655 POSTER

Pre-clinical studies of concomitant taxanes and ionizing radiation therapy: local and systemic anti-tumor effects

L. Liebes[1], B. Ng[1], S. Demaria[2], M. Devitt[3], J. Babb[4], S. Formenti[3]. [1]*New York University Medical Center, NYU Cancer Institute, New York, USA;* [2]*New York University Medical Center, Pathology, New York, USA;* [3]*New York University Medical Center, Radiation Oncology, New York, USA;* [4]*New York University Medical Center, Radiology, New York, USA*

Background: A pre-clinical breast cancer model was used to test whether a taxane combination and local radiation can induce protective anti-tumor immunity in tumor-bearing hosts. The hypothesis tested is that local treatment by ionizing radiation (RT) or concomitant chemotherapy and RT, may have systemic anti-tumor effects.
Methods: The effects of combined RT and taxanes chemotherapy used an immune competent, syngeneic murine mammary carcinoma model. On day 0, BALB/C mice were inoculated with 1×10^5 TSA murine mammary carcinoma cells (left flank, "*primary*" tumor, PT). On day 5, 1×10^5 TSA cells were inoculated in the right flank ("*secondary*" tumor, ST). Tumor-bearing mice were randomized on day 11 into 6 treatment groups: untreated control, 2Gy RT, 8 mg/kg i.v. Docetaxel $\pm$ 2 Gy RT and 8 mg/kg i.v. Paclitaxel $\pm$ 2 Gy RT. Each group consisted of 5 animals. The taxanes were administered intravenously IV 48hr prior to the ionizing radiation treatment. The RT was delivered at a single dose of 2 Gy on day13 exclusively to the PT. Tumor dimensions were measured every third day, over 6-weeks. A mixed model regression statistical analysis was used to assess anti-tumor effects.
Results: Both paclitaxel and docetaxel showed similar effects in slowing the growth of the irradiated PT compared with the un-treated control. The combination of RT and either taxane treatment showed additive effects on tumor growth delay when compared to either modality alone. With respect to the growth of the ST, the RT to the primary tumor did not show any effect and was comparable to that of the untreated controls. Both docetaxel and paclitaxel chemotherapy treated groups showed comparable growth delay responses of the non-irradiated tumors. The effect of left-side RT on ST growth did have a significant effect in the paclitaxel group ($p = 0.0138$). Relative to animals without radiation exposure, the animals with PT RT had lower mean tumor volumes initially but tended to grow at a faster rate, resulting in comparable mean tumor volumes for the two groups by study termination. Chemo and RT had significant additive effects on tumor growth rate and the estimated rates of increase in log tumor volume for the 3 RT treatment regimens were 0.144 (no RT), 0.106 for paclitexel and 0.113 for docetaxel.
Conclusions: These results suggest that concurrent chemoradiation exerts an effect outside of the RT field that is superior to that of chemo alone.
Supported by a grant from Aventis.

656 POSTER

Influence of Irinothecan and SN-38 on the irradiation response of WHO3 human esophageal tumour cells under hypoxic conditions

L. Bohm, C. van Rensburg. *University of Pretoria, Department of Pharmacology, Pretoria, South Africa*

Background: Irinothecan and its metabolite SN-38 are known for their cytostatic properties against lung, colorectal, cervical and ovarian carcinomas. The 2 drugs have also been reported to be effective radiation sensitizers in various carcinoma cell lines under aerobic conditions. The performance of the drugs under anaerobic conditions has not been investigated and is the subject of the present investigation.
Materials and Methods: WHO3 human esophageal carcinoma cells were plated in 5 ml glass tubes at 600 cells per tube and incubated under standard cell culture conditions. After 24 hours, 3.1uM Irinothecan or 0.046 nM SN-38 were added and the tubes were incubated for 30 minutes in a modular shaker/chamber under a constant stream of 5% CO2/95% air or 5% CO2/95% N2 to generate aerobic conditions, when the gas supply was terminated. After a further incubation period of 1 hour the tubes were irradiated with 1–10 Gy of 8 MeV photons and incubated for another 4 hours when the medium was replaced and cells were grown under aerobic conditions for 6 days. Cell survival was determined by the MTT assay using appropriate solvent controls and correction for drug toxicity alone. Survival data were fitted to the linear quadratic equation to obtain mean inactivation doses which were then used to calculate dose modifying factors.
Results and Conclusions: The oxygen enhancement factor (OER) for WHO3 cells (controls) was found to be 2.1 indicating that the cell system responds in the expected manner. Presence of the drugs under hypoxic conditions restored the radiosensitivity of WHO3 cells in a dose dependant manner by factors of 1.5–2.1 suggesting an additive cytotoxic effect for Irinothecan which was also seen under aerobic conditions. Addition of SN-38 in the subtoxic range of 10–2 nM produced dose modifying factors of 1.6–2.1 and a marked sensitisation of anoxic tumour cells to irradiation approximating the radiation sensitivity under aerobic conditions. It is concluded that the topoisomerase inhibitors Irinothecan and in particular the metabolite SN-38 may be clinically useful in the radiotherapy of tumour pathologies which are notoriously hypoxic.

