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Sex- and age-related nephrotoxicity due to 1,2-dichloropropane in vitro

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Abstract. Sex- and age-related nephrotoxicity due to 1,2-dichloropropane was studied in vitro by means of renal cortical slices obtained from Wistar rats. Reduced glutathione content, organic anion accumulation (p-aminohippurate), and release of malondialdehyde (to measure the extent of lipid peroxidation), aspartate aminotransferase, γ-glutamyltransferase and lactate dehydrogenase into the incubation medium were determined. Sex differences in naive rats parameters were slight, but male were more susceptible to toxic effects of 1,2-dichloropropane than female rats; glutathione depletion, lipid peroxidation, and loss of organic anion accumulation were higher in male than in female slices. During senescence, naive male rats showed a progressive decrease of glutathione content (statistically significant from 7–9 months of age), increase of spontaneous lipid peroxidation from the same age, and increase of signs of cytotoxicity (release of aspartate aminotransferase and lactate dehydrogenase into the incubation medium) from 3–4 months of age. A loss of organic anion accumulation started from 7–9 months of age. Slices from rats of 3–4 months old showed the apparently highest susceptibility to 1,2-dichloropropane but depletion of glutathione content and loss of organic anion accumulation were at the same level in the oldest rats. The age decrease of control values caused the differences in the percentage ratio and then, apparently, a lower DCP effect. On the contrary, the increase of aspartate aminotransferase released in the incubation medium by DCP-treated slices corresponded to the age-related increase in cytotoxicity.

Key words: 1,2-Dichloropropane – Sex – Senescence – Nephrotoxicity

Introduction

Age and sex may be factors influencing xenobiotic toxicity in laboratory animals. In particular, kidney toxicity is more related than liver toxicity to age and sex differences; studies with chloroform in mice show that only males exhibit a nephrotoxic response, while the hepatotoxic response is similar in both sexes (Clemens et al. 1979; Smith et al. 1983). Furthermore, aging rats show a number of spontaneous diseases, and chronic progressive nephropathy is one of the most common (Gray 1977). These findings show an increased susceptibility of aging rats to xenobiotics such as salicylate (Kyle and Kocsis 1985), acetaminophen and p-aminophenol (Beierschmitt et al. 1986; Tarloff et al. 1989), and cephaloridine (Goldstein et al. 1986).

The aim of the present research is to study the in vitro effects of 1,2-dichloropropane (DCP) on the kidney in relation to sex and age of Wistar rats. An age-related significant decrease in reduced glutathione content (GSH) and cell viability with an increase of lipid peroxidation had also been previously shown (Trevisan et al. 1992).

Renal cortical slices are an interesting model to study in vitro kidney metabolism and toxicity of xenobiotics. This model was used by our research group to study metabolism and nephrotoxicity of the common aliphatic solvent DCP. Previous studies showed that the solvent is metabolized by the kidney in situ and that nephrotoxicity is mediated by a thiol formed during mercapturic acid metabolism (Trevisan et al. 1991).

Materials and methods

Albino, male, Wistar rats of different ages (1–2, 3–4, 7–9, and 12–14 months old), and albino, female, Wistar rats 3–4 months old were purchased from S. Morini, S. Polo d' Enza (RE), Italy, maintained in cages with a natural dark-light cycle, fed with standard diet (Nuova Zoofarm, Padova, Italy) and water "ad libitum".

