was implanted intraperitoneally. Live images were collected immediately. Next day curcumin (200 ug/ mouse) was injected ip. Images were collected after 2nd and 4th days with fluorescent stereomicroscope. Upon necropsy peritoneal wash was collected for culture and further examination to assess the cell death relative to the imaging data.
Results: In vitro results showed all treated cancer cells displayed green fluorescence. Viability test showed 100% cells were dead that were GFP-positive. In vivo images showed curcumin incorporation into the implanted cancer cells that were was imaged with GFP filter. On day 2 more GFP signal was detected than 4, since cells were killed by curcumin.
Conclusions: Therapeutic effect of curcumin was detected by fluorescent stereomicroscopy in a non-invasive way. This natural product can be used as a preventive medicine against cancer.

569 POSTER

A new antitumor compound, ECO-04601: preclinical evaluation and in vivo efficacy in glioma

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ECO-04601 is a structurally novel farnesylated diazepinone (MW 462) discovered using Ecopia's genomic platform through analysis of actinomycete loci encoding bioactive compounds. ECO-04601 is being developed as an antitumor agent as it shows suitable pharmaceutical properties, including in vivo efficacy, low toxicity, rapid absorption and bioavailability in target tissues. We have recently shown that ECO-04601 strongly inhibits proliferation of several human cancer cell lines in vitro, including low and high-grade human glioma cells (IC50 = 1 to 8 microM). To demonstrate in vivo efficacy, nude mice were inoculated with rat C6 glioma cells (5 millions/ml) either subcutaneously (6/group), or orthotopically in the caudate putamen (10/group). Daily treatment (10 to 30 mg/kg, i.p.) was initiated 24 hrs following glioma cell inoculation. When tumors cells were implanted subcutaneously, treatment with ECO-04601 resulted in a significant decrease of the tumor volume by 60%. In the orthotopic model, mice were treated daily with ECO-04601 until spontaneous death. Initial results indicate efficacy of ECO-04601 in this model as we observed a seven-day increase in the median survival of treated mice as compared to the vehicle-treated group. No significant loss of body weight was observed during the chronic treatment regimen of tumor-bearing mice suggesting a favorable toxicity profile of the compound that has been further confirmed by acute and subchronic administration of ECO-04601 in healthy animals. Female CD-1 mice tolerated single intravenous doses of 100 mg/kg of ECO-04601 and repeated subcutaneous or oral doses of 225 mg/kg. Preliminary pharmacokinetic experiments suggest rapid absorption and tissue distribution of the compound following administration by various routes of administration. These data highlight the promising therapeutic potential of ECO-04601 in aggressive tumors like gliomas.

570 POSTER

In vitro and in vivo characterizations of naturally occurring BBIs in reversal of p-gp mediated multidrug resistance

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Background: Multidrug resistance (MDR) is one of the major obstacles limiting the efficacy of cancer chemotherapy. The overexpression of the membrane associated P-glycoprotein (P-gp), which acts as an energy-dependent drug efflux pump, is believed to play a critical role in MDR. A promising strategy to conquer drug resistance is to develop functional MDR modulators that can specifically inhibit the P-gp activity. Through screening a series of natural products, we have recently identified six bisbenzylisoquinoline alkaloids (BBIs) that possess potent activity to reverse P-gp-mediated drug resistance. In this study, we characterized and evaluated the ability of these newly identified MDR Modifiers in reversal of P-gp-mediated drug resistance caused by different antineoplastic agents.
Materials and Methods: T wo human MDR cells (MCF-7/adr and KBv200) and their drug-sensitive parental cells were used for *in vitro* evaluation and characterization. The *in vivo* activity of these promising BBIs was evaluated through establishment of xenograft tumor models.
Results: In vitro assays indicated that these natural BBIs showed potent activities to restore sensitivity of resistant tumor cells, such as MCF-7/adr and KBv200 cells, to many antitumor drugs including doxorubicin, vincristine and paclitaxel. Further analyses by measurement of radioactive [^{3}H]-vincristine and [^{3}H]-paclitaxel indicated that these BBIs increased intracellular drug accumulation in MDR cells, but had little effect on drug-sensitive cells. Through establishment of xenograft models bearing the intrinsically resistant KBv200 tumors, we also tested one of these compounds (FF0019) and demonstrated that this class of naturally occurring MDR modifiers could also significantly potentiate the antitumor activity of VCR and paclitaxel *in vivo*.

