THE REACTIVITY OF THE METABOLIC PROCESSES OF THE HEp-2p TUMORAL CELLS TO THE ACTION OF SOME ACTIVE CYTOSTATIC BIOPREPARATIONS OF POLYPHENOLIC NATURE

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Abstract: The in vitro cytostatic treatment of the HEp-2p tumoral cell cultures with some autochthonous original polyphenolic 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 polyphenolic 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; Chiricuță, 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 preclinical studies – performed on experimental models adequate to the in vitro and in vivo pharmacodynamic investigation both on neoplastic cell cultures and on animals with different tumoral systems – were relevant for the appreciation of some autochthonous, original biopreparations of polyphenolic type, extracted from phytomass, as potential cytostatic drugs with possible biomedical significance (Rotinberg et al., 1998; Rotinberg et al., 2000; Rotinberg et al., 2000).

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 natural vegetable polyphenolic 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 behaviour of the HEp-2p tumoral cells in the conditions of the in vitro cytostatic treatment with POLYAS I and POLYAS II autochthonous polyphenolic extracts.

MATERIALS AND METHODS

The aromatic extracts of polyphenolic type, which were used in the in vitro experiments, have been the following:

– POLYAS I, representing a total polyphenolic biopreparation separated and purified from a crude alkaline extract obtained from the October harvested leaves of *Asclepias syriaca* after the removal of the hemicellulosic structures and the readjustement of the pH at 7.0-7.1;

– POLYAS II, which is a biopreparation similar to the former presenting also a readjusted pH (7.0-7.1). However, unlike POLYAS I, it contains no waxes, latex, alcohols, fatty acids and terpenoids that have all been removed from its composition by a pre-extraction with cyclohexane.

The chemical compounds remaining in the supernatants after the exclusion of the above mentioned substances are of a phenolic nature. Polyphenol concentration was determined with a spectrophotometer and the total content was expressed in terms of gallic acid (Kren and Martinkova, 2001). The polyphenolic biopreparations with a total polyphenolic content of 12.0% and 15.0%, respectively, have been obtained by dissolving some known quantities of dry substance – resulted from the evaporation of the final supernatants – in appropriate volumes of bidistilled water. Stock solution concentrations were established.

The biological material used in the in vitro investigations was represented by the control and treated HEp-2p cellular cultures of human neoplastic origin (laringeal carcinoma). The test tubes have been inoculated with 1 x 105 tumoral cells in Eagles' MEM growing medium supplemented with 10% calf serum, they being incubated at 37°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 polyphenolic biopreparations in a dose of 10 mg/ml. The cultures were incubated again at 36.5–37°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 HEp-2p tumoral cells submitted to the short cytostatic treatment with the POLYAS I and POLYAS II natural polyphenolic biopreparations. 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 short antitumoral 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.

Table 1. The effect of active cytostatic polyphenolic biopreparations, in dose of 5 mg/ml upon the contents of glycogen, glucose and lactic acid (mg/g cellular mass), from HEp-2p tumoral cell cultures of 72 hours, submitted to the in vitro short 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
Control	30.5 ± 1.3 (5)	–	4.50 ± 0.21 (5)	–	1.53 ± 0.05 (5)	–
POLYAS I	23.5 ± 1.4 (5)	<0.01	4.05 ± 0.25 (5)	N.S.	0.92 ± 0.03 (5)	<0.001
POLYAS II	22.0 ± 1.1 (5)	<0.01	3.85 ± 0.18 (5)	<0.05	0.80 ± 0.03 (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: 23%, 10% and respectively 39.9% for glycogen, glucose and respectively lactic acid in the case of the HEp-2p treated cells with POLYAS I, as well as of 27.9%, 14.5% and respectively 47.7% in the case of neoplastic cells submitted to the action of POLYAS II.

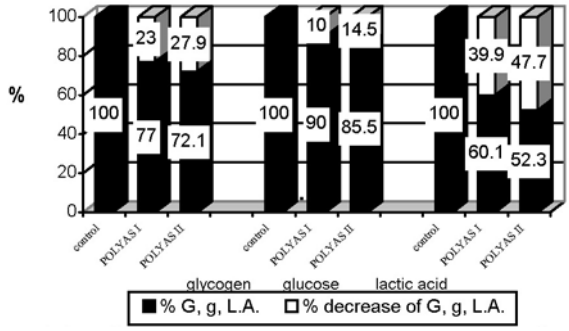

Fig. 1. Percentage variation of the glycogen, glucose and lactic acids concentrations induced by the in vitro short cytostatic polyphenolic treatment of the HEp-2p neoplastic cells.

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 polyphenolic cytostatic extracts 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 HEp-2p tumoral cells incubated with POLYAS I or POLYAS II (5 mg/ml) polyphenolic biopreparations. Figures in brackets indicate the number of experimental cultures for each type.

Experimental group	Total lipids		Free fatty acids	
	X ± SE	p	X ± SE	p
Control	20.56 ± 1.20 (5)	–	6.05 ± 0.35 (5)	–
POLYAS I	16.50 ± 0.90 (5)	<0.05	4.79 ± 0.22 (5)	<0.02
POLYAS II	15.54 ± 0.85 (5)	<0.01	4.32 ± 0.20 (5)	<0.01

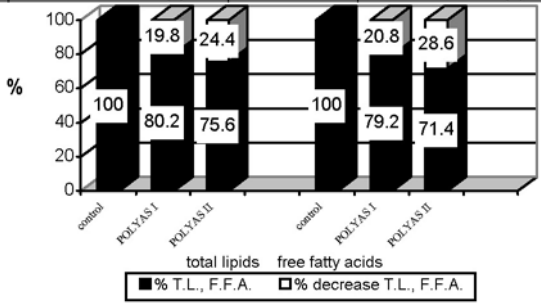

Fig. 2. The sense and the amplitude of the lipidic metabolism modulation, in the malignant HEp-2p cells, by the active cytostatic polyphenolic agents.

