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OXIDATIVE AND MITOCHONDRIAL TOXIC EFFECTS OF CEPHALOSPORIN ANTIBIOTICS IN THE KIDNEY

A COMPARATIVE STUDY OF CEPHALORIDINE AND CEPHALOGLYCIN*

BRUCE M. TUNE,† DORIS FRAVERT and CHIEH-YIN HSU
Laboratory of Renal Pharmacology, Department of Pediatrics, Stanford University, School of Medicine, Stanford, CA 94305, U.S.A.

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Abstract—Cephaloridine and cephaloglycin are the two most nephrotoxic cephalosporins released for human use. Cephaloridine has been shown to produce both oxidative and mitochondrial respiratory injury in renal cortex in patterns of dose (or concentration) and time that are consistent with pathogenicity. Cephaloglycin also produces respiratory toxicity, and recent studies have provided evidence that this injury results from an inactivation of mitochondrial anionic substrate transporters. The abilities of cephaloglycin to produce oxidative changes and cephaloridine to block mitochondrial substrate uptake have not been examined yet. We therefore compared these two cephalosporins with one another and with cephalexin, which is not nephrotoxic, in the production of the following: (1) several components of oxidative stress or damage [depletion of reduced glutathione (GSH) and production of oxidized glutathione (GSSG) in renal cortex, inhibition of glutathione reductase *in vitro*, and production of the lipid peroxidation products malondialdehyde (MDA) and conjugated dienes (CDs) in renal cortex]; and (2) renal cortical mitochondrial toxicity [to both respiration with, and the transport of, succinate]. Cephaloridine depleted GSH and elevated GSSG in renal cortex, inhibited glutathione reductase, and increased both MDA in whole cortex and CDs in cortical microsomes and mitochondria. While cephaloglycin depleted GSH at least as much as did cephaloridine, it produced one-fifth as much GSSG and had little or no effect on glutathione reductase activity or on cortical MDA or microsomal CDs; cephaloglycin caused a transient small increase of mitochondrial CDs. Cephalexin produced no oxidative changes except for a slight increase of mitochondrial CDs comparable to that produced by cephaloglycin. Both cephaloridine and cephaloglycin, but not cephalexin, decreased the unidirectional uptake of, and respiration with, succinate in cortical mitochondria. We conclude that cephaloridine and cephaloglycin are both toxic to mitochondrial substrate uptake and respiration, but differ significantly in their generation of products of oxidation.

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 antibiotic imipenem [2], produce acute proximal tubular necrosis and acute renal failure 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 compare these two nephrotoxic cephalosporins with one another and with cephalexin, which does not produce tubular necrosis even after very large doses [6], in the production of two recognized components of cephalosporin toxicity: oxidative damage [7], which has been studied with cephaloridine but not cephaloglycin, and mitochondrial toxicity [8, 9], which has been studied in greater detail with cephaloglycin than cephaloridine. The following components of oxidative change were examined: depletion of reduced glutathione (GSH) and production of oxidized glutathione (GSSG) in renal cortex, inhibition of glutathione reductase *in vitro*, and production of the lipid peroxidation products malondialdehyde, (MDA) and conjugated dienes (CDs) in renal cortex. The following components of mitochondrial function were investigated: respiration with, and the net uptake and efflux of, succinate by renal cortical mitochondria.

MATERIALS AND METHODS

Female New Zealand white (NZW) 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. Cephaloridine (supplied by the Lilly Research Laboratories, Indianapolis, IN)

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

was dissolved in 0.9% saline, and cephaloglycin and cephalexin (Lilly) were dissolved in 1 mEq/ml of sodium bicarbonate, in solutions of 100 mg of antibiotic base/ml. The antibiotics, or their vehicles, were infused i.v. at 100 mg/min into animals anesthetized i.p. with pentobarbital, 45–60 mg/kg body wt. Except where otherwise indicated, all reagents were purchased from the Sigma Chemical Co. (St. Louis, MO).

Most studies involved the production of nephrotoxicity *in vivo*, with tissues obtained from animals 1–2 hr after administration of vehicle, 300 mg/kg of the nephrotoxic cephalosporins, or 1000 mg/kg of cephalexin. The toxicity of cephaloridine to mitochondrial functions was studied further *in vitro*, by measuring respiration and substrate uptake in control mitochondria without and with the addition to the respiration medium of 2000 μg/ml of the antibiotic. This concentration produces about as much respiratory toxicity as the 300 mg/kg used to produce mitochondrial toxicity *in vivo*.

