Pergamon

Biochemical Pharmacology, Vol. 49, No. 5, pp. 727–734, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(94)00497-8

TOXICITY OF CEPHALOSPORINS TO FATTY ACID METABOLISM IN RABBIT RENAL CORTICAL MITOCHONDRIA

BRUCE M. TUNE* and CHIEH-YIN HSU

Laboratory of Renal Pharmacology, Division of Nephrology, Department of Pediatrics, Stanford University School of Medicine, Stanford, CA 94305–5119, U.S.A.

(*Received* 15 *July* 1994; *accepted* 20 *September* 1994)

Abstract—Cephaloglycin (Cgl) and cephaloridine (Cld) are acutely toxic to the proximal renal tubule, in part because of their cellular uptake by a contraluminal anionic secretory carrier and in part through their intracellular attack on the mitochondrial transport and oxidation of tricarboxylic acid (TCA) cycle anionic substrates. Preliminary studies with Cgl have provided evidence of a role of fatty acid (FA) metabolism in its nephrotoxicity, and work with Cld has shown it to be a potent inhibitor of renal tubular cell and mitochondrial carnitine (Carn) transport. Studies were therefore done to examine the effects of Cgl and Cld on the mitochondrial metabolism of butyrate, the anion of a short-chain FA that does not require the Carn shuttle to enter the inner matrix, and the effects of Cgl on the metabolism of palmitoylcarnitine (PCarn), the Carn conjugate of a long-chain FA that does enter the mitochondrion by the Carn shuttle. The following was found: (1) Cgl reduced the oxidation and uptake of butyrate after *in vitro* (2000 μg/mL, immediate effect) and after *in vivo* (300 mg/kg body weight, 1 hr before killing) exposure; (2) Cld caused milder *in vitro* toxicity, and no significant *in vivo* toxicity, to mitochondrial butyrate metabolism; (3) like Cld, Cgl reduced PCarn-mediated respiration after *in vivo* exposure, but, unlike Cld, it did not inhibit respiration with PCarn *in vitro*; (4) the Carn carrier was stimulated slightly by *in vitro* Cgl but was unaffected by *in vivo* Cgl; (5) *in vivo* Cgl had no effect on mitochondrial free Carn or long-chain acylCarn concentrations in the *in situ* kidney; (6) Cgl increased the excretion of Carn minimally compared with the effect of Cld; and (7) cephalexin, a nontoxic cephalosporin, caused mild reductions of respiration with butyrate and PCarn during *in vitro* exposure, but stimulated respiration with both substrates after *in vivo* exposure. Conclusions: Cgl has essentially the same patterns of *in vitro* and *in vivo* toxicity against mitochondrial butyrate uptake and oxidation that both Cgl and Cld have against TCA-cycle substrates. Cld has little or no *in vivo* toxicity to mitochondrial butyrate metabolism, whereas *in vivo* Cgl is as toxic as Cld to respiration with PCarn. The greater overall *in vivo* toxicity of Cgl to mitochondrial FA metabolism, with lower cortical concentrations and AUCs than those of Cld, supports earlier evidence that Cld is less toxic than Cgl at the molecular level.

Key words: beta-lactam; cephalosporin; fatty acid; mitochondrion; nephrotoxic; transporter

Certain of the beta-lactam antibiotics are nephrotoxic in several mammalian species. When the most toxic of these are given to rabbits, which are approximately as susceptible as humans or nonhuman primates [1], single doses in the therapeutic range cause necrosis of proximal tubular cells, starting as early as 5 hr after administration [2]. Cgl† and Cld, the most thoroughly studied of the nephrotoxic cephalosporins, and imipenem, a nephrotoxic carbapenem, share two important properties: (1) their nephrotoxicity can be prevented by inhibitors of organic anion (hippurate) secretion [3–5]; and (2) they are toxic to respiration with substrates and precursors of the mitochondrial TCA cycle [4, 6, 7], through inactivation of mitochondrial anionic substrate carriers [7–9].

An important feature of Cld sets it apart from the other toxic cephalosporins: it has a quaternary nitrogen in an R_2 pyridinium substituent [10]. As a result, although Cld is freely transported into the tubular cell by a contraluminal secretory carrier, its net movement into the tubular fluid is limited severely [11], with tubular cell concentrations more than twice those of Cgl and Clx [12]. The unusual structure of Cld has also been implicated in its unique ability among the toxic beta-lactams to cause oxidative injury [13, 14], and in its inhibition of mitochondrial and tubular cell Carn carriers [15].

Although respiration with TCA-cycle intermediates and their amino acid precursors is important in the proximal tubule [16, 17], the effects of the beta-lactams on another group of tubular cell substrates, the FAs, have received limited attention. Preliminary work in this laboratory has provided indirect evidence of an involvement of altered FA metabolism in Cgl nephrotoxicity [1]. Cgl is significantly more toxic to the rabbit kidney after

* Corresponding author: Bruce M. Tune, M.D., Division of Nephrology, Department of Pediatrics, G-306, Stanford University School of Medicine, Stanford, CA 94305–5119. Tel. (415) 723–7903; FAX (415) 723–2137.

† Abbreviations: AUC, area under the curve (of concentration and time); Carn, carnitine; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Cgl, cephaloglycin; Cld, cephaloridine; Clx, cephalexin; FA, fatty acid; ND_{50}, 50%-nephrotoxic dose (producing tubular necrosis); PCarn, palmitoylcarnitine; and TCA, tricarboxylic acid.

