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Ceftriaxone Protects against Tobramycin Nephrotoxicity

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The effect of ceftriaxone on tobramycin-induced nephrotoxicity was investigated. Female Sprague-Dawley rats were treated during 4 and 10 days with saline (NaCl, 0.9%), ceftriaxone at a dose of 100 mg/kg of body weight/12 h subcutaneously, tobramycin at doses of 40 and 60 mg/kg/12 h intraperitoneally, or the combination ceftriaxone-tobramycin. Creatinine levels in serum were significantly higher in animals treated with tobramycin alone given at 60 mg/kg/12 h during 10 days, compared with control animals ($P < 0.01$) or animals receiving the combination tobramycin-ceftriaxone ($P < 0.01$). After 10 days of treatment, ceftriaxone did not accumulate in renal tissue but did reduce the renal intracortical accumulation of tobramycin ($P < 0.05$). Tobramycin given alone at either 40 or 60 mg/kg/12 h induced a significant inhibition of sphingomyelinase activity compared with control animals ($P < 0.05$). However, this enzyme activity was significantly less inhibited when tobramycin was injected in combination with ceftriaxone ($P < 0.05$). Ceftriaxone alone had no effect on the activity of this enzyme. The [^{3}H]thymidine incorporation into the DNA of renal cortex was also significantly lower in animals treated with tobramycin-ceftriaxone compared with animals receiving tobramycin alone ($P < 0.05$). The 24-h urinary excretion of β-galactosidase was significantly reduced in animals treated with the combination tobramycin-ceftriaxone compared with the administration of tobramycin alone at 40 and 60 mg/kg/12 h after 5 and 10 days ($P < 0.05$). Histologically, ceftriaxone induced very few cellular alterations and reduced considerably the presence of typical signs of tobramycin nephrotoxicity. This investigation demonstrates that ceftriaxone protects animals against tobramycin-induced nephrotoxicity.

Ceftriaxone is a broad-spectrum cephalosporin. As with all other β-lactams, the antibacterial activity of ceftriaxone is due to inhibition of mucopeptide synthesis in the cell wall (2, 28, 32). Ceftriaxone possesses a broad spectrum of antimicrobial activity including aerobic gram-positive and gram-negative bacteria and also a few anaerobic bacteria (2, 36, 41). The drug is widely used in the treatment of infections caused by microorganisms resistant to conventional therapy or as an alternative to antibiotics with a low therapeutic index. In combination with aminoglycosides, ceftriaxone has been shown to be additive or synergistic against several gram-negative pathogens (1, 45). The efficacy and safety of the combination ceftriaxone-aminoglycosides have already been evaluated in experimental models of infections (18, 20) as well as in patients (9, 25, 29, 34, 43).

Aminoglycosides are eliminated essentially by glomerular filtration and partially reabsorbed by proximal tubular cells by a mechanism of pinocytosis into small vesicles that fuse with lysosomes where they accumulate (3, 24, 42). They induce a lysosomal phospholipidosis characterized by the inhibition of sphingomyelinase and phospholipase A_1 activity and by phospholipid accumulation into lysosomes (30). This phospholipidosis is accompanied by cellular necrosis and postnecrotic cell regeneration (23, 31, 33).

Different compounds or drugs used concomitantly with aminoglycosides may either increase (vancomycin, cisplatinum, and hydrocortisone [6, 7, 16, 38, 48]) or decrease (ticarcillin, carbenicillin, cephalothin, latamoxef, daptomycin, and poly-L-aspartic acid [4–6, 10, 14, 17, 22, 27, 28, 47]) their toxicity. There is no direct correlation between the toxicity of aminoglycosides, whether used alone or in combination, and their renal concentrations. In fact, in combination with cisplatinum, daptomycin, poly-L-aspartic acid, and ticarcillin, renal aminoglycoside levels increased (4, 5, 16, 17, 22, 47), while they decreased with concomitant cephalothin, latamoxef, and vancomycin (14, 21, 27, 48). Some studies even report no influence of daptomycin, vancomycin, cephalothin, and carbenicillin on aminoglycoside cortical accumulation (6, 10, 38).

