that impacts metastasis, KISS1 (products) are likely drug-able and may be useful for inhibiting colonization of tumors at secondary sites.
Support: CA87728, National Foundation for Cancer Research Center for Metastasis Research, DAMD17-02-1-0541, NIH Medical Scientist Training Program Grant T32 GM08361

254 INVITED

Tumour vascular diversity and its translation into targeted therapeutics

R. Pasqualini, W. Arap. *USA*

Abstract not received.

255 INVITED

Targeting the invasion-association integrin alphavbeta6 as an anti-carcinoma strategy

I. Hart, D. Dicara, J.F. Marshall. *Queen Mary's School of Medicine and Dentistry at Barts and The London, Institute of Cancer & CR-UK Clinical Centre, Centre for Tumour Biology, London, United Kingdom*

The epithelial-specific integrin αvβ6 usually is not expressed in resting tissues but is upregulated in wound-healing, inflammation and in many different cancers. Indeed our own immunohistochemical analyses have shown that in oral squamous cell carcinoma, for example, around 90% of tumours express high levels of αvβ6, often at their invasive margins. Interestingly the induced expression of αvβ6 in experimental systems has been shown to increase carcinoma cell invasion, possibly explaining why strong expression of αvβ6 correlates with a 69% reduction of patient survival in colon cancer (Bates RC, et al. J Clin Invest 2005; 115: 339–347). As an integrin hetrodimer αvβ6 obviously is expressed at the cell surface and thus represents a potential target for imaging and, possibly, therapy. Characterised as the prime receptor for Foot-and-Mouth-Disease virus αvβ6 is known to recognise the Arg-Gly-Asp (RGD) motif present in the G-H loop of the VP1 structural protein. We have designed peptide probes, based around this core RGD sequence, and shown that longer (20 mer) peptides are better antagonists of αvβ6 activity than shorter peptides (lead peptide A20FMDV2; IC_{50} = 1 nM). Structural analysis by NMR has shown that efficacy of these longer peptides corresponds with the presence of a helix immediately C-terminal to this critical RGD motif. Non-adjacent residues, brought into juxtaposition as a linear array on the outer face of the helix, also appear to interact with αvβ6. Using engineered human tumour cell lines which differ only in their expression of αvβ6 we were able to demonstrate specificity of binding using biotinylated peptides. *In vivo* specificity was demonstrated using nude mice bearing αvβ6-positive and -negative xenografts which were injected with ^{18}F-FBA-A20FMDV2; positive: negative tumour ratio >4:1 with MicroPET showing selective accumulation in size-matched β6-positive tumours. These data indicate that carcinoma-specific αvβ6 may represent a suitable target for these peptides; a possibility currently under investigation in this laboratory.

256 INVITED

Targeting hypoxia as an anti-metastatic strategy

K. Williams. *University of Manchester, School of Pharmacy and Pharmaceutical Sciences, Manchester, United Kingdom*

Metastases are the primary cause of cancer-related deaths. Several pre-clinical and clinical studies have demonstrated that the development of metastatic disease is linked with the extent of hypoxia in the primary tumour. From the data that has been amassed so far, it would appear that hypoxia, through a number of discreet mechanisms, can "breed" an aggressive disease phenotype. This is problematic given the fact that hypoxia has been identified in all solid tumours analysed to date and that hypoxic cells are resistant to standard radiotherapy and many forms of chemotherapy. On the other hand hypoxia is a tumour-specific physiological abnormality and as such it can be exploited as a difference between tumours and normal tissues. The association between hypoxia and the development of metastases suggests there may be a potential therapeutic window of opportunity in targeting primary tumour hypoxia to reduce metastatic dissemination. There are two ways to approach this. The first is to target the condition of hypoxia per se, using bio-reductive agents that are selectively cytotoxic towards hypoxic cells. The second is to identify hypoxia-dependent changes in gene expression that are pivotal in the transition to a metastatic phenotype. Examples will be given where these approaches have led to a significant reduction in metastatic burden in experiments.

