IN VITRO CYTOSTATIC EFFECT OF SOME NON-IONIZING ELECTROMAGNETIC FIELDS

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Abstract: The *in vitro* action of some kinds of electromagnetic fields, generated by a magnetodiaflux apparatus, upon the proteinsynthesis of HeLa cancerous cells and of RM healthy renal cells, implicitly, as well as upon the development of the corresponding cellular cultures was investigated. The significant perturbation of proteic biogenesis, the modification of the protein dynamics, the inhibition of the cell cultures development and the existence of a dose-response relationship argue the behaviour of the low intensity and frequency electromagnetic field as *in vitro* active cytostatic agent. The registered data have revealed that the intensity of the induced biological effects of EMFs is dependent on the *in vitro* model, intensity and type of electromagnetic field, exposure time, metabolic state and type of the exposed cells. This primary characterization of the low intensity and frequency fields as cytostatic agent justifies the study of their effect upon cell proliferation and viability in order to enlarge the reasoning basis for the introduction of this physical agent in the *in vivo* antitumoral screening program on different experimental tumoral systems.

INTRODUCTION

At present, the *in vivo* or *in vitro* effects of the low frequency and intensity electromagnetic fields aren't totally known, unanimously accepted and discussed from the point of view of their probable action mechanism. This fact is caused by the great number of experimental data from biomagnetic research and by their contradictory and heterogeneous character. The results of the studies – performed on different animal organisms or animal normal cellular systems – have shown both positive (with neurological, endocrinological, immunological, hematological, locomotor expression) and negative (fertility diminution, memory deficiency, growth and development disorders, involvement in the carcinogenetic process) impacts, without the elucidation of the cellular, subcellular or molecular substratum with which the electromagnetic field interacts in the starting of these effects [Abbro et al., 2004; Ailiesie, 1996; Brune, 2003; Chionna et al., 2003, 2005; Dini and Abbro, 2005; Guimaraes and Linden, 2004; Jitariu, 1987; Karasek and Lerchl, 2002; Marinelli et al., 2004; Moreira and Barcinski, 2004; Pagliara et al., 2005; Rosen, 2003; Tarantino et al., 2005; Tenuzzo et al., 2006; Teodori et al., 2002; Tofani et al., 2003; Wartenberg, 2001; Zamfirescu et al., 2000].

The reactivity of some cytophysiological processes of the healthy animal cells and its capitalization in different therapeutic purposes – especially in the locomotory disorders – [Ailiesei, 1996; Jitariu, 1987; Zamfirescu et al., 2000] suggested to us the utility and importance of a complex research of the interaction between the low frequency and intensity electromagnetic fields and the neoplastic cells, in obtaining the scientific basis adequate to conceive some new and efficient antineoplastic therapeutic strategies.

Thus, in the previous [Rotinberg et al., 2007] work we have investigated the *in vitro* reactivity of some membranary and metabolic processes of the HeLa tumoral cells to the action of two electromagnetic field types (continuous and discontinuous) of low frequency (100 Hz) and intensity (5.5 mT). Our experimental results, registered after an unique short lasting treatment of HeLa cells with continuous or discontinuous electromagnetic field, in relation to the control cytophysiological profile, have highlighted: the modulation of the membrane Na^+–K^+–ATP–ase activity; the modification of ionic transmembranary fluxes; the establishment of some new values of the extra- and intracellular ratios, as well as new conditions for the diverse intracellular enzymatic systems' activity; the intensifications of te glycogenogenesis, lipogenesis and proteinosynthesis; the stimulation or inhibition of the nucleic acids biosynthesis; the attenuation of the cholesterolgenesis; the activation of the intracellular metabolic utilization of the glucose, lactic acid, free fatty acids and aminoacids biomolecules as anabolic sources and fuel resources. This specific membranary and metabolic behaviour of HeLa neoplastic cells, submitted to EMF action, can be the consequence of the primary interaction of the physical agent either with the plasmatic membrane or with subcellular, intracellular and molecular structures (from organelles, nucleus) which modifies the gene pattern expression, the oxygen free radicals production as well as the activity of some metabolic key enzymes [Brune, 2003; Chionna et al., 2003, 2005; Dini and Abbro, 2005; Marinelli et al., 2004; Stevens, 2004; Tenuzzo et al., 2006].

