COMMUNICATIONS

Protective effects of cyclodextrin sulphates against gentamicin-induced nephrotoxicity in the rat

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Abstract—The effects of cyclodextrin sulphates on the development of rat renal dysfunction induced with gentamicin, an aminoglycoside antibiotic, were studied. Daily subcutaneous injection of gentamicin (100 mg kg^{-1}, 14 days) developed nephrotoxicity in the rat as assessed by an increase in serum urea nitrogen and histopathological changes in the renal cortex. When cyclodextrin sulphates were given intraperitoneally at 300 mg kg^{-1} at 6 h intervals after gentamicin administration, they protected the rat against the drug-induced renal impairment, while the parent cyclodextrins were ineffective. Since post-administration of cyclodextrin sulphates did not reduce the total amount of gentamicin accumulated in the kidney, the protection may occur through interference with intracellular events leading from the drug accumulation to nephrotoxicity. These results suggest that cyclodextrin sulphates are particularly effective in preventing renal failure associated with aminoglycoside treatment.

Aminoglycoside antibiotics, including gentamicin, are widely used in the clinical treatment of Gram-negative infections, but their use is sometimes complicated by the development of drug-induced acute renal failure (Humes et al 1982; Bennett 1983). Accumulation of aminoglycosides within the renal cortex is known to be intimately related to the pathogenesis of nephrotoxicity. Aminoglycosides are thought to interact with negatively-charged phospholipids of lysosomal membranes in the proximal tubular cells, the interaction of which may lead eventually to lysosomal dysfunction, resulting in necrosis of the cells (Laurent et al 1990).

The value of molecular encapsulation of drugs with cyclodextrins and their derivatives in pharmaceutical formulations has been well recognized (Szejtli 1988; Uekama et al 1991). Introduction of sulphate groups onto the hydroxyl groups of cyclodextrins confer heparin-mimicking activity on such derivatives (Folkman et al 1989). Cyclodextrin sulphates are highly hydrophilic amorphous mixtures with distributions of the degree of substitution by sulphate groups (Pitha et al 1991), and are less toxic than the parent cyclodextrins when given parenterally in the rat (Shiotani et al 1992). Recent studies have shown that polyanions such as dextran sulphates are able to interact electrostatically with gentamicin and to reduce the drug entry into the renal cortex (Kikuchi et al 1988). These findings led us to examine the potential use of cyclodextrin sulphates as alternatives to dextran sulphates for preventing gentamicin-induced nephrotoxicity. Our preliminary studies (Shiotani et al 1992) have shown that cyclodextrin sulphates protected the rat against gentamicin-induced nephrotoxicity. Cyclodextrin sulphates showed protective effects, even when they were given separately after gentamicin had been distributed to the kidney. Thus, the present paper deals with the effects of post-administration of α-, β, and γ-cyclodextrin sulphates on gentamicin-induced nephrotoxicity in the rat compared with those of the parent cyclodextrins.

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Materials and methods

Gentamicin sulphate (696 μg gentamicin base mg^{-1}, Sigma Chemical Co., MO, USA) was used as supplied. Cyclodextrins were donated by Nihon Shokuhin Kako Co. Ltd (Tokyo, Japan). Cyclodextrin sulphates were prepared by a non-regioselective method as described previously (Pitha et al 1991). The average degrees of substitution of sulphate groups in α-, β- and γ-cyclodextrin sulphates were confirmed to be 11·2, 12·0 and 7·8, respectively, by fast-atom bombardment mass spectrometry (Pitha et al 1991).

