accompained by an important apoptotic fraction of all cell lines after treatment with either of extracts. The sub-G1 accumulation of the target cells reached values greater than 25% at 17–34 μl/ml *uman*[b] and at 14–28 μl/ml *uman*[c], depending on the individual cell line.
Conclusion: Two investigated aqueous-ethanol extracts showed significant cytotoxic activity on all neoplastic cell lines, after 72 h of continuous treatment, and point to the need for further characterization of the extracts, their phytochemical analysis, to provide the information about the compounds responsible for the antitumor action of investigated herbal mixtures.

225 PUBLICATION

Inhibition of the endothelin-1/endothelin A receptor axis by green tea polyphenol Epigallocatechin-3-Gallate in ovarian carcinoma

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Background: Green tea polyphenols are reported to possess anti-cancer properties. However, the molecular mechanisms leading to tumor growth inhibition are not fully understood. The endothelin A receptor (ET_AR)/endothelin-1 (ET-1) autocrine pathway is overexpressed in ovarian carcinoma and triggers tumor growth, survival, neoangiogenesis, and invasion indicating that ET_AR-inhibitory agents may be of therapeutic value. In the present study, we investigated whether green tea polyphenol epigallocatechin-3-gallate (EGCG) could act by inhibiting ET-1/ET_AR axis and signaling pathway in ovarian carcinoma cell lines.
Materials and methods: The effects of EGCG and green tea on ET_AR-mediated actions were tested by RT-PCR, Northern and Western blot, ELISA, chemoinvasion assay and immunohistochemical analysis in HEY and OVCA 433 ovarian carcinoma cell lines and in HEY xenografted nude mice.
Results: EGCG and green tea treatment inhibited ET_AR and ET-1 expression, at mRNA and protein levels. These effects resulted in reduction of the basal and ET-1-induced cell proliferation and invasion. Remarkably, EGCG treatment resulted in a reduction of basal and ET-1-induced mediators of angiogenesis, such as cyclooxygenase (COX)-1, COX-2, prostaglandin E_2, vascular endothelial growth factor (VEGF) expression and matrix-metalloproteinase activity. The EGCG-induced inhibitory effects were associated with a reduction of ET_AR-dependent activation of the p42/44 and p38 mitogen-activated protein kinases and phosphatidylinositol-3-kinase pathway. Finally, tumor growth was significantly reduced by oral administration of green tea *in vivo*. This effect was associated with ET-1, ET_AR, and VEGF mRNA and protein expression reduction, as well as with a decreased in the microvessel density and proliferation index.
Conclusions: These results provide a novel insight into the mechanism by which EGCG, affecting multiple ET_AR-driven pathways may inhibit tumor growth suggesting that EGCG may be useful in preventing and treating ovarian carcinoma.
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226 PUBLICATION

Investigation of some quinols and epoxyquinols as potential antitumor agents

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Background: The search for new antitumor agents is the imperative in modern oncology. The aim of this work was to investigate the antiproliferative activity of several newly synthesized quinols and epoxyquinols against five human tumor cell lines *in vitro*.
Material and methods: Stock solutions of investigated compounds were dissolved in DMSO at concentrations of 10 mM, and afterwards diluted by nutrient medium to various final concentrations. Target cells used were malignant human breast adenocarcinoma MDA-MB-361 and MDA-MB-453, cervix carcinoma – HeLa, melanoma – Fem-x and myelogenous leukemia – K562 cells. Normal human peripheral blood mononuclear cells (PBMC) were used as control cells. Antiproliferative activity of investigated compounds was assessed indirectly, measuring cell survival in standard, 72 h MTT test. In order to determine the mode of HeLa cell death induced by the investigated compounds, microscopic examination of morphological characteristics of acridine orange and ethidium bromide stained cells was performed.
Results: Investigated quinols and epoxyquinols exerted a dose dependent antiproliferative action towards investigated cell lines with good selectivity in their action to tumor cells in comparison to normal immunocompetent cells. Concentrations inducing 50% decrease in cell survival (IC50) obtained from three independent experiments, and on mononuclear cells, were given on table.

