

## SHORT COMMUNICATION

### Urinary excretion of glutamine transaminase K as an early index of mercuric chloride-induced nephrotoxicity

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The possibility that urinary glutamine transaminase K activity might be a marker of a proximal tubule segment-specific response to mercuric chloride was investigated in male rats after a single i.p. injection in time-course and dose-response experiments. Urinary total proteins and angiotensin converting enzyme activity were determined simultaneously. Urinary indices showed an early increase (within 5 h of treatment) of total proteins and angiotensin converting enzyme, whereas glutamine transaminase K increased 10 h after treatment. The peak of all these indices was observed 24 h after mercuric chloride injection. The lowest dose that induced a significant increase in proteins and enzymes was  $0.25 \text{ mg kg}^{-1}$ ; in addition, a dose-response effect was observed. Glutamine transaminase K appeared to be an early and sensitive index of response of mercuric chloride effects, similar to total proteins and angiotensin converting enzyme. It is suggested that this enzyme is mainly localized in the 'pars recta' of the proximal tubule. Therefore glutamine transaminase K might be a segment-specific marker for the detection of damage localized in this portion of the proximal tubule.

**Keywords:** mercuric chloride, urinary glutamine transaminase K, urinary angiotensin converting enzyme, urinary proteins.

### Introduction

The acute effects of mercuric chloride ( $\text{HgCl}_2$ ) on the proximal tubule are well known and the straight portion ('pars recta',  $S_1$  segment) has been defined as the site of damage (Rodin and Crowson 1962, Biber *et al.* 1968).

Enzymuria (Price 1982) is an important means of evaluating kidney toxicity caused by xenobiotics and several studies have correlated urinary enzyme excretion and  $\text{HgCl}_2$  treatment (Robinson *et al.* 1967, Stroo and Hook 1977, Braun *et al.* 1978, Lock and Ishmael 1979, Diericks 1980).

Glutamine transaminase K (GTK) is a kidney enzyme which is mainly localized in the cytosol (90%) but which also

occurs in mitochondria (Cooper and Meister 1981); it is identical to kidney-cytosolic cysteine conjugate  $\beta$ -lyase (Stevens *et al.* 1986). Some researchers have attempted to find the location of the enzyme along the proximal tubule. Jones *et al.* (1988), using immunohistochemical techniques, showed no marked difference in the distribution of GTK along the tubule; conversely MacFarlane *et al.* (1989), using a similar technique, found that cytosolic  $\beta$ -lyase was localized mainly in the 'pars recta', though cytosolic  $\beta$ -lyase proteins are also present in the 'pars convoluta'.

The present research studied the effects of different doses of  $\text{HgCl}_2$  on the proximal tubule of male Wistar rats and the time-course and dose-related excretion of GTK in urine.

### METHODS

#### Animals

Albino, Wistar male rats (3 months old) (Morini, S. Polo d'Enza, RE, Italy) which were kept in a natural dark-light cycle and fed with standard diet (Nuova Zoonfarm, Padova, Italy) and water *ad libitum* were used.

#### Chemicals

L-Phenylalanine, D(+)-sucrose, 2-amino-2-methyl-1,3-propanediol (ammediol) were purchased from Fluka (Buchs, Switzerland);  $\alpha$ -keto- $\gamma$ -methiolbutyric acid and glycyl-histidyl-glycine were obtained from Sigma Chemical Co. (St Louis, USA);  $\text{HgCl}_2$  and other chemicals were obtained from Merck (Darmstadt, Germany).

#### Experimental design

##### Time course of urinary indices excreted after $\text{HgCl}_2$

Six adult male rats were treated with  $\text{HgCl}_2$  ( $1.0 \text{ mg Hg}^{2+}$  per kg b.w., i.p.). To obtain a good separation between urine and faeces, single metabolic cages were used. Urine was collected separately in collectors plunged in an ice bath as follows: 6 pm–8 am (before treatment), 8 am–1 pm (0–5 h after treatment), 1 pm–6 pm (5–10 h after treatment), 6 pm–8 am (10–24 h after treatment), 8 am–6 pm (24–34 h after treatment), 6 pm–8 am (34–48 h after treatment), 8 am–6 pm (48–58 h after treatment), and 6 pm–8 am (58–72 h after treatment).

