EXPERIMENTAL MODULATION OF HEp-2p TUMORAL CELLS REACTIVITY TO THE ACTION OF SOME AUTOCHTHONOUS POLYPHENOLIC CYTOSTATICS BY CERTAIN MEMBRANOTROPIC AND METABOLIC AGENTS (I)

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Abstract: The *in vitro* short-lasting treatment of the HEp-2p human tumoral cells, initially sensitized by some standard cytophysiological agents, with the autochthonous POLYAS cytostatic biopreparation has been correlated with quantitative and qualitative modification of the reactivity profile of some membrane phenomena. The sense and amplitude of the modulation of the POLYAS characteristic effects upon membrane enzymatic (Na^+–K^+–ATP-ase) and bioelectrical (RMP) activities have revealed the functional synergism, antagonism or complementarity between the agents used in an associated type treatment. This different functional relations between POLYAS and K^+ or Ca^{2+} ions, ouabaine or chemically modified nystatine, adrenaline or ascorbic acid, are due to their interactions with similar or different membrane receptors and can influence the cytostatic potential of the POLYAS polyphenolic biopreparation.

INTRODUCTION

The numerous, various and profound structural alterations – which address to the plasmatic membrane, glycocalix, extracellular matrix, cytoscheleton, cytoplasma, nucleus, nucleols, endoplasmic reticulum, Golgi apparatus, mitochondria, peroxisomes, centrosome, cell topochemistry, enzymatic and izoenzymatic biomolecules, lisosomes – and cytophysiological perturbations – expressed by membrane permeability, transmembranary transport, cellular signalization, genetic message transmission and exteriorization, energetic conversion, cell metabolism, biomolecules sorting and transport in intracellular compartment, cell motility, intercellular and cell-matrix adhesion, cellular proliferation, cell regulation mechanisms – of the cell, subcell and molecular components of the tumoral dedifferentiated cells – induced by erroneous functioning of the selfregulation and control cellular genetic apparatus – reveal the space-temporale organization simplifying, ectoplasmic undevelopment, antigenic attenuation, total or partial lost of the specific function, irreversible diminution of oxidative processes and compensatory intensification of glycolysis, lack of competence face to the regulation factors of multiplication, the raised degree of the autonomy (Adams, 2002; Alberts and Johnsons, 1998; Anderson, 2002; Bannasch, 1998; Benga, 1985; Cruce, 1999; Karp, 1996; Miron, 2000; Seethala, 2001).

This morphological, structural, functional, biophysical, biochemical and genetical anaplasia of the malignant cell – which apparently seems to be primitive and vulnerable – ensures it some “qualities” which make of it, in reality, a type of vigorous and adapted viable cell, full of vitality and relatively resistant to the physical, chemical and biological therapeutic agents. Thus, can be explained the reduced effectiveness of the antineoplastic therapy, generally and of the chemotherapy, particularly.

Consequently, are necessary extending and thorougoing researches of molecular and cellular biology, structural and functional genomics, proteomics and metabolomics, pharmacogenomics and toxicogenomics for elucidation the numerous mysteries of the enigmatical tumoral cell. The new scientific informations will be capitalized by conceiving of new therapeutic ways of action upon carcinogenesis process, of new strategies and programs of anticancerous chemotherapy, which will improve the effectiveness of the therapy against one of the most deadly, aggressive disease of humanity, the neoplasm, a very important desideratum of the contemporary medical practice.

In the light of the above research directions, we can mention that our studies have revealed – on adequate experimental models – *in vitro*, on some tumoral cells cultures, and *in vivo*, on several experimental tumoral systems of solide type, cytostatic property of an original, autochthonous biopreparation of polyphenolic nature obtained from fitomass. Its pharmacodynamic effect – conditioned by action mechanisms at membrane and metabolic level – was reproducible and significant from the point of view of antineoplastic preclinical chemotherapy (Rotinberg et al., 1998; Rotinberg et al., 2000;Rotinberg et al., 2004; Rotinberg et al., 2005).

