Studies on the Nephrotoxicity of Aminoglycoside Antibiotics and Protection from These Effects (4)

Effects of Tobramycin Alone and in Combination with Latamoxef on the Stability of Rat Kidney Lysosomal Membranes

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Abstract—Effects of tobramycin (TOB) alone and in combination with latamoxef (LMOX) on the stability of rat kidney lysosomal membranes were investigated. Rats were injected with doses of TOB (90 mg/kg/day, s.c.) alone, LMOX (2,000 mg/kg/day, s.c.) alone or TOB (90 mg/kg/day, s.c.) and LMOX (2,000 mg/kg/day, s.c.) for 5 consecutive days. The rat kidney lysosomes were isolated on the 1st, 3rd and 5th days and incubated in a 0.25 M sucrose solution containing 1 mM EDTA (pH 7.0) at 37°C for 20 min. After incubation, the activity of N-acetyl-β-D-glucosaminidase (NAG) released from lysosomes was measured, and the percent NAG release was calculated as an index of the stability of lysosomal membranes. The percent releases of NAG from lysosomes of TOB alone-treated rats were 40 and 50% greater than those of normal rats on the 1st and 3rd days, respectively. On the other hand, treatment with TOB and LMOX suppressed the NAG release from lysosomes with TOB alone by about 80 to 100%. There were insignificant slight increases in the percent NAG release in LMOX alone-treated rats on the 3rd and 5th days. In addition, the in vitro study indicated that incubation of the lysosomal fraction from kidneys of normal rats with TOB (30 μg/ml) significantly increased the NAG release, compared with that of the non-treated lysosomal fraction. However, the preincubated mixture of TOB (30 μg/ml) and LMOX (50 μg/ml) in vitro significantly suppressed the release of NAG from lysosomes by 85%. These results suggest that the suppression of the releases of NAG from lysosomes by the combination of TOB with LMOX may contribute to the protective effect of LMOX against TOB nephrotoxicity.

We have already demonstrated that combination with latamoxef (LMOX), an oxacephem antibiotic, protects rat kidneys from the nephrotoxicity of tobramycin (TOB), one of the aminoglycoside antibiotics (AGs), and the protective effect of LMOX may be partially due to the suppression of TOB accumulation in kidneys (1). Just and Habermann (2) and Silverblatt and Kuehn (3) reported that gentamicin (GM) binds to rat renal brush border membrane and is accumulated within the lysosomes of proximal tubular cells by pinocytosis. Recently, it has been proposed that the pathogenesis of AGs nephrotoxicity may involve membrane injuries of lysosomes accumulating AGs or myeloid bodies and the subsequent releases of various hydrolytic lysosomal enzymes into the cytosol may lead to renal tubular cell necrosis (4). Viotte et al. (5) reported that treatment with dibekacin increased the membrane permeability of lysosomes prepared from rat renal cortex in vivo. It has also been demonstrated that GM increases the release of lysosomal enzymes such as N-acetyl-β-D-glucosaminidase (NAG), acid phosphatase...
(ACP) and β-glucuronidase from non-treated rat kidney lysosomes in vitro (6, 7). In addition, Fry and Plummer (8) and Ngaha (9) reported that cephaloridine (CER), a β-lactam antibiotic, stabilized rat kidney lysosomal membranes in vitro and in vivo. These several lines of evidence suggest that the release of enzymes from rat kidney lysosomes by TOB may be suppressed in vivo when LMOX, having a β-lactam structure in the molecule, is given to rats simultaneously with TOB. Therefore, the present study was designed to determine whether the release of NAG from renal cortical lysosomes by TOB would be suppressed by combination with LMOX in rats.

Materials and Methods

Animals: Male Sprague-Dawley strain rats weighing approx. 230 g (Shizuoka Laboratory Animal Center) were used. These animals were housed in an air-conditioned room at 23±1°C.

Drugs: Drugs used were tobramycin (TOB, Shionogi Co., Ltd.) and latamoxef (LMOX, Shionogi Co., Ltd.). Both TOB and LMOX were dissolved in saline.