In vitro short time incubation of the HEp-2p with POLYAS I and POLYAS II cytostatic extracts 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 HEp-2p tumoral cells, submitted to the action of the vegetable polyphenolic biopreparations, 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 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 (mg/g cellular mass), of the 72 hours HEp-2p tumoral cells cultures, incubated for 3 hours with the cytostatic polyphenolic biopreparations (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
Control	45.06 ± 2.1 (5)	–	23.15 ± 2.1 (5)	–	68.21 ± 2.8 (5)	–
POLYAS I	34.02 ± 1.7 (5)	<0.01	14.00 ± 1.7 (5)	<0.01	48.02 ± 2.6 (5)	<0.001
POLYAS II	30.10 ± 1.1 (5)	<0.001	10.49 ± 1.5 (5)	<0.002	40.59 ± 2.1 (5)	<0.001

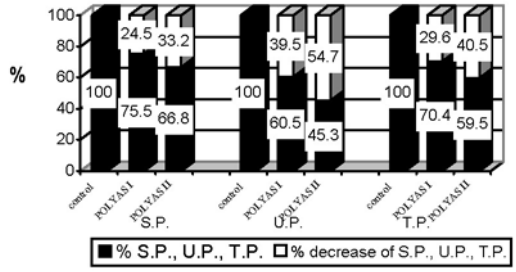

Fig. 3. Percentage variation of the soluble, unsoluble and total proteins concentrations induced by the in vitro short cytostatic polyphenolic treatment of the HEp-2p neoplastic cells.

It also can be seen that the HEp-2p cellular cultures treated with the POLYAS I or POLYAS II 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: 24.5%, 33.5% and respectively 29.6% in the case of POLYAS I and 33.2%, 54.7% and respectively 40.5% in the case of POLYAS II. It is confirmed the inhibitory impact of the polyphenolic biopreparations upon the proteinsynthesis.

In order to obtain supplementary information about the interference of polyphenolic extracts with the tumoral cell metabolism we proposed ourselves to investigate some aspects of nucleic acids metabolism in the HEp-2p cells in the presence of cytostatic agents of polyphenolic nature.

The cytophysiologic behaviour of the nucleic acids, in the HEp-2p malignant cells submitted to the cytostatic treatment with the biologically active polyphenolic extracts, 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 HEp-2p tumoral cells incubated with POLYAS I or POLYAS II (5 mg/ml) polyphenolic biopreparations. 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
Control	2.19 ± 0.095 (5)	–	2.26 ± 0.100 (5)	–	4.45± 0.28 (5)	–
POLYAS I	1.76 ± 0.065 (5)	<0.01	1.81 ± 0.075 (5)	<0.01	3.57 ± 0.22 (5)	<0.05
POLYAS II	1.66 ± 0.070 (5)	<0.01	1.71 ± 0.065 (5)	<0.002	3.37 ± 0.15 (5)	<0.01

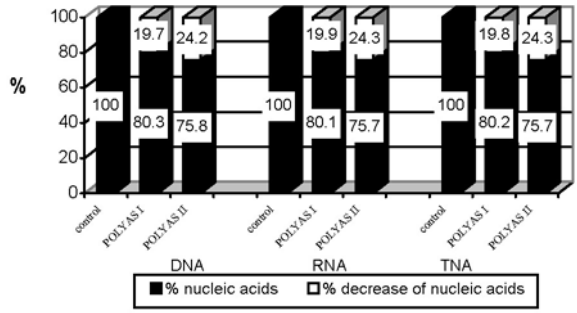

Fig. 4. The sense and the amplitude of the nucleic acids metabolism modulation, in the malignant HEp-2p cells, by the active cytostatic polyphenolic agents.

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 HEp-2p cultures. Thus, an interaction between the polyphenolic cytostatic agents and the metabolic events of the nucleic acids, it can be assumed, this materializing itself in an inhibitory impact (of about 20% for POLYAS I and 24% for POLYAS II) 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; swiching 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 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 struggle.

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 two bioactive autochthounous vegetable polyphenolic extracts, POLYAS I and POLYAS II.

The comparative analysis of our data, in relation to the control metabolic profile of the untreated HEp-2p cultures, highlights quantitative variations – always of negative sense 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 polyphenolic 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 polyphenolic structures to stimulate the energetic metabolism of the HEp-2p tumoral cells. Thus, we must to prove this hypothesis, as soon is possible, by the investigation of the effect of the polyphenolic cytostatics upon cellular respiration of the HEp-2p tumoral cultures.

Finally, it can be revealed that the antitumoral polyphenolic 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 polyphenolic perturbation of the activity of the disordered genetic apparatus and of the diverse enzymatic systems involved in catalyzing the biochemical reactions.

CONCLUSIONS

The antitumoral polyphenolic biopreparations influence 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 polyphenolic structures 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 vegetable polyphenolic biopreparations confirming their cytostatic property.

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