In this context, we have decided to try a sensibilization of the tumoral cells to the action of our polyphenolic cytostatic by a cytophysiological modulation of the cancerous cells, induced by some reference membranotropic or metabolic agents. The final purpose of this study is to elaborate a strategy of associated chemotherapy which would optimize the tumor suppression effectiveness of some standard cancerostatic drugs of clinical use.

In the present paper there are included and discussed our results registered in the *in vitro* investigation of the

reactivity of some membranary processes of the HEp-2p malignant cells, submitted to the successive action of certain cytophysiological modulators and respectively, of POLYAS polyphenolic biopreparation with cytostatic property.

MATERIALS AND METHODS

The active biological compounds used in the *in vitro* short-lasting, singular or associated, treatment of the tumoral cells cultures were:

- the POLYAS cytostatic agent, which represents a total polyphenolic biopreparation, separated and purified from a crude alkaline extract obtained from the October harvested leaves of *Asclepias syriaca* (Rotinberg et al., 2004). This aromatic extract of polyphenolic type (pH 7.0-7.1) was used in a dose of 5 mg/ml.
- the membranotropic modulators: K^+ and Ca^{2+} ions, ouabaine and respectively chemically modified nystatine, added, in doses of 30 mM, 6 mM, 0.05 mM and respectively 2 mg/ml, to the culture medium;
- the metabolic modulators: ascorbic acid and adrenaline, in doses of 25 µg/ml and respectively, 1 µg/ml;

The biological material used in the *in vitro* investigations was represented by the control and treated HEp-2p cellular cultures of human neoplastic origin (laryngeal carcinoma). The test flasks or the Petri dishes with a Noble agar solid substratum were 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 of some cultures - they will become the treated cultures – was replaced with a medium containing one or two from bioactive agents in the anterior mentioned doses. The control and treated cultures were incubated again at 36.5–37^0 C for 240 minutes in the presence of the drugs. In the case of the associated treatment from the whole time interval (4 h) one hour has represented the period of incubation with the cytophysiological modulator and the last 3 hours have represented the cytostatic treatment duration with POLYAS.

After the singular or associated short-lasting treatment, both kinds of cultures were comparatively analyzed in order to follow the unfolding of the membrane processes of the normal and treated tumoral cells. The membrane bioelectrical effect of the active biological agents has been appreciated by the comparative analysis of the experimental values of the membrane resting potential (RMP; -mV), which was recorded on control and treated cellular cultures, performed on the Petri dishes with a Noble agar solid substratum, by the method of the glass intracellular microelectrodes (Neacșu et al., 1996). The layer of tumoral cells, developed in the culture flasks, was washed with PBS and then subjected to the steps of different methods of obtaining cell clarified homogenates. Adequate aliquots were used for the biochemical determination of the membrane enzyme implied in the transmembranary active transport of Na^+ and K^+ ions. The activity of these cations–depending electrogenic pump was assessed by the spectrophotometrical quantitative analysis of inorganic phosphate (mg Pi/g of cell protein) from the cell homogenates, after Ca^{2+}–ATP-ase blocking with EDTA and ATP hydrolysis by Na^+–K^+–ATP–ase action (Artenie and Tănase, 1981).

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

We have investigated, on 72 hours old HEp-2p cultures, the interference of the bioactive agents with some membrane phenomena and the possibility of influencing of POLYAS cytostatic interaction with the membrane structures – suggested by the membrane ATP-ase activity and bioelectrical activity – of the tumoral cells by its association with membranotropic and metabolic modulators.

A pattern to appreciate the unfolding of the membranary processes of the HEp-2p tumoral cells, in the conditions of singular or associated actions of POLYAS polyphenolic cytostatic or/and of cytophysiological modulators was represented by the analysis of the membrane Na^+-K^+– ATP-ase activity.

In an initial experiment we have followed the functionality of this membranary enzymatic complex of the neoplastic cells, singular or associated treated with the cytostatic, membranotropic and metabolic agents, the Na^+-K^+ electrogenic pump activity values – quantitatively and percentage expressed – of the normal and treated HEp-2p tumoral cells being included and graphical illustrated in Table 1 and Figure 1.

