Results. After i.v. IDN 5390 was rapidly distributed and eliminated in a dose dependent manner with clearance of 2.6, 1.4 and 0.9 L/Kg h at the doses of 60, 90 and 120mg/Kg, respectively and terminal half-life (T/2) less than 1 h. The drug was found mainly distributed in kidney, liver, heart and lung and in minor amount in brain. After the oral doses, IDN 5390 was rapidly absorbed (Tmax, 15 min), distributed and eliminated with half-lives of 17–42 minutes. At the dose of 60 mg/Kg, bioavailability resulted 43% and lower, 30% at the highest doses. The exposure to the drug diminished after one week of repeated oral administrations being the AUC determined on days 7 about 50% than the AUC determined after single administration. Incubation experiments conducted with mouse microsomes showed the formation of at least seven metabolites that are products of oxidation, mono- and dihydroxylation reactions. The presence of these metabolites and other related structures were found in faeces and urine. The faecal excretion of the parent drug and of the main identified metabolites amounted to about 20% of the administered dose. Less than 2% of the dose was recovered in urine.
Conclusions. IDN 5390 is rapidly absorbed after oral administration, it possesses good bioavailability that seems reduced after repeated administrations. The drug is mainly distributed in liver, kidney and heart and rapidly eliminated with half-life of less than 1 hour mostly via faecal excretion. As reported for paclitaxel and docetaxel, the metabolism of the drug seems to play a relevant role in the elimination of the drug.

572 POSTER

An investigation of the anticancer mechanism of citrus flavonoids tangeretin and nobiletin

K. Morley, J. Koropatnick. *University of Western Ontario, London, Canada*

Flavonoids are a large group of polyphenolic compounds found in all living plants. Tangeretin and nobiletin are among the most effective flavonoids, from the citrus group, at inhibiting cancer cell growth *in vitro* and *in vivo*. However, relatively little is known about the antiproliferative mechanism of these compounds. We investigated the antiproliferative activity and mechanism of action of tangeretin and nobiletin in human breast cancer cell lines MDA-MB-435 and MCF-7, the human colon cancer line HT-29 and an untransformed human endothelial cell line (HUVEC). Tangeretin and nobiletin inhibited the proliferation of all four cell lines in a dose-dependent manner. Their capacity to inhibit proliferation of the untransformed cell line was equal to that of the tumour cell lines. DNA flow cytometry, using propidium iodide labelled nuclei, revealed that tangeretin and nobiletin blocked cell cycle progression at G_1 in MDA-MB-435, MCF-7 and HT-29 cells. Flow cytometry using Annexin-V and propidium iodide labelling to determine apoptosis revealed that neither flavonoid caused apoptosis or necrosis in any of the tumour cell lines, at concentrations at which proliferation was significantly inhibited. Global analysis of gene expression using Affymetrix oligonucleotide arrays revealed downregulation of cyclin E2 expression (a G_1 phase cyclin) in MDA-MB-435 following short-term exposure to tangeretin, consistent with the flavonoid-induced cell cycle block. In total there were 22 genes with two-fold or greater changes in expression; they have functions in signal transduction, transcription, apoptosis and phospholipid metabolism. These results suggest that at the concentrations used in this study, tangeretin and nobiletin are cytostatic but not cytotoxic, and that cytostasis is the mechanism by which these compounds inhibit human tumour cell growth. Inhibition of proliferation of human cancers without inducing cell death may be an advantage in treating human tumours in the context of normal, untransformed tissues.
Supported by CIHR and KGK Synergize Inc.

573 POSTER

A mechanism for cancer prevention by carotenoids: the role of Nrf2 transcription factor

J. Levy, A. Ben-Dor, L. Gheber, M. Danilenko, M. Steiner, N. Dubi, K. Linnewiel, Y. Sharoni. *Ben Gurion University, Clinical Biochemistry, Beer Sheva, Israel*