Oxidative effects

GSH depletion, GSSG production, and lipid peroxidation. Animals were killed by decapitation 1–2 hr after administration of the vehicle or a cephalosporin, and their kidneys were removed immediately. GSH was determined by measurement of free sulfhydryl using the method of Ellman [10] as modified by Van Doorn *et al.* [11], GSSG as described by Kuo *et al.* [7], and MDA concentration by the method of Buege and Aust [12] in whole cortical homogenates. Cortical microsomes and mitochondria [13] were prepared for measurement of CDs as described by Kuo *et al.* [7].

GSSG reductase. Glutathione reductase (Sigma type VII), 0.15 μg, was added to a mixture containing 300 μmol Tris (pH 7), 10 μmol EDTA, 1.6 μmol oxidized glutathione, and 0.1 μmol NADPH in 3 ml. Enzyme activity was measured at 25° by the change of extinction through oxidation of NADPH at 340 nm on a Perkin–Elmer Spectrophotometer, Five-cell Programmer and Recorder (models 552 and 561, Coleman Instruments, Oak Brook, IL). The absorbancy index of NADPH used in these calculations was 6.22×10^3 cm^2 per mole [14].

Mitochondrial toxicity

Animals were killed 1–2 hr after administration of the vehicle or a cephalosporin, and their kidneys were removed immediately. Renal cortical mitochondria were prepared as previously described [8] in a pH 7.4 solution containing 260 mM sucrose, 5 mM Tris–HCl, and 0.2 mM EDTA. To maintain comparable conditions for measurement 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 mitochondrial protein [15], 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.

Succinate uptake. Uptake was measured in separate aliquots of mitochondria, using [2,3-^{14}C]-succinic acid, 42 mCi/mmol, obtained from the Amersham Corp. (Arlington Heights, IL). 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. Mitochondria (0.5 to 1 mg protein) were incubated for 0.25 to 5 min in 2 ml of respiration medium at 20°, then trapped on Millipore DAWP 025 00 (0.65 μm) 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 [U-^{14}C]sucrose, Amersham) 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 were 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 (E. I. du Pont de Nemours & Co., North Billerica, MA) 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 protein was determined as the difference between the total water and sucrose spaces.

Substrate efflux. The washout of [^{14}C]succinate from normal and *in vivo*-cephaloridine-intoxicated mitochondria was measured by incubating separate aliquots with the substrate for 5 min, then trapping the suspensions on 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.

Analytical

All data are presented as means ± SE. Statistical comparisons were made by analysis of variance where multiple comparisons were made against a single control, or by Student's *t*-test with a two-tailed table of significance where each measurement was

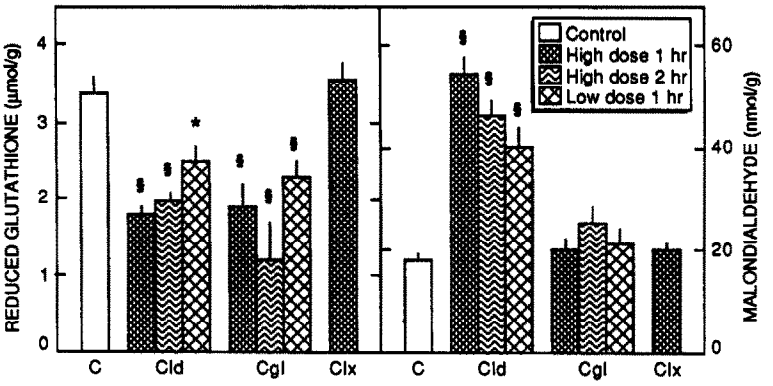

Fig. 1. Effects of cephalosporins on renal cortical reduced glutathione and malondialdehyde concentrations. Rabbits were injected i.v. with 1000 mg/kg body wt of the nontoxic cephalosporin cephalexin (Clx), or 300 (high dose) or 100 (low dose) mg/kg of the toxic cephalosporin cephaloridine (Cld) or cephaloglycin (Cgl) 1 or 2 hr before being killed. Reduced glutathione and malondialdehyde concentrations are expressed as μmol and nmol, respectively, per g of wet tissue. Data are presented as means ± SE (N = 6–10 each). Significance levels by analysis of variance comparing cephalosporin-treated to control animals: 99% (*) or 99.9% (§).

made with a new control. Differences were judged to be significant where the confidence level was >95% by ANOVA, or where P was < 0.05 by *t*-test.

