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Reduction of gentamicin-induced enzymuria by dextran sulfate

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Summary

The capacity of dextran sulfate to prevent enzymuria induced by gentamicin, an aminoglycoside, was investigated in rat. Rats were administered gentamicin intraperitoneally and dextran sulfate orally at the same time for 7 days. The urinary excretion of the enzymes, *N*-acetyl-β-D-glucosaminidase and alkaline phosphatase, increased as a result of gentamicin administration. The increments of both enzymes were suppressed when dextran sulfate was administered along with gentamicin. The effect of dextran sulfate on renal injury due to gentamicin was further investigated histochemically. The alkaline phosphatase activity of renal proximal tubular cells significantly decreased as a result of gentamicin administration, and this decrease was lessened by dextran sulfate. Gentamicin caused proximal tubular epithelium to swell as indicated by periodic acid-Schiff staining. This too was prevented by dextran sulfate. It thus appears that dextran sulfate may be capable of reducing, but not preventing gentamicin-induced nephrotoxicity.

Introduction

Nephrotoxicity is a serious side-effect of aminoglycosides (AGs), and is caused primarily by the accumulation of drugs within renal proximal tubular cells (Kaloyanides and Pastoriza-Munoz, 1980). Many investigators have found AGs to enter these cells through the brush-border membrane (BBM) (Silverblatt and Kuehn, 1979; Wedeen et al., 1983; Aramaki et al., 1986). Consequently, the reduction in this accumulation may be an effective means for avoiding the nephrotoxicity. Lipsky et al. (1980) reported gentamicin (GM) uptake by the renal BBM to be inhibited by spermine, a polyamine, and postulated that GM and spermine may share a common transport system in renal proximal tubular cells. Josepovitz et al. (1982) found the uptake of GM by rat renal cortex to decrease through the co-administration of a polycation, such as spermine or tetra-lysine.

AGs, which are polycationic drugs, have been reported to bind easily with substances such as phosphatidylinositol (Feldman et al., 1986; Sastrasinh et al., 1982), ATP (Inaba et al., 1986), and acidic mucopolysaccharides (Deguchi et al., 1978; Aramaki et al., 1987) through ionic interactions between the amino group(s) of AGs and phosphate, carboxyl, or sulfate groups.

The effects of AGs on enzyme excretion into the urine are well documented. The urinary excre-

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tion of lysosomal enzymes, *N*-acetyl-β-D-glucosaminidase and glucosidase has been noted to significantly increase following the administration of GM (Patel et al., 1975; Beck et al., 1977; Meisner, 1981).

In a previous report, we found GM to bind to dextran sulfate (DS), and that this compound, when co-administered with GM, causes the renal distribution of the GM to decrease (Kikuchi et al., 1988).

In the present study, biochemical and histochemical investigations were carried out to clarify the effects of DS on repeated administration of GM.

Materials and Methods

Materials

GM was provided through the courtesy of Shionogi Pharmaceutical Ind. Co. (Osaka, Japan). DS (mean mol. wt. = 5000, sulfur content was 15%) was purchased from Sigma Chemical Co. (St. Louis, MO). Male Wistar rats weighing 220–250 g were purchased from Shizuoka Agriculture Co. (Shizuoka, Japan).

Inducement of nephrotoxicity

The rats were acclimated for at least 3 days before beginning the experiments. They were administered GM (120 mg/kg/day) intraperitoneally and immediately following this, 50% of DS solution (1 g/kg/day) or physiological saline, orally for a period of 7 days. Two kinds of controls were carried out; saline was administered intraperitoneally and DS orally (DS control group), and saline administered by both routes (saline control group). Each rat was housed in a metabolic cage from which urine was collected immediately before the first time administration and then on days 2, 4, 6 and 8 after the beginning of the experiment. One day after the last administration, all rats were sacrificed and their kidneys isolated.

