1215 POSTER

Involvement of GAPDH in DNA adduct recognition: implication for a DNA destabilizing compound

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Background: Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a glycolytic enzyme catalyzing the formation of 1,3-bisphosphoglycerate from glyceraldehyde-3-phosphate. Beside this cytoplasmic function, many other were identified such as a role in microtubule organization and apoptosis induction through different activation processes among which the release of mitochondrial pro-apoptotic factors. In parallel, GAPDH also localizes in the nucleus where it is implicated in RNA, double-stranded (dsDNA) and single-stranded (ssDNA) DNA binding, properties which afford GAPDH to have a role in DNA repair, transcription and replication. Interestingly, GAPDH recognizes DNA alkylated by saframycin A (Xing et al., 2004), a DNA minor groove alkylating agent that forms covalent bond with the N2 group of guanines and is structurally related to the natural marine alkaloid ecteinascidin 743 (ET-743) used in clinic.
Results: With the objective to identify proteins involved in the recognition of minor groove DNA adducts from comparison with major groove targeting drugs, we focused on the benzo-*b*-acronycine derivative S23906−1. This minor groove alkylating agent presents the original ability to locally open the double helix of DNA.
Protein chromatographic purification followed by 2D-electrophoresis and MALDI-TOF analysis identify GAPDH as a S23906−1/DNA binding protein. We validated the direct binding of GAPDH to alkylated ds- and ss-DNAs using EMSA, both DNAs being generated upon S23906−1 treatment. Comparison of the results obtained using different ds- or ss-DNAs exemplifies the possible sequence selectivity of GAPDH binding to DNA alone as well as to S23906−1/DNA adduct. Interestingly, GAPDH failed to bind to ET-743/DNA adduct, eventhough it recognizes the structurally-related compound saframycin A, suggesting DNA/adduct selectivity.
Conclusion: Very little is known about the way the minor groove DNA lesions are processed in cells and how they interfere with the cellular machinery. We will evaluate the involvement of this protein in S23906−1 cytotoxic activities to better understand the mechanism of action of this original DNA alkylating agent. This work enlightens some sequence-specific binding of GAPDH to native DNA or alkylated one which we currently assessing to better understand its nuclear mechanism of action.

1216 POSTER

Pharmacokinetics studies in balb/c treated with docetaxel and trans-resveratrol

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Background: Docetaxel belong to the taxane family of antineoplastic agents that are widely accepted as evidence-based components of therapy for many advanced malignancies, including breast, lung, ovarian and hormone-refractory prostatic cancer. The following pharmacokinetics studies have been carried out in order to evaluate possible variations in blood concentration of docetaxel and trans-resveratrol a polyphenol extracted from *Vitis vinifera L.* when administered concomitantly in mice.
Materials and Methods: Study experiments have been performed on blood samples of male balb/c mice, aged 6−8 weeks, enrolled in 4 diet-controlled groups of 21 mice each (a, b, c) and treated as follows: trans-resveratrol alone ip 4 mg/Kg, trans-resveratrol ip 4 mg/Kg + docetaxel and docetaxel alone.
For pharmacokinetics study, blood samples were withdrawn at times 0, 3, 5, 10, 15, 30 and 60 minutes. Each blood sample of 100 μl was added to 200 μl of CH_3CN and centrifuged at 800 (rpm) for 10 minutes. 200 μl of water was added to an equal volume of supernatant; the resulting samples were strored at a temperature of −20ºC until analysis.
Qualitative and quantitative analyses of docetaxel, trans-resveratrol and its metabolites were carried out by means of both nano flow liquid chromatography and MS/MS detection. Separation was achieved with a linear gradient water-HCOOH/CH_3CN on a 43 mm C18 chip with a 40 nL trapping column. Detection of docetaxel, and trans-resveratrol together with metabolites, occurred in the positive and in the negative ion mode, respectively, following ionization in the electrospray interface. The quantitative analysis was performed by selecting the MRM transitions for docetaxel, trans-resveratrol and its metabolites.
Results: In mice treated with concomitant trans-resveratrol and docetaxel, pharmacokinetics profiles showed a docetaxel blood concentration increase of nearly 100% in all of the three phases α, β and γ. Moreover, an increase in docetaxel phase γ half-life was observed as a result of peripheral blood circulation return.
Conclusions: For the detection of docetaxel and trans-resveratrol with its metabolites, we have developed an innovative analytical method which allows for high sensitivity and specificity, due to the coupling of a nano-HPLC system with MS/MS detection.
The pharmacokinetics results are really interesting as they pave the way to new clinical trials with lower doses of docetaxel and reduced side effects at the same therapeutic dosage.

