METABOLIC PROFILE OF THE NEOPLASTIC CELLS TREATED *IN VITRO* WITH ANTITUMORAL FUROSTANOLIC-GLYCOSIDE BIOPREPARATIONS

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Key words: glycosidic cytostatics agents; tumoral cells; intermediary metabolism; nucleic acids biochemistry; mechanism of anticancerous action.

Abstract: The *in vitro* short-lasting cytostatic treatment of the HeLa and HEp-2p tumoral cell cultures with some original furostanolic-glycoside biopreparations has conditioned the perturbation of the glucidic, lipidic and proteic intermediary metabolism processes and of the nucleic acids biochemistry.

The metabolic profile of the treated cells seems to be of catabolic type, being outlined by enhancement of the glicogenolysis, glycolysis, lipolysis and proteolysis, of intensification of intracellular consumption of the glucose, lactic acid, free fatty acids and aminoacids, of inhibitory effect upon nucleic acids biosynthesis. These metabolic events were appreciated on the basis of the reduced contents of glycogen, glucose, lactic acid, total lipids, free fatty acids, soluble and unsoluble proteins, DNA and RNA biomolecules. The new tumoral cell metabolic behaviour induced by furostanolic-glycoside cytostatics – analyzed in comparison with that of the control untreated tumoral cells – can be consequence of an interaction between the bioactive agents either with the membrane receptors or with intracellular receptors.

INTRODUCTION

The morphological, structural, physiological, genetical, biochemical, biophysical and antigenic features of the tumoral cells – although assure yet their relative invulnerability – provide the numerous targets for chemotherapy, immunotherapy, genic therapy and biochemical therapy of the malignant diseases (Benga, 1985; Bianchi et al., 1986; Chiricuţă, 1988; Rusu et al., 1988; Stroescu, 1998; Miron, 2000; Owens, 2001).

Despite the fact that there has been continuous progress in cancer diagnosis and treatment as a result of recent discoveries in cellular and molecular oncobiology, structural and functional genomics, pharmacogenomics and toxicogenomics, proteomics and metabolomics, antineoplastic therapy is still of little effectiveness (Karp, 1996; Cruce, 1999; Lyden et al., 2001; Weinstein, 2001; Adams, 2002; Anderson et al., 2002; Habeck, 2002; Wong, 2002).

One of the most significant objectives of contemporary studies in pathology consists in improving the efficacity of means to control the carcinogenesis. In the fight against cancerous diseases, chemotherapy holds pride of place, but it is still of small effectiveness, a fact explained especially by its negative impact on the normal cells of the organism under neoplasm aggression and by the development of a resistance phenomenon of the tumoral cells to the cytostatic drugs action. Consequently, for the improvement of the oncochemotherapy there are necessary the extending and thoroughgoing researches for: the discovery and design of new oncolytic agents that should specifically target the tumoral cells; the identification of new therapeutic ways of action upon carcinogenesis process; the conceiving of new strategies and programs of anticancerous chemotherapy; the use of different drug monithorized delivery and transport systems; the discovery of agents which can potentiate the antitumoral effect of the oncochemoterapeutic drugs (Leiter et al., 1965; DeVita, 1991; Stroescu, 1998; Weinstein, 2001).

The identification of a new antitumoral agent and its introduction in clinical practice – the main purpose of the screening chemotherapeutic programs – are the result of some complex preclinical and clinical pharmacological investigations according to appropriate experimental patterns, which use various testing biological systems having different degrees of reactivity (Leiter et al., 1965; Jungstandt et al., 1971; Boyd, 1989; Bissery and Chabot, 1991; Phillips et al., 1991; Stroescu, 1998; Seethala et al., 2001).

Our previous studies – performed on experimental models adequate to the *in vitro* investigation on neoplastic cell cultures – were relevant for the appreciation of some original biopreparations of furostanolic-glycoside type, extracted from *Digitalis purpurea* leaves, as potential cytostatic drugs with possible biomedical significance (Gherghel et al., 2004; Rotinberg et. al, 2005).