To our knowledge, there are no data on the influence of ceftriaxone on aminoglycoside-induced nephrotoxicity. Since ceftriaxone is often combined with an aminoglycoside and this combination was shown to be very effective as the first-line antibiotic combination in the treatment of several severe infections including those observed in febrile neutropenic patients (9, 29, 34), we have evaluated the nephrotoxic potential of this combination.

MATERIALS AND METHODS

Concentrations of ceftriaxone in serum, urine, and renal cortex. Thirty-six female Sprague-Dawley rats weighing between 175 and 200 g were used. Animals were given a single subcutaneous injection of ceftriaxone (100 mg/kg of body weight), and levels of ceftriaxone were measured in serum, urine, and renal cortex from 1 to 24 h following the injection. Rats were anesthetized with pentobarbital sodium (45 mg/kg) 75 min before sacrifice; 15 min later, the bladders were emptied by suprapubic puncture. At the time of sacrifice (i.e., 1, 2, 4, 6, 12, or 24 h after ceftriaxone injection), urine was collected from each rat as described above. Six animals per group were killed by decapitation, the blood was taken and

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centrifuged, and the serum was collected. Urine and serum were frozen for further antibiotic determination. For each animal, a midline abdominal incision was performed, and the right kidney was removed. Kidneys were bisected, and cortices were dissected. Pieces of cortex were weighed, homogenized in phosphate buffer (pH 7.4) at 0°C with a Tissue-Tearor, and then diluted to obtain a ceftriaxone concentration in the range of the standard curve. The standard curve was linear from 0.78 to 50 μg/ml, with a coefficient of correlation of 0.997. The limits of sensitivity of the microbiological assay were 0.78 μg/ml for serum and urine and 3.67 μg/g for renal tissue. The recovery of ceftriaxone in renal cortex homogenate was 80.0% ± 3.2%. The interday coefficients of variation of the assay were 12.68% at 3 μg/ml and 16.33% at 25 μg/ml. Standard solutions were prepared in normal serum for ceftriaxone assays in serum, in physiological saline for urine, and in normal cortex for renal tissue. Ceftriaxone concentrations were measured by a microbiological assay with *Escherichia coli* EC-596 (Laboratoire de Microbiologie, Centre Hospitalier de l'Université Laval) suspended in Trypticase soy agar-nutrient agar media. This strain of *E. coli* is sensitive to ceftriaxone and resistant to tobramycin.

Toxicity study: animals and treatment. Another group of 72 female Sprague-Dawley rats was treated for 4 and 10 days either with ceftriaxone at a dose of 100 mg/kg per 12 h subcutaneously, with tobramycin at doses of 40 and 60 mg/kg/12 h intraperitoneally, with the combination ceftriaxone-tobramycin at doses described above, or with saline (NaCl, 0.9%). Animals were injected at 0800 and 2000 h. Each animal was killed 15 h after the last injection. One hour before sacrifice, all animals received an intraperitoneal injection of [^{3}H]thymidine (200 μCi) for the measurement of cellular regeneration. Six animals per group were sacrificed by decapitation, the blood was collected, and both kidneys were rapidly removed and bisected. Blood was centrifuged, and the serum was frozen for measurement of creatinine levels. The renal cortex of each kidney was dissected. One part was cut into small blocks in a drop of 2% glutaraldehyde–0.1 M phosphate buffer and further processed for plastic section examination and electron microscopy. The remaining cortical tissue was quickly frozen for further determination of [^{3}H]thymidine/DNA ratio, tobramycin and ceftriaxone levels in tissue, and sphingomyelinase activity.

Antibiotic assays. Ceftriaxone was measured in renal cortex as described above. The renal cortical accumulation of tobramycin was measured by a fluorescence polarized immunoassay (TDX System; Abbott Laboratories) as recently described (6). The interday coefficients of variation were 3.6% at 1 μg/ml and 3.4% at 8 μg/ml for the tobramycin assay.

