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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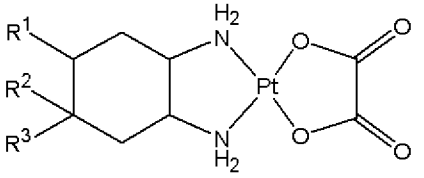
Introduction: Oxaliplatin has been the first platinum drug to prove clinical activity in an inherently cisplatin-resistant malignancy, i. e. colorectal cancer. Although the genuine pharmacodynamic effects of oxaliplatin and the specific properties of its DNA adducts apparently result from the presence 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

interaction with GSH/GST pathway. Interestingly, its sequence-specificity is completely different from that of tallimustine whose DNA interaction is not affected by the presence of GSH/GST. The DNA interaction and the sequence-specificity of PNU-571077 are superimposable to that of brostallicin. These findings further support the role of GSH in the mechanism of action of brostallicin.

544 POSTER

Atomic force microscopy study of structural transitions of supercoiled DNA in response to Poly(ADP-ribose)polymerase-1 protein binding

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Background: Poly(ADP-ribose) polymerase (PARP-1) is a multifunctional nuclear DNA-binding protein that interacts with single and double stranded DNA breaks as well as with secondary structures in undamaged, supercoiled DNA. Secondary structures of DNA, such as cruciforms, can play a role in transcription by creating new protein binding sites, to which various factors bind and restrict movement of the transcription-elongation complex. Modulating the level of DNA supercoiling has been proposed as a possible mechanism for regulating gene expression at a distance. PARP-1 has been shown to affect transcriptional regulation of specific genes.
Materials and methods: Supercoiled topoisomers of the cruciform structure containing plasmid, pUC8F14C, were used as substrate recombinant human PARP-1 binding. Atomic force microscopy (AFM) images were obtained using the NanoScope IIIa instrument equipped with an E-scanner (Digital Instruments, Santa Barbara, CA) and analyzed by using the computer program accompaning the imaging module.
Results: We observed that PARP-1 binds to the ends of the hairpin arms of the topoisomers of pUC8F14C DNA. This DNA contains a 106-bp F14C inverted repeat with predicted cruciform arm length of 53 bp. Analysis of the volume distributions of PARP-1 molecules in DNA-PARP-1 complexes revealed that PARP-1 forms a few dimers on interaction with cruciform structure. We determined that when PARP-1 binds to one segment of the supercoiled plasmid DNA in these complexes, it appears partially relaxed. Whereas, when PARP-1 interacts with nodes, it makes a DNA but, the level of supercoiling in the surrounding plasmid does not decrease.
Conclusions: Proteins that bind to enhancer elements and interact with the transcriptional machinery regulate transcription. Protein binding may alter local DNA structure through the change of DNA superhelicity. In previous work, we found that PARP-1 repressed transcription when it binds to the PARP promoter. This suggests that the affinity of PARP-1 to secondary DNA structures and the changes in the topology of supercoiled DNA generated by PARP-1 binding across poly(ADP-ribosyl)ation reactions can play a role in the regulation of gene expression. Accordingly, PARP inhibitors may be therapeutic agents capable of gene regulation.

545 POSTER

Biological effects of G-Quadruplex binding agents in various human cancer cell lines

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Findings that expression of telomerase and the maintenance of telomere length in the overwhelming majority of tumours together with the absence of such features in normal somatic cells, have created much interest in targeting the enzyme and telomeres, as a new cancer drug discovery strategy. An approach developed by this laboratory involves targeting the 3′-single stranded overhang telomeric DNA substrate, by inducing it to fold into a four stranded guanine-quadruplex structure (G4) that is incompatible with telomerase extension, and which itself may serve as a signal for DNA damage responses.
A series of small molecules that have been designed and synthesised to stabilise G4 structures, have been previously reported by us. These compounds have shown inhibitory effects against telomerase, detected by the TRAP assay. Many of these compounds have demonstrated selective potency against human carcinoma cell lines in short-term cytotoxicity studies while presenting low toxicity against normal human cells. Further, an initial lead compound, the 3,6,9-trisubstituted acridine BRACO-19, has displayed long term growth arrest in carcinoma cell lines and replicative senescence in vitro as well as in vivo activity in a tumour xenograft model. These in vitro cellular effects are both dose and time dependent. The loss of chromosomal integrity by generating end-to-end chromosomal fusions produced by BRACO-19 is consistent with its rapid induction of telomere uncapping.
We report here on detailed cellular and molecular studies for a new set of 3,6,9-trisubstituted acridines, with the goal of establishing structure-activity relationships and identifying optimal candidate telomere maintenance inhibitor molecules for subsequent in vivo studies. A panel of carcinoma cell lines, representing prostate, breast, non-small-cell lung and ovarian cancers, has been established, together with a series of evaluation criteria. Possible reasons for the observed differences in responses will be presented, together with details of the structure-activity relationships. The evaluations involve comparison of quadruplex affinity, telomerase inhibition, with potency in long-term inhibition-of-proliferation studies and measurements of apoptosis and senescence.

