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Attenuation of Gentamicin-Induced Nephrotoxicity in Rats by Fleroxacin

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The effect of fleroxacin on gentamicin-induced nephrotoxicity was evaluated with female Sprague-Dawley rats. Animals were injected during 4 or 10 days with saline (NaCl; 0.9%), gentamicin alone at doses of 10 and 40 mg/kg of body weight/12 h (subcutaneously), fleroxacin alone at a dose of 25 mg/kg/12 h (intraperitoneally), or the combination gentamicin-fleroxacin in the same regimen. Gentamicin induced a dose- and time-dependent renal toxicity as evaluated by gentamicin cortical levels, sphingomyelinase activity in the renal cortex, histopathologic and morphometric analysis, blood urea nitrogen and serum creatinine levels, and cellular regeneration ([^{3}H]thymidine incorporation into DNA of cortical cells). The extent of these changes was significantly reduced when gentamicin was given in combination with fleroxacin. Although the mechanisms by which fleroxacin reduces the nephrotoxic potential of gentamicin are unknown, we propose that the fleroxacin-gentamicin combination enhances exocytosis activity in proximal tubular cells, as suggested by the higher excretion of urinary enzymes and lower cortical levels of gentamicin observed in animals treated with the combination fleroxacin-gentamicin compared with those treated with gentamicin alone. The protective effect of fleroxacin on gentamicin nephrotoxicity should be investigated further.

Fleroxacin is a synthetic broad-spectrum antibiotic belonging to the class of fluoroquinolones (1). It shows a good antimicrobial activity against many gram-negative bacteria, especially members of the family *Enterobacteriaceae*, and less activity against gram-positive bacteria (2, 39). In humans, fleroxacin reaches peak serum levels within 2 h after its injection and has a serum half-life of about 10 h (8, 32, 39). In contrast with several other quinolones, fleroxacin is not metabolized and is mainly excreted unchanged in the urine. However, a fraction of fleroxacin eliminated by the kidney is reabsorbed at the proximal level of renal tubules (24, 32, 39). In fact, high concentrations of fleroxacin have been measured in the kidneys of rabbits with healthy and *Escherichia coli*-infected thigh muscles (16) and in the human kidneys (15). Moreover, the accumulation of fleroxacin was higher in the kidneys of patients with symptomatic complicated urinary tract infection (14).

Like all other aminoglycosides, gentamicin is essentially eliminated by glomerular filtration. Gentamicin also undergoes partial reabsorption by proximal tubular cells as a consequence of adsorptive endocytosis. Gentamicin-loaded endocytic vacuoles fuse with lysosomes where the drug accumulates (7, 19, 38). This accumulation leads to the development of a lysosomal phospholipidosis characterized by an impairment of phospholipase A_1 and sphingomyelinase activities and by phospholipid accumulation within the lysosomal compartment (25). This phospholipidosis eventually results in tubular necrosis, which in turn leads to tubular regeneration (18, 26, 27).

Several drugs or polypeptides have been shown to alleviate or enhance the nephrotoxicity of aminoglycosides when given in combination. Recently, we have observed that the concomitant injection of daptomycin (6, 11, 40), poly-L-aspartic acid (4), or ceftriaxone (5) reduces significantly the renal toxicity of aminoglycosides in experimental animals. In view of the particular distribution of fleroxacin within the kidney, we report here results concerning the effect of this quinolone on gentamicin-induced nephrotoxicity.

MATERIALS AND METHODS

Animals and treatment. Female Sprague-Dawley rats ($n = 6$ to 10 individuals per experimental group) weighing 175 to 200 g were used in the present study. The animals were purchased from Charles River Breeding Laboratories, Inc. (Montréal, Québec, Canada). They were dosed during 4 or 10 days with saline (NaCl; 0.9%), gentamicin alone at doses of 10 and 40 mg/kg of body weight/12 h (subcutaneously), fleroxacin alone at a dose of 25 mg/kg/12 h (intraperitoneally), or the combination gentamicin-fleroxacin in the same regimen.

Urine collection. Urine analysis was performed separately for experimental animals individually housed in metabolic cages where they had free access to food and water. Urine was collected over a period of 24 h (under mineral oil to prevent evaporation) on day 0 of treatment and 2, 4, and 8 days after starting drug administration. The volume of collected urine was measured to evaluate diuresis. Urine was centrifuged ($1,430 \times g$) for 15 min, and enzymatic activities were assayed in supernatants on the day of urine collection.

Sacrifice and tissue sampling. Animal groups other than those employed for urine analysis were killed 15 to 18 h after the last injection of gentamicin (days 5 and 11). One hour before sacrifice, all animals received an intraperitoneal injection of [^{3}H]thymidine (200 μCi). At the time of sacrifice, animals were killed by decapitation and blood was collected from the stump for the assay of serum creatinine and blood urea nitrogen (BUN). Both kidneys were rapidly removed and bisected. The renal cortex of one-half of the left kidney was separated by sharp dissection and snap frozen in dry ice for further determination of the [^{3}H]thymidine/DNA ratio. The cortex of the other half was also dissected. One part was snap frozen for gentamicin assay. The other part was immediately immersed in drops of ice-cold electron microscopy fixative (2% glutaraldehyde, 4% sucrose, 0.1 M phosphate buffer, pH 7.4), minced to obtain small (approximately 1 mm^3) tissue blocks, and kept overnight at 4°C in the same fixative. The cortices of both halves of the right kidney were also dissected, snap frozen in dry ice, and stored at −20°C until biochemical analysis.

Drug assay and biochemical analysis. Measurement of gentamicin accumulation in renal cortex was performed by a fluorescence polarized immunoassay

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(TDX System; Abbott Laboratories) as recently described (40). The lower limit of sensitivity of the assay was 1.26 μg/g of tissue. The percent gentamicin recovery in renal cortex homogenates was 78.0 ± 2.9. The interday coefficients of variations were 3.44% at 1 μg/ml and 2.72% at 8 μg/ml of tissue homogenate. Fleroxacin levels in renal cortex were not measured since previous data from our laboratory showed that levels of fleroxacin in tissue were below the detection limit of our microbiological assay 12 h after drug injection.

Serum creatinine and BUN levels, used as markers of renal function, were measured by automated enzymatic assays on a Hitachi 737 analyzer. Enzymuria related to drug administration was documented by the assays of *N*-acetylglucosaminidase (NAG), β-galactosidase (β-Gal), and γ-glutamyl-transpeptidase (γ-GT) activities according to established procedures (28, 35). The effect of gentamicin and/or fleroxacin on phospholipid metabolism in renal tissue was assessed by measuring sphingomyelinase activity according to a methodology published previously (25). The proliferative response associated with nephrogenic repair was estimated by pulse-labeling with [^{3}H]thymidine and by measuring the specific radioactivity of DNA extracted from renal cortex as described previously (26).