Eight rats per group (males 3–4 months old matched for sex differences studies) were sacrificed and kidneys, quickly removed, placed in cold saline. Thin freehand renal cortical slices (100 ± 10 mg wet tissue, approximately 25 mg/slice, thickness approximately 0.5 mm), prepared with a scalpel according to Berndt (1976), were transferred into the

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Table 1. DCP-effects on renal cortical slice of male and female rats

		Male (M)	Female (F)	% CF vs CM
GSH[a]	C	0.16±0.02	0.15±0.03	94
	T	0.06±0.01*	0.10±0.01*	
% T vs	C	37	64	
MDA[b]	C	3.8 ±0.6	3.3 ±0.4#	87
	T	6.1 ±0.9*	4.1 ±0.9*	
% T vs	C	161	124	
AST[a]	C	1.0 ±0.1	0.9 ±0.1#	90
	T	1.5 ±0.1*	1.1 ±0.1*	
% T vs	C	150	122	
GGT[a]	C	2.7 ±0.2	2.4 ±0.5	89
	T	3.2 ±0.2*	2.5 ±1.1	
% T vs	C	119	104	
LDH[a]	C	3.9 ±0.6	4.8 ±0.9#	123
	T	5.3 ±0.4*	5.3 ±1.2	
% T vs	C	136	110	
PAH[c]	C	35.2 ±4.1	36.7 ±3.1	104
	T	18.8 ±1.5*	25.3 ±5.9*	
% T vs	C	53	69	

Legend: C = control, T = DCP treated. [a] µmol/100 mg tissue; [b] nmol/100 mg tissue; [c] S/M ratio (mean ± standard deviation). * p <0.05 or more T vs C; # p <0.05 or more female vs male controls. Male and female rats aged 3–4 months. Four rats per group were used, and assays were performed in duplicate

incubation medium composed of 97 mM NaCl, 40 mM KCl, and 0.74 mM $CaCl_2$, and sodium phosphate buffer 7.4 mM, pH 7.4, until all slices could be prepared (maximum 30 min). Slices were rinsed free of blood and/or enzymes released from damaged cells during the slicing process. After the preparation, the slices were transferred to 25 ml Erlenmeyer flasks containing 4 ml of the incubation medium. The flasks were stoppered, gassed with 100% O_2 for 5 min and then treated or not with 25 mM (≈250 nmol, final concentration) of DCP (Fluka, Buchs, CH, purity >99%), added with a 10 µl Hamilton microsyringe. The flasks were then incubated at 37° C for 90 min in a Dubnoff metabolic shaker (100 cycles/min).

After incubation, the slices were gently blotted and prepared to measure GSH content as non protein sulfhydryl groups according to Sedlak and Lindsay (1968), or placed into the DCP-free incubation medium for up to 15 min. Then, the slices were incubated in a medium (4 ml) containing additionally 10^{-3} M lactate (Fluka, Buchs, CH) and 7.5×10^{-5} M p-aminohippurate (PAH, Fluka, Buchs, CH) at 25° C for 90 min in a Dubnoff metabolic shaker (100 cycles/min) under 100% O_2 to study organic anion accumulation. After incubation, the slices were homogenized with trichloroacetic acid (TCA) 3% (10 ml/100 mg of tissue). A 1 ml aliquot of the incubation medium was treated with 4 ml 3% TCA. After centrifugation, the supernatant was assayed for PAH (Smith et al. 1945). The organic anion accumulation was expressed as the slice/medium (S/M) ratio, where the PAH concentration (µg/g tissue) of the slices was divided by the PAH concentration (µg/ml) of the medium.

The DCP incubation medium was centrifuged and aspartate aminotransferase (AST, E. C. 2.6.1.1), γ-glutamyltransferase (GGT, E. C. 2.3.2.2), and lactate dehydrogenase (LDH, E. C. 1.1.1.27) were determined using commercial kits (Boehringer, Mannheim, Germany). Malondialdehyde (MDA) was assayed as thiobarbituric acid-reactive substances (TBARS) according to Younes and Siegers (1981), as modified by Kornbrust and Bus (1984), to measure lipid peroxidation in renal cortical slices.

Finally, cysteine conjugate β-lyase (β-lyase) activity was determined in male naive rats during senescence according to Dohn and Anders (1982) using S-(2-benzothiazolyl) cysteine as a substrate, synthesized from 2-chloro-benzothiazole (Colucci and Buyske 1965).

Spectrophotometric determinations were carried out using a UV-Vis. spectrophotometer Perkin-Elmer lambda 5 model.

