Studies on the Nephrotoxicity of Aminoglycoside Antibiotics and Protection from These Effects (9): Protective Effect of Inositol Hexasulfate against Tobramycin-Induced Nephrotoxicity

Ryoji KOJIMA, Mikio ITO and Yoshio SUZUKI

Department of Pharmacology, Faculty of Pharmacy, Meijo University, 15 Yagoto Urayama, Tenpaku-ku, Nagoya 468, Japan

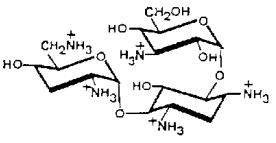
Accepted March 30, 1990

Abstract—We examined the protective effect of inositol hexasulfate (IS_6) against tobramycin (TOB)-induced nephrotoxicity. In the electrophoretic analysis, TOB alone and IS_6 alone were observed as single spots on the cathode and anode sides, respectively. However, in the mixture of TOB and IS_6 preincubated at 37°C for 3 hr, the tailing of the spots of TOB and IS_6 were observed from the origin to the cathode and the anode sides, respectively, and the overlapping of the spots of TOB and IS_6 was recognized at the origin. These results indicated that TOB directly interacted with IS_6 in vitro. Assay of TOB binding to rat kidney brush border membranes (BBMs) indicated that IS_6 inhibited the binding of TOB to BBMs through an interaction of TOB and IS_6. No significant reduction in intrarenal TOB level was observed in the rats given TOB (90 mg/kg, s.c.) and IS_6 (153 or 610 mg/kg, s.c.). However, the treatment of rats with a combination of TOB and IS_6 reduced the degree of necrosis of renal tubular cells and also suppressed the increases in urinary protein, urinary enzyme activities, blood urea nitrogen and plasma creatinine induced by TOB. Additionally, we detected a complex of TOB and IS_6 in the urine of rats given both compounds simultaneously. These results indicate that IS_6 protects against TOB-induced nephrotoxicity and that the protective action of IS_6 may be due to the inhibition of TOB binding to BBMs through an interaction of TOB with IS_6.

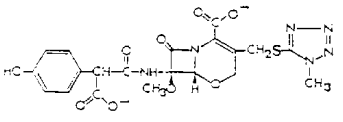
Aminoglycoside antibiotics (AGs) are known to induce severe nephrotoxicity in humans (1) and experimental animals (2). Recently, it has been reported that oral calcium supplementation (3, 4), thyroxine administration (5) and parathyroidectomy (6) prevent nephrotoxicity induced by AGs in experimental animals.

We have also reported that latamoxef (LMOX), an oxacephem antibiotic, protects rats from TOB-induced nephrotoxicity, and that the protective effect of LMOX is due to a reduced intrarenal TOB level (7) and inhibition of TOB binding to brush border membranes (BBMs) isolated from rat kidney cortex (8). More recently, we have found that pyridoxal-5′-phosphate (PALP) also protects against TOB nephrotoxicity by means of mechanisms similar to those by which LMOX prevents TOB nephrotoxicity (9).

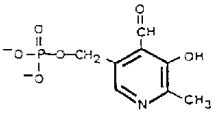
As shown in Fig. 1, LMOX and PALP have carboxyl and phosphate groups, respectively, and thereby both these compounds have negative charges at physiological pH. Recently, since it has been reported that AGs bind to negatively charged phospholipids such as phosphatidylinositides of BBMs (10), our studies using LMOX and PALP suggested that the negative charge of the compounds may play a role in inhibiting the TOB binding to BBMs in vitro, in reducing the renal accumulation of TOB and in preventing the nephrotoxic action of TOB in vivo. Thus, in the present study, according to the methods used in the studies with LMOX and PALP, we investigated the ability of inositol hexasulfate (IS_6), which has six negatively charged sulfate groups in its molecular structure to



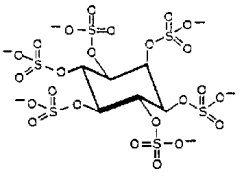
Tobramycin (TOB)



Latamoxef (LMOX)



Pyridoxal-5'-phosphate (PALP)



Inositol hexasulfate (IS_6)

Fig. 1. Chemical structures of tobramycin, latamoxef, pyridoxal-5'-phosphate and inositol hexasulfate.

prevent TOB nephrotoxicity, and examined the importance of the negative charge in the molecules that can prevent TOB nephrotoxicity.

Materials and Methods

Drugs: TOB (free base) and IS_6 (sodium salt) were provided by Shionogi Co., Ltd. (Osaka, Japan) and Mitui Co., Ltd. (Tokyo, Japan), respectively.

Animals: Male Sprague-Dawley rats weighing about 230 g (Japan SLC, Shizuoka, Japan) were used in all experiments. These animals were housed in an air-conditioned room at 24±1 °C.

Electrophoretic analysis: To detect the interaction of TOB and IS_6, electrophoretic analysis was carried out as previously described (11). In brief, TOB alone, IS_6 alone and a mixture of both compounds (molar ratio, TOB : IS_6=1:2) were dissolved in H_2O, followed by adjustment of the pH to 7.4 by adding HCl or NaOH. The solution containing the compounds was incubated for 10, 30 and 60 min at 37 °C, and aliquots sampled at a suitable time of incubation were subjected to paper and cellulose acetate membrane electrophoresis for detecting TOB and IS_6, respectively. The spots associated with TOB and IS_6 were visualized by the ninhydrin (0.2 w/v % acetone solution) reaction and alcian blue (0.5 w/v % acetic acid (3%) solution) staining, respectively.