Biochemical analysis. The cellular regeneration was evaluated as described previously by Laurent et al. (31). Sphingomyelinase activity was assayed in the renal cortex as described by Laurent et al. (30). Creatinine levels were determined by using a Hitachi 737 analyzer with serum samples obtained at the time of sacrifice.

Enzymuria. Five animals of each group treated during 10 days were individually housed in metabolic cages and allowed 3 days to adapt. All animals had free access to food and water throughout the experiment. Twenty-four-hour urine collection was made on days 0, 5, and 10. Collected urine was centrifuged at 2,500 rpm (model G5-6R centrifuge; Beckman Instruments, Inc., Mississauga, Ontario, Canada) for 15 min. Urine diuresis was measured for each animal. β-Galactosidase, a lysosomal enzyme, was evaluated by the method described by Maruhn (35).

Histology. At the time of sacrifice, a portion of the renal cortex was cut in small blocks of 1 mm^3 and left overnight in a solution of 2% glutaraldehyde–0.1 M phosphate buffer at 4°C. After being washed with phosphate buffer (0.1 M, pH 7.4), cubes were fixed in 2% osmium tetroxide-1% sucrose at room temperature, dehydrated in ascending grade of alcohol, and embedded in Araldite 502 resin. Thick sections of the renal cortex were cut with an ultramicrotome (Ultracut E; Leica Canada Inc., Québec, Canada), stained with toluidine blue, and examined to identify gross lesions. Ultrathin sections stained with uranyl acetate and lead citrate were examined with a Philips 300 electron microscope at 60 kV.

Microscopic renal lesions were scored on plastic sections at a magnification of ×400. Each slide was coded so that identification of the groups was not possible for the observer (G.T.). Slices came from three different pieces of renal cortex for each rat, and four rats per group were used. The following lesions in the renal cortex were scored: isolated cell necrosis, proximal tubule with large lysosomes, abnormal proximal tubules (proximal tubule with more than 50% of necrotic cells), tubular desquamation (proximal tubule with 100% of necrotic cells), metachromatic materials in the tubular lumina, and the number of interstitial cells (no specification of cell type was made). The total number of proximal tubules was also measured on each slice. The number of isolated necrotic cells, the number of proximal tubules with large lysosomes, the number of abnormal proximal tubules, the number of desquamated tubules, and the number of proximal tubules with metachromatic materials in the tubular lumina were recorded as percentages of the total number of proximal tubules on each respective slice and assigned a score as follows: 0 to 9%, 1; 10 to 19%, 2; 20 to 29%, 3; etc. A score for the interstitial cells was obtained by dividing the total number of interstitial cells by the total number of proximal tubules on each respective slice. The lesion scores were summed to produce a single toxicity score for each animal.

Statistics. Statistical analysis of the difference between groups was performed by analysis of variance using a least-squares method. For a P value under 0.05, a group comparison was done by using the Fisher protected less significant difference test. A P value of less than 0.05 was considered significant.

TABLE 1. Ceftriaxone levels following a single subcutaneous injection of ceftriaxone (100 mg/kg)

Time (h)	Mean ± SD[a]		
	Serum (μg/ml)	Urine (μg/ml)	Cortex (μg/g of tissue)
1	111.61 ± 7.89	2,231 ± 1,269	71.92 ± 19.72
2	82.99 ± 11.85	1,068 ± 232	38.28 ± 3.05
4	8.08 ± 1.44	376 ± 148	5.41 ± 1.39
6	1.61 ± 0.65	49.0 ± 19.8	<3.67*
12	<0.78*	3.4 ± 1.3	<3.67*
24	<0.78*	<0.78*	<3.67*

[a] Six animals per group. *, lower than the limit of detection of the microbiological assay.