In the light of the above affirmations, supplementary researches have been required in order to enlarge our data base necessary both for the confirmation of the cytostatic property of the biosynthetic and semisynthetic furostanolic-glycoside biopreparations and for the establishment of their action mechanism at cellular, subcellular and molecular level involved in the global expression of the antitumoral pharmacodynamic effect.

Thus, the purpose of the present paper is to investigate the metabolic behavior of the HeLa and HEp-2p tumoral cells in the conditions of the *in vitro* cytostatic treatment with furostanolic-glycosides B1 and E5.

MATERIALS AND METHODS

The active cytostatic compounds of furostanolic glycoside nature which were used in the *in vitro* experiments have been the following:

– B1, a biosynthesis product, specifically extracted and purified from leaves of *Digitalis purpurea*;

– E5, which is a semisynthesis derivative, performed from the former by partial oxidation until to obtaining of some aldehydic groups.

The biological material used in the *in vitro* investigations was represented by the control and treated HeLa and HEp-2p cellular cultures of human neoplastic origin (cervix carcinosarcoma and laringeal carcinoma, respectively). The test flasks have been inoculated with 1 x 10^5 tumoral cells in Eagles' MEM growing medium supplemented with 10% calf serum, they being incubated at 37^0 C for a period of 72 hours of culture development**.** When the monolayer stage was attained, the initial medium was replaced with a medium containing one of the two furostanolic-glycoside biopreparations in a dose of 5 mg/ml. The cultures were incubated again at 36.5–37^0 C for 180 minutes in the presence of the drugs.

At the end of this short *in vitro* antitumoral treatment, the medium was discarded from the test tubes. The layer of tumoral cells was washed with PBS and then subjected to the steps of obtaining of the cell clarified lyzates. Adequate aliquots were used for the biochemical determination of some metabolic indices (Artenie and Tănase, 1981): glycogen (G), glucose (g) and lactic acid (L.A.); total lipids (T.L.) and free fatty acids (F.F.A); soluble (S.P.), unsoluble (U.P) and total proteins (T.P.); deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and total nucleic acids (TNA).

Five tubes of cultures have been employed for each culture type, the results being analyzed statistically by means of Student' „t" test (Snedecor, 1968).

RESULTS AND DISCUSSIONS

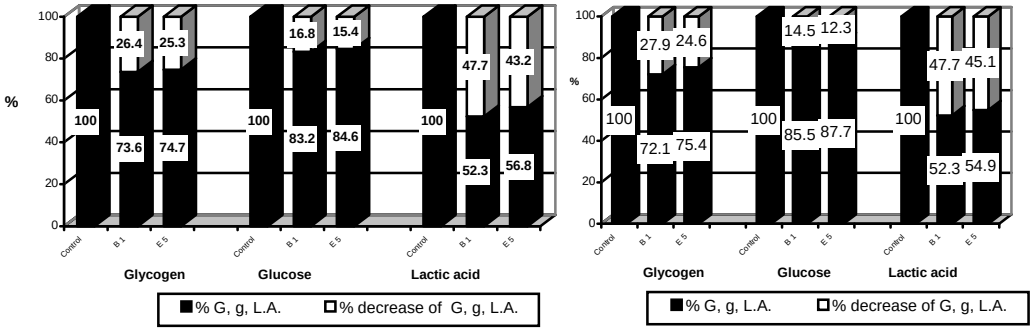
In a first step of the research, we have followed the reactivity of the glucidic intermediary metabolism of the HeLa and HEp-2p tumoral cells submitted to the short-lasting cytostatic treatment with the B1 and E1 bioactive furostanolic-glycoside preparations. The sense and the intensity of the metabolic processes have been expressed by the quantitative values of some glucidic biochemical parameters: glycogen, glucose and lactic acid.

