

## Effects of Daptomycin and Vancomycin on Tobramycin Nephrotoxicity in Rats

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Received 4 May 1989/Accepted 7 October 1989

Daptomycin is a new biosynthetic antibiotic which belongs to a new class of drugs known as lipopeptides. The objective of this study was to evaluate the effects of daptomycin and vancomycin on tobramycin-induced nephrotoxicity. Female Sprague-Dawley rats were treated during 4 and 10 days with either saline (NaCl, 0.9%) or tobramycin at doses of 4 and 40 mg/kg per day (given every 12 h [q12h] intraperitoneally). Each treatment was combined with saline, daptomycin at a dose of 20 mg/kg per day (given q12h subcutaneously), and vancomycin at a dose of 50 mg/kg per day (given q12h subcutaneously). Daptomycin and vancomycin had no effect on the intracortical accumulation of tobramycin. Daptomycin did not accumulate in renal tissue even after 10 days of treatment. Tobramycin given at a dose of 40 mg/kg per day during 10 days induced a significant inhibition of sphingomyelinase activity in the renal cortex ( $P < 0.01$ ) and increased cellular regeneration ( $P < 0.01$ ), as measured by the incorporation of [ $^3$ H]thymidine into DNA of the renal cortex. These changes were minimal when daptomycin was combined with tobramycin. Histologically, signs of tobramycin toxicity were also less severe in the presence of daptomycin. The intracortical accumulation of vancomycin was not modified by tobramycin. The sphingomyelinase activity was significantly more inhibited ( $P < 0.01$ ) when vancomycin was associated with tobramycin (4 and 40 mg/kg) without affecting the rate of [ $^3$ H]thymidine incorporation into DNA. Histologically, signs of tobramycin toxicity were not affected by vancomycin, but the cellular vacuolizations which were also observed in vancomycin-treated animals were still present in the proximal tubular cells of animals that were treated with the combination vancomycin-tobramycin. This study strongly suggests that daptomycin protects animals from tobramycin-induced nephrotoxicity but that vancomycin may enhance the effect of tobramycin. We conclude that daptomycin is safe and protects kidney cells from tobramycin-induced nephrotoxicity.

Daptomycin (LY146032) is a new biosynthetic antibiotic which belongs to a new class of drugs known as lipopeptides. The antibacterial activity of daptomycin is due to inhibition of an early step in cell wall biosynthesis (1). Daptomycin is very active against methicillin-resistant staphylococci and a variety of clinically important aerobic, facultative, and anaerobic gram-positive bacteria, offering a potentially useful alternative to vancomycin (5, 9, 28).

Miniter et al. (25) have shown that daptomycin is as effective as vancomycin or vancomycin-gentamicin for the treatment of enterococcal pyelonephritis, while Sapico et al. (29) showed that the combination of daptomycin plus gentamicin gives better results than no treatment or treatment with a single antibiotic. Moreover, Bush et al. (8) compared daptomycin with vancomycin without and with gentamicin for treatment of an experimental enterococcal endocarditis and concluded that daptomycin-gentamicin significantly reduced bacterial counts of vegetation compared with daptomycin alone but was significantly less effective than vancomycin-gentamicin. Since daptomycin will usually be administered in combination with aminoglycosides, a better understanding of its interaction with these drugs is of crucial importance.

Vancomycin is a glycopeptide antibiotic with potent activity against gram-positive organisms. When the drug was marketed in the 1950s, nephrotoxicity was the most prominent side effect of this drug and was partly responsible for its replacement by less toxic semisynthetic antistaphylococcal

penicillins and cephalosporins. The emergence of methicillin-resistant staphylococci has prompted a resurgence of its use.

The nephrotoxicity of aminoglycosides has been intensively studied, but there are limited data on the mechanism of vancomycin toxicity. The nephrotoxicity of vancomycin given alone has been demonstrated in animals by using sensitive parameters of nephrotoxicity (22). It has also been shown in both human (11, 32) and animal (22, 23, 36, 38) studies with different animal strains and drug doses that nephrotoxicity is increased when vancomycin and an aminoglycoside are injected concomitantly. However, this phenomenon is not reproduced in pediatric populations (27, 33). Moreover, it has also been observed that vancomycin and aminoglycosides accumulate within the renal cortices of experimental animals (22, 23, 38).

The objectives of the present study were to evaluate the nephrotoxicities of daptomycin and vancomycin alone and to compare their effects on tobramycin-induced nephrotoxicity.

### MATERIALS AND METHODS

**Concentrations of daptomycin in serum, urine, and kidneys.** Female Sprague-Dawley rats (weight, between 175 and 200 g) were used throughout the experiment. A first group of 36 animals was used for the measurement of daptomycin concentrations in urine, serum, and kidney cortex and medulla from 1 to 24 h after a single subcutaneous (s.c.) injection of a 10-mg/kg dose. Briefly, rats were anesthetized with pentobarbital sodium (45 mg/kg) 75 min before sacrifice; 15 min

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later, the bladder was emptied by suprapubic puncture. At the time of sacrifice (i.e., 1, 2, 4, 6, 12, and 24 h after daptomycin injection), urine was collected from each animal as described above. Animals were killed by decapitation, the blood was taken and centrifuged, and the serum was collected. Urine and serum were quickly frozen ( $-20^{\circ}\text{C}$ ) for further antibiotic determination. A midline abdominal incision was made, and the right kidney of each animal was removed. Kidneys were bisected with a longitudinal incision and separated into cortical and medullary components under a dissecting microscope. Each entire cortex and medulla was weighed individually, homogenized in phosphate buffer solution (pH 7.4) at  $0^{\circ}\text{C}$  with a Tissue-Tearor (Biospec Products, Bartlesville, Okla.), and then diluted to obtain concentrations in the range of the standard curve (0.4 to 100  $\mu\text{g/ml}$ ). Standard solutions were prepared in normal serum for assays of daptomycin in serum, in physiological saline for urine, and in blank cortex and medulla homogenates for renal tissue. Daptomycin concentrations were measured by a microbiological assay with *Micrococcus luteus* ATCC 9341 suspended in antibiotic medium 1.