1217 POSTER

Dithiarsolanes in the treatment of glioma: in vitro activity on U87 cell line and brain concentrations on a mouse model

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Background: Arsenic trioxide (Trisenox®) is used in the treatment of patients with acute promyelocytic leukemia (APL) who relapsed after initial therapy with chemotherapy and *all-trans* retinoic acid. Additively, an organoarsenical compound (melarsoprol, Arsobal®) has been tested clinically on refractory leukemia; the clinical trial was prematurely closed due to toxicity [1].
Interestingly enough, the less known arsthinol inhibits growth of U937 and K562 at lower concentration than As_2O_3 and melarsoprol. Similarly to melarsoprol, arsthinol is a dithiarsolane and it was used in the 1950's in the treatment of amebiasis and considered as "highly tolerated" [2]. In this work, we have investigated the activity of arsthinol on human U87 glioma cell lines as compared with As_2O_3 and melarsoprol. This study was completed by a pharmacokinetics study of the organoarsenicals (mouse model) in the brain.
Material and Methods: The classical MTT test was used to determine growth inhibition and cytotoxic activity on human U87 glioma cells after treatment with arsthinol, As_2O_3 or melarsoprol (0.01 μmol/l to 1 mmol/l, 24 h or 48 h, 37ºC, 5% CO_2).
Pharmacokinetics studies were conduction in a mouse model (female CD1, Charles Rivers, 24−28 g). Formulations were administered intravenously at a dose of 0.2 or 0.056 mmol/kg via the caudal vein. The amount of total arsenic in the brain was assessed using a colorimetric method [3] after digestion with nitric acid (HNO_3; 65%) and H_2O_2 30%.
Results: Arsthinol was found to be more effective (IC_{50} = 10.4±3.7 μmol/l) than As_2O_3 (IC_{50} = 16.3±8.1 μmol/l) and melarsoprol (IC_{50} = 25.3±9.2 μmol/l) after 24 h. Similar results were obtained after 48 h. Moreover, after injection of 0.2 mmol/kg significant concentrations were obtained in the brain (0.15 μmol/g of brain after 1 h) but the drug was well tolerated [4].
Conclusion: Compared with As_2O_3 and melarsoprol, arsthinol, which was used in the 1950's has a better anticancer activity on U87 glioma cell line. Moreover, the drug passes through the hemato-encephalic barrier and is considered as "highly tolerated" among organoarsenicals. The drug has now to be tested on a mouse model of glioma to confirm these results.

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1218 POSTER

In vivo antitumor activity of platinum(II) complexes with thiosemicarbazones derived from 2-formyl and 2-acetyl pyridine and containing ring incorporated at N(4)-position

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Background: Thiosemicarbazones (TSCs) are among the most potent inhibitors of ribonucleotide reductase (RR) activity and possess a wide