546 POSTER

A novel aureolic acid antibiotic analogue has potent anti-proliferative activity and induces multiple changes in gene expression in ovarian cancer cells

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Aureolic acid antibiotics are interesting lead compounds for drug development because of their ability to bind to GC-rich sequences in DNA, block binding of Sp1-family transcription factors and inhibit transcription of Sp1-regulated genes preferentially. Aureolic acid antibiotics, such as mithramycin and chromomycin, are active against several types of cancer but their clinical use is limited by severe side effects. In the attempt to identify compounds with improved activity and therapeutic index, we have evaluated the activity of a new aureolic acid analogue, SDK, which had been generated by genetic manipulation of the mithramycin biosynthetic pathway in *S. Argillaceus*. SDK was an effective inhibitor of proliferation of several ovarian cancer cell lines with IC_{50} concentrations ranging between 50 and 250 nM. Flow cytometry analysis of A2780 ovarian cancer cells showed cell cycle alterations and induction of massive apoptotic cell death as indicated by the appearance of a prominent sub-G1 peak after 24 and 48 hours of drug treatment. To determine the mechanisms involved in the response of ovarian cancer cells to SDK, we evaluated its effects on gene expression after 6 hours of incubation of A2780 cells using Affymetrix U133 GeneChips. Multiple genes involved in transcription regulation, DNA repair, cell cycle, proliferation, apoptosis and angiogenesis, were n egatively modulated by SDK. Gene expression analysis by RT-PCR and Western blotting confirmed that SDK induced down-regulation of genes, such as c-myc, c-src, hTERT, Bcl-XL, Ets2 and VEGF at low concentrations (50–100 nM) and early time points (<24 hours). The ability of SDK to inhibit cell proliferation and modulate expression of critical cancer promoting genes is an important feature for further development of this compound as a cancer therapeutic agent.

547 POSTER

DNA adduct formation by C-1748, a potent antitumor 4-methyl-1-nitroacridine of lowered toxicity

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4-Substituted 1-nitroacridines represent a new group of acridine derivatives synthesized at Gdansk University of Technology. In contrast to parent 1-nitroacridines, these compounds exhibit low toxicity and enhanced antitumor efficacy. The leading derivative 4-methyl-1-nitroacridine denoted C-1748 is being prepared for phase I clinical evaluation. The introduction of an electron donating methyl group into position 4 (para to 1-nitro) decreased the susceptibility of 1-nitro substituent to reduction. 1-Nitro group is crucial for biological activity and also for the ability of 1-nitroacridines to form DNA adducts. In the present study, we investigated DNA binding properties of C-1748 in comparison to the parent 4-unsubstituted analogue – C-857. Two methods were used: the elaborate [32P]-post-labelling technique and a newly developed by us simple and rapid method exploiting restriction enzymes to the detection of covalent modification of PCR-amplified DNA fragment. The latter approach enabled us to demonstrate covalent binding of C-1748 to DNA in different activating systems and to study kinetics of this reaction. The one involving DTT as a reducing agent required long exposures of DNA to C-1748 (17 h), while C-857 modified DNA within 1 h. In the presence of microsomes, short incubation times (1–3 h) were required for both compounds. For both acridines, the level of binding was concentration- and time-dependent. Another difference revealed by this method was the base pairs preference; C-857 was clearly GC specific, while C-1748 seemed to bind with similar efficiency to both GC and AT base pairs. In parallel, the detection of DNA adducts was carried out by [32P]-post-labelling technique. The maps of [32P]-labelled adducts formed by C-1748 displayed more chromatographic