**Toxicity study: animals and treatment.** Another group of 120 rats received either saline (NaCl, 0.9%; control) or tobramycin at doses of 4 and 40 mg/kg per day (every 12 h administered intraperitoneally during 4 and 10 days). Both the control and tobramycin groups were given saline (same volume as the volume of drug, approximately 0.2 ml), daptomycin at a dose of 20 mg/kg per day (every 12 h administered s.c.), or vancomycin at a dose of 50 mg/kg per day (every 12 h administered s.c.). Six animals per group were killed 15 h after the last injection. One hour before sacrifice, all animals received an intraperitoneal injection of [ $^3\text{H}$ ]thymidine (200  $\mu\text{Ci}$ ).

At the time of sacrifice, animals were killed by decapitation, and both kidneys were rapidly removed and bisected. One half of the left kidney was dissected and quickly frozen in dry ice for further determination of the [ $^3\text{H}$ ]thymidine/DNA ratio. The cortex of the other half was dissected. One part was quickly frozen for tobramycin assays. The other part was cut into small blocks (approximately 1  $\text{mm}^3$ ) in a drop of 2% glutaraldehyde–1% sucrose–0.1 M phosphate buffer and further processed for plastic section examination and electron microscopy. The cortex of both parts of the right kidney was also dissected. Both parts were quickly frozen in dry ice, and one half was used for antibiotic determination and the other half was used for further biochemical analysis.

**Daptomycin assays.** Samples of the kidney cortex were homogenized as described above, except that phosphate buffer was replaced by sodium polyanetholesulfonate (2%; Liquoid; Roche Diagnostics, Div. of Hoffmann-La Roche Inc., Nutley, N.J.), to inhibit tobramycin activity in the homogenate of the renal cortex. Polyanetholesulfonate had no effect on daptomycin activity. Daptomycin concentrations were evaluated as described above. Standards were prepared in blank cortex homogenized in sodium polyanetholesulfonate (2%). The percent daptomycin recovery in renal homogenate was  $91.3 \pm 12.2\%$ . The interday coefficients of variation were 2.3% at 20  $\mu\text{g/ml}$  and 8.6% at 5  $\mu\text{g/ml}$ . The standard curve was linear from 0.4 to 100  $\mu\text{g/ml}$ , with a coefficient of correlation of 0.993.

**Vancomycin and tobramycin assays.** The renal cortical accumulation of vancomycin and tobramycin was measured by a fluorescence polarized immunoassay (TDX System; Abbott Laboratories, North Chicago, Ill.). Briefly, samples of kidney cortex were homogenized in distilled water with a

Tissue-Tearor. The homogenates were sonicated with a sonicator (model 375; Bionetics Ltd., Montreal, Quebec, Canada) and diluted in the TDX buffer (Abbott Laboratories) to obtain concentrations between 0 and 10  $\mu\text{g/ml}$  for tobramycin assays and between 0 and 100  $\mu\text{g/ml}$  for vancomycin assays. Standards were prepared in blank cortex homogenates at the same tissue dilution as was used for tissue assays. Preliminary experiments showed that neither drug assay was affected by the presence of different concentrations of the other drug when they were mixed together in the same tissue homogenate. The percent recoveries of vancomycin and tobramycin in renal homogenate were  $81.9 \pm 2.5$  and  $99.1 \pm 6.9$ , respectively. The interday coefficients of variation were 3.6% at 1  $\mu\text{g/ml}$  and 3.4% at 8  $\mu\text{g/ml}$  for tobramycin and 4.6% at 7  $\mu\text{g/ml}$  and 2.0% at 75  $\mu\text{g/ml}$  for vancomycin.

**Biochemical analysis.** Sphingomyelinase (EC 3.1.4.12) activity was assayed in the cortex by previously published procedures (19). The measurement of DNA specific radioactivity was performed as described by Laurent et al. (20) on purified DNA obtained from the cortical tissue of the left kidneys. Only one kidney from each animal was used since it has been shown that the rates of DNA synthesis are similar in both kidneys of the same animal (20). Blood urea nitrogen and creatinine levels were determined with a blood urea nitrogen-creatinine analyzer (ABA-100; Abbott) from serum samples obtained at the time of sacrifice.

**Histology.** Cubes of 1  $\text{mm}^3$  were taken from the cortex and left overnight in the same fixative at  $4^{\circ}\text{C}$ . After washing with phosphate buffer (0.1 M, pH 7.4), cubes were further fixed in osmium tetroxide (2%) for 1 h at  $4^{\circ}\text{C}$ , dehydrated in ascending grades of alcohol, and embedded in Epon 812 resin. Thick sections (1  $\mu\text{m}$ ) were cut with a microtome (Ultracut E; Reichert-Jung, Cambridge Instruments Canada Inc., Montreal, Quebec, Canada), stained with hot toluidine blue, and examined by using a blind code to identify gross lesions. Thin sections that were double stained with uranyl acetate and lead citrate were examined on an electron microscope (EM 300; Phillips, Scarborough, Ontario, Canada).

Microscopic renal lesions were scored on plastic sections at a magnification of  $\times 400$ . Slides were coded so that it was impossible for the observer to identify groups. Slices came from four different pieces of the renal cortex from each rat, and four rats per group were used. Only the renal cortices of animals that were killed after 10 days of treatment were analyzed. The following lesions in the renal cortices were recorded: isolated cell necrosis, abnormal proximal tubular shape and cytoplasmic disorganization, interstitial cells (no specific identification of cell type was made), and the number of proximal tubules with metachromatic material in their lumina. This last material was only seen in the presence of acute toxicity induced by an aminoglycoside, was composed essentially of myeloid bodies, and probably came from dead cells. The number of isolated necrotic cells, the number of abnormal proximal tubules, and the number of proximal tubules which had metachromatic material in their lumina were recorded as percentages of the total number of proximal tubules on the respective slices. Scores for the interstitial cells were obtained by dividing the total number of interstitial cells (excluding endothelial capillary cells) by the total number of proximal tubules on the respective slices. The lesion scores were summed to produce a single nephrotoxicity score for each animal.

