after i.v. administration of the vehicle or 300 mg/kg of imipenem at a rate of 5 ml/min. All animals were anesthetized with 45–60 mg/kg body wt of i.p. pentobarbital, to maintain consistency with our recent studies using cephaloridine and cephaloglycin [3, 4, 7, 9]. The use of anesthesia has not altered nephrotoxic doses or patterns of production of either tubular necrosis or mitochondrial toxicity in these studies compared to earlier ones [5, 8].

Mitochondrial toxicity

Animals were killed 1 or 2 hr after administration of imipenem or its vehicle, and their kidneys were removed immediately. Renal cortical mitochondria were prepared as previously described [6] in a pH 7.4 solution containing 260 mM sucrose, 5 mM Tris–HCl, and 0.2 mM EDTA. To maintain comparable condition for measurements of respiration and substrate uptake, all studies of mitochondrial function used a standard pH 7.4 respiration medium: 220 mM sucrose, 20 mM Tris–HCl, 10 mM sodium/disodium phosphate, and 5 mM potassium chloride—plus the substrates, inhibitors and tracers indicated in individual protocols below.

Respiration. Mitochondrial oxygen consumption was measured at 20° with a Clarke platinum electrode assembly in 1.7 ml of respiration medium containing 1.7 mg of mitochondtrial protein [11], 10 mM succinate and 5 μg/ml of rotenone (to block electron transport proximal to succinate entry into the respiratory chain), both in the presence of 0.125 mM ADP (State 3 respiration), and after the consumption of the ADP (State 4 respiration). The respiratory control ratio (RCR) was calculated as the ratio of State 3-to-State 4 rates.

Substrate uptake. The net uptakes of succinate and ADP were measured by the method of sieve filtration [9] in separate aliquots of mitochondria, using [^{14}C]-succinic acid or [^{3}H]ADP. For measurement of succinate uptake, the incubation medium contained 1.2×10^{-6} M succinate (0.05 μCi/ml), without ADP. Incubation and rinsing medium contained 5 μg/ml of antimycin A, to block succinate metabolism. For the study of ADP uptake, the incubation medium contained 4.4×10^{-10} M ADP (0.01 μCi/ml), with no substrate. In each case, mitochondria (0.5 to 1 mg protein) were incubated for 5 min in 2 ml of respiration medium at 20°, then trapped on Millipore DAWP 025 00 (0.65 nm) filters (Millipore Corp., Bedford, MA) using a Hoeffer model FH 225V 10 Place Manifold (Hoeffer Scientific Instruments, San Francisco, CA), and washed twice with 5 ml of iced respiration medium. Two washes were established in preliminary studies as necessary to clear contaminating extramitochondrial isotope (using [^{14}C]sucrose) while causing minimal reduction of transported substrate.

Samples were placed in Aquasol Universal LSC (NEN Research Products, Boston, MA) overnight to allow clarification of the filters, and then counted in a Beckman LS 7500 liquid scintillation counter (Beckman Instruments, Inc., Mountain View, CA). Mitochondrial succinate was calculated from the total counts per filter and identically quenched standards of known specific activity. Contamination by extramitochondrial medium, tested by occasional sampling of mitochondria incubated with [^{14}C]-sucrose, was consistently small, equivalent to an average of 1% of substrate counts.

For measurement of intramitochondrial water content, mitochondrial pellets were suspended for 5 min at 20° in respiration medium containing trace quantities of [^{3}H]water and [^{14}C]sucrose and then recentrifuged for 5 min at 15,000 *g*. Separate aliquots of the recentrifuged pellets were counted or assayed for protein, and the intramitochondrial water content per gram of protein was determined as the difference between the total water and sucrose spaces.

Succinate efflux. The washout of [^{14}C]succinate from normal and *in vivo* imipenem-intoxicated mitochondria was measured by incubating separate aliquots with the substrate for 5 min, then trapping the suspensions of Millipore filters and subjecting them to three different times of washing, ranging from 3 to 9 min, by continuous application of 20° respiration medium containing antimycin but no succinate, and counting the radioactivity remaining on the filters. Efflux rates were calculated by the method of least squares as the slopes of the logarithms of concentration against time.

