EFFECT OF SOME ACTIVE CYTOSTATIC VEGETABLE EXTRACTS OF POLYPHENOLIC NATURE UPON MEMBRANE BIOELECTRICAL POTENTIAL

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Abstract: The in vitro short lasting antitumoral treatment of the HEp-2p and HeLa human tumoral cells with some active cytostatic polyphenolic biopreparations has induced a decrease in the resting membrane potential. This depolarization effect of the POLYAS I and POLYAS II vegetable extracts, as well as, their inhibitory impact upon the membranary $Na^+ – K^+$–ATP-ase, argue the interference between these autochthonous antitumoral agents and some membrane processes. Therefore, it can be suggested that the interaction of the polyphenolic biopreparations with membranary receptors represents the most probable mechanism involved in the expression of their cytostatic property.

INTRODUCTION

The activation or the perturbation of the molecular mechanisms of the cellular functional regulation is mostly dependent on transformation of extracellular information in an action of normal or abnormal cellular response. In this case, the starting molecular event is logically localized at the level of the environment–cell interface, meaning in the cellular membranes. After this primary interaction between an active biological agent and a cell membrane receptor, there takes place the extracellular signal's transfer and traducing. Consequently, the intracellular mechanisms of control and the activity of the enzymatic systems will be influenced. These specific modulations would stimulate and inhibate the different cell processes which will represent, the subcellular and molecular substratum implied in the global expression of a pharmacological effect (Benga 1985; Alberts et al., 1998; Stroescu, 1998; Cruce, 1999, Karp, 1996).

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 both for the characterization 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) and for preliminary appreciation of their mechanism of action which seems to be one of membranotropic type (Rotinberg et al., 2004).

Consequently, it has been imposed the extending and thoroughgoing of the research regarding the membranary mechanisms involved in tumor suppressor impact inducing.

Thus, the purpose of the present work is to investigate the behaviour of the resting membrane potential in the conditions of the in vitro action of POLYAS I and POLYAS II cytostatic agents of polyphenolic nature upon the HEp-2p and HeLa tumoral cells.

MATERIALS AND METHODS

The POLYAS I and POLYAS II aromatic extracts of polyphenolic type – separated and purified from *Asclepias syriaca* phytomass – which were used in the in vitro experiments have been described in the precedent papers (Rotinberg et al., 1998; Rotinberg et al., 2000; Rotinberg et al., 2000; Rotinberg et al., 2004).

The biological material used in the in vitro investigations was represented by the control and treated HEp-2p and HeLa cellular cultures of human neoplastic origin (laringeal carcinoma and cervix carcinosarcoma, respectively).

The HeLa and HEp-2p cultures were performed in Petri dishes with a Noble Agar solid substratum, by inoculation of the Eagles' MEM growing medium supplemented with 10% calf serum with 1 x 105 tumoral cells. The cultures were incubated at 37° C for a period of 48 hours.

After 48 hours of their development, when the monolayer stage was attained, the initial medium was replaced with a medium containing the polyphenolic biopreparation 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.

The normal and short lasting treated cultures were studied, at the room temperature, from point of view of the membrane bioelectrical activity. The membrane bioelectrical effect of the active biological biopreparations has been appreciated by the comparative analysis of the control and experimental values of the membrane resting potential (RMP; -mV), which was recorded by the method of the glass intracellular microelectrodes (Neacşu et al., 1996).

Five cultures of each type have been employed for statistical analysis of the results, by means of Student' „t" test (Snedecor, 1968).

REZULTS AND DISCUSSIONS

In an initial experiment we have investigated, on 48 hours old HEp-2p cultures, the interference of the active cytostatic polyphenolic extracts with the membrane bioelectrical activity in relation to control one, the normal and experimental RMP values – quantitative and percentage expressed – being included and graphical illustrated in Table 1 and Figure 1.

Table 1. The resting membrane potential (-mV) of the in vitro short lasting treated HEp-2p tumoral cells with the POLYAS I and POLYAS II active cytostatic polyphenolic biopreparations (10 mg/ml). Figures in brackets indicate the number of cultures for each type.