RESULTS

Oxidative effects

GSH depletion, GSSG production, and lipid peroxidation. Renal cortical GSH was depleted significantly by both cephaloridine and cephaloglycin, but not by cephalexin (Fig. 1). Cortical GSSG concentrations at 1 hr were (nmol/g wet tissue): control, 0.06 ± 0.04; 300 mg/kg cephaloridine, 4.20 ± 0.18; and 300 mg/kg cephaloglycin, 0.78 ± 0.13 (N = 8 each, P < 0.001 comparing control to each cephalosporin and comparing cephaloridine to cephaloglycin). Both MDA in whole renal cortex (Fig. 1) and CDs in cortical microsomes and mitochondria (Fig. 2) were elevated significantly by cephaloridine at the fully toxic dose of 300 mg/kg at 1 and 2 hr. These products of lipid peroxidation were not elevated significantly by the same quantity of cephaloglycin, which has a one-third lower dose threshold (~60 mg/kg) for producing tubular necrosis than that of cephaloridine (~90 mg/kg) [16], except for a slight elevation of mitochondrial CDs at 1 hr—which was also produced by cephalexin (Fig. 2). Even the marginally nephrotoxic dose of 100 mg/kg of cephaloridine produced more elevation of these two measures of lipid peroxidation than did the fully nephrotoxic dose of 300 mg/kg of cephaloglycin.

Inhibition of GSSG reductase. Cephaloridine, but not cephaloglycin or cephalexin, inhibited GSSG reductase activity *in vitro* at 300 and 3000 μg/ml (Fig. 3), concentrations which span the threshold for producing tubular necrosis *in vivo* (1000–2000 μg/g wet tissue [17]). When NADP was added to the reaction chamber instead of NADPH, the same con-

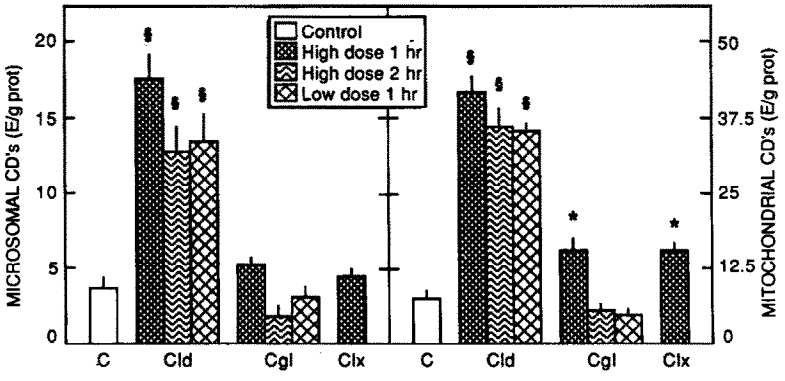

Fig. 2. Effects of cephalosporins on conjugated diene content of renal cortical lipids. Treatment protocols were identical to those described in the legend of Fig. 1. Conjugated dienes (CDs) are expressed as extinction units (E) at 240 nm per g of mitochondrial or microsomal protein [7]. Data are presented as means ± SE (N = 6–10 each). Significance levels by analysis of variance comparing cephalosporin-treated to control animals: 99% (*) or 99.9% (§).

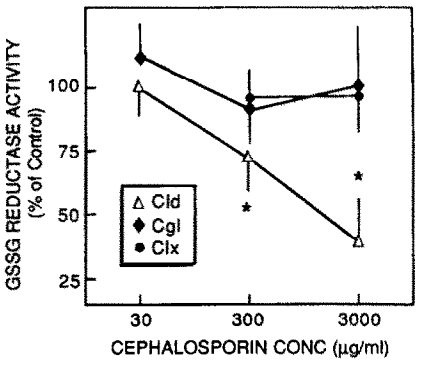

Fig. 3. Effects of cephalosporins on *in vitro* glutathione reductase activity. Glutathione (GSSG) reductase activity was measured in a pH 7, 300 mM Tris buffer at 25° as the decrease of absorbancy at 340 nm due to oxidation of NADPH (0.1 μM) in the presence of GSSG (1.6 μM). Activities are expressed as ratios (%) of enzyme activities in cephalosporin-/vehicle-exposed samples; activities in controls were 18.4 ± 1.2 μmol NADPH consumed per min per mg protein. Data are presented as means ± SE (N = 8–12 each). P values derived from *t*-tests comparing cephalosporin-exposed ratios to 100%: (*) < 0.02 at 300 μg/ml and < 0.01 at 3000 μg/ml cephaloridine.

centrations of cephaloridine did not reduce the oxidized nucleotide, ruling out a simple reducing effect of the cephalosporin subsequent to the enzymatically catalyzed generation of 2 NADP and 2 GSH from 2 NADPH and GSSG.

