Effects of Energy Metabolism Modifiers on Cyclophosphane Toxicity for *Daphnia Magna*

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The effects of various energy metabolism modifiers and their combinations on cyclophosphane toxicity are examined in the fresh-water crustacea *Daphnia magna*. The role of acute energy deficiency in the development of paralyzing effect of cyclophosphane is demonstrated. Transporting forms of succinic acid and combinations of carbonic acids with NAD exhibit high protective activity, while glucose reduces protective activity of NAD and sensitizing activity of amino acids.

Key Words: *cyclophosphane; nicotinamide; succinate; energy deficiency*

Neurotoxic effects of alkylating agents hamper their therapeutic application as cytostatics [5,9]. Acute intoxication by these substances shortens life span of some laboratory animals [8]. The manifestations of such intoxication [8] resemble those typical of acute energy deficiency in the nervous system, while the ability of dichloroethyl sulfide to lower NAD concentration in the cytoplasm [10] implies that this effect is exerted at the cellular level. In the present study we evaluated the role and explored the possibility of preventing energy deficiency upon paralyzing effect of cyclophosphane (CP) in the fresh-water crustacean *Daphnia magna*.

MATERIALS AND METHODS

Neurological damage was modeled by adding CP to the incubation medium in concentrations suppressing motor activity of daphnia within a 5-h period. A short-term exposition allowed us practically to rule out cytostatic effect of CP as a potential cause of death of daphnia, while a 600-fold excess of incubation medium provided constant concentration of CP throughout the experiment. The resistance to CP was estimated by EC_{50}, which was calculated by probit analysis (Hewlett-Packard software) of data obtained by increasing CP concentration in the incubation medium from the minimal to the absolutely lethal.

Modifiers of the resistance to CP, which had no effect on motor activity of daphnia, were added 10 min before CP. The animals were deprived of food for 24 h before the experiment. The concentrations of energy substrates in the incubation medium were close to their average tissue levels in mammals [2]. The effects of modifiers were expressed as the ratio between EC_{50} of CP in the presence and in the absence of the modifier (factor of dose modification, FDM). The measurements were performed 5 times. The significance of differences was evaluated by Student's t test for paired samples [4].

RESULTS

Nicotinamide (NA), a precursor of NAD, increased the resistance of daphnias to CP 1.12- to 1.14-fold (Table 1). Glucose and the NAD-dependently oxidized carbonic acids pyruvate and citrate did not modify cytotoxic effect of CP and markedly altered the protective effect of NA. This effect was abolished by glucose and potentiated by carbonic acids. The amino acids glutamate and aspartate did not abolish the effect of NA and sensitized the animals to CP.

Succinate, a NAD-independently oxidized substrate, increased the resistance of daphnias to CP by

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TABLE 1. Effects of Some Energy Biosubstrates on the Effectiveness of NA Protection against CP Cytotoxicity for *Daphnia magna* (*M±m*, *n*=5)

Substrate, mM		Parameters of resistance to CP	EC_{50}/5 h, mg/liter FDM
Glucose	Control	6604±133	—
	NA, 0.5	7338±178	1.12±0.02*
	Glucose, 0.5	6373±184	0.97±0.02
	Glucose, 5.0	6598±113	1.00±0.02
	NA, 0.5+glucose, 0.5	6630±62	1.00±0.02
	NA, 0.5+glucose, 5.0	6541±112	0.99±0.01**
Carbonic acids	Control	6487±211	—
	NA, 0.5	7400±240	1.14±0.03*
	Pyruvate, 0.5	6617±168	1.02±0.02
	Citrate, 0.5	6525±174	1.00±0.02
	NA, 0.5+pyruvate, 0.5	7874±174	1.22±0.04**
	NA, 0.5+citrate, 0.5	7749±401	1.19±0.05*
Amino acids	Control	6224±11	—
	NA, 0.5	7012±140	1.13±0.02*
	Glutamate, 0.5	5133±475	0.82±0.07*
	Aspartate, 0.5	5354±521	0.86±0.97*
	NA, 0.5+glutamate, 0.5	5747±107	0.92±0.02*
	NA, 0.5+aspartate, 0.5	5203±326	0.83±0.05*

