563 Typhonium flagelliforme induces apoptosis through mitochondrial pathway

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Background: The plant *Typhonium flagelliforme*, commonly known as 'rodent tuber' in Malaysia, is often used as traditional remedy for cancer, including leukemia. Due to the lack of enough literature available on the mechanism of *Typhonium flagelliforme* and its effects on leukemia, the anti-leukemia effect of *Typhonium flagelliforme* was investigated *in vitro.*
Materials and Methods: Extraction and fractionation using organic solvents were applied to obtain good fractions from *T. flagelliforme* and subsequent chemical analysis was done using GC-MS. The Annexin V assay, TUNEL assay and cell cycle analysis were done using flowcytometry. DNA laddering was employed to detect DNA fragments resulting from apoptosis. Levels of caspase-3 and 9 were evaluated using a colourimetric assay. The expression of proteins associated with apoptosis such as cytochrome c, BcL-2, PARP, FasL and β-actin were analyzed using imnunoblot analysis.
Results: Phytochemical analysis using GC-MS revealed that the DCM/F7 fraction contains 51.2% linoleic acid. The Annexin V assay revealed apoptotic induction in CEMss cells exposed to DCM/F7 fraction at 6 μg/ml. TUNEL assay further revealed apoptotic induction in CEMss cells exposed to the DCM/F7 at 3 μg/ml (IC_{50}) in a time-dependent manner, whilst DNA fragmentation of CEMss cells (1×10^6 cells) were detected using 1.0% agarose gel electrophoresis after exposing at 3, 10 and 20 μg/ml of DCM/F7 for 48 h. The DCM/F7 fraction significantly stimulated both caspases 3 and 9 activities, whereby more than one fold increase in both the enzymes activities were observed in all three concentrations used as compared to control. The immunoblot results revealed that cytochrome c gradually increased as the DCM/F7 concentration increases. The present study also found that DCM/F7 caused the release of mitochondrial cytochrome c, cleaved 116 kDa PARP into 85 kDa fragments in CEMss cells. The Bcl-2 protein was found to decrease during treatment. Meanwhile, FasL, a type II transmembrane protein did not exhibit up or down regulation on treatment. Cell cycle analysis revealed that there is significant ($p < 0.05$) G1 phase arrest in a time-depended manner.
Conclusion: Collectively, results presented in this study demonstrate that *T. flagelliforme*, a local herbal medicinal plant in Malaysia inhibiting the proliferation of leukemia *in vitro* selectively, leading to programmed cell death, which was later confirmed to be through mitochondrial pathways.

564 NOX4-dependent ROS production by stromal mammary cells modulates epithelial MCF-7 cell migration

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Background: The influence of the stromal microenvironment on the progression of epithelial cancers has been demonstrated. It is well accepted that among epithelial and stromal compartments, an active signaling exchange is established with functional consequences to both cell types. Unraveling the mechanisms by which stromal cells determine epithelial behavior will contribute to understand a key element of malignancy.
Material and Methods: We analyzed in a co-culture system, the effect of RMF-EG mammary stromal cells on the migratory capacity of the human mammary cell line MCF-7. To test whether the NOX-dependent stromal redox environment plays a role in the epithelial migratory behavior, we knocked-down the expression of NOX4 using a siRNA strategy. The effect of TGF-beta 1 on NOX4 expression and activity was analyzed by qPCR, and intracellular ROS production measured by a fluorescent method.
Results: We found that migration of MCF-7 breast epithelial cells was stimulated when co-cultured with RMF-EG cells. This effect depends on stromal NOX4 expression that, in turn, is enhanced by epithelial soluble factors. Pretreatment of stromal cells with TGF-beta 1 enhanced this migratory stimulus by elevating NOX4 expression and intracellular ROS production. TGF-beta 1 seems to be a major component of the epithelial soluble factors that stimulate NOX4 expression.
Conclusions: Our results have identified that an increased stromal oxidative status, mainly provided by an elevated NOX4 expression, is a permissive element in the acquisition of epithelial migratory properties. This property depends on the production of soluble epithelial factors among which TGF-beta 1 can play a decisive role.
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565 In vitro effects of doxorubicin and deracoxib on oxidative stress-related parameters in canine mammary carcinoma cells

