Pergamon

Biochemical Pharmacology, Vol. 50, No. 4, pp. 562–564, 1995.

0006-2952/95 $9.50 + 0.00

0006-2952(95)00169-7

Short Communication

EFFECTS OF L-CARNITINE ON THE RENAL TUBULAR TRANSPORT OF CEPHALORIDINE

BRUCE M. TUNE*

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

(*Received* 20 *January* 1995; *accepted* 13 *March* 1995)

Abstract—It has been demonstrated recently that cephaloridine (Cld) inhibits the tubular reabsorption of filtered carnitine (Carn) in the rabbit kidney. This interaction has suggested that the limited net cell-to-luminal fluid movement of Cld following its secretory transport across the antiluminal membrane might result from a balance of active Cld reabsorption by a Carn carrier at the brush border approximately equal to its secretion into the tubular fluid, rather than the previously proposed luminal membrane block. Studies were done to determine the effects of L-Carn, 750 mg/kg, i.v. on the tubular secretion and cortical concentrations of Cld, infused i.v. at a dose of 28 mg/kg (Carn : Cld molar ratio = 70 : 1). The fractional excretions of Cld during three consecutive periods of 10 min each, one before and two following the bolus infusion of Carn, were (means ± SEM): 1.18 ± 0.14, 1.20 ± 0.14, and 1.16 ± 0.11, respectively (N = 6 each; differences NS). Cortex-to-serum concentration ratios of Cld in control and Carn-treated rabbits were 10.43 ± 0.32 and 10.16 ± 0.86, respectively (NS). The data provide evidence against the reabsorptive transport of Cld by a Carn carrier, and do not support a model of balanced secretion and reabsorption as the cause of limited clearance despite significant secretory transport of Cld into the tubular cell.

Key words: carnitine; cephaloridine; kidney; nephrotoxicity; transport

Cld†, a nephrotoxic cephalosporin, has two properties not found with other cephalosporins: (1) although rapidly transported into the proximal tubular cell at the antiluminal side [1], it undergoes limited net secretion into the luminal fluid [2] and, therefore, reaches uniquely high intracellular concentrations [2–4]; and (2) it inhibits the renal tubular reabsorption of Carn and acylCarns [5]. Both of these properties are attributed to the zwitterionic charge of Cld, resulting from the pyridinium ring in its C-3 substituent side-group.

The recognition of a competitive interaction between Cld and Carn for tubular cell Carn carriers raised the question of whether the limited net cell-to-tubular fluid movement of Cld might result from a balance of secretion and reabsorption rather than the luminal membrane block previously proposed [2, 5]. Therefore, studies were done to determine the effects of L-Carn on the renal tubular secretion and cortical concentrations of Cld.

Materials and Methods

Except where otherwise noted, reagents were purchased from the Sigma Chemical Co. (St. Louis, MO). Cld was supplied by Lilly Research Laboratories (Indianapolis, IN); inulin was obtained from the J. T. Baker Chemical Co. (Phillipsburg, NJ).

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. The animals were anesthetized with 45–60 mg/kg body wt of intraperitoneal pentobarbital (Abbott Laboratories, North Chicago, IL). An intravenous line was then inserted into an ear vein for infusions of Cld, inulin, and Carn; a femoral artery was cannulated for collection of blood; and the urinary bladder was catheterized for the timed collections of urine.

The animals were given a priming load of 20 mL of 0.9% saline, plus 200 mg inulin and 12 mg/kg Cld in 2.2 mL of 0.9% saline, followed by a 0.1 mL/min 1-hr infusion of saline containing 100 mg/mL inulin and 16 mg/kg of Cld. Thirty minutes after the start of the infusion, the bladder was emptied, and clearances of Cld and inulin [2] were measured using three consecutive 10-min urine collections; three 2-mL mid-collection blood samples were replaced with the same volumes of saline. FE Cld was calculated as the ratio of Cld clearance-to-inulin clearance for each period.

