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Report

Effect of Dextran Sulfate on Renal Accumulation of Gentamicin

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The effect of dextran sulfate of three molecular weights (1000, 5000, and 90,000) on the accumulation of gentamicin in rat kidney was investigated using a continuous infusion technique. During the infusions of both gentamicin and gentamicin–dextran sulfate mixtures, the gentamicin plasma concentration was maintained at 10 μg/ml. The renal cortical accumulation of gentamicin was significantly lower when dextran sulfate (1000, 5000) was coadministered. The inhibition of cortical gentamicin accumulation increased with increasing dextran sulfate dose, and it was proportional to the amount of dextran sulfate excreted into the urine. Analysis by electrophoresis on cellulose acetate membrane indicated that gentamicin binds to dextran sulfate in rat urine. Therefore, gentamicin–dextran sulfate binding within the lumen of the proximal tubules may reduce the renal reabsorption and possibly the renal toxicity of gentamicin.

KEY WORDS: gentamicin; dextran sulfate; nephrotoxicity; infusion.

INTRODUCTION

Nephrotoxicity of aminoglycosides (AGs) is closely related to the concentration of AGs within the proximal cells of the renal tubules, where they accumulate after passing through the brush-border membrane (BBM) during reabsorption (1–3). The interaction between AGs and BBM may thus be a key factor in nephrotoxicity, and its inhibition would reduce AG accumulation of AGs within the renal proximal tubular cells, thereby preventing nephrotoxicity.

According to Lipsky *et al.* (4), polyamines inhibit the binding and uptake of gentamicin (GM) by BBM vesicles, and AGs and polyamines may share a common transport system in renal proximal tubular cells. Josepovitz *et al.* (5) have shown that polyamines, such as tetralysine, spermine, and cadaverine, inhibit GM uptake in rat renal cortex *in vivo*. However, the polyamines are also nephrotoxic, and therefore, can not serve to reduce AG toxicity.

Previously, we reported that GM binds to dextran sulfate (DS) through ionic interactions, and DS inhibits the binding of dibekacin, an aminoglycoside, to rat renal BBM vesicles (6). Bennett *et al.* (7) indicated that a high frequency of GM administration, leading to sustained GM plasma levels, was more important than peak GM serum concentrations in the onset of nephrotoxicity. The present study was thus conducted to determine the effect of continuously infused DS on the renal accumulation of GM in rat.

MATERIALS AND METHODS

Materials. Gentamicin (GM) was provided by Shionogi Pharmaceutical Co. (Osaka, Japan). Dextran sulfate (DS) with an average MW of 5000 (DS-5000, S cont. ca. 15%) was obtained from Sigma Chemical Co. (St. Louis, MO), and DS with an average MW of 1000 (DS-1000) and 90,000 (DS-90000) were kindly supplied from Kowa Pharmaceutical Co. (Tokyo). Cellulose acetate membrane (Separax) was purchased from Jookoo Co., Ltd. (Tokyo). All other reagents were of the best grade available. Male Wistar rats (200–220 g) were purchased from Shizuoka Agricultural Co. (Shizuoka, Japan) and acclimatised for at least 3 days prior to conducting the experiments.

Infusion. The continuous infusion of a GM and GM-DS mixture was carried out according to the procedure of Giuliano *et al.* (8). With the rats under light ether anesthesia, a polyethylene tube (PE-50, Clay Adams) filled with saline was inserted into the right femoral vein. Another tube filled with heparin solution (20 units/ml) was inserted in the left femoral artery. Each rat treated was placed in a Bollmann cage. Following full recovery from anaesthesia, the rats received intravenously loading doses of GM (0.2 mg) or GM-DS mixture (0.2 mg GM and various amount of DS) dissolved in 0.3 ml saline. The cannula was connected to an infusion pump (KN, Natsume) filled with GM or GM-DS in saline solution (1.0 mg/ml) and infused at a rate of 8.86 μl/min so that the concentration of GM in the plasma would be maintained at 10 μg/ml. The amount of infused GM was equivalent between GM and GM-DS administered groups. The rats were sacrificed at indicated time after the infusion and kidneys were removed for assay of the renal cortical concentration of GM.

Blood samples (0.25 ml) were withdrawn from the femoral artery at every hour of infusion up to 3 hr. Urine samples were collected in the Bollman cage to determine the cumulative amount of DS excreted in the urine.