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

Results

GSH content, PAH accumulation and GGT released into the incubation medium from renal cortical slices showed slight (not significant) sex differences. On the contrary, release of MDA (p <0.05) and AST (p <0.02) was higher from male than female slices, whereas female slices showed a higher release of LDH (p <0.05).

DCP effects were always higher in male than in female slices. DCP had no effects on female brush border (GGT release from female slices after solvent treatment was only slightly increased), and cell viability. Data are summarized in Table 1.

The effects of senescence on renal cortical slice targets and the related effects of DCP are summarized in Table 2.

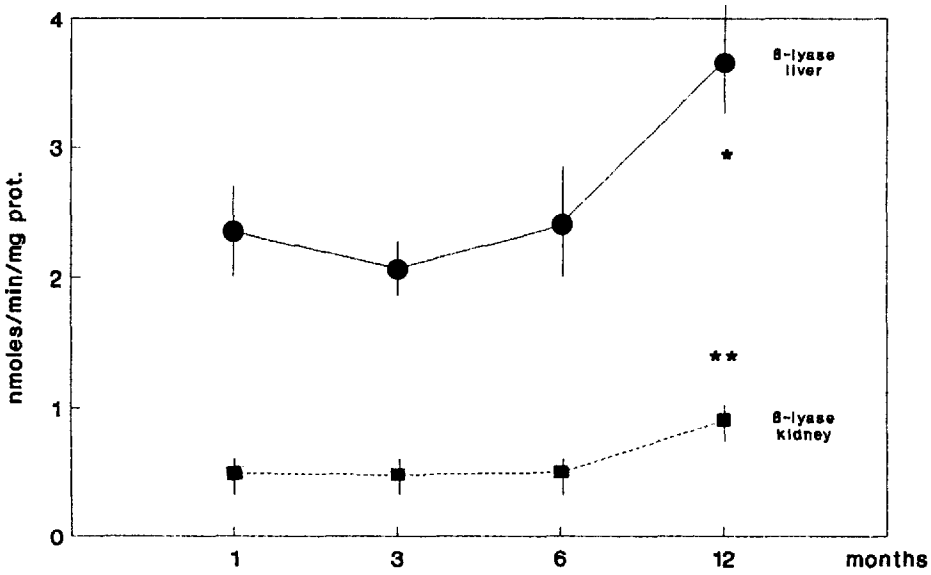

Fig. 1. Age-dependent β-lyase activity in liver and kidney of naive male rats. Mean ± standard deviation was determined in five animals per group. * P <0.01, ** p <0.001

Table 2. DCP-effects on renal cortical slice of male rats of different age

Age	(months)	1–2	3–4	7–9	12–14
GSH[a]	C	0.17±0.02	0.16±0.02	0.14±0.02	0.12±0.01#
	T	0.09±0.02*	0.06±0.01*	0.05±0.01*	0.05±0.01*
% T vs	C	53	38	36	42
MDA[b]	C	3.0 ±1.1	3.8 ±0.6	5.3 ±0.8#	6.5 ±0.8#
	T	4.6 ±0.9*	6.1 ±0.9*	7.0 ±0.5*	8.3 ±1.2*
% T vs	C	153	161	132	128
AST[a]	C	0.8 ±0.1	1.0 ±0.1#	1.1 ±0.1#	1.2 ±0.1#
	T	1.2 ±0.1*	1.5 ±0.1*	1.6 ±0.1*	2.0 ±0.2*
% T vs	C	150	150	145	167
GGT[a]	C	2.5 ±0.2	2.7 ±0.2	2.7 ±0.3	2.8 ±0.2#
	T	2.7 ±0.3	3.2 ±0.2*	3.2 ±0.2*	3.2 ±0.2
% T vs	C	108	119	119	114
LDH[a]	C	3.3 ±0.2	3.9 ±0.6#	5.0 ±0.6#	5.9 ±0.7#
	T	4.0 ±0.4*	5.3 ±0.4*	6.7 ±0.6*	7.7 ±0.9*
% T vs	C	121	136	134	131
PAH[c]	C	37.8 ±5.9	35.2 ±4.1	30.7 ±2.8#	28.8 ±5.9#
	T	24.8 ±1.3*	18.8 ±1.5*	18.7 ±1.7*	18.6 ±1.6*
% T vs	C	66	53	61	65