Histochemical study

Active staining of alkaline phosphatase was carried out as follows: the isolated kidney was fixed with 10% formaline–1% calcium chloride solution at 4°C for 24 h, and then washed several times with 30% sucrose containing arabic gum (1%) at 4°C. Cortex sections, 4 μm in thickness, were incubated for 10 min at room temperature in a reaction mixture containing 0.05 M 2-amino-2-methyl-1,3-propandiol (pH 9.75), 0.05% (w/v) naphthol AS-GR (3-hydroxy-2-anthranoic acid 2-methylanilide) phosphate and 0.1% (w/v) Fast blue RR salt. The sections were thoroughly washed with distilled water and post stained with 1% Methyl green in 0.1 M acetate buffer (pH 4.2). They were then dehydrated by a series of ethanol and embedded in gelatin. In the case of periodic acid-Schiff (PAS) staining of the renal cortex, the sections were incubated in 0.5% periodic acid solution for 10 min at room temperature followed by thorough washing with distilled water. They were subsequently incubated in Schiff's (fuchsin) solution for 10 min and washed 3 times with 10% sodium disulfite in 1 N HCl and then with distilled water. The sections were dehydrated, embedded and were observed by light microscope.

Analytical methods

Protein concentration was determined by the method of Lowry et al. (1951), using bovine serum albumin as the standard. Alkaline phosphatase and *N*-acetyl-β-D-glucosaminidase activities were assayed with *p*-nitrophenyl phosphate (Bessey et al., 1946) and *p*-nitrophenyl-β-D-glucosaminide (Niebes and Ponard, 1975) as the substrates, respectively. Before measurement of enzyme activity, the urine was dialysed against saline for 24 h at 4°C to avoid the effect of GM, which excreted in urine, on the enzyme activity (Aramaki et al., 1983; Takahashi et al., 1987). GM accumulation within the kidney cortex was determined by bioassay using *Bacillus subtilis* ATCC 6633 as the test organism following extraction of GM by the method of Ruben et al. (1984).

Results

Enzyme excretion into the urine

In the amounts of urine (Fig. 1A) and protein excretion (Fig. 1B), there were no significant dif-

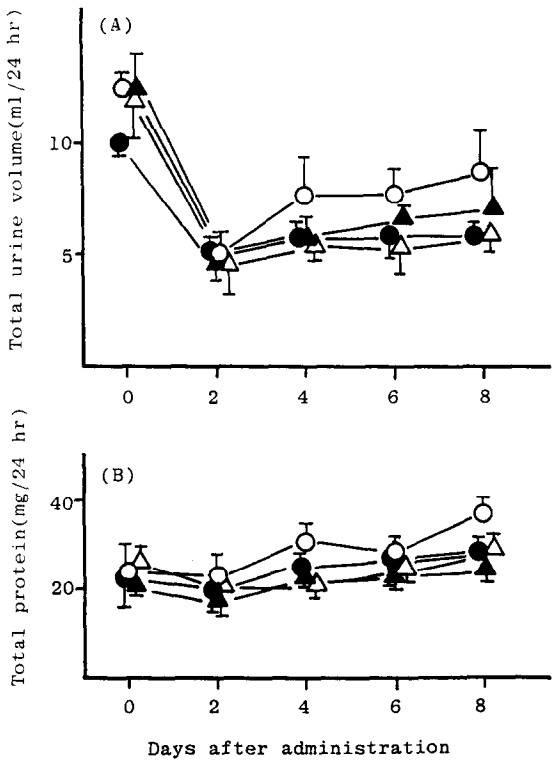

Fig. 1. Effect of gentamicin, and gentamicin with dextran sulfate on the urine volume (A) and urinary protein excretion (B). ○, gentamicin; △, gentamicin with dextran sulfate; ▲, dextran sulfate control; ●, saline control. Values shown represent the mean ± S.D. of 5 experiments.

ferences between GM and control groups or between GM with DS and control groups. As shown in Fig. 2, alkaline phosphatase activity in urine was gradually increased by GM administration, but this increase was suppressed by the oral administration of DS. These data were subjected to analysis of variance (ANOVA), and the significant differences were obtained between GM and GM with DS administered groups, and between control and GM with DS administered groups. In the two kinds of control groups, alkaline phosphatase activity in the urine was quite low and almost the same throughout the experiments. GM administration significantly elevated the activity of *N*-acetyl-β-D-glucosaminidase in the urine, but when GM was administered along with DS, this increase was suppressed. In the control groups of

