583 Antiapoptotic effect of aminoguanidine on doxorubicin induced apoptosis

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Background: Doxorubicin (DOX) is a broad-spectrum anthracycline that has cardiotoxicity as a major side effect. ROS and nitrogen species (RNS) generation have been proposed to be an important mechanism of DOX induced cardiotoxicity and cardiomyocyte apoptosis, a processes that may be mediated by the p53 protein. Aminoguanidine (AMG) is an effective antioxidant and free radical scavenger which has long been known to protect against ROS formation.
Material and Methods: A549 lung cancer cell line were incubated different concentration of AMG (100 to 1000 μM) in the presence or absence of 0.25 μM DOX for 24 hours. The expresssion of p53 and of its transcriptional target p21 were analysed by western blot. Apoptosis was analysed with Annexin V assay. JC1 and H2ax immunofluorescence were used to assess mitochondrial and nuclear DNA damage, respectively.
Results: Results demonstrate that AMG has a dose-dependent antiapoptotic effect on doxorubicin-induced apoptosis.
Conclusions: Thus, these data further identify AMG as a chemopreventive agent with great potential to reduce ROS and NOS damage generated by DOX.

584 Induction of invasion in an organotypic oral cancer model by cobalt chloride, a hypoxia mimetic

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Background: Invasion is one hallmark of malignancy. The aim of this study was to develop an *in vitro* model that can be used for experimental studies of cancer cell invasion.
Material and Methods: The organotypic oral cancer (OTOC) model was constructed by growing oral squamous cell carcinoma (OSCC) cells (PE/CA-PJ49 clone E10) on a collagen matrix in which normal human fibroblasts were incorporated. In order to mimic hypoxia, $CoCl_2$ was added for a short period of time. The pattern of invasion was evaluated in sections from models with and without $CoCl_2$. By use of immunohistochemistry, the expression of selected molecules connected to invasion was studied in the models and compared to oral squamous cell carcinomas.
Results: Treatment of the model with cobalt chloride to mimic hypoxic conditions, increased cancer cell invasion defined as the appearance of cancer cell islands protruding into the matrix ($P = 0.001$). The expression of invasion-related molecules such as phosphorylated extracellular signal-regulated kinases 1 and 2 (p-ERK1/2), cyclooxygenase-2 (COX-2), $p75^{NTR}$ and hepatocyte growth factor receptor (Met) was similar to that seen in OSCC. Models treated with $CoCl_2$ showed increased expression of $p75^{NTR}$ ($P = 0.05$) and laminin-5 in the cancer cells, and a more pronounced fragmentation of collagen IV in the basal membrane area, in contrast to models that were left untreated.
Conclusion: The results indicate that the present model is well suited for studies on cancer cell invasion in the matrix and that addition of $CoCl_2$ is indicated because it markedly increases the invasion and improves the model.

585 Absence or low expression of Fas-associated protein with death domain in acute leukemia and lymphoma cell lines

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The dominant paradigm of tumour biology is that evasion from apoptosis is one of the crucial features of malignant diseases and that the efficiency of cancer therapy depends on p53-dependent apoptosis. Because of the importance of apoptotic pathways in protecting cells against malignant transformation, disruption of apoptosis is extremely common in cancer cells.
In acute leukemia and lymphoma apoptotic death receptor signalling pathway is disrupted. We predicted that absence or low expression of Fas-associated death domain (FADD) protein could be found in leukemic and lymphoma cell lines. FADD is an adapter protein that is required for apoptosis induced by all known death receptors, expression of FADD was analyzed by Western blot in two types of leukemic and two types of lymphoma cell lines.
In our experiment we used MOLT-4 (human acute lymphoblastic leukemia), Jurkat (human T cell leukemia), RAJI (Burkitt's lymphoma) and U-937 (histiocytic lymphoma) cell lines. Cells were maintained by the addition of fresh medium or replacement of medium and were cultured at 37ºC in a 5% CO_2 atmosphere. Cell density was between 4×10^5 and 1.5×10^6 viable cells/ml in complete RPMI 1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin.
Cultured cells were collected together with medium. After centrifugation, the pellet was washed with cold PBS and then lysed in a lysis buffer. Samples were agitated on ice for 1 hour. After centrifugation, supernatants were collected, and the protein extracts were quantified using the BCA protein assay kit (Pierce BCA Protein Assay Kit). Equal amounts of protein (30 μg/lane) were separated by SDS-PAGE and transferred to nitrocellulose membranes using XCell Blot Module. Nonspecific binding was blocked by TBST with 5% nonfat milk overnight at 4ºC. Incubation with a rabbit polyclonal antibody FADD (H-181, sc-5559, Santa Cruz Biotechnologz Inc.), diluted 1:200 lasted for 2 hours at room temperature with agitation. As a secondary antibody was used anti-rabbit, HRP-linked whole antibody from donkey (Amersham Biosciences) diluted 1:5000. Visualization was done by Lumi-light Western Blotting Substrate (Roche).
The results indicated that in two cell lines we found absence, in one low and in second normal expression of FADD protein. The data presented here suggest that apoptotic death receptor signalling pathway in leukemic and lymphoma cells is disrupted due absence or low expression of FADD protein.