Tissue embedding and sectioning. Tissue specimens fixed in glutaraldehyde were postfixed in 1% osmium tetroxide and dehydrated by passage through graded ethanol solutions. The specimens were then transferred to propylene oxide and finally embedded in Araldite 502 epoxy resin (J.B.EM. Service Inc., Pointe-Claire, Québec, Canada). One-micrometer-thick plastic sections were cut with an Ultracut S Reichert-Jung ultramicrotome (Leica Instruments GmbH, Montréal, Québec, Canada) equipped with a diamond knife, mounted on glass slides, and stained with 1% toluidine blue in 2% $Na_2B_4O_7$.

Single-blind evaluation of tissue injury. Histopathological abnormalities were scored on plastic sections at ×400 magnification. Each slide was coded so that the identification of the group was not possible for the observer (L.G.). Sections came from three different regions of renal cortex for each rat, and four rats per group were used. The following lesions in renal cortex were scored: single-cell necrosis, tubular necrosis (proximal tubule profile with more than 50% necrotic cells), complete desquamation (proximal tubule profile with a denuded basement membrane), metachromatic materials in the tubular lumina, and the number of interstitial cells (no specific identification of cell type was made). The total number of proximal tubule profiles was also determined on each section. The number of tubule profiles with single-cell necrosis, extensive necrosis, complete cellular desquamation, or the presence of metachromatic material in lumen was expressed as the percentage of the total number of proximal tubule profiles on each respective section. Scores were assigned as follows: 0 to 9%, 1; 10 to 19%, 2; 20 to 29%, 3; etc. The score for interstitial cell infiltration was obtained by dividing the total number of interstitial cells by the total number of proximal tubules on each respective slice. All these histopathological scores were finally summed up to produce a single toxicity score for each tissue slice.

Morphologic evaluation of lysosome alterations. Morphometric analysis of lysosomes relied on the fact that they exhibit a deep blue appearance after staining with toluidine blue. As described in earlier reports (33, 41), the relative size of lysosomes in proximal tubular cells was estimated by computer-assisted morphometry. Briefly, the analysis was carried out in a single-blind fashion and performed in a system specifically designed for size and color assessment in light microscopy (Système d'Analyses Microscopiques à Balayage Automatique; Alcatel TINT Answare, Grenoble, France). The setting consisted of a high-resolution JVC video camera fitted on a Zeiss Axioplan microscope and connected to a Compaq IBM-compatible microcomputer. Cortical tissue sections (one per experimental animal) were scanned at 400-fold magnification, and at least 10 profiles of proximal tubules were picked up at random for analysis. Each profile was delineated in the interactive mode, and lysosomes were detected by gray-level discrimination (green channel). Tubule section surface and the aggregated area of lysosome profiles were calculated with software developed by one of us (J.Z.). Approximately 25,000 μm^2 of proximal tubule profiles was thus analyzed per experimental animal, and the data were expressed as the aggregated area of lysosomes relative to the surface of tubular epithelium.

Statistics. Results are expressed as means ± standard deviations. Statistical analysis of the differences between groups was first performed by analysis of variance. If P values were <0.05, group comparisons were done by the Fisher protected least-significant-difference post hoc test. A P value less than 0.05 was considered significant. Calculations were made with Statview software.

Materials. Gentamicin was kindly donated by Schering Canada Inc. (Pointe-Claire, Québec, Canada). Fleroxacin was kindly provided by Hoffmann-La Roche Inc. (Mississauga, Ontario, Canada). [*methyl*-^{3}H]thymidine (47 Ci/mmol) and [*N*-methyl-^{14}C]sphingomyelin (58 mCi/mmol) came from Amersham Canada Ltd. (Oakville, Ontario, Canada). Sphingomyelin (from bovine brain) came from Sigma Chemical Co. (St. Louis, Mo.). Other reagents were of analytical grade and were purchased from Fisher Scientific Ltd. (Québec, Québec, Canada) and Sigma.

RESULTS

Accumulation of gentamicin in renal cortex. Figure 1 shows the cortical accumulation of gentamicin after 4 and 10 days of treatment with the aminoglycoside given alone or in combination with fleroxacin. At day 4, significantly higher levels of gentamicin were measured in the renal cortex of animals treated with gentamicin alone at a dose of 40 mg/kg compared with animals treated with gentamicin at a dose of 10 mg/kg ($P < 0.01$). Similar results were observed in animals treated with the gentamicin-fleroxacin combination. Fleroxacin had no effect on gentamicin cortical accumulation over 4 days of treatment. In animals exposed for 10 days to gentamicin alone, the extent of aminoglycoside accumulation within renal cortex was also dose related ($P < 0.01$). Interestingly, fleroxacin reduced significantly the accumulation of gentamicin in the renal cortex of animals treated at 40 mg/kg compared with animals treated with gentamicin alone at the same dosage ($P < 0.01$). At the lower dose of 10 mg/kg, fleroxacin also tended to reduce gentamicin accumulation, though the difference did not appear significant.

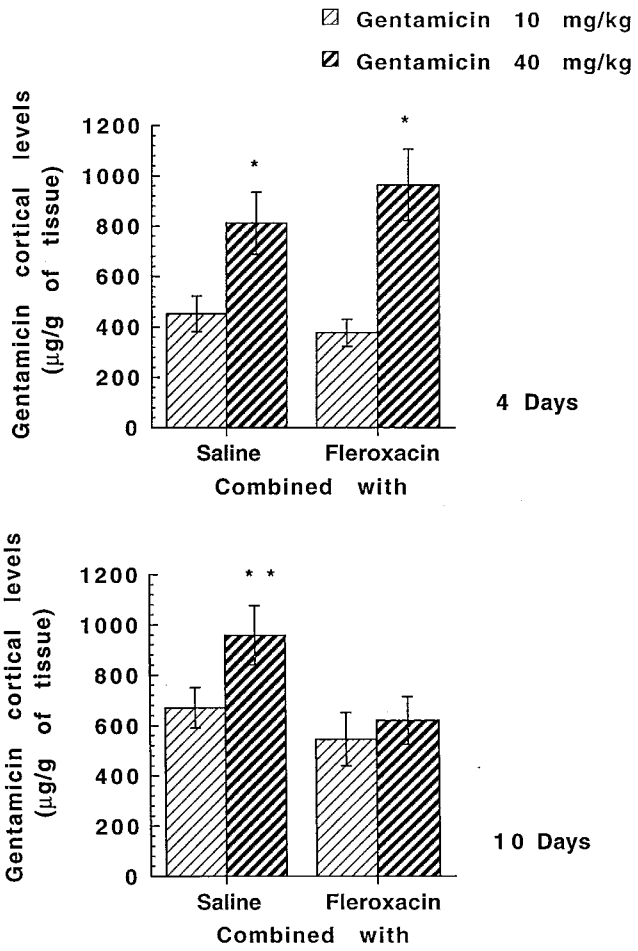

FIG. 1. Effects of fleroxacin on the accumulation of gentamicin (micrograms per gram of tissue) in the renal cortex. Animals were treated with gentamicin at doses of 10 and 40 mg/kg/12 h during 4 and 10 days combined with either saline or fleroxacin at a dose of 25 mg/kg/12 h. *, significantly different from gentamicin at 10 mg/kg ($P < 0.01$); **, significantly different from all other groups ($P < 0.01$).