Epidemiological studies have found an inverse association between tomato products consumption and the risk of many types of cancers. The mechanism for cancer prevention by tomato phyto-nutrient is not clear yet. However, Induction of phase II detoxification enzymes is a possible pathway for reduction of cancer risk. Expression of phase II enzymes, such as NAD(P)H:quinone oxidoreductase (NQO1) and γ-glutamylcysteine synthetase (GCS), is regulated by the antioxidant response element (ARE), which is found in the promoters of genes encoding these proteins. The transcription factor that binds to ARE and induces the expression of phase II enzymes is Nrf2. We found that in transiently transfected cancer cells lycopene (the main tomato carotenoid) transactivated the expression of a reporter gene fused with ARE sequences. Other carotenoids such as phytoene, phytofluene, beta-carotene and astaxanthin had a much lesser effect. An increase in NQO1 and GCS protein as well as mRNA levels was observed in non-transfected cells after carotenoid treatment. Ethanolic extract of lycopene containing yet unidentified hydrophilic derivatives of the carotenoid activated ARE with similar potency to lycopene, suggesting that also oxidized derivatives of the carotenoids are effective. The potency of the carotenoids in ARE activation did not correlate to their effect on intracellular reactive oxygen species (ROS) and GSH level, which may indicate that ARE activation is not solely related to their antioxidant activity. The increase in phase II enzymes was abolished by a dominant negative Nrf2, suggesting that carotenoid induction of these proteins depends on a functional Nrf2 and the ARE transcription system. Moreover, Nrf2, which is found predominantly in the cytoplasm of control cells, translocated to the nucleus after treatment with some carotenoids. Our results imply that the ARE-regulated induction of phase II enzymes is a novel molecular mechanism for the cancer-preventive action of a diet rich in carotenoids.

574 POSTER

Comparative study of anticancer and apoptosis-inducing activity of stilbene derivatives in HL-60 human promyelocytic leukemia cells

Z. Horvath[1], N. Handler[2], P. Saiko[1], S. Madlener[1], C. Illmer[1], M. Murias[2], M. Fritzer-Szekeres[1], T. Erker[2], W. Jäger[2], T. Szekeres[1]. [1]*General Hospital of Vienna, Clinical Institute of Medical and Chemical Laborat, Vienna, Austria;* [2]*University of Vienna, Institute of Pharmaceutical Chemistry, Vienna, Austria*

Resveratrol (3,4′,5-trihydroxystilbene, figure 1), a polyphenol mainly present in the skin of grapes and red wine, possesses antioxidant and antitumor effects in vitro against a multitude of human cancer cell lines. Recently, a polyhydroxylated derivative of resveratrol, 3,4,5,3′,4′,5′-hexahydroxystilbene (M8, figure 2) was synthesized, which was also shown to exhibit antioxidant properties. However, the effects of M8 on tumor cells have not been investigated yet. In the present study, we compared the effects of resveratrol and M8 in HL-60 promyelocytic leukemia cells with respect to cytotoxicity, induction of apoptosis and influence on cell-cycle phase distribution as well as their effects on the intracellular concentrations of deoxyribonucleosidetriphosphates (dNTPs); dNTPs are crucial substrates for de novo DNA synthesis and were shown to be altered due to resveratrol incubation. In growth inhibition assays, we determined the IC_{50}-values of the compounds, which were 12 μM and 6.25 μM for resveratrol and M8, respectively. Using a specific double staining method, we found that M8 induced apoptosis in HL-60 cells at concentrations significantly lower than those of resveratrol. After incubation with 12.5 and 25 μM of the drugs, 19.3% and 44.9% of cells treated with resveratrol underwent apoptosis, whereas M8 could induce programmed cells death in 89.4% and 100% of the cells under the same conditions. Using HPLC methods, 12.5 μM resveratrol significantly depleted all dNTP pools (69%, 61%, 26% and 30% of control for dCTP, dTTP, dATP and dGTP, respectively), whereas 12.5 μM M8 caused an increase of dCTP pools and significantly decreased dTTP and dATP concentrations (137%, 72% and 27% of control, respectively). Resveratrol and M8 altered the cell cycle phase distribution in HL-60 cells, indicating cell cycle specific activity of both compounds.
Our data demonstrate that resveratrol and M8 are both potent antileukemic compounds in vitro. M8 is more active regarding cytotoxicity and induction of apoptosis, which indicates, that introduction of additional hydroxy-groups on the stilbene rings might increase and/or alter the biological activity of resveratrol.

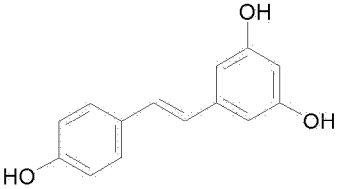

Figure 1. Structural formula of resveratrol.

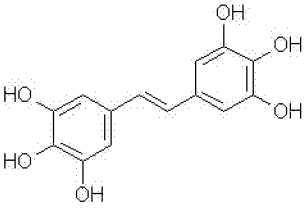

Figure 2. Structural formula of 3,4,5,3′,4′,5′-hexahydroxystilbene (M8).