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Mammary tumours are the most common malignant neoplasm in the bitch representing approximately 50% of all tumours. Clinical management of this neoplastic disease is complicated because, excluding surgery, there are few effective therapeutic options. In the treatment of mammary cancer conventional chemotherapeutic drug doxorubicin is considered to be one of the most effective agent in dogs. But the side effects and the development of resistance limits its usefulness. In recent years reports have documented the chemopreventative and antitumour activity of non-steroidal antiinflammatory drugs (NSAI), against osteosarcoma, breast and bladder cancer as an effect that may be additive to that of standard chemotherapy. Although mechanism of their antitumour effects remains unclear it is suggested that their pharmacological action exerts via cyclooxygenase (COX) dependent and independent mechanisms. Also, NSAI drugs have been shown to be powerful free radical scavengers and it has been suggested that many NSAI drugs might exert part of their action by scavenging oxidants in cancer chemotherapy. Therefore, the present study was aimed to evaluate the efficiency of doxorubicin and deracoxib a selective COX inhibitor, as single agents and in combination treatments, on antioxidant parameters in canine mammary carcinoma cell line CMT-U27.
The cells were seeded and exposed to doxorubicin and deracoxib for 24, 48 and 72 h. The viability of the cells (% of control) was measured using the colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Nitric oxide (NO), lipid peroxidation (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GSHPx) activities were determined.
The IC50 concentration of doxorubicin was found as 0.9 μM in 72 h period. This concentration of doxorubicin was used in the combination experiments. Our results showed increased oxidative status, as revealed by increased lipid peroxidation, NO and decreased GSH, SOD, CAT, GSHPx levels in CMT-U27 cells exposed to doxorubicin compared to control cells. In contrast, there were no significant changes in deracoxib treated groups. Moreover, the combination treatment of 0.9 μM doxorubicin and 1000 μM deracoxib resulted in significant increase in CAT, GSH and GSHPx levels compared to doxorubicin alone.
Our results suggest that deracoxib may be involved in oxidative/antioxidative processes of CMT-U27 cells. The effect of doxorubicin/deracoxib combination on modulations of the antioxidant status by changing the enzymatic and non-enzymatic components in this cell line was more potent than their use as a single agent. In conclusion, the use of COX inhibitor and chemotherapeutic agent may provide basis for new concepts of cancer treatment with the help of systematic modulations of the antioxidant defence systems in mammary cancer of animals.

566 Correlation between TLR signaling and mTOR activity in HNSCC

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Objectives: The signaling cascades of the innate immune system are often triggered by Toll-like receptors (TLR), whereas mTOR (mammalian target of Rapamycin) is a key regulator for cell proliferation, growth and mortality. Furthermore it is an Akt-PI(3)K downstream mediator.
This work shows our recent investigations concerning the impact of TLR 2, 3, 4, 7 and 9 on mTOR mediated signaling cascades as well as on cytokine secretion profiles.
Methods: Cytokine secretion profiles were measured using Cytometric Bead Arrays. Proliferation and cell cycle analysis were determined by using SDS-Page/Western-Blot, Flow cytometry and MTT assays. Different permanent HNSCC cell lines, primary tissue of healthy mucosa and solid HNSCC were analyzed.
Results: Our data indicate mTOR as a regulator of cytokine secretion and cell cycle function in HNSCC. TLR mediated IL6 production increased in the presence of mTOR inhibitors. Furthermore we demonstrate the influence of Rapamycin on growth in permanent HNSCC cell lines. Inhibition of mTOR by Rapamycin leads to a cell cycle arrest in G_0/G_1 phase.
Conclusion: In summary, our data demonstrate a clear correlation between TLR activation and the requirement of mTOR signaling regarding the cytokine secretion patterns. In addition we can display a difference between solid HNSCC, the corresponding metastasis and permanent cell lines with respect to cytokine secretion and mTOR phosphorylation. These heterogeneous characteristics corroborate the need for an individualization in anti-tumour therapeutic approaches.