Aminoglycoside-induced phospholipidosis. Development of a lysosomal phospholipidosis is known to be one of the early manifestations of aminoglycoside nephrotoxicity. Therefore, we examined the activity of sphingomyelinase, a key enzyme in phospholipid catabolism, in the renal cortex of rats exposed to gentamicin with or without fleroxacin. Data appear in Fig. 2. After 4 days of treatment, gentamicin given with saline at doses

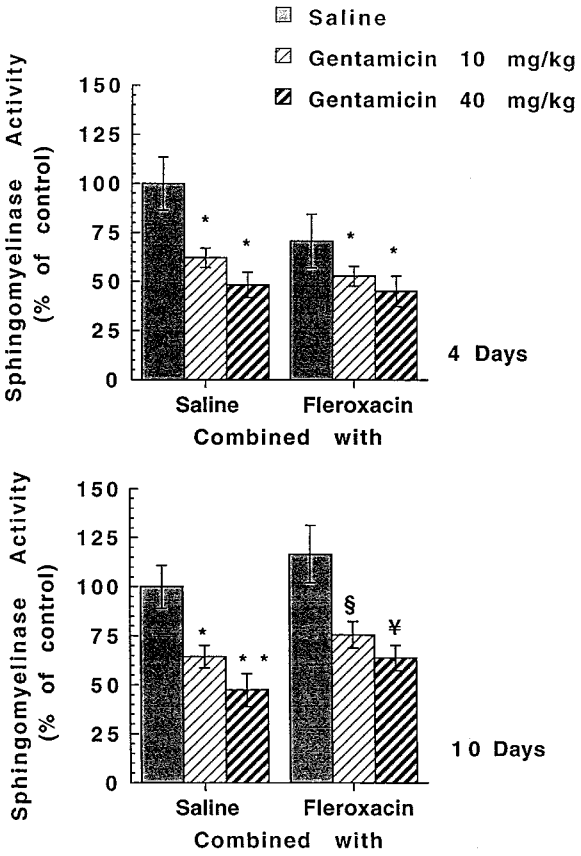

FIG. 2. Effects of fleroxacin on gentamicin-induced inhibition of sphingomyelinase activity. Animals were treated with saline (NaCl; 0.9%) or gentamicin at doses of 10 and 40 mg/kg/12 h combined with either saline or fleroxacin at a dose of 25 mg/kg/12 h during 4 and 10 days. *, significantly different from saline-treated animals ($P < 0.01$); **, significantly different from animals treated with saline and with gentamicin alone (10 mg/kg/12 h); §, significantly different from animals treated with saline, fleroxacin (25 mg/kg/12 h), and gentamicin alone (10 mg/kg/12 h) ($P < 0.05$); ¥, significantly different from animals treated with saline, fleroxacin (25 mg/kg/12 h), and gentamicin alone (40 mg/kg/12 h) ($P < 0.05$).

of 10 and 40 mg/kg induced a significant decrease of sphingomyelinase activity compared with control animals ($P < 0.01$). A similar reduction was observed in animals treated with the combination fleroxacin-gentamicin. Thus, fleroxacin had no effect on gentamicin-induced inhibition of sphingomyelinase activity after 4 days of treatment. After 10 days of treatment, the sphingomyelinase activity measured in animals treated with gentamicin at a dose of 40 mg/kg was significantly lower than that in animals treated with gentamicin at a dose of 10 mg/kg ($P < 0.01$). Fleroxacin administered alone increased by 15% the sphingomyelinase activity compared with control animals ($P < 0.05$). The enzyme activity was significantly less inhibited in animals given the combination fleroxacin-gentamicin compared with rats treated with gentamicin alone at doses of 10 and 40 mg/kg ($P < 0.05$).

Phospholipid accumulation resulting from exposure to gentamicin was also assessed by the morphological analysis of proximal tubules and in particular by the morphometric analysis of lysosomes. Figures 3 and 4 illustrate the aspect of renal cortex in treated animals and controls, as revealed by light micrographs of plastic sections. Following gentamicin treatment, lysosomes in proximal tubular cells enlarge, develop irregular shapes, and frequently exhibit a heterogeneous content (Fig. 3C), a well-known consequence of phospholipid overloading (33, 41). Lysosomal abnormality evolves with the duration of treatment with gentamicin (Fig. 4A) and persists until the emergence of tubular necrosis (Fig. 4B). In contrast, exposure to fleroxacin alone does not influence the appearance of lysosomes (Fig. 3A and B). Strikingly, lysosomal alteration also appears less severe in animals receiving the gentamicin-fleroxacin combination compared to those receiving gentamicin alone (Fig. 3C and D).

The seemingly protective effect of fleroxacin on the lysosomal phospholipidosis induced by gentamicin was explored further by the morphometric analysis of these organelles according to an objective, computer-assisted procedure (Fig. 5). Although the administration of fleroxacin in association with gentamicin did not diminish the enlargement of lysosomes in proximal tubules of rats receiving 10 mg of gentamicin per kg, fleroxacin significantly alleviated the lysosomal alteration caused by gentamicin at 40 mg/kg.

Figure 6 shows the mean histopathologic nephrotoxicity score for animals treated during 10 days. Scores for single-cell necrosis, tubular necrosis, complete desquamation, metachromatic material, and interstitial cells were summed up to produce these mean histopathological scores. The total nephrotoxicity score was significantly higher in animals treated with gentamicin alone at a dose of 40 mg/kg than in control animals ($P < 0.01$) and animals treated with gentamicin at a dose of 10 mg/kg. By contrast, the total nephrotoxicity score was significantly lower in animals given the combination fleroxacin-gentamicin than in those given gentamicin alone at 40 mg/kg ($P < 0.05$).

