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.

References

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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