

Research Article

Biochemical Mechanisms of Specific Antitumor Activity of the Novel Medicinal Preparation Deglutame

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Abstract

Metabolic mechanisms of realizing the biochemical activity of antitumor low-molecular weight peptide Deglutame (Dg), L-Glutamine (Gln) derivatives, were studied on W-256, SM-1, SM-45 and PC tumor-bearers rats. Dg on its enteral and parenteral injection (50 mg/kg) was proved to induce the glycolysis suppression and gluconeogenesis activation and induces the impoverishment of free amino acids pool in tumor-bearer's liver. Gln on it's per oral injection at isomolar Dg dose exerted the single-oriented, but less expressed, influence upon the induce studied. After Dg or Gln injection, Gln concentration decreases while in SM-1 and PC-1 tumor bearers this decrease most evidently is displayed after Dg injection in liver, blood plasma and in the tumor itself, and in tumorous tissue only. Moreover, in blood plasma and liver of W-256 tumor-bearers the total amino acids content, competing with Gln for general systems of transmembranous transport decreases, but it practically does not change in the tumor itself. In liver and blood plasma of SM-1 and PC-1 tumor bearers the total amino acids content, on the contrary, does not change significantly and decreases in tumorous tissue only. After Gln injection the negative correlative relation existing in intact tumor-bearers and confirming the selective Gln absorption by the tumorous tissue between the Gln levels in liver and tumor disappears. According to the data of correlative analysis the "antiglutamine" mechanism of Dg influence is realized only on it's per oral injection depending on real endogenic (physiological) Gln and amino acids concentrations.

Key words: amino acids, antitumor effects, deglutame.

Introduction

It is common knowledge that L-Glutamine (Gln) derivatives related to low-molecular weight peptides of natural origin produce a pronounced antitumorous effect and their deficiency in the body may create a condition for proliferation of malignant cells. These compounds are suggested to affect the mitotic processes in tumorous tissue, causing the anomalous cells to be converted into differentiated ones [1-5].

Transport of Gln through the cellular membrane is proved to be regulated by its conjugation with phenilacetate, more than 90% of which is utilized for phenilacetileglutamine or phenilacetilzoglutamine formation which are natural products of metabolism and are to be found in physiologic fluids [6]. That's why one of the most probable mechanisms of antitumorous antineoplaston influence is the induction of intracellular Gln insufficiency, since their competition for the system of active transmembranous transport may induce the Gln deficiency and realization of "antiglutamine" effect [2,4-6].

Preclinical studies of antineoplastones as for today have been carried out according to NCI requirements. In the USA they already finished I and II stages of the clinical studies on more than 2000 patients suffering from tumors of different localizations. Their therapeutic efficiency was proved to make 40-60% with good durability, slightly pronounced and short-termed side effects. Alongside with per oral and intravenous, they use intramuscular, subcutaneous, rectal, intrapleural, intravascular as well as local kinds of injections of the above mentioned preparations into the tumor [1,3,5,6].

At the same time the peculiarities of metabolism from the point of

view of probable Gln influence upon the neoplastic processes are the subject of recent discussions. Thus, on one hand in vitro it is evidently shown that Gln is necessary for the malignant cells to enter the phase of the cellular cycle. That is why it is necessary for their growth and reproduction. Clinical studies showed that since tumor is "the trap" for Gln, its concentration decreases in the body of the tumor-bearer [1,5-6].

On the other hand, additional administration of Gln together with the diet demonstrated the decrease of tumor weight by 40 % in model situation of MTF-breast cancer. It also increases natural killing lymphocyte activity. On usage of 3% Gln solution by MCA-3 tumor bearing animals the growth of tumor and the processes of their metastasizing are inhibited. At the same time Gln is known to be an effective immune modulator. Its usage causes increase of T-lymphocytes number in oncologic patients, and combined usage of this amino acid with chemical preparations produces a pronounced antitumorous effect [7-13].

Purpose of the study

The aim of this study is the bring to light on metabolic activity of novel medicinal preparation Deglutame.