Immediately after the first urine collection (Period 1), 750 mg/kg of Carn in 10 mL of water was given i.v. over 2 min. After the second and third collections (Periods 2 and 3), the animals were killed by decapitation and their kidneys were removed for measurement of Cld and inulin concentrations in renal cortex and serum, and calculation of C/S ratios, as previously described [2]. Kidneys were also obtained from animals not infused with Carn for the measurement of control C/S ratios.

Results

Clearances and fraction excretion. Serum Cld concentrations (μg/mL) were (means ± SEM) 32 ± 3 during Period 1, 35 ± 3 during Period 2, and 36 ± 2 during Period 3, confirming the steady-state conditions of the study. The

* Correspondence: 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: Carn, carnitine; Cld, cephaloridine; C/S, cortex-to-serum; FE Cld, fractional excretion of Cld; and PAH, *p*-aminohippurate.

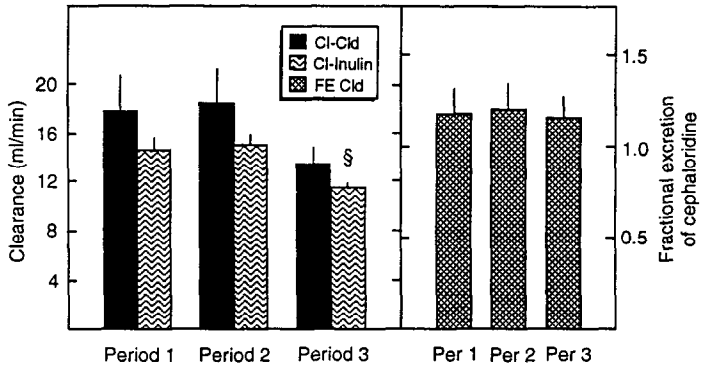

Fig. 1. Effects of carnitine on the renal transport and excretion of cephaloridine. Rabbits were given a loading dose followed by a constant infusion of cephaloridine (total 28 mg/kg over 1 hr) and inulin. After 30 min of equilibration, a single 10-min control collection was made (Per 1 = Period 1); 750 mg/kg of carnitine was infused over 2 min; and two additional 10-min collections (Periods 2 and 3) were made, starting with the beginning of the carnitine bolus. Cl = clearance, Cld = cephaloridine, In = inulin, and FE Cld = the fractional excretion of Cld, or the ratio of Cl-Cld to Cl-In. Values are means ± SEM, N = 6 each. Key: (§) Cl-In, Period 3 vs Period 2, significance level by ANOVA > 99%.

patterns of renal excretion of Cld and inulin before and after the administration of Carn are shown in Fig. 1. Cld and inulin clearances, and Fe Cld, were essentially the same before and in the 10 min following Carn infusion. Cld and inulin clearances were comparably decreased in the final collection period. Therefore, Fe Cld was not affected significantly.

Cortical concentrations. Neither the concentrations of Cld in serum nor the C/S ratios of Cld were affected significantly by Carn. C/S Cld was 10.43 ± 0.32 and C/S inulin was 1.40 ± 0.10 in control rabbits (N = 6). C/S Cld was 10.16 ± 0.86 and C/S inulin was 1.27 ± 0.03 in Carn-treated animals (N = 6).

Discussion

Cld, one of the most nephrotoxic of the cephalosporin antibiotics, produces a selective proximal tubular cell necrosis that can be prevented by probenecid and PAH [6–8]. Like most other cephalosporins [9], Cld is transported into the proximal tubular cell by a probenecid- and hippurate-inhibitable basolateral secretory carrier [1, 7]. However, unlike any other cephalosporin, Cld undergoes very little net secretion into the tubular fluid [2, 10], despite having uniquely high intracellular concentrations [2, 4].

In contrast to the simplified model of PAH transport across the proximal tubular cell schematized in Fig. 2A [11], the cellular uptake of Cld without rapid secretion into the tubular fluid has been attributed to a failure of movement across the luminal cell membrane (Fig. 2B) [2]. Several lines of evidence have been presented in support of this model: (1) unusually slow Cld efflux from *in vivo* renal cortex after secretory uptake is blocked by intravenous probenecid [2]; (2) absence of cortical binding of Cld [1]; (3) failure of cortical Cld concentrations to increase after ureteral obstruction, compared with concentrations of PAH and the more typically secreted cephalosporins cephaloglycin and cefaclor [2, 12]; (4) a uniquely high cortical, compared with serum, Cld half-life [4]; and (5) minimal inhibition of brush border organic anion transport by Cld compared with the effect of cephalothin, a rapidly secreted anionic cephalosporin [13].