Mitochondrial toxicity

Respiration. Cephaloridine inhibited State 3 respiration and reduced the respiratory control ratio at 1 and 2 hr *in vivo* (Fig. 4) and was slightly more toxic to mitochondrial respiration at 2000 μg/ml *in vitro* (Fig. 5). The same dose of cephaloglycin produced greater respiratory toxicity than cephaloridine *in vivo*, while cephalexin showed no *in vivo* toxicity (Fig. 5).

Succinate uptake. Cephaloridine reduced net succinate uptake by approximately 50% after 1 and 2 hr of *in vivo* exposure (Fig. 6), and 2000 μg/ml of cephaloridine *in vitro* produced slightly greater inhibition of substrate uptake (Fig. 7). As previously noted [9], the same dose of cephaloglycin reduced succinate uptake similarly at 5 min, whereas *in vivo* cephalexin had no toxic effect on substrate uptake (Fig. 7).

Substrate efflux. Cephaloridine exposure for 1 hr *in vivo* had no effect on succinate efflux, whereas 2 hr of exposure reduced efflux by approximately one-half (Table 1). These findings support the conclusion previously reached with cephaloglycin [9] that the reduction of net succinate uptake produced by cephalosporin toxicity is a result of decreased entry, rather than increased efflux caused by a nonspecific injury to the mitochondrial membrane.

DISCUSSION

Several mechanisms of cephalosporin nephrotoxicity have been proposed, but studies of three of these have provided the greatest supportive evidence: (1) concentrative uptake into the tubular cell by the organic anion secretory carrier [18]; (2) the production of cell membrane lipid peroxidative injury [7]; and (3) the production of respiratory toxicity through acylation and inactivation of the mitochondrial transporters for anionic substrate uptake [8, 9]. Evidence for the role of tubular cell transport is as follows. Cytotoxicity, seen as acute cellular necrosis, affects only the proximal renal tubule [19], occurs in approximate proportion to cortical concentrations of the individual cephalosporins [17, 20], and can be prevented completely by inhibitors of organic anion secretion [16, 17, 21].

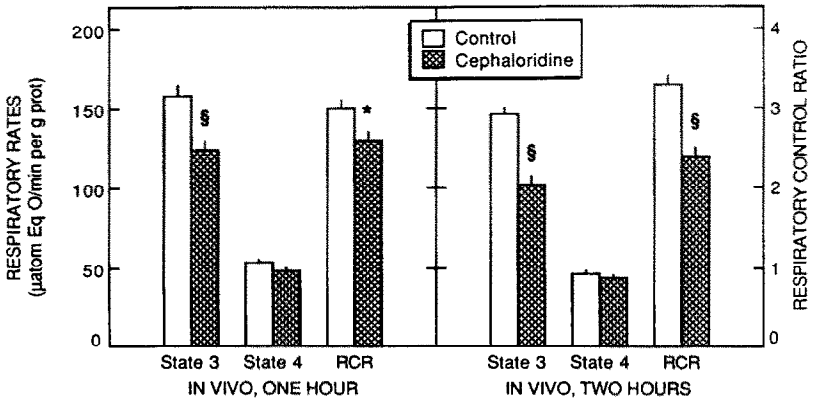

Fig. 4. Mitochondrial respiratory toxicity of cephaloridine. Rabbits were injected i.v. with 300 mg/kg body wt of cephaloridine or its vehicle (control) 1 or 2 hr before being killed. Respiratory rates are expressed as μatom equivalents oxygen consumed per min per g protein, with 10 mM succinate as substrate in the presence of 0.125 mM ADP (State 3) or after the consumption of ADP (State 4); RCR (the respiratory control ratio) = the ratio of State 3-to-State 4 rates. Data are presented as means ± SE (N = 10–15 each). P values derived from *t*-tests comparing control to cephaloridine-intoxicated mitochondria: < 0.01 (*) or < 0.001 (§).

