(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.