Methodology

We studied possible mechanisms of realizing the metabolic

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activity of Dg compound. Dg compound is the analogue of AS2-1 antineoplastone (Burzynski Research Institute, Houston, USA). It has been synthesized at the Institute of Physics and Organic Chemistry of the National Academy of Sciences of Belarus [14]. In the experiments in vitro on the culture of HeLa cells we demonstrated the pronounced dose-dependant inhibiting Dg influence upon the cells reproduction (their number decreased almost to 0) 3-4 days after it had been added into the incubation media, the concentration being 0.5-2.5 mg/ml [3,5,15].

The studies were carried out on W-256-, SM-1-, SM-45- and PC-bearing rats Wistar CRL: (WI) WU BR. Dg screening in vivo experiments was carried out on for varieties of tumor bearing rats (Worker W-256, sarcoma SM-1 and SM-45, alveolar liver cancer PC). To clear up possible mechanisms of realization of Dg biologic activity in the processes of malignant growth we carried out the research by means of cross-comparison of Dg and Gln metabolic influence.

Except the basic criteria (determination of tumor-bearers' lifespan, evaluation of growth inhibiting influence of preparations depending on the tumor weight, morphologic and physiochemical characteristics) [15], the studies included the determination of metabolic target data which, to greater extent, characterize the processes of malignant growth [16,17]:

- activity of reactions in mitochondria, blood cytoplasm;
- levels and correlation of concentrations lactate/pyruvat in blood;
- activity of glycolysis and glyconeogenesis processes in liver: hexokinase, glycoso-6-phosphotase, glucose level, glycoso-6-phosphate;
- activity of limiting reactions of energy forming and substrate utilization reactions in the tricarbon acids cycle of liver: piruvatdehydrogenase in mitochondria, cytoplasmatic and mitochondrial forms of izocytratdehydrogenase, cytoplasmatic malagehydrogenese;

The tumors were implanted to white not thoroughbred rats from the tumor-bearers in accordance with the standard methods [15]. The studies of Dg influence were carried out in dependence on the type of tumor 15-25 days after the implantation. Dg was injected daily (50 mg/kg of body weight in 1 ml of aquary solution, per os or intraperitoneally in comparison to the tumor bearing rats which were injected H₂O or Gln watery solution in isomolar Dg concentration [15]. The biologic material was studied the decapitation of animals.

Results and discussion

Regarding the survival criteria and tumor weight, Dg inhibits W-256 and SM-1 tumor growth (percent of inhibition being 67.2% and 52.4% respectively). It also increases the lifespan of tumor bearing rats (by 39 and 23% respectively), efficiency index being 4,1. On morphologic analysis it turned out that the animals which received Gln showed the I grade injures of tumor cells, and those which were Dg injected-III grade. These symptoms were most distinctly expressed in W-256 and SM-1 tumor-bearers, which also demonstrated the morphologic symptoms of therapeutic oncomorphosis (decrease of mitosis number and increase of necrosis area in tumor).

Besides, Dg administration to W-256 tumor bearers induces the glycolisis inhibition and glyconeogenesis activation. It also induces the impoverishment of free amino acids pool in liver. Gln on it's per oral injection produced though less expressed, but the same as Dg directed influence. Here, after the injection of two substances to W-256 tumor bearers Gln concentration in liver, blood plasma and tumor decreases. At the same time in SM-1 and PC-1 tumor-bearers this decrease is most evidently expressed at the result of Dg injection only in timorous tissue.

At the same time, total amino acids content competing with Gln for the general active transmembraneous transfer (Ala, Ser, Thr, Val, Leu, Ile, Phe, Tyr, Asn, His, Met, Gly) A-system in blood plasma and liver of tumor-bearers decreases, and this index practically does not change in the tumor itself. Vica versa, in liver and blood plasma of SM-1 and PC-1 tumor-bearers the total content of the amino acids mentioned does not change significantly and authentically decreases in tumorous tissue. The enumerated changes in the concentrations of the free amino acids studied after the Gln injection are followed by the disappearance of highly authentic negative correlative relation ($r=-0.98$) peculiar for target tumor-bearers between Gln levels in liver and tumor and it also confirms its selective accumulation by the tumor.

