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LUNG-SELECTIVE IMPAIRMENT OF CYTOCHROME P-450-DEPENDENT MONOOXYGENASES AND CELLULAR INJURY BY 1,1-DICHLOROETHYLENE IN MICE

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The acute toxic effects of 1,1-dichloroethylene (DCE; 125 mg/kg, i.p.) on mouse lung, liver and kidney were investigated 24 hr after its administration. DCE caused a reduction of cytochrome P-450 levels and related monooxygenases in lung microsomes with no corresponding changes in liver and kidney. Examination of the lung tissue by light microscopy revealed necrosis restricted to the Clara cells. In contrast, liver and kidney were relatively unaffected by DCE treatment, as indicated by lack of changes in microsomal monooxygenase activities and morphology.

1,1-Dichloroethylene (DCE) is a volatile liquid which is widely used in the plastics industry and, as a result, human exposure usually occurs by inhalation. Acute toxicity studies in the rat revealed that DCE can cause cellular damage to liver (1,2) as well as kidney (3). Surprisingly, little attention has been paid to the possible acute pulmonary toxicity, even though investigations into the disposition of DCE in rats (4,5) revealed that after oral, intraperitoneal or intravenous administration, pulmonary exhalation of the unmetabolized compound accounted for a significant part of the administered dose.

Forkert and Reynolds (6) reported microscopic evidence of lung injury in mice after oral administration of DCE. At a dose of 100 mg/kg DCE, a selective necrosis of the non-ciliated bronchiolar cells (Clara cells) was observed. Increasing the dose of DCE to 200 mg/kg resulted, however, in damage to other pulmonary cell types.

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In addition to DCE, several other chemicals such as 4-ipomeanol (7), bromobenzene (8), carbon tetrachloride (9) and naphthalene (10) have been reported to preferentially damage Clara cells. Since these compounds require cytochrome P-450-dependent metabolic activation in order to become toxic, these observations suggest that Clara cells contain a relatively high concentration of cytochrome P-450. By selective destruction of these cells one might expect that the overall activity of the microsomal cytochrome P-450 system of the lung would be dramatically diminished.

In the present preliminary study in the mouse, morphological changes 24 hr after i.p. injection of DCE are compared with changes in microsomal cytochrome P-450 and P-450-dependent enzyme activities in lung, liver and kidney. The results demonstrate an organ-specific effect of DCE on pulmonary cytochrome P-450-dependent monooxygenase activity, concomitant with selective damage to the Clara cells.

MATERIALS AND METHODS: Male C57BL/6J mice (The Jackson Laboratory, Bar Harbor, Maine) (20-28 g), given free access to water and laboratory chow (Zeigler Bros, Inc., Gardner, PA), were injected intraperitoneally with 1,1-dichloroethylene (99% purity, Aldrich Chemical Co., Milwaukee, WI) dissolved in olive oil, 125 mg/kg body weight. Control animals received olive oil only (5 ml/kg). Twenty four hours after administration, mice were killed by cervical dislocation, and microsomes were isolated by differential centrifugation in 150 mM KCl -50mM Tris-HCl buffer, pH 7.4 (11) (35-40 lungs, 5 livers, and kidneys of 12-16 mice were pooled for each microsomal sample). Protein concentration was estimated by the method of Lowry *et al* (12), with bovine serum albumin as standard.

Enzyme assays: Cytochromes P-450 and b_5 were determined by the methods of Omura and Sato (13,14). NADPH-cytochrome c reductase activity was measured as described by Williams and Kamin (15). 7-Ethoxyresorufin O-deethylase was estimated according to the method of Burke and Mayer (16), as previously described by Tong *et al* (17). Benzphetamine N-demethylase activity was estimated by measuring the production of formaldehyde according to the method of Nash (18). Benzo(a)pyrene hydroxylase was estimated according to the fluorimetric method of Nebert and Gelboin (19). Because of the low renal hydroxylase activity, the concentration of the components of the incubation mixture were doubled and the incubation time was extended to 60 minutes for kidney microsomes.

Morphology: Lung, liver and kidney tissues were examined histologically. Mice (5 controls and 5 DCE-treated) were killed 24 hr after treatment by cervical dislocation. Lungs were infused in situ with Karnovsky's fixative (20) following tracheal cannulation; the organs of interest were removed and immersed in fixative. Sections of the fixed tissues were stained with hematoxylin and eosin, and evaluated double-blind by light microscopy.