Renal dysfunction and enzymuria. Serum creatinine and BUN levels appear in Fig. 7. After 4 days of treatment, no significant difference was observed between groups (data not shown). Gentamicin given for 10 days at 40 mg/kg induced a significant increase in serum creatinine levels and BUN compared with control animals and animals treated with gentamicin at the dose of 10 mg/kg. Concomitant administration of fleroxacin with gentamicin reduced significantly the elevation of serum creatinine and BUN caused by gentamicin at 40 mg/kg ($P < 0.01$) although both parameters were still significantly higher in the rats receiving both drugs in combination than in control animals ($P < 0.05$) and animals treated with fleroxacin alone ($P < 0.05$).

Regenerative hyperplasia. Figure 8 shows the effects of fleroxacin on gentamicin-induced increase of cell turnover in renal cortex. After 4 days of treatment, a significantly higher incorporation of [^{3}H]thymidine was observed only in the renal cortex of animals treated with gentamicin at a dose of 40 mg/kg with or without fleroxacin compared with control animals ($P < 0.01$). After 10 days of treatment, gentamicin given alone induced a significant and dose-dependent increase in the incorporation of [^{3}H]thymidine into DNA ($P < 0.01$). The nephrogenic repair was significantly lower in animals treated with the combination fleroxacin-gentamicin compared with animals treated with gentamicin alone at doses of 10 and 40 mg/kg ($P < 0.01$).

Figure 9 illustrates the effect of drug administration on the urinary excretion of NAG on days 0, 2, 4, and 8 of treatment. In animals treated with low doses of gentamicin, no significant difference was observed between groups (upper panel). By contrast, NAG activity in urine was significantly higher in animals treated with gentamicin at 40 mg/kg on day 8 compared with control animals or rats exposed to fleroxacin alone. Increased excretion of NAG caused by gentamicin was even greater when fleroxacin was administered in combination with the aminoglycoside ($P < 0.01$). The assay of two other enzymes, β-Gal (Fig. 10) and γ-GT (Fig. 11), in urine of treated animals gave patterns similar to that observed for NAG. Thus, fleroxacin administration in combination with gentamicin at

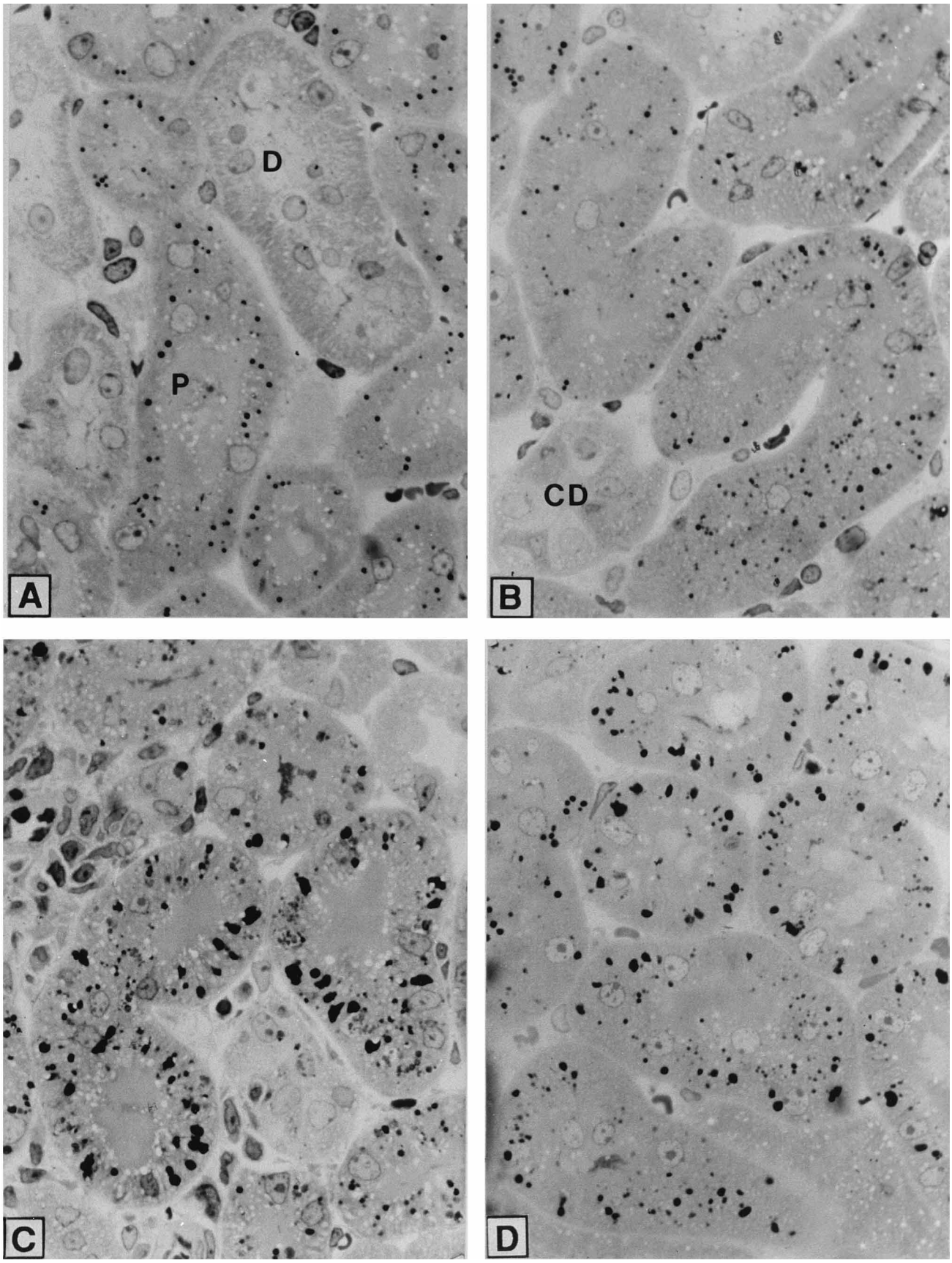
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FIG. 3. Light microscopy appearance of renal cortex in rats treated for 4 days with fleroxacin (25 mg/kg/12 h) (B), gentamicin (40 mg/kg/12 h) (C), or a combination of both antibiotics (D). A control animal is included for comparison (A). Dosing protocols and preparation of morphological specimens are detailed in Materials and Methods. Lysosomes are particularly conspicuous in proximal tubules (P in panel A) and much less apparent in distal tubules (D in panel A) or collecting ducts (CD in panel B). No visible change in lysosome morphology can be seen after exposure to fleroxacin alone (compare panel B with panel A). By contrast, gentamicin administration causes a marked enlargement of lysosomes, which also become heterogeneous and irregular in shape (C). Fleroxacin administered in combination with gentamicin substantially reduces the degree of lysosomal alteration caused by the latter antibiotic (compare panel C with panel D). Plastic sections were stained with toluidine blue. Magnification, ca. ×724.

low or high doses tended to exacerbate the enzymuria induced by the aminoglycoside.