No matter the cellular level of the primary interaction mechanism, the low frequency and intensity electromagnetic fields have induced obvious, significant and indubitable modifications of some membranary and metabolic processes, which perturb "the new" steady-state of the tumoral cell, suggesting even an own cytostatic property, dependent on the electromagnetic field type.

Consequently, we have decided, in the present paper, to evidence and to evaluate the cytostatic action of the continuous and discontinuous, low frequency and intensity electromagnetic field by investigating its effect upon the

proteinbiosynthesis and development of tumoral cellular cultures, in comparison to the behaviour of the healthy monkey renal cells.

MATERIALS AND METHODS

The biological material used in the *in vitro* investigations was represented by stabilized, normal RM cells, obtained from monkey's kidney, and by HeLa cellular cultures of human neoplastic origin (uterine cervix carcinosarcoma) [Bissery and Chabot, 1991; Buskirk et al., 1973; Phillips et al., 1991; Seethala and Prabhavathi, 2001]. The test flasks of 75 cm^2 have been inoculated with 1 x 10^6 normal or tumoral cells in Eagles' MEM growing medium supplemented with 2% (for the RM cells) or 10% calf serum (for the HeLa cells), penicillin and streptomycin solution 100 I.U./ml and nystatin antimycotic solution 10.000 U/ml. The cells were incubated at 37^0 C for a period of 72 hours of culture development**.** When the monolayer stage was attained, the cultures were divided into control and treated cell cultures.

The electromagnetic field (EMF) was generated by a magnetodiaflux apparatus, which has two coils of 29 cm diameter, situated at 14.5 cm one in front of the other and arranged on a cardboard tube, this being the precinct were the biological material was placed during the treatment. The electromagnetic field produced has the frequency of 100 Hz, the intensity of 5,5 mT and was applied continuously or discontinuously (with a cycle on/off by 3/1 seconds) all along the treatment.

At the end of this *in vitro* short term electromagnetic treatment, the cell layer was detached with a solution of trypsin and EDTA and then resuspended in Eagles' MEM supplemented with 2% or 10% calf serum, the control and treated cellular suspensions being used for the inoculation of test tubes, with $1x10^5$ cells/per tube. The test tubes were incubated for 24, 48 and 72 hours respectively at $36,5^0$ – 37^0 C. In these three periods of the RM and HeLa cultures evolution, the medium was discarded from the test tubes. The layer of tumoral cells was washed with phosphate buffered saline solution and then subjected to the biochemical determination of total proteins (expressed in µg protein/culture) through the successive steps of the Lowry method modified by Oyama [Oyama and Eagle, 1956]. Appreciation of the *in vitro* cytostatic effect has involved a comparative analysis of the evaluation indices values (proteinsynthesis intensity, protein dynamics and culture development degree) recorded by us with those imposed by the selection criteria of antitumoral agents, established by the preclinical screening program of the National Institute for the Cancer of USA for this preliminary selection step [Boyd, 1989, Leiter et al., 1965].

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

RESULTS AND DISCUSSIONS

In an adequate experimental model we have investigated the *in vitro* cytophysiological behaviour of the healthy and tumoral cells cultures– performed from 72 hours cultures, submitted or not to the single continuous and discontinuous EMF treatment, for a period of 30 or 60 minutes – during their evolution up to 24, 48 and respectively 72 hours after the inoculation of the test tubes with those two cell types.

The evaluation indices of the daughter cell cultures reactivity to the electromagnetic treatment of the parental cultures were: the proteinsynthesis intensity; proteic dynamics and the cell cultures development degree, the registered experimental date being included and graphically illustrated in Table 1, Fig. 1 and 2.

It can be observed, both in the case of the untreated RM healthy cells cultures and in the case of the control HeLa neoplastic cell cultures, a progressive augmentation of about 10 – 20% of the total protein content from 24 hours age up to 72 hours age, the cellular proteic values being somewhat greater at the normal cells than those of the tumoral cells, in relation to their metabolic peculiarities.

