Arch Toxicol (1998) 72: 531–535

ORGAN TOXICITY AND MECHANISMS

Andrea Trevisan · Patrizia Cristofori · Gianluca Fanelli
Fabio Bicciato · Emanuela Stocco

Glutamine transaminase K intranephron localization in rats determined by urinary excretion after treatment with segment-specific nephrotoxicants

Received: 16 February 1998 / Accepted: 30 March 1998

Abstract Glutamine transaminase K(GTK) excretion assessed in urine and by kidney histology was evaluated in rats after single treatment with 1.0 mg/kg i.p. of mercuric chloride, 100 mg/kg i.p. of hexachloro-1:3-butadiene (both S_3, pars recta, segment-specific nephrotoxicants) and 25 mg/kg s.c. of potassium dichromate (S_1–S_2, pars convoluta, segment-specific nephrotoxicant). The aim was to correlate segment-specific injury and enzyme excretion in order to assess, using non-vasive methods, localization of GTK along the proximal tubule. Mercuric chloride and hexachloro-1:3-butadiene produced early focal damage in the pars recta (focal necrosis was shown 10 h after treatment, and diffuse necrosis appeared later at 34 and 24 h after treatment). Changes of the pars convoluta were occasional and delayed (72 h after treatment for both substances). On the contrary, potassium dichromate induced damage of the pars convoluta (vacuolar degeneration and focal necrosis were evident 24 h and 48 h after treatment, respectively), whereas the pars recta was affected later (focal vacuolar degeneration was observed 72 h after treatment). Increase urinary GTK excretion was early after treatment with mercuric chloride and hexachloro-1:3-butadiene (significant increase was observed within 10 h), with a peak for both substances 24 h after treatment, in agreement with the necrosis of the pars recta. Potassium dichromate induced a significant increase of enzyme excretion in urine also 24 h after injection, according to histological features showing vacuolar degeneration of the pars convoluta; the peak of excretion was reached 48 h after treatment (delay was due, probably, to s.c. administration). The results show that GTK increased in urine after treatment with S_3 and S_1–S_2 specific nephrotoxicants; the combination of histological examination and urinary enzyme supports the evidence that the enzyme is distributed along the whole of the proximal tubule.

Key words Glutamine transaminase K · Proximal tubule segments · Hexachloro-1:3-butadiene · Mercuric chloride · Potassium dichromate

A. Trevisan (✉) · G. Fanelli · F. Bicciato · E. Stocco
Laboratory of Industrial Toxicology,
Institute of Occupational Health, University of Padova,
Via Giustiniani 2, I-35128 Padova, Italy

P. Cristofori
Medicine Safety Evaluation, Pathology Department,
GlaxoWellcome S.p.A., Verona, Italy

Introduction

Glutamine transaminase K (GTK) is a cytosolic pyridoxal-phosphate-dependent enzyme, which catalyses β-elimination and transamination reaction with a cysteine-conjugate in the presence of α-keto-γ-methiolbutyrate. The enzyme was identified as soluble cysteine-conjugate β-lyase (Stevens et al. 1986), and plays a pivotal role in the activation of cysteine-conjugates derived from some haloalkenes to nephrotoxic thioketenes (Elfarra and Anders 1984), pyruvate and ammonia.

GTK localization along the proximal tubule is still under discussion. The first results obtained by Jones et al. (1988) with immunohistochemical methodology demonstrated the ubiquitary distribution of the enzyme. Subsequently, MacFarlane et al. (1989), using a similar technique, showed that GTK is localized in the pars recta only. Recently, Kim et al. (1997) using microdissection techniques, showed that the enzyme is localized prevalently in S_2 and S_3, as well as in S_1 segments. Moreover, cysteine-conjugate β-lyase in the proximal tubule shows two different migration bands (Abraham and Cooper 1991): peak I corresponding to a high molecular weight β-lyase ($M_r \cong 300\,000$), and peak III corresponding to cytosolic GTK ($M_r \cong 90\,000$). A third peak (peak II, $M_r \cong 240\,000$) corresponds to L-amino-acid oxidase.