It can be seen, in Table 1, that the *in vitro* antitumoral treatment of the 72 hours old HeLa and HEp-2p cell cultures has induced statistically significant decreases of the glycogen, glucose and lactic acid contents, as compared to the control level.

Table 1. The effect of the cytostatic glycosides, in dose of 5 mg/ml upon the contents of glycogen, glucose and lactic acid (mg/g cellular mass), from HeLa and HEp-2p tumoral cell cultures of 72 hours, submitted to the *in vitro* short-lasting antitumoral treatment. Figures in brackets indicate the number of experimental cultures for each type.

Experimental group	Glycogen		Glucose		Lactic acid	
	X ± SE	p	X ± SE	p	X ± SE	p
HeLa						
Control	29.6 ± 1.30 (5)	–	4.35 ± 0.21 (5)	–	0.88 ± 0.05 (5)	–
B1	21.8 ± 1.10 (5)	<0.002	3.62 ± 0.18 (5)	<0.05	0.46 ± 0.03 (5)	<0.001
E5	22.1 ± 1.25 (5)	<0.01	3.68 ± 0.33 (5)	<0.05	0.50 ± 0.04 (5)	<0.001
HEp-2p						
Control	30.5 ± 1.30 (5)	–	4.50 ± 0.21 (5)	–	1.53 ± 0.05 (5)	–
B1	22.0 ± 1.10 (5)	<0.01	3.85 ± 0.18 (5)	<0.05	0.80 ± 0.03 (5)	<0.001
E5	23.0 ± 1.18 (5)	<0.01	3.95 ± 0.11 (5)	<0.05	0.84 ± 0.04 (5)	<0.001

From the Figure 1, it is observed that the amplitude of the quantitative diminutions reaches – in comparison with 100% control value – percentage levels of: 26.4% or 27.8%, 16.8% or 14.5%, and respectively 47.7% for glycogen, glucose and respectively lactic acid in the case of the HeLa or the Hep-2p treated cells with B1 agent , as well as of 25.3% or 24.9%, 15.4% or 12.8% and respectively 43.2% or 45.1% in the case of neoplastic cells submitted to the action of E5 derivative.



HeLa HEp-2p

Fig. 1. Percentage variation of the glycogen, glucose and lactic acids concentrations induced by the *in vitro* short cytostatic furostanolic-glycoside treatment of the HeLa and HEp-2p neoplastic cells.

These quantitative and procentual variations of the glucidic biochemical indices reveal the modulations of the cellular metabolic events. Thus, it can be highlighted an intensification of the glycogenolysis, glicolysis and the intracellular consumption of the glucose and lactic acid.

Another intermediary metabolism which was investigated is the lipidic one, the pattern of unfolding of the biochemical processes in the tumoral cells treated with the biosynthetic and semisynthetic furostanolic-glycoside agents being illustrated by some parameters: total lipids and free fatty acids (Table 2 and Figure 2).

Table 2. Total lipids and free fatty acids concentrations (mg/g cellular mass) of the HeLa and HEp-2p tumoral cells incubated with B1 or E5 (5 mg/ml) preparations. Figures in brackets indicate the number of experimental cultures for each type.

Experimental group	Total lipids X±SE	t	p	Free fatty acids X±SE	t	p
HeLa						
Control	18.05 ± 1.20 (5)	–	–	5.05 ± 0.35 (5)	–	–
B1	14.01 ± 0.80 (5)	2.80	<0.05	3.86 ± 0.20 (5)	2.97	<0.02
E5	14.50 ± 0.75 (5)	2.51	<0.05	3.98 ± 0.21 (5)	2.67	<0.05
HEp-2p						
Control	20.56 ± 1.20 (5)	–	–	6.05 ± 0.35 (5)	–	–
B1	15.54 ± 0.85 (5)	3.41	<0.01	4.32 ± 0.20 (5)	4.32	<0.01
E5	15.95 ± 0.96 (5)	2.99	<0.02	4.55 ± 0.26 (5)	3.49	<0.01