586 Esophageal epidermal carcinoma: a novel model associated with thermal injury

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Background: Esophageal squamous cell carcinoma (ESCC) is one of the most lethal types of cancer in the world. Risk factors include alcohol and tobacco usage and exposure to nitrosamines. The Southern tip of South America presents the highest incidence in Americas and epidemiological studies have shown that chimarrão (a hot maté infusion) consumption adds as a risk factor in this region. This seems to be related to a thermal lesion resulting from the temperature and volume at which it is consumed. We developed an experimental model in mice to analyze the contribution of the thermal lesion to esophageal carcinogenesis.
Materials and Methods: Injury was triggered by intra-esophageal administration of water at different temperatures (25 to 70°C) three times a week and/or N-nitrosodiethylamine (NDEA) in the drinking water at different doses for up to 32 weeks.
Results: Animals treated only with water at 70°C did not develop tumours but presented an initial necrosis, which evolved towards a recurrent inflammation that was resolved at 8 weeks of treatment. However, animals treated with water at 70°C and NDEA at 1, 10 or 40 ppm developed more ESCC tumours than those treated only with NDEA. This group developed chronic inflammation and epithelial regeneration was delayed. The group treated with water at 60ºC and NDEA at 1 or 10 ppm did not develop tumours or inflammation.
Conclusion: Mice treated with water at 70°C for up to 32 weeks do not develop tumours, but have a recurrent inflammation with pre-neoplastic lesions which synergize with NDEA to induce tumours. The reduction of 10ºC (70 to 60ºC) in the water temperature associated with NDEA at 1 or 10 ppm prevents the induction of esophageal tumours, which suggests that perhaps a reduction of 10 degrees in the temperature at which of hot maté is consumed, may turn a high incidence into a medium incidence area of esophageal cancer. This experimental model will aid to the comprehension of the molecular mechanisms and the role of inflammation in esophageal tumourigenesis.

587 Differential susceptibility to Urethane-induced lung cancer among mouse strains: a redox imbalance issue

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The mechanisms involved in the initiation of lung carcinogenesis are not yet well understood. Oxidative stress is suggested to play a significant role in this process and in the present study our purpose was to investigate the role of redox imbalance induced by urethane exposure, a known adenocarcinoma inducer, using resistant and susceptible mice models of lung cancer.
Female C57/B6 and A/J 8−12 weeks-old mice were treated with weekly intraperitoneal injections of 1 mg/g of animal weight of Urethane in 0.1 ml of saline for 4 weeks. The control mice were saline-injected under the same weekly regimen. Total lung tissue was collected at different time points of the experiment of both control and urethane-injected mice from both mice strains. Lung tissue was either processed histologicaly or stored as a homogenate for further biochemical measurements.
Measurements performed at resistant C57/B6 mice treated with urethane have shown a decreased catalase activity that correlated to accumulation of carbonylated proteins 2 weeks after the end of urethane treatment. Such parameters are regarded as a sign of oxidative stress. However, from third