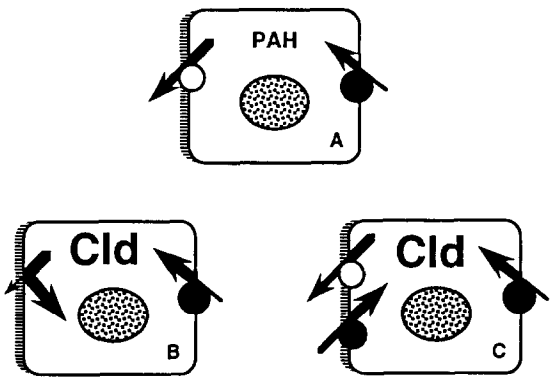

Fig. 2. Schematic representation comparing the transport of *p*-aminohippurate (PAH) across the proximal tubular cell (A), contrasted with two possible models of cephaloridine (Cld) transport: (B) active Cld transport into the cell at the antiluminal membrane with limited subsequent movement into the luminal fluid; and (C) similar antiluminal transport with comparable cell-to-luminal fluid secretory flux and simultaneous luminal-side reabsorption of Cld by a carnitine carrier. Both models could result in the previously measured high and prolonged intracellular concentrations of cephaloridine compared with PAH and rapidly secreted cephalosporins. The data in the present study fail to support the mechanism depicted in diagram (C).

The recent demonstration of two effects of Cld on Carn transport {competitive inhibition of cortical mitochondrial Carn uptake *in vitro* (Carn : Cld molar ratios = 5 : 1 to 1 : 1) and increased fractional renal excretion of endogenous Carn (serum Carn : Cld molar ratio ≈ 1 : 30, after 300 mg/kg of intravenous Cld*) [5]} suggested a possible alternative mechanism for the limited net movement of Cld from cell-

* The same dose of cephaloglycin, which is more toxic and more rapidly toxic than Cld [3], has little or no effect on the tubular reabsorption of Carn at the same time after administration [14]. Therefore, it is unlikely that the effect of Cld on Carn reabsorption is a result of tubular cell injury.

to-tubular fluid: simultaneous and nearly equal luminal-side secretion through an organic anion (or cation) carrier or channel and active reabsorption by a Carn carrier (Fig. 2C). The present study was designed to examine this possibility by measuring the effects in the rabbit of a large intravenous load of Carn on renal Cld excretion and cortical concentrations. Cld was infused in a quantity (28 mg/kg) well below that producing toxicity (100 mg/kg) [7] or saturation of its cortical uptake (≥200 mg/kg) [8, 15, 16]. Carn was given in a 750 mg/kg bolus (Carn:Cld molar ratio = 70:1).

The model depicted in Fig. 2C would predict three consequences of inhibition by Carn of Cld reabsorption at the luminal membrane: (1) an acute disequilibrium, with FE Cld above its new steady-state levels, resulting from the runout of Cld into the tubular fluid; followed by (2) a decline to the new steady-state FE Cld, higher than that of Period 1 and possibly as high as 4.0 (the fractional excretion of PAH [2]); and (3) a reduction of C/S Cld. The rate at which these events could evolve is predictable from the cortical transport pool of Cld in control rabbits and the rates of Cld and inulin clearance in Period 1. In the ~12 *g* of renal cortex in each rabbit, containing a mean of 376 μg/g of the cephalosporin, total cortical Cld averaged 4.5 mg (85–90% intracellular [2]). Between 88 and 95% of circulating Cld is filterable [2, 17]. Therefore, at a mean serum concentration of 32 μg/mL, between 1.2 and 1.6 mg of Cld was secreted during Period 1.