DISCUSSION

This study strongly suggests that fleroxacin protects proximal tubular cells of renal cortex against gentamicin-induced nephrotoxicity. This assumption is based on various criteria such as the alleviation of gentamicin-induced lysosomal phospholipidosis, the modification of renal dysfunction caused by gentamicin at a high dose, and a lesser extent of postnecrotic tubular regeneration. A possible protective effect of fleroxacin is also substantiated by a lower accumulation of gentamicin and less severe histopathological lesions in the renal cortex of animals receiving the combination fleroxacin-gentamicin compared with animals treated with gentamicin alone. However, discrepant results were obtained with urinary excretion of enzymes (β-Gal, NAG, and γ-GT), for which a significantly higher excretion was observed in animals treated with the combination fleroxacin-gentamicin compared with animals treated with fleroxacin or gentamicin alone.

Quinolones have a nephrotoxic potential. Among possible untoward effects, crystalluria is probably the most important one since no nephropathologic changes occur in its absence (10). However, since it is a phenomenon related to the lower solubility of quinolones at alkaline pH, crystalluria occurs mainly in animals which have urine more alkaline than that of humans (10). Crystalluria and other nephrotoxic effects including elevation of serum creatinine, cylindruria, hematuria, and increased renal weight occur in less than 2% of treated patients (10, 34, 37). Furthermore, quinolones have no direct effect on glomerular and tubular functions. Our results show indeed that fleroxacin alone at the dosage regimen that we used affects neither renal function nor urinary enzyme excretion. However, the presence of many small vesicles within the lysosomes of proximal tubular cells and a small increase in the activity of the renal cortex sphingomyelinase might be indicative of minor metabolic disturbance caused by fleroxacin. Also, the slight

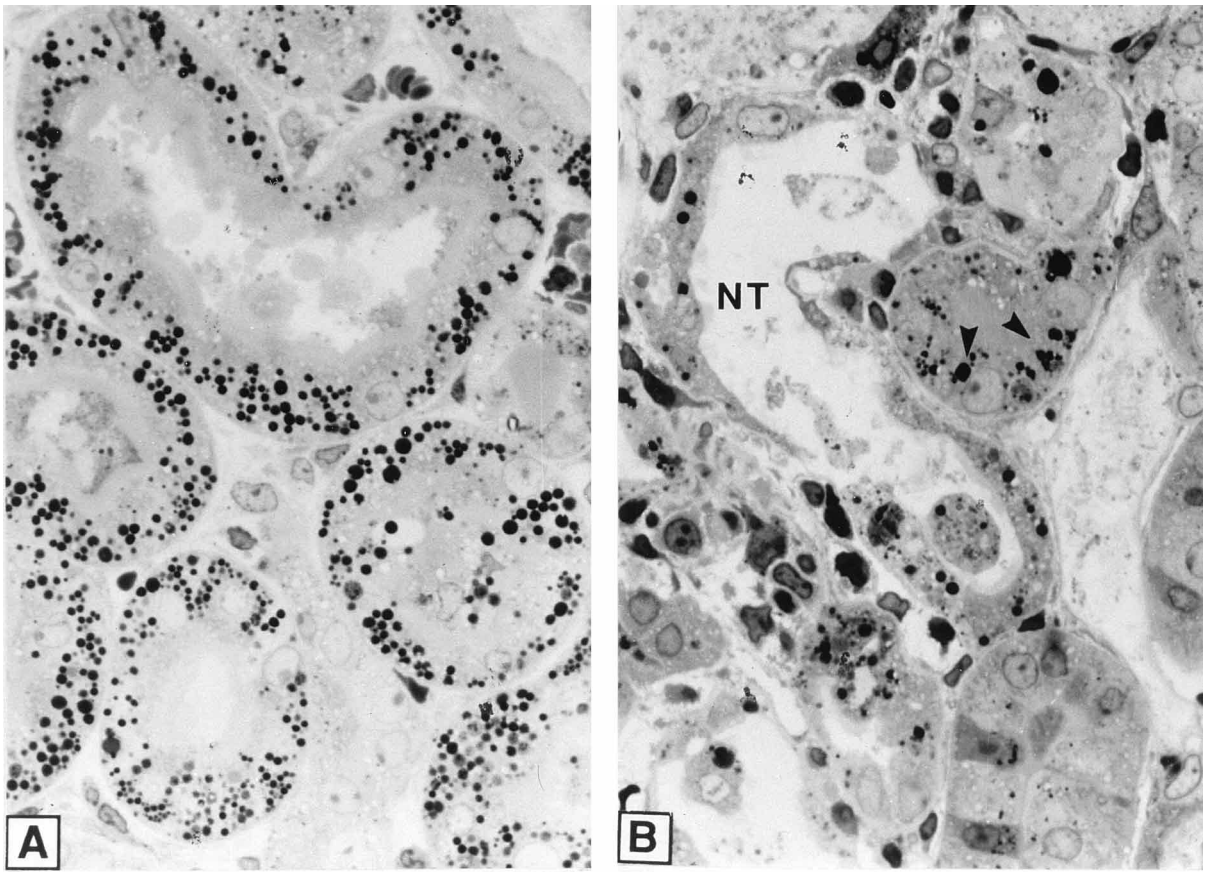

FIG. 4. Severe phospholipidosis (A) and acute tubular necrosis (B) induced by a 10-day exposure to gentamicin (40 mg/kg/12 h). Comparison of panel A with Fig. 3C clearly reveals the progression of lysosomal alteration with the duration of treatment. In panel B, prominent signs of phospholipidosis (arrowheads) can still be observed in the vicinity of a necrotic tubule (NT). Plastic sections were stained with toluidine blue. Magnification, ×746.