The controversy about the localization of cysteine-conjugate β-lyase (or GTK) is remarkable, because the predominant nephrotoxic effects of haloalkenes are on the S_3 segment (pars recta) of the proximal tubule. The

possibility of (1) metabolic activation, (2) accumulation of toxic metabolites and (3) renal lesions resulted from ischaemia as a consequence of altered renal haemodynamics may explain the greater sensitivity of the pars recta to nephrotoxic haloalkenes (Ishmael et al. 1982). In the present work, we studied the effects of segment-specific nephrotoxicants on urinary excretion of GTK. Histological examination was performed to evaluate correspondence between urinary excretion of the enzyme and morphological changes in different proximal tubule segments. The aim was to indicate segmentary localization along the proximal tubule of GTK by means of urinary measurement after treatment with segment-specific nephrotoxic substances.

Materials and methods

Chemicals

Hexachloro-1:3-butadiene (HCBD, purity ≥97%), potassium dichromate ($K_2Cr_2O_7$) and L-phenylalanine were purchased from Fluka (Buchs, Switzerland); mercuric chloride ($HgCl_2$) was purchased from Merck (Darmstadt, Germany); α-keto-γ-methiolbutyrate was purchased from Sigma Chemical Co. (St. Louis, Mo., USA). A commercial kit for creatinine determination was purchased from Boehringer (Mannheim, Germany).

Animals and animal treatment

Albino male Wistar rats (Charles River, Italy), of 2 months in age and 200 ± 10 g body weight were kept in metabolic cages with standard diet (Nuova Zoofarm, Italy) and water provided ad libitum. Urine of five rats for each group was collected in vials plunged in an ice bath for 14 h before treatment (6.00 p.m. – 8.00 a.m., base collection) and every 10 h (8.00 a.m. – 6.00 p.m., diurnal collection) or 14 h (6.00 p.m. – 8.00 a.m., nocturnal collection) until 96 h, after single injection with (i) HCBD 100 mg/kg body wt., i.p., (ii) $HgCl_2$, 1 mg/kg body wt., i.p., as Hg^{2+}, and (iii) $K_2Cr_2O_7$, 25 mg/kg body wt., s.c., as salt.

Enzyme activity assay in urine

Immediately after collection, urine samples were centrifuged to discard debris and prepared for GTK activity measurement according to Cooper and Meister (1985), using α-keto-γ-methiolbutyrate and L-phenylalanine as substrates. Enzyme activity and urinary creatinine were determined using a Perkiu Elmer lambda 5 model UV-Vis. spectrophotometer. Enzyme activity was expressed as μmoles of phenylpyruvate produced/min per mmole of creatinine.

Histopathological studies

An ancillary group of rats treated with the same chemical doses was killed at the same time of urine collection until 96 h after treatment for histological examination. Immediately afterwards the kidneys were removed and fixed in 10% neutral phosphate-buffered formalin. The fixed kidneys were processed in paraffin wax, cut at 5 μm in thickness and stained with haematoxylin/eosin and periodic acid-Schiff (PAS) stains.

Statistics

Statistical evaluation of the results was done by means of variance analysis with $P < 0.05$ as the level of significance.

Results

Urinary GTK excretion began to increase significantly within 10 h and reached a peak at 24 h after single injection of HCBD or $HgCl_2$. Treatment with $K_2Cr_2O_7$ showed a delayed, but significant, increase of enzyme excretion in urine within 24 h, with a peak of excretion 48 h after treatment, probably due to the s.c. route of administration. The time-course of urinary enzyme excretion *in urine* is summarized in Table 1.

Histological examination showed that HCBD produced early (10 h after treatment) and selective necrosis in the pars recta, described as focal necrosis, followed by diffuse necrosis 24 h later (Fig. 1). Necrotic features were less evident 82 h later, at which time there was an association to regeneration findings, and were almost complete at the end of the observation period (96 h after treatment). Involvement of the pars convoluta, described as vacuolar degeneration, was an occasional observation 72 h after treatment.

$HgCl_2$ showed histological features similar to HCBD, though diffuse necrosis of the pars recta appeared later, at 34 h after treatment (Fig. 2). Multifocal necrosis in the pars convoluta was sometimes observed 72 h after treatment; in addition, occasional minimal changes, such as slight tubular dilatation, were observed in this segment 24 h after injection. Regeneration findings and disappearance of necrosis were evident (similar to HCBD) at the end of the observation period.

