METABOLIC BEHAVIOUR OF THE HEp-2p NEOPLASTIC CELLS TO THE ACTION OF A BIOACTIVE FUNGAL EXOPOLYSACCHARIDIC EXTRACT

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Abstract: The *in vitro* cytostatic treatment of the HEp-2p tumoral cell cultures with an autochthonous original glucanic biopreparation has conditioned the perturbation of the glucidic, lipidic and proteic intermediary metabolism processes, of the nucleic acids biochemistry and of the cell respiration. The metabolic profile of the treated cells seems to be of catabolic type, being outlined by the enhancement of the glicogenolysis, glycolysis, lipolysis and proteolysis, of intensification of intracellular consumption of the glucose, lactic acid and aminoacids, of inhibitory effect upon nucleic acids biosynthesis and by the intensification of the respiration. These metabolic events were considered on the basis of the reduced contents of glycogen, glucose, lactic acid, total lipids, soluble and unsoluble proteins, DNA and RNA biomolecules and by the route and amplitude of the cellular respiration dynamics. The new tumoral cells metabolic behaviour induced by the glucanic extract – analyzed in comparison with that of the control untreated tumoral cells – can be the consequence of an interaction between the bioactive agent either with the membrane receptors or with intracellular receptors.

INTRODUCTION

One of the most significant objectives of contemporary research in pathology consists in improving the efficiency 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 optimizing of the oncochemotherapy, the extending and thoroughgoing investigations on several directions are necessary 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 (DeVita, 1991; Stroescu, 1998; Weinstein, 2001; Adams, 2002; Anderson&Chiplin, 2002; Habeck, 2002; Wong, 2002; Lyden et al., 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; Owens, 2001).

Our previous studies – performed on experimental models adequate to the *in vitro* investigation on neoplastic cell cultures – were relevant for the characterization of four original biopreparations of glucanic type, extracted from different submerged strains of fungus *Claviceps purpurea*, as potential cytostatic drugs with possible biomedical significance.

In the light of the above affirmation, a supplementary research has been required in order to enlarge our database necessary both for the confirmation of the cytostatic property of the natural fungal glucanic biopreparations and for the suggesting 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 behaviour of the HEp-2p tumoral cells in the conditions of their *in vitro* cytostatic treatment with GE37.1 autochthonous glucanic extract, the most powerfull bioactive agent selected by us in the initial experiment.

MATERIALS AND METHODS

The glucanic extract used in our *in vitro* experiments was GE37.1, the biopreparation being separated and partially purified (by ethanolic precipitation, centrifugation, sediment resuspendation in distilled water, dialization, ethanolic reprecipitation and final centrifugation) from the cultivation medium supernatant of six days old submerged strain of the fungus *Claviceps purpurea,* codified Cl.p.-37.1.

The biological material used in the *in vitro* investigations was represented by the control and treated HEp-2p cellular cultures of human neoplastic origin, derived from a laringeal carcinoma. The tumoral cells, were cultured in DMEM medium supplemented with 10% fetal bovine serum, 100 µg/ml streptomycin, 100 IU/ml penicillin and 50 µg/ml amphotericin B, at a density of $5x10^5$ cells in 75 cm^2 flasks, in a humidified 5% CO_2 atmosphere at 37ºC (Doyle&Griffiths, 1998). When the monolayer stage was attained, the initial medium was replaced with a medium containing the glucanic biopreparation in a dose of 1.5 mg/ml. The cultures were incubated again at 36.5–37^0 C for 180 minutes in the presence of the drug.

At the end of this short lasting *in vitro* antitumoral treatment, the medium was discarded from the test flasks, the layer of tumoral cells was washed with PBS, was detached from the flask with 0.25% trypsin + 0.02% EDTA in the normal medium, centrifuged at 1800 rpm for 2 minutes and then subjected to the steps of obtaining the cell clarified lyzates.

Adequate aliquots were used for the biochemical determinations 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).

The evaluation of the *in vitro* antineoplastic impact of the glucanic treatment upon energetic metabolism was performed through the comparative analysis of the control and treated tumoral cells respiration intensities, the cytophisiologic process being expressed by the oxygen consumption registered through Warburg microrespirometric method (Dohr, 1961) .

Five flasks 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 studied the reactivity of the glucidic intermediary metabolism of the HEp-2p tumoral cells submitted to the short lasting cytostatic treatment with the GE37.1 natural glucanic biopreparation. 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, these being included in the synthetic Table 1.

