remarkable selectivity to MCF-7, T24 and Col2 cells with an IC50 of 16, 17, and 17μg/ml. The isolate however showed no cytotoxicity to the normal cell line, CHO-AA8. Morphological changes like cytoplasmic membrane blebbing, detachment of cells from the substrate and neighboring cells, nuclear condensation, formation of apoptotic bodies and reduction in cell size were observed in treated cells. Observations using the DAPI, a fluorescent stain complement the morphological analysis made under the bright-field microscope. Further, terminal deoxynucleotidyl transferase-mediated d-UTP nick end labeling (TUNEL) indicated that DNA fragmentation was by apoptosis which suggest its potential as a chemotherapeutic agent. Spectral characterization of the isolate showed that f6l contained polyunsaturated aliphatic esters or carboxylic acids.

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Interferon alpha/beta in rat liver after partial hepatectomy as growth modulator of hepatocytes

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Interferon (IFN)-α treatment is a common therapy for chronic viral hepatitis and contributes to preventing hepatocarcinogenesis. Besides, IFN is prescribed as a prolong course after surgical removal of tumors, combined with chemotherapy and radiotherapy. Along with IFN-sensitive cancers (kidney adenocarcinoma, lung sarcoma, malignant melanoma, neuroblastomas, cancers of limphoid, endocrine and generative organs), there are more resistant ones - cancers of stomach, liver and colon. Moreover, high and low concentrations of IFN can cause different answers. Our aim was to assess the expression of IFN alpha and beta in nontransformed liver after partial hepatectomy (PHE) and laparotomy. The expression of interferon α/β was assessed at RNA level by RT-PCR and as content of the protein in biological test in 0.5, 1, 3, 6, 12 h after operations. The cell specificity of IFN α/β production was assessed in isolated hepatocytes and KC. IFN α and its mRNA is detected in the intact liver. PHE induces increase of IFN α protein and mRNA content during first 3 hours after operation with further decrease till 6–12 hours. This increase is less than maximally possible liver response to the injection of PolyI-PolyC inducer. IFN β is detected neither in intact nor in regenerating rat liver. IFN α-specific mRNA was shown to be produced by KC and not by hepatocytes. The laparotomy is characterized by sharp decrease of IFN α content to null in the liver. That is why the increased IFN α production in regenerating liver is not linked with acute phase response. Laparotomy is a model for acute phase response and possibly the same processes occur after surgical interference. We assume that IFN α and its targets are essential for hepatocytes to leave quiescent state and proceed to proliferation. Liver sensitivity to IFN treatment may be different at pre- and postoperational periods.

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In-vitro antiproliferative effect of Gonatanthus pumilus lectin on various human cancer cell lines

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Gonatanthus pumilus lectin (GPL) is known to polyclonally activate human T-cells. GPL agglutinated rabbit, rat, guinea pig and sheep erythrocytes but was unable to agglutinate human ABO blood group erythrocytes. N-acetyl-D-lactosamine and serum glycoprotein asialofetuin were found inhibitory in the hemagglutination inhibition assay. The lectin was purified by affinity chromatography using asialofetuin linked amino activated silica gel. The lectin had no requirement for divalent metal ions like Ca2+ and Mn2+ for its activity. GPL has a carbohydrate content of 4.1%. Chemical modification of GPL with pyridoxal, Diethylpyrocarbonate and Bis-dithionitrobenzoic acid did not affect its activity, suggesting the absence of arginine, histidine and cysteine respectively in or near the ligand-binding site of the lectin. Modification of tyrosine with N-acetylimidazole led to 50% inactivation of GPL. However, total inactivation was observed only upon N-bromosuccinimide modification of tryptophan residues of the lectin. In vitro antiproliferative activity of GPL was tested on seven human cancer cell lines DU145 (Prostate), PC-3 (Prostate) A549 (Lungs), HCT15 (Colon), 502713 (Colon), KB (Oral) and IMR32 (Neuroblastoma). A 50% inhibition of proliferation was observed in DU145, PC3 and KB at the lowest concentration (10 mg/ml or less) of the lectin tested. HCT15 and 502713 cell lines showed 50% inhibition at 50 mg/ml of GPL. While very less inhibitory effect of GPL was observed on the proliferation of A549 and IMR32. The inhibitory effect of GPL was not associated with toxicity to the cell lines.

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Cysteine proteases as target for anticancer therapy and tumor prevention

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Human lysosomal cysteine proteases of the papain superfamily have received increasing attention as promising novel therapeutic targets (Bromme et al., 2005). Progression of human tumors is accompanied by the increased expression and activity of cysteine, aspartyl, serine and metalloproteases. A correlation was found between tumor growth and increase of cysteine proteases in humans.

Aim: to evaluate the role of inhibitor of cysteine proteases in treatment and prevention of experimental murine tumors.