The *in vitro* short-lasting cytostatic treatment of the cancerous cells with POLYAS

preparation has revealed, as compared with control cultures, a powerful inhibition of Na^+ - K^+

Table 1 The behaviour of the Na^+-K^+-ATP-ase (mg Pi/g protein) from the membranes of the HEp-2p neoplastic cells submitted to short-lasting treatment with POLYAS cytostatic or/and with one of the membranotropic and metabolic agents. Figures in brackets indicate the number of experimental cultures for each type.

Experimental group	X±SE mg Pi /g protein	p
Control	55.94±1.73 (5)	–
POLYAS	21.45±0.95 (5)	<0.001
K (30mM)	62.43±1.95 (5)	<0.05
K + Polyas	29.90±1.35 (5)	<0.001
Ca (6mM)	59.13±1.70 (5)	NS
Ca + Polyas	38.76±1.35 (5)	<0.001
Ouabaine (0.05 mM)	32.29±1.27 (5)	<0.001
Ouab. + Polyas	26.44±1.15 (5)	<0.001
NsMC (2 mg/ml)	36.02±1.25 (5)	<0.001
NsMC + Polyas	17.62±0.75 (5)	<0.001
ASC. (25 µg/ml)	42.15±1.57 (5)	<0.001
ASC. + Polyas	86.21±2.25 (5)	<0.001
ADR. (1 µg/ml)	59.39±1.87 (5)	NS
ADR.+ Polyas	99.24±2.75 (5)	<0.001

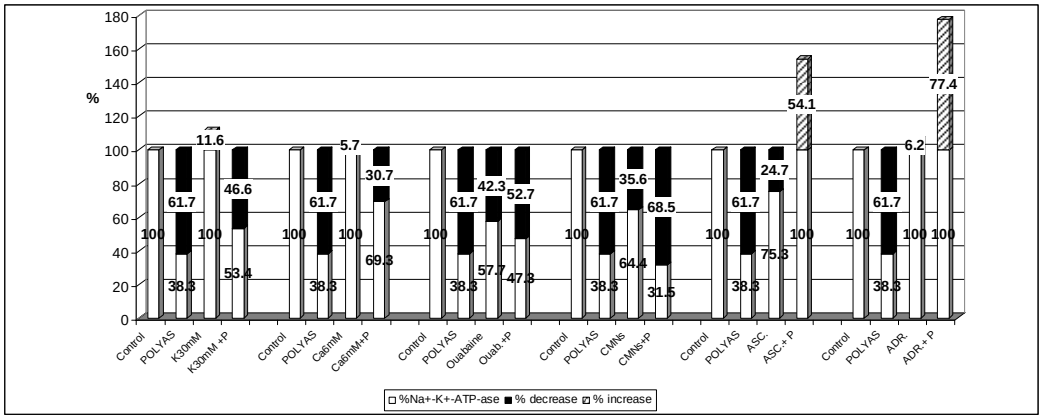

Figure 1 Modulation of the Na^+–K^+–depending electrogenic pump's activity (%) from the HEp-2p tumoral cells singular or associated treated with the POLYAS cytostatic or/and cytophysiological modulators, in comparison with the control cultures.

electrogenic pump activity (61.7%), suggested by a very significant quantitative decrease of the inorganic phosphate in the cellular homogenates.

Reduced contents of Pi released by enzymatic hydrolysis of the ATP, which have highlighted a lower degree of membrane ATP-ase functionality, were registered on the HEp-2p cells subjected to the impact of some membranotropic and metabolic reference agents. This significant inhibitory effect upon Na^+ and K^+ membrane pump reaches successively intensities of 42.3% (Ouab.), 35.6% (CMNs) and 24.7% (ASC).

On the other hand, in the presence of the hyperpotassic medium, of the Ca^{2+} excess, as well as of ADR, we have assisted to the membrane ATP-ase activation with different degrees of expression (in successive order: 11.6%, 5.7% and 6.2%).

