and alveolar cells of the lung. NE disintegrates extracellular matrices and impairs tissue, thereby causing lung injury. In this study, we irradiated the murine lung and analyzed the inhibitory effects of Sivelestat, an NE inhibitor, on lung injury in mice.
Materials and Methods: Twelve-week-old female C57BL/6J mice were used. A dose of 12 Gy, with a 4MV photon beam was delivered to the whole lung in a single fraction via a posterior field with a linear accelerator under Nembutal anesthesia. Sivelestat (3 mg/kg) was administered through intraperitoneal injection immediately, 3 hrs, 6 hrs, and 12 hrs after irradiation in groups RE-0, RE-3, RE-6, and RE-12, respectively. A control group (group C) and a group receiving radiation without sivelestat (group R) were also used. NE activity was measured 24 h and 48 h after irradiation. The lungs were simultaneously extirpated and stained with hematoxylin-eosin. Histopathological features of these cross-sections were analyzed under an optical microscopy.
Results: 1. NE activity: NE activity increased in the groups in which murine lungs were irradiated. There was no increase in NE activity in group C. Among the sivelestat-administered groups, NE activity was slightly elevated in the group RE-0 and was suppressed compared to the group R in groups the RE-3, RE-6, and RE-12 at 24 hours after irradiation. 2. Histopathological features: In the irradiated groups, intra-alveolar neutrophil infiltration, perivascular edema, and alveolar wall thickness were found, but these changes were mild in the sivelestat-administered groups.
Conclusion: NE plays an important role in the development of radiation-induced lung injury. Sivelestat is thus expected to decrease radiation-induced lung toxicity by suppressing NE release from neutrophils.

389 POSTER

Docosahexaenoic acid (DHA) enhances the effect of docetaxel in prostate cancer cells:Modulation of apoptotic pathways

I. Shaikh[1], I. Brown[1], K.G. Wahle[2], A. Schofield[1], S.D. Heys[1], S. Chaturvedi[1]. *[1]University of Aberdeen, Department of Surgery, Aberdeen, United Kingdom; [2]Robert Gordon University, School of Life Sciences, Aberdeen, United Kingdom*

Background: Prostate cancer is one of the most common male cancers. The chemotherapeutic agent, docetaxel, is currently treating hormone refractory prostate cancer. However there are a proportion of patients who cannot tolerate docetaxel either due to toxicity or due to premorbid conditions. Recently, omega-3 fatty acids have been shown to enhance the anti-tumour effect of docetaxel against human cancers. We aim to assess the effect of adding an omega-3 fatty acid, docosahexaenoic acid (DHA), along with docetaxel in prostate cancer cell lines on cell viability and apoptosis.
Materials and Methods: LNCaP and PC3 prostate cancer cells were treated with DHA and docetaxel, alone or in combination. We studied the drug interaction concurrently and sequentially at a range of drug concentrations. Drug response was determined by standard MTT assay to measure cell viability and drug interaction was analyzed by combination index (CI) method. To assess apoptosis and cell cycle, flow cytometry was performed using PI, Annexin V and JC-1 staining protocols.
Results: DHA enhanced, in a synergistic manner, the anti-tumour effect of docetaxel in LNCaP cells but not in PC3 cells. The IC50 of docetaxel showed a 3-fold decrease upon concurrent treatment with 25micromolar DHA and a 2-fold decrease upon sequential treatment with 100micromolar DHA. Flow cytometry analysis by JC-1 staining showed significant increase ($p < 0.018$) in apoptosis and PI staining showed increase in sub-G1 population, but did not reach statistical significance. Annexin V staining did not show a significant increase in apoptosis, however the results showed increased cells with necrosis upon concurrent treatment.
Conclusion: DHA enhances synergistically the anti-tumour effect of docetaxel in LNCaP cells but not in PC3 cells. This may suggest that the response may be cell specific or may be related to androgen-sensitivity. DHA may act with docetaxel through the apoptotic pathway.

