Renal Cortical Slices: An *In Vitro* Model for Kidney Metabolism and Toxicity

Andrea Trevisan, Stefano Maso and Paola Meneghetti

Istituto di Medicina del Lavoro, Laboratorio di Tossicologia Industriale, Universitá di Padova, via Facciolati 71, I-35127 Padova, Italy

Summary — The *in vitro* renal cortical slice model was used to study: 1) the effects on the kidney of some haloalkanes and haloalkenes using 3-month-old male Wistar rats; 2) influence of age and sex on renal cortical slice indices in non-treated rats; and 3) effects of 1,2-dichloropropane on the slices after pretreatment of 3-month-old male Wistar rats with DL-butathionine-[S,R]-sulphoximine. The most nephrotoxic chemical used was 1,3-dichloropropene, which caused a total depletion in the levels of reduced glutathione, a high peroxidation of lipid (about three thousand-fold with respect to control), a significant release of tubular enzymes into the medium, and loss of organic anion (*p*-aminohippurate) accumulation. All the chemicals affected the cytosol more than the brush border. The most remarkable age-related differences in the untreated slices were the progressive decrease of reduced glutathione ($p<0.05$ from three months of age), and an increase in lactate dehydrogenase release into the medium ($p<0.05$ from six months of age). By contrast, sex differences were slight. The treatment with 1,2-dichloropropane of slices prepared from rats pretreated with DL-butathionine-[S,R]-sulphoximine significantly increased the depletion of glutathione content ($p<0.05$) and malondialdehyde release in the medium ($p<0.001$) caused by the solvent alone.

Key words: renal cortical slices, haloalkanes, haloalkenes, nephrotoxicity.

Introduction

Renal cortical slices have been used as a technique suitable for the study of kidney metabolism and of mechanisms of nephrotoxicity caused by xenobiotics (1).

The nephrotoxicities of vanadate (2), chloroform (3), metal salts (potassium dichromate and mercuric chloride; 1), solvents (carbon tetrachloride and hexachlorobutadiene; 1), antimicrobial agents (1), S-(1,2-dichlorovinyl)-cysteine (4), and 1,2-dichloropropane (5), were investigated using a renal cortical slice model. Kidney metabolism was also investigated using an inhibitor and an activator of the metabolic pathways of the chemicals. Inhibition of the oxidative pathway with carbon monoxide prevented the toxicity of chloroform (3) and the depletion of reduced glutathione content (GSH) induced by 1,2-dichloropropane (5). Moreover, the *in vitro* effects are comparable to *in vivo* effects using the same concentration of chemicals (1).

The present work investigates age and sex differences of renal cortical slice indices, effects of GSH depletion on nephrotoxicity induced by 1,2-dichloropropane, and the comparative effects of haloalkanes and haloalkenes at the same concentration on renal cortical slice targets.

Materials and Methods

Effects of donor age on renal cortical slices

Untreated male albino Wistar rats (Morini, S. Polo d'Enza, RE, Italy), 1, 3, 6 and 12 months old, were killed by decapitation under light ether anaesthesia (two rats of each age). The kidneys were removed rapidly and placed in ice-cold 0.9% NaCl. Thin, free-hand renal cortical slices (100 ± 10mg wet tissue, approximately 25mg/slice) prepared according to the method of Berndt (6), were placed in the incubation medium (1) until all slices could be prepared (no longer than 45 minutes). After gassing with O_2 for five minutes and incubation for 90 minutes in a Dubnoff metabolic shaker (100 cycles/minute) at 37°C, the slices were prepared for the determination of GSH content, measured as non-protein sulphydryl groups, according to the method of Sedlak & Lindsay (7), or reincubated with *p*-aminohippurate (PAH) for 90 minutes in a

Dubnoff metabolic shaker (100 cycles/minute) at 25°C under O_2 atmosphere. PAH in slices and medium was determined according to Smith *et al.* (8), and the slice/medium ratio (S/M) was determined. In the first incubation medium, malondialdehyde (MDA) was determined according to the method of Younes & Siegers (9), and enzymes of tubular origin — aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH) from cytosol (10), and gamma-glutamyltransferase (GGT), alkaline phosphatase (ALP) and angiotensin converting enzyme (ACE) from brush border (10) — were also determined. ACE activity was determined according to the method of Summary (11); the activity of the other enzymes was determined using commercial kits (kinetic method at 37°C by Boehringer Mannheim, Penzburg, FRG). All spectrophotometric analyses were performed using a UV-Vis. spectrophotometer Perkin-Elmer lambda 5 model.