Note. $p<0.05$: *compared with control, **compared with NA.

almost 30%; however, this effect was blocked by the competitive inhibitor of succinate malonate (Table 2). Both sodium salt and methyl ester of succinic acid exhibited pronounced protective activity in the presence of cellular respiration coupled to oxidative phosphorylation. The addition of small amounts of 2,4-dinitrophenol, which had no effect on the resistance of daphnias, abolished protective effect of succinates (Table 3).

Thus, energy substrates protect daphnias against paralyzing effect of CP under conditions providing participation of these substrates in terminal biological oxidation. Such conditions for pyruvate and citrate are provided by an essentially high level of NAD. In the present study this was attained by the addition of NA, which is capable of crossing plasma and mitochondrial membranes [7] and increasing NAD pool in mammalian tissues [6]. Presumably, CP initiates NAD degradation by activating the processes of poly-ADP-ribosylation just as it occurs in keratinocytes under the action of dichlorodiethyl sulfide [10]. The presence of dehydrogenase cofactor and dehydrogenase substrates may eliminate energy deficiency resulting from activation of poly-ADP-ribosylation, thus normalizing oxidizing resynthesis of ATP.

It can be hypothesized that substrate activation of glycolysis shifts the intracellular NA pool toward cytosolic compartment and inhibits NAD-dependent oxidizing resynthesis of ATP. This hypothesis is supported by the finding that NAD content in mammalian tissues drops after administration of glucose [6].

NAD-dependently oxidized amino acids can also participate in energy metabolism; however, the pathways of their metabolism include greater number of endergonic reactions [1]. Therefore, sensitization of animals to CP by glutamate and aspartate can be related to increased ATP demand upon incorporation of the amino group nitrogen in glutamine, purines, and pyrimidines, synthesis of peptide bonds, and

TABLE 2. Effects of Succinate and/or Sodium Malonate on the Resistance of *Daphnia magna* to CP (*M±m*, *n*=5)

Substrate, mM	Parameters of resistance to CP	
	EC_{50}/5 h, mg/liter	FDM
Control	6774±351	—
Succinate, 0.5	8697±229	1.29±0.05*
Malonate, 0.5	7417±640	1.09±0.06
Succinate,0.5+malonate, 0.5	7436±275	1.10±0.03**

Note. Here and in Table 3: $p<0.05$: *compared with the control, **compared with succinate.

Table 3. Effect of 2,4-Dinitrophenol on Protective Activity of Sodium Succinate and Methyl Esters Succinic Acid against CP Toxicity for *Daphnia magna* (*M±m*, *n*=5)

Substrate, mM	Parameters of resistance to CP	
	EC_{50}/5 h, mg/liter	FDM
Control	6709±146	—
Succinate, 0.5	8379±171	1.25±0.04*
2,4-Dinitrophenol, 0.01	6545±92	0.98±0.03
Succinate, 0.5+2,4-dinitrophenol, 0.01	6966±306	1.04±0.03**
Control	6593±183	—
Monomethylsuccinate, 0.5	8021±172	1.22±0.02*
2,4-Dinitrophenol, 0.01	6918±217	1.05±0.02
Monomethylsuccinate, 0.5+2,4-dinitrophenol,0.01	7052±134	1.07±0.01**
Control	6371±137	—
Dimethylsuccinate, 0.5	7075±66	1.11±0.02*
2,4-dinitrophenol, 0.01	6041±86	0.95±0.02
Dimethylsuccinate, 0.5+2,4-dinitrophenol, 0.01	6371±137	1.00±0.01**

active transport of amino acids into cells, i.e., in the processes whose intensify is high under the chosen experimental conditions.

From our results it can be concluded that the use of transporting forms of succinic acid and combinations of NAD-dependently oxidized carbonic acids with NA is a prospective approach to the development of strategies aimed at eliminating neurotoxic effects of CP.

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