range of biological activity depending on the parent aldehyde or ketone heterocyclic TSCs have aroused considerable interest in chemistry and biology due to their antibacterial, antimalarial, antineoplastic and antiviral activities. In this study we report the toxicity and antitumor activity of new platinum (II) complexes: the TSCs of 2-formyl and 2-acetyl pyridine, containing azepane ring incorporated at N(4) position, HL^1 (**1**) and HL^2 (**2**) with platinum (II) afforded the complexes, [Pt(L^1)Cl] (**3**) and [Pt(L^2)Cl] (**4**).
Material and Methods: Stock solutions of the compounds **1**-**4** were prepared immediately before use. They were suspended in corn oil following initial dissolution in 10% DMSO. BDA/2 mice were used for toxicity studies. Lymphoid leukemia L1210 bearing BDF1 mice were used to determine the antitumor effect. The tumor was maintained in ascetic form by injection of 1×10^5 cells at 7-day intervals intraperitoneally in DBA/2 mice. The antitumor activity of the compounds was assessed from the oncostatic parameter T/C %. Treatments were given as a single LD10 dose on day 1.
Results: The LD10 therapeutic dose was 40, 37, 53 and 76 mg/Kg for compounds **1**-**4**.
The ligands **1** and **2** cause acute toxicity and display some antitumor activity, while the compounds **3** and **4** show reduction of the toxicity and high increase of survival time of drug-treated leukemia bearing mice. The ligand **2** is achieving a T/C % value of 117 and the platinum (II) complex **4**, of 384. The ligand **1**, is achieving a T/C % value of 141, while the platinum (II) complex **3**, of 301. One of six mice of complex **3**-treated animals was cured and was considered as long-term survivor, i.e. mice alive 90 days.
Conclusions: The replacement of a methyl group at position 7 (HL^2) by a C(7) H group (HL^1) in the 2-pyridil position, causes marked differences in the biologic results as significant increase of life-span of the drug-treated leukemia bearing mice (T/C % 141 and 117 for HL^1 and HL^2, respectively). Probably the Pt (II) complexes [Pt(L^1) Cl] (**3**) and [Pt(L^2) Cl] (**4**), may interact easier with DNA or proteins than the other compounds. Complex **4** was proved to be the most potent antileukemic agent, comparing the T/C% value. Compound **3** yielded a high T/C% of 301 and afforded additionally one of six cures. Compounds **3** and **4** had decreased toxicity combined with impressive potency.

1219 POSTER

Trans-resveratrol reverse drug resistance to docetaxel: a preliminary in vivo study

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Background: Chemotherapeutic drug resistance remains a significant obstacle in the control of prostate cancer in particular of the Docetaxel, that represents the major drug in human prostate cancer treatment. Previous "in vitro" studies showed the capacity of trans-resveratrol to reverte drug resistance to docetaxel in human prostate cancer cells (DU-145). Here, we investigate the sensitization effects of trans-resveratrol in xenograft nude mice models to improve the efficacy of this treatment in prostate cancer.
Material and Methods: Docetaxel-resistant human androgen-independent prostate carcinoma cell line **(**DU-145) were developed by cell culture in medium containing Docetaxel in a dose escalation manner (starting from 1 nM until to 10 nM). Docetaxel-resistant DU-145 cells were injected subcutaneously in posterior legs of 5 nude mice (experimental group) at concentration of 1×10^6 cells/100 μL. After tumor establishment, animals received Docetaxel (5 mg/Kg/4 d) trans-resveratrol by Alzet osmotic pumps placed subcutaneously in such a way to reach a constant blood concentration of 10 μg/mL for 1 mounth. Control group (5 nude mice) were treated only with Docetaxel (5 mg/Kg/4 d) subcutaneously. Weekly, tumor growth were monitored by micro-ultrasound in vivo imaging method and levels of tumor angiogenesis were studied by molecular biomarker (VEGFR2).
Results: Docetaxel-resistant human androgen-independent prostate carcinoma cell line **(**DU-145) was successfully implanted in nude mice, developing a xenograft cancer model for the "in vivo" study of Docetaxel drug resistance. Tumor volume measurements by micro-ultrasound method showed that in experimental group a marked and statistically significative regression of tumor evaluated when nude mice were treated with Docetaxel and trans-resveratrol respect to control group treated only with Docetaxel. Moreover, also tumor angiogenesis in experimental group were less marked respect to control group.
Conclusions: Further experiments are necessary for a better evaluation of the combined effect of trans-resveratrol and Docetaxel. However, our data showed that trans-resveratrol was able to revert drug resistance to Docetaxel in a xenograft model, opening the new way to a clinical trials for the combined use in the therapy of human prostate cancer.