Comparatively to the membrane ATP-ase behavior of control cellular cultures or that ones treated singularly with K^{30mM}, Ca^{6mM}, Ouab. and CMNs, the chronological submissions of the HEp-2p cells to the action of POLYAS cytostatic and one from the cytophysiological modulators have been correlated with a depletion of the Pi contents from the clarified cell lysates. This fact suggests an attenuated enzymatic hydrolysis of the ATP, therefore a significant inhibition of the Na^+ - K^+ depending membrane electrogenic pump activity caused by the reduced energetic state, which results at this level.

In all the above mentioned cases, it can be observed the utterance of the POLYAS capability to block the Na^+ - K^+ – ATP-ase activity, even if the associated agents have the same (Ouab., CMNs) or another (K^+; Ca^{2+}) singular effect upon this enzyme.

However, the degree of manifestation of the inhibitory impact upon Na^+ - K^+ – ATP-ase activity is different in relation to the one which characterizes the polyphenolic cytostatic (61.7%). With one exception, the intensity of the enzymatic hydrolysis of ATP was attenuated (K^{30mM}+POLYAS – 46.6%; Ca^{6mM}+POLYAS – 30.7%; Ouab.+POLYAS – 52.7%). The exception was registered in the case of the HEp–2p cancerous cells successively treated with CMNs and POLYAS, when we have highlighted an augmented inhibitory effect upon Na^+ - K^+ – ATP-ase activity, superior (68.5%) to the corresponding to polyphenolic (61.7%) and respectively, nystatinic (35.6%) singular treatments.

Contrary, the *in vitro* associated treatment of the HEp-2p tumoral cells with POLYAS and ASC or ADR has lead to a modulation of the membrane Na^+ - K^+ – ATP-ase activity, but of positive sense in comparison to the functional profile of this membranary enzyme, highlighted in the case of the control and singular treated with POLYAS, ASC and respectively ADR (partially) cultures. Thus, there were registered increased contents of Pi in the corresponding cellular homogenates, which have justified a major potentiation of the Na^+ - K^+ depending electrogenic pump functionality (54.1% for ASC + POLYAS and 77.4% for ADR + POLYAS), in comparison to the inhibitory effect of the POLYAS, ASC and with the readily stimulatory impact of the ADR. Therefore, we have assumed that the new conditions, created by the successive actions of the ASC, ADR and respectively, POLYAS, have deeply modified the interaction pattern of these agents with the cell membrane structures.

Consequently, we assist either to a disappearance of the specific effect of the POLYAS and ASC – which have blocked the membrane Na^+ - K^+ – ATP-ase – or to an amplification of the adrenalinic impact, which has induced a Na^+ - K^+ – ATP-ase activity stimulation.

The modulation Na^+ - K^+ – ATP-ase activity, highlighted in the above experiments, has justified the opportunity to investigate the effects of the experimental treatment association of the POLYAS cytostatic with some reference membranotropic and metabolic agents upon the membrane bioelectrical phenomena of the HEp-2p neoplastic cells. Thus, in another experiment has been followed the resting membrane potential of the neoplastic cells in the conditions of the singular or associated treatments with these bioactive agents, the quantitative and percentage RMP values of the control and treated HEp-2p tumoral cells being included and graphical illustrated in Table 2 and Figure 2.

It is observed that the *in vitro*, short lasting, singular or associated treatment of the HEp-2p tumoral cell cultures with the studied agents has induced modifications of RMP, various as sense and amplitude. Thus, in the case of POLYAS it was registered a significant decrease of the RMP value in comparison to the control mean value, confirming the strong depolarization effect (almost 60%) of this cytostatic biopreparation.

Also, it can be seen that the K^+ excess and CMNs have conditioned a similar depolarizing

effect, but the diminutions of RMP values are of smaller amplitudes (48% and respectively, 34.8%) comparatively to the control and even POLYAS values.