Urine (pre- and post-dosing) were immediately centrifuged (10 min, 3000 rpm) to remove debris and clean supernatant was used to measure excretion of total urinary proteins (TP) according to Pesce and Strande (1973), angiotensin converting enzyme (ACE) activity according to Summary (1976) with glycyl-histidyl-glycine as substrate, and GTK according to Cooper and Meister (1985) with L-phenylalanine and  $\alpha$ -keto- $\gamma$ -methiolbutyric acid as substrates. All urinary indices were related to creatinine concentration, determined with a commercial kit (Boehringer Mannheim, Germany) based on the basic picrate reaction.

#### Dose-dependent relationship

Male rats were subdivided into five groups (five animals each) and injected with a single dose of  $\text{HgCl}_2$  (0, 0.125, 0.25, 0.5, or  $1.0 \text{ mg Hg}^{2+}$  per kg b.w., i.p.) dissolved in water. A pre-dose urine collection was carried out for 12 h (8 pm–8 am). Afterwards, the rats were treated and distributed into the metabolic cages 12 h after injection. Urine was collected for the nocturnal period (12 h) and TP, ACE and GTK were determined. The choice of this collection period follows from the results of the time-course experiment.

#### Equipment

A spectrophotometer (Perkin-Elmer Lambda 5 model) was used for analytical determinations.

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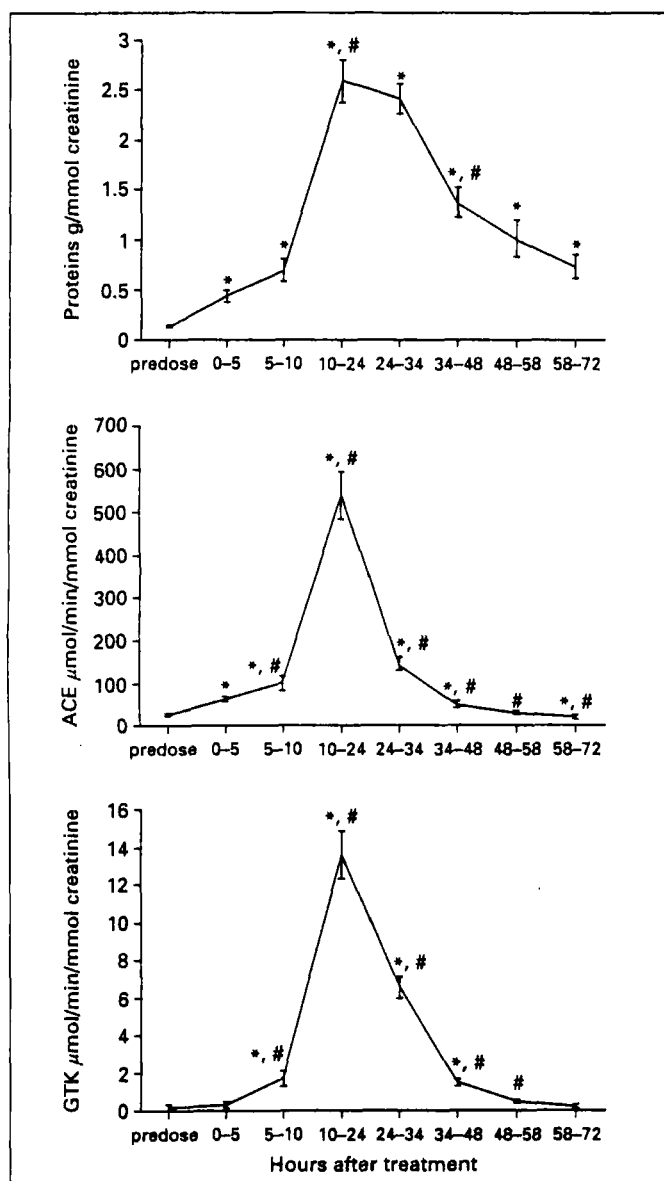
## Statistics