Methods: CBA mice were used; lymphosarcoma LS (106 cell/ml) was implanted into tight muscles. Cyclophospamide, in the doses of 25,30, 50 and 100 mg/kg, recombinant TNF-α

(Vector Best, 1×10^{-5} U, Koltsovo, Novosibirsk Region) or selective inhibitor of cysteine proteases – Ep-475, 80 mg/kg (kind gift of Prof. Hanada K., Japan) were used. Cysteine protease activity was measured by fluorometrical method against Z-L-Phe-L-Arg-MCA and Z-L-Arg-L-Arg-MCA as substrates (Barrett, Kirshke, 1980) with specific inhibitor for cathepsin B – CA-074. Cathepsin B concentration was measured with ELISA kits (KRKA, Slovenia).

Results: Ep-475 in intact CBA mice induced dramatic inhibition of cathepsin B in liver 1 h (about 98% from the control), 3 h and 24 hrs (50% from the control) after the single Ep-475 administration with restoration of activity 48 hrs after. Similar inhibition of cathepsin L was registered (in less degree). There was no changes of aspartic protease cathepsin D. Lymphosarcoma LS development was followed by mild increase of cysteine proteases activity in tumor tissue and liver. Cyclophosphamide, CPA (30 mg/kg) treatment increased activity of cathepsin B and cathepsin L in tumor tissue and caused tumor regression, the most significant at 5th day after the single administration of antitumor drug. The effect was dependent from dose of CPA used: high dose of CPA (50-100 mg/kg) induced 4-6-fold increase of cysteine proteases activity in tumor tissue and practically total regression of tumor. Pretreatment by selective inhibitor of cysteine proteases – Ep-475 slightly stimulated tumor growth in control group and significantly reduced antitumor effect of CPA.

Conclusion: Antitumor effect of CPA in lymphosarcoma LS is related to apoptosis of tumor cells. Probably, cathepsins B and L in combination or independently from caspases are involved into the effector phase of apoptosis induced by CPA.

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Antisense-mediated downregulation of anti-apoptotic proteins induces apoptosis and sensitizes head and neck squamous cell carcinoma cells to chemotherapy

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Inhibition of apoptosis in head and neck squamous cell carcinomas (HNSCC) is because of upregulated expression of Bcl-2, Bcl-XL and Survivin. Hence, we addressed the question whether antisense approach towards these inhibitors of apoptosis could restore the apoptosis in HNSCC. Further, we wanted to see whether chemotherapeutic efficacy of Cisplatin and Etoposide could be enhanced by using these drugs in combination with antisense oligonucleotides in human laryngeal carcinoma HeP2 and tongue carcinoma Cal27 cells. The effect of these antisense oligonucleotides was examined on the mRNA expression by RT-PCR and on protein expression by Western blotting. Apoptosis was measured by flowcytometry, TUNEL assay and caspase-3 activity assay. Treatment of HeP2 and Cal27 cells with 400nM antisense oligonucleotides against Bcl-2, Bcl-XL and Survivin for 48 hrs decreased their expression both at the mRNA as well as at the protein level, resulting in the induction of apoptosis. Treatment of HeP2 and Cal27 cells with these antisense oligonucleotides augmented Cisplatin and Etoposide induced apoptosis. Our findings emphasize the importance of Bcl-2, Bcl-XL and Survivin as survival factors in HNSCC cells. Antisense treatment against these survival factors in combination with lower doses of chemotherapy offers potential as a less toxic chemoadjuvant therapy.

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Active oxygen species contribute to n-nitrosodiethylamine mutagenicity

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Nitrosamines are stable compounds and biologically and chemically inert unless activated. NDEA is present in major dietary sources, like cured meats, salami, millet flour and dried cuttle fish. In biological systems, N-nitrosodiethylamine (NDEA) can be activated by a variety of enzymes, which oxidize them to aldehydes and intermediates which are themselves alkylating agents. It has been shown that NDEA causes reactive oxygen species (ROS) production. Oxidative stress is known to be one of the most important causative agents of mutagenesis, carcinogenesis, aging and a number of diseases. The cell defense seeks to neutralize ROS that escape the primary defense mechanisms (antioxidants). In the present work, using vitamins C or E as ROS scavengers, we evaluated the genotoxicity and DNA repair in Escherichia coli mutants at low NDEA concentrations under exogenous and endogenous metabolic activation. Vitamin C was shown to scavenge NDEA induced metabolites generated by endogenous (in the absence of metabolic activation) bacterial enzymes. Vitamin C protects uvrB and fpg deficient cells against NDEA cytotoxicity in the presence of S9 mix. These data suggest that products of NDEA metabolism are ROS and can be scavenged by vitamin C, by requiring UvrB and FPG to repair the induced DNA lesions. No protective effect was detected for uvrA and uvrC deficient cells. Vitamin E protects E.coli cells proficient or deficient in DNA repair genes from cytotoxic effects, at low NDEA concentrations, both in the presence and in the absence of metabolic activation. Our results support the role of scavengers molecules such as vitamins C or E in the diet.