P-I-26

EFFECTS OF LOW INTENSITY STATIC ELECTROMAGNETIC FIELDS (SEMF) ON SARCOMA CELL LINES IN VITRO.

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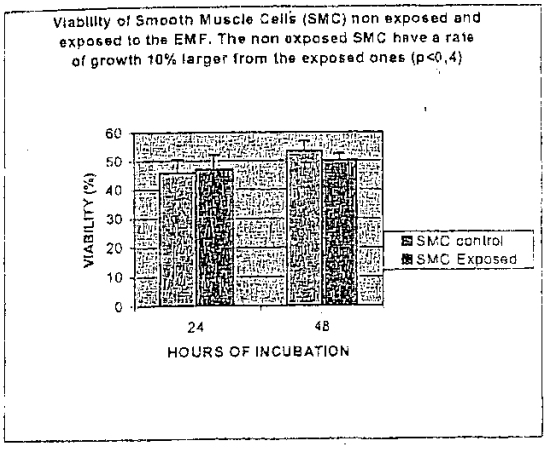
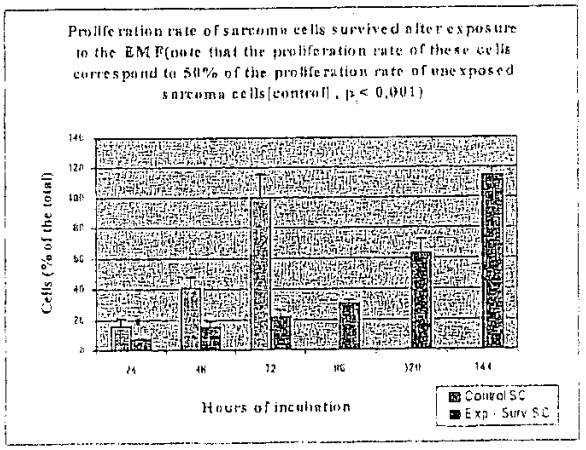
In this study we have investigated the effects of Static Electro-Magnetic Fields (SEMF) on sarcoma cells, isolated from tumors of Wistar rats. The tumors were developed by 3,4-benzopyrene (B[α]P) injection in the rats. The cancer cells were exposed to SEMF for 45 minutes, applying frequencies between 10 kHz to 1 MHz, of the radiowave spectrum. During a 24-hours period after cancer cell exposure to SEMF, no inhibition of cell proliferation appeared. In contrast, at the end of 48 hours incubation time, the cancer cell proliferation was dramatically decreased at a level of 98%.





In addition the survived sarcoma cells, which are 2% of the total cell population, after its exposure to SEMF, showed a significant decrease of proliferation, under the same culture conditions. These cells were then exposed once again to SEMF for 45 minutes (totally 4 sessions of exposure) and tested using a Flow Cytometer. It was found that a great percentage of these cells (45%), doubly exposed to SEMF, was apoptotic and only a small percentage of them was found under mitosis (2 %). Additionally, the cells were counted and tested, by using an aggregometer for their ability to aggregate the platelets (an indicator of their metastatic potential) and they didn't show any difference, compared to the sarcoma cells not exposed to SEMF (control cells).

P-I-27

DIFFERENTIATION EXPERIMENTAL EFFECTS OF LOW INTENSITY SEMF, ON PHEOCHROMOCYTOMA CELLS, TYPE PC-12.

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Herein we investigate the effects of the Static Electro-Magnetic Fields (SEMF) on the pheochromocytoma cell line PC-12, isolated from a rat's pheochromocytoma. The PC-12 cells colony was incubated for 10 days at 37 °C (O2 95%, CO2 5%) and then exposed to SEMF, 12 hours every day, in 3 consecutive sessions, by using Radio Wave Frequencies, between 10-200 kHz of radiowave spectrum. Radio frequency measurements and SEMF exposure of cells were performed by MULTI CHANNEL DYNAMIC EXITER 100-V1, a device certified by the International Committee of Atomic Energy-EKEFE-DEMOKRITUS, for its safe use in humans and animals. It consists of two main parts: a) a diagnostic part with EPR-spectrometer characteristics and b) a SEMF generator of varying intensities (1.1- 1.11 ± 0.01 V/m for electric field and 0.0027-0.0029 ± 0.00005 A/m for magnetic field), along with radio frequencies (1kHz to 1MHz), monitored by a sophisticated software. To use this software, first it is necessary to record the biological target system's frequencies and then, by using a specific algorithm, to calculate the appropriate SEMF frequencies, that are needed for the exposure of living target systems or cells.