Statistical evaluation of the results was done by means of paired t-test (time-course) and variance analysis (dose-response experiments) and  $p < 0.05$  was considered significant. Values were expressed as mean  $\pm$  standard error of the mean (SEM).

## Results

### Time-course of urinary indices excreted after $\text{HgCl}_2$

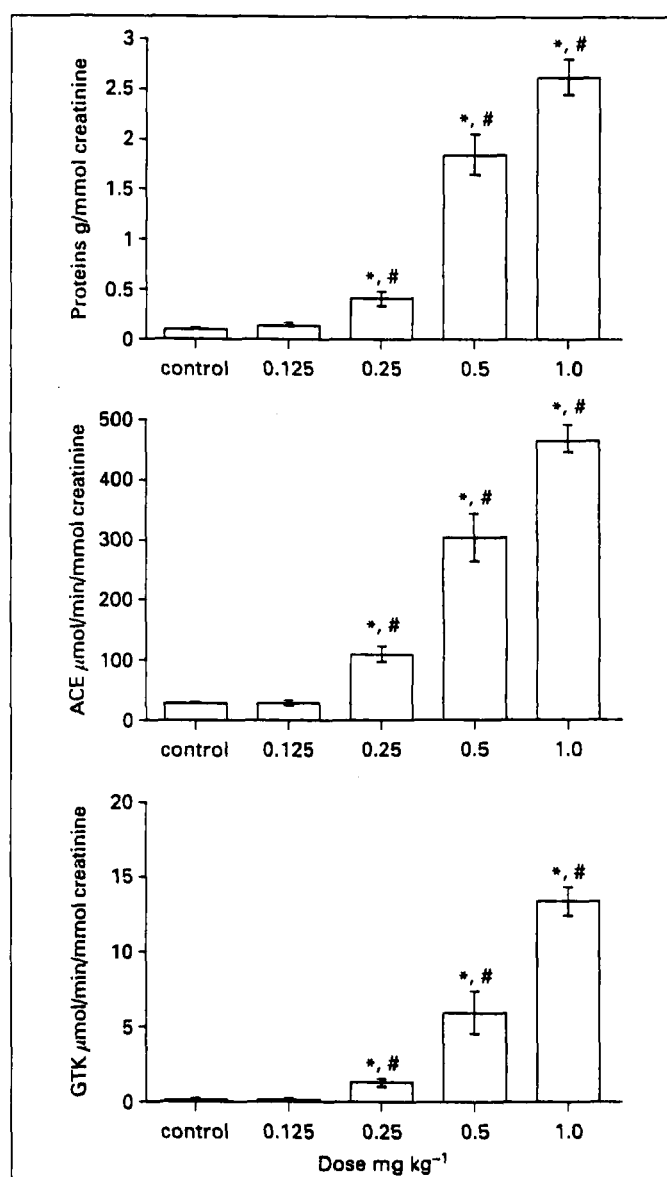
The time-course of urinary indices excreted after doses of  $\text{Hg}^{2+}$  ( $1.0 \text{ mg kg}^{-1}$ ) to male rats is shown in Figure 1. TP



**Figure 1.** Time-course of urinary indices of nephrotoxicity after treatment with an acute dose of  $\text{HgCl}_2$  ( $1.0 \text{ mg kg}^{-1}$  as Hg). Results are expressed as mean  $\pm$  SEM and values are expressed relative to creatinine concentration. \* $p < 0.05$  or more with respect to control; # $p < 0.05$  or more with respect to the previous value.

significantly increased ( $p < 0.001$ ) within 10 h of treatment and reached a maximum after 24 h. Decrease in the level was slow and a significantly increased level of excretion was observed at the end of the experiment. ACE and GTK showed similar behaviour: early significant increase ( $p < 0.005$ ) was observed, with a peak excretion 24 h after that. The decrease in enzyme excretion was fast, and 48 or 58 h after treatment, respectively, was at pretreatment levels. In addition, at 58 and 72 h after treatment, ACE excretion was significantly lower than in pretreatment urine.