Legend: C = control, T = DCP treated. [a] µmol/100 mg tissue; [b] nmol/100 mg tissue; [c] S/M ratio (mean ± standard deviation). * $p < 0.05$ or more T vs C; # $p < 0.05$ or more vs 1–2 months old. Four rats per group were used, and assays were performed in duplicate

Senescent rats showed a significant age-related decrease of GSH content ($p < 0.005$). Rats 7–9 month of age demonstrated a decrease of GSH content of 19%, while at 12–14 months this decrease is 31% as compared with young animals. In addition, PAH accumulation showed an age-related loss (significant, $p < 0.01$, from 7–9 months of age) up to 24% with respect to young rats. AST and LDH release into the incubation medium increased with age, and MDA release into the incubation medium, a measure of lipid peroxidation, increased during senescence; spontaneous lipid peroxidation was two-fold higher in oldest than in young rats.

Sensitivity of renal cortical slice targets to DCP is lower in young (1–2 month old) that in old rats. GSH content and PAH accumulation reach a maximum depletion and loss (respectively) in 3–4 month old rats, following which no other variation was detectable with age. The apparently minor effect in older rats could be due to the age-related decrease of control values. Greater lipid peroxidation was DCP induced in 3–4 month-old rats (compared to the controll), whereas LDH release showed only slight differences after treatment with DCP at different ages. On the contrary, AST release was more affected by DCP treatment in the oldest rats, whereas GGT release showed only slight variation with age and after DCP treatment.

Figure 1 shows the behaviour of β-lyase activity in liver and kidney during senescence. The enzyme activity increases significantly ($p < 0.01$ and $p < 0.001$, respectively) in both organs only in the oldest rats.

Discussion

Sex differences

The results demonstrate only slight differences in renal cortical slice targets between naive male and female rats, whereas DCP affects renal cortical slice targets of male more than of female rats. In particular, the solvent causes a higher GSH content depletion, lipid peroxidation, and loss of organic anion accumulation.

Several sex-related differences of response to nephrotoxic xenobiotics are described in the literature. Male rats (Hook et al. 1983) and mice (Lock et al. 1984) are less susceptible to hexachloro-1:3-butadiene-induced nephrotoxicity than females of both species. In contrast, dichlorovinyl-cysteine induced nephrotoxicity in adult mice was more pronounced in the female after the lower dose and in the male after the higher dose (Darnerud et al. 1991). Male mice (Smith et al. 1983) and rats (Harber and Jennings 1965; Atkinson et al. 1966) are more susceptible than females to nephrotoxic effects of xenobiotics like chloroform (Smith et al. 1983), mercuric chloride (Harber and Jennings 1965) or cephaloridine (Atkinson et al. 1966). All these data show that there is no consistent sex response to nephrotoxic effects of xenobiotics.

Some explanations have been attempted, such as the different capability of the mouse female kidney to form reactive metabolites from chloroform (Smith et al. 1983) or differences in hepatic and renal enzymes responsible for detoxification and activation of foreign compounds (Hook et al. 1983). However, renal glutathione S-transferase activities are higher for several substrates in female than male rats (Clifton et al. 1975), and therefore neither hypothesis explains the variability in sex-related response to different nephrotoxic xenobiotics. Only a hypothetical difference in xenobiotic-related metabolism could explain these discrepancies.

The metabolic fate of DCP is similar in both sexes (Bartels and Timchalk 1990; Timchalk et al. 1991), and information on sex-related nephrotoxicity is not available in the literature. In male rats DCP causes liver hyperplasia (Trevisan et al. 1989) and mesangio-proliferative nephrop-

athy with a dose-dependent proximal tubule impairment after subchronic treatment (Trevisan et al. 1988).