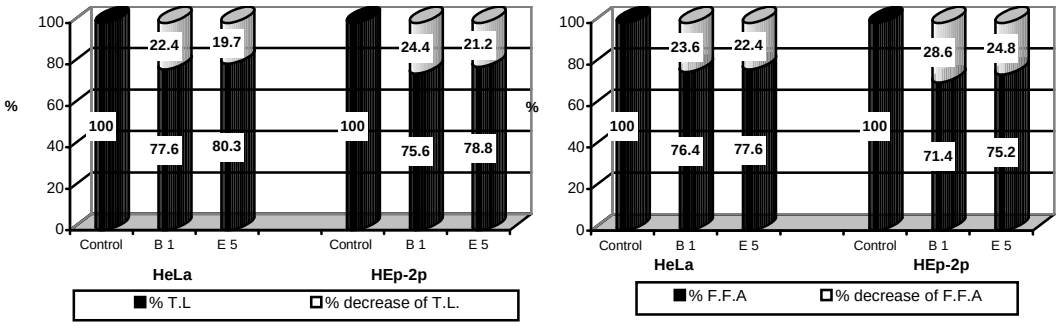


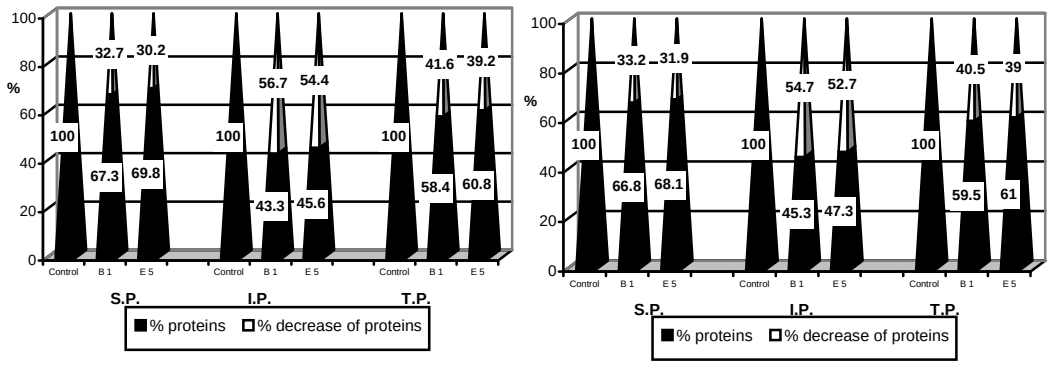
Fig. 2. The sense and the amplitude of the lipidic metabolism modulation, in the malignant HeLa and HEp-2p cells, by the active cytostatic furostanolic-glycoside agents.

In vitro short time incubation of the HeLa and HEp-2p with B1 and E5 cytostatic agents has conditioned – as can be observed from Table 2 and Figure 2 – the perturbation of the lipidic metabolism processes which were materialized by intracellular depletions of the lipidic reserves. Thus, as compared with the control values, the contents of the total lipids and free fatty acids have registered significant quantitative and procentual decreases. The variations of the lipidic parameters – of negative sense and moderate degrees – have emphasized the intensification of the intracellular lipolysis and metabolic utilization of the free fatty acids.

The study of the intermediary metabolism of the HeLa and HEp-2p tumoral cells, submitted to the action of the natural and semisynthesis furostanolic-glycosidic preparations, was extended by the investigation of the protidic metabolism biochemistry in the conditions of the cytostatic treatment. The reactivity of the metabolic events was analysed on the basis of the soluble proteins, unsoluble proteins and total proteins variations evidenced in comparison to the control values, these being inserted in Table 3 and Figure 3.