Assay of TOB binding to BBMs: The isolation of BBMs from rat kidney cortex and the assay of TOB binding to BBMs were carried out by the method of Ca precipitation (12, 13) and the method of Ishikawa et al. (14), respectively. In the assay of TOB binding to BBMs, 200 μl of membrane suspension (200–250 μg protein) was incubated at 37 °C for 10 min with 200 μl of 0.2 mM TOB in 20 mM HEPES/Tris buffer (pH 7.4) containing 100 mM mannitol, followed by centrifugation at 45,000×g for 30 min at 4 °C. The supernatant was discarded, and 200 μl of H_2O and 200 μl of 5% trichloroacetic acid were added. After centrifugation at 1,600×g for 20 min, the resultant supernatant was used for the measurement of TOB concentration by means of a substrate-labeled fluorescent immunoassay (SLFIA, Ames TDA tobramycin assay kit, Miles-Sankyo Co., Ltd., Tokyo, Japan) (15). TOB (final concentration: 0.2 mM) and IS_6 (final concentration: 0.4, 0.8, 2, 4 and 10 mM) were dissolved in 20 mM HEPES/Tris buffer (pH 7.4) containing 100 mM mannitol. To correct for any effect of sodium, in the addition of IS_6, on TOB binding to BBMs, TOB binding in the treatment with TOB alone was assayed in the presence of NaCl solution containing the same sodium content as that in

each concentration of IS_6.

Measurement of the intrarenal TOB concentration: Rats were given TOB (90 mg/kg, s.c.) alone or TOB and IS_6 (153 or 610 mg/kg, s.c.) simultaneously for 3 days. On the 1st and 3rd days, TOB levels in the left kidney were determined by the previously reported method (7), except for centrifugation of the kidney homogenate at 9,000×g for 15 min and the measurement of TOB concentration by SLFIA. When TOB was given alone, subcutaneous injection of NaCl solution was also carried out to correct for any sodium effect on intrarenal TOB level caused by the IS_6 injection.

In vivo nephrotoxicity studies: Animals were given TOB (90 mg/kg, s.c.) alone or TOB and IS_6 (153 or 610 mg/kg, s.c.) simultaneously for 15 consecutive days. After the beginning of drug treatment, protein content, and N-acetyl-β-D-glucosaminidase (NAG) and alkaline phosphatase (ALP) activities were measured in the twenty-four hour urine on days 1, 4, 7, 10 and 15, as described in our previous reports (7). On the 10th, 13th and 15th days, urea nitrogen and creatinine contents in the blood obtained from the tail vein were assayed by the method of Searcy and Cox (16) and by using a CRE-EN Kainos kit (Kainos Laboratories, Tokyo, Japan), respectively. In addition, rat kidneys fixed with formalin, sectioned, and stained with periodic acid-Schiff (PAS) were observed under light microscopy, as described previously (7).

Detection of a complex of TOB and IS_6 in urine: Ureteral catheterization and urine collection were carried out by slightly modifying the methods of Stizer and Martinez-Maldonado (17) and Cojocel et al. (18). The rats given H_2O (8 ml, p.o.) were anesthetized with sodium pentobarbital (32.4 mg/kg, i.p.), placed on a temperature-regulated hot plate, and subjected to tracheostomy. The left kidney was exposed by midline abdominal incision, and the left ureter was catheterized with polyethylene tubing (PE-10) for urine collection. After an equilibration period of 30 min, the rats were given TOB (90 mg/kg, s.c.) alone, IS_6 (153 mg/kg, s.c.) alone or both compounds simultaneously. In other rat groups, IS_6 was injected 5 hr before or after TOB injection. At various times after drug injection, 1 μl of the urine was obtained directly from the ureteral catheter to avoid sampling any complex of TOB and IS_6 formed within the bottle used for urine collection, and was subjected to cellulose acetate membrane electrophoresis. The cellulose acetate membrane electrophoresis of the urine was performed for 1 hr with 70 mM Tris-HCl buffer (pH 7.4), and then the cellulose acetate membrane (7×10 mm) was placed on an agar (Pearlcore, Hart Infusion Agar, Eiken, Japan) plate containing about 1×10^9 CFU/ml of *Pseudomonas aeruginosa* SR-24 (Shionogi Co., Ltd., Japan). After the incubation of the plate for 18 hr at 37°C, the inhibition zone of the bacterial growth caused by TOB was assessed. In addition, IS_6 on the cellulose acetate membrane was also visualized by alcian blue (0.5%) staining.

Statistical analysis: The data were analyzed by the one-way analysis of variance and the Duncan multiple range test or non-parametric statistics. When only two groups were used, the data were analyzed by Student's *t*-test. The data in the text, figures and tables are expressed as the mean±S.D. Differences were considered significant if the P value was <0.05.

Results

1. Analysis of an interaction of TOB and IS_6 by paper and cellulose acetate membrane electrophoresis

Figure 2 illustrates the interaction of TOB and IS_6 in vitro. The spot of TOB alone was observed as a single spot on the cathode side (Fig. 2A). However, the spot corresponding to TOB alone almost disappeared, and the ninhydrin reaction was observed from the cathode side to the origin, when the mixture of TOB and IS_6, which was incubated at 37°C for various times, was subjected to the electrophoresis. As shown in Fig. 2B, the alcian blue staining for IS_6 showed that the spot of IS_6 was observed on the anode side, and in the mixture of TOB and IS_6, the spot associated with IS_6 was detected from the origin to the anode side. As demonstrated by Fig. 2, A and B, the spots of TOB and IS_6 overlapped at the origin. In addition, IS_6 seemed to interact with TOB immediately