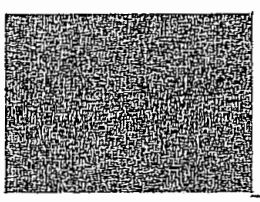

Fig. 1

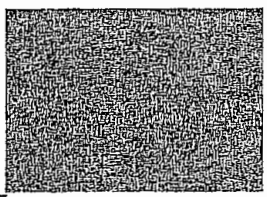

Fig. 2

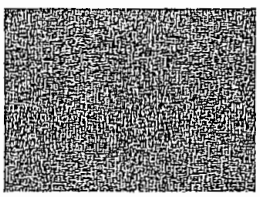

Fig. 3

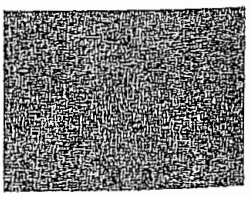

Fig. 4

A slight inhibition of cell proliferation rate was observed after exposition to SEMF. After first exposition a small number of PC-12 cells, presented morphological characteristics of nervous cell differentiations, (Fig. 1, Fig. 2). At the end of the third exposure session, a high percentage (>50%) of the PC-12 cells presented conspicuous morphological characteristics, of nervous cells and a formation of well-described neuronal networks (Fig. 3). It's not yet clear if this

P-I-36

DETERMINATION OF ANTICANCER ACTIVITY OF RESVERATROL ON CANCER CELLS, BASED ON THE CYTOMETRIC MONITORING OF THE NK LYMPHOCYTES STIMULATION

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Introduction: Natural Killer Cells (NK Cells - NKCs) are a subpopulation of lymphocytes that play an important role in immunotherapy. Resveratrol (3,4,5-trihydroxy-stilbene-3-β-D-glucoside) is an ingredient of many plants, with antioxidant properties.

Purpose: The investigation of possible anticancer actions of resveratrol, by stimulation of NK cells.

Material and Methods: 18 healthy volunteers participated in the study. The methodology of quantification of cytotoxicity of NKCs was used in the in vitro study, which included four stages: a) isolation of NKCs from blood and their quantification, b) quantification of cancer cells (leiomyosarcoma - Wistar rats), which used as cancer target cells (CTCs), c) incubation of NKCs with CTCs in CO_2 chamber in the ratios 12.5:1, 25:1, and 50:1 and d) determination of cytotoxicity by flow cytometer Epics XL-MCL of Beckman-Coulter Co. The same trials were repeated after the addition of resveratrol during stage c.

Results: The cytotoxicity of NKCs against CTCs indicated an increase at 320%, 440%, 67% average rate in the ratios 12.5:1, 25:1 and 50:1 respectively.

Conclusions: Resveratrol seems to be an important anticancer substance and therefore further clinical studies should be performed, for more convenient prevention and therapy of cancer.

P-I-37

AGGREGOMETRIC DETERMINATION OF ANTITUMOR AND ANTIPLATELET POTENCY OF CARVACROL, FOR THE PREVENTION OF CANCER AND THROMBOEMBOLIC DISEASES

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Introduction: Carvacrol is a basic ingredient of ethereal oil of *Origanum Vulgaris sbsp Hirtum.*

Purpose: The investigation of possible anticancer and antiplatelet actions of carvacrol (5-isopropyl-2-methylphenol).

Materials and Methods: i) anticancer action: in 28 healthy volunteers the cytotoxicity of NK cells was checked with the methods of cytotoxicity assay and flow cytometry with the use of carvacrol (10^{-3} M), ii) platelet aggregation (PA): in the isolated platelet rich plasma (PRP) of 28 healthy volunteers, trials of PA were performed with the stimulators ADP, PAF and arachidonic acid (ArA) in the presence and absence of carvacrol in concentrations 10^{-4} to 6.5×10^{-3} M, in aggregometer Ca-500 of Chronolog Co.

Results: i) the increase of cytotoxicity observed was 110% in average in the ratio 25:1, while the cytotoxicity in the ratios 12.5:1 and 50:1 remained stable, ii) the PA caused by ADP, PAF, and ArA was completely inhibited when carvacrol was added in concentrations such as 6.5×10^{-3}, 4.5×10^{-3} and 6.5×10^{-4} M respectively.

Conclusions: Carvacrol, a substance with antioxidant properties develops anticancer and antiplatelet actions. Therefore, with the addition of clinical trials, it could be useful in the therapy and prevention of cancer and thromboembolic diseases.

