Volume 48, Supplement 5, July 2012 ISSN 0959-8049

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EUROPEAN JOURNAL OF CANCER

THE OFFICIAL JOURNAL OF

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membrane. After several days of culturing the tissue was fixed and cut into 10 μm slices. The embedding medium was removed and the slides were rehydrated for Raman measurements. A second model with Glyoblastoma spheres was generated and treated the same way. Results were verified on a tissue model composed of Neuronal cells, Neuronal stem cells and Glyoblastoma cells. Analysis of Raman spectra was performed by Principal Component Analysis (PCA) and a Support Vector Machine (SVM) was trained to classify the unknown cells within a mixed population.

Results: Spectral analysis of neuronal cells and Glyoblastoma cells revealed significant differences in the PCA. Spectral differences can be assigned to changes in protein and DNA content of the cells. With the aid of the Support Vector Machine a model could be generated for the classification of unknown spectra in the mixed neuronal and glyoblastoma cell model.

Discussion: Raman spectroscopy is a new tool for cell analysis. The technology provides valuable information about various kinds of cells and tissue. The purely laser light based method is reliable and efficient for cell and tissue characterization especially when standard methods lack the ability for safe identification. In stem cell research and tissue engineering Raman spectroscopy may become a powerful supportive technology.

1191 Diet and DNA Damage – in Vivo Effects of Dietary Compounds on Isolated Colonocytes and Lymphocytes From Rats

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Introduction: Diet is a very important factor in colon carcinogenesis. High fat diets are considered a risk factor for the development of colon cancer. One of the consequences of high fat diets is the increased content of bile acids in the colon. Bile acids are considered promoters of colon carcinogenesis and have shown to induce the formation of reactive oxygen and nitrogen species, and these, in turn, induce DNA damage. A diet rich in fruits and vegetables has been shown, on the other hand, to have preventive effects on colon cancer. A recent study from our lab has shown chemopreventive effects of natural compounds through protection against oxidative DNA damage and stimulation of DNA repair.

Material and Method: In this study, we evaluated the effects of *in vivo* consumption of two natural compounds (ursolic acid (UA) and epigallocatechin gallate (EGCG)) and a bile acid, deoxycholic acid (DCA), on DNA damage in colonocytes and lymphocytes isolated from Fischer 344 rats. These compounds were provided in the diet and administered daily for two weeks. After colonocyte and lymphocyte isolation, endogenous DNA damage (strand breaks, oxidized and alkylated bases) was evaluated using the Comet assay. Colonocytes were also treated with H_2O_2 and MMS, *ex-vivo*, to investigate the potential of our natural compounds to protect against oxidative and alkylating damage, respectively.

Results and Discussion: Our study showed that endogenous DNA damage in colonocytes was slightly higher than in lymphocytes. UA and EGCG decreased the levels of endogenous DNA damage in colonocytes, while in lymphocytes, only UA had preventive effects. *In vitro* H_2O_2 treatment significantly increased DNA damage in colonocytes isolated from control rats. We found that both UA and EGCG protected against H_2O_2-induced damage. On the other hand, treatment with MMS showed a tendency to increase DNA damage but was not significant and of our compounds, only UA protected cells from this type of damage. According to the literature, DCA induces DNA damage *in vitro*, however after two weeks of *in vivo* DCA treatment, increase of endogenous DNA damage in colonocytes or lymphocytes was not observed in this study.

Conclusion: UA and EGCG demonstrated a protective role against DNA damage. These results suggest that UA can protect DNA from both endogenous and induced DNA damage in colonocytes and lymphocytes. EGCG was found to protect only against endogenous and H_2O_2-induced DNA damage in colonocytes. Further studies are undergoing to verify the potential of these compounds on induction of DNA repair systems, specifically base excision repair, mismatch repair, and direct repair by O^6-methylguanine DNA methyltransferase.

Acknowledgements: DP is supported by the FCT grant SFRH/BD/64817/2009.

1192 Investigation of Raman Spectroscopy and Optical Coherence Tomography to Aid in Diagnosis of Oral Cancer

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Introduction: The diagnosis of oral cancer (OC) currently requires surgical biopsy and histological analysis which is invasive and time consuming. The prognosis is far better if OC is diagnosed early. Therefore, technologies that aid detection, improve diagnostic accuracy and are non-invasive, particularly if the results are available at the point of care and in real-time, will be of great patient benefit.