24 hr of fasting, which increases dependence upon FA metabolism [18]. Moreover, this augmentation of injury is prevented by intravenous glucose, which decreases dependence on FA metabolism [18], but not by an identical volume and osmolar infusion of saline [15].

FAs are metabolized in tubular cell mitochondria through the sequential oxidation of carboxyterminal two-carbon fragments [19]. To enter into this process, the long-chain FAs must first undergo conjugation to Carn and be transported by the Carn carrier across the mitochondrial inner membrane [20]. Short-chain FAs can enter the inner matrix for oxidation independent of the Carn carrier [20], possibly through a monocarboxylic acid transporter. Therefore, the present protocols were designed to compare: (1) the effects of different cephalosporins on the metabolism in renal cortical mitochondria of a short-chain (butyric, 4-carbon) and a long-chain (palmitic, 16-carbon) FA; and (2) the effects of Cgl on (a) the mitochondrial Carn carrier, and (b) the renal cortical and mitochondrial content and urinary excretion of free Carn and long-chain acylCarns in the *in situ* kidney.

MATERIALS AND METHODS

Except where otherwise noted, reagents were purchased from the Sigma Chemical Co. (St. Louis, MO). The following isotopes were used: L-[methyl-^{3}H]carnitine hydrochloride, 80 Ci/mmol (Amersham Corp., Arlington Heights, IL); [1-^{14}C]acetyl-coenzyme A, 60 mCi/mmol (Amersham); and [1-^{14}C]-*N*-butyric acid, sodium salt, 50 mCi/mmol (ICN Biomedicals, Irvine, CA).

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 before the cephalosporin infusions (*in vivo* toxicity), or before being killed if they were not pretreated with an antibiotic (for studies of *in vitro* toxicity).

Cgl or Clx (Lilly Research Laboratories, Indianapolis, IN) was dissolved in 1 mEq/mL of sodium bicarbonate, and Cld (Lilly) was dissolved in 0.9% saline, in solutions of 100 mg of antibiotic base per mL, to be infused intravenously at a rate of 0.5 to 1.0 mL/min. In studies of *in vivo* toxicity, 300 mg/kg body weight of the antibiotics, or their vehicles, was administered 1 hr before the animals were killed. In most studies of *in vitro* toxicity, 2000 μg/mL (~5 mM) of the cephalosporin, or its vehicle, was added to the incubation medium immediately before the indicated measurements. In studies to determine the IC_{50} values of Cgl and Cld to butyrate- and PCarn-mediated respiration *in vitro*, 1000 μg/mL increments (up to 7000 μg/mL) were used, and the IC_{50} values were calculated from the linear slope of the highest concentration causing <50% and the lowest concentration causing >50% inhibition.

The vehicles had no measurable effects on control measurements after either *in vivo* or *in vitro* exposure. Therefore, in protocols involving more than one cephalosporin, controls represent the pooled results of appropriate numbers of measurements with each vehicle.

Mitochondrial respiration

Animals were killed by decapitation, their kidneys were removed immediately, and renal cortical mitochondria were prepared as previously described [9] in a pH 7.4 solution containing 260 mM sucrose, 5 mM Tris–HCl, and 0.2 mM EDTA. Mitochondrial oxygen consumption was measured at 30° with a Clarke platinum electrode assembly. The respiration chamber contained 1.7 mg of mitochondrial protein in 1.7 mL of a pH 7.2 respiration medium containing 230 mM mannitol, 70 mM sucrose, 20 mM Tris–HCl and 0.2 mM EDTA, to which was also added 1 μM CCCP [21]. The effects of the antibiotic on respiration with either 30 μM PCarn or 5 mM butyrate were studied after *in vitro* or *in vivo* exposure as described above.

Butyrate uptake

The uptake of butyrate was studied at 20° in the same medium used to measure respiration, with 2 μg/mL rotenone, 5 μg/mL oligomycin, and 1 μg/mL antimycin A added to block oxidative metabolism. Mitochondria (0.5 mg protein) were added to 2 mL of medium containing 0.2 μCi of [^{14}C]butyrate together with 2000 μg/mL of Cgl, Cld, or Clx, or their vehicles, or 5 mM unlabeled butyrate. After 0.25, 1.0 and 2.0 min of incubation, the mitochondria were 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 medium containing the respiratory inhibitors. The filters were placed in Aquasol Universal LSC (NEN Research Products, Boston, MA) overnight to allow clarification, and then counted in a Wallac LKB model 1219 liquid scintillation counter (Wallac Inc., Gaithersburg, MD).

Carnitine–acylcarnitine antiport

The antiport of acetylCarn and Carn was measured by the method of Pande [22], using pH 7.4 incubating and washing media containing 220 mM sucrose, 20 mM Tris–HCl, 10 mM sodium/disodium phosphate, and 5 mM potassium chloride, plus 2 μg/mL rotenone, 5 μg/mL oligomycin, and 1 μg/mL antimycin A. Mitochondria (30 mg protein) were preloaded for 4 min at 28° with 10 μCi[^{3}H]carnitine, then twice washed, and recentrifuged in 30 mL of iced medium containing the respiratory inhibitors.