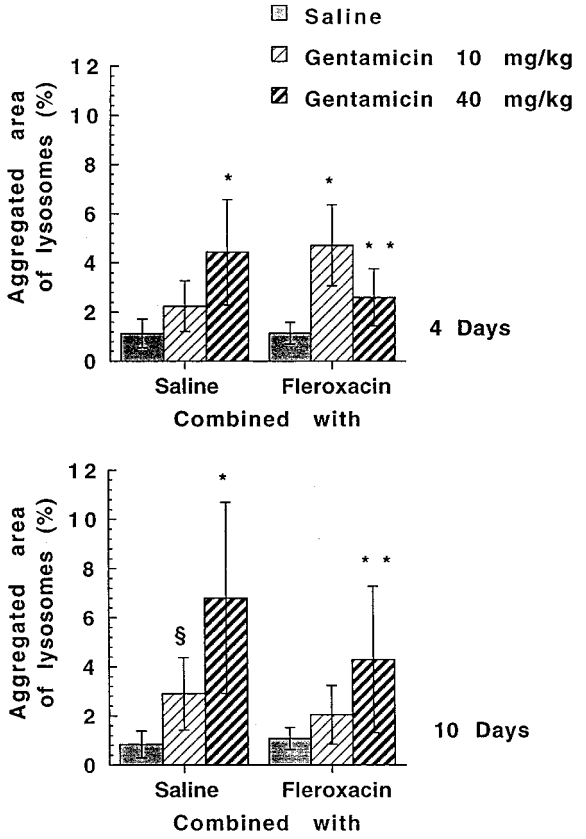

FIG. 5. Morphometric analysis of proximal tubular cell lysosomes. Animals were treated with saline (NaCl; 0.9%) or gentamicin at a dose of 10 or 40 mg/kg/12 h combined with either saline or fleroxacin at a dose of 25 mg/kg/12 h during 4 and 10 days. *, significantly different from all groups ($P < 0.01$); **, significantly different from saline, fleroxacin, and gentamicin (10 mg/kg)-fleroxacin ($P < 0.05$); §, significantly different from saline and fleroxacin ($P < 0.01$).

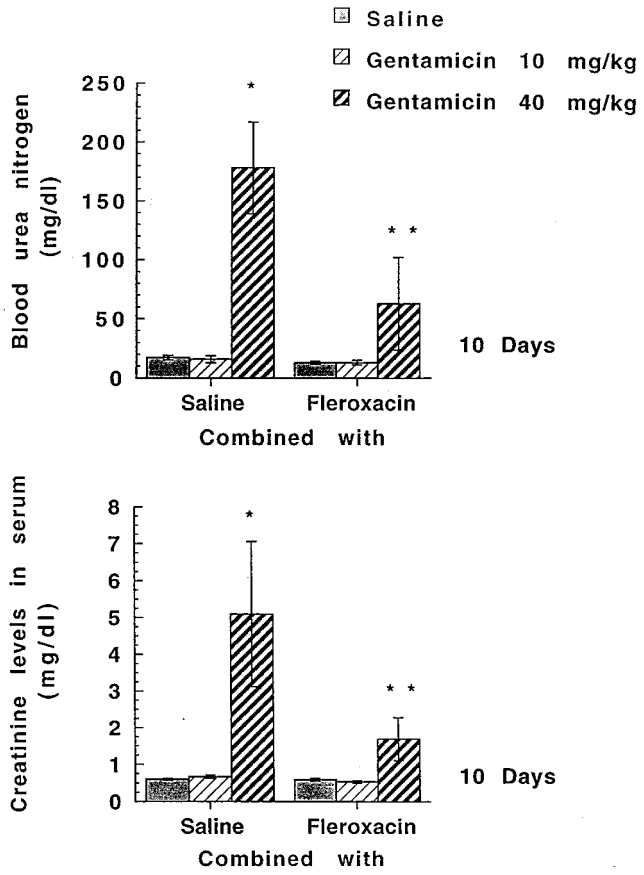

FIG. 7. Effects of fleroxacin on gentamicin-induced renal dysfunction as measured by BUN and serum creatinine. Animals were treated with saline (NaCl; 0.9%) or gentamicin at doses of 10 and 40 mg/kg/12 h combined with either saline or fleroxacin at a dose of 25 mg/kg/12 h during 10 days. *, significantly different from all groups ($P < 0.01$); **, significantly different from all groups ($P < 0.05$).

decrease of DNA synthesis in the renal cortex of animals treated with fleroxacin alone compared to sham-treated rats might be due in part to an effect of fleroxacin on the eucaryotic topoisomerase II, a gyrase-like enzyme found in mammalian cells. It has been reported elsewhere that quinolones inhibit the replication of mitochondrial DNA in mammalian cells in a dose-related manner (29). However, Barrett et al. (3) and Hussy et al. (22) have demonstrated that the affinity of fleroxacin for eucaryotic topoisomerase II is much lower than that for bacterial gyrase, despite the fact that fleroxacin affinity for DNA gyrase is one of the highest among quinolones and surpassed only by that of ofloxacin (21). Nonetheless, a possible effect of fleroxacin on unscheduled DNA synthesis in renal cortex might occur and cause an apparent or actual decrease in the rate of cell proliferation associated with tubular regeneration.

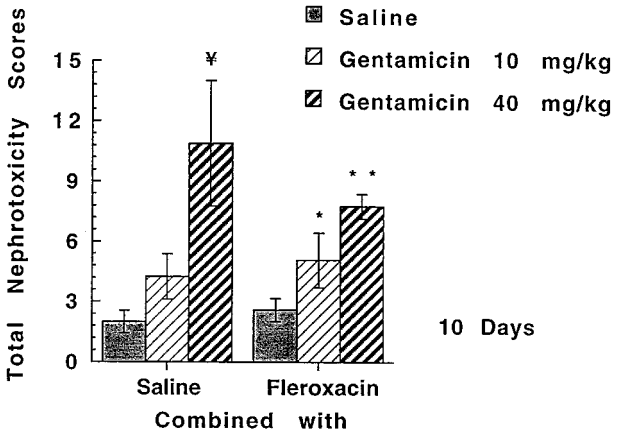

FIG. 6. Total histopathologic nephrotoxicity scores (calculations are detailed in Materials and Methods). Animals were treated during 10 days with saline (NaCl; 0.9%) or gentamicin at a dose of 10 or 40 mg/kg/12 h combined with either saline or fleroxacin at a dose of 25 mg/kg/12 h during 10 days. *, significantly different from saline- and fleroxacin-treated rats ($P < 0.05$); **, significantly different from all groups ($P < 0.05$); ¥, significantly different from all groups ($P < 0.05$).

Protection against aminoglycoside-induced nephrotoxicity has been demonstrated for many antibiotics or chemical compounds. Actually, several antimicrobials, including ceftriaxone (5), ticarcillin (12), carbenicillin (9), latamoxef (23), and daptomycin (6, 11, 40, 42), as well as compounds such as poly-L-aspartic acid (4, 17), act as modifiers of aminoglycoside nephrotoxicity. This protection occurs with or without a reduction in the uptake of the aminoglycosides, suggesting different mechanisms of protection. Studies performed in our laboratory have revealed that daptomycin might reduce tobramycin nephrotoxicity by forming an electrostatic complex with the aminoglycoside (6, 11, 40), whereas ceftriaxone seems to prevent tobramycin from binding to the brush border membrane, consequently