Table 2 The resting membrane potential (-mV) of the HEp-2p tumoral cells submitted to the *in vitro* singular and associated short-lasting treatment with POLYAS cytostatic and one of the cytophysiological modulators. Figures in brackets indicate the number of cultures for each type.

Experimental group	X±SE	p
Control	34.62±3.48 (12)	–
POLYAS (5mg/ml)	13.93±1.43 (12)	<0.001
K^{30mM}	18.04±1.95 (12)	<0.001
K + Polyas	10.90±1.15 (12)	<0.001
Ca^{6mM}	36.80±3.15 (12)	NS
Ca + Polyas	25.51±2.15 (12)	<0.05
Ouabaine (0.05 mM)	31.74±3.15 (12)	NS
Ouab.+ Polyas	17.03±1.67 (12)	<0.001
NsMC (2 mg/ml)	22.57±2.50 (12)	<0.02
NsMC + Polyas	10.21±1.15 (12)	<0.001
ASC. (25 µg/ml)	36.87±2.75 (12)	NS
ASC.+ Polyas	21.78±1.97 (12)	<0.01
ADR. (1 µg/ml)	32.16±2.95 (12)	NS
ADR.+ Polyas	21.55±1.98 (12)	<0.01

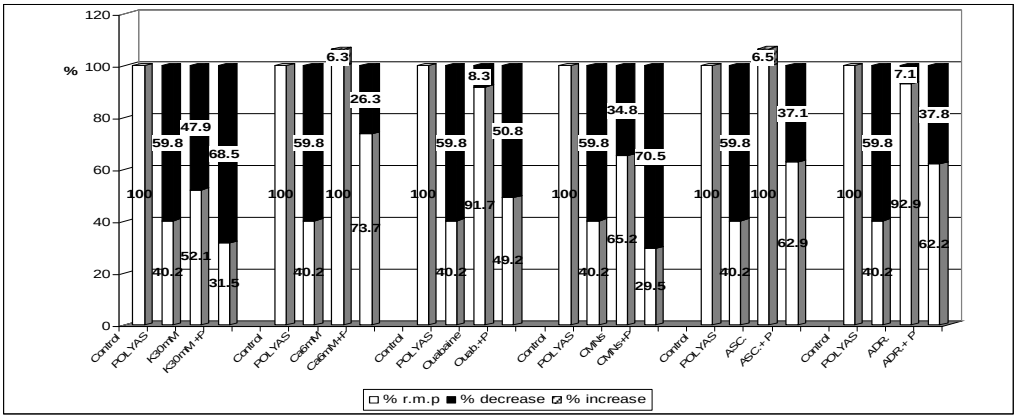

Figure 2 Percentage variations of resting membrane potential (RMP) of the HEp-2p neoplastic cells treated with POLYAS biopreparation and one of the cytophysiological modulator agents

Although the ouabaine and adrenaline interact with the membrane structures of the healthy human or animal cells, they have a minimal influence upon membrane bioelectrical activity. In our experiment, performed on neoplastic cells, the RMP decreases, also illustrate unsignificant depolarizations of 8.3% and respectively, 7.1%.

Contrary, the incubation of the cancerous cells in the hypercalcic medium or in the presence of ascorbic acid (ASC) has been followed by an easy perturbation of the biomembrane bioelectrical activity, but the registered hyperpolarizations were of reduced degrees (6.3% or 6.5%), without statistical significance.

It must be also revealed that the HEp-2p tumoral cells submitted to the associated treatments with POLYAS and one from the reference cytophysiological modulators have presented a

changed RMP, both as sense and as amplitude in relation to control value and to the one corresponding to the singular treated cultures.