Excretion of urinary indices in control rats showed no significant variation and the values were omitted.



**Figure 2.** Dose-response relationship of urinary indices of nephrotoxicity in male rats treated with different doses of  $\text{HgCl}_2$ . Results are expressed as mean  $\pm$  SEM and values are expressed relative to creatinine concentration. \* $p < 0.05$  or more with respect to control; # $p < 0.05$  or more with respect to previous dose.

### Dose-response relationship

A significant increase in TP ( $p < 0.001$ ), ACE ( $p < 0.005$ ) and GTK ( $p < 0.001$ ) was observed at doses of  $0.25 \text{ mg kg}^{-1}$  and above and showed a dose-dependent increase. Figure 2 shows the results of urinary indices.

## Discussion

The evaluation of GTK, a new urinary enzyme of mainly cytosolic origin, identical to cysteine conjugate  $\beta$ -lyase (Stevens *et al.* 1986), as an index of nephrotoxicity, was the main objective of this study. The importance of this enzyme is the possibility that it may be a marker for the segment-specific effect of chemicals, because GTK appears to be localized mainly in the  $S_3$  segment (MacFarlane *et al.* 1989).

Acute exposure to doses of  $\text{Hg}^{2+}$  lower than  $2 \text{ mg kg}^{-1}$  produces highly selective necrosis of the  $S_3$  segment (Eknoyan *et al.* 1982, Dobyan and Bulger 1984), preceded by damage to the cell membrane (Gritzka and Trump 1968, Ganote *et al.* 1975, Trump *et al.* 1980, 1989). The necrosis, at higher doses ( $5\text{--}15 \text{ mg kg}^{-1}$ ), also extends to the 'pars convoluta' (Weinberg *et al.* 1982). The rate of cell death is accelerated by extracellular  $\text{Ca}^{2+}$  (Smith *et al.* 1987, Ambudkar *et al.* 1988). The earliest damage is fragmentation of the brush border microvilli, starting 3 h after treatment. Three days later, the damage becomes less severe (Kempson *et al.* 1977). A dose-dependent regeneration is probably completed within 4–7 days of exposure (Nielsen *et al.* 1991).

Urinary excretion of GTK appears early and is sensitive, with a similar behaviour to ACE (brush border enzyme) and TP. Additionally, comparing our data with the literature concerning enzymes studied as indices of the effect of  $\text{HgCl}_2$  on the kidney, GTK and ACE appear to be more sensitive than maltase (Stroo and Hook 1977), leucine aminopeptidase (Planas-Bohne 1977),  $\gamma$ -glutamyl transferase (Braun *et al.* 1978), or alkaline phosphatase (Planas-Bohne 1977, Stroo and Hook 1977). Fifty-eight hours after treatment, ACE (not GTK) significantly decreased to below base values; these results may be related to a loss of brush border microvilli during acute  $\text{Hg}^{2+}$  poisoning (Kempson *et al.* 1977, Kyle *et al.* 1983).

In conclusion, GTK in urine appears to be a good index of tubular impairment after doses of  $\text{HgCl}_2$  that cause  $S_3$  segment specific injury and it could be a good marker of  $S_3$  segment ('pars recta') damage. In our opinion, segment-specific injury of the tubule might be monitored with selected urinary enzymes, as they are determined till now to evaluate damage at different subcellular structures.

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