On the grounds of the results of correlative analysis the possibility of "antiglutamine" mechanism of Dg influence is realized only on its per oral injection, because exactly in this case we register highly authentic positive correlative dependence ($r=0.96$) between Gln content in blood plasma, liver and tumorous tissue. In its turn on parenteral Dg injection such dependence is preserved between Gln levels in blood plasma and liver, but not in the tumorous tissues.

Thus, the results of the studies give us the grounds to suggest that "antiglutamine" mechanism of Dg influence may exist in dependence upon the activity of Gln synthesis and Gln utilization (glutaminase, glutathione biosynthesis), which determine real concentrations of this compound as well as amino acids concentrations competing with it for general systems of intracellular transport and thus exerting the influence upon the peculiarities of intratissue distribution and endogenic Gln metabolism.

Conclusions

According to the data of correlative analysis the "antiglutamine" mechanism of Dg influence is realized only on it's per oral injection depending on real endogenic Gln and amino acids concentrations.

References

1. Brusilov I (1994) TriButiratetm - a Revolutionary, Non-Toxic Cure and Prevention for Cancer. KalmaSund, Sweden 35.
2. Nefyodov L (2001) Amino Acids and Their Derivatives. Proc of Internat. Symp; Grodno. 124 p.
3. Souba WW (1993) Glutamine and cancer. *Ann Surg* 218: 715-728. [[crossref](#)]
4. Nefyodov L (1999) Biological Activity and Transport of Drugs. Proc of Internat. Symp. Grodno. 189 p.
5. Sugita Y, Tsuda H, Maruiwa H, Hirohata M, Shigemori M, et al. (1995) The effect of Antineoplaston, a new antitumor agent on malignant brain tumors. *Kurume Med J* 42: 133-140. [[crossref](#)]
6. Bartlett DL, Charland S, Torosian MH (1995) Effect of glutamine on tumor and host growth. *Ann Surg Oncol* 2: 71-76. [[crossref](#)]
7. Liao MC, Szopa M, Burzynski B (1987) Chemo-surveillance: a Novel Concept of the Natural Defence Mechanism Against Cancer. *Drugs under Experimental and Clinical Research* 13: 71-76.
8. Nefyodov L, Uglyanitsa K, Karavay A (1997) Antineoplastons – New Antineoplastic Drugs. *Terra Medica* 1: 19-21.
9. Newsholme E (1996) The Possible Role of Glutamine in Some Cells of the Immune System and the Possible Consequence for the Whole Animal. *Experientia* 52: 455-459.
10. Klimberg VS, Kornbluth J, Cao Y, Dang A, Blossom S, et al. (1996) Glutamine suppresses PGE2 synthesis and breast cancer growth. *J Surg Res* 63: 293-297. [[crossref](#)]
11. Pastores SM, Kvetan V, Katz DP (1994) Immunomodulatory effects and therapeutic potential of glutamine in the critically ill surgical patient. *Nutrition* 10: 385-391. [[crossref](#)]

12. Rohde T, Ullum H, Rasmussen JP, Kristensen JH, Newsholme E, et al. (1995) Effects of glutamine on the immune system: influence of muscular exercise and HIV infection. *J Appl Physiol* 79: 146-150. [[crossref](#)]
13. Calder PC, Yaqoob P (1999) Glutamine and the immune system. *Amino Acids* 17: 227-241. [[crossref](#)]
14. Soldatov V, Nefyodov L (1998) Method for Production of Phenylacetyl-L-Glutamine as Na⁺ Salt," State Patent Committee of Belarus 768.
15. Sofina Z, Sirkin B, Goldin C (1980) Experimental Evaluation of Antitumor Drugs in the USSR and the USA. Moscow. 234.
16. Nefyodov L (1993) Formation of The Pool of Free Amino Acids and Their Derivatives Under Conditions of Metabolic Imbalance. M.D. dissertation, Institute of Radiation Biology, Minsk. 234.
17. Nefyodov LI, Karavay PA, Karavay NL (2014) Regulatory action of free amino acids and development on the basis of highly of substances infusion solutions with pathogenetic deterministic composition. *Laboratory diagnosis Eastern Europe* 3: 111-115.