The successive graphical transposition of the total proteins values, obtained at different time intervals of cell cultures evolution, traces the proteinosynthesis dynamics, which in the case

of the untreated healthy and tumoral cellular cultures, are characterized by an ascendant route with progressive increased amplitude. These characteristics of the untreated cell cultures are the expression of an inherent protein synthesis enhancement conditioned by the cell proliferation process, which assures the normal development of the control cultures, considered by us as reference procentual value (100%). (SEE APPENDIX)

In comparison with the control RM cellular cultures, it can be seen that the evolution of electromagnetic treated monkey's kidney cell cultures is characterized – at 24, 48 and 72 hours ages – by lower total protein concentrations. This negative quantitative variation has different degrees, it being nonsignificant in the case of the 30 or 60 minutes continuous EMF exposure and statistically significant at the action of discontinuous EMF treatment of 30 and 60 minutes.

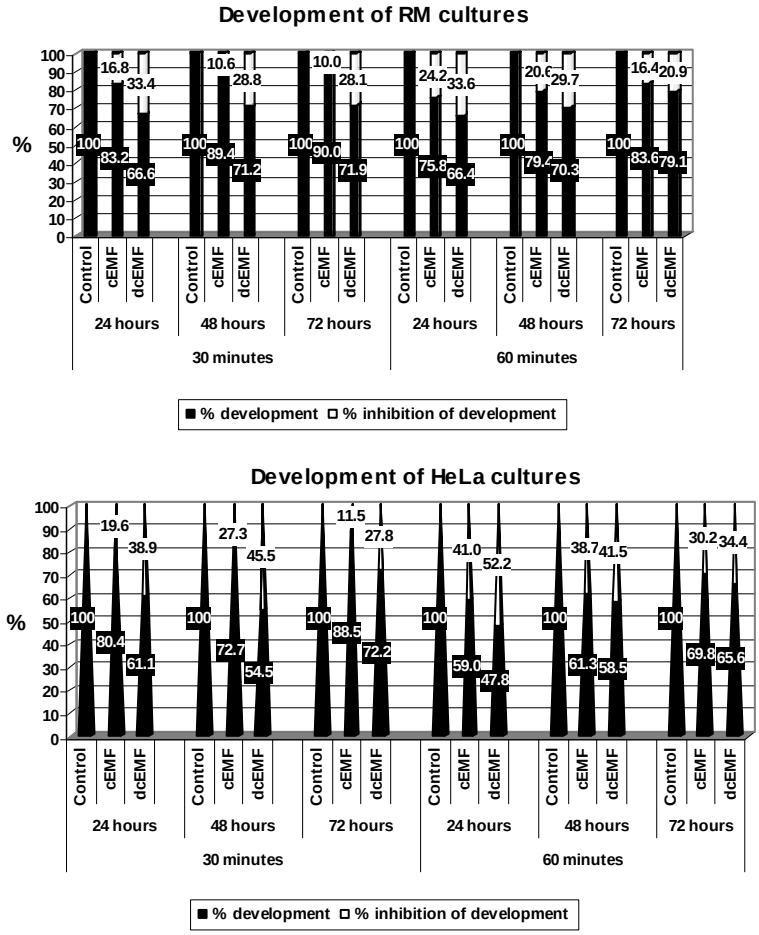

Fig. 2. The development degree of healthy and cancerous cell cultures, during their evolution, in the conditions of the short time action of some non-ionizing electromagnetic fields.

Similar results as direction but not as amplitude were registered with the tumoral HeLa

cellular cultures performed from the electromagnetically treated parental cultures. Thus, comparatively with the corresponding proteic values, total protein contents of the continuous or discontinuous EMF treated HeLa cultures – registered at the different time intervals from cultures evolution–were nonsignificantly smaller in the case of the continuous electromagnetic application and significantly decreased in the case of treatment with discontinuous electromagnetic field.

Certainly, in this context, the protein biosynthesis dynamics has presented modifications both of direction and of amplitude, the route and level being descendent and reduced.

Finally, the comparative analysis of the total protein values, estimated at the diverse ages of the electromagnetic treated cultures, confronted with those of control, has highlighted that the electromagnetic fields disturb the culture development process. The electromagnetic exposures have been correlated to different degrees of development. Thus, the treated RM and HeLa cultures have reached the following development levels:

– in the case of the 24, 48 and respectively 72 hours healthy renal monkey's cellular cultures: 83.2%, 89.4% and respectively 90%, as well as 66.0%, 71.2% and respectively 71.9%, after the 30 minutes treatment with cEMF and dcEMF; 75.8%, 79.4% and respectively 83.6%, as well as 66.4%, 70.3% and respectively 79.1%, after the 60 minutes treatment with continuous or discontinuous EMFs;

– in the case of the various ages neoplastic HeLa cellular cultures: 80.4%, 72.7% and respectively 54.5% as well as 61.1%, 54.5% and respectively 72.2% after the 30 minute application of continuous or discontinuous electromagnetic fields; 59.0%, 61.3% and respectively 69.8% as well as 47.8%, 58.5% and respectively 65.6% after the 60 minutes exposure to continuous or discontinuous EMFs.