On the contrary, $K_2Cr_2O_7$ produced signs of damage in the pars convoluta: tubular dilatation 10 h after treatment, followed at 24 h by multifocal vacuolar degeneration (Fig. 3); diffuse vacuolar degeneration and focal necrosis after 48 h; diffuse vacuolar degeneration and diffuse necrosis after 58 h. Only later (from 72 h after treatment), were minimal changes (focal vacuolar degeneration) seen in the pars recta (Fig. 4). Necrosis of the pars convoluta was well evident until 96 h after treatment, without signs of regeneration. The data of

Table 1 Time-course of glutamine transaminase K (GTK) excretion in urine until 96 h after single treatment with HCBD (100 mg/kg, i.p.), $HgCl_2$ (1 mg/kg, i.p.) and $K_2Cr_2O_7$ (25 mg/kg, s.c.). Enzyme activity is expressed in μmoles/min per mmole of creatinine and results are presented as mean ± SEM. (*HCBD* Hexachloro-1:3-butadiene)

Time (h)	HCBD	$HgCl_2$	$K_2Cr_2O_7$
Base values	0.10 ± 0.06	0.16 ± 0.12	0.13 ± 0.07
0–10	2.72 ± 0.25*	1.46 ± 0.21*	0.10 ± 0.04
10–24	4.65 ± 0.42*,†	12.20 ± 0.69*,†	0.51 ± 0.05*,†
24–34	0.78 ± 0.07*,†	7.02 ± 0.49*,†	2.66 ± 0.41*,†
34–48	0.55 ± 0.25	1.77 ± 0.07*,†	3.69 ± 0.36*
48–58	0.52 ± 0.06*	0.74 ± 0.02*,†	2.06 ± 0.28*,†
58–72	0.39 ± 0.06*	0.69 ± 0.12*	2.55 ± 0.38*
72–82	0.46 ± 0.01	0.55 ± 0.29	1.27 ± 0.09*,†
82–96	0.34 ± 0.05*,†	1.03 ± 0.65	0.39 ± 0.24†

* $P < 0.05$ or more vs base values; † $P < 0.05$ or more vs the previous values

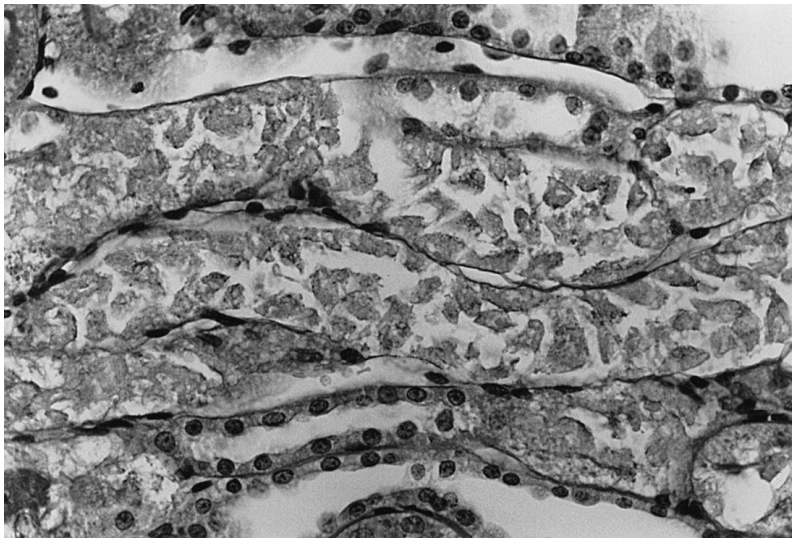

Fig. 1 Histological features 24 h after i.p. treatment with hexachloro-1:3-butadiene (HCBD) at 100 mg/kg body wt. Diffuse necrosis of the pars recta is well represented. Periodic acid-Schiff (PAS), ×400