RESULTS AND DISCUSSION: Morphology: Histological sections of lung, liver and kidney from control mice were normal, indicating that the olive oil used as the vehicle for DCE did not cause significant morphologic alterations. In contrast, all lung specimens from mice receiving DCE were characterized by a highly specific and nearly complete loss of Clara cells from the linings of the bronchioles (Figures 1 and 2). This effect was extremely uniform, and encompassed the entire bronchiolar tree from the trachea to the alveolus and involved every segment observed. Characteristically, in tissues from DCE-treated mice the nuclei of Clara cells were pyknotic and extremely dark-stained, whereas the cytoplasm of such cells was only faintly eosinophilic. Rarely were Clara cells still attached to bronchiolar walls; more typically, they were detached, accumulating as luminal debris. Ciliated bronchiolar epithelial cells were not morphologically affected by the DCE.

In the liver sections examined from DCE-treated mice there were occasional vacuolated hepatocytes, but no evidence of irreversible injury, nor were there any histologically apparent changes in kidney sections from mice receiving DCE.

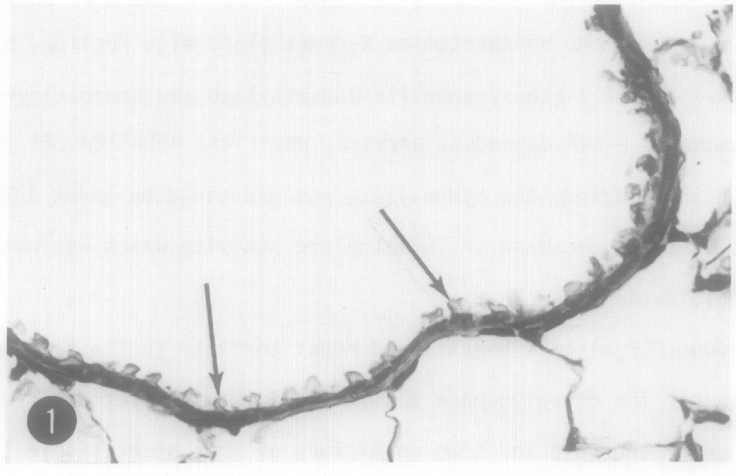

Fig. 1 Photomicrograph of bronchiole (paraffin embedment, H & E stain) from control mouse. Clara cells (arrows) are abundant and appear normal. (Magnification 409.6x)

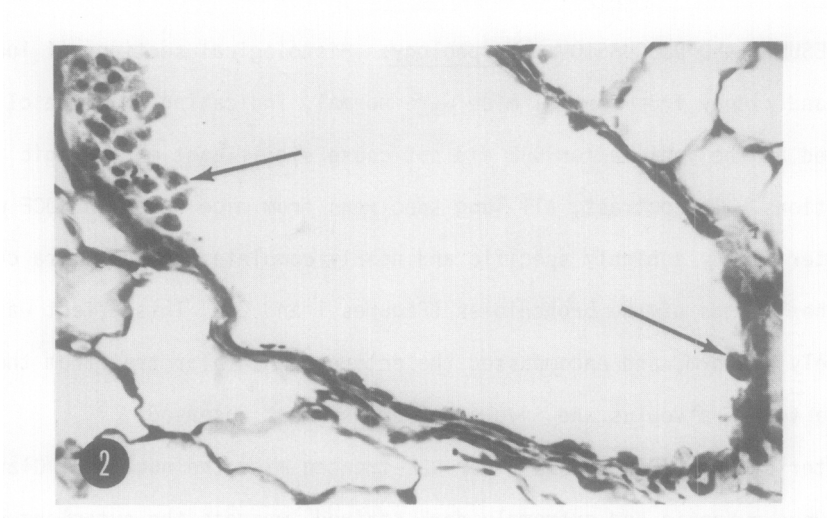

Fig. 2 Photomicrograph of bronchiole from mouse treated with DCE (125 mg/kg, ip) 24 hrs prior to sacrifice. Clara cells (arrows) uniformly have pyknotic, heavily stained nuclei, and appear to be completely separated from the walls of the bronchioles, forming clumps of cellular debris in the lumen. (Magnification 409.6x)

Microsomal monooxygenase activities: Twenty-four hours after the intraperitoneal administration of DCE to mice (125/kg, i.p.), a significant decrease was observed in both the pulmonary microsomal cytochrome P-450 content and the related enzyme activities. By contrast, these activities were essentially unaffected in liver and kidney (Table 1).

The concentrations of cytochromes P-450 and b_5 in lung microsomes were reduced to approximately 50 and 60% of control, respectively. NADPH cytochrome c reductase and benzphetamine N-demethylase also declined by about 50%. Activities of 7-Ethoxyresorufin O-deethylase and benzo(a)pyrene hydroxylase, cytochrome P-448-dependent enzymes, were less affected, at least at the time point studied; the hydroxylase was decreased by about 30%, while there was a slight decrease in O-deethylase activity which was not statistically significant.