P-I-38

AGGREGOMETRIC - CYTOMETRIC MONITORING OF THE ACTION OF VARIOUS ANTIOXIDANTS ON PLATELET ACTIVITY, FOR THE CLINICAL PREVENTION OF THROMBOEMBOLIC DIDEASES

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Introduction: Membranic platelet receptor GpIIb-IIIa contributes to platelet aggregation (PA) by binding fibrinogen. Administration of antioxidants (AO) to platelets inhibits PA.

Purpose: To investigate the possible inhibition of: a) PA and b) the operation of GpIIb-IIIa ex vivo, through administration of AO.

Materials and Methods: 28 healthy volunteers participated in the study as blood donors. The following AO were administered in their rich platelet plasma (PRP) in concentration 3 x 10^{-3} M per substance: 2,3-diphosphoglyceric acid (2,3-DPG), carvacrole, gloutathione (GSH), azoulene, bismuthiole, 2-methyl-2-nitroso-propane (MNP) and N-tert-butyl-α-phenylnitrone (PBN). The GpIIb-IIIa receptors were measured by ADIAflo Platelet Occupancy kit, American Diagnostica Inc. and the flow cytometer Epics XL-MCL-Beckman Coulter. PA tests to the PRP were implemented with epinephrine (EPN), thrombine (THR), arachidonic acid (AA), PAF and ADP as activators. Same tests took place after administration of the under study AO in the PRP at 3 x 10^{-3} M concentration per substance. PA was calculated in a PICA Chronolog Co aggregometer.

Results: After the administration of the AO 2,3- DPG, carvacrole, GSH, azoulene, bismuthiole, MNP and PBN: a) the operation of the receptor GpIIb-IIIa decreased by 92, 99.4, 93, 91.5, 90, 95, and 89 % respectively and b) inhibition of PA was provoked at 87, 94, 88, 84, 83, 91 and 82% respectively.

Conclusions: Antioxidants contained in many plants and fruits and known to be free oxygen radicals scavengers, are possible to act at the level of platelet receptors GpIIb-IIIa inhibiting their function and in this way averting the configuration of platelet clotting. Based on these given facts, they could be used complimentary to the prevention of thromboembolic diseases.

P-I-44

FLOW CYTOMETRIC MONITORING OF THE APIGENIN ACTIVITY ON THE INDUCTION OF NK CELLS' FUNCTIONALITY, FOR THE CLINICAL PREVENTION OF CANCER

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Introduction: Natural killer cells (NK Cells – NKCs) are a subpopulation of lymphocytes that play an important role in immunotherapy.

Purpose: The investigation of possible induction of functionality of NK cells by the use of apigenin (4′,5,7-trihydroxyflavone).

Materials and Methods: 18 healthy volunteers participated in the study. The methodology of quantification of cytotoxicity of NKCs was used in the in vitro study, which included four stages: a) isolation of NKCs from blood and their quantification, b) quantification of cancer cells (leiomyosarcoma - Wistar rats), which were used as cancer target cells (CTCs), c) incubation of NKCs with CTCs in CO_2 chamber in the ratios 12.5:1, 25:1, and 50:1 and d) determination of cytotoxicity by flow cytometer Epics XL-MCL of Beckman-Coulter Co. The same trials were repeated after the addition of apigenin during stage c.

Results: The cytotoxicity of NKCs against CTCs indicated an increase of 320%, 480%, average rate in the ratios 25:1 and 50:1, while no increase in cytotoxicity observed in the ratio 12.5:1.

Conclusions: Apigenin seems to have important anticancer properties against cancer cells and its use in clinical trials should be seriously considered in the future.

P-I-46

CYTOMETRIC MONITORING OF PHYTOESTROGENES EFFECTS ON PLATELET ACTIVITY

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Introduction: Membranic platelet receptor GpIIb-IIIa participates in platelet aggregation (PA) as fibrinogen's receptor. Administration of phytoestrogens (PO) to platelets inhibits PA.

Purpose: To investigate the possible inhibition of a) PA and b) the operation of GpIIb-IIIa ex vivo, through administration of PO.

