effects of BPs on GAPD mRNA expression, and to explore the suitable of GAPDH as housekeeping gene in gene expression studies. Cells were treated for 48 h with BPs with doses of 10, 50 and 100 μM. 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 powerfull 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.

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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 threonin 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 abbrogated 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, abbrogated 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 αvβ5, 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.

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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-dihydorxypyrimidine (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.

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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 damge-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.

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Cytotoxic and cell cycle effects induced by two aqueous-etanol 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 diferent 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 20μl 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 μl/ml), the extracts did not exert any significant cytotoxicity toward healthy human PBMC. *In vitro* antitumor activites was