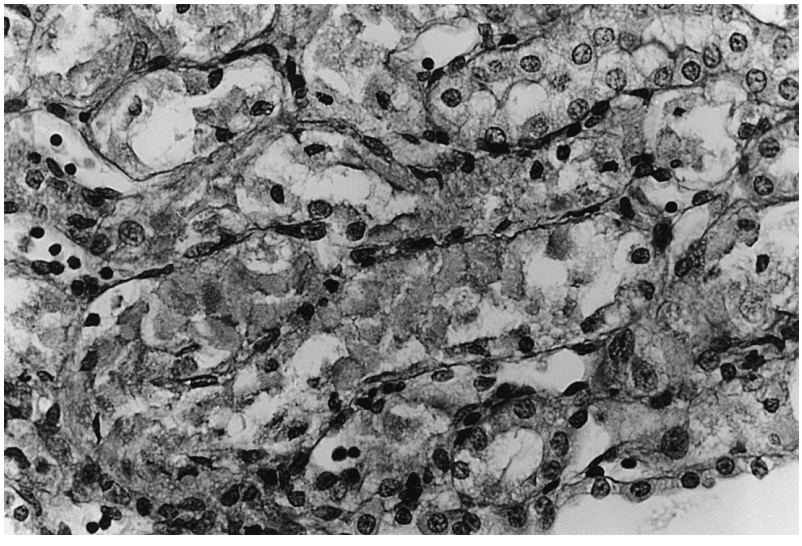

Fig. 2 Histological features 34 h after i.p. treatment with $HgCl_2$, 1.0 mg/kg body wt. Histological pattern shows diffuse necrosis of the pars recta. PAS, ×400

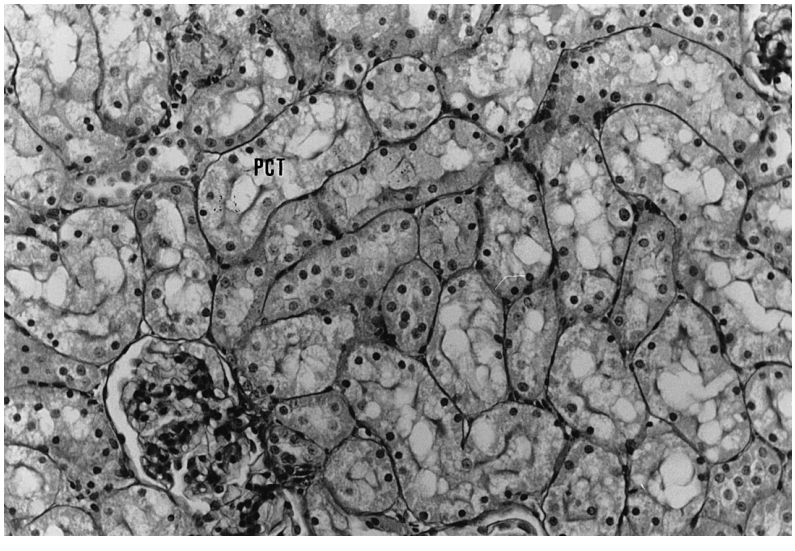

Fig. 3 Histological features 24 h after s.c. treatment with $K_2Cr_2O_7$, 25 mg/kg body wt. Multifocal vacuolar degeneration of the pars convoluta is well evident. PAS, ×200. (*PCT* Proximal convoluted tubule)