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Electrophoresis on Cellulose Acetate Membrane. The binding between GM and DS in rat urine was investigated by electrophoresis using cellulose acetate membrane. GM, DS-5000, and a mixture of both compounds (GM:DS = 1:1 by mole) were dissolved in rat urine and preincubated at 37°C for 30 min, and then the aliquot (10 μl) of each sample was subjected to electrophoresis. Following the electrophoresis using 50 m*M* phosphate buffer (pH 7.0), DS was stained with 2% toluidine blue and GM was detected by bioassay described under Analytical Methods.

Analytical Methods. The extraction of GM from the rat kidney cortex was carried out by the method of Ruben *et al.* (9). In the plasma and renal cortex, GM concentrations were determined by bioassay using the *Bacillus subtilis* ATCC 6633 as the test organism. DS concentration in urine was determined by the method of Dodgson and Price (10). Student's *t* test was employed for the statistical analysis of the data.

RESULTS

Plasma Level of Gentamicin. In the continuous infusion experiments, the blood levels of GM were examined in the groups to which GM and the mixture of GM and DS with three different molecular weights (molar ratio, 1:0.25) had been administered. The amount of administered GM was equivalent in four experimental groups. Figure 1 indicates that the blood concentration of GM was unaffected by DS, and it was maintained at about 10 μg/ml during the 3-hr infusion period.

Renal Accumulation of Gentamicin. Following the continuous infusion of GM and the GM-DSs mixtures (1:0.5) for 3 hr, GM accumulation in the renal cortex was examined. It was significantly reduced by the infusion of GM along with DS-1000 or DS-5000, and the highest reduction was observed with DS-5000. On the other hand, the addition of DS-90000 did not affect GM accumulation (Fig. 2), and there was no urinary excretion of DS-90000. These results suggest an inverse relationship between urinary excretion of DS and GM accumulation.

The time course of GM accumulation with or without coadministration of DS-5000 was examined. In both groups, given GM only and the mixture of GM-DS-5000 (1:0.25), GM accumulation reached a plateau after 3 hr and was noted to be significantly reduced by DS-5000 throughout the experimental period (Fig. 3). The effect of the molar ratio of GM to DS-5000 on this accumulation of GM was studied at 3 hr of continuous infusion. GM accumulation decreased by 40% with increasing DS content of GM-DS (1:0.5) infusion (Table I).

Urinary Excretion of Dextran Sulfate. The urinary excretion of DS and renal accumulation of GM were compared in the continuous infusion experiment. As shown in Fig. 4, within 3 hr GM renal accumulation decreased inversely proportional to DS urinary excretion, and a good correlation was observed ($r = -0.8447$).

Electrophoresis. Figure 5 shows the binding between GM and DS in rat urine by electrophoresis using a cellulose acetate membrane. When GM or DS-5000 was subjected to electrophoresis, each compound was detected as a single spot on the cathode and anode side, respectively. On the other hand, the mixture of GM-DS-5000 in rat urine gave a new spot close to the origin, which was positive for both toluidine blue staining and bioassay using *Bacillus subtilis*. These results indicate that GM binds tightly to DS in rat urine.

DISCUSSION

AG nephrotoxicity is strongly related to the AG accu-

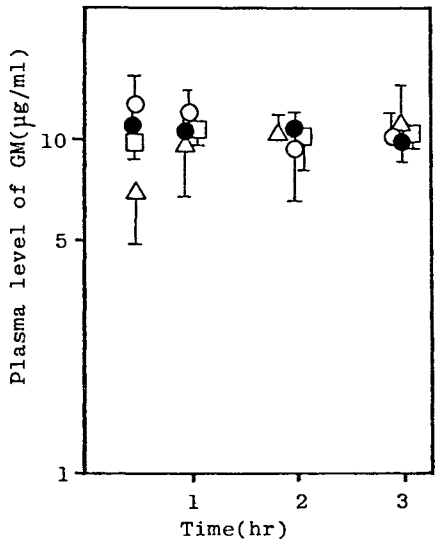

Fig. 1. Plasma GM concentration after continuous infusion. Loading dose and infusion rate of maintenance dose were 0.2 mg and 0.54 mg/hr, respectively. The molar ratio of GM and DS was 1:0.25. (●) GM; (○) GM- DS (1000); (□) GM-DS (5000); (△) GM-DS (90000). Each point represents the mean ± SD of three experiments.

