

C2-60**Radiation-induced antitumor action of genistein. Experiments *in vitro***J. Hartmann¹ and N. Getoff²¹Medical University of Vienna, Waehringer Gürtel 18-20, 1090 Vienna, AUSTRIA, ²Section of Radiation Biology, Department of Nutrition Sciences, University of Vienna, Althanstr. 14, UZA II., 1090 Vienna, AUSTRIA

Isoflavones and related compounds are natural products having multiple biological properties. Genistein (5, 7, 4'-trihydroxyisoflavone) belongs to this group of substances and is a very potent phytoestrogen. Investigations *in vitro* demonstrated the broad spectrum of the biological properties of genistein, e.g. acting as anticancer agent, inhibitor of protein tyrosine- and histidine kinases, inhibitor of DNA topoisomerase and many other health benefits. The genistein pharmacological activity very likely arises from its antioxidant properties. Experimental results on the radiation induced antitumor action of genistein were observed in the frame of experiments *in vitro* using human cancer cells (MCF-7) as a model. Based on the course of the obtained survival curves, taken as a function of the absorbed radiation dose (Gy) in oxygenated as well as airfree media it is concluded that: In oxygenated media the N/N_0 ratio (N_0 = number of cells before -, N = number of cells after treatment) as a function of absorbed radiation dose shows a strongly pronounced antitumor effect. With increasing genistein concentrations the antitumor effect is correspondingly enhanced. Combinations of genistein (5 μ M or 40 μ M) with the cytostatic agent Mitomycin-C (2.5 μ M) show a significant synergism. In air-free media these effects are essentially pronounced. Obviously electron transfer processes from genistein to Mitomycin-C take place. The obtained experimental data demonstrate the antitumor action of genistein as well as its synergism to MMC. The results of these studies are of special interest in respect to anticancer treatment.

C2-61**Comparative effects of boric acid and calcium fructoborate on breast cancer cells**R. N. Ciubar¹, R. I. Scorei², C. M. Ciofrangeanu¹, V. Mitran¹, A. Cimpean¹ and D. Iordachescu¹¹Faculty of Biology, Bucharest University, Bucharest, ROMANIA, ²Craiova University, Craiova, ROMANIA

Recent studies suggested that boron has a chemopreventive role in prostate cancer. We investigated the effects of boric acid (BA), the naturally occurring form of boron circulating in human plasma, and calcium fructoborate (CF), a nutritional supplement which has a chemical structure similar to the natural forms of boron found in edible plants, on the MDA-MB-231 human breast cancer cell line. Exposure to BA and CF inhibited the proliferation of breast cancer cells in a dose-dependent manner. To understand the mechanism of cell growth inhibition, Ki67 cell proliferation marker, p53 and apoptosis related proteins expression following BA and CF treatment were evaluated. We found down-regulation of Ki67 expression which may partly explain the chemopreventive effect of boron compounds. Treatment with CF but not BA resulted in a decrease of p53 and bcl-2 protein levels. Furthermore up-regulation of pro-caspase-3 protein, increase in cytosolic cytochrome c and caspase-3 activity were observed after treatment with CF indicating apoptotic cell deaths. Staining with annexin-V and propidium iodide showed that the number of apoptotic cells increased with higher doses of CF. From these results we conclude that while both BA and CF inhibit the growth of breast cancer cells, only CF induces apoptosis. Further studies will be needed to determine if BA and CF will be suitable for clinical application in breast cancer patients.

C2-62**Molecular mechanisms of tumour cell growth inhibition by curcumin treatment**

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Numerous reports suggest that curcumin, a natural polyphenol exerts anti-cancer properties and may be useful for dietary prevention of cancer. We investigated the effects of curcumin on proliferation and apoptosis-related protein expression of two breast cancer cell lines with different *in vivo* tumorigenicity (MDA-MB-231 < MDA-MB-435). Curcumin reduced cell growth by 50% after 2 h in 231 cells. The response of 435 cells to curcumin is also rapid, but much weaker (only 10% decrease of viability), although the level of proliferation markers-PCNA and Ki67 remain unchanged comparative with 231 cells were their reduction was observed after 24 h treatment. Following curcumin treatment cell apoptosis occurred after 2 h and the number of annexin V positive cells was much higher in 231 cell line than in 435. We have analyzed the protein expression of p53, Bcl-2, pro-caspase-3 by western blot, cytochrome c release and the caspase-3 activity by ELISA. The curcumin treatment of 231 cells decreased the level of Bcl-2 and pro-caspase-3 proteins; increased the caspase 3 activity with 100% after 24 h of treatment and induced a significant release of cytochrome c into the cytoplasm. In both cell lines curcumin had no effect on the p53 protein expression. We conclude that curcumin inhibits the growth breast cancer cells with stronger effects in less tumorigenic cells and induces apoptosis through a p53-independent pathway.

C2-63**Anticancer and cytotoxic activities of olive oil phenolic compounds**F. Paiva-Martins^{1,2}, V. Rodrigues^{1,2}, J. G. Coelho^{1,2}, R. Calheiros³ and M. P. M. Marques^{4,5}¹Faculdade de Ciências Universidade do Porto, Porto, PORTUGAL, ²CIQ-UP, Porto, PORTUGAL, ³Química-Física Molecular, Universidade de Coimbra, Coimbra, PORTUGAL, ⁴Química-Física Molecular, Coimbra, PORTUGAL, ⁵Departamento de Bioquímica, Universidade de Coimbra, Coimbra, PORTUGAL

Direct evidence for the protective role of olive oil against cancer has been published. Diets containing 15% olive oil were found to significantly reduce induced pre-cancerous lesions in rat breast and colon, and olive oil phenolic extract has shown to improve the barrier function of CACO2 cells as well as inhibition of HT115 cell attachment and invasion. Oleuropein, the main polyphenol found in olive fruit, completely regress tumors in 9–12 days, when administered to mice with spontaneous tumors. Hydroxytyrosol, one of the components of the phenol extract of olive oil, was shown to inhibit the proliferation of both human promyelocytic leukaemia cells HL60 and colon adenocarcinoma cells HT29 and HT20 clone 19A, by arresting the cell cycle and inducing apoptosis and to prevent an increase in L-isoaspartyl residues, a marker of protein damage in human melanoma cells (M14) exposed to UV. However, studies for the most abundant polyphenols in the oil, are inexistent. The present work reports the *in vitro* evaluation of the anti-proliferative and cytotoxic activity of the most important secoiridoids present in olive oil, the 3,4-DHPEA-EDA and 3,4-DHPEA-EA, against distinct human cancer cell lines (namely C32 melanoma cells). Different growth-inhibition and viability determination methods were used: Trypan Blue exclusion method and Sulphorodamine (SRB) test. The toxicity of these compounds towards non-neoplastic cells was also evaluated, through similar experiments.