Oxidative effects

Animals were killed by decapitation 1 or 2 hr after administration of imipenem or its vehicle, and their kidneys were removed immediately. GSH was determined by measurement of free sulfhydryl using the method of Ellman [12] as modified by van Doorn *et al.* [13], and GSSG as described by Kuo *et al.* [10]. Cortical microsomes [14] were prepared for measurement of CDs [10].

Analytical

All data are presented as means ±SE. Statistical comparisons were made by an analysis of variance (ANOVA). Differences were judged to be significant where confidence levels were >95%.

RESULTS

Mitochondrial toxicity

Respiration. Imipenem reduced State 3 mitochondrial respiration by 24–26% and ATP-generating respiration (the difference between State 3 and State 4) by 29–37% (Fig. 1). Toxicity was slightly greater at 2 hr than at 1 hr.

Succinate uptake and efflux. Imipenem reduced the net uptake of succinate by 45–49% in mitochondria at 1 and 2 hr, but had no significant effect on succinate efflux (Fig. 2). These findings fit the pattern of mitochondrial toxicity of cephaloglycin [9] and cephaloridine [7], which indicate that the reduction of net succinate uptake is a result of decreased entry, rather than increased efflux caused by a nonspecific injury to mitochondrial membranes.

ADP uptake. The uptake of ADP was reduced by 16% in imipenem-intoxicated mitochondria at 1 hr, but this effect was gone by 2 hr (Fig. 3).

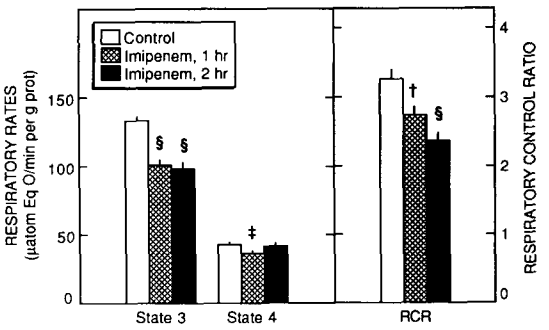

Fig. 1. Mitochondrial respiratory toxicity of imipenem. Rabbits were injected i.v. with vehicle (control) or 300 mg/kg body wt of the thienamycin 1 or 2 hr before being killed. Respiratory rates are expressed as μatom equivalents oxygen consumed per min per g protein, with 10 mM succinate as substrate in the presence of 0.125 mM ADP (State 3) or after the consumption of ADP (State 4). The respiratory control ratio (RCR) = the ratio of State 3-to-State 4 rates. Data are presented as means ±SE (N = 8–10 each). Significance levels comparing imipenem-intoxicated to control mitochondria: >97.5% (†), >99% (‡), or >99.9% (§).

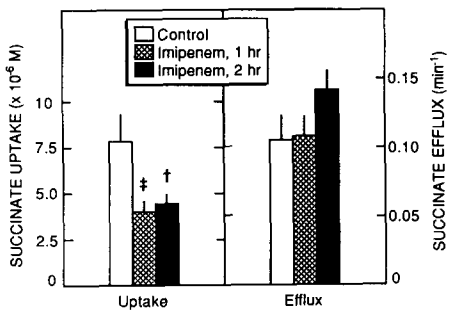

Fig. 2. Effects of imipenem on succinate transport in renal cortical mitochondria. Rabbits were injected i.v. with vehicle (control) or 300 mg/kg of imipenem 1 or 2 hr before being killed. The incubation medium contained 1.2×10^{-6} M succinate. Incubation and rinsing medium contained 5 μg/ml of antimycin A, to block succinate metabolism. Uptakes are expressed as 10^{-6} M succinate in mitochondrial water, with water contents of 3.04 ± 0.30 and 2.92 ± 0.22 ml/g protein in control and imipenem-intoxicated mitochondria respectively. Effluxes are calculated from washout studies as the slopes of the logarithms of concentration against time. Data are presented as means ±SE (N = 10–12 each). Significance levels comparing imipenem-intoxicated to control mitochondria: >97.5% (†), or >99% (‡).