Preparation of lysosomes: Renal cortical lysosomes were prepared by slightly modifying the methods described by Shibko and Tappel (10) and Harikumar and Reeves (11). Rats were anesthetized with sodium pentobarbital (32.4 mg/kg, i.p.), and a midline abdominal incision was made to expose both kidneys. The kidneys were perfused with 0.25 M sucrose containing 1 mM EDTA adjusted to pH 7.0 (sucrose/EDTA) to wash out the blood. The renal cortical tissues were separated, weighed, minced and diluted 1:8 with sucrose/EDTA. The tissues were then homogenated using a glass/teflon homogenizer, followed by centrifuging at 650×g for 2.5 min. The resulting supernatant was recentrifuged at 5,900×g for 5 min. The resultant pellets were resuspended in 100 μl of sucrose/EDTA and layered on the top of the preformed Percoll gradient and centrifuged at 24,500×g for 20 min. The densest zone of turbidity was collected, suspended in a 10-fold excess of sucrose/EDTA and centrifuged at 1,100×g for 12 min to sediment the lysosomes. The final pellet was used for measuring the stability of the lysosomal membranes. In addition, the grade of preparation of the lysosomal fraction was estimated by calculating the ratio of the specific activities of lysosomal enzymes, NAG and ACP, in the lysosomal fraction to those of these enzymes in the homogenate.

Stability of lysosomal membranes: The prepared lysosomal fraction was resuspended in sucrose/EDTA, divided into two aliquots and incubated at 37°C for 20 min. After incubation, the lysosomal fractions were quickly cooled in an ice bath, and then one aliquot was centrifuged at 15,000×g for 10 min. The resultant supernatant was used for measuring NAG activity and protein content released from the lysosomes. The total NAG activity and protein content also were measured in the other aliquot of the lysosomal fraction. The total NAG activity was determined after the treatment of lysosomes with Triton X-100 at a final concentration of 0.1%. The stability of lysosomal membranes was estimated by calculating the percent NAG release as follows: percent NAG release= (free specific activity of NAG in supernatant / total specific activity of NAG in supernatant / total specific activity of NAG)×100.

Protein content and NAG (EC 3.2.1.30) and ACP (EC 3.1.3.2) activities: Protein content was measured by the method described by Lowry et al. (12) using bovine serum albumin as a standard. NAG activity was assayed by the method of Hasebe (13) using p-nitrophenyl-N-acetyl-β-D-glucosaminide as a substrate. ACP activity was measured using Acid Phosphate-Color-Test (Boehringer). One milliunit of activity of NAG and one unit of activity of ACP were defined as the amount of enzyme liberating 1 μmole of p-nitrophenol/ml/hr and 0.06 mmole of p-nitrophenol/ml/hr at 37°C, respectively. The specific activities of NAG and ACP were expressed as mU/mg protein and U/mg protein, respectively.

Effects of TOB alone and in combination with LMOX on the stability of lysosomal membranes in vivo and in vitro: In vivo, rats were given daily s.c. doses of TOB (90 mg/
kg) alone, LMOX (2,000 mg/kg) alone or TOB (90 mg/kg) and LMOX (2,000 mg/kg) for 5 consecutive days. Normal rats were given daily s.c. with saline. Rats of each group were sacrificed on the 1st, 3rd and 5th days. The kidney lysosomes were isolated, and the stability of lysosomal membranes was measured by the method described above. Additionally, urinary NAG activities in normal and LMOX (2,000 mg/kg/day, s.c.) alone-treated rats were measured at various times by the method described previously (1).

In the in vitro test, the lysosomal fraction was prepared from the renal cortex of normal rats by the method described above. TOB and LMOX were separately dissolved in sucrose/EDTA. Then, TOB (30 μg/ml) alone and the mixture of TOB (30 μg/ml) and LMOX (50 μg/ml) were incubated at 37°C for 15 min. After incubation, the drug solutions were added to the lysosomal fraction, and the lysosomal fractions were incubated at 37°C for 20 min. The subsequent procedures were performed by the method described above.

**Statistical analysis:** Results in the text, tables and figures are expressed as the mean±S.E. Student's t-test was used for statistical analysis. The percent suppression in results was derived from the following formula: \((T-L)/(T-N)\times100\) (where \(T=\text{TOB}\), \(L=\text{TOB+LMOX}\) and \(N=\text{Normal}\)).