Table 1. The effect of GE 37.1 cytostatic, in dose of 1.5 mg/ml, upon the cellular contents of glycogen, glucose and lactic acid, total lipids and FFA, soluble, unsoluble and total proteins, nucleic acids (mg/ 100 g cellular mass), from HEp-2p tumoral 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	X ± ES	p	X ± ES	p	X ± ES	p
	Glucidic metabolism					
	Glycogen		Glucose		Lactic acid	
Control	2.86 ± 0.15 (5)	–	4.95 ± 0.35 (5)	–	1.09 ± 0.025 (5)	–
GE37.1	1.95 ± 0.13 (5)	<0.001	2.33 ± 0.21 (5)	<0.001	0.49 ± 0.018 (5)	<0.001
	Lipidic metabolism					
	Total lipids		Free fatty acids			
Control	5.20 ± 1,10 (5)	–	1.21 ± 0.025 (5)	–		
GE37.1	4.70 ± 0,80 (5)	N.S.	2.07 ± 0.035 (5)	<0.001		
	Proteic metabolism					
	Soluble proteins		Unsoluble proteins		Total proteins	
Control	5.02 ± 0.32 (5)	–	1.59 ± 0.14 (5)	–	6.61 ± 0.50 (5)	–
GE37.1	1.93 ± 0.21 (5)	<0.02	0.89 ± 0.08 (5)	<0.001	2.82 ± 0.35 (5)	<0.02
	Nucleic acids metabolism					
	DNA		RNA		TNA	
Control	144.0 ± 8.0 (5)	–	159.0 ± 7.9 (5)	–	303.0 ± 10.2 (5)	–
GE37.1	74.0 ± 7.2 (5)	<0.001	76.9 ± 6.4 (5)	<0.001	150.9 ± 7.6 (5)	<0.001

It can be seen that the *in vitro* glucanic treatment of the 72 hours old HEp-2p cell cultures has induced statistically significant decreases of the glycogen, glucose and lactic acid contents, as

compared to the control level. The amplitude of these quantitative diminutions reaches – in comparison with 100% control value – percentage levels of: 31.8%, 52.9% and respectively 55.1% for glycogen, glucose and respectively lactic acid in the case of the HEp-2p treated cells. The quantitative and percentage variations of the glucidic biomolecules reveal the modulations of the cellular glucidic intermediary metabolism events by the bioactive agent. Thus, an intensification of the glycogenolysis, glicolysis and the intracellular consumption of the glucose and lactic acid can be highlighted.

Another followed intermediary metabolism was the lipidic one, the pattern of unfolding of the biochemical processes in the tumoral cells treated with the polysaccharidic cytostatic extract being illustrated by some parameters: total lipids and free fatty acids (Table 1 and Figure 1).

In vitro short time incubation of the HEp-2p with GE37.1 cytostatic has conditioned – as can be also observed from Table 1 and Figure 1 – the perturbation of the lipidic metabolism processes, which were materialized by intracellular depletions of the total lipidic reserves and increases of the intracellular amounts of the free fatty acids. Thus, as compared to the control values, the contents of the total lipids and free fatty acids have registered significant quantitative and percentage decreases or increases. The variations of the lipidic parameters – of negative or positive sense and moderate degrees – have emphasized the intensification of the intracellular lipolysis, without a metabolic consumption of free fatty acids, which aren’t used as energetical resources.

The study of the intermediary metabolism of the HEp-2p tumoral cells, submitted to the action of the GE 37.1 glucanic biopreparation, was extended by the investigation of the protidic metabolism biochemistry to the cytostatic treatment. The reactivity of the metabolic events was analysed on the basis of the soluble proteins, unsoluble proteins and total proteins quantitative and procentual values, evidenced in comparison to the control values, the protein contents and their variations being inserted in the same Table l and Figure 1.

It also can be seen that the HEp-2p cellular cultures treated with the GE37.1 cytostatic have been characterized, as compared to control, by significantly reduced stocks of the soluble, unsoluble and respectively total proteins, which reach levels of 61.6%, 44.1% and respectively 57.4%. The diminution of the cellular protein content can be the consequence either of an inhibitory impact of this glucanic biopreparation upon the proteinsynthesis - effect signaled by us in the previous work – or of its stimulating effect of proteolysis released aminoacids use as intracellular energetic fuels, or of both its actions.

