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In-Vitro Mechanisms of 1,2-Dichloropropane Nephrotoxicity using the Renal Cortical Slice Model*

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1 Renal cortical slices isolated from the kidneys of male Wistar rats were used as an experimental model for studying the nephrotoxicity induced by 1,2-dichloropropane.

2 The solvent causes a depletion of renal reduced glutathione content and slight, but significant, lipid peroxidation. The block of the oxidative pathway with carbon monoxide prevents glutathione content depletion, and shows that this conjugation is the major step in 1,2-dichloropropane metabolism.

3 Loss of organic anion accumulation and release into the incubation medium of tubular enzymes, mainly from the soluble fraction, are the toxic effects of the solvent. The brush border is only slightly affected.

4 The mechanism of nephrotoxicity appears to occur via mercapturic acid metabolism. Acivicin and aminooxyacetic acid, inhibitors of gamma-glutamyltransferase and ß-lyase activity, respectively, partially but significantly prevent the loss of organic anion accumulation induced by 1,2-dichloropropane. Furthermore, α-ketobutyrate, an activator of ß-lyase, enhances the effects of 1,2-dichloropropane on the target, but is itself toxic for organic anion accumulation.

Introduction

In subchronically treated rats, 1,2-dichloropropane (DCP), a chlorinated alkane, causes liver hyperplasia[1] and mesangio-proliferative nephropathy with late involvement of the proximal tubule which results in damage to the brush border.[2] The biochemical changes are reversible.[3]

The effects on the kidney are different during acute poisoning in man; the solvent induces acute tubular necrosis without involvement of the glomerulus.[4]

The metabolism of DCP, studied by Jones and Gibson,[5] follows a major oxidative pathway with dechlorination to 1-chloro-2-hydroxypropane and then to 1,2-epoxypropane. The latter intermediate reacts with reduced glutathione (GSH) and follows the mercapturic acid pathway to N-acetyl-S(2-hydroxypropyl)cysteine. The conversion of 1,2-epoxypropane to 1,2-propanediol and then to pyruvate and CO_2 is a reaction characteristic of epoxides *in vivo*. An undefined amount of parent compound could interact directly with GSH as shown by *in-vivo*[6,7] experiments.

The aim of this research was to study the DCP mechanisms of nephrotoxicity using the renal cortical slice model. This model allows the study of kidney metabolism and kidney toxicity independently from variables such as pharmacokinetics and toxicity to other target organs.[8]

Methods

Chemicals

DCP (purity > 99%), α-ketobutyrate sodium salt (KBT), aminooxyacetic acid (AOAA), lactic acid sodium salt (lactate), and 4-aminohippurate (PAH) were purchased from Fluka (Buchs, CH); acivicin (AT-125) was purchased from Sigma Chemical Co. (St. Louis, USA). Other chemicals were purchased from Merck (Darmstadt, FRG).

Toxicity studies

Albino, male, Wistar rats (Morini, S. Polo d'Enza, Reggio Emilia, Italy) weighing 300±10 g with free access to feed (standard diet, Nuova Zoofarm, Padova, Italy) and water, were killed by decapitation and their kidneys were removed rapidly and placed in ice-cold 0.9% NaCl. Thin handfree renal cortical slices (100±10 mg wet tissue, approx. 25

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mg per slice) prepared according to Berndt,[9] were placed into incubation medium (see below) until all the slices could be prepared. Slices were rinsed 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 composed of sodium phosphate buffer 7.4 mM, pH 7.4 enriched with 97 mM NaCl, 40 mM KCl, and 0.74 mM $CaCl_2$. The flasks were stoppered, gassed with 100% O_2 for 5 min and then treated with 5–25×10^{-3} M of DCP (≈50–250 nmol, final concentration), added by means of a 10 µl Hamilton microsyringe. The flasks were incubated at 37°C for 90 min in a Dubnoff metabolic shaker (100 cycles per min). After incubation, the slices were gently blotted and prepared in order to measure GSH content according to the method of Sedlak and Lindsay.[10] At the same time, the medium was centrifuged and GSH as above, aspartate aminotransferase (AST, E.C. 2.6.1.1), lactate dehydrogenase (LDH, E.C. 1.1.1.27), and gamma-glutamyltransferase (GGT, E.C. 2.3.2.2) were determined using a commercial kit (Boehringer, Mannheim, FRG). Moreover, malondialdehyde (MDA) in the medium was determined according to the method of Younes and Siegers,[11] as modified by Kornbrust and Bus,[12] to measure lipid peroxidation in renal cortical slices.