390 POSTER

Proliferation of human lung cancer in orthotopic transplantation model, comparing with in subcutaneous transplantation model of nude mice

Y. Kang[1], M. Omura[1], A. Suzuki[1], Y. Nagashima[2], T. Inoue[1]. *[1]Yokohama city unversity, Radiology, Yokohama, Japan; [2]Yokohama city unversity, Molecular Pathology, Yokohama, Japan*

Purpose: Previously we have established an orthotopic transplantation model of lung cancer in nude mice. The objective of this study is to analyze the proliferation of human lung cancer growing in mouse lung tissue and compare it with tumors implanted in subcutaneous (s.c.).
Experimental design: Human lung adenocarcinoma A549 cell line and squamous cell carcinoma SQ5 cell line were used. In orthotopic lung cancer model tumor suspensions were directly injected into the main bronchi of anesthetized athymic nude mice (7−9 week old) with simultaneous administration of 0.01 M EDTA. In s.c. model tumor suspensions were injected into the flank. To label the proliferation tumor cells mice were intraperitoneal injected with 60 mg/kg of body weight bromodeoxyuridine (BrdUrd) in PBS at 20 min before sacrifice. Lung tissue with tumor nodules and s.c. tumor were fixed with formalin and conformed by histology. Proliferation tumor cells were stained by anti-BrdUrd and labeling index (LI) were counted.
Results: Tumor formation rate in mouse lung of A549 cell line and SQ5 cell line were 80% and 100%, respectively. Tumor nodules occurred more frequently in the right lung than the left lung, and in the frequency order of the upper, lower, and middle lobes. The nodular xenografts were numerous, of various sizes. Histological expression showed that adenocarcinoma A549 tumor nodules were distributed primarily in alveoli, showing an abortive glandular arrangement. The squamous cell carcinoma SQ5 tumor nodules mainly invaded the bronchioles and terminal bronchioles. LI in orthotopic implanted lung cancer was 28% per field. Proliferation cells distributed as several groups. LI in s.c. implanted lung cancer was 22% per field. Proliferation cells distributed separately.
Conclusion: Our results showed that in orthotopic lung cancer model, tumor grew in a suitable position which resembles the lung cancer position in humans. Proliferation of tumor cells in orthotopic transplantation model showed different pattern with s.c. transplantation model. These data suggest that this orthotopic lung cancer model may be suitable for analyzing biological behavior of lung cancer.

391 POSTER

Mitochondrial effects of combination cetuximab and ionizing radiation in head and neck squamous carcinoma cells

M. Rebucci[1], N. Rezvoy[1], N. Wattez[1], E. Lartigau[2], A. Lansiaux[1]. *[1]Centre Oscar Lambret, antitumor pharmacology unit, Lille, France; [2]Centre Oscar Lambret, Departement of Radiation Oncology, Lille, France*

The combination of Epidermal Growth Factor Receptor (EGFR) inhibitors and conventional cancer therapies has become the subject of intensive investigations. Overexpression of EGFR is involved in carcinogenesis of several types of cancer (specially in head and neck squamous cell cancer). Recently, a phase III clinical trial had shown that the treatment of locoregionally advanced head and neck cancer with concomitant high-dose radiotherapy plus cetuximab improves locoregional control and reduces mortality without increasing the common toxic effects.
The aim of this study is to explain radiosensitizing properties of anti-EGFR treatments and to clarify the molecular mechanisms involved in the combination.
To explore this question, we focused our attention on cell death pathways and especially on mitochondrial effects. The combination of cetuximab and ionizing radiation has been studied on different head and neck cell lines (CAL27, CAL33, SQ20B). Several conditions of treatment were tested. Standard proliferation studies were performed. Cell cycle analysis, mitochondrial changes (mitochondrial membrane potential and mitochondrial mass) and cellular modifications were performed by flow cytometry, confocal and electronic microsopies.
Our data shown that CAL27 cell line is sensitive to ionizing radiation and cetuximab and the combination increases markedly this sensitivity of cells in vitro. Cell cycle analysis shows characteristics modifications and apoptotic cell population induced by ionizing radiation. Conversely, cetuximab with or without ionizing radiation has no effect on cell cycle. Cell cycle perturbations can be connected with changes in mitochondrial membrane potential. Ionizing radiation induces marked G2 arrest and concomitantly provokes a collapse in mitochondrial membrane potential, the depolarized population increases with the dose of irradiation, cetuximab alone induces a stronger depolarization and the combination increases proportionally the depolarized population cells. In a very interesting way, an increase of the mitochondrial membrane potential was also observed on cells treated with cetuximab with or without ionizing radiation. This apparent increase corresponds to an increase of mitochondrial mass which is not observed with ionizing radiation and is amplified with the combination of cetuximab and ionizing radiation.
These findings provide insight into a specific role of the mitochondria and may help explain the potency of the combination.