Male Wistar rats, 180–200 g, were given free access to water and food throughout the study. Renal failure was induced by daily subcutaneous injection of gentamicin in 0·9% NaCl (saline) (40 mg mL^{-1}) at a dose of 100 mg kg^{-1} for a period of 14 days; choice of the dose of gentamicin was based on published data (Wellwood et al 1976). Cyclodextrins were dissolved in saline (60 mg mL^{-1}) and injected intraperitoneally at a dose of 300 mg kg^{-1} at 6-h intervals after the gentamicin administration based on our preliminary studies (Shiotani et al 1992). The only exception was β-cyclodextrin which was given at a dose of 100 mg kg^{-1} day^{-1} due to limited solubility (18·5 g L^{-1} in water at 25°C). The body weight gain of each rat was recorded and a blood sample (0·2 mL) was drawn from the caudal vein once a day for 14 days. Serum urea nitrogen was measured by a modified urease-indophenol method (Mizuho Medy Co. Ltd, Saga, Japan). On the final day of the experiment, the rat was killed with anaesthetic ether and the kidney was weighed. Part of the kidney served as a sample for histological examination. The kidney was fixed in 20% buffered formalin, embedded in paraffin, and stained with haematoxylin and eosin. The extraction of gentamicin from the renal tissue homogenates was performed according to the method described by Agarwal (1989) with slight modification. The concentration of gentamicin was determined using homogeneous enzyme immunoassay (Emit Gentamicin Assay, Syva Co., CA, USA). Student's t-test was used for statistical evaluation of the data; $P < 0.05$ was considered to be statistically significant.

Results and discussion

Fig. 1 shows changes in serum levels of urea nitrogen in the rat treated with gentamicin or in combination with β-cyclodextrin sulphate for 14 days. The daily subcutaneous administration of gentamicin at 100 mg kg^{-1} caused renal dysfunction in the rat. Serum urea nitrogen in the rat treated with gentamicin rose gradually after day 7, reaching a maximum increase of 53·2 ± 9·8 mg dL^{-1} on day 11, along with reduction in body weight gain and increase in kidney weight. The decline in serum urea nitrogen after day 11 may be due to the biochemical effects of gentamicin on urea metabolism and the reduced food uptake in the rat. Furthermore, the advanced renal impairment due to gentamicin was inferred to militate against the reabsorption of urea from the renal tubules, a situation which may lead to the decline in serum urea nitrogen.
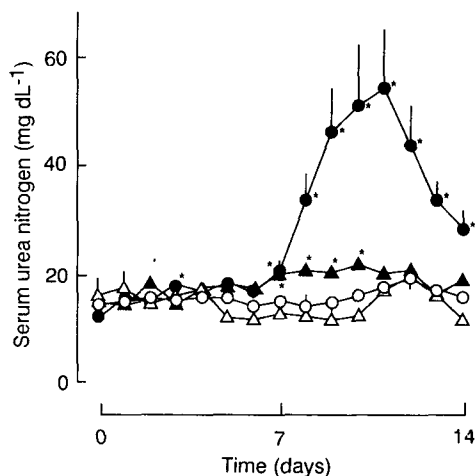
FIG. 1. Effect of β-cyclodextrin sulphate (300 mg kg^{-1} day^{-1}, i.p., 6 h post-administration) on the serum levels of urea nitrogen in the rats treated with gentamicin (100 mg kg^{-1} day^{-1}, s.c.) for 14 days. O Saline control, $\triangle$ β-cyclodextrin sulphate alone, $\bullet$ gentamicin alone, $\blacktriangle$ gentamicin with β-cyclodextrin sulphate. Each value represents the mean$\pm$s.e. of 3–12 rats. *$P < 0.05$ vs saline control.

In this study, cyclodextrins were designed to be administered separately after gentamicin had been distributed to targetted tissues in the expectation of minimizing their interference with the therapeutic efficacy of the drug. Following subcutaneous administration, the serum level of gentamicin attained a maximum within 15 min and fell to below the detection limit of 1 μg mL^{-1} within 6 h (Shiotani et al 1992). Thus, cyclodextrins were given intraperitoneally at 300 mg kg^{-1} at 6-h intervals after the gentamicin administration. As shown in Fig. 1, β-cyclodextrin sulphate significantly protected the rat against the gentamicin-induced renal dysfunction. Under the present conditions, β-cyclodextrin sulphate alone did not affect the serum levels of urea nitrogen in the rat; the values for β-cyclodextrin sulphate alone were comparable with those for saline control over a 14-day period. All cyclodextrin sulphates prevented the increase in serum urea nitrogen induced with gentamicin (Table 1), the efficacy of which may depend on the degree of substitution of sulphate groups rather than the size of the cavity in cyclodextrins used. On the other hand, the parent cyclodextrins failed to prevent gentamicin-induced renal dysfunction. In our preliminary studies, other hydrophilic cyclodextrin derivatives such as dimethyl-β-cyclodextrin and 2-hydroxypropylated cyclodextrins were ineffective for this purpose (data not shown).