Table 3. Contents of the soluble, unsoluble and total proteins (g/g cellular mass), of the 72 hours HeLa and HEp-2p tumoral cells cultures, incubated for 3 hours with the cytostatic B1 and E5 preparations (5 mg/ ml). Figures in brackets indicate the number of experimental cultures for each type.

Experimental group	Soluble proteins		Unsoluble proteins		Total proteins	
	X ± SE	p	X ± SE	p	X ± SE	p
HeLa						
Control	25.64 ± 1.10 (5)	–	15.13 ± 1.50 (5)	–	40.77 ± 2.80 (5)	–
B 1	17.25 ± 2.10 (5)	<0.05	6.55 ± 1.70 (5)	<0.01	23.80 ± 2.10 (5)	<0.002
E 5	17.90 ± 1.30 (5)	<0.002	6.90 ± 1.20 (5)	<0.01	24.80 ± 2.00 (5)	<0.002
HEp-2p						
Control	45.06 ± 2.10 (5)	–	23.15 ± 2.10 (5)	–	68.21 ± 2.80 (5)	–
B1	30.10 ± 1.10 (5)	<0.001	10.49 ± 1.50 (5)	<0.002	40.59 ± 2.10 (5)	<0.001
E5	30.68 ± 1.25 (5)	<0.001	10.96 ± 1.62 (5)	<0.02	41.64 ± 2.18 (5)	<0.001



HeLa Hep-2p

Fig. 3. Percentage variation of the soluble (S.P.), unsoluble (U.P.) and total proteins (T.P.) concentrations induced by the *in vitro* short cytostatic furostanolic-glycosidic treatment of the HeLa and HEp-2p neoplastic cells.

It also can be seen that the HeLa or HEp-2p cellular cultures treated with the B1 or E5 active cytostatic agents have been characterized, as compared to control, by significantly reduced contents of the soluble, unsoluble and respectively total proteins, which reach levels of: 32.7% or 33.2%, 56.7% or54.7% and respectively 41.3% or 40.5% in the case of B1 and 30.2% or 31.9%,

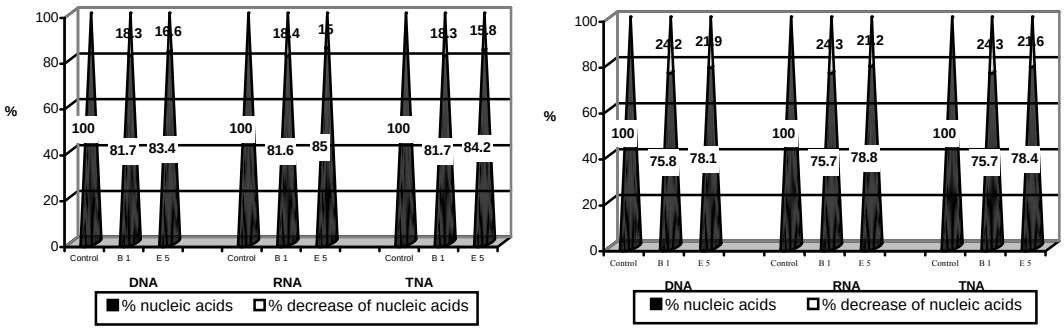
54.4% or 52.7% and respectively 39.2% or 39.7% in the case of E5. It is confirmed the inhibitory impact of the furostanolic-glycoside preparations upon the proteinsynthesis.

In order to obtain supplementary information about the interference of biosynthesis or semisynthesis glycosides with the tumoral cell metabolism we proposed ourselves to investigate some aspects of nucleic acids metabolism in the HeLa and HEp-2p cells in the presence of cytostatic agents of glycosidic nature.

The cytophysiologic behavior of the nucleic acids, in the HeLa and HEp-2p malignant cells submitted to the cytostatic treatment with the biologically active furostanolic-glycoside agents, can be appreciated from the direction and intensity of display of the metabolic processes illustrated by the data included in Table 4 and Figure 4.

