URINARY PHOSPHOLIPIDS PATTERNS AFTER TREATMENT WITH AMINOGLYCOSIDE AND CIS-PLATINUM

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INTRODUCTION

Oto- and nephro-toxicity are the two main limiting factors in the clinical use of aminoglycoside antibiotics. The earliest renal alteration induced by aminoglycosides is the development of a phospholipidosis in proximal tubular cells related to the inhibition of the activities of lysosomal phospholipases and sphingomyelinase (see Tulkens, 1986 for review). Josepovitz et al. (1986) reported that large doses of aminoglycosides, the use of which is associated with the rapid onset of widespread tubular necrosis and kidney dysfunction, induce a marked urinary excretion of phospholipids in rats. We have observed that this excretion already occurs in animals treated at low, clinically relevant doses (Ibrahim & Tulkens, 1986), suggesting that it was not solely due to tubular necrosis and shedding of phospholipid-overloaded cell casts in the lumen. We have therefore examined the phospholipid excretion in humans treated with normal doses of an aminoglycoside, in the absence of significant alteration of the renal function. In parallels we have examined the phospholipid excretion in rats treated with another nephrotoxin acting on proximal tubular cells but which does not cause phospholipidosis, namely cis-platinum.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats (200-250 mg) were injected with either gentamicin (supplied as the gentamicin complex GEOMYCIN used in human clinical practice in Belgium; Essex Belgium s.a., subsidiary of the Schering Corporation, N.J.) at 10 mg/(kg.day) for 10 days, or with cis-platinum (supplied as an sample for non-clinical investigations by Bristol Laboratories, Syracuse, N.Y.) at 2 mg/(kg.day) for 4 days. The daily doses were administered in one injection per day (qD). Controls received 0.9% NaCl on the same schedule. 24h urines were collected by placing animals individually in metabolic cages.

Patients. Nine young women suffering from pelvic inflammatory disease (who had not received an aminoglycoside before being referred to our hospital) were enrolled in the present study. Spot urine was obtained at day 0 (prior therapy) and 24h urines were collected at days 1, 4 and 7 during therapy. The antibacterial treatment consisted of netilmicin...
NETROMYCIN, Essex Belgium s.a., subsidiary of the Schering Corporation, N.J.) at an initial dose of 6.6 mg/(kg.day) given either in 1 injection (QID) or 3 injections (TID) per day, ampicillin (4 g/day, BID) and tinidazole (0.8 g/day, QID). For the patients treated on a TID schedule with netilmicin, dosage was readjusted if necessary to maintain the serum peak concentration (extrapolated at t=0) between 5 and 7 mg/l.

**Assay of phospholipids.** The whole 24h rat urine sample (7-15 ml) of each animal, or a 100 ml aliquot of each human urine sample was centrifuged at 25,000 rpm for 1h and the pellet suspended in 0.5-1.0 ml of water. Total lipid phosphorus was extracted and measured as described by Laurent et al. (1982). Individual phospholipids were separated by unidimensional thin-layer chromatography on silical gel 60 (Merck AG, Darmstadt, W.-Germany). For the separation of phosphatidylethanolamine (PE) and sphingomyelin (SM), elution was performed with chloroform:methanol:ammonia:water (24:16:2:1, v/v). Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were separated by two successive developments in chloroform:methanol:acetic acid:water (65:50:1:4, v/v). The position of each phospholipid on the plates was established by comparison with reference standards chromatographed on the same plates and visualized by iodine vapour. The zones of the plates corresponding to the phospholipids to be measured were scrapped off and the samples mineralized with 60% perchloric acid at 210°C for 90 min.

**Other analyses.** At the end of the treatment, the animals were killed and the renal cortex collected, fixed and treated for histopathological examination using standard procedures.

**RESULTS**

Table I shows the excretion of phospholipids in rats. After 4 days of treatment with gentamicin, or 2 days with cis-platinum, the excretion of all measured phospholipids was increased approximately 2- to 3-fold compared to baseline values (4-fold for phosphatidylethanolamine). When the treatment was continued for gentamicin, phospholipiduria became more pronounced for all phospholipids, reaching a 6- to 8-fold increase, except for sphingomyelin. Earlier studies have demonstrated that a 10 days treatment with gentamicin at 10 mg/(kg.day) induces a conspicuous lysosomal phospholipidosis (Laurent et al., 1982), but no widespread cortical necrosis (Kosek et al., 1974), even though a 5- to 6-fold increase in DNA cortex synthesis can be evidenced (Laurent et al., 1983), partly corresponding to a tubular repair process following focal tubular necrosis (Toubeau et al., 1986). In comparison to gentamicin-treated animals, rats receiving cis-platinum for 4 days showed considerably less phospholipid excretion. Yet, histopathological examination revealed that this treatment with cis-platinum induced widespread necrosis of proximal tubules, as previously described by Maids & Harrington (1978).

Table II shows the urinary excretion of phospholipids in women treated with netilmicin. Whereas all phospholipids measured were increased, their pattern was different from that observed in gentamicin-treated rats, with a highly predominant excretion of phosphatidylinositol. As in rats, however, sphingomyelin excretion was the least enhanced. During treatment, no patient experienced increase in serum creatinine over 0.1 mg %, and creatinine clearance was not significantly decreased (data not shown). Only minor dosing readjustment was needed to maintain patients assigned to the TID schedule within acceptable peak and through serum concentrations (5-7 mg/l and less than 1 mg/l, respectively). Patients treated on a qD schedule showed peak levels comprised between 16 and 24 mg/l. In all cases, the serum half-life of the aminoglycoside remained comprised between 1.5 and 2h.