**Results**

1. **Preparation of kidney lysosomes:** Table 1 summarizes the specific activities of NAG and ACP in the lysosomal fraction and homogenate. When the lysosomal fraction was prepared by differential centrifugation, the specific activities of NAG and ACP in the lysosomal fraction were about 8- and 6-fold greater than those in the homogenate, respectively. Furthermore, the preparation with Percoll density gradient centrifugation augmented the specific activities of NAG and ACP in the lysosomal fraction by about 32- and 14-fold as compared to the respective values in the homogenate.

Table 2 summarizes the percent release of NAG from intact lysosomes incubated at 37°C for 20 min and 40 min. Since an incubation time which produced about 50% release of NAG from intact lysosomes was considered to be suitable, all of the lysosomal fractions were incubated at 37°C for 20 min.

2. **Effects of TOB alone and in combination**

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| Enzyme                        | Specific activity | Homogenate | Lysosomal fraction |
|-------------------------------|-------------------|------------|-------------------|
| **Differential centrifugation** |                   |            |                   |
| NAG (n=10)                    | 2.82±0.19         | 21.37±1.68 |                   |
| ACP (n=10)                    | 0.038±0.004       | 0.245±0.017|                   |
| **Percoll density gradient centrifugation** |   |            |                   |
| NAG (n=15)                    | 2.37±0.14         | 73.61±5.39 |                   |
| ACP (n=15)                    | 0.033±0.001       | 0.457±0.024|                   |

The values are expressed as the mean±S.E. NAG: N-acetyl-β-D-glucosaminidase, ACP: Acid phosphatase. The specific activities of NAG and ACP were given as mU/mg of protein and U/mg of protein, respectively.

| Table 2. Percent N-acetyl-β-D-glucosaminidase release from lysosomes after incubation at 37°C for 20 min or 40 min |
|---------------------------------------------------|-------------------|
| 20 min                                            | 40 min            |
| Percent NAG release                               |                   |
| 53.5±1.9                                          | 76.2±2.3          |

The values are expressed as the mean±S.E. NAG: N-acetyl-β-D-glucosaminidase.
with LMOX on release of NAG from rat kidney lysosomes in vivo: As shown in Fig. 1, the percent release of NAG from the kidney lysosomes of rats receiving TOB (90 mg/kg/day, s.c.) significantly increased (44%, $P<0.01$), compared with that of normal animals on the 1st day. On the other hand, treatment with TOB and LMOX (2,000 mg/kg/day, s.c.) suppressed the increase in the percent NAG release with TOB alone by about 75% ($P<0.05$). On the 3rd day, the percent NAG release with TOB alone was about 50% ($P<0.01$) greater than normal, while the percent NAG release in combination with LMOX resulted in complete suppression ($P<0.01$) of the percent NAG release with TOB alone. No significant differences between all treatment groups were observed on the 5th day. The percent NAG release with LMOX alone was similar to that of normal rats on the 1st day. Then, it slightly (though not significantly) elevated on the 3rd and 5th days. In addition, treatment with LMOX alone showed no obvious changes in urinary NAG activities throughout the experimental period (Table 3).

3. Effects of TOB alone and in combination
with LMOX on release of MAG from rat kidney lysosomes in vitro: The addition of TOB (30 μg/ml) to the lysosomal fraction significantly increased the release of NAG from intact lysosomes by 43% (P<0.01) as shown in Fig. 2. However, when the lysosomal fraction was incubated with the preincubated mixture of TOB (30 μg/ml) and LMOX (50 μg/ml), the percent NAG release from lysosomes was suppressed by 85% (P<0.05).

Discussion

Recently, it has been proposed that the lysosomes in renal tubular cells may play a role in the pathogenesis of AGs nephrotoxicity via the release of hydrolytic lysosomal enzymes into the cytosol, because striking alterations such as myeloid bodies in renal tubular lysosomes of animals (14) and humans (15) or in lysosomes of cultured cells (16) are observed.