Duplicate samples (with and without 0.1 mM mersalyl to block the Carn/acylCarn transporter) containing 1.0 mg of mitochondrial protein were then incubated for 0.25 to 2.0 min at 28° in the same medium containing 10 mM unlabeled acetylCarn. The mitochondria were trapped on Millipore filters as described above and washed twice with iced incubation medium containing mersalyl and the respiratory inhibitors. The filters were placed in LSC and counted.

Mitochondrial Carn/acetylCarn exchange was

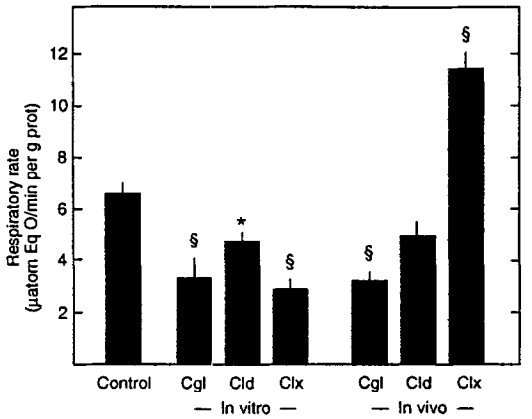


Fig. 1. Effects of cephalosporins on butyrate-mediated respiration by rabbit renal cortical mitochondria. *In vitro* toxicity: mitochondria were isolated from untreated animals and studied in the presence of 2000 µg/mL of cephaloglycin (Cgl), cephaloridine (Cld), cephalexin (Clx), or an equivalent volume of their vehicles (control). *In vitro* toxicity: animals were infused with 300 mg/kg of the cephalosporins or their vehicles (control) 1 hr before being killed, and subsequently isolated mitochondria were studied *in vitro*. Respiration (µatom equivalents oxygen consumed per minute per gram protein) was measured in mitochondria incubated with 5 mM butyrate. Values are means ± SEM; N = 12 control and 6–10 experimental measurements for each comparison. Significance level by ANOVA comparing experimental with control mitochondria: (*) >95%; and (§) >99.9%.

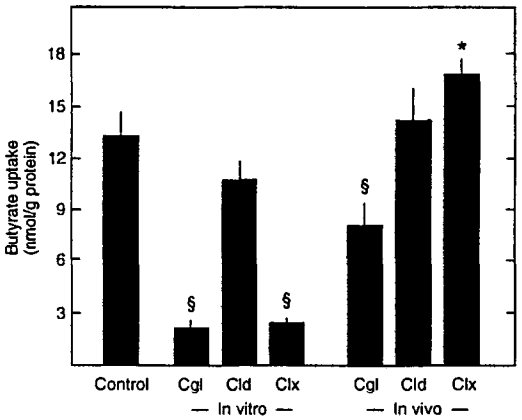


Fig. 2. Effects of cephalosporins on the uptake of butyrate by rabbit renal cortical mitochondria. Protocols for *in vivo* and *in vitro* exposure were the same as described for Fig. 1. Butyrate uptake was measured after 0.25 min of incubation in a medium containing trace quantities of [^{14}C]butyrate (2 µM). Values are means ± SEM; N = 12 control and 6–8 experimental measurements for each comparison. Significance level by ANOVA comparing experimental with control mitochondria: (*) >95%; and (§) >99.9%.

calculated as the percent of [^{3}H]carnitine leaving through the antiport (counts lost from samples incubated without mersalyl minus those lost in the presence of mersalyl). The *in vitro* effects of Cgl on the antiport were determined by adding the cephalosporin, or its vehicle, to the incubation medium immediately before the acetylCarn. The *in vivo* effects were measured in mitochondria harvested 1 hr after intravenous administration of the cephalosporin, or its vehicle.

In situ *renal distribution and excretion of carnitine and acylcarnitines*

Concentrations of total Carns, free Carn, and long-chain acylCarns were measured using a radioenzymatic method described by de Sousa *et al.* [23] in homogenates of renal cortex, cortical mitochondria, plasma, and urine of rabbits infused with 300 mg/kg of Cgl 1.25 hr before sampling. Urine samples were obtained at the time of killing from the urinary bladder, which had been emptied 1 hr after antibiotic infusion. Preliminary tests, in which Cgl was added to control specimens at concentrations known to occur under these conditions (300–6000 µg/mL), ruled out a methodological artifact caused by the presence of the antibiotic in the samples.

Creatinine concentrations were measured in plasma and urine samples using a Beckman Creatinine Autoanalyzer (Beckman Instruments Inc., Mountain View, CA), and fractional excretions were calculated as follows:

$$FE_x = (U_x/P_x)/(U_{Cr}/P_{Cr})$$

where FE = fractional excretion, U = urine concentration, and P = plasma concentration of the Carn fractions (x) or creatinine (Cr). As noted for the radioenzymatic Carn assay, Cgl did not influence measurements of creatinine concentrations in these samples.

Analytical

All data are presented as means ± SEM. Statistical comparisons were made using Student's *t*-test where one experimental group was compared with its control, or by an ANOVA where multiple comparisons were made. Differences were judged to be significant where P values were <0.05 (*t*-test) or confidence levels were >95% (ANOVA).