Separate experiments were carried out to provide information on the mechanism of toxicity. All these experiments were performed with a DCP concentration of 25×10^{-3} M (≈250 nmol, final concentration). Before treatment, the slices were gassed with carbon monoxide (CO) 100% for 5 min to inhibit oxidative metabolism or preincubated for 30 min at 37°C with AOAA (10^{-3} M), a selective renal ß-lyase inhibitor both *in vitro* and *in vivo*[13,14] or KBT (5×10^{-3} M), an activator of renal cytosolic and mitochondrial ß-lyase activities,[15] or AT-125 (2.5×10^{-4} M), inhibitor of GGT activity.[16,17] The chemicals were also added during the incubation period.

Organic anion accumulation

After preincubation and incubation with chemicals (see above), the slices were blotted, placed in the incubation medium for 15 min, and then incubated in medium (4 ml) containing 10^{-3} M of lactate and 7.5×10^{-5} M of PAH at 25°C for 90 min in a Dubnoff metabolic shaker (100 cycles per min) under 100% O_2 to study organic anion accumulation. After incubation, the slices were homogenated with trichloroacetic acid (TCA) 3% (10 ml 100 mg^{-1} of tissue). A 1-ml aliquot of the incubation medium was treated with 4 ml of TCA 3%. After centrifugation, the supernatant was assayed for PAH.[18] Organic anion accumulation was expressed as the slice/medium (S/M) ratio, where the PAH concentration (µg g^{-1} of tissue) of the slices was divided by PAH concentration (µg ml^{-1}) of the medium.

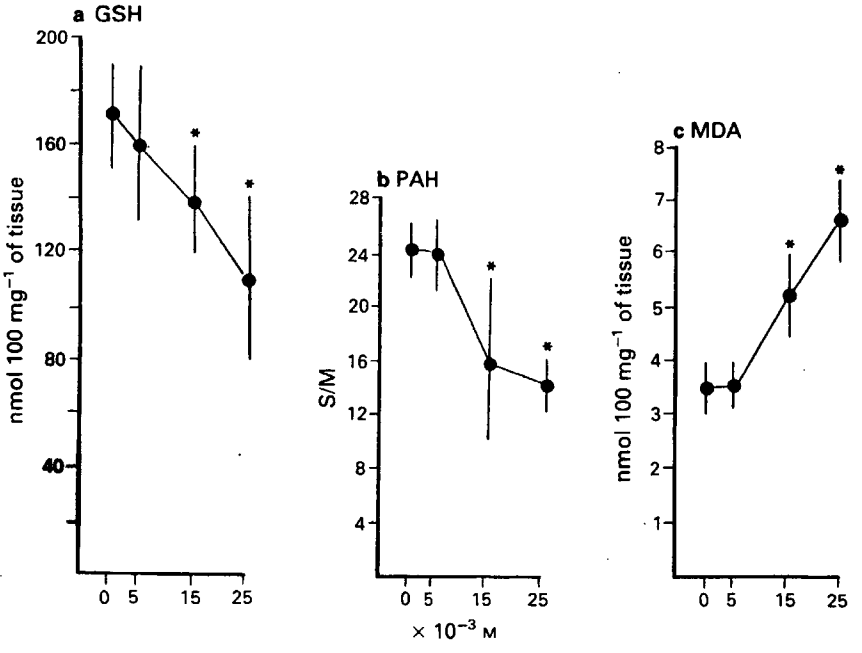

Figure 1 GSH content (a), PAH accumulation (b), and MDA release in the medium (c) after treatment with DCP. Mean ± s.d. was determined from five proofs. *P < 0.05 or more with respect to the control.