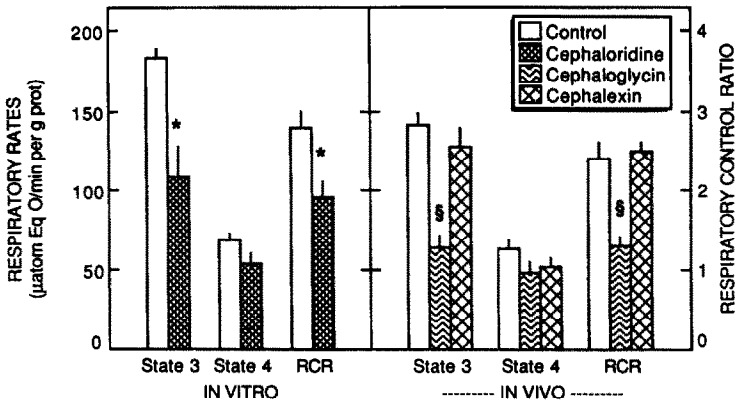

Fig. 5. Mitochondrial respiratory toxicity of cephalosporins. *In vitro* toxicity of cephaloridine was measured in mitochondria from untreated animals immediately upon exposure to 2000 μg/ml of the antibiotic in the respiration chamber. In studies of *in vivo* toxicity, rabbits were injected i.v. with vehicle (control), or 300 mg/kg body wt of cephaloglycin or 1000 mg/kg of cephalexin i.v. 1 hr before being killed. Respiratory rates and the respiratory control ratio (RCR) are presented as described for Fig. 4. Data are presented as means ± SE (N = 8–10 each). P values derived from *t*-tests comparing control to cephalosporin-intoxicated mitochondria: < 0.02 (*) or < 0.001 (§).

The models of oxidative injury and mitochondrial toxicity, therefore, incorporate concentrative uptake as the first step in producing tubular cell necrosis.

Kuo and associates [7] first observed that cephaloridine depletes GSH and induces lipid peroxidation (measured as microsomal CDs) in renal cortex. This and later work measuring tissue MDA [9, 22, 23], showed patterns of antibiotic dose, concentration and time of administration appropriate for a pathogenic role of lipid peroxidation in producing tubular necrosis. The suggested involvement of the pyridinium ring of cephaloridine in redox cycling of electrons [7], supported by the demonstration of its exchange of electrons with NADPH *in vitro* [22], fits the lack of comparable oxidative effects of cephaloglycin (Figs. 1 and 2), which has no pyridinium ring or comparable structure [24].

By the same reasoning, one could not have anticipated that cephaloglycin would deplete cortical GSH. Its decrease by cephaloglycin (Fig. 1), accompanied by comparatively little peroxidative change (Figs. 1 and 2), raises a question as to how the two cephalosporins decrease GSH concentrations. The significantly greater production of GSSG with cephaloridine compared to cephaloglycin suggests that they deplete GSH by different mechanism: a direct oxidative attack by cephaloridine and possibly an indirect oxidative action of the mito-

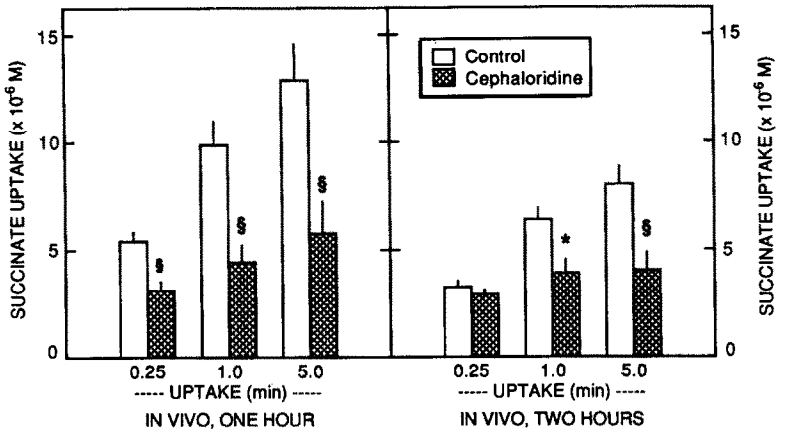

Fig. 6. Effects of cephaloridine on substrate uptake by renal cortical mitochondria. Rabbits were injected i.v. with vehicle (control) or 300 mg/kg of cephaloridine 1 or 2 hr before being killed. The incubation medium contained 1.2×10^{-6} M succinate. Uptakes are expressed as 10^{-6} M succinate in mitochondrial water, calculated from water contents of 5.80 ± 0.79 and 5.92 ± 0.74 ml/g protein in control and cephaloridine-intoxicated mitochondria respectively. Data are presented as means ± SE (N = 10–12 each). P values derived from *t*-tests comparing control to cephaloridine-intoxicated mitochondria: < 0.01 (*) or < 0.005 (§).