Oxidative effects

Imipenem depleted GSH and increased GSSG and CDs at 1 hr (Fig. 4). These effects were considerably less than those of a comparably nephrotoxic dose of cephaloridine, which causes 2.5- and 3-fold higher levels of GSSG and CDs respectively [7]. All three parameters of imipenem-induced oxidative stress had returned to normal by 2 hr (Fig. 3).

DISCUSSION

Imipenum (*N*-formimidoyl thienamycin), the first

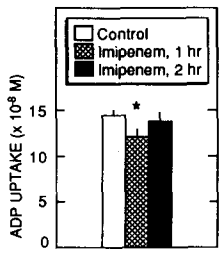

Fig. 3. Effects of imipenem on ADP uptake by renal cortical mitochondria. Rabbits were injected i.v. with vehicle (control) or 300 mg/kg of imipenem 1 or 2 hr before being killed. The incubation medium contained 4.4×10^{-10} M ADP, with no succinate. Uptakes are expressed as 10^{-8} M ADP in mitochondrial water. Data are presented as means ±SE (N = 8 each). Significance levels comparing imipenem-intoxicated to control mitochondria: >95% (*).

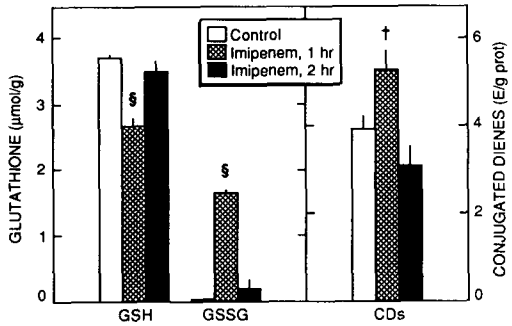

Fig. 4. Effects of imipenem on renal cortical reduced (GSH) and oxidized (GSSG) glutathione and conjugated diene (CDs) concentrations. Rabbits were injected i.v. with vehicle (control) or 300 mg/kg body wt of imipenem 1 and 2 hr before being killed. GSH and GSSG concentrations are expressed as μmol/g of wet tissue; CDs are presented as extinction units (E) at 240 nm per g of microsomal protein [10]. Data are presented as means ±SE (N = 8–12 each). Significance levels comparing imipenem-intoxicated to control kidneys: >97.5% (†), or >99.9% (§).

of the carbapenems, a new group of extremely broad-spectrum antibiotics, has been released only recently for human use [2]. Although structurally distinct from the cephalosporins [2, 8], imipenem is a beta-lactam antibiotic and therefore shares with them certain biochemical properties [2]. It is also nephrotoxic to monkeys and rabbits, producing acute proximal tubular necrosis in almost exactly the same dosages at which cephaloridine causes this injury [2]. As with the nephrotoxic cephalosporins [1], the renal toxicity of imipenem is prevented by prior administration of the proximal tubular transport inhibitor probenecid [2].

It seems logical to anticipate that imipenem and the cephalosporins could be nephrotoxic through similar subcellular or molecular mechanisms, but there are no published data testing this assumption. The present study was therefore done to compare the toxicologic properties of imipenem with the known efects of cephaloridine and cephaloglycin [7, 9].

Several mechanisms of beta-lactam antibiotic nephrotoxicity have been proposed. Three of these, studied mainly with the toxic cephalosprins, have

received the most support: (1) concentrative uptake into the tubular cell by the organic anion secretory carrier [15]; (2) production of respiratory toxicity [6, 16] through acylation and inactivation of the mitochondrial transporters for anionic substrate uptake [7, 9]; and (3) production of cell membrane lipid peroxidative injury [10].