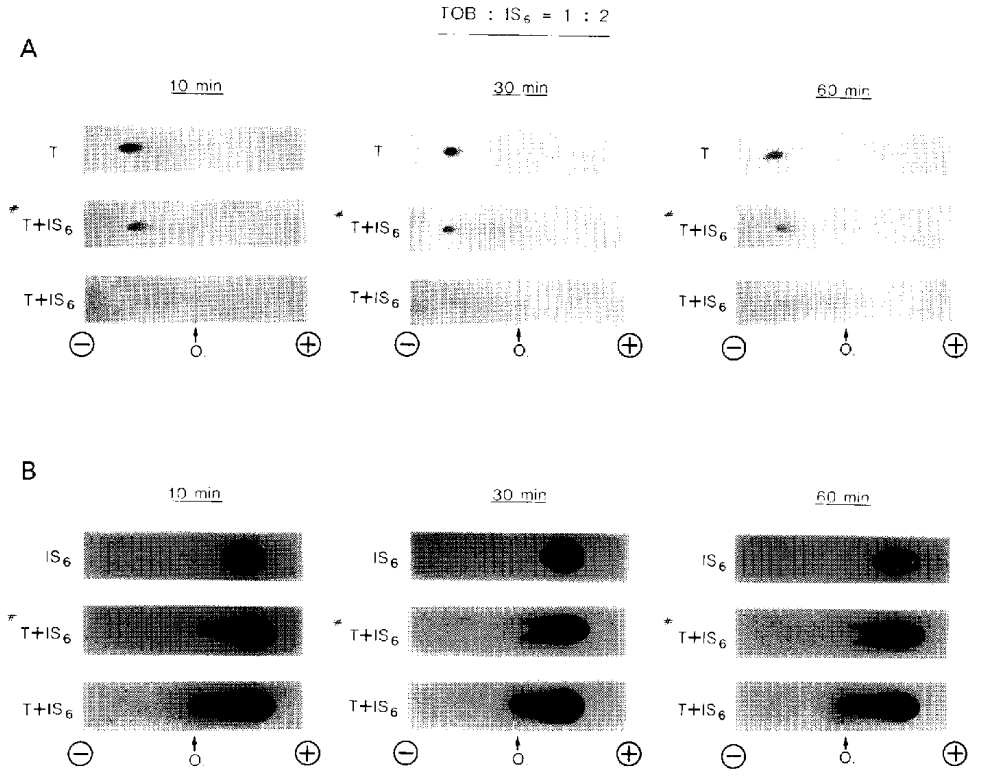


Fig. 2. Analysis of the interaction of TOB and IS_6 by electrophoresis. A: Detection of TOB by the ninhydrin reaction. B: Detection of IS_6 by alcian blue staining. Black spots and circled areas on the paper indicate the spots corresponding to TOB and IS_6, respectively. The mixture of TOB and IS_6 (molar ratio, TOB: IS_6=1:2) was incubated for 10, 30 and 60 min at 37°C. The electrophoreses using paper or cellulose acetate membrane were carried out with 70 mM Tris-HCl buffer (pH 7.4). O.: origin, T: tobramycin, T+IS_6: incubated mixture, #: the compounds were separately spotted at the origin.

after the mixing of the two compounds.

2. Assay of TOB binding to BBMs

Figure 3 illustrates the effect of IS_6 on TOB binding to BBMs. The simultaneous addition of TOB (0.2 mM) and IS_6 (0.4 to 10 mM) to the BBMs fraction resulted in a significant inhibition (20–60%) of TOB binding to BBMs (Fig. 3A). The addition of the reaction mixture of TOB and IS_6 (0.4 to 10 mM), which was preincubated for 3 hr at 37°C, did not have inhibitory effect on TOB binding to BBMs (Fig. 3B). Pretreatment of BBMs with IS_6 showed that the amounts of TOB which bound to the membranes were similar to those observed in simultaneous treatment with TOB and IS_6 (Fig. 3C). No significant differences in the TOB binding to BBMs were observed between IS_6-treated and non-treated (Fig. 3D).

3. Effect of IS_6 on intrarenal TOB concentration

Table 1 summarizes the effect of IS_6 on TOB level in the kidney. IS_6 was ineffective in reducing the intrarenal TOB level.

4. In vivo nephrotoxicity study

1) Effect of TOB alone and in combination with IS_6 on biochemical parameters in urine and blood: Although urinary protein content and urinary NAG and ALP activities were increased by the injection of TOB, the combination of TOB and IS_6 significantly suppressed the elevation in those parameters caused by TOB (Fig. 4). Additionally, BUN and creatinine contents elevated by TOB were also reduced by combination with IS_6 (Table 2).