Thursday 9 November

Poster Sessions

Bioreductive agents

257 POSTER

Tirapazamine disrupts vascular endothelial-cadherin, suggesting a mechanism behind its ability to cause central vascular dysfunction

L. Huxham, A. Kyle, A. Minchinton. *BC Cancer Research Centre, Medical Biophysics, Vancouver, Canada*

By mapping the microregional effects in HCT-116 tumour xenografts, we have shown that the hypoxic cytotoxin tirapazamine (SR 4233: 3-amino-1,2,4-benzotriazine 1,4-dioxide) unexpectedly causes central vascular dysfunction 1 day after treatment with a progression over the following 1–3 days to necrosis. Similar effects, but with different kinetics, have been seen after treatment with known vascular targeting agents such as combretastatin A4 phosphate which is a microtubule disrupting agent and has also been shown to interfere with the endothelial cell-specific junctional molecule vascular endothelial-cadherin (VE-cadherin).
To investigate the mechanism of action behind the vascular dysfunction caused by tirapazamine we have examined human umbilical vein endothelial cells under oxic and hypoxic conditions. Cells were seeded in 4 well glass slide chambers and grown until confluent. Chamber slides were then gassed with specific levels of oxygen. The confluent monolayers were treated with 100 μM tirapazamine for 1.5 hours and stained to show hypoxia (pimonidazole), DNA double strand breaks (γH2AX), microtubule fine structure (β-tubulin), and vascular endothelial cell adhesion junctions (VE-cadherin).
Under hypoxic conditions tirapazamine treated cells showed labeling for pimonidazole and an increase in γH2AX compared to cells in an oxic environment. Microtubule disruption was not seen after exposure to tirapazamine in either the oxic or hypoxic groups. However, under hypoxic conditions tirapazamine did cause disruption of VE-cadherin as seen by an absence of pseudopodia and by fragmentation of the structured cell membrane junctions.
We propose that this activity of tirapazamine *in vivo* is related to its effect on hypoxic tumour vasculature located in the centre of tumours. The observed central vascular dysfunction may be due to disruption of the cell adhesion junctions between endothelial cells in hypoxic regions, thereby damaging the vessel and leading to cessation of perfusion along the vessel.
This research is supported by the Canadian Institutes of Health Research and the Michael Smith Foundation for Health Research. Animals were maintained in accordance with the Canadian Council on Animal Care guidelines.

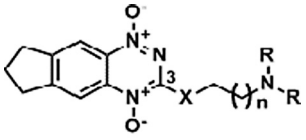
258 POSTER

Tricyclic triazine 1,4-dioxides: a new class of hypoxia-selective cytotoxins with improved extravascular transport compared to tirapazamine

M.P. Hay, K.O. Hicks, B.G. Siim, F.B. Pruijn, H.H. Lee, S. Yang, K. Pchalek, A. Blaser, W.R. Wilson, W.A. Denny. *The University of Auckland, Auckland Cancer Society Research Centre, Auckland, New Zealand*

Pharmacokinetic/pharmacodynamic (PK/PD) modelling has shown the in vivo activity of tirapazamine (TPZ), a bioreductive cytotoxin currently in Phase III clinical trial in combination with chemo-radiotherapy, to be limited by poor extravascular transport (EVT). EVT limitations result from slow diffusion and high bioreductive metabolism.
We present a new class of tricyclic triazine 1,4-dioxides (TTOs) as hypoxia-selective cytotoxins with improved EVT compared to TPZ. The indanetriazine core was designed to increase EVT by increasing lipophilicity and decreasing hypoxic metabolism through lowered electron affinity. The addition of a lipophilic amine side chain, attached via the 3-NH position, provided increased solubility while maintaining EVT. Replacement of the 3-NH linker with an alkyl linker improved diffusion by increasing lipophilicity and removing H-bond donors. The 3-alkyl substituents contributed to increased rates of metabolism which were balanced by the electron-donating nature of the indanetriazine core.
TTOs were screened for in vitro hypoxic cytotoxicity (IC_{50}) and hypoxic selectivity (HCR = aerobic IC_{50}/hypoxic IC_{50}) in human HT29 colon carcinoma cells. Diffusion coefficients (D_{MCL}) were calculated from diffusion studies in HT29 multicellular layers and rates of hypoxic metabolism (k_{met}) measured in single cell suspensions. Calculation of a 1-D transport parameter (X_*) allowed comparison of EVT between TTOs. PK/PD modelling predicted the plasma AUC required for 1 log of hypoxic cell killing

(AUC_p) and the in vivo hypoxic selectivity (HCD). TTOs with favourable predictions of EVT, AUCp and HCD were tested in vivo.
Maximum tolerated doses (MTD) given i.p. were determined in CD-1 nu/nu mice and plasma PK parameters (AUC, C_{max}) measured at 75% of the MTD. Lipophilic TTOs with strongly basic amine sidechains were found to be considerably more toxic in vivo, and had lower AUC values, than moderately lipophilic TTOs with weakly basic side chains. TTOs with favourable PK parameters were assessed for anti-tumour activity in combination with radiation by excision assay of HT29 xenografts 18 h after treatment. Administration of the lead compound, SN30000 at 75% of MTD, 5 min after RAD, gave an additional 1.4 logs of cell kill above RAD alone, compared with TPZ (0.6 additional logs).
The combination of IC_{50} and HCR with PK/PD modelling successfully identified a novel TTO, SN30000, with improved in vivo activity compared to TPZ.