Materials and Methods: 28 healthy volunteers participated in the study as blood donors. The PO 1) apigenine (4',5,7-trihydroxyflavone), 2) β-naphthol (β-hydroxynaphthalene), 3) quercetin (3,3',4',5,6,7-hexahydroxyflavone), 4) resveratrol (3,4',5-trihydroxy-stilbene-3-β-D-glucoside), 5) thymol (3-hydroxy-p-cymene), 6) genistein (4,5,7-trihydroxy-isoflavone) and 7) origan oil were administered in their rich platelet plasma (PRP) at a concentration of 3×10^{-3} M per substance. The GpIIb-IIIa receptors, were measured by ADIAflo Platelet Occupancy kit, American Diagnostica Inc. and the flow cytometer Epics XL-MCL-Beckman Coulter. PA trials to the PRP were implemented with epinephrine (EPN), thrombine (THR), arachidonic acid (AA), PAF and ADP as stimulants. Same trials took place after administration of the under study PO in the PRP at a 3×10^{-3} M concentration per substance. PA was calculated in a PICA Chronolog Co. aggregometer.

Results: After the administration of the PO apigenine, β-naphthole, quercetin, resveratrol, thymol, genistein and origan oil: a) the operation of the receptor GpIIb-IIIa decreased by 91, 98.1, 93, 91, 92, 89 and 99.5 % respectively, b) each substance studied caused a 100% inhibition of PA.

Conclusions: Phytoestrogenes contained in many plants and fruits and known to be free oxygen radicals scavengers, are possible to act at the level of platelet receptors GpIIb-IIIa, inhibiting their function and in this way averting the configuration of platelet clotting. Based on these facts, they could be used complimentary, to prevent thromboembolic diseases.

P-I-47

KINETIC AND POTENTIOMETRIC ASSAY OF FORMALDEHYDE IN REAL SAMPLES, MONITORED BY COPPER SOLID ION SELECTIVE ELECTRODE, AFTER ITS REACTION WITH $[CU\{(CH_2NH_2)_2\}_2]\cdot SO_4$.

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Many publications have been reported so far, regarding electroanalytical methods for HCHO determination[1]. HCHO reacts quickly, quantitatively, irreversibly and stoicheiometricaly (2/1), with aquatic solutions of the stable complex Bis-(Ethylenediamino)-Cu(II)-Sulfate, thus demasking Cu(II) cations and producing the soluble ethylenediimine, according to the reaction sheme[2]:

$$[Cu\{(CH_2NH_2)_2\}_2]\cdot SO_4 + 4HCHO \rightarrow Cu^{2+}_{(sol)} + SO_4^{2-}{}_{(sol)} + (CH_2)_8N_{4(sol)} + 4H_2O$$

The Cu(II)-solid membrane ISE, type OP-CU-0711P RADELKIS[3] is monitoring Cu(II) cations, being released and kinetic curves are taken. Their initial slopes, as well as their limiting potentials linearly correlate with HCHO concentrations. Calibration graphs are taken in concentration range of 50-250 ppm HCHO, at an optimum pH 7. The main advantages of the proposed method are the simplicity, the low cost and the speed of the measurements. It is recommended for HCHO assay, in relatively concentrated samples 0.02-0.04 M, as well as in diluted solutions 0.001 M. It can, also, be successfully applied in colored and turbid samples or emulsions, where other methods fail.

P-I-54

KINETIC-CATALYTIC MICRO-ASSAY OF FE(III), ON HUMAN SERUM AND PHARMACEUTICAL SAMPLES, BASED ON THE PERBROMATE - DIPHENYLAMINE REACTION, IN A MIXED ACID MEDIUM.

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Perbromic acid ($HBrO_4$) is a strong monobasic acid and very strong oxidant at 100 °C, but almost inactive at room temperature. Thermodynamic and kinetic limitations make synthesis of perbromates[1] almost inevitable, under usual conditions. Due to their lack, a few papers have been reported so far among the scientific community, concerning the development of spectrophotometric[2], kinetic[3] and potentiometric methods[4]. The water insoluble Diphenylamine (DPA), becomes soluble in concentrated mineral and strong organic acids. Here in we describe the development of a novel kinetic spectrophotometric determination of Fe(III), based on its catalytic effect on the reaction of perbromates with diphenylamine in a mixed acid medium.