**Statistics.** Statistical analysis of the differences between groups was performed first by analysis of variance by a least-squares method. If the  $P$  value was  $<0.05$ , a group

TABLE 1. Concentrations of daptomycin in serum, urine, and kidney cortex and medulla from 1 to 24 h after a single subcutaneous injection of 10 mg/kg

Time (h)	Daptomycin concn in <sup>a</sup> :			
	Serum	Urine	Cortex	Medulla
1	13.6 ± 2.8	357 ± 119	1.82 ± 0.49	<1.0
2	19.2 ± 4.4	1,258 ± 553	3.06 ± 0.55	2.96 ± 0.03
4	24.7 ± 2.2	1,903 ± 702	4.07 ± 0.81	3.49 ± 0.78
6	18.7 ± 2.2	3,015 ± 1,457	2.88 ± 0.63	2.88 ± 0.45
12	0.93 ± 0.45	89 ± 45	<1.0 <sup>b</sup>	<1.0
24	<0.4 <sup>b</sup>	<0.4	<1.0	<1.0

<sup>a</sup> Daptomycin concentrations in serum and urine are means ± standard deviations in micrograms per milliliter. Daptomycin concentrations in cortex and medulla are means ± standard deviations in micrograms per gram.

<sup>b</sup> Lower than the limit of sensitivity.

comparison was done by using the Waller-Duncan multiple range test with the Kramer adjustment for unequal frequencies (18). A *P* value of less than 0.05 was considered significant. Calculations were made by using Statistical Analysis System software (SAS Institute Inc., Cary, N.C.).

**Materials.** Rats were purchased from Charles River Inc. (Montreal, Quebec, Canada). Daptomycin, vancomycin, and tobramycin were kindly donated by Eli Lilly Canada Inc. (Scarborough, Ontario, Canada). [*N*-methyl-<sup>14</sup>C]sphingomyelin (58 mCi/mmol) and [*methyl*-<sup>3</sup>H]thymidine (49 Ci/mmol) came from Amersham Canada Ltd. (Oakville, Ontario, Canada). DNA (from salmon testis type III) and 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. (Quebec, Quebec, Canada) and Sigma.

## RESULTS

Concentrations of daptomycin in serum, urine, and renal cortex and medulla measured from 1 to 24 h after a single s.c. injection of a dose of 10 mg/kg are shown in Table 1. A peak level in serum of 24.7 ± 2.2 µg of daptomycin per ml was obtained 4 h after the s.c. injection. Twelve hours after the injection, a concentration of 0.93 ± 0.45 µg/ml was still detectable in serum. At 24 h the concentration of daptomycin was lower than the limit of sensitivity of the microbiological assay (<0.4 µg/ml). Concentrations of daptomycin in urine reached very high values from 2 to 6 h postinjection, with a peak level occurring at 6 h. At 24 h no daptomycin was detectable in urine. Cortical and medullary levels of daptomycin were similar in both parts of the renal tissue. In fact, 4 h after the injection, renal concentrations of daptomycin reached levels of 4.07 ± 0.81 and 3.49 ± 0.78 µg/g in the cortex and medulla, respectively. Daptomycin was undetectable in renal tissue 12 h after the injection.

Daptomycin was undetectable in the renal cortices of animals 15 h after the end of 4 and 10 days of treatment at a dose of 20 mg/kg per day (given every 12 h s.c.). We did not observe any accumulation of daptomycin in the renal tissue during therapy. Figure 1 shows the influence of daptomycin and vancomycin on the intracortical accumulation of tobramycin. The accumulation of tobramycin in renal cortex was dose dependent (*P* < 0.01). Daptomycin and vancomycin had no significant effect on tobramycin levels in the cortex after either 4 or 10 days of treatment.

Similar levels of vancomycin were observed in the renal cortices of animals treated with vancomycin alone or in combination with vancomycin and tobramycin after either 4

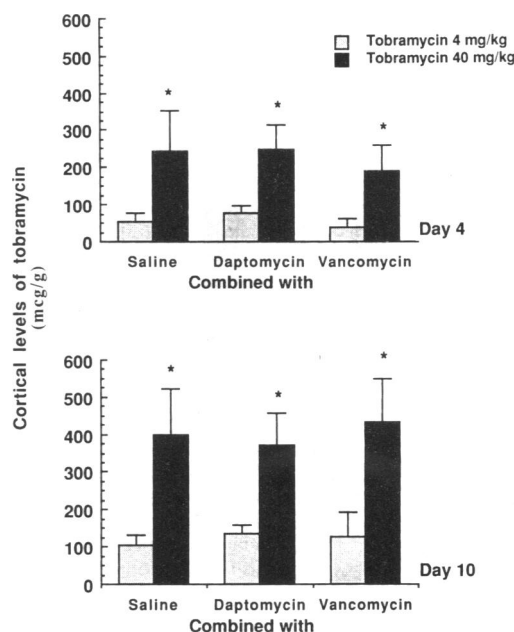


FIG. 1. Concentrations of tobramycin in the renal cortices of rats treated with tobramycin at doses of 4 and 40 mg/kg for 4 and 10 days. Tobramycin was combined with saline (NaCl, 0.9%), daptomycin (20 mg/kg), or vancomycin (50 mg/kg). Mean concentrations ± standard deviations are given. \*, *P* < 0.01 for tobramycin at 40 mg/kg versus tobramycin at 4 mg/kg.

or 10 days of treatment (Fig. 2). Vancomycin concentrations in the renal cortices were significantly higher in rats treated with the combination of vancomycin and tobramycin after 10 days of treatment compared with those in animals that were treated similarly for 4 days (*P* < 0.05).

Figure 3 shows the influence of daptomycin and vancomycin on tobramycin-induced inhibition of sphingomyelinase activity after 10 days of treatment. At day 4, no significant inhibition of sphingomyelinase activity was observed in any of the groups (data not shown). At day 10, tobramycin

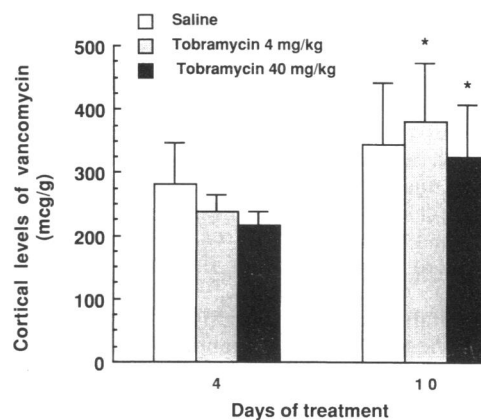


FIG. 2. Concentrations of vancomycin in the renal cortices of animals treated for 4 and 10 days. Vancomycin (50 mg/kg) was combined with saline (NaCl, 0.9%) and tobramycin at doses of 4 and 40 mg/kg. Mean concentrations ± standard deviations are given. \*, *P* < 0.05 for tobramycin at 40 mg/kg and vancomycin at 4 versus 10 days and *P* < 0.01 for tobramycin at 4 mg/kg and vancomycin at 4 versus 10 days.

