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# Effects of dextran sulphate on renal dysfunctions induced by gentamicin as determined by the kidney perfusion technique in rats

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**Abstract**—The effect of dextran sulphate on accumulation of gentamicin within the renal cortex and on renal functional changes induced by gentamicin has been investigated using the rat renal perfusion technique. Gentamicin accumulation decreased by the co-administration of dextran sulphate, the degree being proportional to dextran sulphate concentration. The filtered load of gentamicin was unaffected by dextran sulphate. The perfusion of kidney with gentamicin brought about a decrease in the reabsorption of $Na^+$ and an increase in the rate of urine flow with no effect on that of glomerular filtration. The addition of dextran sulphate in the perfusate resulted in the resumption of these levels to those of the control. Dextran sulphate thus apparently interacts with gentamicin in the lumen of proximal tubules to inhibit the reabsorption of gentamicin by proximal tubular cells, and prevents the renal dysfunctions induced by gentamicin.

Aminoglycoside accumulation within the renal proximal tubular cells occurs during the reabsorption process (Silverblatt & Kuehn 1979; Wedeen et al 1983). Theoretically, reduction of the nephrotoxicity should be possible by blocking this process. The inhibitory effects of polyamines such as polylysine, spermine, and spermidine for interactions between brush-border membrane vesicles and aminoglycosides have been previously investigated (Lipsky et al 1980), but polyamines are nephrotoxic (Rosenthal et al 1952). Aminoglycosides, which are polycationic antibiotics, have been reported to interact ionically with substances possessing negatively charged groups (Deguchi et al 1978; Sastrasinh et al 1982).

Recently, we indicated that gentamicin interacts with dextran sulphate, and their co-administration by single intravenous administration (Kikuchi et al 1988) and the continuous infusion method (Kikuchi et al 1990) significantly reduces gentamicin accumulation in the renal cortex.

The present study has been conducted to determine gentamicin accumulation and the renal functional changes during the co-administration of dextran sulphate using rat isolated perfused kidney.

## Materials and methods

*Renal perfusion.* Kidneys of male Wistar rats, 250–280 g, were perfused according to Nishiitsutsuji-Uwo et al (1967) and Hori et al (1988). The rats were anaesthetized with sodium pentobarbitone (50 mg $kg^{-1}$, i.p.), saline (1·0 mL), containing heparin (200 units) and mannitol (80 mg), was injected into the femoral vein, and a polyethylene tube (PE-10, Cray Adams) inserted into the ureter to collect the urine. Another polyethylene tube (PE-240) was then inserted into the lower inferior vena cava, following cannulation of the right renal artery with a 20 gauge needle via the superior mesenteric artery. The kidney was immediately perfused at 37°C at an arterial pressure of 90–100 mmHg, maintaining the perfusate flow at 4–6 mL $min^{-1}$. Reconstituted blood containing bovine red blood cells, glucose, amino acids, mannitol, and inulin served as the renal perfusate (haematocrit value: 13–15%). The perfusate for the drug-administered group was supplemented with gentamicin (final concentration; 10 μg $mL^{-1}$) or gentamicin:dextran sulphate (molar ratio 1:0·25 or 1:0·5, gentamicin concentration 10 μg $mL^{-1}$).

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*Analytical methods.* After 20 min equilibration, clearance periods were started every 10 min and perfusate samples were collected at the midpoint of each clearance period. Urine samples were collected in pre-weighed vials and volume was determined gravimetrically (as d = 1·0). The glomerular filtration rate (GFR) was estimated from the clearance of inulin. $Na^+$ concentration was determined by flame photometry, and glucose by a Glucose C-test Kit (Wako Pure Chemicals, Tokyo, Japan). Extraction of gentamicin from rat kidney cortex was carried out by the method of Ruben et al (1984), and its concentration was determined by bioassay using *Bacillus subtilis* ATCC 6633 as the test organism. Statistical analysis was performed by one way analysis of variance (ANOVA). *P* values of 0·05 or less were considered significant.

## Results

After 60 min perfusion, the renal cortical accumulation of gentamicin decreased dependently in relation to the molar ratio of dextran sulphate perfused with gentamicin (Fig. 1A). At a ratio of gentamicin:dextran sulphate = 1:0·25, gentamicin accumulation significantly ($P < 0·05$) decreased to 66%, and in the gentamicin:dextran sulphate = 1:0·5 perfused group, the accumulation was significantly ($P < 0·01$) reduced to 50% of the gentamicin perfused group. On the other hand, filtered loads of gentamicin which were calculated from GFR and its concentration in the perfusate were unaffected by addition of dextran sulphate (Fig. 1B).

