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Proximal Tubule Brush Border Angiotensin Converting Enzyme Behaviour and Nephrotoxicity Due to 1,2-Dichloropropane

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Introduction

1,2-Dichloropropane (1,2-D) is an aliphatic halogenated hydrocarbon largely used as a domestic stain remover, as a paint solvent, and as a decreaser in industry. The acute toxicity of 1,2-D has recently been reported in man (Pozzi et al. 1985) and is characterized by liver damage, renal failure, hemolytic anemia, and disseminated intravascular coagulation. Immunofluorescence and electron microscopy have shown that renal failure is due to acute tubular necrosis without alterations of the glomeruli.

Low doses (50 ppm) of 1,2-D in rats and mice induce slight signs of chronic toxicity (Parker et al. 1982); there is no renal impairment. The present study reports the preliminary results in respect of renal changes induced in male rats after repeated treatment with 1,2-D.

Methods

Fifty albino, male, outbred Wistar rats (Morini, S. Polo d'Enza, Italy), starting weight 200 $\pm$ 10 g, were used. Groups of five rats each were caged and allowed to eat and drink ad libitum. The treatment scheme was as follows: 1,2-D (Merck, Darmstadt, FRG, purity 97%) dissolved in corn oil was given i.p. for 5 days (once a day) or for 4 weeks (once a day, 5 days/week). Dose levels (ten animals per group) were 0, 50, 100, 250, and 500 mg/kg body wt. Twenty-four hours after the last treatment, overnight fasted rats were killed by decapitation; the kidneys were removed and weighed. From one kidney the renal proximal tubule brush border was prepared according to Booth and Kenny (1974); the ACE activity and proteins were measured according to Summary (1976) and to Miller (1959) respectively. Concurrently, the other kidney was subdivided into three

parts for histological examination. One specimen was frozen in liquid nitrogen and kept at −80°C for immunofluorescence (the slices were treated with fluorescein-conjugated rabbit-anti-rat immunoglobulins fluorescinate obtained from Dakopatts, Glastrup, Denmark. The second specimen was fixed in 10% buffered formaldehyde and prepared for light microscopy. The third specimen was fixed in 2% buffered glutaraldehyde for electron microscopy.

Statistical evaluation was by means of variance analysis.

Results and Discussion

Kidney/body weight ratio was not significantly different among the groups. Proximal tubule brush border angiotensin converting enzyme (ACE) activity varied according to the treatment (Table 1). 1,2-D, 250 mg/kg for 5 days, caused a significant increase ($P < 0.005$) in ACE activity, while 500 mg/kg for 5 days caused a significant decrease ($P < 0.01$). When the treatment was prolonged over a 4-week period, a dose-dependent decrease in the enzyme activity was observed. The histological examination supported the biochemical results. 1,2-D given for 5 days produced an enlargement of the microvilli of the brush border with destruction and fraying at higher doses. 1,2-D given for 4 weeks caused a dose-dependent fraying of the microvilli, with epithelial coagulative necrosis of the brush border at the higher doses. It is hypothesized that 1,2-D causes a functional alteration of the brush border which is followed by anatomical destruction and a consequent loss of the enzyme activity. The glomerular changes are clear and more pronounced than those of the tubule. They are characterized by mesangial proliferative glomerulonephritis (Fig. 1), with mesangial and subepithelial granular deposition as shown by immunofluorescence. These alterations are also present at the low dose (50 mg/kg), but the severity of the change increases with the dose.

Sprecace (1963) first suggested an association between glomerulonephritis and hydrocarbon exposure, reporting two cases of Goodpasture's syndrome after exposure to gasoline vapors. Twelve cases of Goodpasture's syndrome related to hydrocarbon exposure have been reported so far. In addition, several cases of glomerulonephritis have also been reported. Recently Bell et al. (1985) suggested that exposure to organic solvents is related to both mesangial and en-

Table. 1. ACE activity of the proximal tubule brush border

Dose (mg/kg)	ACE activity (V/mg prot.)	
	5 days	4 weeks
0	4.3 ± 0.2	4.6 ± 0.2
50	4.0 ± 0.3	4.1 ± 0.3
100	4.1 ± 0.4	3.8 ± 0.5*
250	5.3 ± 0.6*	2.9 ± 0.6*
500	3.6 ± 0.4*	2.7 ± 0.2*

* $P < 0.025$ between group indicated and controls

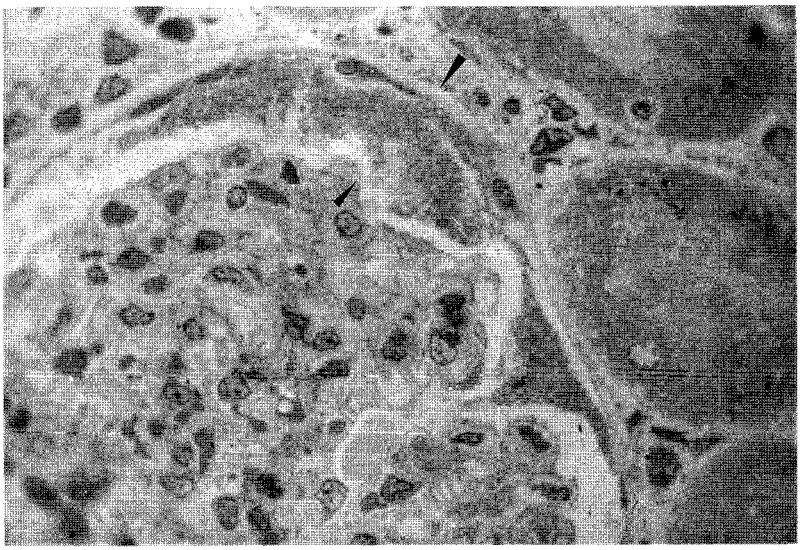

Fig. 1. Subepithelial deposits with epithelial and mesangial hyperplasia after treatment with 1,2-D for 5 days. Semithin preparation; toluidine blue, × 100. Arrows show the deposits

docapillary proliferative glomerulonephritis, the latter being more frequent in patients who were apparently more exposed.

In conclusion, 1,2-D causes kidney alterations in experimental animals after administration of sublethal doses. At lower doses glomeruli seem to be the only target, as shown by the mesangial proliferative glomerulonephritis. Tubular changes are evident only at doses of 250 mg/kg for 5 days and higher. These changes consist in a significant enlargement of the brush border and an increase in ACE activity, followed by functional and anatomical impairment. This impairment is more evident after prolonged treatment.

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