If the singular treatment with POLYAS, K^{30mM}, Ouab., CMNs and respectively, ADR has induced RMP decreases of 59.8%, 47.9%, 6.3%, 34.8% and respectively 7.1%, the cellular action of Ca^{6mM} or ASC was correlated with RMP moderate increases of 6.3% or 6.5%, these percentages having role of reference values. In the light of the depolarization effect amplitudes – assessed in comparison to the control RMP value – registered in the polyphenolic, hyperpotassic, ouabain, nystatine and respectively adrenalinic singular treatments, the association of POLYAS cytostatic agent with one from the mentioned modulators has determined an amplification of the depolarizing impact (68.5% for K^{30mM} + POLYAS, 50.8% for Ouab. + POLYAS, 70.5% for CMNs + POLYAS and 37.8% for ADR. + POLYAS), which has exceeded the reference values.

Thus, we have appreciated an enhancement of the depolarizations induced by this kind of combined treatment in relation to the singular treatments with POLYAS, K^{30mM}, ouabine, nystatine and adrenaline. Sometimes (K^{30mM} + POLYAS – 68.5%; CMNs + POLYAS – 70.5%) the depolarization amplitude is greater than that corresponding to reference values (for POLYAS 59.8%; for K^{30mM} – 47.9%; for CMNs – 34.8%). In this case we can assume a complementarity between actions, which can be expressed by a summation or by an emphasis of the depolarizing effects. In the other cases (Ouab. + POLYAS; ADR. + POLYAS), we have pointed out that the extent of the registered depolarizations is greater than the one corresponding to ouabain (8.3%) and adrenalin (7.1%) and, in the same time, smaller than that correlated to POLYAS (59.8%).

In the context of the light hyperpolarizing effect – estimated in comparison to the control RMP value – registered in the hypercalcic and ascorbic acid treatments, the association of POLYAS cytostatic agent with one from the mentioned modulators has canceled the hyperpolarizing impact of the Ca^{6mM} or ASC and has induced the depolarizing effect of the POLYAS, but its action was attenuated as intensity (26.3% for Ca^{6mM} + POLYAS and 37.1% for ASC + POLYAS) in comparison to the one of the polyphenolic singular treatment.

The lack of poise between the structural components of the tumoral cell membranes, the decrease of the membranary fluidity, the modification of the packing degree of the membrane overmolecular structures, the different topographical location and activity of the membrane ATP-ases are functionally expressed by perturbation of the membranary permeability. The modification of the ionic fluxes leads to the appearance of the transmineralization phenomenon. This specific feature of the neoplastic cells consists in an abnormal distribution of the ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^- etc.) correlated with other ionic ratios in extra- and intracellular compartments and with a decrease of the membrane resting potential. Among other membranary peculiarities of the tumoral cells, it is important to mention the powerful enhancement of the activity of the Na^+–K^+–depending electrogenic pump (Benga, 1979; Chaubal and Firket, 1979; Binggeli and Cameron, 1980; Bianchi et al., 1986; Rusu et al., 1988; Bannasch et al., 1998; Cruce, 1999; Olbe, 1999; Miron, 2000; Owens, 2001; Wong, 2002). Despite these structural and functional alterations of the cell membrane, the diversity of the glycolipoprotein complex molecules from the structure of the tumoral cells membranes – similar to normal, healthy cells – represent numerous and important targets of the action of the chemotherapeutic agents. Thus, a primary membranotropic action mechanism – with inherent membrane and intracellular negative consequences, principally upon the tumoral cells cytophysiology – is implied in expression of a pharmacodynamic antineoplastic effect.

In previews papers (Rotinberg et al., 2004) we have demonstrated, on adequate experimental models, that the POLYAS polyphenolic biopreparation interacts with membrane structures of the

neoplastic cells, involving a preferential actions mechanism of membranary type in inducing the cytostatic effect. In our attempt to optimize the tumorsuppression effectivness of the POLYAS cytostatic, by an associated type therapy, we have preliminarly followed to promote its interactions with the cell membrane by an initial cytophysiological modulation of the tumoral cells with some standard membranoropic or metabolic agents.