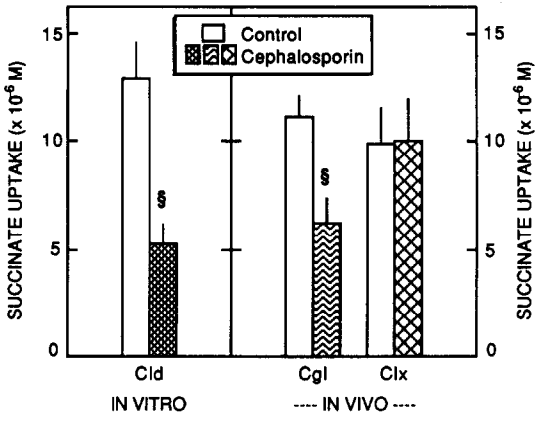

Fig. 7. Effects of cephalosporins on substrate uptake by renal cortical mitochondria. *In vitro* toxicity of cephaloridine (Cld) was measured in mitochondria from untreated animals. In studies of *in vivo* toxicity, rabbits were injected i.v. with vehicle (control), or 300 mg/kg body wt of cephaloglycin (Cgl) or 1000 mg/kg of cephalexin (Clx) i.v. 1 hr before being killed. Measurements of succinate uptake are presented as described for Fig. 6. Data are presented as means ± SE (N = 6–8 each). P values derived from *t*-tests comparing control to cephalosporin-intoxicated mitochondria: < 0.005 (§).

chondrial toxicity of cephaloglycin. The *in vitro* studies with glutathione reductase (Fig. 3) may not explain the *in vivo* mechanism of GSH depletion, but inhibition by cephaloridine and not by cephaloglycin provides further evidence of difference between the two toxic cephalosporins.

Table 1. Succinate efflux from cephaloridine-intoxicated mitochondria

Treatment*,†	Washout‡	Half-life‡
One hour, *in vivo*		
Control	−0.14 ± 0.02§	2.6 ± 0.4
Cephaloridine	−0.13 ± 0.02	2.5 ± 0.4
	NS‖	NS‖
Two hours, *in vivo*		
Control	−0.15 ± 0.02	2.2 ± 0.3
Cephaloridine	−0.07 ± 0.02	6.1 ± 1.1
	P < 0.02‖	P < 0.01‖

* NZW rabbits were given 300 mg/kg of cephaloridine, or its vehicle, i.v. 1–2 hr before being killed. Mitochondrial protein (0.5 mg) was incubated at 20° in 5 ml of respiration medium for 5 min, then trapped on Millipore filters, and washed continuously for 3–9 min with 20° respiration medium.

† Incubation medium contained 1.2×10^{-6} M succinate, without ADP; incubation and rinsing medium contained 5 μg/ml of antimycin A.

‡ Slopes of the logarithms of concentration against time, calculated by linear regression. Half-life (min) = log 0.5/slope of washout.

§ Means ± SE, N = 6–8 each.

‖ P values derived from *t*-tests comparing cephaloridine to control.

While the production of direct oxidative damage appears to be a particular property of cephaloridine, *in vivo* mitochondrial toxicity has been shown with both nephrotoxic cephalosporins [8, 16]. The following evidence supports a pathogenic role of mitochondrial injury in the development of tubular necrosis. Respiratory toxicity is produced *in vivo* only by the nephrotoxic cephalosporins, 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 [25]. The ultrastructural damage of the cephalosporins evolves more slowly (1–5 hr after administration [19]) and follows a pattern that closely resembles acute ischemic injury [26].