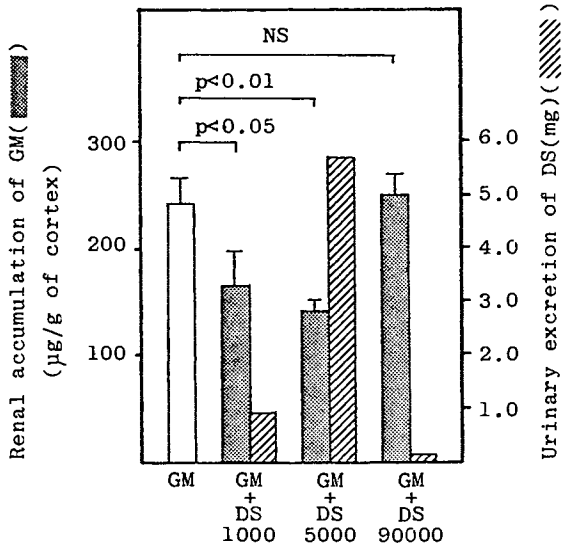

Fig. 2. Effect of DS of different molecular weights on the renal accumulation of GM. After a 3-hr continuous infusion of GM or a GM-DS mixture (molar ratio, 1:0.5), the renal cortical accumulation of GM was compared. Each value represents the mean ± SD of three experiments.

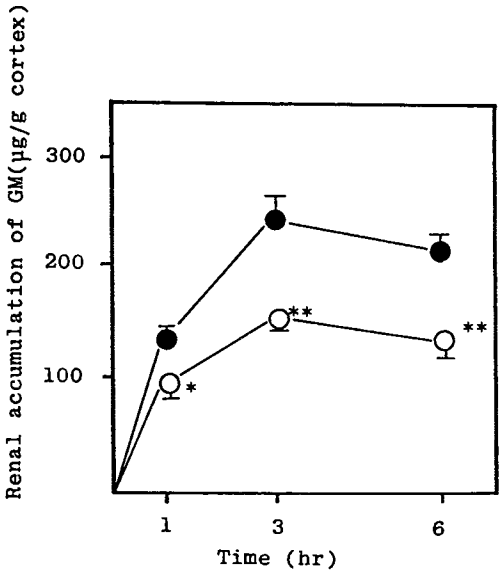

Fig. 3. Time course of GM accumulation within the rat renal cortex. (●) GM; (○) GM:DS-5000 (molar ratio, 1:0.25). Each point represents the mean ± SD of three experiments. (*) $P < 0.02$ and (**) $P < 0.01$; significant difference from GM-administered group.

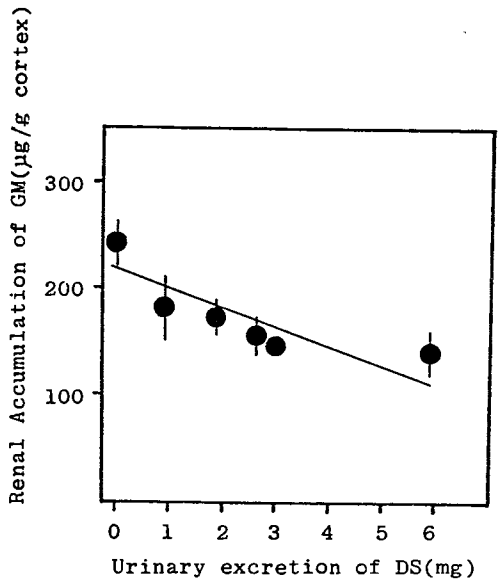

Fig. 4. Correlation between renal accumulation of GM and urinary excretion of DS-5000. The solid line was the linear regression line, whose correlation coefficient (r) was −0.8447.

mulation in renal cortex, predominantly in the proximal convoluted tubules and pars recta (11). Decreasing the renal AG accumulation could therefore enhance the safe use of AGs. We investigated here the effect of DS on renal GM accumulation during continuous infusion in rats.

