

effects of BPs on GAPD mRNA expression, and to explore the suitability of GAPDH as housekeeping gene in gene expression studies. Cells were treated for 48 h with BPs with doses of 10, 50 and 100 μ M. For each concentration three experiments were performed. The housekeeping gene B2M was used to normalized GAPDH mRNA expression. Our results show a significant dose-dependent downregulation of GAPDH gene expression after treatment of different cancer cells line with different amino-BPs. Zoledronate resulted the most powerful bisphosphonate, whereas Clodronate, a non-amino BP, exerted significant effect on GAPDH expression only with the highest concentration tested. In conclusion, the use of GAPDH as a control gene, in particular in studies investigating the effects of BPs on bone or cancer cells, should be inappropriate and RT-PCR data on the effects of BPs in cancer cell should be reviewed, utilizing a different house keeping gene, i.e. B2M. On the other hand, this gene could be considered as a novel target gene for BPs on cancer cells.

221

PUBLICATION

Changes in the regulatory mechanism of protein synthesis induced by the combined antimigratory action of borrelidin and CGP57380

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Background: Borrelidin an inhibitor of threonyl-tRNA synthase inhibits angiogenesis, induces apoptosis and reduces tumor metastasis. Present studies addressed the questions whether cluster- or amoeboid-type of tumor cell migration are similarly affected and whether translational factors are implicated in this activity. Furthermore the modulation of the antimigratory potency of borrelidin by threonine and CGP57380 was tested. **Materials and methods:** Migration of HT-1080 fibrosarcoma cells and ZR-75-1 mammary adenocarcinoma cells were studied both in 3D tissue culture, containing matrigel allowing cluster-type of migration and wound-healing assay, resulting in amoeboid cell-movements in monolayer. Western immunoblot technique was used to detect phosphorylated and non phosphorylated molecules participating in signal transduction, actin was studied by applying immunocytochemical technique.

Results: Migration of HT-1080 fibrosarcoma cells could be inhibited both in 3D cell-culture and in wound healing assay. Interestingly the antimigratory action of borrelidin was abrogated in the wound-healing assay, by threonine which offered protection against the cell-kill action of borrelidin and also by CGP57380 an inhibitor of MNK-1. In fact both compounds enhanced the antimigratory action of borrelidin in the 3D cell-culture. Inhibitory action of borrelidin against global protein synthesis was further reduced by CGP57380 and also by inhibitors of SAPKp38 and PI3-K, however, abrogated by threonine. Borrelidin enhanced the phosphorylation of SAPKp38 and eIF-4E, increased the expression of HSP-27, reduced the activity of MMP-9 and MMP-2 and the expression of integrin α v β 5, and in addition the cellular localization of F-actin was redistributed.

Conclusions: It may be assumed that the proapoptotic and antimigratory action of borrelidin are the consequence of dysregulated cross-talks among the translational factors, preferentially eIF-4E and HSP-27 with the subsequent alterations in the functions of the cytoskeletal system. The opposite response of the same tumor cell population, participating in cluster- or in amoeboid-type of migration to the inhibitory action of borrelidin in the presence of CGP-57380 or threonine indicates that two different molecular mechanisms are implicated in these two migratory processes.

222

PUBLICATION

Hepatic arterial injection with 5-fluorouracil and dihydropyrimidine dehydrogenase inhibitor for the metastatic liver tumor in rabbits

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Backgrounds: Hepatic metastasis is one of the most important prognostic factors of cancer in digestive organs, especially gastric and colorectal cancer. In many cases of multiple hepatic metastases, surgical resection is impossible and hepatic artery injection (HAI) with fluoropyrimidine anticancer drugs such as 5-fluorouracil (5-FU) is commonly performed using a reservoir. However, 5-FU is rapidly degraded to α -fluoro- β -alanine after contact with dihydropyrimidine dehydrogenase (DPD) which is mainly present in the liver. Recently, a novel oral fluoropyrimidine anticancer drug, S-1 was developed and contains fluorinated pyrimidine and 5-chloro-2,4-dihydropyrimidine (CDHP) as a DPD inhibitor. We investigated the pharmacokinetics of HAI with 5-FU and CDHP in the experimental model.

Materials and methods: VX2 tumor cells were inoculated into the hepatic parenchyma at single site of the rabbits. Two weeks later, the rabbits were divided into two groups. Group A: 10 mg/kg of 5-FU was continuously administered into the hepatic artery for one hour. Group B: 10 mg/kg of 5-FU and 4.5 mg/kg of CDHP were continuously administered into the hepatic artery for one hour. In each groups, samples were collected from the plasma, normal liver tissue and liver tumor tissue in the same lobe, at 0, 1, 2, 4, 8, 12 hours after intra-hepatic arterial infusion. The levels of CDHP and 5-FU in the plasma, normal liver tissue and liver tumor were measured. The levels of DPD activity, thymidylate synthase inhibition rate (TSIR) and 5-FU incorporated into the RNA fraction (F-RNA) in the normal liver and liver tumor were investigated.