Aim: To investigate two novel methods for the diagnosis of OC. The first of these is Raman spectroscopy, a technique able to determine the specific chemical bonds present in a sample and their relative quantities. The second technique is optical coherence tomography (OCT), a non-invasive imaging technique which is commonly used in ophthalmology. Here we investigate the potential of this technology to visualise the oral epithelium.

Materials and Methods: A DXR Raman microscope was used to obtain spectra from healthy, pre-cancerous and cancerous tissue sections. These sections were from either tissue engineered models of OC and the oral mucosa or from archived patient samples. Raman spectra (RS) were also obtained from different cell lines (cancer and dysplastic), healthy oral cells isolated from excised patient tissue and tissue engineered models of mucosa and OC. Tissue engineered models were cultured as described previously (Colley *et al., Br J Cancer* 2011). OCT images of tissue engineered models and healthy human volunteers were obtained using an OCT device.

Results and Discussion: It is possible to obtain RS from tissue sections, cells and tissue engineered models, and differences were observed between normal and cancerous samples. OCT could non-invasively image over 500 μm into oral tissue in real-time. It was possible to identify the epithelial-connective tissue architecture and superficial connective tissue structures such as blood vessels and minor salivary glands.

Conclusion: RS of *in vitro* samples and *ex vivo* sections show variations between healthy tissue and cancerous tissues which could have potential as a diagnostic aid for OC. Further studies are required to determine the potential use in patients. OCT images show differences between tissue engineered models of OC and normal mucosa. Real-time images of healthy volunteers were easily obtained and showed the epithelial connective tissue boundary. Studies in patients with potentially malignant lesions will further test the clinical benefits of OCT.

1193 Searching for Biomarkers in Silica Early Exposure

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Introduction: Silica is formed by the elements silicon and oxygen under high pressure and heat. It can be found in the amorphous and crystalline forms. Inhalation of free crystalline silica results in a spectrum of lung diseases known as silicosis. Silicosis is characterized by lung fibrosis and it appears to be a major cause of occupational lung disease in exposed workers. It has a latency of 10 to 30 years, although the disease can develop earlier in workers exposed to high quantities of fine silica dust over a relatively short period of time. Identification of silicosis at a population level in workers exposed to silica containing dust has until recently been carried out by conventional chest radiographs, classified according to the International Labour Organization guidelines. Moreover, the interpretation of standard radiographs in accordance with international agencies, has proven problematic in regards to the reliability of an inter and intra reading. Silicosis is a disease that there is no cure, and can result besides its severe symptoms, tuberculosis and lung cancer associated. Moreover, the whole process of the disease is still unknown. The fact that in most cases the detection of the disease can only be possible in an advanced stage leads us to look for biomarkers that could detect it early, and contribute to a greater knowledge about the disease process. Thus, the aim of this work was to evaluate the silica genotoxicity through comet assay, and catalase activity, as possible markers of early-stage disease detection.

Material and Methods: Mice were treated with silica instillation (10 mg/50 μl NaCl solution) and 3 and 7 days after, blood samples were analyzed by Comet assay, besides catalase activity determination.

Results and Discussion: Comet assay showed enhanced level of DNA damage ($p = 0.0102$), in treated cells as compared with control, after 7 days from the exposure, indicating that the DNA damage could be due to stress caused by silica instillation. There was also significant difference in the level of DNA damage between the exposed groups of 3 and 7 days showing a possible increased effect. Moreover the statistical analysis showed no difference between control and exposed groups after 3 days ($p = 0.0817$). Since the p value is found near the significance level, a new assay, with increasing in sample size, could clarify this issue. The catalase activity showed no significant difference between control and treated animals ($p > 0.05$). This result could be due to the fact that the concentration of peroxide, generated in the inflammatory process of silica exposure, did not reach the Km of the enzyme, which would be necessary to observe differences in its activity.

Conclusion: Treatment of mice with silica exerted effect on cells as observed mainly after 7 days from exposure, when compared treated and control groups. So, comet assay appears to be a promising assay to be used in early-stage silicosis detection.

Supported by: UERJ, FAPERJ, CAPES