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Delayed Ethanol Elimination from Rat Blood after Treatment with Thiram, Tetramethylthiuram Monosulfide, Ziram, or Cyanamide

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Therapy with the alcohol deterrent disulfiram (tetraethylthiuram disulfide, TETD) caused a delayed ethanol elimination from the blood of human subjects (Peachey et al. 1981). The aim of the present study was to evaluate in an experiment with rats to which extent other dithiocarbamate-derivatives retard the elimination of ethanol. To this end the following compounds were tested: tetramethylthiuram disulfide (thiram, TMTD), tetramethylthiuram monosulfide (TMTM), bis(dimethyldithiocarbamato) zinc (ziram), (ethylene bis(dithiocarbamato)) zinc (zineb) and cyanamide (metabolized in the human organism to compounds of the dithiocarbamate type (Hauschild 1953)). Calcium cyanamide is used as fertilizer, all the other substances are used as fungicides or rubber accelerators. Human contact especially occurs at the workplace.

MATERIALS AND METHODS

Adult female SPF Wistar rats, weighing 200 - 220 g, were obtained from the Central Breeding Station of the University of Heidelberg / FRG. The animals were housed in Macrolon cages (53 x 32 x 19 cm, 6 rats per cage) under standardized conditions (temperature 22°C, relative humidity approx. 50%; 12-hour neon lighting for simulation of light-dark cycles; standard diet from Altromin, Lage / FRG, and tap water ad libitum).
The following chemicals were used: TMTM (ICN Pharmaceuticals Inc., Life Science Group, Plainview, N.Y. / USA), TMTD, ziram, cyanamide and methyl cellulose (Fluka, Neu-Ulm / FRG), zineb (Roehm and Haas Comp., Philadelphia / USA), ethanol (Merck, Darmstadt / FRG), Chromosorb WAW DMCS 80/100 mesh and Flexol 8N8 (WGA, Griesheim / FRG).
16 µmol/kg b.w. of TMTD, TMTM, ziram, zineb, or cyanamide or 256 µmol /kg b.w. of zineb or cyanamide were given in 0.75 % (w/v) aqueous methyl cellulose suspension (10 ml/kg b.w.) by gavage; the pretreatment interval prior to i.p. administration of ethanol (2 g/kg b.w. in 5 ml physiol. saline/kg b.w.) was 90 min. The animals of the control group received 10 ml/kg b.w. of 0.75% (w/v) aqueous methyl cellulose suspension by gavage.
Blood (0.02 ml) was collected repeatedly within 4 hours after ethanol dosing from the retro-orbital plexus using heparinized disposable pipettes.

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The blood ethanol content was determined by gas chromatography (Sigma 1 with HS-6 head space sampler, Perkin-Elmer, Ueberlingen / FRG) using a modified standard method (Machata 1967). The following analytical conditions were observed: glass column 180 cm by 2 mm (i.d.) packed with Chromosorb WAW DMCS on 80/100 mesh and 10 % Flexol 8N8. Operating temperatures were: injector port, 115°C; oven, 50°C; FID-detector, 125°C; head space HS-6, 70°C. Equilibration time: 10 min; pressure build-up time: 1 min. Preparation of samples: The pipettes containing the blood samples were transferred into autosampler vials containing 0.5 ml aqueous t-butanol (0.1 %, v/v) as an internal standard. The vials were closed with butyl rubber septa, shaken vigorously, and then placed into the HS-6 head space sampler. Calibration curves were determined in blood for prevention and compensation of matrix effects. The coefficients of variation for the 6 control analyses included in the daily series were found to be maximally 1 %.