2) Histological observations: Figure 5

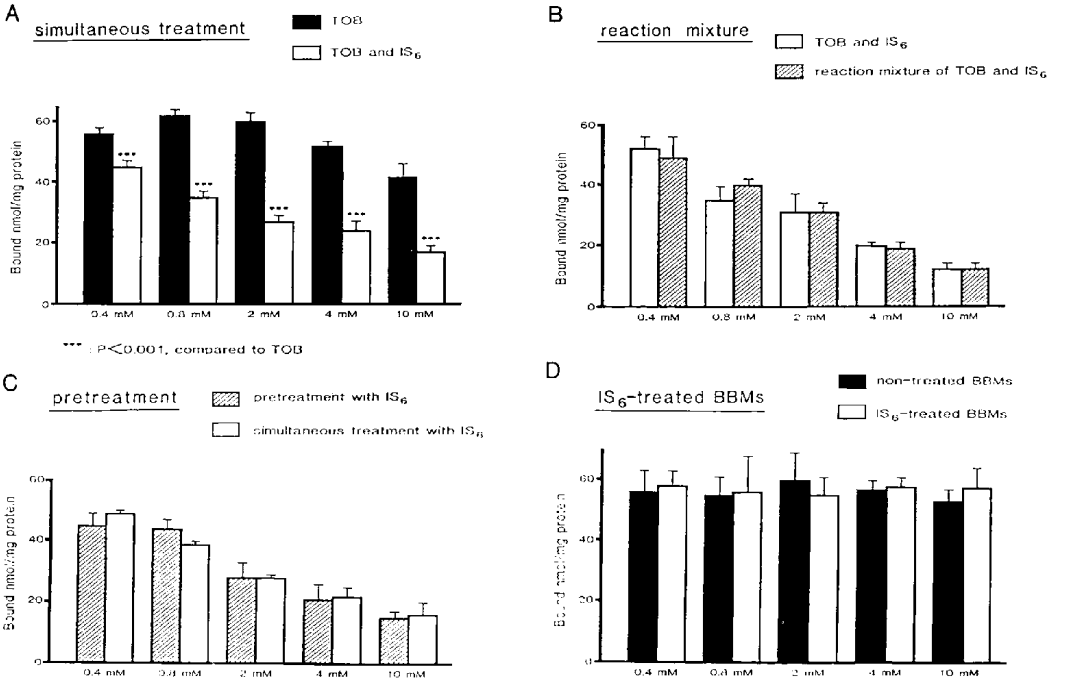


Fig. 3. Effect of simultaneous treatment (A) and pretreatment (C) with IS_6, and the reaction mixture of TOB and IS_6 (B) on TOB binding to BBMs, and the binding of TOB to the IS_6-treated BBMs (D). A: TOB and IS_6: IS_6 (0.4, 0.8, 2, 4 and 10 mM) and TOB (0.2 mM) were added simultaneously to the BBM fraction and incubated for 10 min at 37°C. TOB: TOB and NaCl were added simultaneously to the BBM fraction and incubated for 10 min at 37°C. To correct for the effect of sodium, in the addition of IS_6, on the TOB binding to BBMs, TOB binding in the addition of TOB alone was monitored in the presence of a NaCl solution containing the same sodium content (2.4, 4.8, 12, 24 and 60 mM) as that in each concentration of IS_6. B: reaction mixture of TOB and IS_6: TOB and IS_6 were mixed and preincubated for 3 hr at 37°C to form the complex of both compounds. The preincubated reaction mixture was added to the BBM fraction and incubated for 10 min at 37°C. TOB and IS_6: TOB and IS_6 were added to simultaneously to the BBM fraction and incubated for 10 min at 37°C as described above. C: pretreatment with IS_6: Pretreatment with IS_6 for 10 min at 37°C, followed by the incubation with TOB for 10 min at 37°C. Simultaneous treatment with IS_6: IS_6 and TOB were added simultaneously to the BBM fraction and incubated for 10 min at 37°C as described above. D: BBMs were preincubated for 10 min at 37°C in a medium (100 mM mannitol, 20 mM HEPES/Tris, pH 7.4) with (IS_6-treated BBM) or without (non-treated BBM) IS_6, centrifuged at 45,000×g for 30 min, and then the membrane pellets were resuspended in the fresh medium (100 mM mannitol, 20 mM HEPES/Tris, pH 7.4). The resuspended BBMs were incubated for 10 min at 37°C with 0.2 mM of TOB. The values are expressed as the mean±S.D. Concentrations shown are the final concentrations.

shows the light micrographs of the kidneys from given TOB or TOB and IS_6 on the 10th day. As shown in Fig. 5B, the treatment with TOB induced severe tubular cell necrosis, cast formation and exposure of the tubular basement membrane. On the other hand, the kidneys from rats receiving TOB and IS_6 showed a reduced degree of cell necrosis (Fig. 5, C and D).

5. Detection of a complex of TOB and IS_6 in urine

Figure 6 illustrates the interaction of TOB and IS_6 in vivo determined by cellulose acetate membrane electrophoresis, microbiological test and alcian blue staining. In the microbiological test, the inhibition zone of cell growth caused by TOB was observed as a single zone on the cathode side. However,

Table 1. Effect of IS_6 on intrarenal TOB concentration

	μg/g tissue wet weight	
	1	3 days
IS_6 153 mg/kg		
TOB+NaCl	130.63±21.02	310.66±52.06
TOB+IS_6	157.92±13.66	313.13±51.89
IS_6 610 mg/kg		
TOB+NaCl	107.24±28.08	223.63±56.32
TOB+IS_6	117.63±19.38	259.56±44.89

The values are expressed as the mean±S.D. from 7 rats.

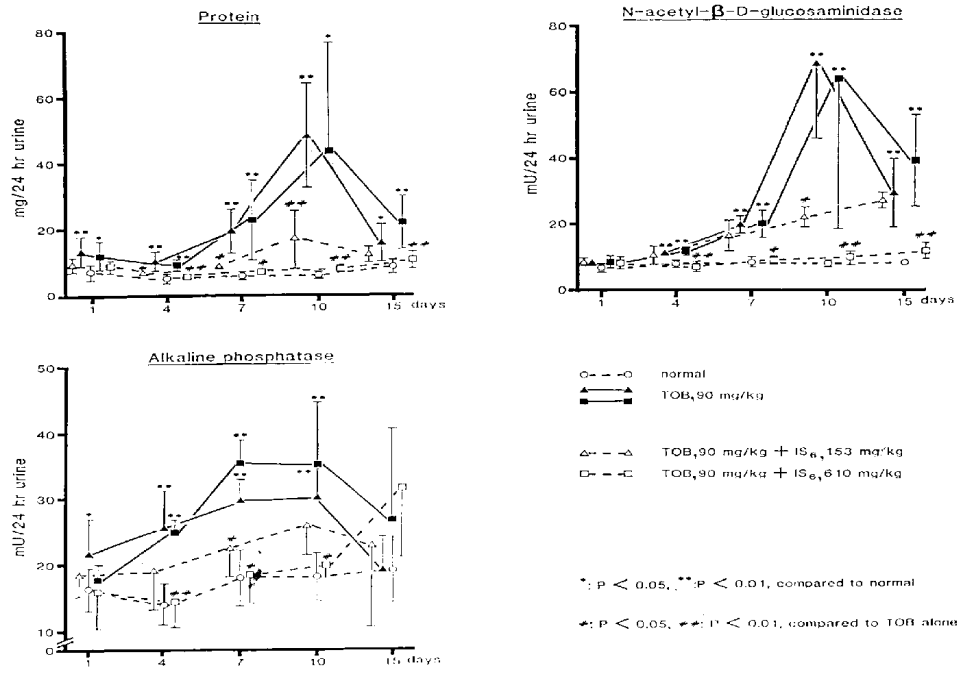


Fig. 4. Effect of TOB and in combination with IS_6 on urinary protein content and urinary N-acetyl-β-D-glucosaminidase and alkaline phosphatase activities. Each point represents the mean±S.D. of 7 rats.

when the aliquot of the urine obtained from a rat given TOB and IS_6 was analyzed, the inhibition zone was detected from the cathode side to the origin (Fig. 6A). As shown in Fig. 6B, alcian blue staining showed that the spot of IS_6 alone was observed as a single spot on the anode side, and that IS_6 was excreted into the urine immediately after the injection. In the analysis of the urine from rats receiving both compounds, alcian blue on the acetate membrane was found from the origin to the anode side.