RESULTS

Concentrations of ceftriaxone in serum, urine, and renal cortex measured from 1 to 24 h after a single subcutaneous injection of 100 mg/kg are shown in Table 1. A peak concentration of 111.61 ± 7.89 μg of ceftriaxone per ml of serum was obtained only 1 h after the injection. Levels decreased with time, and ceftriaxone concentrations were under the limit of detection of the microbiological assay (0.78 μg/ml) after 12 h. The highest concentration of ceftriaxone in the urine was also

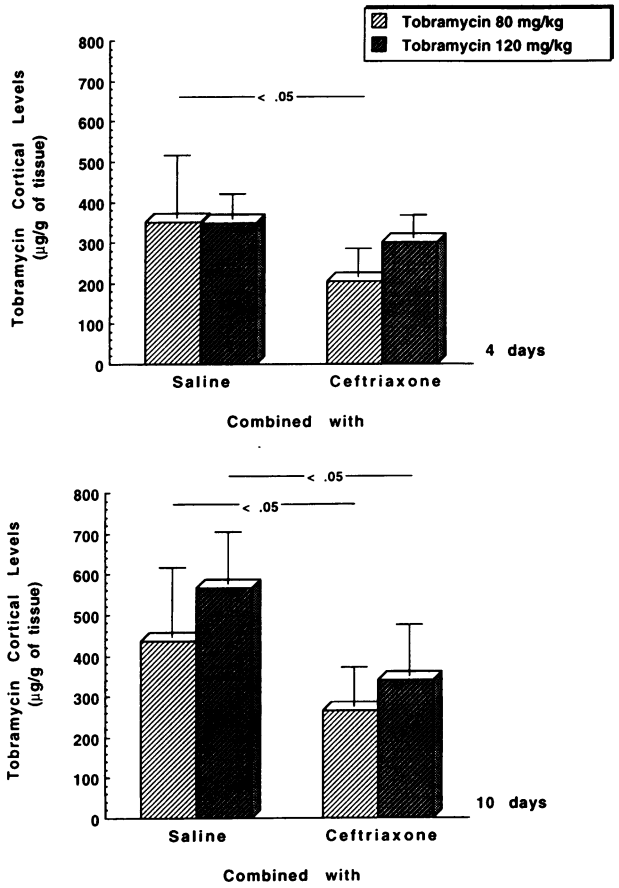

FIG. 1. Concentrations of tobramycin in the renal cortices of rats treated with tobramycin at doses of 80 and 120 mg/kg/day during 4 and 10 days. Tobramycin was administered in combination with saline (NaCl, 0.9%) or ceftriaxone (200 mg/kg/day). Means and standard deviations (bars) are given (n = 6).

observed 1 h after the injection (2,231 ± 1,269 μg/ml). Ceftriaxone was not detectable in urine 24 h after the injection. Ceftriaxone reached a peak level of 71.92 ± 19.72 μg/g of cortex 1 h after its administration. Ceftriaxone concentrations were under the range of detection in renal cortex after 6 h.

Fifteen hours after the end of 4 and 10 days of treatment, ceftriaxone (100 mg/kg/12 h) was undetectable in the renal cortices of animals treated with ceftriaxone alone or combined with tobramycin. No accumulation of ceftriaxone was observed in the renal tissues of any treated rats. The effect of ceftriaxone on tobramycin cortical accumulation is shown in Fig. 1. After 10 days of treatment, ceftriaxone significantly reduced the intracortical accumulation of tobramycin administered at 40 and 60 mg/kg/12 h ($P < 0.05$). After 4 days, the same effect was observed when ceftriaxone was given in combination with tobramycin at 40 mg/kg/12 h ($P < 0.05$).

Figure 2 shows creatinine levels in sera of animals of all groups after 10 days of treatment. There was no significant difference between animals treated during 4 days (data not shown). A significant rise in creatinine levels in sera of animals treated with tobramycin alone at a dose of 60 mg/kg/12 h compared with all other groups was observed ($P < 0.01$). However, serum creatinine levels were no different from control groups when tobramycin was given in combination with ceftriaxone, suggesting, once again, that the combination between these two drugs is less toxic than tobramycin given alone.

