had grade 2 or 3 lymphopenia at baseline. Data on 23 of the 32 patients show that 9 had stable disease and 14 had progressive disease as best response; 1 prostate cancer patient had a drop in PSA level of 88% from baseline. PK data available from 35 patients up to 42.5 mg/m^2 indicate that Cmax and AUC (0–6) appeared to be dose-proportional. Clearance (~19 L/h/m^2), half-life (~5 h), and volume of distribution at steady state (~91 L/m^2) were consistent with the PK seen in adult leukemia patients.
Conclusions: Clofarabine has been administered weekly for 3 weeks (days 1, 8, and 15) every 28 days to adult pts with solid tumors. Patients have been treated with doses up to 53 mg/m^2 and MTD has not been reached. Enrollment is ongoing in the 66 mg/m^2 cohort.

541 POSTER

Effects of bisintercalating DNA threading agents on global gene expression

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We are investigating the capacity of 12 novel DNA bisintercalating threading agents and related compounds, together with recognized transcription poisons (including actinomycin D, echinomycin and nogalamycin), to affect gene expression. The novel agents are dimers of 9-aminoacridine carboxamide in which the linker is attached at the 9 position and bearing different 4-carboxamide threading side chains (Wakelin LPG, *J Med Chem* 46: 5790–5802, 2003). We studied the effect of each of the available agents on the expression of 6000 sequence-verified human genes by cDNA microarray analysis following treatment of cultured CEM cells using a 5 × IC50 concentration for 24h. Cube root plots of array fluorescence intensity values indicated that changes in global gene expression could be represented by three separate populations of genes which respond differently to the various agents. The largest population was comprised of genes which were expressed in control preparations, and whose expression was altered by treatment. Ratiometric analysis, involving comparison of the distribution of log2 of the ratio of fluorescence from both channels on each cDNA microarray, indicated that, for each agent, this population exhibited a near-Guassian distribution and it was further invesigated using heirarchical clustering and Significance Analysis of Microarray (SAM) procedures. Within this set of genes, expression profiles suggestive of common effects attributable to the various agents were not immediately apparent. Thus, despite similarities of structure and DNA interaction, in the context of a common cytotoxic response, a state of 'transciptional chaos' was indicated 24h after toxic insult by these agents. However, expression of a separate gene population (>1000 in each treatment) was eliminated completely in response to the respective agents, despite expression of these genes in control cells. A major proportion of genes in this cluster appear to be common between the agents used and the response will be discussed with reference to mechanisms of action of the DNA interactive agents. The third population of genes, which are silent in control preparations, is expressed following treatment with the various agents. Initial examination using Gene Ontology database searches of the latter population indicates a considerable proportion of these genes is associated with stress response and apoptotic mechanisms.

542 POSTER

Synthesis, lipophilicity and cytotoxicity of new oxaliplatin derivatives

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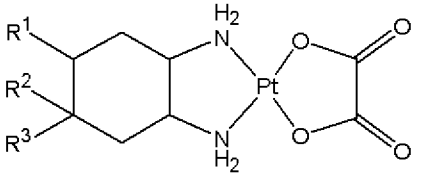
Introduction: Oxaliplatin has been the first platinum drug to prove clinical activity in an inherently cisplatin-resistant malignancy, i. e. colorectal cancer. Although the genuine pharmacodynamic effects of oxaliplatin and the specific properties of its DNA adducts apparently result from the presence of the sterically demanding, hydrophobic cyclohexane ring, structure-activity relationships with regard to modifications of this part of the molecule have not been systematically investigated. In order to fill this gap and to explore possibilities of improving antitumor activity, we have synthesized ring-substituted cyclohexanediamine derivatives and prepared the oxalatoplatinum complexes depicted in the figure.
Methods: Lipophilicity of these complexes has been estimated by means of microemulsion electrokinetic chromatography (MEEKC), and their cytotoxicity in human colon carcinoma and other tumor cell lines has been determined in colorimetric microculture assays (resazurin assay, MTT assay).
Results: The following structure-activity relationships can be deduced from these studies: (1) Compared to oxaliplatin, potency is increased in subsets of cell lines, particularly in leukemia and some colon carcinoma cells, by introduction of small substituents (methyl, ethyl) on C4 of cyclohexanediamine, but tremendously affected in all cell lines by bigger substituents (propyl, tert-butyl, phenyl). (2) Within a panel of five colon carcinoma cell lines, the activity profile of the 4,4-dimethyl-substituted complex most closely resembles that of oxaliplatin, while that of the *cis*-4,5-dimethyl-substituted complex, which on average exhibits a lower potency, contrasts sharply. (3) No simple correlation is found between lipophilicity and cytotoxicity.
Conclusions: These findings warrant testing in a greater panel of cell lines in order to further explore the possibility of improving antitumor activity and of altering the spectrum of activity compared to oxaliplatin.



R^1	R^2	R^3
H	H	CH_3 (methyl)
H	H	C_2H_5 (ethyl)
H	H	C_3H_7 (propyl)
H	H	C_4H_9 (tert-butyl)
H	H	C_6H_5 (phenyl)
H	CH_3 (methyl)	CH_3 (methyl)
CH_3 (methyl)	H	CH_3 (methyl)

543 POSTER

In vitro evidences on the role of the halogenoacrylic moiety in modulating brostallicin mechanism of action

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Brostallicin (PNU-166196) is a α-bromoacrylic distamycin-like derivative DNA minor groove binder (MGB), currently in Phase II clinical evaluation. Unlike other cytotoxics, this drug has the peculiarity of showing enhanced antitumor activity in cells with high glutathione-S-transferase (GST) and/or glutathione (GSH) content. In order to better characterize its mechanism of action, molecules with different acrylic moieties have been synthesized and tested for in vitro cytotoxic activity on tumor cells, chemical reactivity *vs* nucleophiles and in vitro DNA binding mechanism.
The in vitro cytotoxicity of brostallicin and its analogs was tested against murine L1210 leukemia. Results showed that the Cl-acrylic analog (PNU-248427) is only 8 times less cytotoxic than brostallicin (IC_{50} = 14.95 nM and IC_{50} = 1.85 nM, respectively) while F-acrylic (PNU-248482) and acrylic (PNU-230858) derivatives were not cytotoxic (IC_{50} = >7000 nM and IC_{50} = 4382.29 nM, respectively).
The chemical reactivity of these compounds against nucleophiles such as GSH, amines and thiols correlates with their in vitro activity. In fact, while brostallicin and the Cl-acrylic derivative react with nucleophiles giving the corresponding adducts, F-acrylic and acrylic analogs do not. Thus, suggesting that the α-halogenoacrylic moiety plays a crucial role in the cytotoxic activity of these new MGBs and supporting the hypothesis that a reactive adduct between brostallicin and a biological nuclephile eg. GSH could lead its antitumor activity.
Finally, to verify the correlation between chemical reactivity and a possible covalent DNA binding, experiments on the interaction of brostallicin and the inactive F-acrylic derivative with plasmid DNA (pUC18) were performed. Both molecules did not interact covalently with DNA by themselves. Conversely, upon incubation with GSH only brostallicin showed a change of the DNA topology from the supercoiled to the circular form (nicking).
In order to better characterize the brostallicin-DNA binding mechanism, Taq Stop assay on topoisomerase IIα cDNA was performed with or without GSH/GST. Brostallicin was tested in comparison with a synthetic GSH-halogenoacrylic-adduct model (PNU-571077) and tallimustine. Data confirmed the ability of brostallicin to bind covalently to DNA only upon