RESULTS

Respiration with butyrate

Respiration with butyrate was examined after *in vitro* and *in vivo* exposure to Cgl, Cld, and Clx (Fig. 1). At 2000 µg/mL, *in vitro* Cgl and Clx caused a 45–50% reduction of respiration, while Cld reduced respiration by 30%. The IC_{50} of Cgl was 2396 ± 196 and that of Cld was 4353 ± 260 µg/mL ($P < 0.001$). *In vivo* Cgl reduced respiration with butyrate by 50%; the apparent mild respiratory toxicity of *in vivo* Cld was not statistically significant, and *in vivo* Clx increased respiration by 70–75%.

Uptake of butyrate

The uptake of butyrate was similarly examined after *in vitro* and *in vivo* exposure to the three

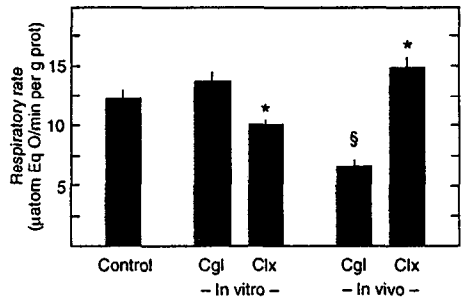


Fig. 3. Effects of cephaloglycin (Cgl) and cephalexin (Clx) on palmitoylcarnitine-mediated respiration by rabbit renal cortical mitochondria. Protocols for *in vivo* and *in vitro* exposure were the same as described for Fig. 1. Respiration (µatom equivalents oxygen consumed per minute per gram protein) was measured in mitochondria incubated with 30 µM PCarn. Values are means ± SEM; N = 12 control and 6–8 experimental measurements for each comparison. Significance level by ANOVA comparing experimental with control mitochondria: (*) >95%; and (§) >99.9%.

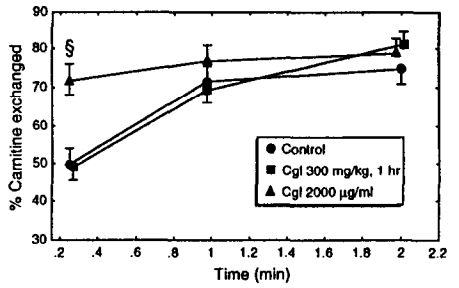


Fig. 4. *In vitro* effects of cephaloglycin (Cgl) on acetylcarnitine/carnitine exchange by rabbit renal cortical mitochondria. Protocols for *in vivo* (■) and *in vitro* (▲) exposure were the same as described for Fig. 1. Mitochondria were preloaded with [^{3}H]carnitine, and the carrier-mediated loss of radioactivity was measured at the indicated times after the addition of 10 mM added acetylcarnitine. Values are means ± SEM; N = 6 each. Significance levels by ANOVA comparing experimental with control mitochondria at each time: (§) >99.9%.

cephalosporins. Uptake in control and cephalosporin-exposed samples after 1 and 2 min of incubation with [^{14}C]butyrate was comparable to corresponding uptake at 0.25 min. Therefore, only the 0.25-min levels are presented (Fig. 2). The effects of the three cephalosporins on butyrate uptake paralleled their effects on respiration with butyrate. *In vitro* Cgl and Clx (2000 µg/mL) caused a 90% reduction of uptake, while *in vitro* Cld had no significant effect. *In vivo* Cgl reduced butyrate uptake by 40%; *in vivo* Cld had no significant effect; and *in vivo* Clx increased uptake by 28%. Nonisotopic butyrate reduced [^{14}C]butyrate uptake by 95%, to 0.57 ± 0.14 nmol/g protein (significance level >99.9%).

Respiration with palmitoylcarnitine

In vitro, 2000 µg/mL (Fig. 3) of Cgl had no toxicity and Clx caused a 15–20% reduction of PCarn-mediated respiration. The IC_{50} value of Cld was 5106 ± 379 µg/mL, while no inhibition of respiration with PCarn was seen with Cgl concentrations as high as 7000 µg/mL. *In vivo* Cgl caused a 45–50% reduction of respiration, while Clx once again stimulated respiration, by 22%. In essentially identical protocols [15], Cld reduced PCarn-mediated respiration *in vitro* by 30–45% and reduced respiration to the same degree as Cgl after *in vivo* exposure.

Carnitine–acylcarnitine antiport

The effects of *in vitro* and *in vivo* Cgl on the Carn carrier are shown in Fig. 4. *In vitro* exposure caused increased antiport in the earliest (15 sec) period of study, but showed no effect in 1- and 2-min measurements. *In vivo* exposure had no effect on the Carn carrier.

In situ *renal distribution and excretion of carnitine and acylcarnitines*

Whole cortical and mitochondrial content. In vivo Cgl (Fig. 5) had no significant effect on mitochondrial

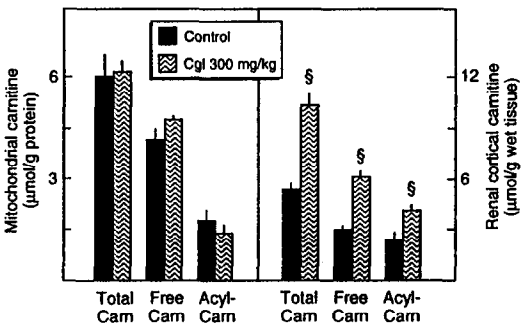


Fig. 5. Effects of *in vivo* cephaloglycin on total, free, and long-chain acylcarnitines (Carn) in renal cortical mitochondria and whole renal cortex. Rabbits were infused with 300 mg/kg of cephaloglycin (Cgl) 1.25 hr before being killed. Concentrations of total, free, and acylcarnitine were measured by radioenzymatic assay. Values are means ± SEM; N = 9 each. Key: (§) $P < 0.001$ (Student's *t*-test).

concentrations of free Carn or long-chain acylCarns, but increased whole cortical concentrations of both fractions by 100 and 60%, respectively.