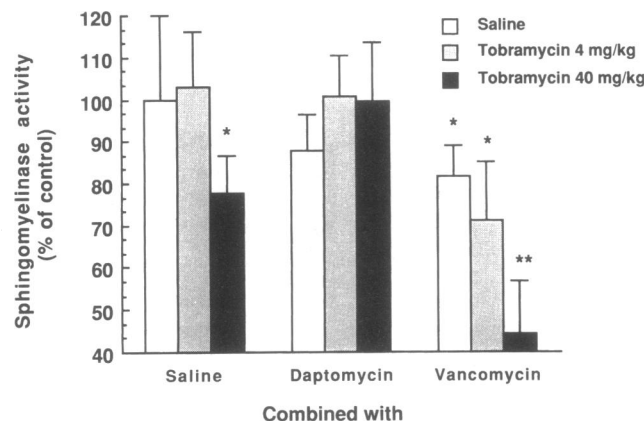


FIG. 3. Sphingomyelinase activity in the renal cortices of rats treated for 10 days with saline (NaCl, 0.9%) and tobramycin at doses of 4 and 40 mg/kg. Tobramycin was combined with saline (NaCl, 0.9%), daptomycin (20 mg/kg), or vancomycin (50 mg/kg). Mean sphingomyelinase activities  $\pm$  standard deviations are expressed as percentages of the activity found in the control group. \*,  $P < 0.01$  for tobramycin at 40 mg/kg and saline versus saline and saline, tobramycin at 4 mg/kg and saline, and tobramycin at 40 mg/kg and daptomycin; for vancomycin and saline versus saline and saline; and for tobramycin at 4 mg/kg and vancomycin versus saline and saline, tobramycin at 4 mg/kg and saline, and tobramycin at 4 mg/kg and daptomycin. \*\*,  $P < 0.01$  for tobramycin at 40 mg/kg and vancomycin versus saline and saline, tobramycin at 40 mg/kg and saline, tobramycin at 40 mg/kg and daptomycin, and tobramycin at 4 mg/kg and vancomycin.

injected at a dose of 40 mg/kg induced a significant inhibition of sphingomyelinase activity compared with that in saline-treated rats and animals injected with tobramycin at a dose of 4 mg/kg ( $P < 0.01$ ). By contrast, the sphingomyelinase activity was significantly less affected when daptomycin was combined with tobramycin ( $P < 0.01$ ). Daptomycin alone had no significant effect on sphingomyelinase activity.

Compared with the control, vancomycin induced a 20% inhibition of sphingomyelinase activity ( $P < 0.01$ ). An additive effect was observed when vancomycin was associated with tobramycin. In fact, the sphingomyelinase activity was significantly lower in the renal cortices of animals receiving the combination of tobramycin and vancomycin compared with that in animals receiving tobramycin at 4 and 40 mg/kg ( $P < 0.01$ ).

Figure 4 shows postnecrotic cell regeneration measured by the incorporation of [ $^3$ H]thymidine into renal cortex DNA in all groups after 10 days of treatment. At day 4, no significant change was observed between groups. At day 10, tobramycin injected alone at a dose of 40 mg/kg induced a twofold increase of DNA synthesis compared with that in control animals ( $P < 0.01$ ). By contrast, when daptomycin was associated with tobramycin, the [ $^3$ H]thymidine incorporation into DNA was similar to the control value, suggesting that the combination of tobramycin and daptomycin is significantly less nephrotoxic than is tobramycin alone ( $P < 0.01$ ). Similar [ $^3$ H]thymidine incorporation into DNA was observed in animals treated with the combination of vancomycin and tobramycin compared with that in animals treated with tobramycin alone. Vancomycin injected alone had no effect on cellular regeneration in the renal cortex. Blood urea nitrogen and serum creatinine were in the normal range throughout the experiment in all groups.

Histologically, plastic sections showed that tobramycin induced typical signs of aminoglycoside nephrotoxicity (17)

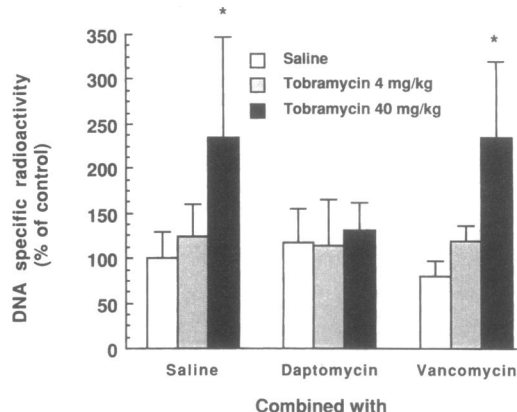


FIG. 4. DNA specific radioactivity in the renal cortices of rats measured after 10 days of treatment with tobramycin (4 and 40 mg/kg). Tobramycin was combined with saline (NaCl, 0.9%), daptomycin (20 mg/kg), or vancomycin (50 mg/kg). Mean DNA specific radioactivities  $\pm$  standard deviations are expressed as percentages of the radioactivity (disintegrations per minute per milligram of DNA) measured in the control group. \*,  $P < 0.01$  for tobramycin at 40 mg/kg and saline versus saline and saline, tobramycin at 4 mg/kg and saline, and tobramycin at 40 mg/kg and daptomycin and for tobramycin at 40 mg/kg and vancomycin versus saline and saline, tobramycin at 4 mg/kg and vancomycin, and tobramycin at 40 mg/kg and daptomycin.

in the renal cortex. The cortices of animals injected with tobramycin at a dose of 4 mg/kg showed minimal alterations, while large lysosomes containing numerous myeloid bodies were observed in animals injected with tobramycin at a dose of 40 mg/kg. Animals injected with daptomycin exhibited large vacuoles in proximal tubular cells which appeared with all of the doses of tobramycin used. Electron microscopic examination showed large membrane-enclosed vacuoles with various internal densities (Fig. 5). Signs of tobramycin nephrotoxicity were also less severe when daptomycin was used in combination with tobramycin. Treatment with vancomycin induced the appearance of numerous small vacuoles throughout the cytoplasm of proximal tubular cells. When tobramycin was used in combination with vancomycin, signs of vancomycin and tobramycin nephrotoxicity were still present in proximal tubular cells.