Evidence for the role of tubular cell transport is as follows. Cytotoxicity, seen as acute cellular necrosis, affects only the proximal renal tubule [17], occurs in approximate proportion to cell concentrations of the individual cephalosporins [18, 19], and can be prevented completely by probenecid and other inhibitors of organic anion secretion [16, 18, 20]. Imipenem is also secreted across the proximal tubule, to which it is selectively toxic, and this toxicity is prevented by either probenecid or cilastatin, both of which inhibit its secretory transport [2]. Models of mitochondrial toxicity and oxidative injury, therefore, have incorporated concentrative uptake as the first step in producing tubular cell necrosis.

Findings that support a pathogenic role of mitochondrial injury in causing tubular necrosis are as follows. Respiratory toxicity is produced *in vivo* by the nephrotoxic but not by nontoxic cephalosporins [9, 16], develops 0.5 to 1 hr after administration, and is augmented at this early time by an aminoglycoside regimen that potentiates nephrotoxicity [5]. Exposure of cortical slices to cephaloridine significantly decreases their ATP content by approximately 1.5 hr [21]. The ultrastructural damage of the cephalosporins evolves more slowly (from a patchy loss of the brush border 1 hr after administration, to multiple alterations of membranous organelles by 5 hr, and finally to cellular necrosis by 10–16 hr [17]), in a pattern that closely resembles acute ischemic injury [22].

The following molecular mechanism has been proposed to account for the respiratory toxicity of the cephalosporins [1]. All cephalosporins, toxic and nontoxic, can fit the carriers for mitochondrial anionic substrate uptake. In the intact kidney, where natural substrates are abundant, this fit causes limited or transient respiratory inhibition with the nontoxic cephalosporins. *In vivo* toxicity, which is seen after isolation and washing of the mitochondria exposed for 0.5 to 2 hr in the intact cell, develops with the comparatively sequestered and reactive cephalosporins that acylate these carriers, causing irreversible injury to substrate uptake.

Several lines of evidence support this hypothesis. *In vitro* exposure of normal mitchondria to either toxic or nontoxic cephalosporins produces an immediate competitive inhibition of respiration that is overcome by increasing metabolic anionic substrate concentrations [23], whereas *in vivo* mitochondrial toxicity is specific to the nephrotoxic cephalosporins [9, 16], evolves more slowly [16], and is irreversible [23]. Acylation by the beta-lactams of functionally important membrane-bound proteins, the mechanism by which they exert their antibacterial action [24, 25], also occurs in tubular cell mitochondria [14]. Cephaloridine, cephaloglycin and imipenem, the most nephrotoxic beta-lactams, are among the most reactive protein acylators, whereas cephalexin and the penicillins, which have little or no nephrotoxic potential, are among the least reactive [2, 8, 26, 27]. Finally, cephaloglycin [9], cephaloridine [7], and imipenem (Fig. 2), like the inhibitor of mitochondrial succinate transport phenylsuccinate [28], reduce the uptake of succinate into the mitochondrion, whereas cephalexin does not [9].

The evidence for a pathogenic role of lipid peroxidation in cephaloridine nephrotoxicity is strong, but has been largely limited to that cephalosporin. Kuo *et al.* [10] showed that cephaloridine decreases GSH and increases GSSG and CDs in renal cortex, whereas selenium- and vitamin E-deficiency, which potentiate oxidative injury, increase cephaloridine nephrotoxicity. Later work measuring cortical malondialdehyde as a by-product of lipid peroxidation showed patterns of cephaloridine dose, concentration and time of administration appropriate for a pathogenic role of oxidative injury in producing tubular necrosis [7, 29, 30].

The suggested involvement of the pyridinium ring of cephaloridine in redox cycling of electrons [10], supported by the demonstration of its exchange of electrons with NADPH *in vitro* [29], fits with the lack of comparable oxidative effects of cephaloglycin [7], which has no pyridinium ring or comparable structure [8]. By the same reasoning, however, one would not have expected the depletion of cortical GSH caused by cephaloglycin [7]. The 5-fold greater production of GSSG by cephaloridine compared to cephaloglycin suggests that the two cephalosporins deplete GSH by different mechanisms, a direct oxidative effect of the pyridinium ring of cephaloridine and possibly an indirect action of cephaloglycin, mediated by its mitochondrial toxicity.