Table 1. Effect of BBIs on reversing MDR[a]

		Fold shift of Dox IC_{50}^b		Fold shift of VCR IC_{50}^c	
		MCF-7/ADR	MCF-7	KBv200	KB
FF0011	5 μM	22.1±6.6	1.2±0.7	12.8±1.5	1.3±0.8
	2.5 μM	19.7±1.9	1.1±0.2	9.5±1.9	1.0±0.2
	1.25 μM	7.6±2.2	0.9±0.3	4.1±1.1	1.0±0.3
	0.625 μM	2.3±1.7	0.9±0.2	1.8±0.2	0.9±0.4
FF0012	5 μM	24.5±3.8	1.1±0.4	17.1±3.5	1.2±0.2
	2.5 μM	17.1±2.3	1.0±0.2	9.4±1.7	1.0±0.1
	1.25 μM	7.5±1.9	1.0±0.4	6.1±2.5	1.0±0.2
	0.625 μM	3.0±0.5	1.4±0.4	3.4±2.9	1.1±0.5
FF0014	5 μM	35.0±7.7	1.9±0.3	18.0±6.4	1.4±0.6
	2.5 μM	13.3±4.8	1.4±0.4	9.4±3.6	1.3±0.4
	1.25 μM	6.9±1.6	1.3±0.1	6.1±2.2	1.0±0.1
	0.625 μM	3.2±0.9	O.9±0.3	27±0.5	0.8±0.4
FF0015	5 μM	43.9±15.0	1.1±0.2	21.9±3.5	1.8±0.6
	2.5 μM	32.0±7.2	0.9±0.4	12.9±5.0	1.3±0.5
	1.25 μM	10.3±7.3	1.0±0.3	7.2±4.0	1.3±0.3
	0.625 μM	5.3±2.0	1.1±0.4	3.9±1.6	1.1±0.2
FF0018	5 μM	42.7±6.8	1.3±0.6	20.1±4.6	1.6±0.5
	2.5 μM	22.9±5.4	1.3±0.4	12.9±3.8	1.1±0.3
	1.25 μM	7.7±2.2	1.0±0.3	5.8±2.3	1.1±0.4
	0.625 μM	3.6±1.1	1.0±0.3	2.8±1.1	1.0±0.4
FF0019	5 μM	49.0±7.9	1.4±0.4	23.0±5.6	1.5±0.6
	2.5 μM	29.4±5.8	1.0±0.3	15.6±6.6	1.3±0.4
	1.25 μM	12.3±3.9	1.0±0.2	8.9±2.5	1.1±0.5
	0.625 μM	3.4±0.7	0.9±0.2	3.2±0.9	0.9±0.2
VRP	5 μM	7.6±3.4	1.0±0.3	6.4±1.4	1.1±0.4

[a]This table is based on three separate experiments and presented as mean±SEM.
[b]The IC_{50} of Dox for MCF-7/adr and MCF-7 cells in the absence of BBIs are 16.712 μM and 0.1718 μM, respectively.
[c]The IC_{50} of VCR on KBv200 and KB cells in the absence of BBIs are 0.054 μM and 4.437 μM, respectively.

Conclusions: These results suggested that the mechanism of these compounds to reverse MDR was associated with the increase in the intracellular drug accumulation through inhibiting the activity of P-gp. These compounds may possess great promising in being developed into novel anticancer drugs as modifiers of MDR (Supported by NIH Grants CA82440 and CA92280).

571 POSTER

Bioavailability and pharmacokinetic study of the novel oral C-Seco taxane derivative IDN 5390 in mice

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Background. IDN5390 is the prototype of C-Seco taxanes, a new class of semi-synthetic taxoids. C-seco taxanes are characterized by an opening of C-ring by cleavage of C7-C8 bond. It was selected from a screening of new molecules with antiangiogenic and antimetastatic properties. It has shown high antitumor activity and good tolerability against a variety of human tumor xenografts including ovarian, colon ca and glioblastoma either sensitive or resistant to paclitaxel. The therapeutic advantages of IDN 5390 over paclitaxel were evident when the drug was administered by protracted oral-treatment schedules.
Aims: To characterize the pharmacokinetic of IDN 5390 after single and repeated administration in mice, we have determined the bioavailability, tissue distribution, faecal and urinary excretion and the *in vitro* (hepatic microsomes) and *in vivo* metabolism.
Materials and Methods. The study was carried out in CDF1 female mice treated with single intravenous and oral doses of 60, 90 and 120 mg/Kg or, for one week with protracted oral daily exposure of 90 mg/Kg. Blood, urine, faeces and tissue samples were taken at different time points. IDN 5390 were determined by HPLC with UV detection in plasma and tissues and by HPLC/MS/MS in urine, faeces and microsomes.

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

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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.
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573 POSTER

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

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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

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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.

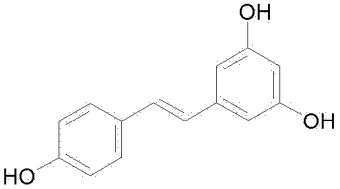

Figure 1. Structural formula of resveratrol.

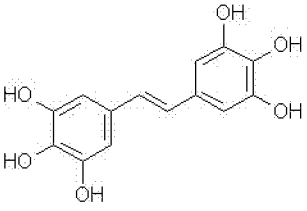

Figure 2. Structural formula of 3,4,5,3′,4′,5′-hexahydroxystilbene (M8).