Influence of sex on renal cortical slices

Six 2-month-old, male and female albino Wistar rats, untreated, weighing 200 ± 10g, were sacrified for the evaluation of sex-related differences. The parameters used above were determined.

Effect of pretreatment with DL-butathionine-[S,R]-sulphoximine (BSO) on 1,2-dichloropropane toxicity

Two 3-month-old, male albino Wistar rats were fasted for 24 hours and then treated i.p. with a 500mg/kg dose of BSO (Sigma Chemical Corp., St Louis, MO, USA) dissolved in alkaline saline. Two control rats were treated with alkaline saline only. Four hours after treatment, the rats were sacrificed as indicated and their kidneys removed for the preparation of renal cortical slices as described above. Some slices from pretreated and untreated rats were incubated with 25×10^{-3}M 1,2-dichloropropane (1,2 DCP; Fluka, Buchs, Switzerland; purity > 99%) for 90 minutes, and some were left untreated. GSH, MDA, PAH, AST, GGT and LDH were determined as above.

Effects of solvents on renal cortical slices

Carbon tetrachloride (TETRA; Merck, Darmstadt, FRG; purity 99.8%), 1,1,1-trichloroethane (1,1,1 TRI; Fluka; purity 90–95%), chloroform (CLO; Merck; purity 99%), 1,2-dichloroethane (DCE; Fluka; purity >99%), 1,2-DCP and 1,3-dichloropropane (1,3 DCP; Merck; purity 97%), haloalkanes, and hexachloro-1,3-butadiene (HCBD; Fluka; purity >97%), 1,3-dichloropropene (1,3 DCPe; Fluka; tech. 75–80%), trichloroethylene (TRI, Fluka; purity >99%), and tetrachloroethylene (PER; Fluka; purity >99%), haloalkenes, were incubated at the same concentration (25×10^{-3}M) with renal cortical slices of 3-month-old, male Wistar rats, and the effects on the targets as above were measured. The chemicals were added directly to the incubation medium via a 10μl Hamilton microsyringe.

Statistical evaluation of the results was carried out by variance analysis and Student's t test.

Results

Table I shows the trend of changes in renal cortical slice markers with age. The statistically-significant results show a decrease of GSH content ($p<0.05$ from 3 months) and an increase of MDA ($p<0.001$ at 12 months), AST and LDH release into the medium ($p<0.05$ from 6 months). ALT release into the medium decreased from 3 months ($p<0.05$) and then showed no other significant variations.

Sex differences (Table II) are slight: renal slices from female rats show a higher PAH accumulation ($p<0.02$) and ALT ($p<0.001$) and LDH ($p<0.05$) release in the medium than renal slices from male rats.

BSO pretreatment and fasting caused a 50% depletion of GSH content in the slices ($p<0.05$) and a significant release of LDH in the medium ($p<0.05$). Treatment of slices with 1,2 DCP significantly increased the depletion of GSH ($p<0.005$) and MDA release into the medium ($p<0.001$) caused by 1,2 DCP or BSO alone. The results are summarised in Table III.

1,3 DCPe appeared to be the most toxic chemical for the slices. The solvent caused complete GSH depletion and high levels of lipid peroxidation. 1,1,1 TRI was least toxic to kidney slices. Table IV summarises the effects caused by chemicals in the slices.

Discussion

Renal cortical slice data show that this technique is of interest in the study of kidney metabolism and toxicity of xenobiotics, but various factors need to be taken into consideration. The age of the donor animals is of importance for some features, such as GSH content, lipid peroxidation and cellular viability. The increase in lipid peroxidation and the decrease of cellular viability, as demonstrated by MDA and LDH release in the medium, respectively, agree with the age-related decrease of GSH content.