In order to obtain supplementary informations about the interference of exopolysaccharidic cytostatic agent with the tumoral cells metabolism we proposed ourselves to investigate some aspects of nucleic acids metabolism in the HEp-2p cells in its presence. The biochemical behaviour of the nucleic acids, in the treated HEp-2p malignant cells can be analysed in relation to direction and intensity of display of the metabolic processes illustrated by the data included in the same Table 1 and Figure 1.

Once again, the experimental results have highlighted significantly smaller amounts of nucleic acids, registered on treated HEp-2p cultures, in comparison to the control values. Thus, an interaction between the glucanic agent and the metabolic events of the nucleic acids, can be assumed, this materializing itself in an inhibitory impact (of 48.6%, 51.6% and respectively 50.2% in the case of DNA, RNA and respectively TNA) upon biosynthesis of the nucleic biomolecules.

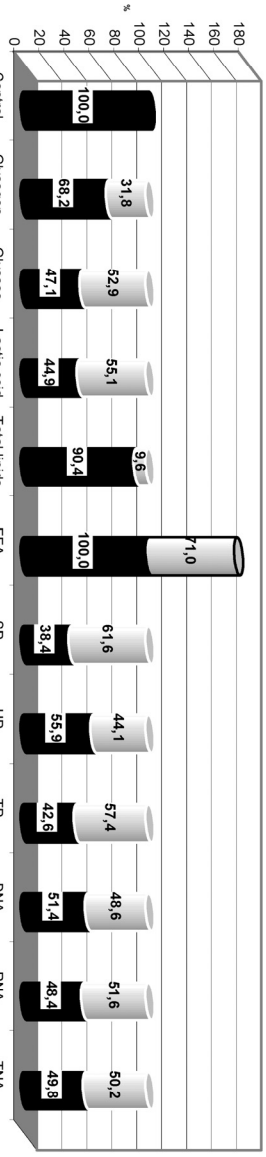

Fig. 1. Percentage variations of the glycogen, glucose and lactic acids, total lipids and free fatty acids, soluble, unsoluble and total proteins, nucleic acids from the HEp-2p neoplastic cells submitted to the. *in vitro* t cytostatic treatment with GE 37.1 glucanic extract.

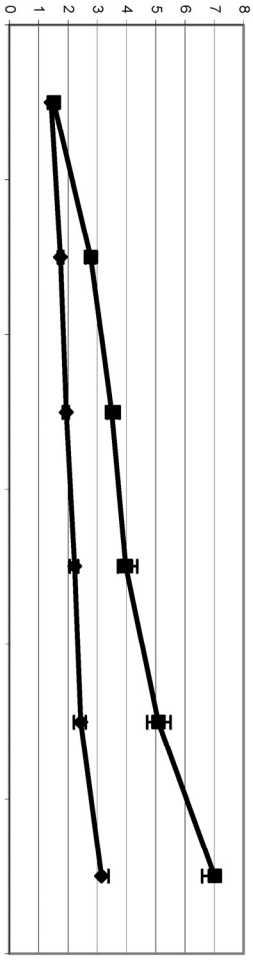

Fig. 2 The dynamics of the respiratory process of the tumoral cells treated with the GE37.1 glucanic extract (in a dose of 1.5 mg/ml) for 15, 30, 45, 60 and 75 minutes ,as compared to the control respiratory dynamics..

Finally, the metabolic comportment of the neoplatic cells treated with the glucanic biopreparation, has not revealed a quantitative augmentation of the intracellular metabolites, with the exception of the free fatty acids, suggesting an intracellular usage of the proteins, aminoacids, glycogen, glucose and lactic acid, in cell energetics. To decipher this problem, we have investigated the cellular respiration process of the tumoral cells HEp-2p in the conditions of the glucanic treatment.

Table 2 The intensity of the respiratory process - expressed by O_2 consumption/g cellular mass - of the tumoral cells HEp-2p treated with the GE37.1 glucanic biopreparation. Figures in brackets indicate the number of experimental cultures for each type.