Table 2 shows the mean nephrotoxicity scores for each treatment group and the mean scores for each of the four renal lesions by treatment group. The scores for isolated cell necrosis were more important in the renal cortices of animals receiving tobramycin alone at a dose of 40 mg/kg and in all vancomycin-treated groups. A lower count of isolated cell necrosis was obtained in animals receiving daptomycin and tobramycin at a dose of 40 mg/kg compared with that in animals receiving tobramycin alone at the same dose. More abnormal tubules were seen when vancomycin was associated with tobramycin at a dose of 40 mg/kg compared with the number of tubules seen when tobramycin was used alone. By contrast, a lower score was observed when tobramycin was combined with daptomycin. No difference was observed in interstitial cell scores among groups. The presence of metachromatic material, which was composed of myeloid bodies in the lumina of proximal tubules, was only present in tobramycin-treated rats and was found more frequently (although not significantly so) in animals treated with tobramycin alone compared with that present in those receiving tobramycin in combination with daptomycin. A lower count was also observed in the group receiving tobra-

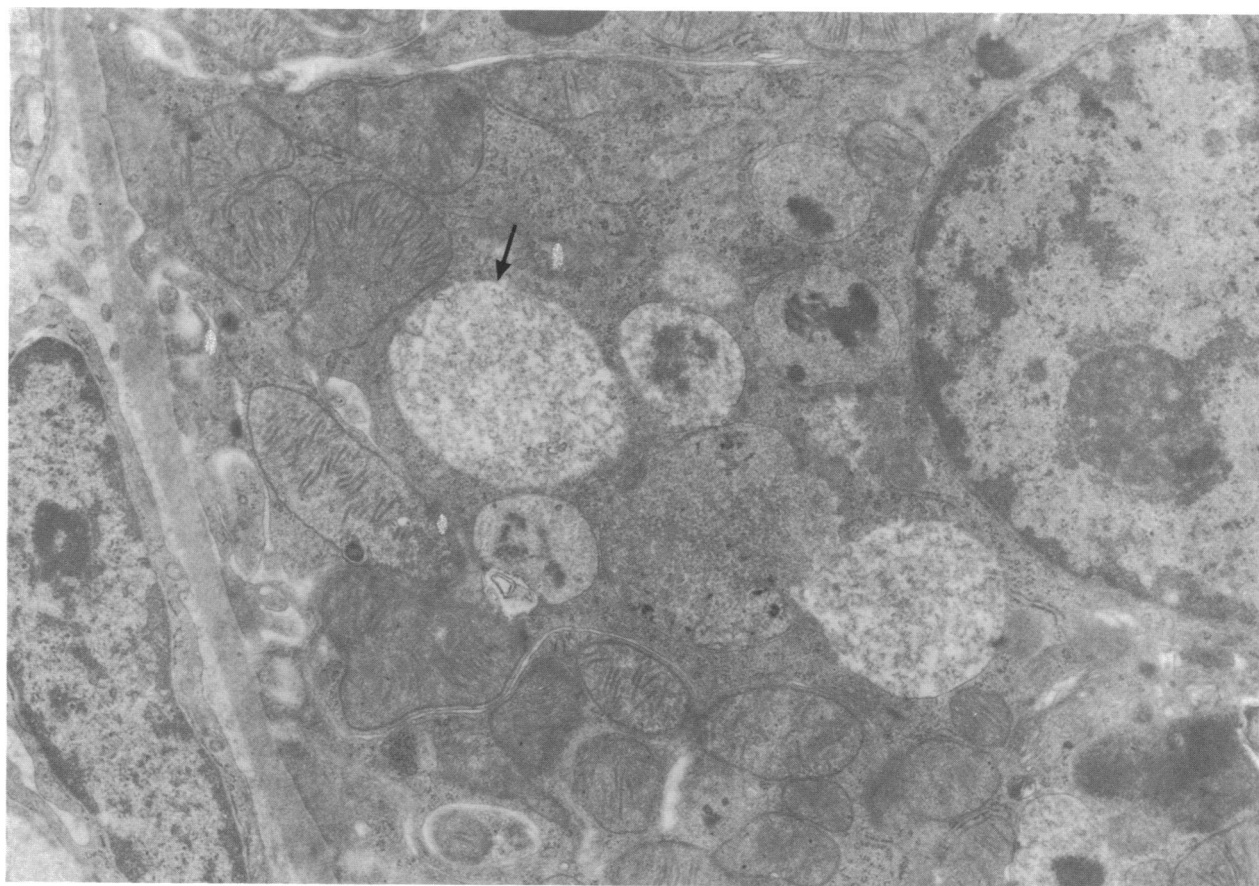


FIG. 5. Typical appearance of proximal tubular cells from animals treated with daptomycin (20 mg/kg) for either 4 or 10 days. The cytoplasm is filled with numerous large vacuoles containing heterogeneous material (arrow). Uranyl acetate and lead citrate stains were used. Magnification,  $\times 14,407$ .

mycin at 40 mg/kg and vancomycin compared with that observed in the group receiving tobramycin alone (not significant).

The highest total nephrotoxicity score was observed in animals that were treated with the combination of tobramycin (40 mg/kg) and vancomycin ( $P < 0.01$ ; compared with that seen in animals treated with tobramycin alone). By contrast, a lower score was obtained when tobramycin given at a dose of 40 mg/kg was used in combination with daptomycin ( $P < 0.01$ ; compared with that in animals treated with tobramycin alone).