	IC50[μM]							
Cell lines	TK4	TK21	TK22	TK23	TK24	TK25	TK26	TK27
HeLa	1.5	47.7	90.7	8.4	49.6	5.5	35.0	3.9
Fem-x	1.2	81.4	> 100	8.2	70.6	5.1	54.7	4.6
MDA-MB-361	3.4	37.7	93.6	9.4	36.4	6.2	28.9	4.7
MDA-MB-543	5.6	53.9	> 100	23.7	43.6	9.4	35.2	16.3
K562	1.3	42.6	92.6	5.7	36.1	3.7	26.7	1.0
PBMC−PHA	16.6	> 100	46.4	> 100	19.0	66.3	19.3	
PBMC+PHA	16.3	> 100	> 100	54.1	> 100	20.0	> 100	18.9

Microscopic examination of the mode of direct cell death induced by the most active compounds, epoxyquinols TK4, TK23, TK25 and TK27, 24 h after continuous agents action in concentrations 2 × IC50, showed morphological appearance of apoptosis (condensed and/or fragmented nuclei).
Conclusions: Results obtained showed that investigated compounds, especially epoxyquinols 4β, 5β-epoxy-10β-hydroxy-17β-propionyloxy-1-estren-3-one (TK4), 4β,5β-epoxy-10β,17β-dihydroxy-1-estren-3-one (TK23), 4β,5β-epoxy-10β,17β-dihydroxy-17α-(phenylmethyl)-1-estren-3-one (TK25) and 17α-butyl-4β,5β-epoxy-10β,17β-dihydroxy-1-estren-3-one (TK27) could be promising agents for the treatment of human tumors, and are candidates for further analyses on experimental animals, *in vivo*.

227 PUBLICATION

The inhibitors of hydroxy-methyl-glutaryl-CoA (statins) induce cell growth arrest and apoptosis in osteosarcoma cell lines

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Osteosarcoma (OS) is an aggresive bone tumor of children and adolescents. The introduction of neoadjuvant chemotherapy has increased the fraction of patients who can cured to about 70%. Nevertheless, subsequent clinical trials of a variety of new treatment have all failed further improve survival. In order to progress in the treatment of OS we have tried to identify new pathways, like the mevalonato pathway, which may be exploited therapeutically.
For this purpose rat (UMR-106) and human (HOS, SaOS, U2OS) OS cell lines were grown under standar conditions. The parameters studied after administration of simvastatin at differents doses and times, with or without mevalonate, FPP, GGPP, FTI or GGTI were: cell growth rate, cell viability, morphologic changes, apoptotic response, cell cycle alterations, p53 and p27(Waf1/Cip1) protein expression and cell motility.
We observed that statins induced: 1. a decreased in cell growth rate; 2. an increased in the number of no-viable cells; 3. morphological alterations characterized by cell rounding and cell detachment from the substrate; 4. a p53-independent apoptotic response, dependent of the mevalonate pathway; 5. cell growth arrest in G1 and G2/M phases, dependent of of an increase in the p27(Cip/Kip/Waf) and decrease wound assay.
In conclusion, Statins, at leasti n vitro, are useful agent in the treatment of osteosarcome. These drugs are able to decrease cell proliferation, induce cell death by apoptosis and affect the cell motility. At present, we are evaluating the *in vivo* effect of these drugs in the osteosarcoma growing in nude mouse.

228 PUBLICATION

The expression of plakoglobin correlates with a favourable outcome of breast cancer patients

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Background: Plakoglobin = γ-catenin is an important protein of cellular adhesion structures in epithelia. It is part of the desmosomal plaque as well as of the adherens junctions. Together, both structures account for more than 90% of total cellular adhesion. During the metastatic process cell-cell adhesion has to be broken before tumor cells are able to disseminate. Since plakoglobin is part of both important adhesive structures it might be a main candidate for downregulation during dedifferentiation and malignant transformation. In a retrospective study we have determined the plakoglobin