Several lines of evidence have suggested that the respiratory toxicity of the cephalosporins could be mediated by a blocking of mitochondrial metabolic substrate uptake. Toxicity following either *in vitro* or *in vivo* exposure affects succinate- more than glutamate-plus-malate-driven respiration [8, 16]. Glutamate and malate contribute electrons to the respiratory chain through NADH dehydrogenase at ADP phosphorylation site 1, while succinate contributes more distally through coenzyme Q near site 2. The greater involvement of succinate-driven respiration therefore suggests an attack on a function outside the respiratory chain. This could involve either substrate transport or the enzymatic steps that feed into the electron transport chain. *In vitro* cephalosporin toxicity [27] causes a competitive inhibition of substrate-driven respiration: toxicity is immediate, occurs similarly with nephrotoxic or nontoxic cephalosporins, and is reversed by substrate excess. In contrast, toxicity after *in vivo* exposure is delayed by 0.5 hr, is specific to the nephrotoxic cephalosporins, and is irreversible [27].

The development of irreversible inhibition after *in vivo* exposure could be explained by the acylating properties of the beta-lactam antibiotics. Acylation and inactivation of functionally important membrane-bound proteins is the mechanism of antibacterial action of the penicillins, cephalosporins, and newer beta-lactams [24, 28]. Cephaloglycin acylates proteins of tubular cell mitochondria over 0.5 to 1 hr of exposure [13]. Cephaloridine, cephaloglycin and imipenem, the most nephrotoxic beta-lactams, are among the most reactive protein acylators, while cephalexin and the penicillins, which have little or no nephrotoxic potential, are among the least reactive [2, 6, 16, 24, 29, 30].

The following model was developed to account for the lack of specificity and the reversibility of *in vitro* toxicity and the specificity and irreversibility of *in vivo* toxicity to mitochondrial respiration. All of the cephalosporins can competitively reduce the carrier-mediated mitochondrial uptake of anionic substrates. However, the nontoxic cephalosporins undergo comparatively less sequestration within the tubular cell, and/or fit the targeted transporter(s) less well, and/or are less reactive than those that are nephrotoxic [1]. They therefore cause limited or transient respiratory inhibition in the intact kidney, where natural substrates are abundant. *In vivo* tox-

icity, which is seen after *in situ* exposure followed by later isolation and washing of the mitochondria, occurs with the more sequestered, high-affinity, and reactive (i.e. nephrotoxic) cephalosporins, because they acylate the transporters and block substrate uptake irreversibly. Studies of the transport of succinate in cephaloglycin-intoxicated mitochondria have supported this proposed model [9].

The decreased respiration (Figs. 4 and 5) and substrate entry (Figs. 6 and 7 and Table 1) caused by cephaloridine parallel closely the effects of cephaloglycin [9]. Cephalexin, which has the expected *in vitro* toxicity to respiration and uptake [9], has no toxicity to either function after *in vitro* exposure (Figs. 5 and 7). The pattern of reduced succinate uptake without increased efflux after *in vivo* cephaloglycin and cephaloridine, and with decreased efflux 2 hr after cephaloridine, is relatively specific, like the effects of phenylsuccinate, an inhibitor of mitochondrial succinate transport [31]. In contrast, succinate uptake is not reduced in mitochondria poisoned by the respiratory chain inhibitors cyanide and oligomycin*.

It is difficult at this point to assign a predominant role to either primary oxidative or direct mitochondrial injury in cephaloridine nephrotoxicity. In favor of oxidative damage, the lipid peroxidation measured after cephaloridine has a lower threshold dose than does toxicity to mitochondrial respiration [32], and diets deficient in selenium or vitamin E, which augment oxidative injury in general, potentiate cephaloridine nephrotoxicity [7]. Primary lipid peroxidation could be expected to cause secondary disturbances of mitochondrial function, although one might expect increased substrate efflux from generalized injury to mitochondrial membranes, as is seen after acute ischemia*.

In support of a pathogenic role of mitochondrial toxicity, both cephaloglycin and cephaloridine reduced unidirectional substrate uptake and respiration early and to a comparable degree. This mitochondrial injury could be the cause of the oxidative stress, seen as GSH depletion, with cephaloglycin and may contribute to that produced by cephaloridine. A strong case has been made [7, 22] that cephaloridine also causes direct oxidative injury, which fits with the higher levels of cortical GSSG and the elevations of MDA and CDs not seen after cephaloglycin. However, there is insufficient early oxidative action of cephaloglycin to suggest that lipid peroxidation comparable to that produced by cephaloridine is a general property of the nephrotoxic cephalosporins. We therefore conclude that the available evidence supports a common involvement of mitochondrial toxicity from cephaloridine and cephaloglycin, with an important contributory role of primary oxidative injury from cephaloridine, in the production of acute tubular necrosis by these two nephrotoxic cephalosporins.

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