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REDUCTION IN THE RATE OF ETHANOL ELIMINATION IN VIVO BY DESFERRIOXAMINE AND DIETHYLENETRIAMINEPENTAACETIC ACID : SUGGESTION FOR INVOLVEMENT OF HYDROXYL RADICALS IN ETHANOL OXIDATION.

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Recent studies have suggested a possible role for hydroxyl radicals ($OH^{\cdot}$) in the microsomal production of acetaldehyde from ethanol (1-4). The generation of $OH^{\cdot}$ is known to require superoxide ($O_2^{\cdot-}$) and H_2O_2 and to involve ferric ions as catalyst (5). Some iron-chelators like desferrioxamine (DFO) and diethylenetriaminepentaacetic acid (detapac) have been reported to inhibit $OH^{\cdot}$ formation in vitro by chelating the catalytic iron (5-6). Consequently, if $OH^{\cdot}$ dependent systems play a significant role in the elimination of ethanol, one would expect these agents to interfere with ethanol metabolism. It has in fact been reported that the microsomal oxidation of ethanol in vitro can be divided into two components, a DFO sensitive and a DFO insensitive (7). The microsomal oxidation of ethanol has also been reported to be slightly inhibited by detapac (8). What remains unclear is the importance of the $OH^{\cdot}$ dependent process of ethanol oxidation in the overall elimination of ethanol in vivo. To ascertain such a role for $OH^{\cdot}$, we studied the effect of DFO and detapac on ethanol elimination in the rat.

Male Sprague-Dawley rats (180-200 g) were injected intraperitoneally with an ethanol dose of 2.3 g/kg body weight (as a 25 % solution in saline, v/v). The concentration of ethanol was measured in rat breath at hourly intervals according to a method previously described (9). Subsequently the value of blood ethanol was derived from the linear correlation ($r = 0.96$) established between breath ethanol values and blood ethanol concentrations measured by the head space technique (10). It was further established that neither DFO nor detapac treatment had any effect on this correlation and that the initial blood ethanol levels were equal in the control and experimental groups (results not shown). Liver alcohol dehydrogenase (ADH) was assayed according to (11), catalase activity was determined in liver supernatant and pellet obtained at 12.000 g as previously described (12) and protein according to (13). DFO and detapac were administred at doses which have been effective in blocking iron-dependent cytotoxic processess (14,15).

DFO (100 mg/kg body wt, i.p.), administered to rats 1 hr prior to the ethanol injection as well at 1,3 and 5 hr after this ethanol load, reduced the rate of ethanol elimination by 19 %. Detapac (250 mg/kg body wt, i.p.), administered once 1 hr prior to ethanol, reduced this rate by 16 % (Table 1).

Table 1

Influence of desferrioxamine or detapac on ethanol elimination in rats *in vivo*

Treatment	Elimination rate (mmoles/kg body wt/hr)
Saline	7.58 ± 0.32
Desferrioxamine	6.11 ± 0.70*
Detapac	6.34 ± 0.18*

All animals received ethanol, 2.3 g/kg body wt, i.p., plus saline, desferrioxamine or detapac as described in the text. The reported values are means ± S E M, with at least seven animals in each group.
* $p < 0.01$ versus saline.

Neither of these two iron-chelators when added *in vitro* (1 mM) had any effect on ADH and catalase activities. DFO administration *in vivo* did not modify these enzyme activities (Table 2). The same results were obtained with animals treated with detapac (250 mg/kg body wt, i.p.) (data not shown).

Table 2

Effect of desferrioxamine administration on liver ADH and catalase activities*

Treatment	ADH activity Ethanol oxidized (μmol/min/g liver wet wt)	Catalase activity U/mg protein	
		Supernatant	Pellet
Saline	2.67 ± 0.39	0.194 ± 0.020	0.200 ± 0.015
Desferrioxamine	2.75 ± 0.20	0.194 ± 0.028	0.202 ± 0.043

*Desferrioxamine (100 mg/kg body wt, i.p.) was injected twice at 2hr interval. The enzyme activities were determined 1 hr after the second injection. The reported values are means ± S E M, with at least four animals in each group.

These data show that the reduction of ethanol elimination by either desferrioxamine or detapac is not due to a direct effect of these two substances on either ADH or catalase. As it has already been shown that cytochrome P450 is also not affected by DFO (4), one can suggest that the reduction in the ethanol elimination rate following the administration of iron-chelators includes the intervention of non-heme iron. So far, ethanol oxidation via $OH^{\cdot}$ radicals is the only mechanism proposed to be iron-dependent and consequently inhibited by iron-chelators. Such a mechanism has in fact been detected and subsequently studied in isolated liver microsomal preparation (1-4, 8). However this microsomal system has been reported to be only partly sensitive to DFO *in vitro* (4,7). Furthermore,

microsomes are known to play only a minor role in the overall ethanol oxidation under the experimental conditions presently used, namely the administration of a single moderate dose of ethanol to naive rats. It can therefore be suggested that the role of OH· radicals in ethanol metabolism is not restricted to liver microsomes, but could be extended to extra-microsomal compartments and eventually to extra-hepatic tissues.

In conclusion, this report suggests that ethanol oxidation via OH· represents a non negligible pathway in the overall ethanol elimination by rats. Considering the present finding, a reappraisal of the metabolic pathway involved in ethanol oxidation after acute and chronic alcohol administration becomes of major interest. This applies especially to the involvement of ADH in ethanol oxidation since conventional studies on this point rely mostly on the utilization of ADH inhibitors (pyrazole and its derivatives) which have been found to be themselves potent scavengers of OH· radicals (16).

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References

(1) A.I. Cederbaum, E. Dicker and G. Cohen, Biochem. 17, 3058 (1978).

(2) A.I. Cederbaum, E. Dicker and G. Cohen in Advances in Experimental Medicine and Biology (Ed R.G. Thurman) 132, p.1, Plenum Press, New York (1980).

(3) G. Cohen and A.I. Cederbaum, Arch. Biochem. Biophys. 199, 438 (1980).

(4) A.I. Cederbaum and E. Dicker, Biochem. J., 210, 107 (1983).

(5) B. Halliwell, FEBS Lett. 92, 321 (1978).

(6) J.M.C. Gutteridge, R. Richmond and B. Halliwell, Biochem. J. 184, 469 (1979).

(7) G.W. Winston and A.I. Cederbaum, J. Biol. Chem. 258, 1514 (1983).

(8) A.I. Cederbaum and G. Cohen, Arch. Biochem. Biophys. 204, 397 (1980).

(9) S.J. Grieve and J.M. Littleton, J. Pharm. Pharmacol. 31, 605 (1979).

(10) G.D. Wendell and R.G. Thurman, Biochem. Pharmacol. 28, 273 (1979).

(11) K.E. Crow, N.W. Cornell and R.L. Veech in Alcohol and Aldehyde Metabolizing Systems (Eds R.G. Thurman, J.R. Williamson, H.R. Drott and B. Chance) 3, p. 335, Academic Press, New York (1977).

(12) C. Ribière, J. Sinaceur, J. Nordmann and R. Nordmann, Pharmac. Biochem. Behav. (in press).

(13) O.H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem. 193, 265 (1951).

(14) M.D. Maines, Int. J. Biochem. 12, 781 (1980).

(15) R.E. Heikkila and F.S. Cabbat, Experientia 38, 378 (1982).

(16) A.I. Cederbaum and L. Berl. Arch. Biochem. Biophys. 216, 530 (1982).