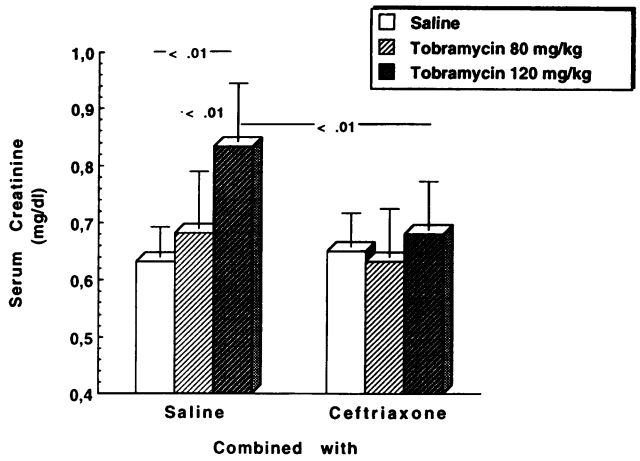

FIG. 2. Creatinine levels in sera of animals treated with saline (NaCl, 0.9%) or tobramycin at doses of 80 and 120 mg/kg/day during 4 and 10 days. Saline and tobramycin were administered in combination with saline (NaCl, 0.9%) or ceftriaxone (200 mg/kg/day). Means and standard deviations (bars) are given (n = 6).

Figure 3A shows the influence of ceftriaxone on tobramycin-induced inhibition of sphingomyelinase activity after 10 days of treatment. At day 10, tobramycin given at 40 and 60 mg/kg/12 h induced a significant inhibition of sphingomyelinase activity compared with the saline-treated group ($P < 0.01$). There was no significant difference between the group receiving tobramycin at a dose of 40 mg/kg/12 h and the group receiving 60 mg/kg/12 h. However, sphingomyelinase activity was significantly less inhibited when tobramycin (40 and 60 mg/kg/12 h) was given in combination with ceftriaxone compared with rats treated with tobramycin alone ($P < 0.05$ to 0.01). Ceftriaxone alone induced a small inhibition of the enzyme activity, which, however, was not significant. After 4 days of treatment, no significant inhibition of the enzyme activity was observed in any groups (data not shown).

Postnecrotic cellular regeneration measured by the incorporation of [^{3}H]thymidine into the DNA of renal cortex after 10 days of treatment is shown on Fig. 3B. Tobramycin given alone at 40 and 60 mg/kg/12 h induced 3.5- and 5-fold increases in [^{3}H]thymidine incorporation, respectively, compared with control groups ($P < 0.01$). By contrast, when ceftriaxone was used in combination with tobramycin, the [^{3}H]thymidine incorporation was similar to that for control animals, suggesting that ceftriaxone protects kidney cells against tobramycin toxicity. There was no significant difference between groups after 4 days of treatment.

Table 2 shows the 24-h urinary excretion of β-galactosidase for each treated group on days 0, 5, and 10 following the beginning of treatment. No significant difference in the excretion of this enzyme was observed between any groups on day 0. By contrast, β-galactosidase excretion was significantly higher on days 5 and 10 in the urine of animals treated with tobramycin alone at a dose of 60 mg/kg/12 h compared with animals given saline ($P < 0.05$). This increase in the urinary excretion of β-galactosidase was significantly smaller when ceftriaxone was given in combination with tobramycin (60 mg/kg/12 h). In fact, the urinary excretion of β-galactosidase showed no significant difference between control animals and animals treated with the combination ceftriaxone-tobramycin (60 mg/kg/12 h).