Discussion

The purpose of the present study was to examine the involvement of the negative charge of compound in protecting against nephrotoxicity induced by TOB by evaluating the ability to prevent TOB nephrotoxicity of IS_6 which has negative charges derived from its sulfate groups.

We have already detected, by paper electrophoresis, the overlapping of the spots of TOB and LMOX (11) and that of TOB and

Table 2. Effects of TOB and in combination with IS_6 on blood urea nitrogen and plasma creatinine

	Blood urea nitrogen mg/dl			Plasma creatinine mg/dl		
	10	13	15 days	10	13	15 days
Normal	24.27±13.03	19.31±1.29	18.65±2.85	0.51±0.05	0.50±0.02	0.44±0.02
IS_6 153 mg/kg						
TOB+NaCl	50.48±10.38**	30.13±6.84**	26.15±3.27**	0.93±0.15**	0.85±0.16**	0.66±0.05**
TOB+IS_6	34.62±3.34#	25.64±5.10	18.81±2.56##	0.63±0.12#	0.65±0.10#	0.54±0.08#
IS_6 610 mg/kg						
TOB+NaCl	40.73±12.56*	32.70±10.75**	24.61±5.23*	0.80±0.33*	0.76±0.21**	0.54±0.05**
TOB+IS_6	27.48±2.31#	23.58±4.36#	20.03±2.68	0.45±0.03#	0.44±0.01#	0.42±0.04##

*: $P<0.05$, **: $P<0.01$, compared to normal. #: $P<0.05$, ##: $P<0.01$, compared to TOB+NaCl. The values are expressed as the mean±S.D. from 7 rats.

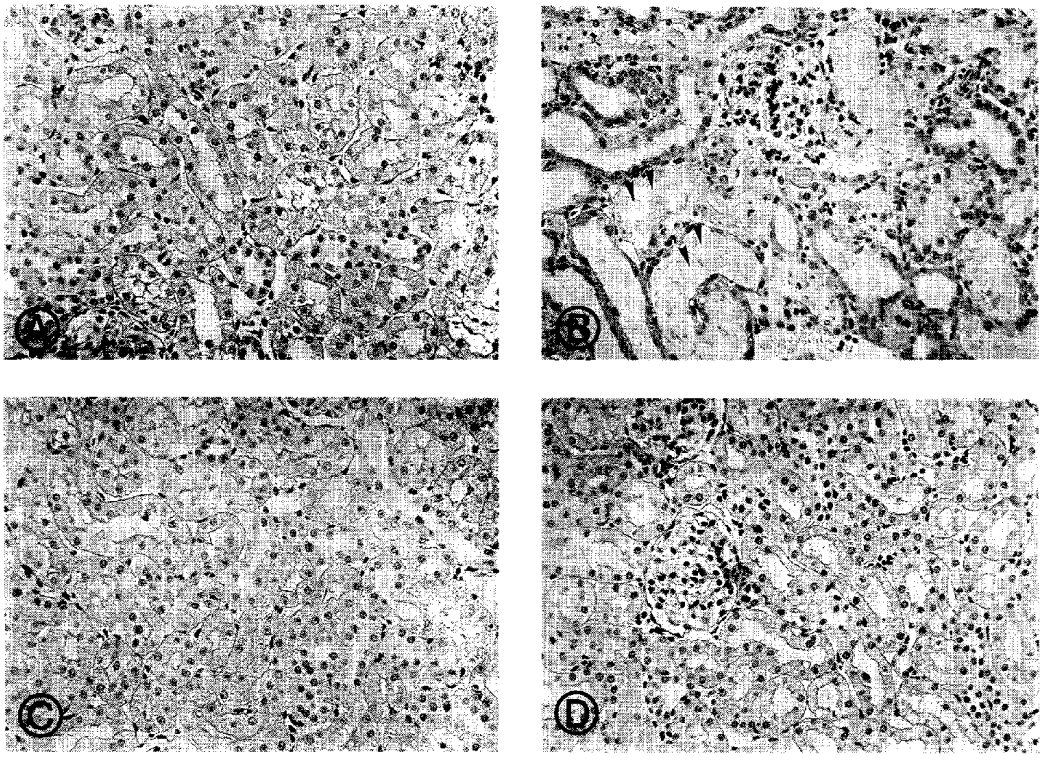


Fig. 5. Light micrograph of rat kidney on the 10th day (PAS stain). A: Normal. B: TOB—note the extended necrosis of proximal tubular epithelial cells (arrow head). C: TOB+IS_6, 153 mg/kg; D: TOB+IS_6, 600 mg/kg—the degree of necrosis of tubular cells was reduced.

PALP (9) as a documentation of the interaction between two compounds. Likewise, in this study, electrophoretic analysis showed that in the mixture of TOB and IS_6, the overlapping of the spots of both compounds was found at the origin. This result indicates that IS_6 directly interacts with TOB in vitro (Fig. 2). With regard to the molecular mechanisms of the interaction between TOB and IS_6, the ionic binding between the positive charge of TOB and the negative charge of IS_6 and other binding forces such as hydrogen bonding may be involved in the interaction between both compounds.

