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Recovery of Biochemical Changes Induced by 1, 2-Dichloro propane in Rat Liver and Kidney

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1 Biochemical changes in male, Wistar rats, treated with different doses of 1,2-dichloropropane (50–500 mg kg^{-1} body weight), were investigated at the end of a 4-week treatment and after a 4-week recovery period.
2 The behaviour of Phase I and Phase II metabolic steps and of the angiotensin converting enzyme activity of the renal proximal tubule brush border were determined.
3 Phase II is more affected by the solvent than Phase I metabolism, and liver metabolism is more affected than the kidney.
4 Angiotensin converting enzyme activity from the proximal tubule brush border appears to be the most sensitive parameter of kidney involvement during treatment.
5 After a 4-week recovery period all the metabolic indices together with angiotensin converting enzyme activity have returned to normal. Only liver reduced glutathione content shows a slight, but significant, increase for the highest dose (500 mg kg^{-1} body weight).
6 The results show that the biochemical changes induced in liver and kidney by 1,2-dichloropropane are reversible.

Introduction

1,2-Dichloropropane (1,2 D) induces liver alterations during the subchronic i.p. treatment of rats.[1] These consist of cell hyperplasia and biochemical changes depending on the reaction of the cell (decrease of Phase I and increase of Phase II metabolic steps). However, the pattern of anatomical and biochemical modification suggests that cell repair occurs. This is in agreement with the reports of several years ago.[2]

Less investigated are the biochemical changes that occur in the kidney after treatment with the solvent. Quite recently, non-protein sulphydryl (NPSH) groups in the kidney cortex have been shown to increase during p.o. treatment with 1,2 D,[3] whereas a dose-dependent decrease of angiotensin converting enzyme (ACE) activity of the proximal tubule brush border, fraying of the microvilli and epithelial coagulative necrosis of the brush border after i.p. treatment with 250 and 500 mg kg^{-1} doses were observed.[4] Alterations of the glomeruli are the earliest changes to be seen.[4]

The aim of the present work is to study the effects of 1,2 D on biochemical markers of the liver and the kidney and to determine whether, after a recovery period, the induced changes are reversible. Only one report[3] shows a disappearance of the effects after a 1-week recovery period following p.o. treatment with 100 and 250 mg kg^{-1} doses.

Methods

Animals
Albino, male, Wistar rats, with a starting weight 200 ± 10 g, were supplied from Stefano Morini (S. Polo d'Enza, Reggio Emilia, Italy); the rats were located in cages with free access to food (standard diet, Nuova Zoofarm, Padova, Italy) and water.

Treatment
Fifty rats were used in the study, and were divided into two groups of 25 animals. One group was sacrificed 24-h after 4-weeks of treatment

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and one group following a 4-week recovery period. The rats (both groups) were treated i.p. once a day, 5 d a week (2 d without treatment) for 4 weeks with 1,2 D (Merck, Darmstadt, FRG, purity 97%) dissolved in corn oil (0.5 ml volume) at doses of 50, 100, 250 and 500 mg kg^{-1}. Two control groups were treated with corn oil only (0.5 ml). In all cases rats were killed by decapitation and blood, liver and kidney were removed.

Plasma transaminases (AST and ALT) were determined with a commercial kit (Boehringer, Mannheim, FRG).

Both liver and kidney cortex were prepared for: the measurement of reduced glutathione content (GSH) in homogenate (200 mg 8 ml^{-1} EDTA), determined as NPSH according to the method of Sedlak and Lindsay;[5] assay of glutathione S-transferase (GST) activities (10 000 xg supernatant, 2 mg ml^{-1} of phosphate buffer 0.1 M pH 6.5), according to the method of Habig *et al.*;[6] aniline hydroxylase (AOH) activity, according to the method Imai *et al.*;[7] aminopyrine demethylase (ADEM) activity (10 000 xg supernatant, 25% w/v), according to McLean and Day;[8] and cytochrome P450 (CYT) activity in microsomes, according to the method of Omura and Sato.[9] These enzyme activities were chosen in order to value the biochemical pattern of cell hyperplasia induced by 1,2 D treatment. The proximal tubule brush border was then prepared using the method of Booth and Kenny[10] and the ACE activity determined according to the method of Summary.[11] Protein concentrations were determined by Miller's method.[12]

Statistics

Variance analysis and Student's *t* test were used for the statistical evaluation of the results. A 0.05 limit was assumed as significant.

Results

Table 1 shows that 1,2 D induces a dose-dependent increase of liver Phase II metabolism (GSH and GST) and a decrease of Phase I metabolism in both organs. Lower doses effected GSH and GST activity too. No variation of AOH activity was measurable.

After a 4-week recovery period (Table 2), only GSH content could be shown to have a slightly significant ($P < 0.05$) increase with the highest doses. All other parameters of the liver did not differ significantly from control values.

ACE activity appears to be a more sensitive index of kidney involvement; the enzyme activity decreased significantly 100 mg kg^{-1} ($P < 0.05$). Both liver and kidney Phase II metabolic steps react at lower doses than those of Phase I. ADEM activity is not dosable in kidney cortex and AOH activity showed no significant variation during treatment. The effects on the kidney are summarized in Table 3.

The 4-week recovery period resulted in the kidney indices returning to normal (Table 4).

According to the histology,[1] there was no significant variation in plasma transaminases during treatment (data not shown).

Discussion

The liver is more sensitive than the kidney and at lower doses of 1,2 D. In both organs, Phase II metabolism is more sensitive than Phase I.

Data from the liver confirm our previous report. From Table 1 it can be seen that no-observed-adverse-effect-level (NOAEL) for the liver is seen below 50 mg kg^{-1} for Phase II, and 50 mg kg^{-1} for Phase I metabolism. The lowest-observed-adverse-effect-level (LOAEL) is 50 mg kg^{-1} for Phase II and 100 mg kg^{-1} for Phase I metabolism.