Hormonal agents

657 POSTER

Effects of Fulvestrant on oestrogen receptor levels during long-term treatment of patients with advanced breast cancer

E. Gutteridge[1], J. Robertson[1], K. Cheung[1], S. Pinder[2], A. Wakeling[3]. [1]*Nottingham City Hospital, Department of Surgery, Nottingham, UK;* [2]*Nottingham City Hospital, Department of Histopathology, Nottingham, UK;* [3]*AstraZeneca Pharmaceuticals, Macclesfield, UK*

Background: Fulvestrant (Faslodex®) is an oestrogen receptor (ER) antagonist with no agonist effects. Fulvestrant (50 mg, 125 mg, 250 mg intramuscular [IM] injection) for 14–21 days prior to surgery for primary breast cancer caused a dose-dependent reduction in ER protein levels (Cancer Res 2001;61:6739–46). Here, we determine the long-term changes in ER levels occurring in patients receiving fulvestrant as first-line endocrine therapy for locally advanced/metastatic breast cancer.
Material and methods: 29 patients with lesions suitable for biopsy were recruited. Fulvestrant was given as a monthly 250 mg IM injection until disease progression (PD). Biopsies were taken pre-treatment (at diagnosis), after 4–6 weeks and 6 months of treatment, and at PD. Changes in ER levels were assessed by immunohistochemistry and analysed using SPSS statistical software. Tumour response was assessed every 3 months according to UICC criteria.
Results: 24 patients had evaluable disease at 6 months: 5 had PD and 19 had clinical benefit (CB; complete response, n=1; partial response, n=5; stable disease $\geqslant$24 weeks, n=13), giving a CB rate of 79.2% (19/24 patients) and an objective response rate of 25.0% (6/24 patients). Overall, ER downregulation was seen at 4–6 weeks ($p=0.016$) and at 6 months ($p=0.011$) compared with pre-treatment samples. There was a trend for continued downregulation from 4–6 weeks (mean H-score 170.8 [range 50–280]) to 6 months (mean H-score 132.33 [range 0–260]). No significant reduction in ER ($p=0.461$) was seen in patients who progressed *de novo* (n=5). In patients achieving CB, ER downregulation was statistically significant at both 4–6 weeks ($p=0.029$) and 6 months ($p=0.011$). In all patients, ER was detectable at the time of PD.
Conclusions: This is the first dataset to show continued downregulation of the ER beyond the presurgical period and demonstrates that fulvestrant can maintain ER suppression for at least 6 months. Fulvestrant showed good clinical efficacy at the current clinical dose (250 mg). The presence of ER at PD supports the use of further endocrine therapies. Ongoing studies are investigating whether alternative dosing regimens result in earlier/increased ER downregulation and further improvement of clinical efficacy.

658 POSTER

An essential role for AIB1 phosphorylation in tamoxifen resistance

J. Shou[1], R. Cook[2], G. Chamness[1], K. Osborne[1], R. Schiff[1]. [1]*Baylor College of Medicine, Breast Ctr, Medicine, Molecular & Cell Biology, Houston, USA;* [2]*Baylor College of Medicine, Department of Immunology, Houston, USA*

AIB1 is a steroid nuclear receptor coactivator. In addition, its effect also extends to unrelated transcription factors such as those involved in the cAMP or cytokine pathways. It is overexpressed in human breast, prostate, and ovarian cancers. There is compelling evidence that overexpression of AIB1 reduces tamoxifen's antagonistic activity in cultured cells. Breast cancer patients with overexpression of AIB1 along with high HER2 signaling are much more clinically resistant to tamoxifen in comparison to patients with only one of these factors elevated. Therefore we hypothesized that signaling from HER2 overexpression leads to phosphorylation and activation of AIB1, which contributes to development of tamoxifen resistance.
In this study, we found that exogenous overexpression of AIB1 can further increase tamoxifen's agnostic activity in HER2-overexpressing MCF7/HER2–18 (HER2–18) cells compared to parental cells. Retardation of AIB1 mobility on Western blots was found in HER2–18 cells treated with estrogen, tamoxifen, or the growth factor heregulin, and in *in vivo* HER2–18 tumors treated with tamoxifen, indicating that tamoxifen treatment may also lead to AIB1 phosphorylation in these cells. Recently we have identified a specific serine residue (S) located between the nuclear receptor interaction domain and CBP interaction domain in AIB1 that can be phosphorylated