The low in vitro nephrotoxicity of DCP in female rats could be related to a lower metabolic activation via β-lyase which produces nephrotoxic thiols. Our unpublished experiments, in fact, show that DCP toxicity on female renal cortical slices was not prevented by in vitro pretreatment with a specific inhibitor of β-lyase activity like aminooxyacetic acid. The opposite effects was demonstrated in vitro in male rats (Trevisan et al. 1991). Some years ago Van Bladeren et al. (1981) found an episulphonium ion metabolite as reactive intermediate in the metabolism of 1,2-dibromoethane, but recent research (Bartles and Timchalk 1990) does not support an episulphonium ion intermediate during DCP metabolism.

Age-related differences

Renal cortical slices of 3–4-month-old male Wistar rats are apparently more susceptible to DCP-induced nephrotoxicity than young adult rats (1–2 months old). In fact the solvent causes GSH depletion and a loss of organic anion accumulation (PAH) higher in 3–4 than in 1–2-month-old rats. Hook et al. (1983), on the contrary, observed that young rats (22 days old) were more susceptible to hexachloro-1:3-butadiene induced nephrotoxicity than adult rats, and attributed this different response to marked differences of pharmacokinetics and disposition of the xenobiotic. Phase II metabolic enzyme systems are known to be immature in young rats, and the enzymes responsible for detoxification are fully functional after 22 days of age. These data were confirmed in the mouse (Lock et al. 1984). However, an age-dependent increase in the number of functionally active nephrons and thereby also the potential sites of xenobiotic metabolite binding and nephrotoxic attack may cause an age-related accumulation of nephrotoxic compounds (McCormack et al. 1981) as dichlorovinyl cysteine (Darnerud et al. 1991).

Organic anion accumulation (PAH) shows a postnatal increase, as supported by many previous studies (Rennick et al. 1961; Hirsch and Hook 1970; Kim et al. 1972; Ecker and Hook 1974). Senescent Sprague-Dawley (Adams and Barrows 1963) and Wistar (Beauchenne et al. 1965), but not Fischer-344 rats (Goldstein et al. 1986), show an age-related decrease of this tubular function. Wabner and Chen (1987) suggested the possibility of an age-related decrease in V_{max} for PAH. Our data partially agree with these studies: in fact a significant loss of organic anion accumulation (PAH) was observed in naive Wistar rats from 7–9 months of age. However, these results were not obtained with high (600 μM) but with low PAH concentration (75 μM) in the incubation medium, showing that senescence does not cause a decrease in V_{max} for PAH, but agree with an in toto impairment of proximal tubule function. This is also supported by age-related GSH content depletion, increase in lipid peroxidation, and decrease of cell viability (increase of LDH release into the incubation medium).

Surprisingly, the GSH content of the slices and PAH S/M ratio after DCP treatment were the same in the oldest and in 3–4-month-old rats. An explanation of these data may be a threshold over which no further effect is measurable. Senescent rats show an apparent lower response to DCP effects, but this is due to an age-related decrease of the baseline (values in naive rat slices). However, the increase of AST release in the incubation medium confirms the higher cytotoxicity of DCP in senescent rats.

Finally, data reported in Fig. 1 make clear that β-lyase activity increases significantly in the kidney of the oldest rats ($p < 0.001$), other than in the liver ($p < 0.01$). These results could be due to a greater availability of β-lyase which forms toxic thiols in naive and DCP-treated slices of old rats.

In conclusion, sex- and age-related differences were observed in naive and DCP-treated slices of Wistar rats. Male slices are more susceptible than female ones to in vitro effects of DCP, whereas senescence is an important factor for expression of the solvent toxicity. The reason of these phenomena is not clear, but sex differences related to toxic activation and spontaneous impairment of normal tubular function during senescence might be a relevant topic of discussion.

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