In the present study, we isolated rat renal cortical lysosomes and determined the effects of TOB alone and in combination with LMOX on the stability of rat kidney lysosomal membranes. Differential centrifugation and Percoll density gradient centrifugation techniques were used to isolate the lysosomal fraction from rat renal cortex. When rat kidney lysosomes were isolated using only differential centrifugation, the specific activities of NAG and ACP in the lysosomal fraction were 8- and 6-fold greater than those in the homogenate, respectively. The use of Percoll density gradient centrifugation to separate lysosomes from mitochondria (11) resulted in a greater ratio of the specific activities of NAG and ACP than those obtained by differential centrifugation (Table 1). Although in the present study, we did not measure mitochondrial marker enzymes such as succinate dehydrogenase to confirm the contamination of mitochondria, the observed specific activities of these two enzymes in the lysosomal fraction indicated that a more purified lysosomal fraction was obtained by the density gradient centrifugation. Thus, density gradient centrifugation was used to prepare the lysosomal fraction.

Morin et al. (6) have reported that the addition of GM (25 μg/ml) to the lysosomal fraction markedly increases the release of enzymes from isolated rat kidney lysosomes in vitro, and treatment with GM (150 mg/kg/day, s.c.) produces a slight but significant enhancement of the lysosomal membrane susceptibility to thermal shock in vivo. In the present study, we also demonstrated that the lysosomal fraction prepared from TOB alone-treated rats produced greater NAG release than the fraction from normal rats did on the 1st and 3rd days. However, the percent NAG release with TOB alone showed nearly a normal level on the 5th day (Fig. 1). No differences between normal rats and those treated with TOB alone on the 5th day may be explained by the following consideration: the lysosomes prepared from renal cortex derive from proximal tubular cells, distal tubular cells, and mesangial cells, endothelial cells and epithelial cells composing glomeruli. Since proximal tubular cells are the main target cells for AGs, it is considered that the release of NAG from the lysosomal fraction in TOB-treated rats mainly reflects that from lysosomes in proximal tubular cells. On the 5th day, however, the lysosomes derived from proximal tubular cells might be disrupted during the lysosome preparation, and consequently, intact lysosomes obtained from cells except for proximal tubular cells might show the normal release of NAG.

The mechanisms of increase in releases of enzymes from lysosomes prepared from AGs-treated rats remain to be fully elucidated. Recently, it has been reported by some investigators that AGs can interact with lipid monolayers containing polyphosphoinositides (17) and liposomes containing acidic phospholipids (18) as well as displace calcium binding to lipid monolayers and biomembranes (19).

Wang et al. (20) demonstrated the fluidizing effect of neomycin on liposomes containing phosphatidylserine and phosphatidylinositol by differential scanning calorimetry. The changes in membrane fluidity alter the activities of membrane-bound enzymes such as 5'-nucleotidase (21), membrane permeability (22) and phase transition temperature (23). Therefore, the binding of TOB transported within lysosomes to lysosomal membranes containing acidic
phospholipids (24) and the subsequent changes in fluidity of the lysosomal membrane may induce membrane rupture or increases in membrane permeability which contributes to the increased release of NAG from lysosomes.

In this study, the combination with LMOX suppressed the release of NAG from lysosomes on the 1st and 3rd days (Fig. 2). It has been reported that the combination of GM with CER reduces the GM-induced nephrotoxicity in rats (25, 26). This protective effect of CER is considered to be partially due to suppression of lysosomal enzyme release into the cytosol via the stabilizing effect on renal lysosomal membranes, because CER is transported from the antiluminal side to proximal tubular cells (27) and stabilizes rat kidney lysosomal membranes in vitro (8) and in vivo (9). If LMOX has the stabilizing effect on rat kidney lysosomal membranes in vivo, the percent release of NAG from isolated lysosomes and urinary NAG excretion would be suppressed by treatment with LMOX. However, the percent NAG release slightly (though not significantly) increased rather than decreased by the treatment with LMOX alone in vivo (Fig. 1), and the urinary NAG activities in rats receiving LMOX alone did not differ from those observed in normal rats throughout the experimental period (Table 2). Additionally, it has been reported that LMOX is filtrated through the glomerulus mainly without transport from the antiluminal side to the cytosol (28), and no accumulation in rat tissues is observed by whole body autoradiography (29). Therefore, LMOX appears to be almost incapable of stabilizing rat kidney lysosomal membranes in vivo.

We previously reported that TOB accumulation in kidneys is reduced by combination with LMOX (1). Thus, the suppression of NAG release by combination with LMOX may result from the reduction of intrarenal TOB concentration, but not from a stabilizing effect of LMOX on kidney lysosomal membranes. The present results of our in vivo study suggest that the suppression of lysosomal enzyme releases by combination with LMOX may contribute to the protective effect of LMOX against TOB-induced nephrotoxicity.