It has been proposed that the first step of accumulation of AGs into renal tubular cells is the binding of AGs to biomembranes such as BBMs (19), and we have already reported that LMOX and PALP were effective in inhibiting the TOB binding to BBMs in vitro. In the present study, we have found that IS_6 also inhibited the binding of TOB to BBMs in a dose-dependent manner (Fig. 3A). Although it is unclear whether IS_6 binds to BBMs in this study, the experiments concerning the pretreatment with IS_6 (Fig. 3C) and the IS_6-treated BBMs (Fig. 3D) indicated that the modification by IS_6 of the membrane properties of BBMs would not, at least, contribute to the inhibitory effect of IS_6 on TOB binding to BBMs. Our previous studies showed that the addition of the reaction mixtures of TOB and LMOX (8), and that of TOB and PALP (9), to the BBM fraction resulted in a significant inhibition of TOB binding to the membranes, and this suggested that the molecular interaction between TOB and LMOX, and that between TOB and PALP, was associated with the inhibition of TOB binding to BBMs caused by LMOX or PALP. However, in this study, the addition of the reaction mixture of TOB and IS_6 to the membrane fraction failed to inhibit the membrane binding of TOB to BBMs more than values

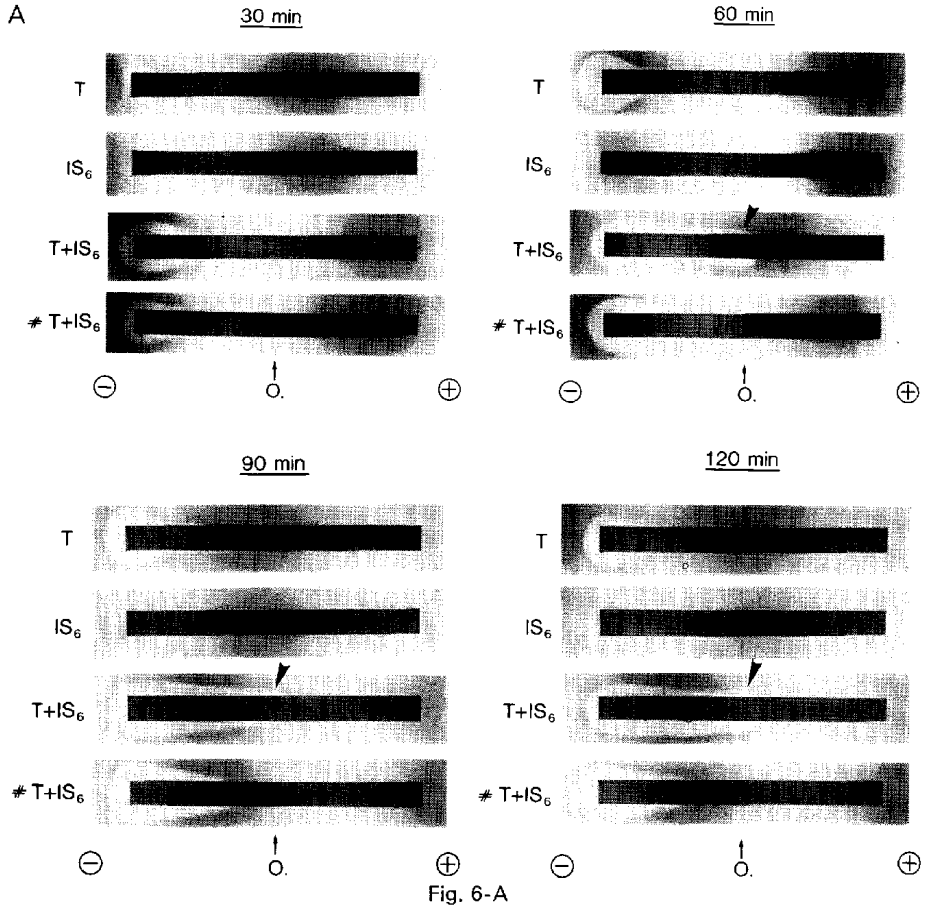


Fig. 6-A

Fig. 6. Detection of the complex of TOB and IS_6 in the urine of rats given both compounds simultaneously. A: Detection of the complex by paper electrophoresis and microbiological test. B: Detection of the complex by cellulose acetate membrane electrophoresis and alcian blue staining. Urine samples were analyzed by the method described in Materials and Methods. O.: origin. T: tobramycin. #: Each urine sample containing TOB or IS_6 was separately spotted at the origin. Arrow heads represent the complex of both compounds.

observed in the case of co-addition of TOB and IS_6 to the BBM fraction (Fig. 3B). This may be explained by the rapid interaction between TOB and IS_6 as suggested by the electrophoretic analysis (Fig. 2, A and B). Based on the observations in the electrophoretic analysis and the membrane binding assay, it is considered that the inhibitory effect of IS_6 on TOB binding to BBMs may result from the molecular interaction between TOB and IS_6. This result was also in agreement with those observed in the experiments with LMOX and PALP, except for the result from the experiment regarding the pretreatment with IS_6.

In the in vivo nephrotoxicity study, we have demonstrated that IS_6 protects against TOB nephrotoxicity, as evaluated by the measurements of the biochemical parameters in the urine and blood (Fig. 4 and Table 2), and the histological findings (Fig. 5).

Generally, it has been assumed that the accumulation of AGs within the proximal tubular cells is related to the pathogenesis of AGs nephrotoxicity (2). In fact, we have found that LMOX (7) and PALP (9), which reduce the intrarenal TOB level, prevent the nephrotoxic action of TOB. However, in spite of having responses similar to those with LMOX and PALP in the experiment concerning the