This evidence confirms that senescence influences kidney function both *in vitro* and *in vivo* (12).

Sex differences are slight with respect to the control values of the parameters measured, but the response to toxicity can be different. Chloroform (3) and 1,2 DCP (5) cause a higher toxicity in male than in female mice and rats.

Renal metabolism and the toxicity of xenobiotics can also be studied using the renal cortical slice model. Inhibition of the oxidative pathway (5), depletion of GSH content

Table I: Influence of age on renal cortical slice markers

Months	1	3	6	12
GSH[a]	0.163±0.009	0.135±0.015*	0.137±0.019*	0.119±0.026*
PAH[c]	18.13±2.46	19.11±2.26	16.81±3.05	15.79±2.01
MDA[c]	4.07±0.38	4.65±1.72	5.16±0.95	6.58±0.77
AST[a]	0.82±0.04	0.91±0.19	1.01±0.09*	1.13±0.14*
ALT[c]	55.9±4.2	25.9±4.4*	25.5±4.8*	20.8±6.5*
LDH[a]	3.23±0.32	3.62±0.63	4.61±1.21*	5.26±1.07*
GGT[a]	2.44±0.35	2.79±0.11	2.84±0.56	2.56±0.53
ALP[a]	1.03±0.24	1.01±0.19	0.91±0.15	0.67±0.12*
ACE[a]	1.00±0.09	1.22±0.37	1.36±0.48	1.16±0.13

[a] *μmoles/100mg of tissue;*

[b] *slice/medium ratio;*

[c] *nmoles/100mg of tissue.*

* *$p<0.05$ with respect to 1 month;*

Data are expressed as mean ± standard deviation on 4 proofs.

Table II: Influence of sex on renal cortical slice markers

	Female	Male
GSH[a]	0.170±0.021	0.153±0.023
PAH[b]	21.26±1.47	17.83±2.56*
MDA[c]	4.22±1.32	5.20±1.50
AST[a]	0.80±0.19	0.80±0.17
ALT[d]	194.2±41.3	64.1±12.3*
LDH[a]	5.27±1.44	3.75±0.71*
GGT[a]	1.87±0.45	1.82±0.17
ALP[a]	1.12±0.28	0.82±0.19
ACE[a]	0.77±0.19	1.02±0.25

[a] *μmoles/100mg of tissue;*

[b] *slice/medium ratio;*

[c] *nmoles/100mg of tissue.*

* *$p<0.05$ with respect to 1 month;*

Data are expressed as mean ± standard deviation on 4 proofs.

Table III: Effects of fasting and pretreatment with BSO on renal cortical slices after treatment with 1,2 DCP

	Control	+1,2 DCP	+BSO	+BSO +1,2 DCP
GSH[a]	0.133 ± 0.008	0.074 ± 0.014*	0.071 ± 0.001*	0.049 ± 0.013#
PAH[b]	18.08 ± 1.50	11.06 ± 1.38*	21.83 ± 2.34	10.89 ± 0.93*
MDA[c]	5.20 ± 1.50	8.31 ± 1.36*	5.80 ± 1.42	14.08 ± 3.73#
AST[a]	1.05 ± 0.09	1.43 ± 0.74*	1.27 ± 0.13*	1.63 ± 0.28*
LDH[a]	4.86 ± 0.78	5.29 ± 0.75	7.17 ± 0.83*	7.23 ± 1.09*

[a] *μmoles/100mg of tissue;*

[b] *slice/medium ratio;*

[c] *nmoles/100mg of tissue;*

* *$p<0.05$ with respect to the control;*

\# *$p<0.05$ with respect to 1,2 DCP alone.*

Data are expressed as mean ± standard deviation on 4 proofs.

with the combined effects of fasting and BSO pretreatment, and the induction or inhibition of mercaptopuric acid metabolic pathway (13), are techniques that, taken together, provide information on xenobiotic metabolism *in situ*. Depletion of GSH content shows an increase of lipid peroxidation caused by 1,2 DCP and indicates that solvent toxicity may be mediated by an oxidative intermediate formed during microsomal metabolism (probably 1,2-epoxypropane). Experiments with carbon monoxide (5) confirm the data; carbon monoxide pretreatment of slices prevents GSH depletion and toxicity of 1,2 DCP.

