

without significantly affecting the maximum plasma concentration (C_{max}) or time to maximum plasma concentration (t_{max}) of CS-7017. There were no deaths, serious adverse events (SAEs) or discontinuations due to AEs in this study. All treatment emergent AEs (TEAEs) were mild in severity. Only two TEAEs (abdominal distension and abdominal discomfort) were considered to be related to study treatment (ketoconazole).

Conclusions: Co-administration of ketoconazole significantly increased the bioavailability of CS-7017. Administration of a single dose oral dose of 0.25 mg CS-7017 either alone or concomitantly with 400 mg ketoconazole was well tolerated in healthy male subjects.

327

POSTER

Activating transcription factor 3 as a novel regulator and predictor of cisplatin response in human cancers

A. O'Brien¹, J. Dimitroulakos¹. ¹Ottawa Hospital Research Institute, Centre for Cancer Therapeutics, Ottawa, ON, Canada

Background: The development of resistance to platinum-based chemotherapy is a major obstacle in the treatment of cancer. Activating transcription factor 3 (ATF3) is a stress-inducible gene that we recently demonstrated to be a regulator of cisplatin induced cytotoxicity. Further characterization of ATF3's role in cisplatin cytotoxicity will lead to novel therapeutic approaches that could improve the efficacy of this important class of chemotherapies.

Methods: A panel of human cancer cell lines was treated with a range of cytotoxic and non-cytotoxic doses of cisplatin. Cell viability was assessed by the MTT assay and a time course of ATF3 protein expression was evaluated by Western blot. The expression of potential ATF3 targets in response to cisplatin treatment was determined by Western blot and confirmed by the chromatin immunoprecipitation assay. ATF3 levels were evaluated in a clinical setting where total RNA was extracted from 51 resected ovarian tumors and basal ATF3 mRNA was evaluated by QPCR. Immunohistochemistry using an anti-ATF3 antibody was performed on ovarian and lung cancer tissue microarrays.

Results: ATF3 protein expression was induced upon cytotoxic doses of cisplatin treatment in all cell lines examined. Two potential targets of ATF3 had altered expression in response to cisplatin treatment: cyclin D1, a cell cycle regulator, showed reduced protein expression and CHOP/gadd153, a pro-apoptotic transcription factor, showed increased protein expression. ATF3 was also found to be bound to the CHOP/gadd153 promoter in response to cytotoxic doses of cisplatin. In a clinical setting, basal ATF3 mRNA levels were variable among ovarian tumors and there was a trend toward higher ATF3 expression correlating with better outcomes. ATF3 protein expression on tissue microarrays containing ovarian and non-small cell lung cancer cases was found to be differentially expressed among tumor subtypes. A higher proportion of ATF3 expression was seen in ovarian mucinous adenocarcinomas and lung squamous cell carcinomas suggesting that stratifying treatment based on tumor subtype might yield better patient outcomes.

Conclusions: ATF3 is a novel regulator of cisplatin cytotoxicity acting through the suppression of cyclin D1 and activation of CHOP/gadd153 protein expression. The basal expression of ATF3 might be predictive of cisplatin treatment response and may present itself as a novel biomarker.

328

POSTER

P-glycoprotein antagonists as opposed to inhibitors of glucosylceramide synthase enhance short-chain ceramide cytotoxicity in human ovarian cancer cells

J.V. Chapman¹, V. Gouazé-Andersson¹, R. Karimi¹, M.C. Cabot¹. ¹John Wayne Cancer Institute, Experimental Therapeutics, Santa Monica, USA

Background: A major focus in the field of drug resistance has been on design and testing of antagonists that limit drug effluxing properties of P-glycoprotein (P-gp). This direction however has shown little clinical benefit. The purpose of this study was to assess the utility of P-gp antagonists as enhancers of ceramide-induced cell death in ovarian cancer, a malignancy in which P-gp occurs with high frequency and correlates with disease progression.

Materials and Methods: A2780 wild-type and the multidrug resistant (MDR) counterpart 2780AD, and MDR NCI/ADR-RES human ovarian cancer cell lines were employed. Cultures were exposed to the ceramide (cer) analog C6-cer (5–10 μ M) in the absence and presence of P-gp antagonists (2.5–5 μ M) tamoxifen, cyclosporin A, and VX-710 (Biricodar[®]), or the glucosylceramide (GC) synthase (GCS) inhibitor, ethylenedioxy-P4 (P4). Cell viability was measured by Cell Titer 96 Aqueous[®]. C6-cer metabolism was followed using N-hexanoyl[1–¹⁴C]-D-erythro-sphingosine, thin-layer chromatography, and liquid scintillation counting. Cell-free GCS assays employed [³H]UDP-glucose and C6-cer.