Culture types	X ± ES (–mV)	p
Control	34.62 ± 3.48 (12)	–
POLYAS I	16.58 ± 1.89 (12)	<0.001
POLYAS II	13.93 ± 1.43 (12)	<0.001

It is observed that the in vitro short antitumoral treatment of the HEp-2p cell cultures has induced a statistically significant regression of the RMP, in comparison with the control mean value.

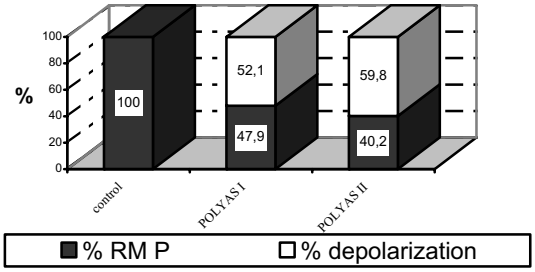

Fig. 1. Percentage variation of resting membrane potential (RMP) of the HEp-2p neoplastic cells submitted to the action of the POLYAS I and POLYAS II vegetable polyphenolic biopreparations.

The Figure 1 reveals that the RMP of the treated cellular cultures reaches levels of 47.9 and respectively 40.2%. Thus, comparatively with the 100% membrane bioelectrical

activity of the control HEp-2p neoplastic cells, we assess a fast post treatment depolarization of 52.1%, in the case of POLYAS I, and of 59.8%, in the case of POLYAS II.

In Table 2 and Figure 2 there are inserted the results regarding the membrane bioelectrical activity – expressed in –mV and in % – registered after the short lasting in vitro cytostatic treatment of the 48 hours old HeLa tumoral cell cultures with the bioactive polyphenolic biopreparations.

Table 2. The values of the resting membrane potential (-mV) recorded at the neoplastic HeLa cell cultures of 48 hours, submitted 180 minutes to the action of the POLYAS I and POLYAS II cytostatic agents, in dose of 10 mg/ml, comparatively with the control cultures. Figures in brackets indicate the number of cultures for each type.

Culture types	X ± ES (–mV)	p
Control	32.58 ± 3.46 (12)	–
POLYAS I	16.00 ± 1.71 (12)	<0.001
POLYAS II	14.70 ± 1.30 (12)	<0.001

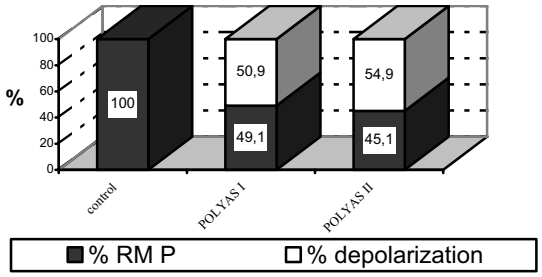

Fig. 2. Modulation of the membranary bioelectrical activity of the HeLa cancerous cell cultures by the POLYAS I and POLYAS II cytostatic agents of polyphenolic nature.

Consequently, it can be said that the cancerous cells submitted to the in vitro antitumoral treatment with POLYAS I and POLYAS II vegetable extracts have again presented a negative modification of the bioelectrical activity. Thus, after the interaction between the cytostatic agents and the membranary structures, the RMP values evidence a significant decrease, in relation with the control one, fact which argues the installation of the same quick membrane depolarization. The amplitude of this effect is of 50.9 and respectively 54.9% for the POLYAS I and respectively POLYAS II treatment.

A greater degree of depolarization can be seen at the HEp-2p and respectively HeLa cultures submitted to the action of POLYAS II.

Generally speaking, the behaviour of the resting membrane potential is relevant for the characterization of the sense and intensity of the passive and active transmembranary ionic flows between the extra- and intracellular compartments, they depending on electrochemical gradient and on membrane Na^+-K^+-ATP-ase activity. Therefore, the bioelectrical activity expresses the state both of supramolecular structural organization and of the permeability of the cell membrane (Rusu et al., 1988; Alberts et al., 1998; Bannasch et al., 1998; Cruce, 1999; Olbe, 1999).