Table 1 Phase I and Phase II metabolic steps of the liver after 4-week's treatment with 1,2 D.

Dose mg kg^{-1}	*ADEM (1)*	*AOH (2)*	*CYT (3)*	*GSH (4)*	*GST (5)*
0	9.1 ± 1.3	0.65 ± 0.08	0.47 ± 0.09	17.0 ± 2.7	143.0 ± 15.9
50	7.2 ± 2.0	0.67 ± 0.06	0.49 ± 0.16	22.1 ± 3.1*	170.1 ± 6.1*
100	5.4 ± 2.5*	0.79 ± 0.29	0.46 ± 0.06	21.1 ± 2.7*	179.7 ± 14.8*
250	4.0 ± 2.5*	0.85 ± 0.19	0.41 ± 0.07	26.2 ± 1.6*	193.1 ± 24.6*
500	3.5 ± 2.1*	0.63 ± 0.05	0.35 ± 0.07*	28.4 ± 2.9*	254.8 ± 24.6*

* $P < 0.05$
(1) nmol mg^{-1} protein
(2) nmol mg^{-1} protein
(3) nmol mg^{-1} protein
(4) nmol mg^{-1} protein
(5) nmol mg^{-1} protein

Table 2 Phase I and Phase II metabolic steps of the liver after 4-week's recovery period

Dose mg kg^{-1}	*ADEM (1)*	*AOH (2)*	*CYT (3)*	*GSH (4)*	*GST (5)*
0	9.9 ± 2.4	0.74 ± 0.11	0.42 ± 0.06	18.4 ± 1.9	210.0 ± 40.3
50	9.2 ± 2.3	0.60 ± 0.10	0.46 ± 0.15	20.7 ± 1.0	242.6 ± 21.5
100	9.2 ± 3.3	0.62 ± 0.10	0.41 ± 0.11	18.7 ± 2.8	267.2 ± 38.1
250	9.6 ± 2.5	0.80 ± 0.13	0.42 ± 0.11	19.8 ± 4.2	270.0 ± 61.1
500	7.6 ± 2.3	0.75 ± 0.14	0.40 ± 0.04	22.3 ± 1.5*	251.8 ± 32.4

For legends see Table 1.

Table 3 Phase I and Phase II metabolic steps of the kidney cortex and ACE activity after 4-week's treatment with 1,2 D

Dose mg kg^{-1}	*AOH (2)*	*CYT (3)*	*GSH (4)*	*GST (5)*	*ACE (6)*
0	0.11 ± 0.04	0.063 ± 0.013	22.7 ± 3.0	82.1 ± 8.0	4.19 ± 0.38
50	0.11 ± 0.01	0.065 ± 0.012	23.1 ± 2.1	82.0 ± 8.4	3.85 ± 0.41
100	0.12 ± 0.02	0.055 ± 0.021	24.5 ± 3.2	83.3 ± 9.1	3.52 ± 0.26*
250	0.12 ± 0.03	0.047 ± 0.016	25.9 ± 1.1*	90.3 ± 9.8	3.01 ± 0.13*
500	0.13 ± 0.04	0.044 ± 0.006*	29.6 ± 3.3*	125.5 ± 13.2*	2.74 ± 0.29*

* see Table 1
(2–5) see Table 1
(6) μmol mg^{-1} protein

Table 4 Phase I and Phase II metabolic steps of the kidney cortex and ACE activity after 4-week's recovery period

Dose mg kg^{-1}	*AOH (2)*	*CYT (3)*	*GSH (4)*	*GST (5)*	*ACE (6)*
0	0.10 ± 0.02	0.062 ± 0.007	21.5 ± 2.0	84.6 ± 9.9	4.90 ± 0.25
50	0.10 ± 0.03	0.060 ± 0.005	20.2 ± 1.1	79.7 ± 15.0	5.03 ± 0.10
100	0.11 ± 0.05	0.055 ± 0.013	19.7 ± 3.1	79.6 ± 12.8	4.88 ± 0.29
250	0.12 ± 0.02	0.052 ± 0.012	19.2 ± 1.8	92.5 ± 22.9	5.03 ± 0.49
500	0.13 ± 0.04	0.051 ± 0.012	19.8 ± 1.4	100.5 ± 12.8	4.68 ± 0.29

For legends see Tables 1 and 3

The NOAEL for kidney is 50 mg kg^{-1}. ACE activity is a more sensitive index of effect than phases of metabolism in the kidney: the LOAEL is 100 mg kg^{-1}. Phase I metabolism is slighlty impaired (LOAEL 500 mg kg^{-1}), whereas Phase II shows a LOAEL of 250 mg kg^{-1}.

The decrease of ACE activity shows that the brush border is more sensitive to the toxic effect of 1,2 D than the cytoplasm; these results confirm our previous observations[4] that demonstrated a decrease in ACE activity after subchronic treatment.

A 4-week recovery period results in normal indices both in the liver and in the kidney; only GSH in the liver shows a slight but significant increase at a dose of 500 mg kg^{-1}. These results show that the effects induced by subchronic treatment with 1,2 D are reversible and agree with Bruckner *et al.*,[3] in spite of the differences in administration (i.p. instead of p.o.) and doses.

In conclusion 1,2 D affects the liver to a greater extent and at lower doses. Furthermore, GSH content and GST activities appear to be the more sensitive indices of liver involvement, whereas ACE activity in the brush border is the best parameter of kidney involvement; the brush border is also the structure of the tubule more affected. Finally, the effects on liver and kidney are reversible after a 4-week recovery period.

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