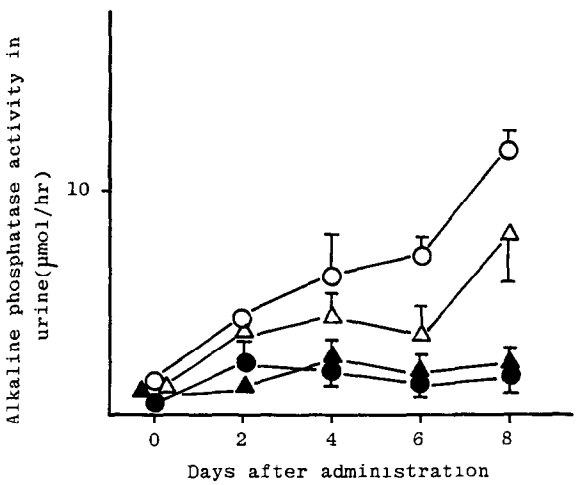

Fig. 2. Effect of dextran sulfate on gentamicin-induced excretion of alkaline phosphatase into urine. ○, gentamicin; △, gentamicin with dextran sulfate; ▲, dextran sulfate control; ●, saline control. Values shown represent the mean ± S.D. of 5 experiments. Statistical analysis was carried out by ANOVA.

saline or DS administration, increased excretion of this enzyme could not be observed throughout the experimental period (Fig. 3).

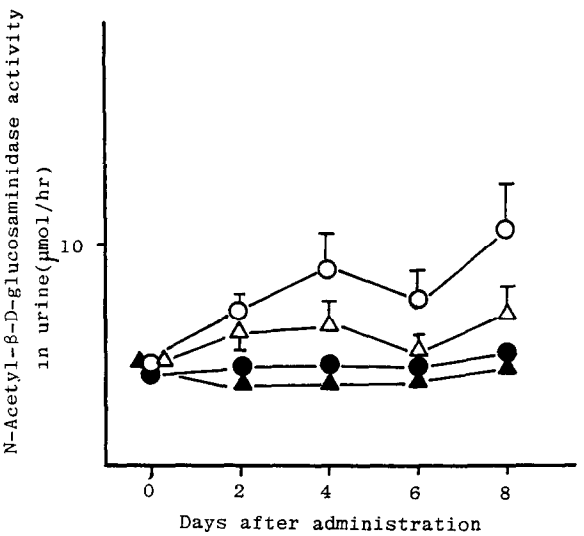

Fig. 3. Effect of dextran sulfate on gentamicin-induced excretion of *N*-acetyl-β-D-glucosaminidase into the urine. ○, gentamicin; △, gentamicin with dextran sulfate; ▲, dextran sulfate control; ●, saline control. Values shown represent the mean ± S.D. of 5 experiments. Statistical analysis was carried out by ANOVA.

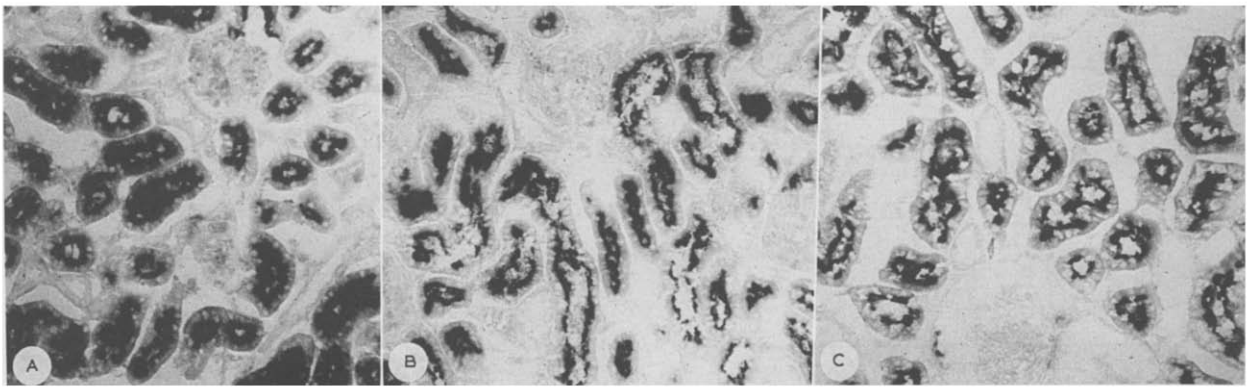

Fig. 4. Light micrographs of alkaline phosphatase activity of kidney cortex (×200). A: control (saline administered). B: gentamicin administered. C: gentamicin with dextran sulfate administered.