Finally, renal cortical slices can be used to study and compare the nephrotoxicities of xenobiotics. The *in vitro* model shows that toxicity is frequently, but not always, related to GSH depletion. The highest toxicity in this study was caused by 1,3 DCPe which completely depletes the GSH content of the kidney, but TETRA, which causes only a slight GSH depletion, was very toxic for organic anion accumulation. According to Smith (1), cytosol enzymes (AST, ALT and LDH) are more sensitive than brush border enzymes (GGT, ALP and ACE) for the determination of the toxic effects.

In agreement with other authors (1–5), we can conclude that the renal cortical slice model appears to be a versatile means of studying nephrotoxicity of xenobiotics. Targets such as GSH content, organic anion accumulation (PAH), and MDA and cytosol enzyme release into the medium, are good indices of toxicity. In particular, organic anion accumulation by slices of kidney cortex *in vitro* is an energy-requiring process that may estimate the capacity for tubular ion transport *in vivo*, and the impairment of this process gives an indication of tubular injury (14).

References

1. Smith, J.H. (1988). The use of renal cortical slices from the Fischer 344 rat as an *in vitro* model to evaluate nephrotoxicity. *Fundamental and Applied Toxicology* **11**, 132–142.
2. Smith, J.H., Braselton, W.E., Tonsager, S.R., Mayor, G.H. & Hook, J.B. (1982). Effects of vanadate on organic ion accumulation in rat renal cortical slices. *Journal of Pharmacology and Experimental Therapeutics* **220**, 540–546.
3. Smith, J.H. & Hook, J.B. (1983). Mechanism of chloroform nephrotoxicity. II.*In vitro* evidence for renal metabolism of chloroform in mice. *Toxicology and Applied Pharmacology* **70**, 480–485.
4. Wolfgang, G.H.I., Gandolfi, A.J., Stevens, J.L. & Brendel, K. (1989). *In vitro* and *in vivo* nephrotoxicity of the L and D isomers of S-(1,2-dichlorovinyl)-cysteine. *Toxicology* **58**, 33–42.
5. Trevisan, A., Maso, S., Troso, O. & Meneghetti, P. (1991). Kidney metabolism and sex related nephrotoxicity of 1,2-dichloropropane *in vitro*. *Pharmacology and Toxicology* **69**, Suppl. II, 9.
6. Berndt, W.O. (1976). Use of the tissue slice technique for evaluation of renal transport