Fig. 2 shows the typical appearance of renal proximal tubules

Table 1. Effects of cyclodextrins (300 mg kg^{-1} day^{-1}, i.p., 6 h post-administration) on the serum levels of urea nitrogen in the rat treated with gentamicin (100 mg kg^{-1} day^{-1}, s.c.) on day 11.

Treatment	Serum urea nitrogen (mg dL^{-1})[a]
Saline control	18.9 $\pm$ 0.8**
Gentamicin alone	53.2 $\pm$ 9.8*
+ α-cyclodextrin	83.8 $\pm$ 15.8
+ β-cyclodextrin[b]	59.7 $\pm$ 20.2
+ γ-cyclodextrin	49.9 $\pm$ 6.7*
+ α-cyclodextrin sulphate	22.7 $\pm$ 2.6**
+ β-cyclodextrin sulphate	21.1 $\pm$ 1.3**
+ γ-cyclodextrin sulphate	23.1 $\pm$ 1.7*.**

[a] Each value represents the mean $\pm$ s.e. of 3–12 rats. [b] The dose of β-cyclodextrin was 100 mg kg^{-1} day^{-1}. *$P < 0.05$ vs saline control. **$P < 0.05$ vs gentamicin alone.

A   B
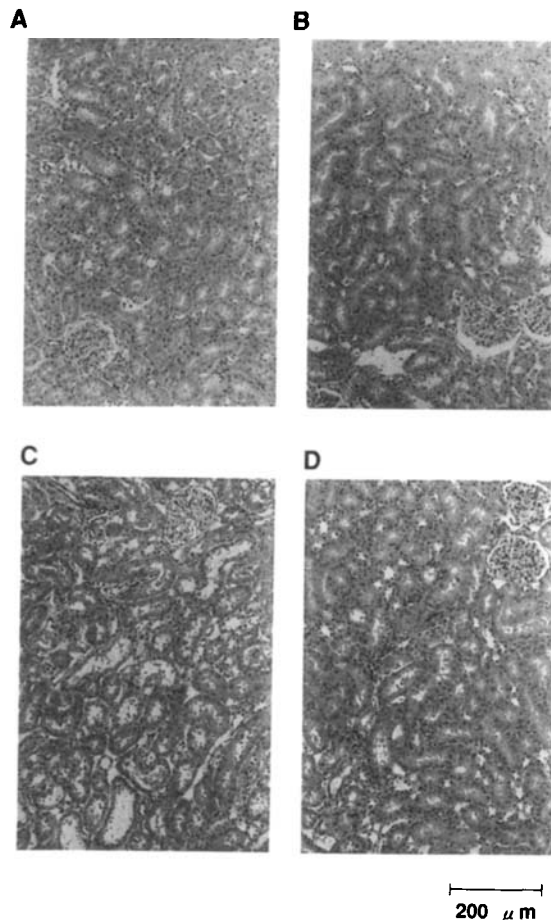

C   D

$\longmapsto$
200 μm

FIG. 2. Micrographs of renal proximal tubules of the rat treated with gentamicin (100 mg kg^{-1} day^{-1}, s.c.) alone or in combination with β-cyclodextrin sulphate (300 mg kg^{-1} day^{-1}, i.p., 6 h post-administration) for 14 days. A Saline control, B β-cyclodextrin sulphate alone, C gentamicin alone, D gentamicin with β-cyclodextrin sulphate.