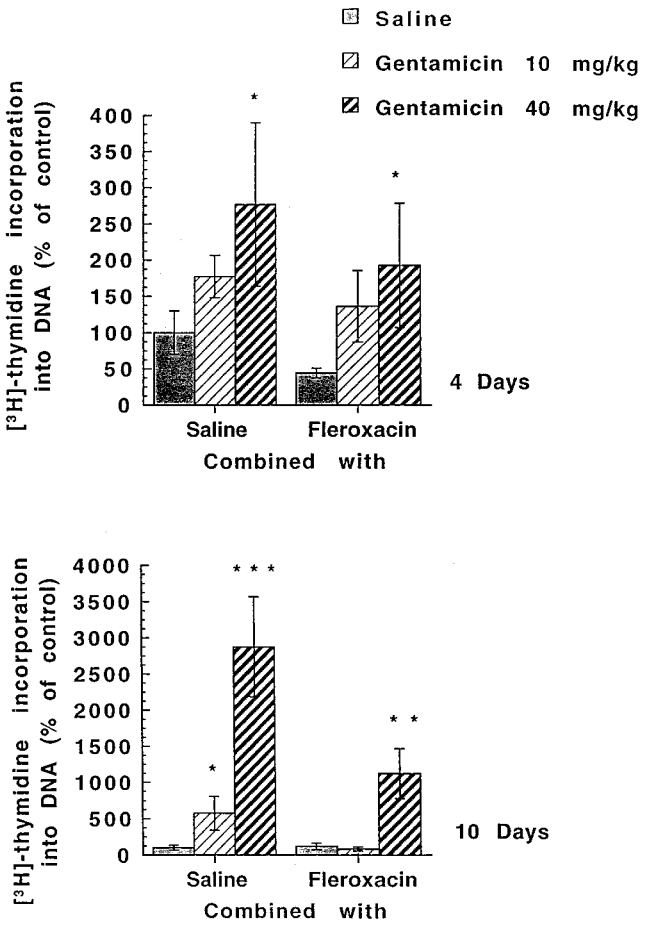

FIG. 8. Regenerative hyperplasia in renal cortex after treatment with gentamicin and/or fleroxacin. Animals were treated with saline (NaCl; 0.9%) or gentamicin at doses of 10 and 40 mg/kg/12 h combined with either saline or fleroxacin at a dose of 25 mg/kg/12 h during 4 and 10 days. *, significantly different from animals treated with saline and fleroxacin ($P < 0.05$); **, significantly different from all groups ($P < 0.01$); ***, significantly different from all groups ($P < 0.01$).

reducing its uptake by the proximal tubular cells (5). On the other hand, poly-L-aspartic acid protects against aminoglycoside nephrotoxicity by forming complexes at acidic pH and preventing phospholipid overloading in lysosomes, even though it does not diminish but rather increases aminoglycoside accumulation (4).

To our knowledge, fleroxacin is the first quinolone antibiotic reported to offer protection against aminoglycoside-induced nephrotoxicity. To some extent, this protection could result from the fact that fleroxacin reduces intracortical accumulation of gentamicin, consequently decreasing cell injury, tubular necrosis, and renal dysfunction induced by gentamicin. The mechanism by which fleroxacin reduces gentamicin accumulation remains unknown, but one can suppose that fleroxacin and gentamicin compete for similar binding sites or interact with each other in such a way that gentamicin reabsorption by proximal tubular cells is decreased or prevented.

It is also conceivable that gentamicin, after its reabsorption, is actively excreted from the proximal tubular cells via a stimulation of exocytosis. It would explain the increase in the urinary excretion of β-Gal, NAG, and γ-GT observed when fleroxacin is administered in combination with gentamicin. One might even consider the possibility that interactions between fleroxacin and gentamicin enhance the exocytic activity in proximal tubular cells, resulting in a higher excretion of urinary enzymes. In this respect, it is worth noting that fleroxacin injected alone into animals increases significantly the activity of sphingomyelinase, a lysosomal enzyme which is less inhibited when the combination fleroxacin-gentamicin is used compared with gentamicin alone. Since fleroxacin and gentamicin are both reabsorbed by proximal tubular cells, an interaction between these drugs at the brush border membrane level may modulate membrane structure and physiological state, causing a depolarization and resulting in an increase of intracytosolic Ca^{2+}. It is well known that this last event triggers the exocytosis process. This hypothesis might provide an explanation for the increased urinary excretion of β-Gal, NAG, and γ-GT observed in the present study. The nature of the interaction between fleroxacin and gentamicin remains unknown even though it can be assumed that their respective anionic and cationic natures are involved. Additional studies are warranted in order to test these assumptions, such as the measurement of gentamicin urinary excretion, the examination of gentamicin and fleroxacin interactions with brush border membranes, and the effect of treatment on intracellular Ca^{2+}.

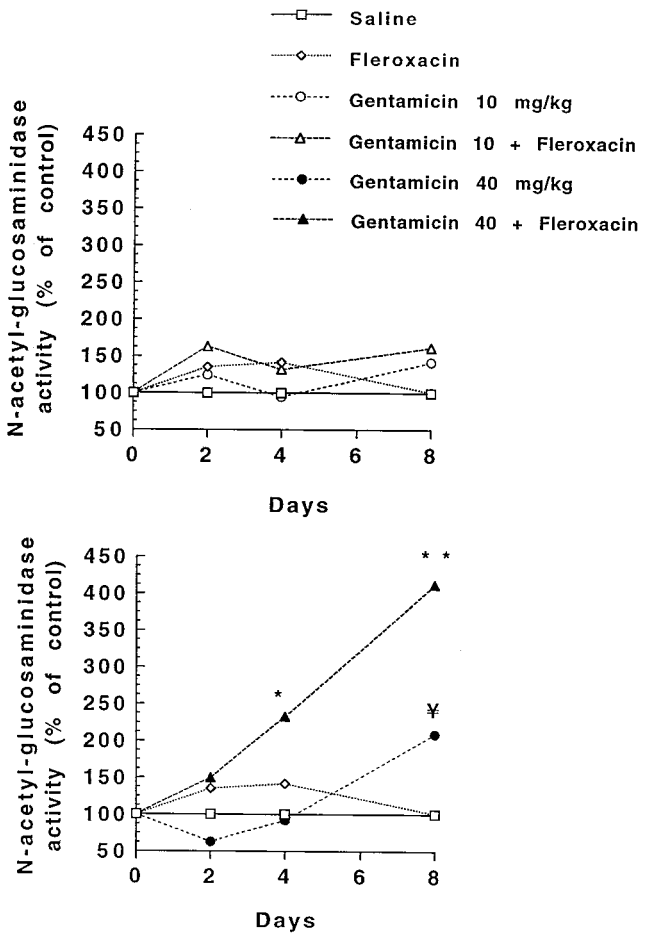

FIG. 9. Effects of fleroxacin on gentamicin-induced urinary excretion of NAG. Animals were treated with saline (NaCl; 0.9%) or gentamicin at doses of 10 (upper panel) and 40 (lower panel) mg/kg/12 h combined with either saline or fleroxacin at a dose of 25 mg/kg/12 h during 4 and 10 days. *, significantly different from saline and gentamicin at 40 mg/kg ($P < 0.05$); **, significantly different from saline, fleroxacin, and gentamicin at 40 mg/kg ($P < 0.01$); ¥, significantly different from saline, fleroxacin, and fleroxacin-gentamicin at 40 mg/kg ($P < 0.05$).