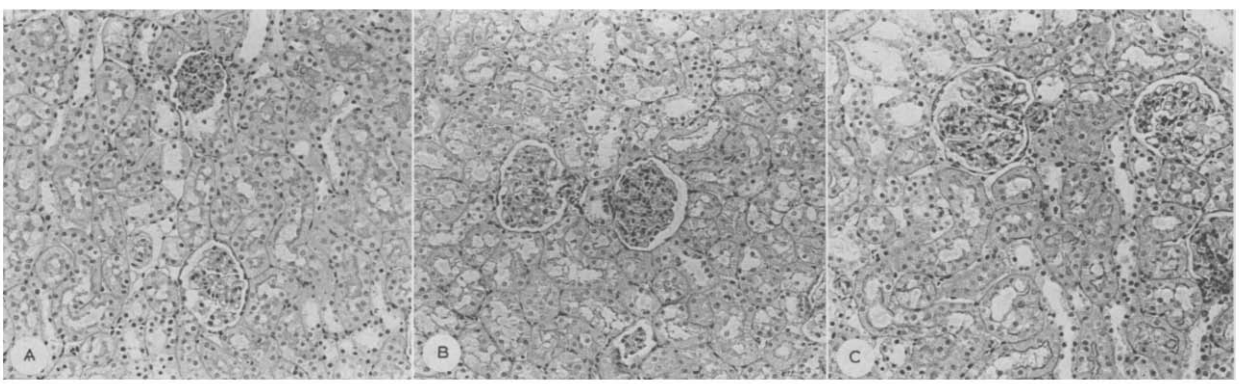

Fig. 5. Light micrographs of PAS-staining of kidney cortex. A: control (saline administered). B: gentamicin administered. C: gentamicin with dextran sulfate administered.

TABLE 1

Semiquantitative evaluations of histochemical observations (n = 3)

	Saline (Control)			Gentamicin			Gentamicin-DS		
	Rat			Rat			Rat		
	1	2	3	1	2	3	1	2	3
Disappearance of alkaline phosphatase at BBM [a]	–	–	–	+ + +	+ +	+	–	±	±
Edema of PTC [b]	–	–	–	+ +	+ +	+	±	±	±
Swelling of PTC [b]	–	–	–	–	+	+ +	–	–	–
Necrosis of PTC [b]	–	–	–	–	–	+	–	–	–
Decrease of BBM at PTC [b]	–	–	–	+ + +	+ +	+	±	±	±

BBM = brush-border membrane; PTC = proximal tubular cells. – = without change (control); ±, very slightly changed; +, slightly changed; + +, medium changed; + + +, strongly changed.

[a] Summarized the observation from Fig. 4.

[b] Summarized the observation from Fig. 5.

Histochemical observation

The effects of GM or GM with DS administration on rat renal alkaline phosphatase activity, a marker enzyme of BBM, were studied histochemically. As shown in Fig. 4A, the activity of this enzyme was noted to be high at the BBM of proximal tubules of the control rat kidneys. In the GM-administered kidney section (Fig. 4B), alkaline phsophatase-positive sites became thin and rough, and exfoliated from the BBM of proximal tubular cells. The disappearance of alkaline phsophatase by GM administration was suppressed by co-administered DS (Fig. 4C), and the intensity of positive staining of the enzyme showed a tendency to recover to that of the section from the control (Fig. 4A).

The effect of DS on GM-induced renal injury was estimated by PAS-staining, which stains mucin, glycoproteins, polysaccharides and the like. In the GM-administered kidney sections, the proximal tubular cells were observed to swell, and PAS-positive staining disappeared at the BBM, particularly in the area of extensive injury (Fig. 5B). This swelling or edema disappeared in the sections from DS co-administered rats, and the intensity of PAS staining recovered to the control level (Fig. 5C). Semiquantitative histochemical estimation obtained by blinded evaluator were summarized in Table 1.