As compared to the 100% reference development value, an inhibitory impact of the EMFs upon the RM and HeLa cultures development can be evidenced and assessed, its intensity being chronological:

– in the case of healthy cellular cultures 16.8%, 10.6% and respectively 10% as well as 34.0%, 28.8% and respectively 28.1% for the 30 minutes continuous or discontinuous electromagnetic treatment; 24.2%, 20.6% and respectively 17.4% as well as 33.6%, 29.7% and respectively 20.9% for the single 60 minutes cEMF or dcEMF application;

– in the case of the tumoral cellular cultures: 19.6%, 27.3% and respectively 11.5% as well as 38.9%, 45.5% and respectively 27.8% after the single shorter electromagnetic exposure with continuous or discontinuous EMFs; 41.1%, 38.7% and respectively 30.2% as well as 52.2%, 41.5% and respectively 34.4% after the single longer electromagnetic exposure after continuous or discontinuous EMFs.

It is clear that any factors influencing cell surface structures, molecules, subcellular and molecular molecules can in turn affect the cytophysiological state of the cells. The EMF can, nonetheless, induce cellular and molecular modifications when interacting with biological materials (whole organisms, cellular systems). Cellular responses depend on the duration of exposure, the tissue penetration, the heat generation, the intensity and frequency of EMF, the type of EMF, the form of the wave, the biological status of the exposed cells, those suggesting a very complicated interaction among the various factors [Abbro et al., 2004; Chionna et al., 2005; Dini and Abbro, 2005, Guimaraes and Linden, 2004; Pagliara et al., 2005; Saffer, 1996; Schenck, 2000; Tarantino et al., 2005; Tenuzzo et al., 2006].

In this context, we have extended the study of the *in vitro* specific interactions of some electromagnetic field types with different cell lines (healthy and tumoral), in order to evidence

their cytostatic property, as a starting point for the investigation of the *in vivo* EMFs therapeutic significance upon the tumorigenesis processes.

Our above presented experimental data, registered on the untreated and treated RM or HeLa cellular cultures, highlight that the continuous or discontinuous electromagnetic exposures – for a time of 30 or 60 minutes – have induced, in the descendant cells, a progressive diminishing of the total protein contents, an alteration of the protein dynamics and an inhibition of the cellular cultures development, explained in this moment only on the basis of the signaled proteinsynthesis inhibitory impact of the electromagnetic fields.

The proteic stock regression is correlated both with the electromagnetic treatment time and with the cell and EMF types. Thus, the shorter EMF application (30 minutes) has conditioned a smaller qualitative and quantitative variation than that induced after the *in vitro* irradiation of 60 minutes. It has been also revealed that the healthy RM cells are less sensitive to the EMF action than the HeLa neoplastic cells. Last but not least, the cellular reactivity is more intensive to the action of the discontinuous electromagnetic field.

The negative impact of the electromagnetic treatment upon cellular protein concentration is positively correlated to the proteinsynthesis dynamics, which is characterized by a descendent route and by decreased amplitude.

These consequences of the electromagnetic fields of low frequency and intensity can be due to an inhibitory effect of the physical agent on the cellular protein biosynthesis intensity. Indeed, the interaction of both kinds of EMF with one or both parameters was materialized by a clear perturbation of the cellular cultures development.

The inhibitory degree of the healthy and tumoral culture development expresses the existent correlation between cellular type, the time and manner of the EMF application. Thus, the inhibitory impact upon the cultures development has reached higher levels in the case of the tumoral culture, of the discontinuous electromagnetic field of low intensity and frequency and of the single longer (60 minutes) electromagnetic treatment.