In the most extreme interpretation of the model of Cld transport depicted in Fig. 2C, antiluminal uptake and unidirectional cell-to-tubular fluid secretion of Cld would equal those of PAH, and Cld reabsorption would be totally inhibited by Carn. If this had occurred, a new steady state after the Carn bolus would have resulted in Cld secretion more than ten times that of Period 1 {(4 minus 0.88)/(1.18 minus 0.88)} and C/S Cld one-half of the control ratio [2]. Under those conditions, FE Cld and C/S Cld would have easily reached their new steady states during Period 2. If Cld secretion after the infusion of Carn had increased significantly, but not by enough to achieve a new steady state, FE Cld would still have increased during Periods 2 and 3, and C/S Cld decreased by the end of the study.

Instead, Carn had no significant effect on the secretion of Cld or on C/S Cld. The calculations for FE Cld and C/S Cld used total serum Cld concentrations, and would be slightly influenced by allowance for a maximum of 12% binding to plasma proteins [17]. However, measurements with and without Carn would be similarly influenced, and the conclusions unaffected. If Carn displaced all Cld bound to plasma proteins, increases of FE Cld and C/S Cld as high as 12% would have resulted. Had such displacement occurred, however, potentially obscuring a reduction of intracellular sequestration of Cld (C/S) after Carn infusion, it would have further augmented any simultaneously occurring increase of FE Cld.

The present data, therefore, fail to support any significant reabsorption of Cld by a luminal Carn carrier. Moreover, no other single effect of Carn on the transport of Cld by the tubular cell can explain the present results.

Acknowledgement—This work was sponsored by a grant from the National Institutes of Health (DK33814).

REFERENCES

1. Tune BM and Fernholt M, Relationship between cephaloridine and *p*-aminohippurate transport in the kidney. *Am J Physiol* **225**: 1114–1117, 1973.
2. Tune BM, Fernholt M and Schwartz A, Mechanism of cephaloridine transport in the kidney. *J Pharmacol Exp Ther* **191**: 311–317, 1974.
3. Tune BM and Fravert D, Cephalosporin nephrotoxicity. Transport, cytotoxicity and mitochondrial toxicity of cephaloglycin. *J Pharmacol Exp Ther* **215**: 186–190, 1980.
4. Tune BM, The nephrotoxicity of cephalosporin antibiotics. Structure–activity relationships. *Comments Toxicol* **1**: 145–170, 1986.
5. 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.
6. Child KJ and Dodds MG, Nephron transport and renal tubular effects of cephaloridine in animals. *Br J Pharmacol Chemother* **30**: 354–370, 1967.
7. 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.
8. Tune BM, Wu K-Y, Longerbeam DF and Kempson RL, Transport and toxicity of cephaloridine in the kidney. Effect of furosemide, *p*-aminohippurate and saline diuresis. *J Pharmacol Exp Ther* **202**: 472–478, 1977.
9. Brogard JM, Comte F and Pinget M, Pharmacokinetics of cephalosporin antibiotics. *Antibiot Chemother* **25**: 123–162, 1978.
10. Child KJ and Dodds MG, Mechanism of urinary excretion of cephaloridine and its effects on renal function in animals. *Br J Pharmacol Chemother* **26**: 108–119, 1966.
11. Tune BM, Burg MB and Patlak CS, Characteristics of *p*-aminohippurate transport in proximal renal tubules. *Am J Physiol* **217**: 1057–1063, 1969.
12. 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.
13. Williams PD, Hitchcock MJM and Hottendorf GH, Effect of cephalosporins on organic ion transport in renal membrane vesicles from rat and rabbit kidney cortex. *Res Commun Chem Pathol Pharmacol* **47**: 357–369, 1985.
14. Tune BM and Hsu C-Y, Toxicity of cephalosporins to fatty acid metabolism in rabbit renal cortical mitochondria. *Biochem Pharmacol* **49**: 727–734, 1995.
15. 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.
16. 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.
17. Welles JS, Gibson WR, Harris PN, Small RM and Anderson RC, Toxicity, distribution, and excretion of cephaloridine in laboratory animals. *Antimicrob Agents Chemother* **1965**: 863–869, 1966.