Discussion

AGs are widely used to treat Gram-negative bacterial infections. Their use, however, occasionally results in nephrotoxicity. Nephrotoxicity is caused by AGs accumulation within the renal cortex, particularly the proximal tubular cells. The highest accumulation is usually found within the lysosomes (Silverblatt and Kuehn, 1979). Consequently, reduction in this accumulation may effectively reduce nephrotoxicity. Enzymuria has been frequently found to be induced by AGs and it is a useful indicator of the early stage of nephrotoxicity (Patel et al., 1975; Beck et al., 1977; Meisner, 1981). We previously reported that GM distribution within rat renal cortex following single dose i.v. administration was decreased by the co-administration of DS (Kikuchi et al., 1988). DS was chosen for the following reasons: (1) the stability of DS is high and its toxicity low (William et al., 1969); (2) the urinary excretion of DS with mol. wt. = 5,000 is high (Wada et al., 1976); and (3) this compound exists in various molecular weights. Thus, in this report, the effect of DS on enzymuria induced by GM was investigated.

As shown in Fig. 3, the urinary excretion of *N*-acetyl-β-D-glucosaminidase was increased by GM administration, and this increase was suppressed by subsequent DS administration. Inaba et al. (1984) reported that the release of lysosomal enzymes, *N*-acetyl-β-D-glucosaminidase and acid phosphatase were increased significantly by dibekacin in vitro. Patel et al. (1975) reported lysosomal enzymuria to be an early manifestation of GM nephrotoxicity and possibly related to lysosomal abnormalities. Thus, DS may possibly cause internalization of GM into lysosomes to decrease.

We previously observed that the activities of *N*-acetyl-β-D-glucosaminidase and alkaline phosphatase were inhibited by GM in vitro (Aramaki et al., 1983; Takahashi et al., 1987) and histochemical activity of alkaline phosphatase in renal cortex also decreased by GM administration (Takahashi et al., 1987). Thus, activities of these enzymes in the urine were measured after dialyzing against saline to eliminate urinary GM. Alkaline phosphatase has been reported to be localized in the BBM, tightly interacting with phospholipid, especially phosphatidylinositol, and to be liberated from the membrane by phosphatidylinositol-specific phospholipase C (Schali et al., 1984). Sastrasinh et al. (1982) reported AGs to have a high affinity towards phosphatidylinositides such as phosphatidylinositol, phosphatidylinositol 4-phosphate and phosphatidylinositol 4,5-diphosphate. These phosphatidylinositides are considered receptors for AGs. Beck et al. (1977) have reported the urinary excretion of γ-glutamyltransferase, another marker enzyme of BBM, in patients receiving AGs. But previously, we observed neither the release of alkaline phosphatase nor γ-glutamyltransferase from BBM vesicles by the addition of GM. Thus, the increase in urinary excretion of alkaline phosphatase by repeated GM

administration (Fig. 2) may result from disruption of BBM on the subsequent GM binding to phosphatidylinositides. The possibility of this is supported by histochemical experiments, and alkaline phosphatase activity and PAS-positive staining in the lumen of the renal proximal tubule were found to decrease by GM administration (Fig. 4B and 5B).

On the other hand, the increase in the urinary excretion of alkaline phosphatase by GM administration was repressed by DS co-administration (Fig. 2). Furthermore, this effect of DS for preventing GM-induced renal injury was observed histochemically; alkaline phosphatase activity in renal proximal tubular cells was recovered (Fig. 4C) and their swelling was virtually eliminated (Fig. 5C).

As mentioned above, AG-induced nephrotoxicity arises primarily from the accumulation of this drug within the renal cortex. Consequently, the effect of DS on the accumulation of GM within the renal cortex following 7 days of DS administration subsequent to that of GM was investigated, but the degree of accumulation at that time was almost similar in the GM (41.20 ± 0.95 μg/g of renal cortex, $n = 3$) and GM with DS (37.09 ± 2.87 μg/g of renal cortex, $n = 3$) administered groups. On the other hand, we have already reported that the rat renal accumulation of GM was reduced by a half following 3 h of the co-administration with DS (Kikuchi et al., 1988). Consequently, due to the long duration of this experiment, this comparatively low level of GM accumulation in the renal cortex of rat without the administration of DS may indicate that further amounts of GM cannot enter due to the necrosis of cells. Essentially the same result was reported by Kojima et al. (1986) using tobramycin. They observed latamoxef to ward off tobramycin-induced nephrotoxicity. The significant difference of tobramycin accumulation was greatest on day 5, but on day 7, no significant difference was evident.

The data of the present research demonstrate DS to be an effective substance for reducing nephrotoxicity by GM. At present, the mechanism for this effect is being investigated in detail by infusion experiments.

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