Tobramycin induced the formation of large lysosomes containing numerous myeloid bodies. When daptomycin was combined with tobramycin, the lysosomes were smaller and were also filled with myeloid bodies that were similar to those observed in the groups receiving tobramycin. However, these myeloid bodies were more frequently associated with clear vacuoles that were similar to those observed in the proximal tubular cells of animals treated with daptomycin alone (Fig. 5). The proximal tubular cells of animals treated with vancomycin showed the presence of numerous small clear vacuoles throughout the cytoplasm. These vacuoles were also present in the proximal tubular cells of animals that were treated with vancomycin and tobramycin. Moreover, large lysosomes containing myeloid bodies were also observed in the animals treated with vancomycin-tobramycin, but these myeloid bodies were smaller and had a

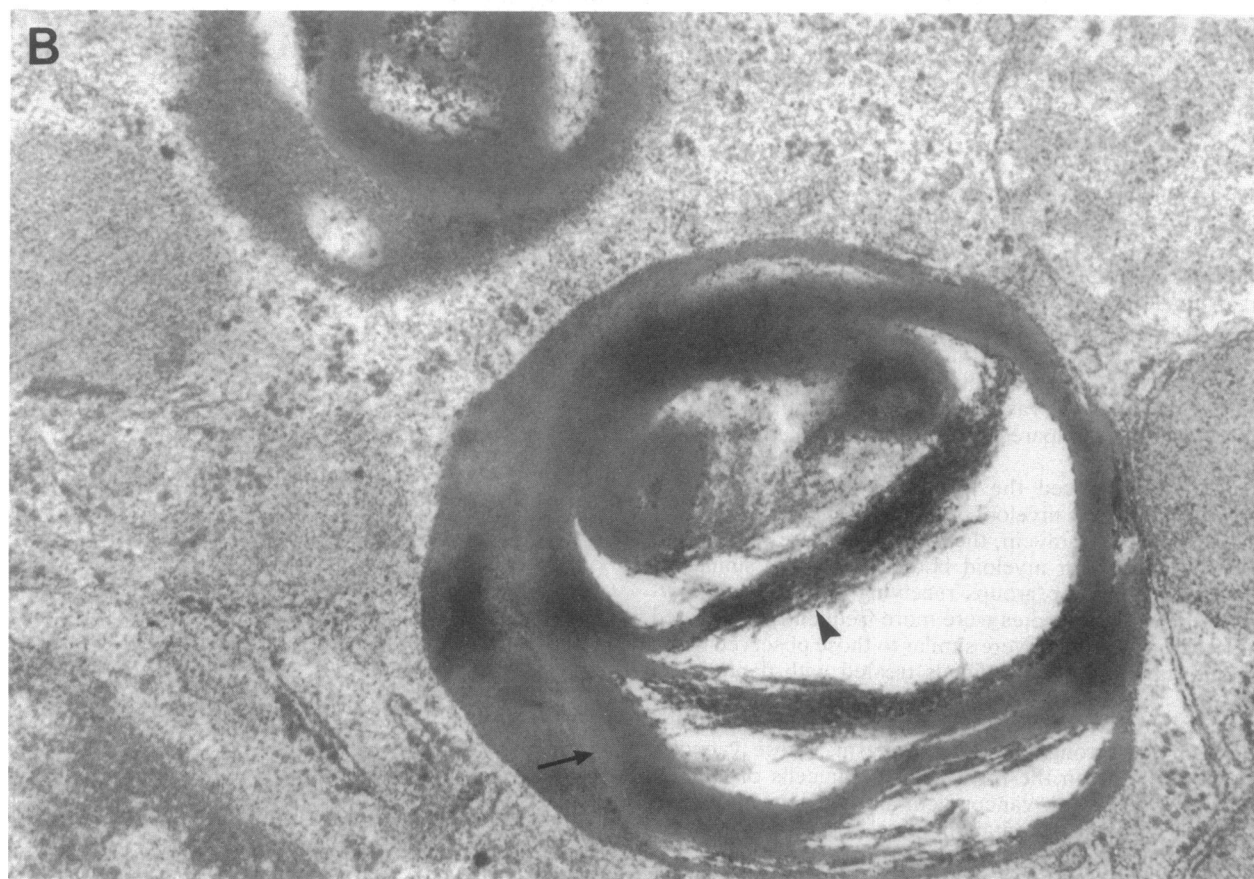
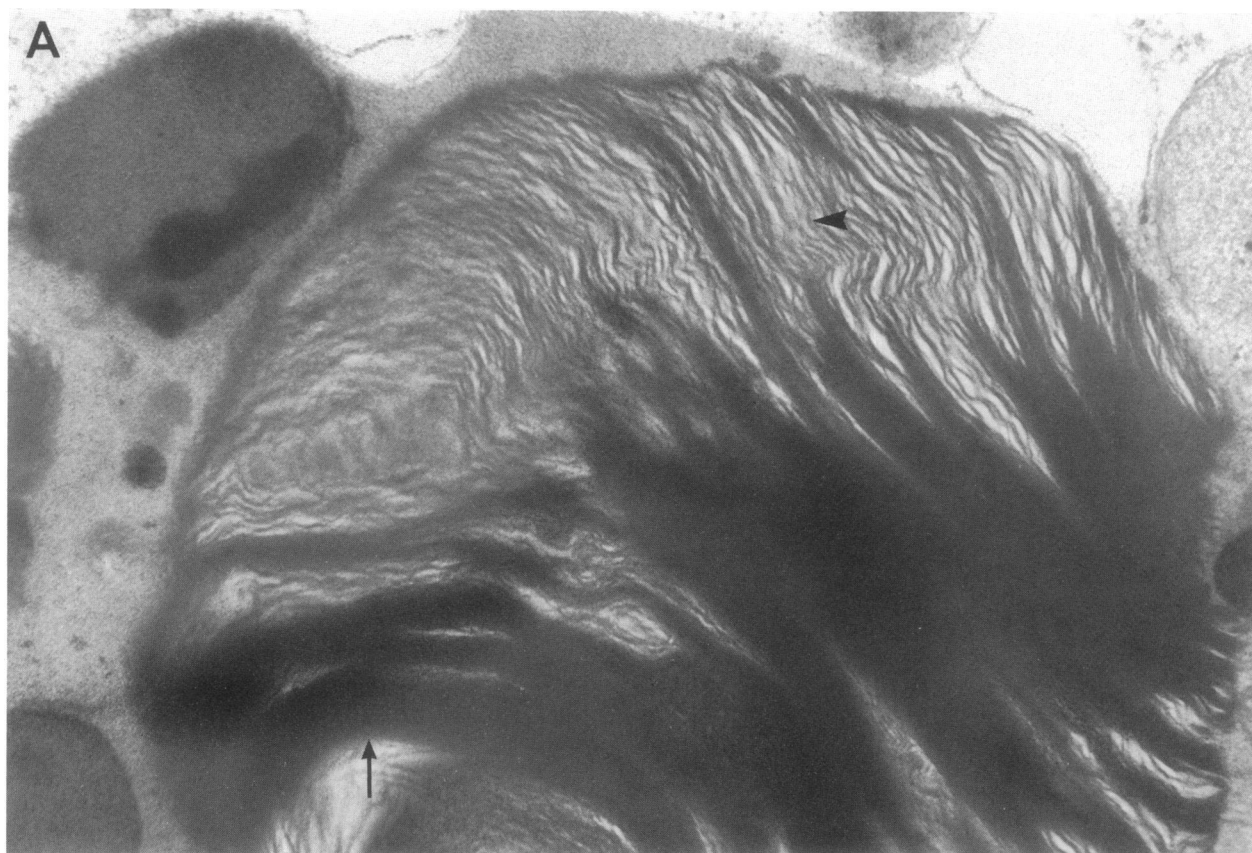
TABLE 2. Mean histopathologic nephrotoxicity scores in rats