1220 POSTER

Antitumor activities of nab-rapamycin (ABI-009) enhanced by combination with kinase inhibitors Erlotinib and Perifosine

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Background: *Nab*-rapamycin (ABI-009) was developed using proprietary *nab*-technology and showed dose-linear pharmacokinetics and safety up to 90 mg/kg in rats. ABI-009 exhibited effective antitumor activity at 40 mg/kg against a panel of human tumor xenografts. The goal of this study was to develop an effective combination regimen for ABI-009 to test the hypothesis that inhibition of mTORC2, in addition to inhibition of mTORC1 by rapamycin, is necessary to achieve complete shutdown of mTOR signaling pathway.
Materials and Methods: Subcutaneous human breast (MDA-MB-231) tumors were grown in athymic nude mice and treated intravenously (IV) with ABI-009 alone at 40 mg/kg (3xwkly/4 wks) and in combination with Erlotinib, Perifosine, Cetuximab, doxorubicin, SAHA, and oxaliplatin.
Results: ABI-009 was highly effective as a single agent against MDA-MB-231 breast tumor xenografts with 75% tumor growth inhibition (TGI). Combination with Erlotinib resulted in TGI of 85% and 95% (50 and 100 mg/kg respectively, daily/4 wks, IP), 92% and 96% for Perifosine (30 and 60 mg/kg respectively, 3×wkly/4 wks, PO), 83% and 87% for Cetuximab (20 and 40 mg/kg respectively, 3×wkly/4 wks, IP), 83% and 90% for doxorubicin (2.5 and 5 mg/kg respectively, wkly/10 wks, IV), 82% and 90% for SAHA (50 mg/kg daily/7 or 14 days respectively, IP), and.82% and 85% for oxaliplatin (5 and 10 mg/kg respectively, wkly/4 wks, IV).
Conclusions: ABI-009 alone was highly effective against MDA-MB-231 human breast tumor xenografts. Antitumor activity of ABI-009 was significantly increased in combination with kinase inhibitors (Erlotinib – EGFR kinase inhibitor, and Perifosine – AKT inhibitor) with both showing significant improvement versus ABI-009 alone. In contrast, combination of ABI-009 with anti-EGFR monoclonal antibody Cetuximab was not effective. Antitumor activity of ABI-009 was significantly increased in combination with doxorubicin – a topoisomerase inhibitor, and SAHA – an HDAC inhibitor, but not in combination with oxaliplatin – a DNA crosslinker. The synergy of these combinations confirmed that rapamycin is active only on TORC1 and that suppression of TORC2 via AKT or PI3K pathways is a means of increasing activity of mTOR inhibitors.

1221 POSTER

Enhanced sensitivity to Bortezomib pro apoptotic effects in human cancer cells with acquired resistance to anti-EGFR TKIs

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Background: Despite great clinical promise the majority of cancer patients show either intrinsic resistance or acquired resistance to EGFR inhibitor therapies. Bortezomib (PS-341; Velcade) is an approved drug for the treatment of haematological neoplasms and is being currently evaluated for the treatment of solid cancers. Recent works showed that bortezomib may play a role as sensitizer for the EGFR inhibitor demonstrating a rationale for the combined use of bortezomib with EGFR inhibitors and thus in cancer cells not anymore responding to the EGFR blockade.
Materials: We developed gefitinib- and erlotinib-resistant non small cell lung cancer (Calu 3) and colon cancer (HCT116) cell lines.
Results: These resistant cell line showed iperactivation of Akt and survivin if compared to parental lines. Bortezomib treatment induced a strong inhibition of cell proliferation and inhibition of Akt and survivin and induction of apoptosis, but in addition to the inhibitory effect on Akt signalling, bortezomib showed a strong ability to induce the expression of GADD153, a well-recognized ER stress-inducible transcription factor, and DR5, in all resistant cell lines, but not in wild type cells. Furthermore, bortezomib induced significant PARP and bid cleavage by caspase 8 activation.
Conclusions: Together, these findings support a mechanistic framework for the induction of apoptosis in resistant cells by bortezomib in which the ER stress-inducible transcription factor, GADD153, is induced, leading to up-regulated DR5 expression and stimulation of the extrinsic apoptotic pathway.