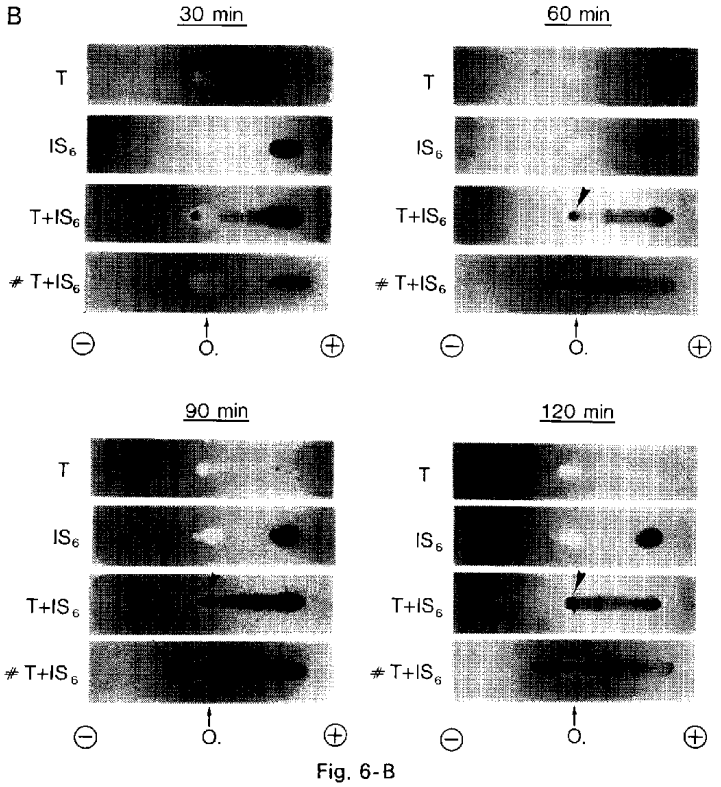


Fig. 6-B

chemical reaction with TOB and the action on TOB binding to BBMs, IS_6 prevented TOB nephrotoxicity without a decrease in the renal TOB level (Table 1). This result raises the possibility that the intrarenal accumulation of TOB may not be involved in the pathogenesis of nephrotoxicity induced by TOB.

It has been reported that AGs including gentamicin (GM) induce striking alterations in membrane properties such as fluidity (20), permeability (21–23), potential (24) and metabolism (25–29). This evidence suggests the importance of the interaction of AGs with biomembranes in the pathogenesis of AGs nephrotoxicity. Thus, on the basis of the evidence from the present study and the results reported by other investigators (20–29), we make the following speculations about the mechanisms of the protective action of IS_6 against TOB nephrotoxicity: IS_6 interacts with TOB in the renal tubular lumen or in the blood, inhibits the TOB binding to BBMs, and consequently prevents the nephrotoxic action of TOB. The documentation of the complex of TOB and IS_6 in the urine from rats treated with TOB and IS_6 simultaneously (Fig. 6, A and B), as well as in the cases of TOB and LMOX or PALP (data not shown), may reflect the molecular interaction of TOB and IS_6 in vivo. Moreover, we demonstrated that the protective effect of IS_6 in vivo and the complex of TOB and IS_6 in the urine were not observed in the rats injected with IS_6 5 hr before or after TOB injection (data not shown). Additionally, we detected by mass spectrometry the non-metabolized IS_6 in the urine from rats receiving IS_6 alone (data not shown). These observations also may support our speculation regarding the mechanism of the protective action of IS_6 against TOB nephrotoxicity. In addition, the possibility that IS_6 may intracellularly prevent the toxic action of TOB should also be examined to clarify the mechanism of the protective action of IS_6.

Recently, several investigators (30–32) have reported that polyaspartic acid (PAA) also protects against GM nephrotoxicity in rats without reducing the accumulation of GM in the renal cortex. Williams et al. (30)

also demonstrated the inhibitory effect of PAA on the binding of GM to BBMs in vitro and suggested the significance of the binding of AGs to BBMs rather than the intrarenal accumulation of AGs in the pathogenesis of AGs nephrotoxicity. Thus, our findings that LMOX (7), PALP (9) and IS_6, which inhibited the binding of TOB to BBMs in vitro, protected against TOB-induced nephrotoxicity in vivo also indicate that the binding of AGs to BBMs may be a crucial factor in the expression of cell toxicity induced by AGs.

A series of studies on LMOX, PALP and IS_6 raises the possibility that a compound which inhibits the binding of AGs to the biomembranes including BBMs may prevent AGs-induced nephrotoxicity. Furthermore, since these three compounds have negative charges derived from the carboxyl, phosphate or sulfate group in their molecules (Fig. 1), our studies suggest the possibility that a compound which has a negative charge in its molecular structure can be used for preventing AGs nephrotoxicity. The observation that negatively charged PAA inhibited the GM binding to BBMs and prevented GM nephrotoxicity (30) may also support our suggestion.

In conclusion, the present studies indicate that IS_6 protects against TOB-induced nephrotoxicity, and that the protective action of IS_6 may be due to the inhibition of TOB binding to BBMs by the interaction between TOB and IS_6. In addition, our studies using LMOX, PALP and IS_6 suggst the possibility that some compounds, but not all, having a negative charge in their molecular structure can be used for preventing TOB nephrotoxicity.

Acknowledgments: We thank Shionogi Co., Ltd. for supplying TOB, and we also thank Mitui Co., Ltd. for supplying IS_6.

References

1 **Bennett, W.M.**: Aminoglycoside nephrotoxicity. Mineral Electrolyte Metab. **6**, 277–286 (1981)

2 **Kaloyanides, G.J. and Pastoriza-Munoz, E.**: Aminoglycoside nephrotoxicity. Kidney Int. **18**, 571–582 (1980)

3 **Humes, H.D., Sastrasinh, M. and Weinberg, J.M.**: Calcium is a competitive inhibitor of gentamicin-renal membrane binding interactions and dietary calcium supplementation protects against gentamicin nephrotoxicity. J. Clin. Invest. **73**, 134–147 (1984)

4 **Bennett, W.M., Elliott, W.C., Houghton, D.C., Gilbert, D.N., DeFehr, J. and McCarron, D.A.**: Reduction of experimental gentamicin nephrotoxicity in rats by dietary calcium loading. Antimicrob. Agents Chemother. **22**, 508–512 (1982)

5 **Cronin, R.E. and Newman, J.A.**: Protective effect of thyroxine but not parathyroidectomy on gentamicin nephrotoxicity. Am. J. Physiol. **248**, F332–F339 (1985)

6 **Pulliam, J.P., Houghton, D.C. and Bennett, W.M.**: Modification of gentamicin nephrotoxicity by selective parathyroidectomy. Am. J. Physiol. **249**, F832–F835 (1985)

7 **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)

8 **Kojima, R., Ito, M. and Suzuki, Y.**: Studies on the nephrotoxicity of aminoglycoside antibiotics and protection from these effects (7): Effect of latamoxef on binding of tobramycin to brush border membranes isolated from rat kidney cortex. Japan. J. Pharmacol. **51**, 465–473 (1989)