The comparative analysis of the present results has pointed out that the HEp-2p cells incubation with one from the reference bioactive agents has conditioned: more or less extensive diminution of the RMP values (POLYAS, K^{30mM}, Ouab, CMNs, ADR) or its minimum increase (ASC) as compared to contro level; the more or less strong inhibition of the membrane Na^+–K^+–ATP-ase activity (POLYAS, Ouab., CMNs, ASC) and the moderate stimulation of this membranary enzymatic activity (K^{30mM}, Ca^{6mM}, ADR). Some reference cytophysiological agents (K^+, CMNs, Ca^{2+}, Ouab.) have induced functional destabilization or stabilization of the cellular membrane, membrane Na^+–K^+–pump activity blockage, membrane permebilization, by the influencing the membrane fluidity and of the packing degree of the membrane supramolecular structures. On the background of these known effects, the specific actions of the POLYAS biopreparation upon some membranary processes – in the case of the associated treatment with one from the standard modulators – registers some deviations. These suggests the modulation of the membrane enzymatic activity and membrane bioelectric activity.

Most of the times, the specific interaction of POLYAS with the membrane structures is preserved, registering only a quantative modification of expressing degree of its effect upon the membrane Na^+–K^+–ATP-ase activity and upon the RMP (resting membrane potential). Thus, we have noticed, on one hand, an enhancement of the Na^+–K^+–pump activity repression and a membrane depolarization in the case of POLYAS with K^{30mM} or CMNs association, and on the other hand, an attenuation of the Na^+–K^+–ATP-ase inhibitory level and of the membrane depolarization, in the case of the POLYAS with Ca^{6mM}, Ouab or ASC double impact. These facts suggest that in the first type of the association there is different membrane situses for the drugs interactions and in the second type of association there is a competition towards the same membranary molecular substratum of the associated agents` interaction.

However, sometimes the treatment association conditions the loss of the POLYAS specific effect, installing a stimulation of the Na^+–K^+–ATP-ase activity, which is a characteristic for the associated agents (ASC, ADR) and not for POLYAS. This new situation is probably due either to the blockage of the POLYAS interactions with its specific membrane structures or to the realization of another interactions which amplifies the ADR or ASC effects.

Therefore, the preliminary modulation of the membrane processes – illustrated by membrane Na^+–K^+–ATP-ase activity and RMP values – by the reference cytophysiological agents influences the interaction conditions of the POLYAS cytostatic with the membrane biomolecules. Consequently, we assist to a conditioning of a new mode of expression of the POLYAS membrane effects by the membranotropic or metabolic standard modulators.

CONCLUSIONS

It is confirmed the membranotropic action mechanism implied in inducing the pharmacodynamic effect of the POLYAS cytostatic of polyphenolic nature.

The sort-lasting *in vitro* cytostatic treatment of the HEp-2p human cancerous cell – initially cytophysiologically sensitized by membranary and metabolic standard association agents – with the polyphenolic biopreparation has been correlated with quantitative and qualitative modulations of its characteristic membrane effects.

The sense and amplitude of the POLYAS effect` influence upon Na^+–K^+–ATP-ase and bioelectric membrane activities by standard cytophysiological modulators represent the consequence of the functional synergism, antagonism or complementarity between the associated agents which interact with similar or different membrane receptors.

The interactions of the POLYAS and respectively, of the standard membranary or metabolic agents with the same or another membrane biomolecules – with inherent consequences upon the membrane permeability, transmembranary transport and intracellular metabolic events – can condition the cytostatic effectiveness of the polyphenolic biopreparate.

