EFFECT OF METHYLURACIL ON OXIDATIVE PHOSPHORYLATION IN THE HEPATIC MITOCHONDRIA OF RATS POISONED WITH DICHLOROETHANE

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The effect of methyluracil (methacil) on oxidative phosphorylation in the hepatic mitochondria was studied in experiments on male rats 3 and 6 days after poisoning with dichloroethane (0.5 ml/kg) by the intragastric route. Besides restoring normal alanine-aminotransferase activity in the blood serum and improving the antitoxic function of the liver, methyluracil also restored oxidative phosphorylation in the liver mitochondria to normal. This action of methyluracil was seen particularly clearly 3 days after poisoning. The positive therapeutic action of methyluracil in toxic lesions of the liver can perhaps be explained by its influence on ATP formation.

Methyluracil (methacil) has a positive therapeutic action in experimental toxic hepatitis [1, 2, 5, 6]. One aspect of the toxic action of chlorinated hydrocarbons is the damage caused to the mitochondria of the hepatocytes [3, 7, 10].

Since the positive therapeutic effect of methyluracil in dichloroethane poisoning may be connected with an improvement in the energy metabolism of the hepatocytes, the investigation described below was carried out to study the effect of this compound on respiration and oxidative phosphorylation in the mitochondria of the rat liver.

EXPERIMENTAL METHOD

Experiments were carried out on 72 male albino rats weighing 200-250 g. Acute poisoning of the rats was produced by administration of a single dose of 20% dichloroethane solution in sunflower oil (0.5 ml/kg body weight) by the intragastric route. The methyluracil was injected through a tube into the stomach (200 mg/kg) and treatment began 24 h after poisoning and continued until the end of the experiments.

Three groups of animals were used: 1) intact rats, 2) rats poisoned with dichloroethane, and 3) rats poisoned with dichloroethane and treated with methyluracil. Some of the rats of each group were decapitated simultaneously 3 and 6 days after administration of dichloroethane and the respiration and phosphorylation were determined in the liver mitochondria by the method described previously [8]. Alanine-aminotransferase activity was determined in blood serum obtained immediately after decapitation of the rats [4]. In the other animals of groups 2 and 3, the antitoxic function of the liver was determined 2 days before and 3 and 6 days after poisoning by the Quick–Pytel' test in the writers' modification [5].

EXPERIMENTAL RESULTS AND DISCUSSION

Alanine-aminotransferase activity in the intact rats was 0.86 ± 0.09 μmole pyruvic acid formed in 1 ml serum during incubation for 1 h. In the course of 4 h the intact animals excreted 64.7 ± 3.4 mg hippuric acid (100%) in the urine.

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TABLE 1. Effect of Methyluracil on Oxidative Phosphorylation in Liver Mitochondria of Rats Poisoned with Dichloroethane ($M \pm m$)

Time after poisoning (in days)	Group of animals	Number of rats	Number of μg-atoms O_2/mg protein	Number of μg-atoms P/mg protein	P/O
—	Intact rats	8	2,62±0,13	4,68±0,25	1,79±0,10
3	Rats poisoned with dichloroethane	8	2,16±0,34	1,68±0,14	0,78±0,09
	Rats poisoned with dichloroethane and treated with methyluracil	10	2,23±0,17 $P^* > 0,1$	2,47±0,18 $P < 0,01$	1,10±0,12 $P < 0,01$
6	Rats poisoned with dichloroethane	8	1,96±0,14	1,87±0,16	0,95±0,07
	Rats poisoned with dichloroethane and treated with methyluracil	8	1,86±0,12 $P > 0,1$	2,03±0,13 $P > 0,1$	1,08±0,09 $P > 0,1$

Note: P* for animals receiving methyluracil was calculated by comparison with rats not receiving methyluracil

Administration of a single dose of dichloroethane to the rats was followed on the 3rd day by a marked increase in alanine-aminotransferase activity (4.3 ± 0.26 μmoles pyruvate) and disturbance of the antitoxic function of the liver: the excretion of hippuric acid was $24.6 \pm 3.1\%$ of its initial level. These findings reflected the development of toxic hepatitis, the clinical severity of which is measured by these tests [9, 11]. On the 6th day after administration of dichloroethane the analine-aminotransferase activity was reduced (1.58 ± 0.18 μmole pyruvate) and the excretion of hippuric acid in the urine was increased ($53.5 \pm 4.6\%$).

Administration of methyluracil to the poisoned rats led to more normal values of the alanine-aminotransferase in the blood serum: 2.8 ± 0.27 and 0.93 ± 0.10 μmole pyruvate on the 3rd and 6th days, respectively. The antitoxic function of the liver was considerably improved: on the 3rd day after poisoning these rats excreted $42.4 \pm 2.8\%$, and on the 6th day $81.3 \pm 5.3\%$ of the original quantity of hippuric acid.

Methyluracil had a positive effect on the survival of the rats after poisoning: of the 36 poisoned rats not receiving methyluracil 12 (33%) died; of the 28 rats treated with methyluracil only 2 (7%) died. Most animals died during the first 4 days after poisoning.

Data on respiration and phosphorylation in the liver mitochondria of the three groups of rats are given in Table 1. Acute dichloroethane poisoning caused a marked disturbance of oxidative phosphorylation in the liver mitochondria. No statistically significant changes were found in the utilization of oxygen by the mitochondria although it showed a tendency to decrease. The phosphorylation coefficient reached its lowest value on the 3rd day.

Administration of methyluracil to the poisoned rats partly abolished these disturbances. Methyluracil had a particularly marked beneficial effect on oxidative phosphorylation 3 days after poisoning. No significant difference in the course of these processes could be seen in the rats receiving and not receiving methyluracil on the 6th day after administration of dichloroethane.

Methyluracil thus restores normal serum alanine-aminotransferase activity, improves the antitoxic function of the liver, and restores normal processes of oxidative phosphorylation in the liver mitochondria in acute dichloroethane poisoning. The positive therapeutic action of methyluracil in toxic lesions of the liver can possibly be explained by its effect on ATP formation.

LITERATURE CITED

1. E. Ya. Arzyaeva, Gig. Truda, No. 2, 33 (1966).
2. I. F. Grekh and M. V. Natsyuk, in: Proceedings of a Conference on the Medical Use of Pyrimidine Derivatives [in Russian], Rostov-on-Don (1961), p. 152.
3. V. V. Lyakhovich, A. V. Dolgov, V. M. Mishin, et al., Farmakol. i Toksikol., No. 2, 243 (1972).
4. V. V. Men'shikov, in: Unified Methods of Clinical Laboratory Investigation [in Russian], No. 1, Moscow (1970), p. 15.
5. M. V. Natsyuk, Data on the Experimental Treatment of Toxic and Traumatic Lesions of the Liver, Author's Abstract of Candidate's Dissertation [in Russian], Kiev (1968).

6. M. V. Natsyuk and F. S. Chernukha, in: Pharmacology and Toxicology [in Russian], No. 7, Kiev (1972), p. 151.
7. A. K. Selezneva, Vopr. Med. Khimii, No. 5, 512 (1969).
8. V. V. Fedorov, Ukr. Biokhim. Zh., No. 5, 489 (1969).
9. A. Fisher, The Physiology and Experimental Pathology of the Liver [in Russian], Budapest (1961).
10. A. Francavilla, O. Albano, et al., Clin. Chim. Acta, 30, 415 (1970).
11. C. Sicot, Cah. Coll. Med. Hop. (Paris), 10, 891 (1969).