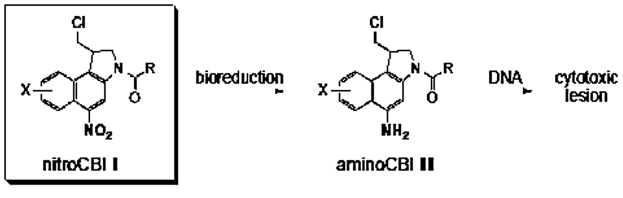
X = NH, CH_2; n = 1,2

259 POSTER

Substituted nitro(chloromethyl)benzindolines (nitroCBIs): a new class of hypoxia selective cytotoxins with in vivo activity

M. Tercel, F.B. Pruijn, G.J. Atwell, S. Yang, J.K. Botting, E. Smith, Y. Gu, S. Valentine, W.A. Denny, W.R. Wilson. *Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, Auckland, New Zealand*

Amino(chloromethyl)benzindolines (aminoCBIs, **II**) are DNA alkylating agents related to the natural products CC-1065 and the duocarmycins. They share many of the same properties (sequence selective alkylation at N3 of adenine, high cytotoxic potency) but in addition are amenable to the preparation of hydrolytically stable prodrug forms. Here we describe the potential of nitro(chloromethyl)benzindolines (nitroCBIs, **I**) to act as prodrugs activated by bioreduction in hypoxic regions of tumours.



R = minor groove binding side chain; X = H, electron withdrawing group

We show that (a) nitroCBIs **I** are considerably less toxic than aminoCBIs **II** under aerobic conditions in vitro, (b) **I** are metabolised to **II** selectively under hypoxic conditions, (c) several nitroCBIs **I** provide hypoxic cytotoxicity ratios (HCRs) of over 100-fold in vitro, and (d) alcohol-substituted nitroCBIs **I** can be converted to water soluble phosphate pre-prodrugs. A lead compound, SN29730, can be formulated in PBS containing $NaHCO_3$ (soluble at 25 mM), is well tolerated (MTD 100 micromol/kg iv in CD1 mice), has favourable pharmacokinetic properties (phosphate rapidly hydrolysed to corresponding alcohol), and is active in vivo as a single treatment but also in combination with radiation in human cervical carcinoma xenografts. In SiHa xenografts in combination with 15 Gy whole body irradiation SN29730 reduces the number of viable clonogens per gram of tumour tissue by over 4 orders of magnitude (radiation alone provides less than 2 logs of cell kill) at doses well below the MTD. These properties strongly suggest that nitroCBIs have potential for development as a new class of hypoxia selective cytotoxins.

260 POSTER

SN 30000: a tricyclic triazine 1,4-dioxide hypoxia-selective bioreductive drug with superior in vivo activity to tirapazamine

B.G. Siim, M.P. Hay, K.O. Hicks, A.M. Fraser, F.B. Pruijn, S. Yang, H.H. Lee, S.P. Valentine, W.A. Denny, W.R. Wilson. *The University of Auckland, Auckland Cancer Society Research, Auckland, New Zealand*