The depletion of GSH and the elevation of GSSG and CDs by imipenem (Fig. 4) are much less than that of cephaloridine but greater than that of cephaloglycin [7]. It is possible that an attack by imipenem on the free sulfhydryl of GSH [31] contributed to the depletion of reduced glutathione, thereby causing the mild oxidative stress reflected by CD accumulation. Studies of the intracellular distribution of GSH, the mitochondrial and cytosolic pools of which may be altered differently by toxic insults [32], may amplify the differences between the effects of these three beta-lactams on its reduced and oxidized forms.

Regardless of mechanism, the variability of oxidative effects of these three nephrotoxins, and the fact that the oxidative stress caused by imipenem is only transient, rule against a primary role of lipid peroxidation in the nephrotoxicity of beta-lactam antibiotics other than cephaloridine. In contrast, the reduction of mitochondrial substrate uptake and respiration by all three antibiotics, developing in advance of mitochondrial ultrastructural changes, indicates a common mitochondrial toxic effect of all of these nephrotoxic beta-lactams.

REFERENCES

1. Tune BM, The nephrotoxicity of cephalosporin antibiotics—structure–activity relationships. *Comments Toxicol* **1**: 145–170, 1986.
2. Birnbaum J, Kahan FM, Kropp H and MacDonald JS, Carbapenems. A new class of beta-lactam antibiotics.