Experimental group	X ± ES	p	X ± ES	p	X ± ES	p
	Energetic metabolism (mm^3 O_2 / g cellular mass)					
	15 minutes		30 minutes		45 minutes	
Control	1.42±0.10 (5)	–	1.74±0,10 (5)	–	1.94±0,09 (5)	–
GE37.1	1.51±0.13 (5)	NS	2.78±0,18 (5)	0,002	3.50±0,16 (5)	0.001
	60 minutes		75 minutes		90 minutes	
Control	2.23±0.12 (5)	–	2.44±0.17 (5)	–	3.15±0.24 (5)	–
GE37.1	3.98±0.27 (5)	0.001	5.09±0.38 (5)	0,001	7.01±0.42 (5)	0.001

As we can see from Table 2 and from Figure 2, the registration of the control neoplastic HEp-2p cells respiration during a 90 minutes period, at 15 minutes intervals, has revealed a relative equal consumption of oxygen, through the entire experiment. The successive graphical transposition of the consumed O_2 volumes imprints to respiratory dynamics an uniform ascendent route of low amplitude. The cellular respiration of the untreated HEp-2p cultures has been considered by us as control value of 100%. Comparatively to the reference respiratory process, the tumoral cells treated continuously (75 minutes) with the glucanic biopreparation present an accentuated intensification of the cell respiration (over 200%), the prolonged stimulatory effect of the fungal extract being highlighted by the progressive increases of the consumed O_2 volumes, by the ascendent route of great amplitude of the respiratory dynamics.

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; Chiricuţă, 1988; Rusu et al., 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; Chiricuţă, 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:

– intracellular 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;

– augmented intracellular 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, correlated with cell energetic profound disorders, 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 previously mentioned factors within the frame-work of the different kinds of antineoplastic therapy. Among these is the cytostatic chemotherapy, which allows interactions drugs–cancerous cells and, therefore, antitumoral effect.

In the light of the above information we will discuss and interpret the results we obtained in the study of the metabolic behaviour of the human HEp-2p neoplastic cells submitted to the *in vitro* cytostatic treatment with the bioactive autochtounous fungal glucanic extract GE37.1.

The comparative analysis of our data, in relation to the control metabolic profile of the untreated HEp-2p cultures, highlights quantitative variations – in most cases 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, DNA and RNA, as well as an augmented level of free fatty acids. Therefore, we can state that the glucanic cytostatic intensifies the glycogenolysis, activates the lipolysis and proteolysis, inhibates the nucleic macromolecule biosynthesis and stimulates the intracellular metabolic consumption of the glucose, lactic acid 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 sustained by energogenetic catabolic reactions, which use the glucose, lactic acid, aminoacids as fuels. Therefore, it is possible for the glucanic biopreparation to stimulate the energetic metabolism of the HEp-2p tumoral cells. Thus, to prove this hypothesis we have investigated the effect of the glucanic bioproduct GE37.1 upon cellular respiration of the HEp-2p tumoral cultures.

The high levels of the spent oxygen, the ascendant route of great amplitude of the respiratory dynamics, as well as the percentage intensification of the respiration, registered in the case of the neoplastic cells treated continuously with the GE37.1 glucanic extract, have argued the induction of a stimulatory effect upon the respiratory process.

The augmentation of the oxygen consumption isn't conditioned by the increase of the total cell number, because the bioactive glucanic extracts have induced, on the tumoral cells, a mitoinhibitory impact (unpublished data), correlated with an inhibitory effect upon the proteinsynthesis and culture development (previous paper).

The intensification of the HEp-2p neoplastic cells oxygen consumption by the cytostatic treatment is positively correlated with the induced glycogenolysis, lipolysis and proteolysis, is associated with the intensification of the consumption of the glucose, lactic acids and aminoacids biomolecules. As we can see, the consumption way is not represented by the biosynthesis reactions of the intracellular metabolites, but by the energetic metabolism processes, which seem to be exacerbated by the glucanic cytostatic.

Finally, it can be highlighted that the fungal antitumoral agent of glucanic nature conditions 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 glucanic perturbation of the activity of the disordered genetic apparatus and of the diverse enzymatic systems involved in catalyzing the biochemical reactions.

CONCLUSIONS

The GE 37.1 antitumoral glucanic biopreparation influences negatively the development of the metabolic processes in the HEp-2p tumoral cells.

The multitude of the metabolic effects can be the consequence of interactions of the glucanic structure either with the cell membrane receptors or with the intracellular ones.

The bulk of the present results globalizes the behavioural spectrum of the HEp-2p tumoral cells to the action of the fungal glucanic biopreparation confirming its cytostatic property, expressed either of a membranotropic action mechanism or of a metabolic action mechanism.

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