Optic and electron microscopy showed typical signs of

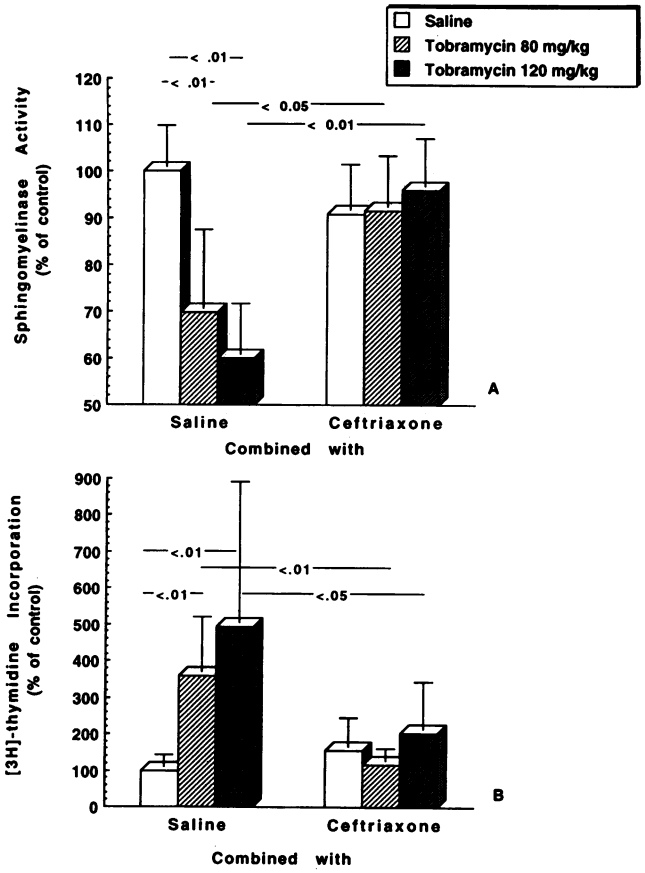

FIG. 3. Activity of sphingomyelinase (A) and [^{3}H]thymidine incorporation (B) in the renal cortices of animals treated with saline (NaCl, 0.9%) or tobramycin at doses of 80 and 120 mg/kg/day during 4 and 10 days. Saline and tobramycin were administered in combination with saline (NaCl, 0.9%) or ceftriaxone (200 mg/kg/day). The data are percentages of the value for the control group with standard deviations (bars) (n = 6).

aminoglycoside nephrotoxicity in the renal cortices of animals treated with tobramycin alone given at either 40 or 60 mg/kg/12 h. Large lysosomes containing myeloid bodies were present in renal cortices of rats treated with tobramycin at both doses. More severe lesions were observed when tobramycin was given at the highest dose. Ceftriaxone given alone had no significant effect on kidney ultrastructure. By contrast, signs of aminoglycoside nephrotoxicity were significantly less important when ceftriaxone was administered concomitantly with tobramycin.

The mean score for each renal lesion and the mean histopathologic nephrotoxicity score in the renal cortex for each group treated during 10 days are shown in Table 3. The scores for isolated cell necrosis were lower in the groups receiving saline (NaCl, 0.9%), ceftriaxone alone, or ceftriaxone in combination with tobramycin at 40 mg/kg/12 h. The highest score was measured in the group of animals receiving tobramycin alone at 40 mg/kg/12 h. The lower score in animals treated with tobramycin at 60 mg/kg/12 h is due to the very high toxicity as measured by the intensity of abnormal tubules in this group. Proximal tubules with large lysosomes were found only in groups treated with tobramycin. Although not significantly different, the number of proximal tubules with large lysosomes was lower when tobramycin was used in combination with ceftriaxone. The highest count of abnormal tubules was observed in rats treated with tobramycin at 60 mg/kg/12 h. By contrast, a lower score was measured in animals treated with the combination ceftriaxone-tobramycin (60 mg/kg/12 h) (P < 0.05). A similar effect was not observed when tobramycin was used at 40 mg/kg/12 h. The presence of tubular desquamation in the proximal tubule, which is a sign of acute tubular necrosis, was observed only in animals injected with tobramycin alone at 60 mg/kg/12 h. When ceftriaxone was administered with the same dose of tobramycin, no sign of tubular desquamation was observed (P < 0.05). Metachromatic material, which was composed of cellular debris and myeloid bodies in the lumina of proximal tubules, was observed only in tobramycin-treated animals. However, the frequency of the presence of metachromatic materials was significantly lower when ceftriaxone was added to tobramycin compared with animals given tobramycin alone (P < 0.05). No significant increase in the peritubular cell infiltration was observed in any group. The highest total mean nephrotoxicity scores were measured in animals treated with tobramycin administered at 40 and 60 mg/kg/12 h (P < 0.01). By contrast, when ceftriaxone was given concomitantly with tobramycin at either 40 and 60 mg/kg/12 h, these scores were significantly lower (P < 0.05).

