THE PATTERN OF EXCRETION OF ENZYMES IN THE URINE OF GENTAMICIN-INJECTED RATS

S.B. Chahwala & E.S. Harpur, Pharmacology Laboratories, Department of Pharmacy, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET

There is much interest in the assay of enzymes excreted in urine as an index of proximal tubular damage caused by nephrotoxic drugs such as the aminoglycoside antibiotic, gentamicin (Patel et al 1975). Although the clinical application of such a procedure may be limited, it has value as an adjunct to the conventional methods of assessment of the nephrotoxic potential of drugs in animal models.

A study was made of changes in the urinary excretion of a cytosolic enzyme, lactate dehydrogenase (LDH), a lysosomal enzyme, N-acetyl-β-glucosaminidase (NAG) and an enzyme localised on the brush border membrane of the proximal tubular cells, alanine aminopeptidase (AAP). Two groups of five Wistar albino rats were injected s.c., daily for seven days, with water (controls) or gentamicin (40 mg/kg/day). The rats were housed individually in metabolic cages with free access to food and water for four days of acclimatization during the injection period and for seven subsequent days. Twenty-four hour urines were collected continuously over ice. Enzyme activities were assayed in triplicate using centrifuged and dialysed urine.

After the first injection (day 1) the activity of all three enzymes in the urine of gentamicin-injected rats was significantly elevated ($p < 0.001$) compared with the controls. LDH activity increased progressively and reached a peak on day 9, i.e. two days after the last injection (Table 1). Although the activity then declined it was still significantly elevated ($p < 0.001$) on day 13. In contrast, the activities of NAG and AAP rose initially but then fell to levels not significantly different from the controls on days 3 - 5 (NAG) and days 3 - 4 (AAP). The excretion of both enzymes then rose again and remained significantly elevated ($p < 0.001$) throughout the course of the experiment.

Table 1. The peak excretion of enzymes in urine of rats following injection of gentamicin (40 mg/kg/day) for seven days

Enzyme	Day of peak activity	Enzyme activity (mU/hr/mg creatinine) Control	Gentamicin-injected
LDH	9	0.33 ± 0.05	3.32 ± 1.57
NAG	9	0.23 ± 0.04	0.57 ± 0.11
AAP	7	1.14 ± 0.11	1.94 ± 0.33

The second phase elevation of the excretion of NAG and AAP was paralleled by a significant increase in the amount of protein in the urine of gentamicin-injected rats and was presumably due to cell necrosis and the appearance of cell debris in urine. The initial rise in the levels of NAG and AAP in urine may have resulted from the binding of the drug to the brush border membrane and subsequent uptake into the proximal tubular cells. These processes have been established by other studies. The early appearance and the progressive increase in the levels of LDH in urine may reflect damage tc the plasma membrane with leakage of cytosol from the proximal tubular cells, later augmented by the appearance of cell debris.

Interpretation of the results of studies of the pattern of enzyme excretion in urine together with information about concomitant functional and histological changes may provide valuable information about the mechanisms of the cell damage caused by drugs such as gentamicin.

Patel, V. et al (1975) Antimicrob. Agents Chemother. 7: 364-369

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