carcinomas capable of distinguishing samples according to clinical course and progression of the disease.

524 Poster
Effects of mycotoxins on apoptosis of human immune system

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Apoptosis is an important process in a wide variety of different biological systems and also in chemical-induced cell death.

The immune system is now recognized as a target organ for many xenobiotics such as drugs and chemicals, which are able to trigger unwanted apoptosis or to alter the regulation of programmed cell death. Reducing the number of immune-competent cells after xenobiotic treatment can lead to immunosuppressive effects, resulting in an increased susceptibility to tumors or infections diseases.

Mycotoxins are secondary metabolites produced by microfungi that are capable of causing diseases and death in humans and animals at low concentrations. The immunotoxic effects can be mediated by direct toxin interactions with lymphocytes and other blood cells, provoking, among others effects, a rapid and strong apoptosis of human lymphoid cells.

Our objective was to explore the effects of deoxynivalenol (DON) and ochratoxin A (OTA) on apoptosis of immune-competent cells using cell lines as model.

The effects of both mycotoxins on apoptosis of lymphocytes at the cellular and molecular level were studied using flow cytometric analysis, western blotting or ELISA system. We found that toxin effects was origin-dependent and dose dependent and both mycotoxins caused inhibition of cell proliferation, mediated by activation of apoptosis pathway. In conclusion DON and OTA by apoptosis-induced in vitro on cells lines model may have some negative effects on human immunosystem that support further investigations

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Lipoxygenase expression and intracellular localization in different types of cancer

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Lipoxygenases (LOX), particularly 5-LOX and 12-LOX, have been implicated in carcinogenesis and several LOX-inhibitory drugs and natural products have been tested in preclinical studies and early drug trials for anti-carcinogenic activity and clinical effects. Knowledge is still lacking on LOX and their products with respect to biological activities, particularly intracellularly. The expression and localization of 5- and 12-LOX was studied by immunohistochemistry in 10 samples from cancers and normal tissue of pancreas, breast, colon, stomach, prostate and lung. Staining intensity, localization and proportion of positive cells were scored. Furthermore, malignant cell lines from pancreas (PANC-1) and breast (T-47D) were synchronized by serum starvation before adding 10% FCS. Cells were stained for 5- and 12-LOX by immunoperoxidase or immunofluorescence after 0, 2 and 6 hours and analysed by light and confocal microscopy. The expression of 5-LOX was more marked in cancer compared with the normal counterpart for most tissues except colon (very little expression) and stomach (marked expression in normal and malignant tissue). The nuclear envelope was the most prominent localization. For 12-LOX the expression was generally more marked than that of 5-LOX; little or no difference was seen between cancer and normal tissue in breast, colon and stomach, but cancer of pancreas, prostate and lung showed increased expression. The nucleus and nuclear envelope stained strongly for 12-LOX. The in vitro experiments showed appearance of 5-LOX in the nuclear envelope following stimulation but 12-LOX was always seen at the nuclear envelope. Nuclear expression of 12-LOX changed following stimulation and showed different behaviour in the two cell lines; a transient increase in PANC-1 but a decrease in T-47D. In conclusion, changes in LOX expression in cancer are variable depending on tissue. Increased expression was seen for 5-LOX in cancer of breast and pancreas and for 12-LOX in cancer of pancreas, prostate and lung. As the activating protein of 5-LOX (FLAP) is located in the nuclear envelope, the shift in intracellular localization in the in vitro experiments might indicate a link to the cell cycle. The considerable differences between cancers of different origin, reflected also in the different behaviour of 12-LOX seen in the two cell lines, will have to be kept in mind when designing strategies for chemoprevention or treatment of cancer based on LOX inhibition.

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Effect of stimuli treatment on proliferation and apoptosis of tumor cells

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The main obstacle against the success of therapy in many cancers seems to be the impossibility of eradication of all tumor cells. Increase of replicative capacity, loss of cell adhesion and angiogenesis process represent aggravating factors of clinical evolution for cancer patients. Breast and ovarian cancers represent some malignancies with high incidence and mortality throughout women, their ethiology involving many genetic, immunological and biochemical factors. Malignant evolution depends on the genetic profile of tumor, which dictates its reaction to citotoxic action exerted by chemotherapeutical agents or contributes to a resistant phenotype. Structural or gene expression alterations are responsible not only for the appearance of cancer, but also for the clinical responses of patients to chemotherapy. The present study focused on the potential influence of stimuli treatment (doxorubicin, cytokines, curcumin) on proliferation by cell cycle phases and apoptosis of breast and ovarian tumor cells. Experiments were performed on human breast and ovarian adenocarcinoma cell lines, levels of resistance being tested by measuring the expression of P-glycoprotein during cultivation of MCF-7, MDA-MB-231 and SK-OV-3 cells with stimuli. Sensibitility of tumor cells to stimuli treatment was evaluated by measuring the cytotoxicity induced by treatment using MTT or XTT colorimetric methods. We have also analyzed by flow-cytometry the influence of stimuli treatment on antigene expression of bcl-2, p53, Ki-67, Fas and P-glycoprotein, correlated with the modifications of apoptosis and cell cycle phases. Progression through cell cycle phases was evaluated by PI technique and flow-cytometry analysis, while percentages of apoptotic cells were detected by using Annexin V - FITC/PI coloration, followed by flow-cytometry. In addition, gene expression of molecules under study was analyzed by RT real-time PCR, and the results correlated with antigen expression detected by flow-cytometry. Data obtained could lead to a selection of patients who might benefit the most of antitumor immunotherapeutical strategies focused on diminishing the primary tumor and controlling/eliminating the metastases. Knowing the modifications induced by chemotherapeutical agents in human tumor cell lines will make possible the identification of new gene alterations associated with the resistant phenotype, which might be taken into account for future gene therapy of breast and ovarian cancers.

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TSA regulates P-glycoprotein and multidrug resistance associated protein expression in cancer cells

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Multidrug resistance (MDR) constitutes a major obstacle for success of cancer treatment. Although several mechanisms could be involved in the acquisition of this phenotype, the role of two different membrane proteins, P-glycoprotein (Pgp) and multidrug resistance associated protein (MRP) has been well established. Both proteins are members of the same ATP-binding cassette superfamily of transport proteins.

We have studied the effects of histone deacetylase inhibitors, such as TSA and SAHA on Pgp and MRP expression in different cancer cell models, including HT-29 and HCT-15 human colon carcinoma cell lines; IMIM-PC-1, RWP-1 and IMIM-PC-2 human pancreatic adenocarcinoma cell lines; MCF-7 and MCF-7/Adr human breast carcinoma; HL-60, HL-60R, K-562 and K562/Adr human leukaemia cell lines. In all these cell lines we