Tirapazamine (TPZ) is a hypoxia-selective bioreductive drug that has showed promising clinical activity in combination with chemo-radiotherapy. However, the activity of TPZ is limited by poor extravascular transport (EVT) restricting diffusion to the target hypoxic regions in tumours and high toxicity limiting the number of doses that can be administered.
We have developed a novel series of tricyclic triazine 1,4-dioxides (TTOs) with improved EVT relative to TPZ. Certain moderately lipophilic TTOs with weakly basic sidechains provided favourable plasma PK while having greater hypoxic potency and hypoxic selectivity than TPZ across a panel of human tumour cell lines.
Antitumour activity was assessed by excision assay of HT29 xenografts 18 hr after treatment. Mice were treated with drug alone (75% of MTD) ± radiation (RAD, 20 Gy whole body). RAD alone gave 1.9 logs cell kill while the TTOs (administered 5 min after RAD) gave up to an additional 1.4 logs kill, compared with TPZ (0.6 logs). The activity of SN 30000, the most active TTO in the HT29 assay, was confirmed in SiHa cervix carcinoma xenografts. Administration of SN 30000 (0.56 mmol/kg) from 2 hr before to 5 min after RAD (15 Gy) provided 1.3−2.9 logs kill in addition to RAD alone. The greatest activity was obtained when SN 30000 was administered 1 hr before RAD. In contrast, TPZ (0.13 mmol/kg) showed no significant time dependence, with lower activity at all times (maximum of 0.8 logs kill in addition to RAD).
Bidaily dosing with SN 30000 provided an MTD of 0.24 mmol/kg/dose over 4 days, allowing administration of a total dose 2.5-fold higher than the single dose MTD. The activity of SN 30000 in combination with fractionated RAD (8 ×–2.5 Gy) was determined by excision assay of HT29 and SiHa xenografts. With fractionated dosing there was no time dependence for administration of either SN 30000 or TPZ (8 × 0.08 mmol/kg) from 1 hr before to 5 min after each RAD dose in SiHa or HT29 xenografts. At all times greater cell killing (up to 2.2 logs in addition to RAD only) was obtained for SN 30000 than for TPZ (up to 1.0 logs in addition to RAD). The increased *in vivo* activity of SN 30000 relative to TPZ was achieved with no increase in host toxicity (weight loss, histopathology of normal tissues). Thus SN 30000 has a higher therapeutic ratio than TPZ as a hypoxic cytotoxin in two human tumour xenograft models.

261 POSTER

Banoxantrone (AQ4N), a tissue CYP 450 targeted prodrug: the results of a Phase I study using an accelerated dose escalation in patients with advanced solid tumors

J. Sarantopoulos[1], A.W. Tolcher[1], A. Wong[2], G. Goel[3], M. Beeram[4], G. Lam[5], K. Desai[3], K. Woody[2], S. Mani[3], K. Papadopoulos[1].
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Background: AQ4N was rationally designed to have anti-tumor activity following bioreduction by tissue cytochrome P450 to AQ4, an active DNA topoisomerase II inhibitor. Preclinical studies demonstrated AQ4N selectively targets lymphoid tissues and hypoxic tumor tissues. This study assessed the maximum tolerated dose (MTD), pharmacokinetic (PK), and pharmacodynamic (PD) of repeated weekly dosing of AQ4N in patients (pts) with advanced cancers.
Methods: AQ4N was administered IV on Days 1, 8, and 15 of a 28-day cycle in the following dose cohorts: 12, 24, 48, 96, 192, 384, 768, and 1200 mg/m^2. Accelerated titration design 2B was employed and the MTD was defined by ⩽ 33% of 6 pts with a drug-related dose limiting toxicity (DLT). Response was assessed every 8 weeks by RECIST.
Results: 16 pts were enrolled. A single pt per cohort was treated up to 384 mg/m^2. At 1200 mg/m^2, 2 of 5 pts experienced a DLT (Grade 5 respiratory distress and Grade 3 fatigue). A total of 5 pts were treated without toxicity at the 768 mg/m^2, and established this dose as MTD. One pt in the 1200 mg/m^2 cohort died during the trial from acute complications of metastatic soft tissue sarcoma and respiratory distress. The most common related adverse events (AE) observed were skin discoloration (81%), chromaturia (75%), fatigue (38%), nausea (28%), vomiting (25%), and diarrhea (25%). 7 pts experienced 8 serious AEs. One pt (48 mg/m^2) with renal cancer has had stable disease for > 20 months. The PK was linear over all doses studied and no accumulation was observed after repeated doses. At 768 mg/m^2 (n = 4), the Day 1 AQ4N C_{max} was 99.8±27.0 μg/mL, AUC $_{0-\infty}$ was 259.5±67.8 μg h/mL, and $T_{1/2}$ was 3.9±0.7 h (range 3.1−4.8 h). Multiple cycles of AQ4N at weekly doses of 768 mg/m^2 or higher demonstrated a mild reduction of both lymphocyte count and ANC.
Conclusions: AQ4N is well-tolerated when administered on a weekly schedule. AQ4N levels sufficient for anti-neoplastic activity in pre-clinical models are achieved with weekly dosing at 768 mg/m^2. AQ4N monotherapy and combination trials with chemo- and radiation therapy are planned.