Although DCE elicits hepatic and renal toxicity in the rat, neither the morphology nor the monooxygenase activities in mouse liver and kidney were affected under the dose and time conditions of this study (Table 1). In the recent study by Forkert and Reynolds (6), also using mice, hepatic damage apparently occurred as indicated by an elevation in SGOT and SGPT levels

TABLE 1: Effects of 1,1-dichloroethylene (125 mg/kg, ip) on microsomal enzyme activities in lung, liver and kidney of mice 24 hours after treatment

	LUNG		LIVER		KIDNEY	
Parameter	control	DCE	control	DCE	control	DCE
Cytochrome P-450[a]	0.13 ± 0.01	0.06 ± 0.003*	0.74 ± 0.03	0.75 ± 0.05	0.20 ± 0.02	0.20 ± 0.02
Cytochrome b_5[a]	0.11 ± 0.004	0.07 ± 0.004*	0.43 ± 0.01	0.35 ± 0.02*	0.26 ± 0.01	0.24 ± 0.01
NADPH-cytochrome c reductase[b]	305 ± 21	134 ± 10*	287 ± 19	284 ± 24	153 ± 8	144 ± 6
Benzphetamine N-demethylase[b]	2.41 ± 0.28	1.30 ± 0.20*	7.33 ± 0.30	7.05 ± 0.47	1.02 ± 0.14	1.30 ± 0.09
7-Ethoxyresorufin O-deethylase[c]	26 ± 5	19 ± 2	412 ± 41	415 ± 22	1.6 ± 0.3	8.9 ± 0.6*
Benzo(a)pyrene hydroxylase[c,d]	3.6 ± 0.1	2.4 ± 0.2*	59.7 ± 4.0	63.4 ± 7.1	27.2 ± 5.0	28.0 ± 3.5

[a] nmoles/mg protein

[b] nmoles/mg protein/min

[c] pmoles/mg protein/min

[d] for kidney microsomes activity is expressed as pmoles/mg protein/hour

* Statistically different from control, $p < 0.01$

Data are given as mean ± S.E.M.; n = 6-12

(5- and 19- fold, respectively), measured 24 hr after oral administration of DCE (100 mg/kg). Unfortunately little else is known about acute toxic effects of DCE in mice. The organ-specific effect of DCE in the present study may be attributed to the pharmacokinetic behavior of the agent and the resulting tissue exposure, since the lung is the major organ of unmetabolized DCE excretion, and a significant part of the administered dose is excreted by this route (4,5).

Interestingly, DCE caused a clear and significant rise in the P-448 dependent 7-ethoxyresorufin O-deethylase activity in kidney microsomes, an effect which was very reproducible. We have no explanation for this finding at present, since no changes were observed either in renal cytochrome P-450 content or in benzo(a)pyrene hydroxylase, also a cytochrome P-448-linked enzyme. Moreover, 7-ethoxyresorufin O-deethylase activites were unchanged in either liver or lung microsomes following DCE treatment.

The specific pulmonary toxic effects of DCE strikingly resemble the effects of naphthalene, which decreased microsomal cytochrome P-450 content and related monooxygenase activities in mouse lung, concomitant with selective Clara cell damage and without any morphological or biochemical indication of damage to the liver (17). Thus, the present results for DCE, like those obtained for naphthalene (17), 4-ipomeanol (7), carbon tetrachloride (9) and bromobenzene (8) strongly suggest that pulmonary toxins, which must be metabolically activated to reactive intermediates to cause tissue damage, may be activated by enzymes localized within the Clara cells.

Abundant work has shown cytochrome P-450 and its related monooxygenase activities to be highly concentrated in Clara cells relative to the 40 other cell types in lung. In studies employing isolated, purified (70-80% pure) lung cell types, the specific activity of several monooxygenases in Clara cells was 3-40 times higher than in type II cells (21). We are thus left with the provocative question: if monooxygenase activity is concentrated in Clara cells which are almost totally destroyed by DCE treatment (Figures 1 and 2), why is the total lung monooxygenase activity reduced by only 50-60%

(Table 1) and not > 95%? The answer may lie in the relative numbers of Clara cells and alveolar type II cells in mouse lung. If type II cells are many times more abundant than Clara cells, then the residual enzyme activity in lung after destruction of Clara cells by DCE may be attributable to that residing in the undamaged type II cells. The answer to this question is under investigation.

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