Renal excretion. In vivo Cgl increased urinary losses of free Carn (Fig. 6), but this effect was only 10–20% of that caused by Cld [15]. Corresponding plasma concentrations, although slightly lower, were not affected significantly.

DISCUSSION

Two properties of the cephalosporin antibiotics have been implicated previously in their widely differing potentials for producing acute renal tubular necrosis: (1) secretory uptake by the proximal tubular cell [4, 24], and (2) reactivity of the beta-

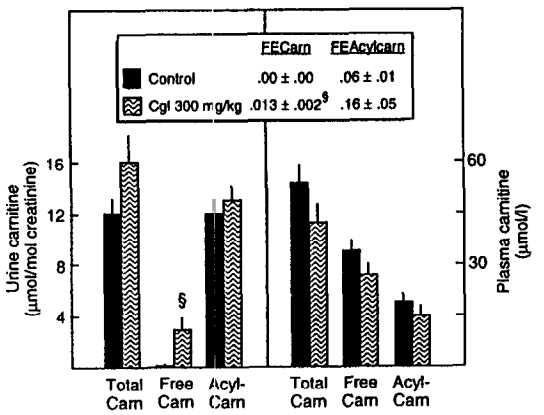


Fig. 6. Effects of *in vivo* cephaloglycin on total, free, and long-chain acylcarnitines (Carn) in plasma and urine, and on the fractional renal excretions of free and acylcarnitines. Rabbits were infused with 300 mg/kg of cephaloglycin (Cgl) 1.25 hr before being killed. The urinary bladder was emptied 1 hr after administration of the antibiotic; urine and plasma were obtained at the time of killing. Concentrations of total, free, and acylcarnitine were measured by radioenzymatic assay. Values are means ± SEM; N = 9 each. Key: (§) $P < 0.001$ (Student's *t*-test).

lactam ring, leading to acylation of intracellular target proteins [25]. The different cephalosporins, and other potentially nephrotoxic beta-lactam antibiotics, vary significantly in their expressions of these two properties, neither of which appears sufficient by itself for the production of tubular necrosis [26]. Cld has the additional effect, unique among the nephrotoxic beta-lactams [9], of causing oxidative injury, which also contributes to its toxicity [13, 27].

Of the cephalosporins used in the present study, single-dose ND_{50} values in the rabbit are 60 mg/kg for Cgl, 100 mg/kg for Cld, and >1000 mg/kg for Clx [1]. In the same species, after 100 mg/kg dosage, peak renal cortical concentrations (2600 μg/g wet tissue) and AUCs (5000 μg·hr/g) of Cld are 2.5 and 5 times, respectively, of those of Cgl, which are approximately the same as those of Clx [12]. Cgl and, to a lesser extent, Cld are among the more reactive cephalosporins, while Clx has extremely low reactivity [28, 29]. Other highly reactive but less toxic cephalosporins have significantly lower renal cortical AUCs [26].

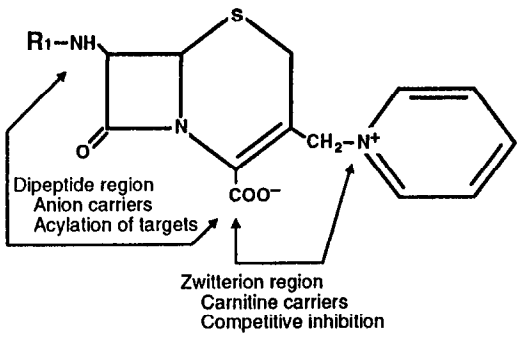
The tubular cell mitochondrial transporters of anionic respiratory substrates have been identified as targets of acylation by the nephrotoxic beta-lactams [7–9] with a probable pathogenic role in the production of tubular cell necrosis [30]. Studies with Cld, Cgl, and imipenem have shown [4, 6–9, 30, 31]: (1) immediately after *in vitro* exposure, reductions in the uptake and oxidative metabolism of various TCA-cycle substrates, with reversal of toxicity by washing or by increasing substrate concentrations; and (2) after 1–2 hr of *in vitro* or *in vivo* exposure, irreversible toxicity to the same functions. Clx has similar *in vitro* toxicity; after *in vivo* exposure, however, Clx produces little or no reduction of TCA substrate transport or metabolism [4, 8, 9, 30]. These observations have led to the hypothesis that various cephalosporins may reversibly inhibit anionic substrate transport, but those that produce tubular cell necrosis in the *in situ* kidney have the additional properties of higher tubular cell concentrations and greater reactivity to acylate the mitochondrial carriers of these substrates [1].