Results

DCP causes a significant dose-dependent depletion of GSH (Figure 1a). The highest concentration (25×10^{-3} M) caused a 35% depletion. GSH was not found in the medium (data not shown). PAH accumulation (S/M ratio) was comparable (Figure 1b). The release of MDA in the medium increased as GSH decreased (Figure 1c).

The amount of enzyme released into the medium is slight (Table 1): a significant increase in AST ($P < 0.005$) and LDH ($P < 0.01$) could only be measured at the highest dose.

Table 1 Effects of DCP on enzyme release in the medium.

Dose (M)	*AST*	*LDH*	*GGT*
	μmol 100 mg⁻¹ of tissue		
Control	0.81± 0.13	2.68± 0.53	2.28± 0.40
5×10^{-3}	0.81± 0.14	2.82± 0.57	2.11± 0.37
15×10^{-3}	0.90± 0.08	3.05± 0.63	2.35± 0.31
25×10^{-3}	1.10± 0.12**	3.80± 0.54*	2.84± 0.60

*$P < 0.01$; **$P < 0.005$ with respect to the control. Mean ± s.d. was determined from five proofs.

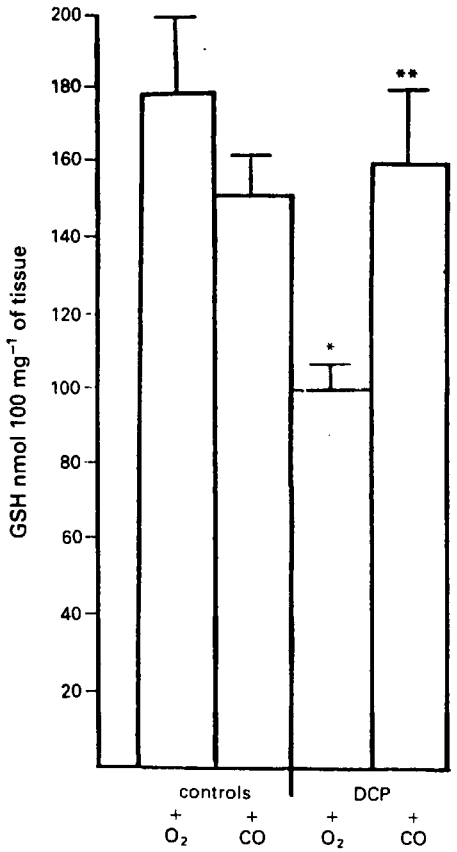

Figure 2 GSH content of slices gassed with O_2 or CO and then treated or not treated with DCP (25×10^{-3} M). Mean ± s.d. was determined from five proofs. *$P < 0.001$ with respect to the control plus O_2; **$P < 0.005$ with respect to DCP plus O_2.

CO completely inhibits the effects resulting from DCP treatment (Figure 2), and shows that oxidation plays an important role during solvent metabolism.

AOAA, a specific inhibitor of ß-lyase activity, a pyridoxal phosphate-dependent enzyme, partially prevents the accumulation of PAH ($P < 0.001$) and MDA release induced by DCP. Comparable behaviour is seen with AT-125, a specific inhibitor of GGT activity. The use of a specific activator of ß-lyase activity, such as KBT, appears to cause kidney toxicity; in fact it causes only a slight increase in MDA release ($P < 0.025$), but a loss of PAH accumulation ($P < 0.005$) to a greater extent than that caused by DCP. Moreover, pretreatment with KBT significantly enhances MDA release in the medium ($P < 0.01$) and loss of PAH accumulation ($P < 0.02$) more than DCP or KBT alone. The effects of all pretreatments are summarized in Table 2.

