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

week on, these parameters were back to control levels. The modulation of oxidative state of C57/B6 lung was not accompanied by histological modifications. Surprisingly, we did not observe the same results in the susceptible A/J mice.
In the present study, we have shown that urethane modulates redox components of the lung and this effect seems to be strain dependent. It is known that urethane-treated A/J mice will develop lung adenocarcinoma within 16−20 weeks after the first injection and that only a very small percentage of C57/B6 will develop lung cancer under the same conditions. Therefore, we put forward the idea that the ability of the resistant mice to up regulate a proper stress response at an initial stage act as a protective mechanism against carcinogenesis. On the other hand, the apparent lack of response observed in susceptible mice might mitigate the establishment of a chronic nocive environment that would contribute to the development of lung adenocarcinoma.

588 Aloe vera and honey solution decreases cell proliferation and increases apoptosis susceptibility in tumour tissue while avoids liver damage

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Background: Cancer is diagnosed in approximately 11 million people and is responsible for approximately 8 million deaths worldwide every year. Researches in cancer control have shown the importance of co-adjuvant therapies. *Aloe vera* may reduce tumour mass and metastasis rates, while honey may inhibit tumour growth.
Materials and Methods: This study verified the influence of *Aloe vera* and honey on tumour growth evolution accessing cell proliferation rate (Ki67-LI) and apoptosis susceptibility (Bax/Bcl-2 ratio) in tumour and liver tissue from adult rats at 7, 14 and 20 days of Walker 256 carcinoma (sc) implant. Tumour-bearing Wistar rats were distributed into two groups: *Aloe vera* and honey-treated group (WA) received a gavage with a 670 ml/kg dose of *Aloe vera* and honey solution daily, while non-treated group (CW) received only 0.9% NaCl solution in the same dose.
Results: The effect of *Aloe vera* and honey against tumour growth was observed through WA versus CW, showing decrease in tumour relative weights (CW-7d = 0.79±0.32; WA-7d = 0.68±0.43; CW-14d = 4.14±2.08; WA-14d = 3.17±1.38; CW-20d = 7.57±2.98; WA-20d = 5.16±2.46 (%)), lower cell proliferative rates (Ki-67 LI: CW-7d = 71.0±10.9; WA-7d = 51.4±18.1; CW-14d = 69.6±13.5; WA-14d = 37.2±16.4; CW-20d = 59.1±22.7; WA-20d = 32.0±3.3), and increase in apoptosis susceptibility (Bax/Bcl-2 ratio: CW-7d = 0.39±0.05; WA-7d = 2.35±0.08; CW-14d = 0.55±0.24; WA-14d = 2.48±2.16; CW-20d = 0.15±0.06; WA-20d = 1.20±0.80). In contrast, we observed that the *Aloe vera* and honey treatment leaded to increase in hepatocytes proliferation in early stages of tumour development (CW-7d = 12.6±3.3; WA-7d = 19.9±1.8; CW-14d = 7.2±1.4; WA-14d = 10.2±1.7; CW-20d = 10.9±1.8; WA-20d = 7.8±1.5) and decrease in their apoptosis susceptibility at 14th day of tumour implant (Bax/Bcl-2 ratio: CW-7d = 0.93±0.53; WA-7d = 0.86±0.62; CW-14d = 4.06±2.39; WA-14d = 0.88±0.63; CW-20d =3.53±3.24; WA-20d = 3.34±0.88), suggesting a possible protective effect in liver tissue, which is commonly harmed by tumour effects.
Conclusion: These data suggest that *Aloe vera* and honey affected tumour and host in a different way, inducing some benefits to host tissue while promoted damages in tumour evolution. Indeed, there are a large number of complex mechanisms involved in tumour growth, apoptosis and host health maintenance that can be modulated by *Aloe vera* and honey.