The amino groups in the AG molecule were protonated at physiological pH, and thereby the AGs were negatively charged (30). This raises a possibility that the negatively charged amino groups in the AG molecule may electrostatically interact with anionic carboxyl groups in the LMOX molecule. Thus, in vitro, the effect of a preincubated mixture of TOB and LMOX was examined on the release of NAG from isolated lysosomes. Morin et al. (6) and Ngaha and Ogunleye (7) reported that GM or TOB increased the release of enzymes from the rat kidney lysosomes in vitro. Likewise, we demonstrated that TOB (30 μg/ml) exposure to the lysosomal fraction resulted in an increase in percent NAG release from the lysosomes. On the other hand, the mixture of TOB and LMOX caused a significant suppression of the NAG release (Fig. 2). Since we have found by paper electrophoresis and thin layer chromatography that TOB can directly interact with LMOX (31), the possibility exists that a direct interaction of TOB with LMOX may contribute to the suppression of NAG release from lysosomes in vitro. Although it is unknown whether LMOX stabilizes lysosomal membranes in vitro, LMOX in this study does not appear to have the stabilizing effect on rat kidney lysosomal membranes, at least in vivo.

In conclusion, these results indicate that the combination of TOB with LMOX suppresses NAG release from rat kidney lysosomes in vivo. This suggests that the protective effect of LMOX against TOB-induced nephrotoxicity is associated with the suppression of enzyme release from lysosomes as well as the reduction of intrarenal TOB concentration as previously described.