Table 2 Effect of pretreatment with AOAA (a), AT-125 (b), and KBT (c) on DCP toxicity

Treatment	*MDA*	*PAH*
	nmol 100 mg⁻¹ of tissue	*S/M*
(a) Control	3.93± 0.67	29.41± 1.78
+ AOAA	3.99± 0.73	28.47± 5.30
+ DCP	5.20± 0.70*	17.64± 1.99*
+ AOAA + DCP	4.66± 0.73	22.78± 1.50*#
(b) Control	3.98± 0.50	25.10± 0.42
+ AT-125	4.18± 0.88	24.72± 2.39
+ DCP	7.35± 0.88*	16.30± 1.92*
+ AT-125 + DCP	6.73± 0.81*	19.26± 1.83*#
(c) Control	3.20± 0.23	29.47± 4.27
+ KBT	3.83± 0.40*	17.81± 0.73*
+ DCP	4.73± 0.71*	19.09± 1.37*
+ KBT + DCP	5.99± 0.46*#	11.57± 3.10*#

*$P < 0.05$ or more with respect to the control; #$P < 0.05$ or more between pretreatment plus DCP and DCP alone. Mean ± s.d. was determined from five proofs. DCP 25×10^{-3} M, AOAA 10^{-3} M, AT-125 2.5×10^{-4} M, KBT 5×10^{-3} M.

Measurement of transaminases (pyridoxal phosphate-dependent enzymes) and GGT activities in the medium was carried out to verify the effects of these chemicals. AOAA causes a 90% decrease, and KBT a 40% increase in transaminase release in the incubation medium; AT-125 a complete inhibition of GGT release.

Discussion

Renal cortical slices were used to investigate the *in-vitro* mechanism of toxicity of known nephrotoxic chemicals such as vanadate,[19] chloroform,[20] metals (potassium dichromate and mercuric chloride), solvents (hexachlorobutadiene and carbon tetrachloride) and antimicrobial agents[8] and S-(1,2-dichlorovinyl)-cysteine.[21]

DCP causes a dose-dependent depletion of slice GSH content that is almost totally prevented by CO. This evidence points to the oxidative metabolism of DCP in the kidney with the formation of a reactive intermediate followed by the conjugation with GSH. Previous studies[22] show that DL-buthionine-[S,R]-sulphoximine, a well known GSH-depleting agent, increases DCP toxicity, and confirms the role of GSH in solvent metabolism. However, in a complex system such as renal cortical slices, CO could bind other cytochromes and interfere with oxidative DCP metabolism indirectly, e.g. by impairing cell energy supplies.

The solvent also causes the dose-dependent loss of organic anion accumulation (PAH). The mercapturic acid pathway appears to play a fundamental role in tubular toxicity. AT-125, an inhibitor of GGT activity that catalyses the second step of the mercapturic acid metabolic pathway, significantly prevents the loss of organic anion accumulation induced by DCP. This evidence might show that toxicity is not related to a glutathione-conjugate but to a cysteine-conjugate. In fact, AOAA, an inhibitor of ß–lyase activity, plays a protective role, whereas an activator of this enzyme such as KBT, that is surprisingly more toxic for organic anion accumulation than DCP, enhances the loss of organic anion accumulation and MDA release in the medium induced by the solvent. Otherwise the enhancement of the toxicity caused by KBT could be related to the synergistic effect of DCP on the previously damaged tubule.

These results show that the toxicity occurs, partially, via a cysteine-conjugate activated by ß-lyase to form a nephrotoxic thioalkane. The haloalkanes may react with GSH and then undergo hydrolysis or alkylate nucleophiles via episulphonium ion intermediates.[23,24] The nephrotoxicity of the 1,2-dihaloalkanes may be the result of alkylation of essential renal macromolecules by episulphonium ions. Recent research[6] does not support an episulphonium ion intermediate in mercapturic acid formation during DCP metabolism, besides, the mechanisms of cytotoxicity appear to be different. Moreover, the nephrotoxicity of the haloalkanes is dependent on activation by renal ß-lyase.[25]

In conclusion, the *in-vitro* renal cortical slice model shows that: the kidney may metabolize DCP to toxic metabolites; conjugation with GSH occurs in the kidney as well as in the liver; nephrotoxicity is caused by a cysteine-conjugate that is formed during mercapturic acid metabolism; it is activated to a thioalkane by renal ß-lyase.

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