The therapeutic range of GM blood concentrations is 2–10 μg/ml (12). We thus targeted a plasma GM concentration of 10 μg/ml and calculated the loading dose and infusion rate of the maintenance dose from pharmacokinetic parameters determined previously in rats (6). Irrespective of molecular weight of DSs, the GM concentrations in rat plasma were unaffected by DS coadministration. In a previous study, we demonstrated that GM bound to DS in Tris–HCl buffer through ionic interaction between the amino group(s) of GM and the sulfate group(s) of DS, but this interaction did not occur in rat serum (6). In a rat kidney perfusion study, the glomerular filtration rate of GM was also unaffected by the addition of DS (unpublished data). These results suggest that GM and DS may exist separately in the blood and are filtered through the glomerulus membrane individually, whereas binding of GM and DS occurs in rat urine.

Table I. Effect of Dextran Sulfate-5000 on Renal Accumulation of Gentamicin After 3 hr of Continuous Infusion

Treatment	Molar ratio (GM:DS)	Renal cortical accumulation (μg)[a]	Inhibition (%)
Gentamicin	1:0	242.8 ± 17.9	
Gentamicin: dextran sulfate	1:0.18	181.9 ± 29.3	25.1
	1:0.25	157.2 ± 6.2*	35.5
	1:0.5	143.4 ± 12.1*	40.9

[a] Mean ± SD ($n = 3$).

* $P < 0.01$, significantly different from the gentamicin-administered group.

The highest decrease in GM accumulation was observed when DS-5000 was coadministered, while DS-90000 did not reduce GM accumulation (Fig. 2). The fenestration of blood capillaries at the glomerulus is 70 to 100 Å in diameter, which limits the molecular size of compounds filtered through the glomerulus. Bohrer *et al.* (13) reported the effect of charge and molecular size on the glomerular filtration of dextran. The relative filtration ratio of negatively charged dextran is very low compared to those of neutral and positively charged dextran, and dextran of a molecular size greater than 30 Å is hardly filtered through the glomerulus membrane. Over a 3-hr infusion, DS was barely detectable in the urine following DS-90000 coadministered with GM (Fig. 2). Consequently, the reduction in GM accumulation by DS may depend on the glomerular filtration of DS.

An AG specific receptor is thought to mediate the saturable transmembrane transport (4,12,14). Since renal accumulation of GM reached a maximum after 3 hr of continuous infusion (Fig. 3), GM uptake into and efflux from renal cortex apparently reached steady state.

Coadministration of GM-DS or GM alone did not affect

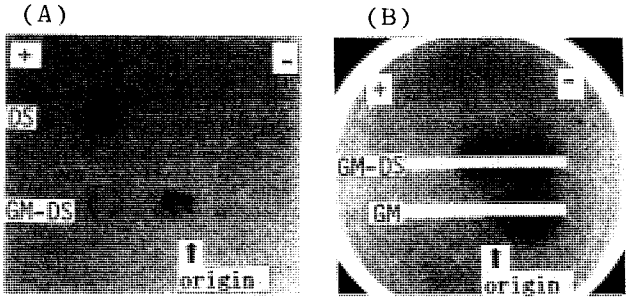

Fig. 5. Photograph of electrophoresis of GM-DS mixture on cellulose acetate membrane. (A) Stained with 2% toluidine blue; (B) the result of a bioassay using *B. subtilis* ATCC 6633. Arrow indicates the origin. The details are described under Materials and Methods.

urine pH (5.8–6.0) or urine flow rate (data not shown). Hence, the difference in GM accumulation within the renal cortex following GM or GM-DS infusion cannot be explained on the basis of the urinary pH and osmotic diuretic effect of DS but, rather, results from GM-DS binding in the urine. Kikuchi *et al.* (6) have shown that the binding of ^{3}H-dibekacin to renal BBM vesicles is noncompetitively inhibited by DS. Sastrasinh *et al.* (14) reported the phosphatidylinositides of renal BBM to have a greater affinity for AG than other phospholipids. Therefore, the decrease in GM accumulation could be caused by the inhibition of GM binding to phosphatidylinositides by the GM-DS complexation during the reabsorption process in the lumen of renal proximal tubles.

Our results indicate that the coadministration of GM and DS decreases GM accumulation in renal cortex, and low molecular weight DS may be useful for reducing the AG nephrotoxicity.

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