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References
1 Kojima, R., Ito, M. and Suzuki, Y.: Studies on the nephrotoxicity of aminoglycoside antibiotics and protection from these effects (3). Protective effect of latamoxef against tobramycin nephrotoxicity and its protective mechanism. Japan. J. Pharmacol. 42, 397–404 (1986)
2 Just, M. and Habermann, E.: The renal handling
of polybasic drugs. 2. In vitro studies with brush border and lysosomal preparations. Naunyn Schmiedebergs Arch. Pharmacol. 300, 67–76 (1977)
3 Silverblatt, F.J. and Kuehn, G.: Autoradiography of gentamicin uptake by the rat proximal tubule cell. Kidney Int. 5, 335–345 (1979)
4 Kaloyanides, G.J. and Pastoriza-Munoz, E.: Aminoglycoside nephrotoxicity. Kidney Int. 18, 571–582 (1980)
5 Viotte, G., Olier, B., Morin, J.P. and Fillastre, J.P.: Renal histological and biochemical changes induced in the rat by dibekacin. J. Pharmacol. Exp. Ther. 226, 226–231 (1983)
6 Morin, J.P., Fresel, J., Fillastre, J.P. and Vaillant, R.: Aminoglycoside actions on rat kidney lysosomes in vivo and in vitro. In Nephrotoxicity, Edited by Fillastre, J.P., p. 253–263, Masson Publishing U.S.A., Inc., New York (1978)
7 Ngaha, E.O. and Ogunleye, I.O.: Studies on gentamicin-induced labilization of rat kidney lysosomes in vitro. Possible protection by selenium. Biochem. Pharmacol. 32, 2659–2664 (1983)
8 Fry, M. and Plummer, D.T.: The stabilization of renal lysosomes by cephaloridine: The role of a membrane-bound phospholipase A₂. In Nephrotoxicity, Edited by Fillastre, J.P., p. 193–211, Masson Publishing U.S.A., Inc., New York (1978)
9 Ngaha, E.O.: Further studies on the in vivo effect of cephaloridine on the stability of rat kidney lysosomes. Biochem. Pharmacol. 31, 1843–1847 (1982)
10 Shibko, S. and Tappel, A.L.: Rat-kidney lysosomes: Isolation and properties. Biochem. J. 95, 731–741 (1965)
11 Harikumar, P. and Reeves, J.P.: The lysosomal proton pump is electrogenic. J. Biol. Chem. 258, 10403–10410 (1983)
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275 (1951)
13 Hasebe, K.: Biochemical studies on synovial fluid. Fukushima J. Med. Sci. 15, 35–44 (1968)
14 Kosek, J.C., Mazze, R.I. and Cousins, M.J.: Nephrotoxicity of gentamicin. Lab. Invest. 30, 48–57 (1974)
15 De Broe, M.E., Daullus, G.J., Verpooten, G.A., Roels, F., Buyssens, N., Wedden, R., VanHoof, F. and Tulkens, P.M.: Early effects of gentamicin, tobramycin and amikacin on the human kidney. Kidney Int. 25, 643–652 (1984)
16 Tulkens, G.A., Hoof, F.V. and Tulkens, P.: Gentamicin-induced lysosomal phospholipidosis in cultured rat fibroblasts. Quantitative ultrastructural and biochemical study. Lab. Invest. 40, 481–491 (1979)
17 Lodhi, S., Weiner, N.D. and Shacht, J.: Interaction of neomycin with monomolecular films of polyphosphoinositides and other lipids. Biochim. Biophys. Acta 557, 1–8 (1979)
18 Chung, L., Kaloyanides, G., McDaniel, R., McLaughlin, A. and McLaughlin, S.: Interaction of gentamicin and spermine with bilayer membrane containing negatively charged phospholipids. Biochemistry 24, 442–452 (1985)
19 Lullmann, H. and Vollmer, B.: An interaction of aminoglycoside antibiotics with Ca binding to lipid monolayers and biomembranes. Biochim. Pharmacol. 31, 3769–3773 (1982)
20 Wang, B.M., Weiner, N.D., Ganesan, M.G. and Schacht, J.: Interaction of calcium and neomycin with anionic phospholipid-lecithin liposomes. A differential scanning calorimetry study. Biochim. Pharmacol. 33, 3787–3791 (1984)
21 Dipple, I., Gordon, L.M. and Housley, M.D.: The activity of 5′-nucleotidase in liver plasma membrane is affected by the increase in bilayer fluidity activated by anionic drugs but not by cationic drugs. J. Biol. Chem. 257, 1811–1815 (1982)
22 Srivastava, S., Phadke, R.S., Govil, G. and Rao, C.N.R.: Fluidity, permeability and antioxidant behaviour of model membranes incorporated with α-tocopherol and vitamin E acetate. Biochim. Biophys. Acta 734, 353–362 (1983)
23 Papahadjopoulos, D.: Effect of bivalent cations and proteins on thermotropic properties of phospholipid membranes. Implications for the molecular mechanism of fusion and endocytosis. J. Colloid Interface Sci. 58, 459–470 (1977)
24 Henning, R. and Heidrich, H.G.: Membrane lipides of rat liver lysosomes prepared by free flow electrophoresis. Biochim. Biophys. Acta 345, 326–335 (1974)
25 Luft, F.C., Patel, V., Yum, M.N. and Kleit, S.A.: Nephrotoxicity of cephalosporin-gentamicin combinations in rats. Antimicrob. Agents Chemother. 9, 831–839 (1976)
26 Harrison, W.O., Silverblatt, F.J. and Turck, M.: Gentamicin nephrotoxicity: Failure of three cephalosporins to potentiate injury in rats. Antimicrob. Agents Chemother. 8, 209–215 (1975)
27 Tune, B., Fernholt, M. and Schwartz, A.: Mechanism of cephaloridine transport in the kidney. J. Pharmacol. Exp. Ther. 191, 311–317 (1974)
28 Yoshida, T., Kimura, Y. and Tochino, T.: Pharmacokinetics of 6059-S in experimental animals. Chemotherapy 28 (s-7), 194-206 (1980)
29 Sugeno, K., Okabe, H., Tanaka, H. and Norikura, R.: Disposition of 6059-S in rats, dogs and monkeys. Chemotherapy 28 (s-7), 207-235 (1980)
30 Josepovitz, C., Pastoriza-Munoz, E., Timmerman, D., Scott, M., Feldman, S. and Kaloyanides, G.J.: Inhibition of gentamicin uptake in rat renal cortex in vivo by aminoglycoside and organic polycations. J. Pharmacol. Exp. Ther. 223, 314-321 (1982)
31 Kojima, R. and Suzuki, Y.: Interaction of tobramycin with latamoxef in vitro. Japan J. Pharmacol. 39, Supp. 316P (1985)