Fig. 2 shows the results of the effect of gentamicin and/or dextran sulphate on the renal functions. By adding gentamicin to the perfusate, urine flow rate gradually increased, and the reabsorption of $Na^+$ decreased as the perfusion period increased. These data were subjected to ANOVA, and the significant differences were obtained between control and gentamicin perfused groups ($P < 0·05$). Following its perfusion with gentamicin:dextran sulphate = 1:0·25 or 1:0·5, urine flow rate

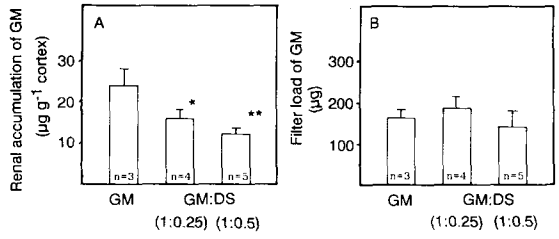


FIG. 1. Effect of dextran sulphate (DS) on the gentamicin (GM) accumulation in renal cortex (A) and filtered load of gentamicin (B). In each case, gentamicin concentration was 10 μg $mL^{-1}$. Dextran sulphate with the mean mol. wt 5,000 was used. Values shown represent mean ± s.d. for 3–5 experiments. Statistical analysis was carried out by ANOVA. * $P < 0·05$, ** $P < 0·01$, significant difference from gentamicin perfused group.

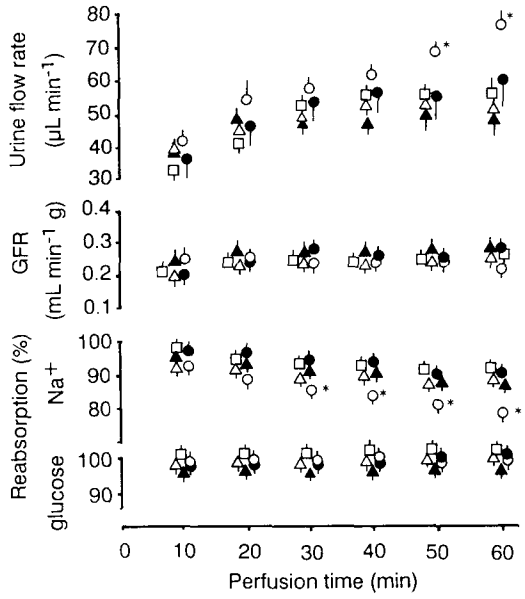


FIG. 2. Effect of gentamicin and dextran sulphate on renal functional properties of perfused rat kidney. In each case, gentamicin concentration was 10 μg mL$^{-1}$. ●, saline (control); ○, gentamicin; ▲, gentamicin-dextran sulphate (1:0·25); △, gentamicin-dextran sulphate (1:0·5); □, dextran sulphate. Values shown represent mean ± s.d. for 4 experiments. Statistical analysis was carried out by ANOVA. * $P<0·05$, significant difference from saline perfused group.

and the reabsorption of $Na^+$ were noted to have resumed control levels, and analysis by ANOVA showed no significant differences between control and gentamicin:dextran sulphate = 1:0·25 or 1:0·5 groups.

However, neither GFR nor glucose reabsorption were observed to undergo any significant change throughout the experimental period.

## Discussion

The isolated kidney perfusion technique is preferable for determining the direct effects of aminoglycosides on renal functions (Cojocel et al 1983; Miura et al 1985); thus, this technique was employed to assess the preventive effects of dextran sulphate on the renal accumulation of gentamicin and inducement of renal dysfunction by gentamicin.

The renal accumulation of gentamicin was found to decrease in proportion to the molar ratio of gentamicin-dextran sulphate with no effect on the filtered load of gentamicin (Fig. 1). Kikuchi et al (1988) reported that gentamicin bound to dextran sulphate in Tris-HCl buffer solution, but no binding could be observed in rat plasma by the equilibrium dialysis method. Consequently, in the perfusate of the present experiment, the two drugs do not bind and may be filtered through the glomerulus membrane individually, and no difference in the filtered load could be observed between gentamicin and gentamicin:dextran sulphate perfused groups. The reabsorption of weak electrolytes at proximal tubules is affected by urine flow rate and pH. Following the perfusion, urine flow rate and pH (5·8–6·0) were essentially the same between saline and gentamicin-dextran sulphate or dextran sulphate administered groups (Fig. 2). Consequently, the reduction in gentamicin accumulation following gentamicin-dextran sulphate perfusion cannot be explained on the basis of urinary pH and osmotic diuretic effect of dextran sulphate. The binding of aminoglycoside to brush-border membrane vesicles was also inhibited non-competitively by dextran sulphate (Kikuchi et al 1988). Furthermore there was an inverse correlation between renal accumulation of gentamicin and urinary excretion of dextran sulphate (Kikuchi et al 1990). Therefore, gentamicin may interact with dextran sulphate in the lumen of proximal tubules, and thus dextran sulphate inhibits gentamicin reabsorption through the brush-border membrane.

Kidney perfusion with gentamicin showed significant decreases in reabsorption of $Na^+$ and increase in urine flow rate with no effect on GFR (Fig. 2). Because aminoglycosides specifically accumulate within proximal tubular cells, gentamicin may inhibit the reabsorption of water or electrolytes which are considered to be reabsorbed at proximal tubules. These findings are consistent with the results that acute exposure to gentamicin reduced water and electrolyte reabsorption by the rat isolated perfused kidney (Cojocel et al 1983; Miura et al 1985). Gentamicin-induced renal functional changes in urine flow rate and $Na^+$ reabsorption were prevented and resumed the control level by addition of dextran sulphate in the perfusate. Using the rat isolated kidney perfusion technique, dextran sulphate in the perfusate may thus prevent an early stage of aminoglycoside nephrotoxicity. This effect may be attributed to the action of dextran sulphate that decreases the renal accumulation of gentamicin.

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