The present studies examined the effects of the toxic cephalosporins on the mitochondrial transport and oxidation of butyrate, a short-chain fatty anion that enters the mitochondrion independent of the Carn carrier [20]. For comparison to the previously demonstrated toxicity of Cld to long-chain FA metabolism [15], studies were also done to examine the effects of Cgl on the following: respiration with the acylCarn conjugate of the long-chain FA palmitic acid, mitochondrial Carn/acylCarn antiport, and the *in situ* renal mitochondrial and cortical content and the renal excretion of free Carn and long-chain acylCarns. Clx was used as a nontoxic control in studies of butyrate- and PCarn-mediated respiration.

In studies with butyrate, a monocarboxylate, Cgl (Figs. 1 and 2) had the same combination of *in vitro* and *in vivo* toxicity to respiration and uptake previously found with the dicarboxylic and tricarboxylic acid-cycle substrates and precursors [1]. Cld was less toxic than Cgl *in vitro*, and was not significantly toxic *in vivo*, to the uptake or oxidation of butyrate (Figs. 1 and 2). In an identical treatment regimen [30], Cld caused no *in vivo* inhibition of respiration with pyruvate, also a monocarboxylate. It is possible that the cationic charge of Cld hinders its access to the monocarboxylate carrier(s), allowing limited, reversible inhibition of transport but not acylation. While the 2.5-fold higher cortical concentrations of Cld compared with Cgl could result in significant inhibition of butyrate oxidation by Cld (IC_{50} = 4400 μg/mL, compared with 2400 for Cgl), it is not clear that this transient effect would be as cytotoxic in the *in situ* kidney as the irreversible attack of Cgl on butyrate transport.

In studies of PCarn-stimulated respiration, Cgl caused no *in vitro* inhibition but had significant *in vivo* toxicity (Fig. 3). In contrast, Cld is toxic to respiration with PCarn by both routes of exposure [15]. The difference between the *in vitro* toxicities of the two cephalosporins is understandable when one considers that the Carn carrier transports a series of zwitterionic fatty acylCarn conjugates. Because of its close structural homology with Carn, Cld is a potent inhibitor of the Carn carrier [15], whereas Cgl is not (Fig. 4). However, because the region of the Cld molecule that is homologous with Carn does not include its reactive beta-lactam ring (Fig. 7), the Cld–Carn competition is reversible [15]. As a result, Cld's inhibition of the Carn carrier, seen during *in vitro* exposure of untreated cortical mitochondria and reflected in its effects on mitochondrial Carn transport in the *in situ* kidney, is not expressed as reductions of Carn or acylCarn transport in mitochondria isolated for study after *in vivo* exposure [15].

Measurements of *in situ* concentrations of free Carn and acylCarns, therefore, also showed very



Cephaloridine

Carnitine

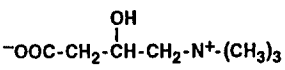


Fig. 7. Molecular structure of cephaloridine, showing the protein-acylating dipeptide region implicated in its irreversible attack on mitochondrial anionic substrate transporters and the zwitterionic region responsible for its competitive inhibition of carnitine transport. Carnitine is also shown for comparison.

different effects of Cgl compared with Cld. The inhibition of *in situ* Carn transport by Cld was reflected in a 100% elevation of mitochondrial free Carn [15], while Cgl had no such effect (Fig. 5). Although Cgl caused small increases of excretion of free Carn (Fig. 6), as occurs in a variety of disorders of FA metabolism [32, 33], Cld causes 5- to 10-fold greater excretion of both Carn fractions [15]. The elevated *in situ* cortical concentrations of free Carn and acylCarns after Cgl (Fig. 5), possibly derived from the circulating plasma pool (Fig. 6), might represent increased uptake in response to a generalized reduction of tubular cell oxidative metabolism. The absence of similar *in situ* cortical elevations of the Carn fractions in Cld-treated rabbits could have resulted from the very large urinary losses caused by Cld [15].

The stimulation of respiration with PCarn (Fig. 3) and the transport and oxidation of butyrate (Figs. 1 and 2) by prior *in vivo* exposure to Clx have precedent in other inhibitors of mitochondrial transport. Preincubation with monocarboxylate transport inhibitors stimulates the mitochondrial uptake of pyruvate through an increased carrier affinity [34, 35], and *in vivo* exposure to Cld augments Carn/acetylCarn exchange in subsequently isolated mitochondria [15]. In contrast, the irreversible attack of Cgl on its target carriers [26] is reflected in significant reductions of both butyrate (Fig. 2) and succinate [8] transport after *in vivo* exposure.

Any explanation of the inhibition after *in vivo* exposure of PCarn-mediated respiration by either Cgl (Fig. 3) or Cld [15] must, therefore, involve a mechanism other than an attack on the Carn carrier. One possibility is a reduction of beta-oxidation, caused by a depletion of TCA-cycle intermediates [1]. Such a process might explain the 1- to 1.5-hr delay after exposure to Cld before ATP depletion begins [36–38]. Anthony *et al.* [39] have measured elevated urine and plasma hydroxybutyrate, acetoacetate and lactate in the 8 hr following Cld administration to rats, consistent with a reduction of renal oxidative metabolism.