Group	Mean score for each renal lesion <sup>a</sup>				Total mean nephrotoxicity score $\pm$ SD <sup>b</sup>
	ICN	AT	IC	MM	
Saline	0	0	1.80	0	1.80 $\pm$ 0.62 <sup>c</sup>
Saline + tobramycin (4 mg/kg)	0.72	0	1.89	0	2.61 $\pm$ 1.45 <sup>c</sup>
Saline + tobramycin (40 mg/kg)	2.64	6.86	2.23	8.39	20.12 $\pm$ 9.67 <sup>d</sup>
Daptomycin	0.57	0.12	1.70	0	2.39 $\pm$ 1.19 <sup>c</sup>
Daptomycin + tobramycin (4 mg/kg)	0.23	0.09	1.78	0.22	2.32 $\pm$ 1.29 <sup>c</sup>
Daptomycin + tobramycin (40 mg/kg)	0.34	4.61	1.97	5.97	12.89 $\pm$ 9.27 <sup>e</sup>
Vancomycin	2.35	4.74	1.61	0	8.70 $\pm$ 3.95 <sup>e</sup>
Vancomycin + tobramycin (4 mg/kg)	3.07	3.75	1.93	0.58	9.33 $\pm$ 5.58 <sup>e</sup>
Vancomycin + tobramycin (40 mg/kg)	3.19	16.65	1.61	5.66	27.11 $\pm$ 13.19 <sup>f</sup>

<sup>a</sup> Abbreviations: ICN, isolated cell necrosis; AT, abnormal tubules (see text); IC, interstitial cells; MM, tubules with metachromatic material in their lumina.

<sup>b</sup> Mean values with a common letter were not significantly different at a level of 1%.





different shape. In fact, the myeloid bodies that were observed in tobramycin-treated animals showed well-defined, tightly and loosely apposed lamellae (Fig. 6A). By contrast, when tobramycin was combined with vancomycin, the loosely apposed lamellae disappeared, but the tightly apposed lamellae were still present (Fig. 6B). Electron-dense, filamentlike material was observed close to the tightly apposed lamellae.

### DISCUSSION

In the present study we showed that daptomycin protects kidney cells from tobramycin-induced nephrotoxicity. Daptomycin did not accumulate in the renal cortex and had no influence on the renal uptake of tobramycin. By contrast, the combination of tobramycin and vancomycin had an additive effect on the inhibition of sphingomyelinase activity and induced more severe cellular lesions than did tobramycin alone. Vancomycin had no effect on the cortical accumulation of tobramycin, and similar cortical levels of vancomycin were measured in vancomycin- and vancomycin-tobramycin-treated rats. The protection of daptomycin against tobramycin-induced nephrotoxicity has been observed previously (37) in male Fisher 344 rats with higher doses of tobramycin (80 mg/kg per day).

Actually, there are no data reported in the literature on any renal adverse effect related to daptomycin treatment. Our results show that daptomycin did not affect renal function (blood urea nitrogen or serum creatinine), sphingomyelinase activity, and cellular regeneration; but histologically, we observed large clear vacuoles with various internal densities in proximal tubular cells of all daptomycin-treated animals throughout the experiment. The shape of these vacuoles was similar to that observed by Maunsbach et al. (24) in proximal tubular cells following intravenous infusion of glucose. In fact, they observed a gradual accumulation of large cytoplasmic bodies with a flocculent content in the cells. Our experiment was not designed to check for the posttreatment disappearance of these vacuoles, but they were still present in proximal tubular cells at the time of sacrifice (15 h after the last injection of daptomycin), at a time when we did not detect any daptomycin accumulation in the renal cortex (limit of sensitivity, 1  $\mu$ g/g of tissue). It remains to be determined whether (i) daptomycin is directly responsible for the formation of these vacuoles, (ii) daptomycin is still present within these vacuoles at low concentration, and (iii) daptomycin interferes with normal endocytosis and lysosome interactions. These vacuoles have not been observed by other investigators (37) in animals.

The mechanism by which daptomycin protects proximal tubular cells from tobramycin nephrotoxicity is unknown and remains to be elucidated. Several investigators have tried to identify inhibitors of aminoglycoside-induced nephrotoxicity, but the clinical interest remains uncertain. Humes et al. (14) and Bennett et al. (4) showed that calcium supplementation protects proximal tubular cells from gentamicin nephrotoxicity without modifying peak levels of the drug for renal control. Nitrendipine, a calcium channel inhibitor, was found to be effective in protecting rats from

gentamicin toxicity by measuring inulin clearance and histopathology (21). The concomitant administration of ticarcillin (10) and carbenicillin (7) also protected kidney cells from tobramycin and gentamicin nephrotoxicity, respectively. More recently, Kacew (16) demonstrated that the simultaneous injection of pyridoxal 5'-phosphate with gentamicin treatment for 2 weeks was effective in blocking aminoglycoside-induced kidney phospholipidosis and elevating urinary enzyme excretion as well as renal phospholipase C and alkaline phosphatase.