Table IV: Effects of haloalkanes and haloalkenes on renal cortical slice targets

	GSH[a]	PAH[b]	MDA[c]	AST[a]	ALT[c]	LDH[a]	GGT[a]	ALP[a]	ACE[a]
Control I	0.14 ± 0.02	24.68 ± 2.27	4.40 ± 1.29	0.81 ± 0.13	41 ± 5	4.36 ± 0.46	2.82 ± 0.44	1.03 ± 0.17	1.31 ± 0.27
Control II	0.12 ± 0.03	22.61 ± 1.97	4.18 ± 1.32	0.96 ± 0.11	47 ± 11	4.30 ± 0.19	2.85 ± 0.72	1.11 ± 0.21	1.23 ± 0.06
Haloalkanes									
TETRA	0.11 ± 0.03	6.10 ± 1.05#	5.73 ± 2.81	1.87 ± 0.30#	110 ± 5#	5.03 ± 0.51#	3.87 ± 0.92	1.35 ± 0.23	1.60 ± 0.11#
1,1,1 TRI	0.12 ± 0.03	22.75 ± 1.86	3.47 ± 2.43	1.21 ± 0.18	68 ± 29	4.55 ± 1.14	2.90 ± 0.46	1.15 ± 0.23	1.24 ± 0.27
CLO	0.12 ± 0.02	20.48 ± 1.67*	4.32 ± 1.35	1.50 ± 0.50*	80 ± 8*	5.62 ± 0.43*	2.87 ± 0.60	1.12 ± 0.35	1.36 ± 0.23
1,2 DCE	0.03 ± 0.02*	8.39 ± 1.23	13.11 ± 1.62*	1.20 ± 0.13*	119 ± 13*	5.22 ± 0.60	3.28 ± 0.49	1.12 ± 0.16	1.61 ± 0.04*
1,2 DCP	0.09 ± 0.03*	14.44 ± 2.23*	6.58 ± 0.80*	1.10 ± 0.12*	66 ± 14*	6.19 ± 0.54*	3.50 ± 0.60	1.23 ± 0.39	1.59 ± 0.30
1,3 DCP	0.04 ± 0.02*	12.34 ± 2.12*	6.16 ± 0.45*	1.38 ± 0.24*	60 ± 5*	4.55 ± 0.45	3.19 ± 0.11	1.02 ± 0.16	1.45 ± 0.11
Haloalkenes									
HCBD	0.09 ± 0.01	15.70 ± 2.10#	6.60 ± 2.98	1.34 ± 0.45	75 ± 6#	5.43 ± 0.10#	3.48 ± 0.18	1.24 ± 0.09	1.38 ± 0.30
1,3 DCPe	0#	9.00 ± 1.45#	147.35 ± 6.94#	2.62 ± 0.25#	218 ± 8#	10.95 ± 1.96#	6.17 ± 0.27#	2.89 ± 0.32#	2.46 ± 0.19#
TRI	0.13 ± 0.02	19.62 ± 1.67	4.21 ± 1.34	1.52 ± 0.06#	115 ± 6#	4.91 ± 1.09	3.19 ± 0.13	1.02 ± 0.18	1.25 ± 0.16
PER	0.10 ± 0.01	15.64 ± 2.34#	6.25 ± 0.69#	1.20 ± 0.25	65 ± 2#	4.87 ± 0.81	3.04 ± 0.33	1.19 ± 0.03	1.42 ± 0.25

[a] *μmoles/100mg tissue;*

[b] *slice/medium ratio;*

[c] *nmoles/100mg tissue;*

* *$p<0.05$ respect to control I;*

\# *$p<0.05$ with respect to control II.*

process. *Environmental Health Perspectives* **15**, 73–88.

7. Sedlak, J. & Lindsay, R.H. (1968). Estimation of total, protein-bound, and non-protein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry* **25**, 192–205.
8. Smith, H.W., Finkelstein, N., Aliminosa, L., Crawford, B. & Graber, M. (1945). The renal clearance of substituted hippuric acid derivatives and other aromatic acids in dog and man. *Journal of Clinical Investigation* **24**, 388–404.
9. Younes, M. & Siegers, C-P. (1981). Mechanistic aspects of enhanced lipid peroxidation following glutathione depletion *in vivo*. *Chemico-Biological Interactions* **34**, 257–266.
10. Piperno, E. (1981). Detection of drug induced nephrotoxicity with urinalysis and enzymuria assessment. In *Toxicology of the Kidney* (ed. J.B. Hook), pp. 31–55. New York: Raven Press.
11. Summary, J.J. (1976). The spectrophotometric determination of human serum carboxypolypeptidase with angiotensin converting enzyme-like activity. *Clinical Science and Molecular Medicine* **50**, 321–327.
12. Goldstein, R.S., Tarloff, J.B. & Hook, J.B. (1988). Age-related nephrotoxicity in laboratory rats. *FASEB Journal* **2**, 2241–2251.
13. Trevisan, A., Meneghetti, P. & Maso, S. (1991). Thiol mediated nephrotoxicity by 1,2-dichloropropane. *Third International ISSX Meeting. Amsterdam 24–28 June 1991*, p. 305.
14. Kluwe, M.W. (1981). The nephrotoxicity of low molecular weight halogenated alkane solvents, pesticides, and chemical intermediates. In *Toxicology of the Kidney* (ed. J.B. Hook), pp. 179–226. New York: Raven Press.