The comparative analysis of the EMFs inhibitory potential upon tumoral cell proteinsynthesis and culture development with the standard value (at least 50 %) - stipulated by the American screening program [Boyd, 1989; Leiter et al., 1965] - for discovery of new antineoplastic agents, allows us to appreciate that although the electromagnetic field of low intensity and frequency used in this experimental model belongs to the moderate EMF category (1 mT – 1 T), has a significant cytostatic property in its discontinuous variant and to a treatment time of 60 minutes. It is necessary to mention that, in the same experimental parameters, the electromagnetic treatment doesn’t have a considerable negative impact upon the healthy cell cultures.

Contrary to the membanary and metabolic interactions of the continuous or discontinuous electromagnetic field, we signaled in a previous paper [Rotinberg et al., 2007], which have been detectable immediately after electromagnetic exposure, the present results have highlighted later effects of this physical agent in the descent treated tumoral cells.

However, the cytostatic effectiveness of the 60 minutes discontinuous electromagnetic treatment decrease in time, the lower level being registered 3 days from the treatment.

Generally speaking, a cytostatic action of a chemical, physical or biological agent represents the expression of the bioactive factor interaction with different cell structures and processes. In our case, this property was qualitatively highlighted and quantitatively estimated by following the proteinsynthesis during the cell cultures evolution with an immediate consequence upon the culture development, process complementarily assured also by cell proliferation.

Therefore, for an objective appreciation of the *in vitro* antitumoral property of the EMFs, the investigation of its effects upon cell mitosis and viability is necessary, this being the purpose of a future work of our research team. This primary characterization of the low intensity and frequency fields as cytostatic agent justifies its introduction in the *in vivo* antitumoral screening program, on different experimental tumoral systems [DeVita, 1991; Leiter et al., 1965].

CONCLUSIONS

The proteinsynthesis intensity attenuation, the modification of the protein dynamics and the inhibition of the cell cultures development, especially of the neoplastic HeLa cultures, outline an important cytostatic property of the low intensity and frequency electromagnetic field, especially of the discontinuous EMF, applied at least 60 minutes.

In relation to the multiplicity of experimental conditions, the registered data have revealed that the intensity of the induced biological effects of EMFs is dependent on the *in vitro* model, intensity and type of electromagnetic field, exposure time, metabolic state and type of the exposed cells.

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APPENDIX

Table I and Figure 1. The total protein contents (µg/culture) and the modulation of the protein synthesis processes of the RM and HeLa cellular cultures submitted, for 30 or 60 minutes, to the action of the electromagnetic field, in a continuous or discontinuous manner, comparatively with control cultures. Figures in brackets indicate the number of experimental cultures for each type.

Culture type	RM						HeLa					
	24 hours		48 hours		72 hours		24 hours		48 hours		72 hours	
	X ± ES	p	X ± ES	p	X ± ES	p	X ± ES	p	X ± ES	p	X ± ES	p
30 minutes												
Control	187.1 ± 8.9 (5)	–	201.4 ± 12.7 (5)	–	224.3 ± 14.5 (5)	–	182.9 ± 19.9 (5)	–	207.1 ± 6.1 (5)	–	228.0 ± 10.2 (5)	
cEMF	155.7 ± 8.9 (5)	<0.05	180.0 ± 11.4 (5)	NS	202.0 ± 13.8 (5)	NS	147.0 ± 9.5 (5)	NS	150.5 ± 10.8 (5)	<0.002	201.8 ±18.1 (5)	NS
dcEMF	133.3 ± 6.4 (5)	<0.002	132.9 ± 10.7 (5)	<0.01	161.4 ± 8.6 (5)	<0.01	111.7 ± 7.8 (5)	<0.02	112.9 ± 9.5 (5)	<0.001	164.6 ±11.0 (5)	<0.01
60 minutes												
Control	178.9±10.5 (5)	-	212.1 ± 9.8 (5)	-	238.7±12.5 (5)	-	142.5±10.0 (5)	-	196.6 ± 9.7 (5)	-	235.5±13.7 (5)	-
cEMF	135.6 ± 8.7 (5)	<0.02	168.4±11.9 (5)	<0.05	199.6±11.9 (5)	NS	84.1 ± 4.6 (5)	<0,001	120.5 ± 9.1 (5)	<0,001	164.4 ± 9.9 (5)	<0.01
dcEMF	118.8 ± 9.4 (5)	<0.01	149.1 ± 8.7 (5)	<0.002	188.9±13.5 (5)	<0.05	68.1 ± 3.9 (5)	<0,001	115.0±11.3 (5)	<0,001	154.5±10.3 (5)	<0.002