Our results showed that the cortical uptake of tobramycin is not modified by daptomycin treatment. This suggests that daptomycin protects proximal tubular cells against tobramycin nephrotoxicity without affecting the intracellular concentration of tobramycin. Similar results have been observed with gentamicin (3, 12) and amikacin (35) in animals treated with poly-L-aspartic acid.

Williams et al. (35) suggested that cortical accumulation of an aminoglycoside is not the determining factor in nephrotoxicity. They proposed that membrane binding of the aminoglycosides is the critical factor in the pathogenesis of nephrotoxicity. Tobramycin binding to the renal membrane in the presence or absence of daptomycin was not measured in the present study, but daptomycin may have reduced tobramycin binding to the renal membrane as poly-L-aspartic acid did, reducing subsequent cellular toxicity. The concomitant use of daptomycin with tobramycin might have delayed the toxic insult induced by tobramycin, but the experiments in the present study were not designed to examine this hypothesis.

Daptomycin and tobramycin were injected at the same time every 12 h. Our findings on daptomycin distribution showed that this drug was still present in serum and urine 12 h after it was injected, when another injection was given to the animals. This means that daptomycin was constantly present in the urinary space at the luminal level of proximal tubular cells throughout the experiment. Daptomycin might exert its protective effect by interacting on brush border receptors, sites where aminoglycosides bind to acidic phospholipids (30).

Autoradiography (6, 15, 31) and cell fractionation studies *in vivo* in animal models (26) and in cultured fibroblasts (2, 34) have demonstrated the lysosomal sequestration of gentamicin. Giurgea-Marion et al. (13) have shown, using subcellular fractionation techniques, that aminoglycosides are associated with lysosomes throughout 9 days of treatment, where they induce well-known specific alterations (19). The presence and persistence of clear vacuoles or pinocytic vesicles in the cytoplasm of proximal tubular cells of rats treated with daptomycin may suggest that a fraction of the tobramycin that is reabsorbed by endocytosis is sequestered within these clear vacuoles, preserving in part lysosomes from aminoglycoside-induced inhibition of phospholipase activity.

Another hypothesis would be that daptomycin also accumulates in the lysosomes, preserving tobramycin-induced lysosomal toxicity by an unknown mechanism. The subcellular localization of daptomycin and tobramycin in the

FIG. 6. Differences observed by electron microscopy between myeloid bodies observed in the lysosomes of rats treated with tobramycin (40 mg/kg) and saline (A) and myeloid bodies observed in the lysosomes of rats treated with the combination of tobramycin (40 mg/kg) and vancomycin (50 mg/kg) (B). Tightly (arrow) and loosely (arrowhead) apposed lamellae are well defined in panel A, but loosely apposed (arrowhead) lamellae are apparently absent in panel B. However, tightly apposed (arrow) lamellae are still present in both groups (A and B) and are easily seen at higher magnification. Uranyl acetate and lead citrate stains were used. Magnifications,  $\times 56,817$ .

presence of daptomycin must be investigated to shed more light on the mechanism of protection of daptomycin on tobramycin nephrotoxicity.

The mechanism of vancomycin nephrotoxicity is unknown. Marre et al. (22, 23) have suggested that there are similarities between the mechanisms of renal damage induced by both vancomycin and aminoglycosides, since renal tolerance of both compounds is increased by D-glucaro-1,5-lactam, sodium chloride, and fosfomycin. In the present study, vancomycin induced a significant inhibition of sphingomyelinase activity, which has already been observed (M. P. Francq-Dufief, G. Laurent, G. Toubeau, J. A. Heuson-Stiennon, and P. M. Tulkens, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 23, 1987), and histological alterations to proximal tubular cells. Although a more severe inhibition of sphingomyelinase activity was induced by the coadministration of vancomycin with tobramycin, this association was not more lethal for the tubular cells compared with the lethality caused by the administration of tobramycin alone. In fact, as shown in Table 2, the isolated cell necrosis scores were similar for rats treated with tobramycin-vancomycin and animals treated with tobramycin, while abnormal tubules were observed in approximately 16% of the proximal tubules of animals treated with the combination of tobramycin and vancomycin. Experiments with different dosages of both drugs used in combination should be done to better understand this toxic interaction. It should also be taken into consideration that the effect of an aminoglycoside on phospholipase activity is probably not the only determining factor that contributes to cell death. Other interactions of both drugs on membrane and cytoplasmic enzyme activities, organic anion and cation transport, mitochondrial function, etc., and on other parameters that have never been investigated could not be excluded.

One important finding of the present study was the difference that was observed in the shape between myeloid bodies found in rats treated with tobramycin and those found in rats treated with the combination of tobramycin and vancomycin. In fact, loosely apposed lamellae were apparently absent in the latter group. We speculate that vancomycin accumulates in the lysosomes and modifies such a condition, favoring the formation of tightly apposed lamellae. The importance and implication of this finding in the pathogenesis of aminoglycoside toxicity is unclear.

Subcellular distribution studies of vancomycin must be done to clarify its transport and site of accumulation. Our electron microscopic and enzymatic studies suggest that both drugs disturb lysosomal function and that although the mechanism of toxicity may be different, the target site may be the same. Additional studies on the biochemical composition of the lysosomes to explore the variation in structure we observed may shed some light on the biochemical interaction between lysosomes and these antibiotics.

In conclusion, we showed that daptomycin is safe and, in terms of toxicity, is a good alternative to vancomycin since daptomycin protects from and vancomycin may enhance tobramycin-induced nephrotoxicity.

#### ACKNOWLEDGMENTS

We thank Danielle Gagné and Lise Villeneuve for secretarial help and Pierre Provencher for statistical analysis.

This work was supported by a grant from Eli Lilly & Co., Indianapolis, Ind., and by grant MA-10157 from the Medical Research Council of Canada. D.B. is scholar of the P.M.A.C. Health

Research Foundation. M. Pellerin was a fellow of the Fonds de la recherche en santé du Québec.

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