TABLE 2. Urinary excretion of β-galactosidase

Treatment[a]	Mean (mU/24 h) ± SD on day[b]:		
	0	5	10
Saline	76.96 ± 18.71	86.50 ± 25.23	77.48 ± 30.23
Tobramycin			
40	81.98 ± 24.73	124.86 ± 25.26	127.32 ± 39.95
60	88.67 ± 27.56	145.40 ± 72.89†	168.93 ± 81.08†
Ceftriaxone	102.25 ± 19.99	76.82 ± 22.54	97.32 ± 29.86
Tobramycin + ceftriaxone			
40	88.95 ± 30.65	89.77 ± 40.84	116.05 ± 52.47
60	90.76 ± 26.05	93.90 ± 8.62‡	111.22 ± 16.34‡

[a] The numbers refer to milligrams of tobramycin per kilogram per 12 h.
[b] Five animals per group. † and ‡, significantly different from the corresponding value for saline or tobramycin at 60 mg/kg/12 h, respectively (P < 0.05).

DISCUSSION

The present investigation demonstrates that ceftriaxone protects proximal tubular cells against tobramycin-induced nephrotoxicity. In fact, tobramycin cortical levels were significantly lower; the creatinine levels in serum were identical to those of saline-treated animals; the inhibition of sphingomyelinase activity was significantly less severe; and the cellular regeneration, the urinary excretion of β-galactosidase, and the histopathologic lesions were significantly less affected in animals treated with the combination ceftriaxone-tobramycin compared with animals given tobramycin alone.

Significant nephrotoxicity has never been reported with any of the broad-spectrum cephalosporins (19). Our results show that ceftriaxone by itself had no detrimental effects on renal function, lysosomal enzymatic profile, or cellular regeneration. Both electron-microscopic observations and mean nephrotoxicity scores demonstrated that renal lesions induced by ceftriaxone administration were rare and that this cephalosporin did not accumulate in the renal cortices of rats even after 4 and 10 days treatment. Furthermore, no specific sign or alteration of

TABLE 3. Histopathologic nephrotoxicities in the renal cortices of animals treated with saline or ceftriaxone (100 mg/kg) every 12 h alone or combined with tobramycin for 10 days

Treatment[a]	Mean score[b]						Total ± SD[c]
	ICN	LL	AT	TD	MM	IC	
Saline	0.50	0	0	0	0	1.63	2.13 ± 0.52
Tobramycin							
40	2.10	4.54	0.64	0	2.18	2.16	11.61 ± 3.81*‡
60	1.00	4.58	3.50	0.67	1.58	2.67	14.00 ± 2.50*
Ceftriaxone	0.55	0	1.00	0	0	2.83	4.37 ± 1.24
Ceftriaxone + tobramycin							
40	0.75	1.13	2.00	0	0.12	2.64	6.64 ± 1.99†
60	1.00	3.91	1.36	0	0.64	2.36	9.27 ± 2.45*§

[a] The numbers refer to milligrams of tobramycin per kilogram per 12 h.

[b] Abbreviations: ICN, isolated cell necrosis; LL, proximal tubules with large lysosomes; AT, abnormal proximal tubules (see the text); TD, tubular desquamation; MM, metachromatic materials in the tubular lumina; IC, interstitial cells.

[c] *, different from saline and ceftriaxone, $P < 0.01$; †, different from saline, $P < 0.01$, and ceftriaxone, $P < 0.05$; ‡, different from tobramycin at 60 mg/kg/12 h, $P < 0.05$, and ceftriaxone plus tobramycin at 40 mg/kg/12 h, $P < 0.01$; §, different from ceftriaxone plus tobramycin at 40 mg/kg/12 h, $P < 0.05$, and tobramycin at 60 mg/kg/12 h, $P < 0.01$.

subcellular structures that could be associated with ceftriaxone administration was identified.

Other β-lactam antibiotics when administered concomitantly with aminoglycosides have been previously shown to be protective against their nephrotoxic potential (8, 14, 17). Cephalothin and carbenicillin protected the kidney against gentamicin (8, 14, 32), while ticarcillin appeared to reduce the renal damage induced by tobramycin (17). Kojima et al. (28) also observed that latamoxef inhibited the binding between tobramycin and brush border membranes and therefore protects against tobramycin-induced nephrotoxicity.