Results: The level of CDHP positively correlated with that of 5-FU and negatively correlated with that of DPD activity. The levels of CDHP, 5-FU, TSIR and F-RNA in group B were higher than those in group A and the level of DPD activity in group B was lower than that in group A ($p < 0.05$). In group B, the levels of CDHP, 5-FU and TSIR in the liver tumor were higher than those in the normal liver tissue and the level of DPD activity in the liver tumor was lower than that in the normal liver tissue ($p < 0.05$).

Conclusion: HAI with 5-FU and CDHP suspected to be effective for unresectable metastatic tumors in the liver in which DPD abound.

223

PUBLICATION

Nuclear translocation of Abl tyrosine kinase in the apoptotic response to DNA damage

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The ubiquitously expressed tyrosine kinase c-abl is localized in the cytoplasm and the nucleus. Nuclear c-Abl is activated by diverse genotoxic agents and induces apoptosis mediated by p73 or hRad9, although the regulation of DNA damage-induced c-Abl activity remains unclear. Here we show that c-Abl accumulates in the nucleus in response to DNA damage. Nuclear targeting of c-Abl is independent of its kinase activity.

The results also demonstrate that 14-3-3 proteins interact with c-Abl predominantly in the cytoplasm. c-Abl phosphorylation at Thr 735 located near the nuclear localization signal (NLS) is responsible for binding to 14-3-3. The mechanism by which genotoxin exposure disrupts sequestration of c-Abl by 14-3-3 in the cytoplasm is supported by the finding that JNK phosphorylates 14-3-3 that no longer associates with c-Abl. In concert with these results, the expression of unphosphorylated mutant of 14-3-3 suppresses nuclear accumulation of c-Abl and induction of apoptosis.

Taken together, these findings demonstrate that 14-3-3 is the essential regulator for c-Abl in the intracellular localization and in the apoptotic response to DNA damage.

224

PUBLICATION

Cytotoxic and cell cycle effects induced by two aqueous-ethanol herbal extracts on human cervix carcinoma and human breast cancer cell lines

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Background: In recent time there is an increasing interest for the anticancer activities of extracts of different plants. The goal of this study was to examine the *in vitro* cytotoxic effects of the aqueous-ethanol extracts, *uman^b* and *uman^c* provided by 17 and 12 herbs respectively, originating from Serbia and Montenegro, to two human breast cancer cell lines (MDA-MB-361, MDA-MB-453), to human cervix carcinoma HeLa cells and to normal human PBMC.

Methods: Antiproliferative action was determined indirectly, measuring target cell survival *in vitro*, by two assays: by metabolic MTT, and by Kenacid BlueR (KBR) dye binding method. Fresh extracts were used for each experiment. The effects of investigated extracts on the cell cycle was measured after PI staining by flow cytometry. The cell cycle distribution was estimated from the DNA frequency histograms of different cell lines after 24 h, 48 h and 72 h of the extracts action. Concentration of the investigated extracts were equal to IC50 or two IC50. Besides, the mode of cell death (apoptosis and/or necrosis) was examined by fluorescence microscopy, using acridine orange and ethidium bromide stained cells.

Results: Examined extracts exerted the antiproliferative action to neoplastic lines IC50 being less than 20 μ l of extract per ml of nutrition medium. The order of sensitivity of various cell lines determined by both assays was: cervix HeLa > MDA-MB-453 > MDA-MB-361, for *uman^b*, and MDA-MB-361 > MDA-MB-453 > cervix HeLa for *uman^c*. At this range of extracts concentrations (<20 μ l/ml), the extracts did not exert any significant cytotoxicity toward healthy human PBMC. *In vitro* antitumor activities was

accompanied 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 μM *uman*^b and at 14–28 μM *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 E₂, 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.

Cell lines	IC ₅₀ [μM]							
	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 $2 \times \text{IC}_{50}$, showed morphological appearance of apoptosis (condensed and/or fragmented nuclei).

Conclusions: Results obtained showed that investigated compounds, especially epoxyquinols 4 β , 5 β -epoxy-10 β -hydroxy-17 β -propionyl-1-estren-3-one (TK4), 4 β , 5 β -epoxy-10 β , 17 β -dihydroxy-1-estren-3-one (TK23), 4 β , 5 β -epoxy-10 β , 17 β -dihydroxy-17 α -(phenylmethyl)-1-estren-3-one (TK25) and 17 α -butyl-4 β , 5 β -epoxy-10 β , 17 β -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 aggressive bone tumor of children and adolescents. The introduction of neoadjuvant chemotherapy has increased the fraction of patients who can be 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 mevalonate pathway, which may be exploited therapeutically.

For this purpose rat (UMR-106) and human (HOS, SaOS, U2OS) OS cell lines were grown under standard conditions. The parameters studied after administration of simvastatin at different doses and times, with or without mevalonate, FPP, GGPP, FTL 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 decrease in cell growth rate; 2. an increase in the number of non-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 an increase in the p27(Cip/Kip/Waf) and decrease wound assay.

In conclusion, Statins, at least *in vitro*, are useful agents in the treatment of osteosarcoma. 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