Discovery and improvement of imipenem/cilastatin. *Am J Med* **78** (Suppl 6A): 3–21, 1985.
3. Browning MC, Wang PL, Hsu C-Y and Tune BM, Interaction of ischemic and antibiotic-induced injury in the rabbit kidney. *J Infect Dis* **147**: 341–351, 1983.
4. Tune BM and Hsu C-Y, Augmentation of antibiotic nephrotoxicity by endotoxemia in the rabbit. *J Pharmacol Exp Ther* **234**: 425–430, 1986.
5. Bendirdjian J-P, Prime DJ, Browning MC, Hsu C-Y and Tune BM, Additive nephrotoxicity of cephalosporins and aminoglycosides in the rabbit. *J Pharmacol Exp Ther* **218**: 681–685, 1981.
6. Tune BM, Wu K-Y, Fravert D and Holtzman D, Effect of cephaloridine on respiration by renal cortical mitochondria. *J Pharmacol Exp Ther* **210**: 98–100, 1979.
7. Tune BM, Fravert D and Hsu C-Y, The oxidative and mitochondrial toxic effects of cephalosporin antibiotics in the kidney. A comparative study of cephaloridine and cephaloglycin. *Biochem Pharmacol* **38**: 795–802, 1989.
8. Tune BM and Fravert D, Mechanisms of cephalosporin nephrotoxicity. A comparison of cephaloridine and cephaloglycin. *Kidney Int* **18**: 591–600, 1980.
9. Tune BM, Sibley RK and Hsu C-Y, The mitochondrial respiratory toxicity of cephalosporin antibiotics. An inhibitory effect on substrate uptake. *J Pharmacol Exp Ther* **245**: 1054–1059, 1988.
10. Kuo C-H, Maita K, Slieght SD and Hook JB, Lipid peroxidation: a possible mechanism of cephaloridine-induced nephrotoxicity. *Toxicol Appl Pharmacol* **67**: 78–88, 1983.
11. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
12. Ellman GL, Tissue sulfhydryl groups. *Arch Biochem Biophys* **82**: 70–77, 1959.
13. Van Doorn R, Leijdekkers Ch-M and Henderson PTh, Synergistic effects of phorone on the hepatotoxicity of bromobenzine and paracetamol in mice. *Toxicology* **11**: 225–233, 1978.
14. Browning MC and Tune BM, Reactivity and binding of beta-lactam antibiotics in rabbit renal cortex. *J Pharmacol Exp Ther* **226**: 640–644, 1983.
15. Tune BM and Fernholt M, Relationship between cephaloridine and *p*-aminohippurate transport in the kidney. *Am J Physiol* **225**: 1114–1117, 1973.
16. Tune BM and Fravert D, Cephalosporin nephrotoxicity. Transport, cytotoxicity and mitochondrial toxicity of cephaloglycin. *J Pharmacol Exp Ther* **215**: 186–190, 1980.
17. Silverblatt F, Turck M and Bulger R, Nephrotoxicity due to cephaloridine: A light- and electron-microscopic study in rabbits. *J Infect Dis* **122**: 33–44, 1970.
18. Tune BM, Wu K-Y and Kempson RL, Inhibition of transport and prevention of toxicity of cephaloridine in the kidney. Dose-responsiveness of the rabbit and the guinea pig to probenecid. *J Pharmacol Exp Ther* **202**: 466–471, 1977.
19. Wang PL, Prime DJ, Hsu C-Y and Tune BM, Effects of ureteral obstruction on the toxicity of cephalosporins in the rabbit kidney. *J Infect Dis* **145**: 574–581, 1982.
20. Child KJ and Dodds MG, Nephron transport and renal tubular effects of cephaloridine in animals. *Br J Pharmacol Chemother* **30**: 354–370, 1967.
21. Goldstein RS, Contardi LR, Pasino DA and Hook JB, Mechanisms mediating cephaloridine inhibition of gluconeogenesis. *Toxicol Appl Pharmacol* **87**: 297–305, 1987.
22. Venkatachalam MA, Bernard DB, Donohoe JF and Levinsky NG, Ischemic damage and repair in the rat proximal tubule: differences among the S_1, S_2, and S_3 segments. *Kidney Int* **14**: 31–49, 1978.
23. Bendirdjian J-P, Prime DJ, Browning MC and Tune BM, The mitochondiral respiratory toxicity of cephalosporins—molecular properties and pathogenic significance. In: *Nephrotoxicity, Ototoxicity of Drugs* (Ed. Fillastre J-P), pp. 303–319. Editions INSERM, Universite de Rouen, France, 1982.
24. Hoover JRE, β-Lactam antibiotics: structure–activity relationships. In: *Antibiotics Containing the Beta-Lactam Structure II* (Eds. Demain AL and Solomon NA), pp. 118–245. Springer, Berlin, 1983.
25. Waxman DJ and Strominger JL, Penicillin binding proteins and the mechanism of action of beta-lactam antibiotics. *Annu Rev Biochem* **52**: 825–869, 1983.
26. Yaman T and Tsuji A, Comparative stability of cephalosporins in aqueous solution: kinetics and mechanisms of degradation. *J Pharm Sci* **65**: 1563–1574, 1976.
27. Indelicato JM, Dinner A, Peters LR and Wilham WL, Hydrolysis of 3-chloro-3-cephems. Intramolecular nucleophilic attack in cefaclor. *J Med Chem* **20**: 961–963, 1977.
28. Palmieri F and Klingenberg M, Direct methods for measuring metabolite transport and distribution in mitochondria. *Methods Enzymol* **56**: 279–301, 1979.
29. Cojocel C, Hanneman J and Baumann K, Cephaloridine-induced lipid peroxidation initiated by reactive oxygen species as a possible mechanism of cephaloridine nephrotoxicity. *Biochim Biophys Acta* **834**: 402–410, 1985.
30. Tune BM and Hsu C-Y, Mechanisms of the endotoxin-cephalosporin toxic synergy and the protective action of saline in the rabbit kidney. *J Pharmacol Exp Ther* **244**: 520–525, 1988.
31. Kahan JS, Kahan FM, Goegelman R, Currie SA, Jackson M, Stapley EO, Miller TW, Miller AK, Hendlin D, Mochales S, Hernandez S, Woodruff HB and Birnbaum J, Thienamycin, a new β-lactam antibiotic. I. Discovery, taxonomy, isolation and physical properties. *J Antibiot (Tokyo)* **32**: 1–12, 1979.
32. Schnellmann RG, Gilchrist SM and Mandel LJ, Intracellular distribution and depletion of glutathione in rabbit renal proximal tubules. *Kidney Int* **34**: 229–233, 1988.