REFERENCES

1. Alberts, B., Bray, O., Johnsons, A., 1998., Garland Publ. Inc. N.Y., London, 193-553
2. Anderson, S., Chiplin, J., 2002**.** *Drug Disc. Today*, 7, 105–107
3. Artenie, V., Tănase, Elvira, 1981., *Practicum de biochimie generală,* Ed. Universităţii „Al. I. Cuza", Iaşi, 128-133
4. Bannasch, P., Kanduc, D., Papa, S., Tager, J.M., 1998., *Cell Growth and Oncogenesis*, Birkhäuser Verlag, Basel
5. Benga, G., 1985.*„Biologie celulară şi moleculară,* Ed. Dacia, Cluj-Napoca, 47-130; 209-277
6. Bianchi, G., Carafoli, E., Scarpa A., 1986., *Membrane pathology,* N.Y. Academy of Sciences, Vol. 488, 430-491
7. Binggeli, R., Cameron, I. L., 1980. *Cancer Res.,* 40, 1830-1835.
8. Boyd, M.R., 1989. *Cancer: Princ. Pract. Oncol. Updates*, 3, 1-12
9. Bustamante, E., Morris, H., Pedersen, P., 1981. *The Journal of Biological Chemistry*, 256, 8699–8704
10. Chaubal, K., Firket, H., 1979. *Sciences Soc. Biol.,* 173, 627-631.
11. Chiricuţă, I., 1988. *Cancerologie,*II, Ed. Med. Bucureşti
12. Cruce, M., 1999.*Biologie celulară şi moleculară,* Ed. Aius, Craiova, 14-25; 33-56, 141-161; 193-300
13. Habeck, M., 2002. *Drug Discovery Today*, 7, 635–637
14. Karp, G., 1996. *Cell and Molecular Biology,* John Wiley and Sons, INC., N.Y., Brisbane, Toronto, 694-725
15. Lyden, D., Hu, Z., Caren, A., Kresty, L.A., 2001**.** *Drug Discovery Today*, 6, 1252–1254
16. Miron, L., 2000. *Oncologie generală*¸ Ed. Egal, Bacău, 9-131
17. Neacşu I., Agrigoroaei Şt., Crăciun M., Crăciun V., Rotinberg P., Kelemen S., Nuţă V., Oiţă N., (1996), Rev. Roum. Biol.-Biol. Anim., 41 (2): 165-169
18. Olbe, L., 1999. *Proton pump inhibitors*, Parnham, M., Bruinvels, J., Eds., Birkhäuser Verlag, Basel-Boston-Berlin
19. Owens, J., 2001. *Drug Discovery Today*, 6, 1203–1206
20. Rotinberg, P., Nuţă, Violeta, Kelemen, Smaranda, Petraşincu, Doina, Rotinberg, Hellen, 1998. *Romanian Journal of Physiology,* 35, 91–98
21. Rotinberg, P., Kelemen, Smaranda, Grămescu, Mihaela, Rotinberg, Hellen, Nuţă, Violeta, 2000. *Romanian Journal of Physiology,* 37, 91–103
22. Rotinberg, P., Kelemen, Smaranda, Grămescu, Mihaela, Rotinberg, Hellen, Nuţă, Violeta, 2000. *Romanian Journal of Physiology,* 37**,** 105–118
23. Rotinberg P., Gherghel D., Neacşu I., Rotinberg H., Mihai C., 2004, Analele Ştiinţifice ale Universităţii "Al. I. Cuza" Iaşi, Secţia Genetică şi Biologie moleculară*,* V: 44–51.
24. Rotinberg P., Gherghel D., Neacşu I., Rotinberg H., Mihai C., 2004, Analele Ştiinţifice ale Universităţii "Al. I. Cuza" Iaşi, Secţia Genetică şi Biologie moleculară*,* V: 51–57.
25. Rotinberg P, Gherghel D., Grămescu M., Mihai C., Neacşu I., Hefco V., Rotinberg H., 2005, Analele Ştiinţifice ale Universităţii „Al. I. Cuza" Iaşi, SII, Genetică şi Bio. Mol., V, 75–82.
26. Rusu, V., Baran, T., Brănişteanu, D., D., 1988., Ed. Medicală, Bucureşti, Vol. I, 63-87; 395-435
27. Seethala, R., Prabhavathi F., 2001. *Drugs Pharm. Sci.,* 114**,** 5–520
28. Snedecor, G.W., 1968.*, Metode statistice aplicate în agricultură şi biologie,* Ed. Did. Ped., Bucureşti
29. Wong, J.M.Y., 2002. *Drug Discovery Today*, 7, 1072–1073

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