The pharmacokinetic data analysis was carried out by computer programs using a one-compartment open model, which takes into account the peculiarities of ethanol kinetics (Wilkinson 1980). First order kinetics is assumed for the invasion phase (i.p. administration of ethanol):

$$\frac{dc}{dt} = k_1 * C_0 * \exp(- k_1 * t) \qquad (1)$$

The time-dependent ethanol concentration in the enzymatically controlled elimination phase is described by the Michaelis-Menten equation ((2) or (3)-intregated form):

$$\frac{dc}{dt} = - \frac{Vm * C}{Km + C} \qquad (2)$$

$$C_0 - C + Km * \ln (C_0 / C) = Vm * t \qquad (3)$$

The complete time courses of ethanol blood concentrations (figure 1) was calculated from the differential equation (4) (a combination of the equations (1) and (2)) by numeric integration:

$$\frac{dc}{dt} = k_1 * C_0 * \exp(- k_1 * t) - \frac{Vm * C}{Km + C} \qquad (4)$$

dc/dt	instanteneous rate of change of concentration with time
C	concentration at time t
C_0	theoretical initial concentration (t = 0)
k_1	invasion rate constant
Vm	maximal velocity (slope of the apparent linear decline of ethanol concentration with time, equal to ß, the Widmark constant)
Km	Michaelis Menten constant

Table 1. Blood ethanol concentration (g . l^{-1}, mean ± SEM, number of animals in parenthesis) 4 h after ethanol administration (2 g/kg b.w. i.p.). The rats were pretreated with dithiocarbamate-derivatives or cyanamide by gavage 90 min before ethanol dosing .

Dose	TMTD	TMTM	Ziram	Zineb	Cyanamide	Control
						0.72 ± 0.03 (41)
16 µmol/kg	1.22 ± 0.08 * (16)	1.23 ± 0.07 * (4)	1.19 ± 0.12 * (7)	0.80 ± 0.06 (4)	1.00 ± 0.05 (4)	
256 µmol/kg	n.d.	n.d.	n.d.	0.69 ± 0.03 (4)	1.38 ± 0.08 * (4)	

* $p < 0.01$ (comparison with the control, Dunnett's test) n.d. = not determined

Table 2. Pharmacokinetic parameters of figure 1 according to equation (4).

	C_0 g . l^{-1}	k_1 h^{-1}	Km g . l^{-1}	Vm g . l^{-1} . h^{-1}	AUC g . l^{-1} . h	number of observations (animals)
control	2.73	14.4	0.0155	0.53	6.8	207 (41)
TMTD 16 µmol/kg	3.01	14.5	0.0122	0.47	9.3	73 (16)

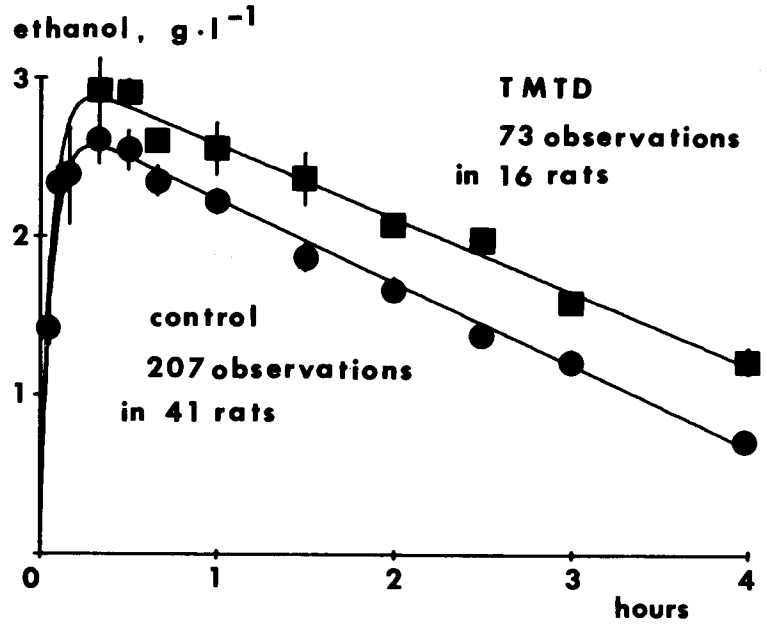

Figure 1. Time courses of blood ethanol concentration after i.p. administration of ethanol (2 g/kg b.w. in 5 ml physiol. saline/kg b.w.).
Upper curve: after pretreatment with TMTD (16 μmol/kg b.w., given in 0.75 % (w/v) aqueous methyl cellulose suspension (10 ml/kg b.w.) by gavage) 90 min before ethanol;
Lower curve: control (the rats received 10 ml/kg b.w. 0.75 % aqueous methyl cellulose suspension by gavage 90 min before ethanol).