There is preliminary evidence that this process is sufficiently advanced under the conditions of the present studies to affect the oxidation of long-chain FAs, even where the Carn transporter is spared. Although *in vivo* Cgl decreased PCarn-mediated respiration (Fig. 3), an identical treatment protocol increased net mitochondrial PCarn uptake [1]. The ^{14}C-label used in the study of uptake, in the palmitoyl carboxyl, could have been retained in the Cgl-intoxicated mitochondria as a result of reduced beta-oxidation, while departing more rapidly from control mitochondria as $^{14}CO_2$.*

The greater concentrative uptake and lesser tubular cell toxicity of Cld compared with Cgl reflects a qualitative or quantitative difference in their molecular toxicities. The oxidative potential of Cld does not provide an explanation for this difference, because Cgl, which causes little or no oxidative injury, is more toxic. Cgl's 2-fold better fit, reflected by its 50% lower IC_{50} values against mitochondrial succinate [40] and butyrate (above) oxidation, falls short of accounting for the 8-fold difference expected from its lower ND_{50} and cortical AUC compared with Cld. The important remaining variable(s) in the production of irreversible injury could be the greater intrinsic reactivity [29, 41] or greater ability of Cgl to acylate mitochondrial anion transporters because it is an anion, whereas Cld is a zwitterion.

The timing and severity of toxicity to mitochondrial substrate uptake and oxidation, and subsequently to ATP production, fit the model of cytotoxicity through injury to respiration with TCA-cycle substrates and short-chain FAs like butyrate. Both substrate groups are important for proximal tubular respiration [16, 17, 42, 43]. The absence of toxicity of Cgl to long-chain FA transport might be taken as evidence against a pathogenic role of mitochondrial injury in cephalosporin toxicity. However, the importance of long-chain FAs as substrates of proximal tubular respiration is uncertain [19]. Moreover, the comparable *in vivo* toxicities of Cgl (Fig. 3) and Cld [15] to PCarn-mediated respiration, and the absence of toxicity of Clx to PCarn oxidation after identical exposure (Fig. 3), indicate equivalent *in situ* injury to long-chain FA oxidation by both toxic cephalosporins.

It is tempting to speculate that the protection against cephalosporin nephrotoxicity by doses of nontoxic beta-lactams insufficient to inhibit the tubular cell uptake of Cld or Cgl [44] may be mediated by a salutary effect on substrate carriers, like the stimulation of butyrate uptake and oxidation (Figs. 1 and 2) and PCarn-mediated respiration (Fig. 3) caused by pretreatment with Clx. By the same reasoning, the stimulation of Carn/acylCarn

* No inhibitors of metabolism other than Cgl were used in that study.

exchange seen after Cld pretreatment [15] could limit the *in situ* toxicity of Cld if its cortical clearance precedes the development of irreversible injury to the tubular cell.