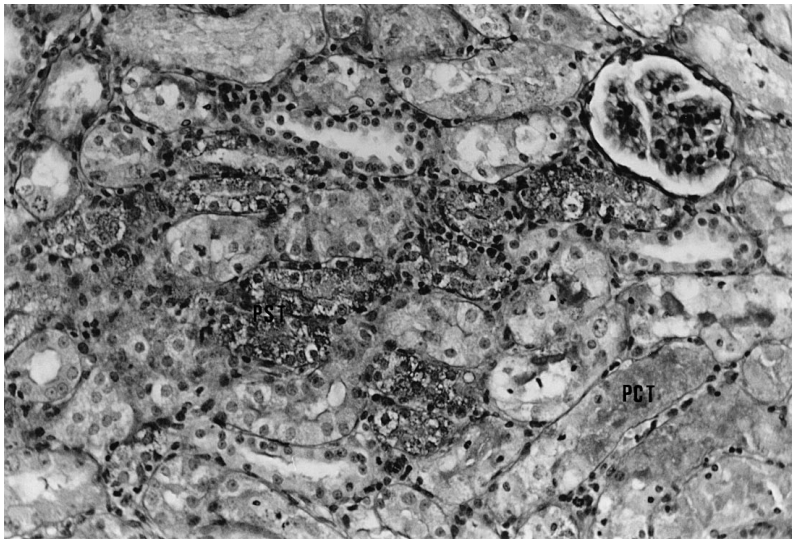

Fig. 4 Histological features 72 h after s.c. treatment with $K_2Cr_2O_7$, 25 mg/kg body wt. Diffuse necrosis of the pars convoluta and focal vacuolar degeneration of the pars recta are well represented. PAS, ×200. (*PST* Proximal straight tubule)

Table 2 summarize the time-course of histological features of the renal proximal tubule after treatment with xenobiotic substances.

Discussion

To ascertain segment-specific localization along the proximal tubule of renal enzymes or segment-specific damage caused by chemicals, measurement of urinary enzymic activity may be a reasonable, non-invasive, technique of investigation. Obviously, the former aim needed to use chemicals with proven segment-specific toxicity, the latter to use enzymes with well known segmentary distribution. In the present study the first approach to the topic, i.e. treatment with segment-specific chemicals to localize enzyme distribution along the proximal tubule, was used. In order to support urinary data, histological examination of the kidney was performed at the same time points.

The selective target site of damage in the renal proximal tubule after treatment with HCBD, $HgCl_2$ and $K_2Cr_2O_7$ has been well defined. HCBD (Ishmael et al. 1982) and $HgCl_2$ (Eknoyan et al. 1982; Dobyan and Bulger 1984) specific to pars recta (S_3), whereas $K_2Cr_2O_7$ (Biber et al. 1968) is a pars convoluta (S_1–S_2) segment-specific nephrotoxicant. GTK, soluble cysteine-conjugate β-lyase (Stevens et al. 1986), was submitted to investigation owing to its involvement in toxic activation of haloalkenes such as HCBD, as well as tri- and tetrachloroethylene to nephrotoxic and, probably, cancerogenic thioketenes (Lock 1988). GTK localization along the proximal tubule was discussed for the pivotal role that the enzyme plays in S_3 segment-specific damage caused by these substances. The distribution of the enzyme along the whole proximal tubule, although prevalently localized in S_2–S_3 segments, was recently demonstrated (Kim et al. 1997).

The results presented support the enzyme distribution along the whole proximal tubule. In fact, both S_3 (HCBD and $HgCl_2$) and S_1–S_2 ($K_2Cr_2O_7$) segment-specific chemicals produce a significant increase of enzyme excretion. Histological examination confirms that the damage of the pars convoluta caused by $K_2Cr_2O_7$, similarly to the damage of the pars recta induced by HCBD and $HgCl_2$, is in agreement with the increase of urinary excretion of GTK. In addition, the histological features show that the chemicals affect the pars convoluta or the pars recta with high specificity, because the involvement of the other segments of the proximal tubule is occasional for HCBD and $HgCl_2$, whereas it is delayed for $K_2Cr_2O_7$, and corresponds to an extension of the damage to the near segment. On the other hand, GTK shows high sensitivity to treatment with $HgCl_2$, which is greater than with HCBD or $K_2Cr_2O_7$. These results confirm our previous observation (Trevisan et al. 1996) reporting that GTK excretion in urine, compared with other urinary enzymes, was an early and good marker of tubular impairment induced by the metal.

In conclusion, our data show that the treatment of rats with proximal tubule segment-specific toxicants supplies sufficient proof for the distribution of GTK along the whole proximal tubule. In addition, the results suggest the possibility: (a) to study the segment-specific damage caused by xenobiotics (in animals and probably in humans also) measuring segment-specific enzymes in urine as a very non-invasive method); and (b) to define (in animals) segment-specific localization of enzymes along the proximal tubule by means of segment-specific toxicants. In addition, according to the experimental evidence, HCBD is suggested as preferred, more specific chemical than $HgCl_2$ to produce pars recta toxicity, and $K_2Cr_2O_7$ to induce specific pars convoluta damage. The necessity must be emphasized to use s.c. route of $K_2Cr_2O_7$ administration to prevent high peritoneal damage, and hence the death of the animals, caused by i.p. injection.