9 **Kojima, R., Ito, M. and Suzuki, Y.**: Studies on the nephrotoxicity of aminoglycoside antibiotics and protection from these effects (8): Protective effect of pyridoxal-5'-phosphate against tobramycin nephrotoxicity. Japan. J. Pharmacol. **52**, 11–21 (1990)

10 **Sastrasinh, M., Knauss, T., Weinberg, J.M. and Humes, H.D.**: Identification of the aminoglycoside binding site in rat renal brush border membranes. J. Pharmacol. Exp. Ther. **222**, 350–358 (1983)

11 **Kojima, R., Ito, M. and Suzuki, Y.**: Studies on the nephrotoxicity of aminoglycoside antibiotics and protection from these effects (5). Interaction of tobramycin with latamoxef in vitro. J. Pharmacobiodyn. **11**, 9–17 (1988)

12 **Evers, C., Haase, W., Murer, H. and Kinne, R.**: Properties of brush border vesicles isolated from rat kidney cortex by calcium precipitation. Membr. Biochem. **1**, 203–219 (1978)

13 **Inui, K., Okano, T., Takano, M., Kitazawa, S. and Hori, R.**: A simple method for the isolation of basolateral plasma membrane vesicles from rat kidney cortex: Enzyme activities and some properties of glucose transport. Biochim. Biophys. Acta **647**, 150–154 (1985)

14 **Ishikawa, Y., Inui, K. and Hori, R.**: Gentamicin

binding to brush border and basolateral membranes isolated from rat kidney cortex. J. Pharmacobiodyn. **8**, 931–941 (1985)

15 **Burd, J.F., Wong, R.C., Feerey, J.E., Carrico, R.J. and Boguslaski, R.C.:** Homogeneous reactant-labeled fluorescent immunoassay for therapeutic drugs exemplified by gentamicin determination in human serum. Clin. Chem. **23**, 1402–1408 (1977)

16 **Searcy, R.L. and Cox, F.M.:** A modified technique for ultramicro estimation of urea nitrogen. Clin. Chim. Acta **8**, 810–812 (1963)

17 **Stitzer, S.O. and Martinez-Maldonado, M.:** Clearance methods in the rat. *In* Methods in Pharmacology, Vol. **4B**, Renal Pharmacology. Edited by Martinez-Maldonado, M., p. 23–40, Plenum Press, New York (1978)

18 **Cojocel, C., Dociu, N., Ceacmacudis, E. and Baumann, K.:** Nephrotoxic effects of aminoglycoside treatment on renal protein reabsorption and accumulation. Nephron **37**, 113–119 (1984)

19 **Just, M., Erdmann, G. and Habermann, E.:** The renal handling of polybasic drugs. I. Gentamicin and aprotinin in intact animals. Naunyn Schmiedebergs Arch. Pharmacol. **300**, 57–66 (1977)

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. Biochem. Pharmacol. **33**, 3787–3791 (1984)

21 **Au, S., Schacht, J. and Weiner, N.:** Membrane effects of aminoglycoside antibiotics measured in liposomes containing the fluorescent probe, 1-anilino-8-naphthalene sulfonate. Biochim. Biophys. Acta **862**, 205–210 (1986)

22 **Au, S., Weiner, N.D. and Schacht, J.:** Aminoglycoside antibiotics preferentially increase permeability in phosphoinositide-containing membranes: A study with carboxyfluorescein in liposomes. Biochim. Biophys. Acta **902**, 80–86 (1987)

23 **Ramsammy, J.S. and Kaloyanides, G.J.:** Effect of gentamicin on the transition temperature and permeability to glycerol of phosphatidylinositol-containing liposomes. Biochem. Pharmacol. **36**, 1179–1181 (1987)

24 **Chung, L., Kaloyanides, G.J., McDaniel, R., McLaughlin, A. and McLaughlin, S.:** Interaction of gentamicin and spermine with bilayer membrane containing negatively charged phospholipids. Biochemistry **24**, 442–452 (1985)

25 **Schacht, J.:** Interaction of neomycin with phosphoinositide metabolism in guinea pig inner ear and brain tissues. Ann. Otol. Rhinol. Laryngol. **83**, 613–618 (1974)

26 **Schibeci, A. and Schacht, J.:** Action of neomycin on the metabolism of polyphosphoinositides in the guinea pig kidney. Biochem. Pharmacol. **26**, 1769–1774 (1977)

27 **Marche, P., Koutouzov, S. and Girard, A.:** Impairment of membrane phosphoinositide metabolism by aminoglycoside antibiotics: Streptomycin, amikacin, kanamycin, dibekacin, gentamicin and neomycin. J. Pharmacol. Exp. Ther. **227**, 415–420 (1983)

28 **Marche, P., Olier, B., Girard, A., Fillastre, J.P. and Morin, J.P.:** Aminoglycoside-induced alterations of phosphoinositide metabolism. Kidney Int. **31**, 59–64 (1987)

29 **Ramsammy, L.S., Josepovitz, C. and Kaloyanides, G.J.:** Gentamicin inhibits against stimulation of the phosphatidylinositol cascade in primary cultures of rabbit proximal tubular cells and in rat renal cortex. J. Pharmacol. Exp. Ther. **247**, 989–996 (1988)

30 **Williams, P.D., Hottendorf, G.H. and Bennett, D.B.:** Inhibition of renal membrane binding and nephrotoxicity of aminoglycosides. J. Pharmacol. Exp. Ther. **237**, 919–925 (1986)

31 **Beauchamp, D., Laurent, G., Maldague, P. and Tulkens, P.M.:** Reduction of gentamicin nephrotoxicity by the concomitant administration of poly-L-aspartic acid and poly-L-aspargine in rats. Toxic Interfaces of Neurones, Smoke and Genes Arch. Toxicol., Supp. **9**, 306–309 (1986)

32 **Ramsammy, L.S., Josepovits, C., Lane, B.P. and Kaloyanides, G.J.:** Polyaspartic acid protects against gentamicin nephrotoxicity in the rat. J. Pharmacol. Exp. Ther. **250**, 149–153 (1989)