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

REFERENCES

1. Tune BM, The nephrotoxicity of beta-lactam antibiotics. In: *Toxicology of the Kidney* (Eds. Hook JB and Goldstein RS), pp. 257–281. Raven Press, New York, 1993.
2. 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.
3. Tune BM and Fernholt M, Relationship between cephaloridine and *p*-aminohippurate transport in the kidney. *Am J Physiol* **225**: 1114–1117, 1973.
4. Tune BM and Fravert D, Cephalosporin nephrotoxicity. Transport, cytotoxicity and mitochondrial toxicity of cephaloglycin. *J Pharmacol Exp Ther* **215**: 186–190, 1980.
5. 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.
6. Tune BM, Wu KY, 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, Thienamycin nephrotoxicity: Mitochondrial injury and oxidative effects of imipenem in the rabbit kidney. *Biochem Pharmacol* **38**: 3779–3783, 1989.
8. 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.
9. Tune B, 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.
10. Hoover JRE, Beta-lactam antibiotics: Structure–activity relationships. In: *Handbook of Experimental Pharmacology* (Eds. Demain AL and Solomon NA), Vol. 67/II, pp. 119-245. Springer, Berlin, 1983.
11. Tune BM, Fernholt M and Schwartz A, Mechanism of cephaloridine transport in the kidney. *J Pharmacol Exp Ther* **191**: 311–317, 1974.
12. Tune BM, The nephrotoxicity of cephalosporin antibiotics. Structure–activity relationships. *Comments Toxicol* **1**: 145–170, 1986.
13. 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.
14. Cojocel C, Hannemann J and Baumann K, Cephaloridine-induced lipid peroxidation by reactive oxygen species as a possible mechanism of cephaloridine nephrotoxicity. *Biochim Biophys Acta* **834**: 402–410, 1985.
15. Tune BM and Hsu C-Y, Toxicity of cephaloridine to carnitine transport and fatty acid metabolism in rabbit renal cortical mitochondria: Structure–activity relationships. *J Pharmacol Exp Ther* **270**: 873–880, 1994.
16. Klein KL, Wang M-S, Torikai S, Davidson WD and Kurokawa K, Substrate oxidation by isolated single nephron segments. *Kidney Int* **20**: 29–35, 1981.
17. Jung KY, Uchida S and Endou H, Nephrotoxicity assessment by measuring cellular ATP content. I. Substrate specificities in the maintenance of ATP content in isolated nephron segments. *Toxicol Appl Pharmacol* **100**: 369–382, 1989.
18. Weidemann MJ and Krebs HA, The fuel of respiration in rat renal cortex. *Biochem J* **112**: 149–166, 1969.
19. Goldstein RS, Biochemical heterogeneity and site-specific tubular injury. In: *Toxicology of the Kidney* (Eds. Hook JB and Goldstein RS), 2nd Edn, pp. 201–247. Raven Press, New York, 1993.
20. Tzagoloff A, Oxidative pathways of mitochondria. In: *Mitochondria* pp. 39–60. Plenum Press, New York, 1982.
21. Pande SV and Parvin R, Characterization of carnitine acylcarnitine translocase system of heart mitochondria. *J Biol Chem* **251**: 6683–6691, 1976.
22. Pande SV, The mitochondrial carnitine acylcarnitine translocase system. *Proc Natl Acad Sci USA* **72**: 883–887, 1975.
23. de Sousa C, English NR, Stacey TE and Chalmers RA, Measurement of L-carnitine and acylcarnitines in body fluids and tissues in children and adults. *Clin Chim Acta* **187**: 317–329, 1990.
24. Tune BM, Effect of organic acid transport inhibitors on renal cortical uptake and proximal tubular toxicity of cephaloridine. *J Pharmacol Exp Ther* **181**: 250–256, 1972.
25. Browning MC and Tune BM, The reactivity and binding of beta-lactam antibiotics in rabbit renal cortex. *J Pharmacol Exp Ther* **226**: 640–644, 1983.
26. Tune BM, The renal tubular transport and nephrotoxicity of beta-lactam antibiotics. In: *Renal Disposition and Nephrotoxicity of Xenobiotics* (Eds. Anders MW, DeKant W, Henschler D, Oberleithner H and Silbernagl S), pp. 249–267. Academic Press, Orlando, 1993.
27. Cojocel C, Lacschke KH, Inselmann G and Baumann K, Inhibition of cephaloridine-induced lipid peroxidation. *Toxicology* **35**: 295–305, 1985.
28. Yamana T, Tsuji A, Kanayama K and Nakano O, Comparative stabilities of cephalosporins in aqueous solution. *J Antibiot (Tokyo)* **27**: 1000–1002, 1974.
29. 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.
30. Tune BM and Hsu C-Y, The renal mitochondrial toxicity of cephalosporins: Specificity of the effect on anionic substrate uptake. *J Pharmacol Exp Ther* **252**: 65–69, 1990.
31. Tune BM and Hsu C-Y, The renal mitochondrial toxicity of beta-lactam antibiotics: *In vitro* effects of cephaloglycin and imipenem. *J Am Soc Nephrol* **1**: 815–821, 1990.
32. Ohtani Y, Nishiyama S and Matsuda I, Renal handling of free and acylcarnitine in secondary carnitine deficiency. *Neurology* **34**: 977–979, 1984.
33. Stanley CA, Berry GT, Bennett MJ, Willi SM, Treem WR and Hale DE, Renal handling of carnitine in secondary carnitine deficiency disorders. *Pediatr Res* **34**: 89–97, 1992.
34. Paradies G and Papa S, The kinetics and substrate specificity of the pyruvate translocator in rat liver mitochondria. *Biochim Biophys Acta* **462**: 333–346, 1977.
35. Paradies G and Papa S, Substrate regulation of the pyruvate-transporting system in rat liver mitochondria. *FEBS Lett* **62**: 318–321, 1976.
36. Goldstein RS, Contardi LR, Pasino DA and Hook JB, Mechanisms mediating cephaloridine inhibition of gluconeogenesis. *Toxicol Appl Pharmacol* **87**: 297–305, 1987.

37. Rush GF and Ponsler GD, Cephaloridine-induced biochemical changes and cytotoxicity in suspensions of rabbit isolated proximal tubules. *Toxicol Appl Pharmacol* **109**: 314–326, 1991.
38. Rush GF, Heim RA, Ponsler RA and Engelhardt J, Cephaloridine-induced renal pathological and biochemical changes in female rabbits and isolated proximal tubules in suspension. *Toxicol Pathol* **20**: 155–168, 1992.
39. Anthony ML, Gartland KPR, Beddell CR, Lindon JC and Nicholson JK, Cephaloridine-induced nephrotoxicity in the Fischer 344 rat: Proton NMR spectroscopic studies of urine and plasma in relation to conventional clinical chemical and histopathological assessments of nephronal damage. *Arch Toxicol* **66**: 525–537, 1992.
40. Tune BM and Fravert D, Mechanisms of cephalosporin nephrotoxicity. A comparison of cephaloridine and cephaloglycin. *Kidney Int* **18**: 591–600, 1980.
41. Yamana T and Tsuji A, Comparative stability of cephalosporins in aqueous solution: Kinetics and mechanisms of degradation. *J Pharm Sci* **65**: 1563–1574, 1976.
42. Balaban RS and Mandel LJ, Metabolic substrate utilization by rabbit proximal tubule. An NADH fluorescence study. *Am J Physiol* **254**: F407–F416, 1988.
43. Harris SI, Balaban RS, Barrett L and Mandel LJ, Mitochondrial respiratory capacity and Na^+- and K^+-dependent adenosine triphosphatase-mediated ion transport in the intact renal cell. *J Biol Chem* **256**: 10319–10328, 1981.
44. Tune BM, Browning MC, Hsu C-Y and Fravert D, Prevention of cephalosporin nephrotoxicity by other cephalosporins and by penicillins without significant inhibition of renal cortical uptake. *J Infect Dis* **145**: 174–180, 1982.