Table 2 Time-course of histological features[a] of the renal proximal tubule up to a total duration of 96 h, after single treatment with HCBD (100 mg/kg, i.p.), $HgCl_2$ (1 mg/kg, i.p.) and $K_2Cr_2O_7$ (25 mg/kg, s.c.). (*PC* Pars convoluta, *PR* pars recta)

	10 h		24 h		34 h		48 h		58 h		72 h		82 h		96 h	
	PC	PR	PC	PR	PC	PR	PC	PR	PC	PR	PC	PR	PC	PR	PC	PR
HCBD:																
Tubular dilatation											+					
Vacuolar degeneration											+	+				
Necrosis		+				+		+		+		+		+		
Regeneration						+		+		+		+		+		+
$HgCl_2$:																
Tubular dilatation			+											+		+
Vacuolar degeneration		+														
Necrosis		+										+				
Regeneration												+				+
$K_2Cr_2O_7$:																
Tubular dilatation	+						+									
Vacuolar degeneration			+		+		+		+							+
Necrosis					+				+		+		+		+	
Regeneration					+		+		+		+		+		+	

[a] Grading is as follows: +, focal; ++, multifocal; +++, diffuse

Acknowledgement The research was supported by a grant of the Board of University and Scientific Technological Research.

References

Abraham DG, Copper AJL (1991) Glutamine transaminase K and cysteine S-conjugate β-lyase activity stain. Anal Biochem 197: 421–427

Biber TUL, Mylle M, Baines AD, Gottshalk CW, Oliver JR, MacDowell MC (1968) A study by micropuncture and microdissection of acute renal damage in rats. Am J Med 44: 664–705

Cooper AJL, Meister A (1985) Glutamine transaminase K from rat kidney. Methods Enzymol 113: 344–349

Dobyan DC, Bulger RE (1984) Partial protection by chlorpromazine in mercuric chloride-induced acute renal failure in rats. Lab Invest 50: 578–586

Eknoyan G, Bulger RE, Dobyan DC (1982) Mercuric chloride-induced acute renal failure in the rat. I. Correlation of functional and morphologic changes and their modification by clonidine. Lab Invest 46: 613–620

Elfarra AA, Anders MW (1984) Renal processing of glutathione conjugates. Role in nephrotoxicity. Biochem Pharmacol 33: 3729–3732

Ishmael J, Pratt I, Lock EA (1982) Necrosis of pars recta (S3 segment) of the rat kidney produced by hexachloro 1:3 butadiene. J Pathol 138: 99–113

Jones TW, Qin C, Schaeffer VH, Stevens JL (1988) Immunohistochemical localization of glutamine transaminase K, a rat kidney cysteine conjugate β-lyase, and the relationship to the segment specificity of cysteine conjugate nephrotoxicity. Mol Pharmacol 34: 621–627

Kim HS, Cha SH, Abraham DG, Cooper AJL, Endou H (1997) Intranephron distribution of cysteine S-conjugate β-lyase activity and its implication for hexachloro-1,3-butadiene-induced nephrotoxicity in rats. Arch Toxicol 71: 131–141

Lock EA (1988) Studies on the mechanism of nephrotoxicity and nephrocarcinogenicity of halogenated alkenes. Crit Rev Toxicol 19: 23–42

MacFarlane M, Foster JR, Gibson GG, King LJ, Lock EA (1989) Cysteine conjugate β-lyase of rat kidney cytosol: characterization, immunocytochemical localization, and correlation with hexachlorobutadiene nephrotoxicity. Toxicol Appl Pharmacol 98: 185–197

Stevens JL, Robbins JD, Byrd RA (1986) A purified cysteine conjugate β-lyase from rat kidney cytosol. Requirement for an α-keto acid or an amino acid oxidase for activity and identity with soluble glutamine transaminase K. J Biol Chem 261: 15 529–15537

Trevisan A, Nicoletto G, Secondin L, Maso S (1996) Urinary excretion of glutamine transaminase K as an early index of mercuric chloride-induced nephrotoxicity. Biomarkers 1: 63–66