of the rat treated with gentamicin alone or in combination with β-cyclodextrin sulphate for 14 days; conditions of the drug administration were the same as those in Fig. 1. Under the present conditions, treatment with saline control or β-cyclodextrin sulphate alone induced no noticeable histological changes in tubular cells of the rat (Fig. 2). Gentamicin produced extensive proximal tubular necrosis in the renal cortex; the tubular cells were flattened and partly discontinuous, and the lumens were widened (Fig. 2). On the other hand, there were no conspicuous alterations in glomeruli, distal tubules, and collecting ducts in the rat treated with gentamicin. These histological findings were similar to those described previously (Bertelli et al 1981). In sharp contrast, the combination of gentamicin with β-cyclodextrin sulphate produced no significant alterations in the renal proximal tubules (Fig. 2).

To avoid the renal toxicity of aminoglycosides, several approaches have been proposed, including the reduction in renal accumulation of the drugs by competitive inhibition with cationic polypeptides (Josepovitz et al 1982) and by complexation with dextran sulphates (Kikuchi et al 1988), and interference with intracellular processes linked to injury cascade by anionic polypeptides (Kishore et al 1990). To gain insight into the mechanism by which cyclodextrin sulphates protect against the gentamicin nephrotoxicity, the effect of cyclodextrin sulphates on the renal accumulation of the drug was examined.
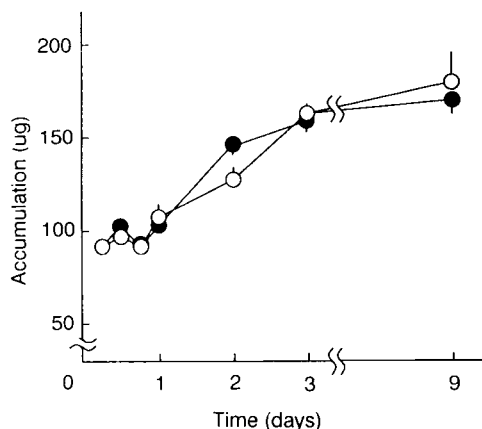
FIG. 3. Effect of β-cyclodextrin sulphate (300 mg kg^{-1} day^{-1}, i.p., 6 h post-administration) on the renal accumulation of gentamicin in rats treated with gentamicin (100 mg kg^{-1} day^{-1}, s.c.) for nine days. ○ Gentamicin alone, ● gentamicin with β-cyclodextrin sulphate. Each point represents the mean ± s.e. of 3–6 rats.

β-Cyclodextrin sulphate, when given 6 h after gentamicin administration, did not reduce the total amount of the drug stored in the kidney for up to nine days (Fig. 3). This suggests that the protection afforded by cyclodextrin sulphates may occur through interference with intracellular processes linked to the injury cascade of the gentamicin toxicity in renal proximal tubular cells. Our preliminary studies have demonstrated that cyclodextrin sulphates interact electrostatically with gentamicin and interfere with the binding of gentamicin to an anionic resin in-vitro (Shiotani et al 1992). It is, therefore, likely that cyclodextrin sulphates may affect the interaction of gentamicin with negatively-charged phospholipids in lysosomes of the renal cortex, which would render the drug less toxic (Kishore et al 1990). It is conceivable that an enhancement of the renal tissue repair could prevent the gentamicin-induced tubular necrosis from leading to functional impairment (Laurent et al 1990). Since cyclodextrin sulphates were found to have anti-inflammatory activity (Szejtli 1988) and have high affinity to growth factors and stabilize them against proteolysis (Kato et al 1989), the effects of cyclodextrin sulphates on the renal regeneration processes should be considered as another possible protective mechanism.

While the pharmacokinetic and toxicological issues on cyclodextrin sulphates should be further investigated before their practical use, the limited data obtained here suggest that cyclodextrin sulphates may serve as potent antidotes against renal failure associated with aminoglycoside treatment.

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