Results: Compared to wild-type A2780 cells, P-gp rich 2780AD cells converted 4-fold more C6-cer to nontoxic C6-GC (C6-GC), whereas cell-free GCS activities were equal. In 24–72 hr viability assays, 2780AD cells demonstrated resistance to C6-cer and A2780 cells were sensitive. Tamoxifen and cyclosporin A inhibited conversion of C6-cer to C6-GC by 70–90% in 2780AD cells. C6-cer resistance in 2780AD cells was reversed by tamoxifen but not by addition of the GCS inhibitor P4, even though P4 inhibited conversion of C6-cer to C6-GC by 90%. For example, cell viabilities measured 78, 84, and 83% of control in C6-cer-, tamoxifen-, and P4-treated cells, respectively, and 15 and 68% in cells exposed to C6-cer/tamoxifen and C6-cer/P4, respectively. Co-administration of C6-cer and P-gp antagonists was also effective in reducing NCI/ADR-RES cell viability; whereas C6-cer, VX-710, and cyclosporin A exposure resulted in viabilities of ~90%, C6-cer/VX-710 and C6-cer/cyclosporin A exposure resulted in viabilities of 22 and 17%, respectively.

Conclusions: Many studies have focused on inhibiting GCS to enhance ceramide-related cytotoxicity, as GCS catalyzes GC synthesis. Here we have taken advantage of the function of P-gp in GC trafficking and shown that targeting P-gp is more effective than inhibiting GCS for increasing ceramide cytotoxicity.

329

POSTER

Insights into the cellular responses against different DNA-binding agents

M. Zihlif¹, L. Wakelin², D. Catchpool³, B. Bernard W. Stewart⁴. ¹University of Jordan, Pharmacology, Amman, Jordan; ²University New South Wales, Pharmacology and Physiology, Sydney, Australia; ³Children Hospital of Westmead, Tumor bank, Sydney, Australia; ⁴Public Health Unit, South East Sydney Public Health Unit, Sydney, Australia

The cell response to anti-cancer agents is largely determined by the expression of the genetic materials into proteins in space and time. Here, we used the cDNA microarray technology to follow the biological responses to different classes of anti-cancer DNA binding agents. Although all chosen agents are a DNA minor groove binding agents, they exert different mechanisms of killing towards the cancer cells. The examined agents include doxorubicin (topoisomerase poison), echinomycin (transcription inhibitor), alkamin (minor groove alkylating agent). The cultured human CEM cells were the model and the 5 \times IC₅₀ for 24 h were the concentration and the time of exposure. The expression of 6000 human genes printed cDNA was the target. Genes that have been up-regulated more than 3 folds were selected and used as a representative for the cell responses against these agents. Applying ontological and pathway analysis results three distinguish profiles. The first profile that representing topoisomerase poisons mainly characterized by the up-regulation of those functions that related to protein metabolic process, regulation of macromolecule metabolic process, regulation of apoptosis, regulation of signal transduction. Importantly the ubiquitination pathway was largely up-regulated suggesting an attempt to remove the trapped cleavable complex by an ubiquitin-dependent mechanism, marking it a substrate for proteolysis by the 26S proteasome. The second resulted profile, which represent the transcription inhibitors, distinguished from the first by the abundance of those functions that related to RNA metabolic process, regulation of signal transduction, protein kinase cascade, mRNA metabolic process. One significant point to be mention here is that echinomycin profile is characterized by many negative impacts on the important cellular functions. It is as if the cells are down regulating many of its essential functions to cope with DNA assault. The third biological profile, the alkamin profile, is dominated by those functions related to transcription activation and RNA biosynthesis and cell cycle. Interestingly, in the alkamin profile the basic transcription factor pathway and the RNA elongation ontological category were largely up-regulated alarming the idea that alkamin mechanism of action may involve transcription inhibition. Finally we concluded that the muted response of the drugs suggests a degree of biological resistance amongst functions that might be expected to be most damaged by drug action.

330

POSTER

P-glycoprotein in doxorubicin resistant MCF-7 breast cancer cells: DPAGT1 approach to N-glycosylation regulation

I. Kuznecovs¹, G. Kuznecova¹, K. Jegina¹, S. Kuznecovs¹. ¹Preventive Medicine Research Society, Cancer Research Laboratory, Riga, Latvia

Background: Dysregulation of DPAGT1 (Dolichyl-phosphate (UDP-N-acetylglucosamine) N-acetylglucosaminophosphotransferase 1 (GlcNAc-1-P transferase) causes disturbances in P-glycoprotein (Pgp) expression in multidrug resistance. The recent results are in favour of the idea that N-glycosylation in cancer cells is limited by Dolichyl Phosphate (DolP). The aim of the present study is to investigate the effect of polyprenol (PP)