

ANTICANCER EFFICACY OF HYDROXYETHYLTHIAMINE DIPHOSPHATE *IN VIVO*

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Aim: The aim of the presented article was investigation of anticancer efficacy of hydroxyethylthiamine diphosphate (HTD) *in vivo*.
Materials and Methods: The study was carried out on 30 C57BL/6J mice subcutaneously transplanted with Ehrlich carcinoma. Animals were treated with intraperitoneal injections of the solution composed from pyruvic acid and thiamine bromide every other day during 10 days and thereafter, every day during 2 weeks. Treatment efficacy was evaluated by tumor growth inhibition. **Results:** In experimental animals treated with HTD, significant tumor growth inhibition has been registered: 73% at day 45th compared to the control group ($p < 0.001$). **Conclusion:** Treatment with HTD demonstrated high anticancer efficacy *in vivo*.
Key Words: carcinogenesis, pH, hydroxyethylthiamine diphosphate, lipoic acid amide.

As it is known, the tumor tissue is composed of atypical cells with unlimited cell proliferation potential requesting great amount of energy. Therefore, oncogenesis should require alterations in energetic apparatus of cell, i.e. in electron-transport chain of mitochondria. We suppose that alterations in electron-transport chain of mitochondria may depend on cytochrome structure (involved in normal functioning of respiratory system), and increased pH in mitochondria, which could affect normal functioning of some enzymes, in particular, pyruvate dehydrogenase, an enzyme involved in the first reaction of oxidative decarboxylation of pyruvic acid, where coenzyme is thiamine diphosphate (TDP). TDP activity depends on pH of medium [1]. TDP is active at low pH level, i.e. in acidic medium, and its activity (oxidative decarboxylation of pyruvic acid) decreases in neutral or alkaline medium. Upon further shift of mitochondrial pH, the aerobic glycolysis may be replaced by anaerobic glycolysis.

In present study we aimed to evaluate the effects of hydroxyethylthiamine diphosphate (HTD) administration on tumor growth *in vivo*.

Experiments were carried out on 30 C57BL/6J male mice, 2–3 months old and with body weight of 18–20 g. All animals were fed standard laboratory chow and given free access to water. The care and use of the animals complied with the Georgian regulations on protection of animals, with Guidelines prepared by the Ethics Committee of the Institutional Animal Care and with the National Institutes of Health Guide for the Care and Use of Laboratory animals.

All experimental animals were subjected to subcutaneous inoculations with Ehrlich carcinoma cells ($1 \cdot 10^6$ cells). Thereafter, they were randomly divided into 2 groups: group 1 was control (untreated mice),

and group 2 was experimental, where mice were subjected to intraperitoneal injections with 0.2 ml of mixture prepared as follows: 0.1 ml of 98% pyruvic acid were diluted with distilled water 1000 times, and 5 mg thiamine bromide was added. Injections of the preparation were started on the 14th day of tumor growth. Tumor volume was measured every 3rd day of cancer growth. Initially, during the first 10 days injections were carried out every other day, and thereafter — every day, during 2 weeks.

Treatment efficacy was evaluated by calculating tumor growth inhibition (%) by the formula:

$$V_{\text{contr.}} - V_{\text{exp.}} / V_{\text{contr.}} \cdot 100$$

Obtained data were analyzed statistically with the use of SPSS 16.0 for Windows. Significance of differences between the data were determined by using the Student's *t*-test. The criterion for significance was set to $p < 0.05$.

Results of experiments have shown that HTD treatment resulted in significant inhibition of tumor growth (Table).

Table. Tumor volumes and tumor growth inhibition in control (group 1) and experimental (group 2) mice treated with HDT

Day	Tumor volume, mm ³		TGI, %
	Group 1	Group 2	
18 th	589 ± 50.5	463 ± 64.6	21
21 st	865 ± 46.6	723 ± 88.5	16
24 th	1156 ± 80.4	805 ± 90.0*	30
27 th	1874 ± 299.8	854 ± 121.4**	54
30 th	2325 ± 354.9	962 ± 151.0**	59
33 rd	2445 ± 277.3	1448 ± 398.5	41
36 th	3316 ± 326.8	2224 ± 618.1	33
39 th	5453 ± 791.4	1611 ± 453.9***	70
42 nd	5539 ± 861.1	1634 ± 459.9**	71
45 th	5374 ± 102.5	1425 ± 397.5***	73

Notes: the difference is significant compared to the control: * $p < 0.01$; ** $p < 0.005$; *** $p < 0.001$.

At the beginning (18th and 21st days of tumor growth) in case of every other day treatment regimen, the TGI in HTD-treated mice 2 was not statistically significant, but at 24th day TGI was 30% ($p < 0.01$). TGI was higher and significant when injections were carried out every day (at 27th, 30th, 39th, 42nd and 45th days).

Supposedly, administration of HTD restores oxidative decarboxylation of pyruvic acid, Krebs cycle and

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Abbreviations used: DNA – deoxyribonucleic acid;

HTD – hydroxyethylthiamine diphosphate; LAA – lipoic acid amide;

TDP – thiamine diphosphate.

functioning of electron-transport chain of mitochondria. Therefore, energy production in malignant cells normalizes and cell proliferation decreases.

Experiments carried out in different countries referring on biological reactions proceeding in mitochondria are somehow similar (not same) to our investigations. For instance, in literature there is information about antitumor efficacy of dichloroacetate in glioblastoma treatment, and 3-bromopyruvate acid in experimental liver cancer [2, 3]. However, information about administration of HTD as an anticancer agent has not been found up today in the available literature.

Although HTD revealed anticancer efficacy and administration of HTD in Ehrlich carcinoma bearing

C57BL/6J mice caused significant inhibition of cancer growth, these results support the need of a further detailed investigation of HTD anticancer properties with the final aim of its possible use as therapeutic agent.

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