The bulk of our results highlights the alteration of the membrane bioelectrical activity, which consist of a decrease of the resting membrane potential, caused by in vitro polyphenolic action upon the HEp-2p and HeLa neoplastic cells. The intensity of the induced depolarization is dependent on the quality of polyphenolic extract. There is a smaller impact in the case of POLYAS I and a greater effect in the case of POLYAS II, the last agent being characterized by a stronger in vitro and in vivo effectiveness (Rotinberg et al., 1998; Rotinberg et al., 2000; Rotinberg et al., 2000).

It can be also emphasized that our RMP control values – registered on the HEp-2p and HeLa tumoral cell cultures of the same age but untreated – are compatible with those which are mentioned in the specialty bibliography (Chaubal et al., 1979; Binggeli et al., 1980; Churchill et al., 1980) and reveal the low level of this membranary functional parameter, as compared with the RMP values of the normal healthy cells.

This peculiarity of the neoplastic cells is result of their structural and functional membrane alterations, which are involved in the inducing of transmineralization phenomenon.

The present data are correlated with the inhibitory effect of the membrane Na^+-K^+-ATP-ase depending electrogenic pump induced by these active cytostatic polyphenolic biopreparations, effect which was signaled in the previous paper from this journal (Rotinberg et al., 2004).

The negative modulation the RMP – determined by the short lasting action of the polyphenolic cytostatics upon the tumoral cells – arises a question. How can we explain the causality of this membranary effect?

The answer is most probably given by the interaction between the bioactive polyphenolic agents with some membranary receptor molecules which leads to a perturbation of the membrane permeability of the treated cancerous cells.

The regression of the resting membrane potential can be the consequence of the enhancement of active or passive cationic transport mechanisms from the extracellular medium to intracellular compartment. Nevertheless, the active transmembranary ionic flows are diminished by the inhibitory impact of the polyphenolic cytostatics upon membrane Na^+-K^+-ATP-ase activity.

Therefore, the augmentation of cationic influx is the result of the increase of membrane passive permeability facilitated by the ionic gradient. In this case, the mechanism of the bioelectrical effect – the membrane depolarization – is probably represented by a reduced packing degree of the membrane overmolecular structures, correlated with concomitantly increased membranary fluidity in a molecular labilization, which determines an intensification of the transmembranary passive ionic fluxes and a consecutive decrease of the ionic gradients.

At the end, we can again appreciate that the primary interactions between the polyphenolic biopreparation and tumoral cells take place at membranary level, they being succeeded by a “cascade” of cellular, subcellular and molecular events, which express finally the cytostatic impact of the polyphenolic agents.

CONCLUSIONS

The in vitro short lasting treatment of the 48 hours old HEP-2p and HeLa tumoral cell cultures – with the POLYAS I and POLYAS II autochthonous cytostatic extracts – has highlighted a decrease in the RMP.

This effect is positively correlated with the inhibitory impact of the vegetable polyphenolic biopreparations upon the membrane Na^+-K^+ electrogenic pump.

The complementary and/or simultaneous membrane double reactivity to the action these biological active products of polyphenolic nature is relevant for the argumentation of the most probable involvement of a mechanism of membranary type in inducing the antineoplastic pharmacodynamic effect.

Without generalizing this main membranary mechanism of action we consider that it is not impossible for some hydrophilicity or lipophylicity compounds – with simple organic structure and small molecular weight – from the composition of the natural polyphenolic extracts, to penetrate into the tumoral cells through the cell barrier. Here, they would interact with the intracellular receptors modifying the cells processes, which will assure the substratum of their cytostatic property.

In order to elucidate the mechanism/ mechanisms responsible for the antitumoral impact of the autochthonous polyphenolic biopreparations further investigations are necessary.

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