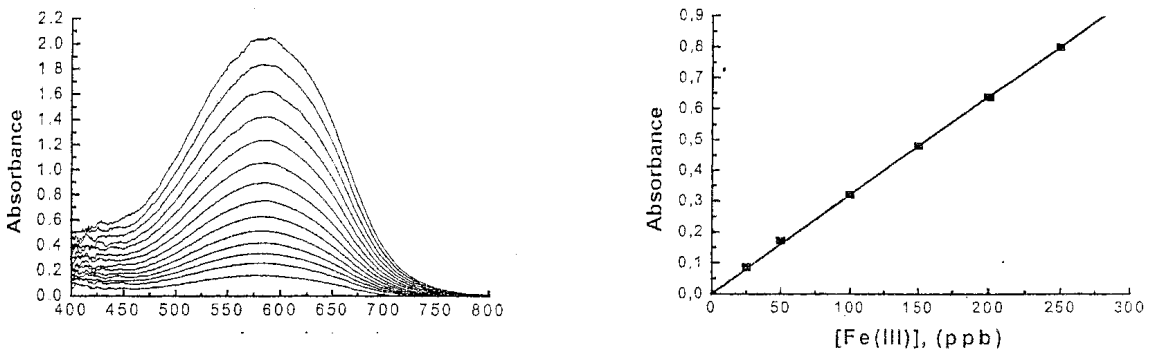

FIGURE: LEFT: The visible spectrum progress of the blue product, every 30 sec. DPA: 50 μl (0.5% stock solution), $KBrO_4$: 10 μl (0.1 M stock solution), Fe(III): 250 ppb, Final volume: 2 ml mixed acid. RIGHT: Calibration curve for the determination of Fe(III), according to the proposed method, in the concentration range 25-250 ppb.

P-I-68

SYNTHESIS, CHARACTERIZATION AND X-RAY STRUCTURE ANALYSIS OF A NOVEL POLYOXO-AZIDE, "BALLERENE" TYPE COMPLEX, WITH MIXED VALENCE STATES OF VANADIUM (IV)-(V).

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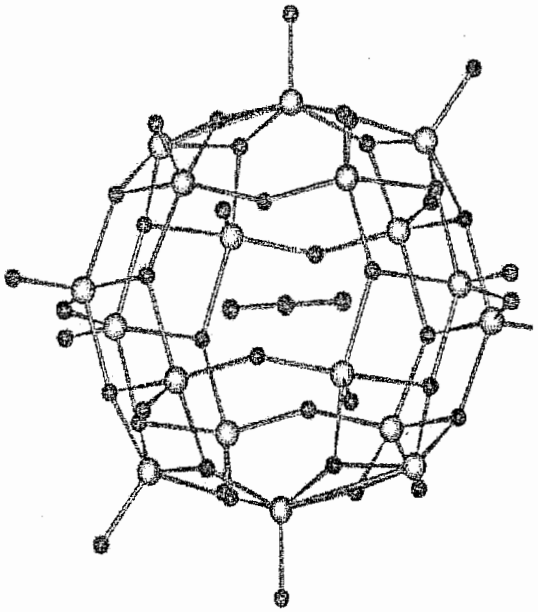
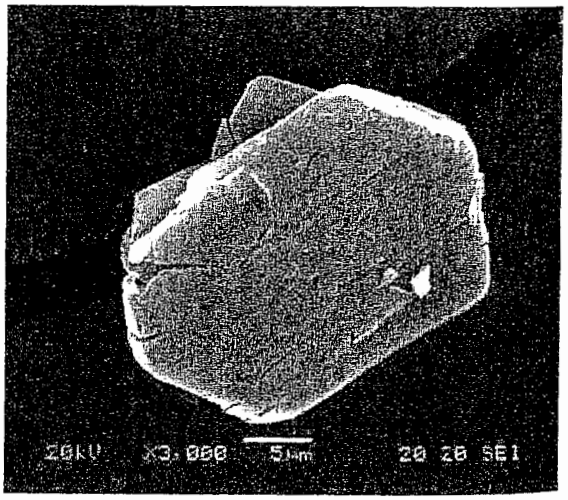
Vanadium is an essential trace element, with relevant biological and therapeutical properties, therefore it posses a special status among all biometals. A variety of vanadium compounds have been synthesized so far in an effort to offer better tolerance, more potent activity, increased selectivity and less toxicity in cancer treatment[1]. Thus many research groups have focused in the synthesis of novel vanadium compounds[2], which have been extensively reviewed[3]. In our effort





to prepare new electroactive amperometric mediators, we study the electrochemical behaviour of the binary system NH_4VO_3-NaN_3, before and after thermal treatment[3]. Finally we managed to isolate for a first time the ballerene-type cluster, with catenes hyphenated by V(IV)-V(V) strong interactions, along with a [$N_3^{\cdot}$] radical located at the center of the cluster, as it can be seen in the picture above. The cluster with the molecular formula $Na_{10}[V_{18}O_{44}N_3]\cdot 33H_2O$, is centrosymmetric and crystallizes in the triclinic P-1 space group, with cell parameters: a=12.019 Å, b=13.114 Å, c=13.425 Å, α=114.77°, β=92.86°, γ=113.93°, V=1693.44 Å^3. There are two