Dellinger et al. (14) suggested that the protective effect of cephalothin was related to the presence of nonreabsorbable anion in the urine. Furuhama and Onodera (21) reported that the reduction of aminoglycoside nephrotoxicity by cephem antibiotics is due to their intrinsic nephrotoxic potential rather than the concentration of aminoglycoside in the kidney. Other investigators speculated that an interaction between tobramycin and ticarcillin which modifies tobramycin and lowers its toxic potential occurs in the renal proximal tubular cell (17). More recently, Ohnishi et al. (39) concluded that the obligatory salt supplementation with ticarcillin is sufficient to induce the protective effect without having to infer a direct chemical interaction of the penicillin with the aminoglycoside. A dietary calcium loading was also shown to delay and attenuate gentamicin-mediated renal dysfunction and structural damage (8).

Fosfomycin was also shown to decrease aminoglycoside nephrotoxicity. Increases in polyuria, enzymuria, blood urea nitrogen, and creatinine levels in serum induced by the administration of dibekacin were less severe when fosfomycin was given concomitantly (26). Neuman (37) showed an improvement in the alanine-aminopeptidase excretion in urine when fosfomycin was injected in combination with amikacin. Furthermore, Palmieri et al. (40) demonstrated a decrease in nephrotoxicity and an increase in the antibacterial activity when fosfomycin was given in combination with gentamicin in patients.

Several mechanisms may be proposed for the observed protective effect of ceftriaxone on renal cells. While there is not necessarily a good correlation between the intracortical levels of aminoglycosides and renal toxicity, in our study we cannot eliminate the possibility that the marked reduction in the intrarenal accumulation of aminoglycoside in the presence of ceftriaxone might have protected renal cells against tobramycin toxicity. We and others have demonstrated that other protective agents such as daptomycin and poly-L-aspartic acid protected proximal tubular cells against tobramycin and gentamicin nephrotoxicity, respectively, without reducing the uptake of the aminoglycosides (4–6, 22). The mechanisms of protection for different antimicrobial agents are most likely different.

The mechanism by which tobramycin uptake by the renal tubule is disturbed is obscure, but on the basis of previous observations with other β-lactams, we cannot eliminate the possibility that ceftriaxone would have bound to tobramycin, thus disturbing its reabsorption in the proximal tubules. In fact, it has been shown that preincubation of aminoglycosides with penicillin antibiotics resulted in the formation of complexes (13, 15). This would be consistent with the work of English et al. (17), who reported that a 24-h preincubation of ticarcillin and tobramycin resulted in a more important decrease in nephrotoxicity than when ticarcillin and tobramycin were injected in combination without preincubation, resulting in lower levels of tobramycin in renal cells. By preventing binding of the aminoglycosides to renal membranes, thus diminishing reabsorption, ceftriaxone could protect the renal tubules against aminoglycoside toxicity (46).

Sodium salt loading is known to be of major importance in the prevention of nephrotoxicity induced by agents such as amphotericin B and glycerol (11, 44). Ticarcillin and its obligatory sodium load were shown to reduce amphotericin B nephrotoxicity (12). Ceftriaxone, like ticarcillin, requires an obligatory salt loading that represents less than half of the salt loading of ticarcillin. However, this may be sufficient to confer the protection against tobramycin-induced nephrotoxicity observed in this study. The mechanisms by which ceftriaxone protects against tobramycin-induced nephrotoxicity remains unclear, and further investigations are needed to better understand the mechanism and extent of this protective effect.

Aminoglycosides are frequently used in combination with ceftriaxone or other broad-spectrum cephalosporins for the treatment of patients with major infections. These patients are specially prone to renal damage caused by aminoglycosides. The potential protective effect of ceftriaxone against aminoglycoside toxicity obtained in the present study might add to the benefit of an additive or synergistic combination of antibiotics

in very sick patients. Further investigations must be done to better understand the mechanisms and the extent of this protective effect.

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