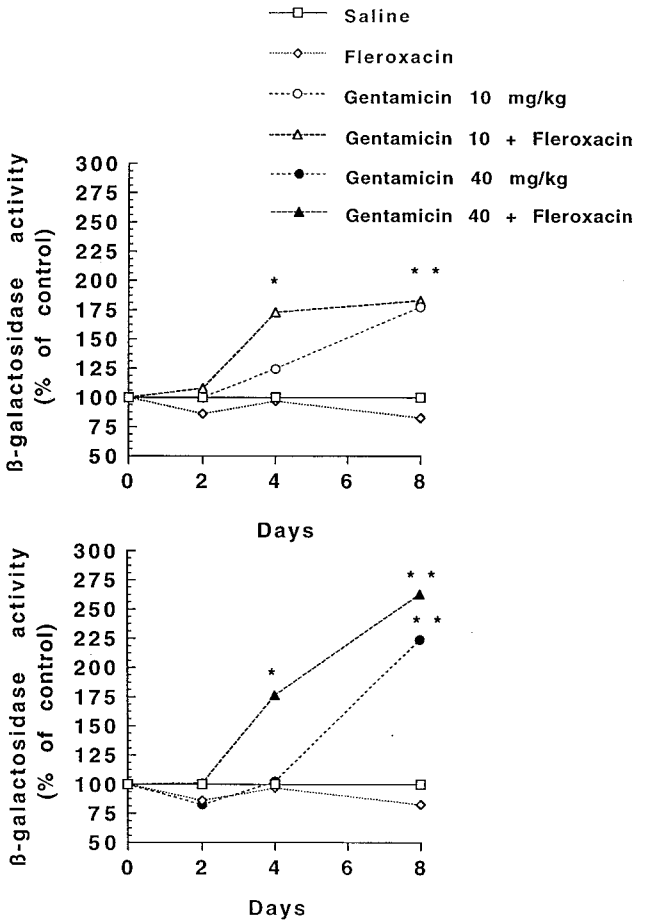

FIG. 10. Effects of fleroxacin on gentamicin-induced urinary excretion of β-Gal. Animals were treated with saline (NaCl; 0.9%) or gentamicin at doses of 10 (upper panel) and 40 (lower panel) mg/kg/12 h combined with either saline or fleroxacin at a dose of 25 mg/kg/12 h during 4 and 10 days. *, significantly different from all groups ($P < 0.05$); **, significantly different from saline- and fleroxacin-treated groups ($P < 0.01$).

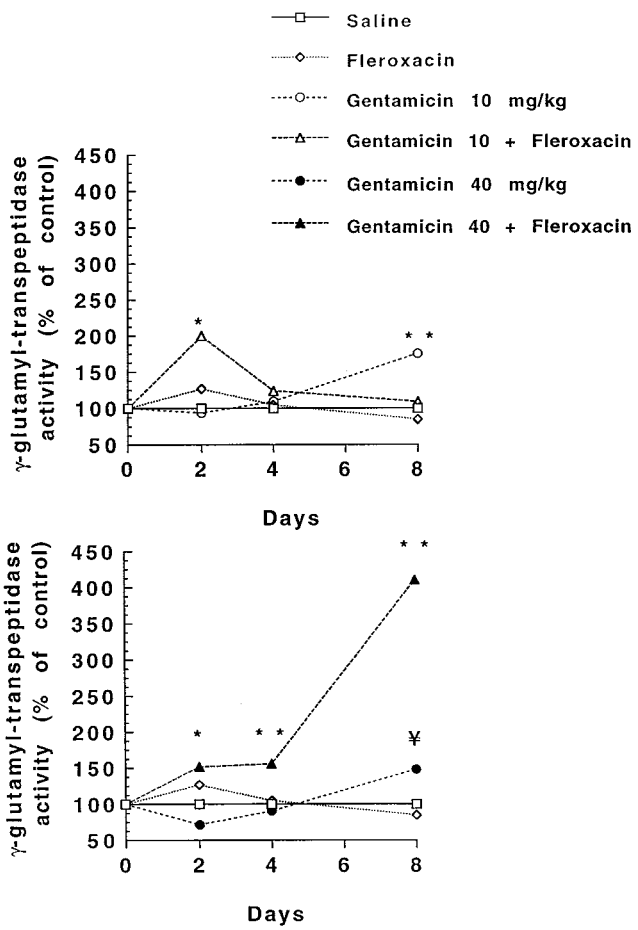

FIG. 11. Effects of fleroxacin on gentamicin-induced urinary excretion of γ-GT. Animals were treated with saline (NaCl; 0.9%) or gentamicin at doses of 10 (upper panel) and 40 (lower panel) mg/kg/12 h combined with either saline or fleroxacin at a dose of 25 mg/kg/12 h during 4 and 10 days. *, significantly different from saline and gentamicin ($P < 0.05$); **, significantly different from saline, fleroxacin, and gentamicin ($P < 0.05$); ¥, significantly different from saline and fleroxacin ($P < 0.05$).

Very little information is available concerning the concomitant use of quinolones and aminoglycosides, and more specifically of fleroxacin and gentamicin in antibiotherapy. In vitro studies have demonstrated that the combination of fleroxacin with gentamicin does not increase antimicrobial activity against *Pseudomonas aeruginosa* (30, 31). A similar absence of synergistic or additive effect against this bacteria has also been noted when quinolones were combined with other aminoglycosides such as sisomycin, amikacin, and tobramycin (20). Similar observations have been reported for most *Enterobacteriaceae*, including *E. coli*, *Klebsiella pneumoniae*, and *Serratia marcescens* (20, 30). Furthermore, combination of gentamicin with other quinolones including temafloxacin and ciprofloxacin does not improve activity against these gram-negative bacteria (30). Finally, methicillin-susceptible and methicillin-resistant staphylococci did not become more sensitive when gentamicin was combined with fleroxacin, as demonstrated by many investigators (13, 31, 36). Even though such combinations of aminoglycosides and quinolones are not additive or synergistic in vitro, they are often used clinically. Besides, aminoglycosides persist within the renal parenchyma for many months after their use, and since they are often used as step-down therapy following intravenous therapy, the chances of exposing the kidney to these combinations are high. Therefore, further investigations should be conducted in order to better characterize the attenuation of gentamicin-induced nephrotoxicity by fleroxacin.

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