Table 4. Deoxyribonucleic acid, ribonucleic acid and total nucleic acids concentrations (mg/g cellular mass) of the HeLa and HEp-2p tumoral cells incubated with B1 or E5 (5 mg/ml) preparations. Figures in brackets indicate the number of experimental cultures for each type.

Experimental group	DNA		RNA		TNA	
	X ± SE	p	X ± SE	p	X ± SE	p
HeLa						
Control	1.799 ± 0.095 (5)	–	1.870 ± 0.120 (5)	–	3.669 ± 0.20 (5)	–
B1	1.470 ± 0.070 (5)	<0.05	1.527 ± 0.065 (5)	<0.05	2.997 ± 0.13 (5)	<0.05
E5	1.500 ± 0.075 (5)	<0.05	1.590 ± 0.070 (5)	≈0.05	3.090 ± 0.15 (5)	=0.05
HEp-2p						
Control	2.190 ± 0.095 (5)	–	2.260 ± 0.100 (5)	–	4.450 ± 0.28 (5)	–
B1	1.660 ± 0.070 (5)	<0.01	1.710 ± 0.065 (5)	<0.01	3.370 ± 0.15 (5)	<0.01
E5	1.710 ± 0.076 (5)	<0.01	1.780 ± 0.080 (5)	<0.01	3.490 ± 0.18 (5)	=0.02



HeLa Hep-2p

Fig. 4. The sense and the amplitude of the nucleic acids metabolism modulation, in the malignant HeLa and HEp-2p cells, by the active cytostatic furostanolic-glycosidic agents.

Once again, the experimental results have highlighted significant smaller amounts of DNA, RNA and respectively TNA in comparison with the control values, registered on untreated HeLa and HEp-2p cultures. Thus, an interaction between the furostanolic-glycoside cytostatic agents and the metabolic events of the nucleic acids, it can be assumed, this materializing itself in an inhibitory impact (of about 18% for B1 and 16% for E5, in the case of HeLa cells and respectively of about 24% for B1 and 21% for E5, in the case of the HEp-2p cells) upon biosynthesis of the nucleic biomolecules.

The numerous, various and profound structural alterations (of the plasmatic membrane; glycocalix; extracellular matrix; cytoskeleton; cytoplasm; nucleus; nucleoli; endoplasmic reticulum; Golgi apparatus; mitochondria; peroxisomes; centrosome; lysosomes; cell topochemistry; enzymatic and isoenzymatic biomolecules) and citophysiological perturbations (of the membrane permeability and transport; cell signaling; transmission and expression of genetic information; energy conversion; cell metabolism; sorting and transport of the biomolecules in intracellular compartment; cell motility; intercellular and cell–matrix adhesion; cell proliferation; molecular regulation mechanisms) of the cellular, subcellular and molecular components of the dedifferentiated tumoral cells induced by erroneous functioning of the cellular genetic apparatus of selfregulation and control, turn the cancerous cells – apparently primitive and vulnerable – into a type of vigorous and viable cell, full of vitality and relative resistance to the chemical, physical and biological factors, this transformed cell being characterized by another homeostatic level (Benga, 1985; Bianchi et al., 1986; Chiricuță, 1988; Karp, 1996; Alberts et al., 1998; Stroescu, 1998; Cruce, 1999; Miron, 2000).

One of the most important features of the neoplastic cell is strongly connected to the qualitative and quantitative modifications of the cellular metabolism processes (Bustamante et al., 1981; Chiricuță, 1988; Bagetto, 1992; Gonzales et al., 1993, Mathupala et al., 1995; Karp, 1996; Bannasch et al., 1998; Cruce, 1999 Miron, 2000). Generally speaking, in comparison with the corresponding normal cell, the tumoral cell presents:

– intracellularly increased concentrations of proteins, aminoacids and nucleosides, due to: the intensified transmembranary transport of these biomolecules; augmented activity degree of the protein synthase kynases; amplified proteinsynthesis and nucleoside biosynthesis; switching of the catabolic reactions of aminoacids and nucleosides in an anabolic pathway of synthesis of the polyaminoacids and polinucleosides (proteins, enzymes, DNA, RNA);

– reduced contents of glycogen, glucose correlated to intracellular lactic acid accumulations, conditioned by: exaggerate, uncontrolled intensification of the hexokinase, phosphofructokinase, piruvatkinase, ATP-ase activity; glycolysis; intracellular quantitative increasing of glucose and other hexoses with membranary determination; depressing of the key gluconeogenesis enzymes activity;

– intracellular augmented amounts of some tumoral lipids (desmosterol, cholesterol, triglycerides) and fatty acids due to: the changed membrane permeability; the quantitative and qualitative modification of the key opposite enzymes of the isoenzyme patterns and of the metabolic pathway.

The biochemical unbalance of the glucidic, lipidic and protidic metabolism and of the nucleic acids metabolism is the result of the reschedule of the corresponding genetic expression in tumoral cell.

However, the structural and functional peculiarities of the tumoral cell assure at the same time the targets of the antitumoral factors within the frame-work of the different kinds of antineoplastic therapy. Among these is the cytostatic chemotherapy, which allows interactions drugs–cancerous cells, conditioning expression of the antitumoral effect.

In the light of the above information we will discuss and interpret the results we obtained in the study of the metabolic behavior of the human HeLa and HEp-2p neoplastic cells submitted to the *in vitro* cytostatic treatment with the two bioactive original products of furostanolic-glycoside nature, B1 and E5.

The comparative analysis of our data, in relation to the control metabolic profile of the untreated HeLa and HEp-2p cultures, highlights quantitative variations – always of negative

sense (meaning) and different amplitudes – of some glucidic, lipidic and protidic biomolecules and of the nucleic macromolecules. Thus, there were assessed reduced intracellular contents of glycogen, glucose and lactic acid, soluble and unsoluble proteins, aminoacids, total lipids and free fatty acids, DNA and RNA. Therefore, we can appreciate that the glycosidic cytostatics accent the glycogenolysis, activate the lipolysis and proteolysis, inhibate the nucleic macromolecule biosynthesis and intensify the intracellular metabolic consumption of the glucose, lactic acid, free fatty acids and aminoacids biomolecules.

Certainly, the intracellular utilization pathway is not represented by anabolic reactions of synthesis of the glucidic, lipidic, protidic and nucleic compounds, but it is probably assured by energogenetic catabolic reactions, which use the glucose, lactic acid, aminoacids and free fatty acids as fuels. Therefore, it is possible for the glycosidic structures to stimulate the energetic metabolism of the HeLa and HEp-2p tumoral cells. Thus, we must to prove this hypothesis, as soon is possible, by the investigation of the effect of the furostanolic-glycoside agents upon cellular respiration of the HeLa and HEp-2p tumoral cultures.

Finally, it can be revealed that the antitumoral glycosidic agents condition a new lack of poise between the two sides of the cell metabolism, inducing an inhibitory impact upon the glucidic, lipidic, protidic and nucleic metabolism and an exacerbate stimulatory effect upon the exergonic metabolic reactions. These metabolic consequences – incompatible with the tumoral cell life – are induced by the furostanolic-glycoside perturbation of the activity of the disordered genetic apparatus and of the diverse enzymatic systems involved in catalyzing the biochemical reactions.

CONCLUSIONS

The antitumoral glycosidic preparations influence negatively the development of the metabolic processes in the HeLa and HEP-2p tumoral cells. The multitude of the metabolic effects can be the consequence of interactions of the glycosidic structures either with the cell membrane receptors or with the intracellular ones. The bulk of the present results globalizes the behavioral spectrum of the tumoral cells to the action of the vegetable glicosydic biopreparation (B1) and its semisynthetic derivative (E5), confirming their cytostatic property.

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