The parameters in Table 2 were determined as followed: The invasion rate constant k_1 was calculated by semi-logarithmic regression from the measured points of the initial invasion phase. The parameters C_0, Vm and Km were calculated by a variation method. Preliminary estimated values of C_0, Vm and Km were improved by variation of C_0, Vm and Km and by minimizing the sum of square errors of the data set from the elimination phase according to equation (3). The ethanol concentration-time curves and the areas under the curves (AUCs) were determined by numeric integration of equation (4) according to the extended trapezoidal rule (Davis and Polonsky 1970).
Dunnett's test (Tallarida and Murray 1981) was used to determine any significant difference of treated groups in comparison with the control group in Table 1. Changes were regarded as significant, if p-values were less than 0.01.

RESULTS AND DISCUSSION

Four hours after administration of ethanol the blood ethanol concentration was markedly increased over the controls in rats which were pretreated (p.o.) with 16 μmol/kg TMTD, TMTM or ziram 90 minutes before ethanol dosing (2 g/kg i.p.) (Table 1).

A statistically significant increase ($p<0.01$) of blood ethanol concentration after cyanamide pretreatment was found after a dose of 256 µmol/kg (Table 1). Zineb had no influence on the blood ethanol level up to a dose of 256 µmol/kg. This may possibly result from the low absorption rate of zineb from the intestinal tract.

The above findings were further characterized by measuring the time course of ethanol concentration in the blood of rats which had been pretreated with TMTD (16 µmol/kg, p.o.). The control animals had just received the aqueous methyl cellulose suspension (Figure 1, Table 2). The TMTD dosed animals displayed higher blood ethanol concentrations during the observation period of four hours. The theoretical initial concentration C_0 was also higher in the TMTD pretreated animals. This results indicates a smaller apparent volume of distribution (Vd) of ethanol after oral application of TMTD ($Vd = D / C_0$, with D = dose of ethanol). The invasion rate constant k_1 with or without TMTD were nearly identical. The parameters of the elimination phase (Km and Vm) were both lower after application of TMTD, resulting in a retarded ethanol elimination. (However, the Km-values could have been determined more exactly by measuring the late phase of the elimination beyond four hours. This was not feasible in the present case). By comparison of the areas under the curves (AUCs) it can be demonstrated that the body burden of ethanol (a parameter for risk assessment) of the TMTD-treated rats is over 35 % greater than in the control rats. The delayed elimination of ethanol caused by TMTD confirms the earlier finding (Freundt and Netz 1977) of a flatter and higher linear branch of the elimination curve of ethanol after pretreatment with this dithiocarbamate-derivative.

The delay of the ethanol elimination may be caused by an inhibition by the tested compounds of the alcohol dehydrogenase (ADH) and / or the aldehyde dehydrogenase (AlDH) (Garcia de Torres et al. 1983) linked with the ethanol metabolism by mass action of the accumulating acetaldehyde.

While recognizing that results from animal experiments cannot be transposed without restriction to the human situation it is concluded that after intake of TMTD, TMTM or ziram (considering into account the fast accumulation after repeated uptake of these substances) and simultaneously consumption of alcohol higher blood level of ethanol have to be taken into account which may bear clinical or forensic significance. The delayed ethanol elimination effected by high doses of cyanamide confirms an earlier finding in human subjects of retarded ethanol elimination under influence of the therapeutical alcohol deterrent calcium cyanamide (Peachey et al. 1981).

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