589 Extracts from endemic plant Helichrysum zivojini suppress survival of malignant cells

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Background: A wide variety of compounds and extracts from medicinal plants are in the center of attention of modern anticancer research as potential bioactive agents which might be used in future for the suppression of initiation, promotion and/or progression of malignant diseases. In this study our main goal was to investigate the anticancer properties of endemic plant species *Helichrysum zivojini* collected in Macedonia.
Material and Methods: The aerial parts of the plant were air-dried, powdered, and successively extracted with solvents of increasing polarity to obtain hexane, dichloromethane, ethyl-acetate, *n*-butanol and methanol extract. The cytotoxic activity of five obtained extracts was tested against selected cancer cell lines: human cervix adenocarcinoma HeLa, human breast adenocarcinoma MDA-MB-361, human malignant melanoma Fem-x, human myelogenous leukemia K562, unstimulated and stimulated for proliferation by phytohemagglutinin normal human immunocompetent peripheral blood mononuclear cells (PBMC) using MTT test. The mode of K562 cell death was analyzed morphologically.
Results: All investigated extracts exerted a selective dose-dependent cytotoxic action against all used target cancer cell lines and to PBMC stimulated for proliferation, but cytotoxic action was not as pronounced to normal, rested PBMC. The very prominent cytotoxic effect was observed against K562 cell line (IC_{50} values ranging from 11.78±0.94 to 74.88±7.57 μg/ml). Moreover, cytotoxicity of different extracts of *Helichrysum zivojini* was significantly stronger toward HeLa, Fem-x and K562 cancer cell lines than toward healthy immunocompetent PBMC stimulated for proliferation. It should be stressed that these extracts in whole exhibited weaker cytotoxic effect against unstimulated PBMC in comparison to stimulated PBMC. Morphological evaluation by microscopic examination of acridine orange and ethidium bromide stained K562 cells pre-treated for 48 h with plant extracts applied at a double $IC50_{72h}$ concentrations, demonstrated that all five extracts induced apoptotic cell death.
Conclusion: Results from this research show that extracts prepared from endemic plant species *Helichrysum zivojini* possess very pronounced anticancer potential, which could be attributed to the observed very selective antiproliferative and apoptotic effect, specially exerted to malignant cells.

590 Anticancer activity screening of Thai medicinal plants in human leukemic cell line MOLT-4

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Many phytochemicals have been proved to be a good candidate for anticancer drug. Eleven Thai plants were thus selected based on local usage for anticancer activity investigation. The 50% ethanol-water crude extract were prepared from *Rhus javanica* (stem), *Pinus kesiya* (branch), *Cratoxylum formosum* (stem), *Acorus tatarinowii* (leave & rhizome), *Tetracera loureirii* (vine), *Abrus pulchellus* (stem), *Catymbium speciosum* (rhizome), *Amomum villosum* (leave & rhizome), *Glochidion daltonii* (stem), *Rhus succedanea* (stem), and *Cladogynos orientalis* (arial part). The anticancer activity was determined from cytotoxicity and apoptosis induction in leukemic MOLT-4 cell and Vero cells. Cytotoxicity was tested by using Neutral red assay. An alkylation reaction with nitrobenzylpyridine (NBP), a nucleophilic DNA model was also examined. Apoptosis induction was evaluated from DNA fragmentation by using gel electrophoresis. Results showed that the plant that showed strong cytotoxic (IC_{50} < 100 μg/ml) and high selectivity (SI > 3.0) at 24 h and 48 h was *T. loureirii* (IC_{50} of 53.9±5.4 and 68.4±7.4 μg/ml, respectively). While *A. pulchellus*, and *P. kesiya* showed strong cytotoxic and high selectivity only at 48 h (IC_{50} of 71.7±4.2 μg/ml and 74.0±7.5 μg/ml, respectively). The plants that showed strong cytotoxic but less selectivity at 24 h and 48 h were *G. daltonii* (95.5±6.4 μg/ml and 61.0±3.9 μg/ml) and *C. speciosum* (99.4±3.6 μg/ml and 86.9±9.1 μg/ml). *C. formosum* possessed strong cytotoxicity (76.2±4.1 μg/ml) only at 48 h. Other crude extracts were found to be moderate cytotoxic (100 μg/ml ⩽ IC_{50} ⩽ 500 μg/ml) or inactive (IC_{50} > 500 μg/ml). The crude extracts illustrated different alkylating activity and only *A. tatarinowii* (leaves) showed no alkylating activity. The first 4 plants, *C. formosum, G. daltonii, R. succedanea, and T. loureirii*, showed high alkylating activity with 36, 22, 16, and 16% compared to melphalan, a positive control. Alkylating activity also indicated the presence of some electrophilic substance in the crude extract which alkylate with the nucleophilic site of NBP. Interestingly, almost of crude extract exhibited DNA ladder at 24 h except *T. loureirii*, *G. daltonii*, and *C. orientalis.* To be concluded, *A. pulchellus*, and *P. kesiya* showed high potential anticancer activity. While, the other plants that exhibited apoptosis induction were also of interest for further study. The active compound contributed to the activity and detailed mechanism of action will be further carried on.

591 Methylation of the mismatch repair genes in head and neck cancer

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Background: The Mismatch Repair System (MMR) plays a crucial role in the maintenance of genomic stability and increases the fidelity of DNA replication by eliminating mismatches which occur during the replication process. The MMR system incorporates several genes and has been conserved from procaryotes to eucaryotes. Aberrant methylation of the CpG islands at the promoter region of the genes is an epigenetic change that leads to transcriptional silencing of tumour suppressor genes. However, transcriptional silencing of the MMR genes in head and neck cancer has not been investigated throughly. In this study we investigated methylation of six MMR genes and the