

---

---

## ABSTRACTS OF SOCIETIES

### Scottish Society for Experimental Medicine

Level 7 Conference Room, Institute of Medical Sciences,  
Forresterhill, Aberdeen

Friday, 11 May 2007

ORAL PRESENTATIONS

#### Identifying candidate genes on chromosome 12p in docetaxel-resistant breast cancer cells.

*J Sangrithi-Wallace, SL McDonald, I Brown, SD Heys, AC Schofield*

Department of Surgery, University of Aberdeen, Aberdeen AB25  
2ZD

**Introduction:** Docetaxel is a chemotherapeutic agent that is very effective in the treatment of breast cancer. However, despite its efficacy, resistance to docetaxel remains a significant problem and the genetic pathways involved in docetaxel resistance are not clear. We have previously used comparative genomic hybridisation (CGH) on docetaxel resistant MCF-7 and MDA-MB-231 breast cancer cell lines to identify the chromosomal regions that are altered in docetaxel-resistant cells relative to their parental docetaxel-sensitive cells. Loss of chromosome 12p was identified in the MCF7 docetaxel-resistant cells by CGH. This region may contain candidate

genes that may be involved in docetaxel resistance. **Methods:** The CGH result was verified by the use of whole chromosome paints for chromosome 12. Bacterial artificial chromosome (BAC) fine-mapping was employed to accurately map the minimal region of loss in MCF7 docetaxel-resistant cells.

Bio-informatics was used to identify candidate genes present in the minimal region of loss that may have a functional role in contributing to docetaxel resistance. RT-PCR was performed to validate candidate genes by assessing mRNA expression in MCF7 docetaxel-resistant cells relative to their parental docetaxel-sensitive cells. A change in expression greater than or equal to 1.2-fold was considered to be 'significant' and candidates were further validated at protein level by western blot analysis. **Results:** The whole chromosome paint of chromosome 12 confirmed the CGH result, showing a loss of chromosome 12p in the MCF7 docetaxel-resistant cells relative to docetaxel-sensitive cells. BAC fine-mapping of chromosome 12p in MCF7 docetaxel-resistant cells relative to their parental docetaxel-sensitive cells, narrowed the region of loss to a minimal region spanning 12p11.21-13.33. Candidate genes in this region include LRP6, DUSP16, BclGL and CYP3A43. DUSP16 mRNA expression in MCF7 docetaxel-resistant cells was found to be decreased 1.4-fold (+/- 0.1 SEM) relative to MCF7 docetaxel-sensitive cells. Other candidates were not significantly different. **Conclusions:** This study confirms the loss of chromosomal regions on 12p in MCF7 docetaxel resistant-cells relative to parental docetaxel-sensitive cells. Pilot data from this study highlights that candidate genes on chromosome 12p may be involved in mediating docetaxel resistance in MCF7 breast cancer cells. Preliminary data suggest that DUSP16 mRNA expression is decreased in MCF7 docetaxel-resistant cells relative to docetaxel-sensitive cells and requires further investigation.

## Can a mitochondrially targeted antioxidant protect against hyperglycaemia induced damage in an *in vitro* model of sepsis?

*J Toner, DA Lowes, NR Webster, MP Murphy, HF Galley*

Academic Unit of Anaesthesia & Intensive Care, School of Medicine, University of Aberdeen, Aberdeen AB25 2ZD

Mitochondrial damage occurs during severe sepsis as a result of oxidative stress. Complications in diabetes are mediated by mitochondrial oxidative damage and hyperglycaemia is common in patients with sepsis due to insulin resistance. Severe oxidative stress is known to initiate inflammatory responses. Coenzyme Q10 (CoQ) is the predominant form of the antioxidant ubiquinone in man and has an important role in defence against mitochondrial oxidative damage. MitoQ is a form of CoQ which has been attached to a lipophilic triphenylphosphonium cation and is selectively accumulated within mitochondria. The aim of this study was to investigate whether MitoQ was able to protect against oxidative damage induced by a combined inflammatory and hyperglycaemic insult *in vitro* to mimic human sepsis. HUVEC-C endothelial cells were exposed to 2 g/ml lipopolysaccharide (LPS) plus 20 g/ml peptidoglycan G (PepG) as an inflammatory insult, in the presence of 0-50mM glucose and 1 M MitoQ or PBS as control. Sodium pyruvate was added as an alternative energy source to cells exposed to zero glucose. After 7 days mitochondrial membrane potential was measured in intact cells using the fluorescent dye JC-1. Mitochondrial membrane potential decreased in cells exposed to 25 or 50mM glucose in the presence of LPS and PepG indicating mitochondrial damage. This decrease was abrogated by MitoQ ( $p < 0.001$ ) suggesting mitochondrial protection. MitoQ may represent novel therapeutic option in preventing oxidative mitochondrial damage in sepsis in the future.

We are grateful to Antipodean Pharmaceuticals Inc., for the gift of MitoQ.

## Regulation of the human AKR1D1 (5 $\beta$ -reductase) gene; a novel role for bile acids in modulating cortisol metabolism in chronic liver disease.

*E O'Flaherty, B Walker, R Andrew*

Endocrinology Unit, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ

AKR1D1 (5 $\beta$ -reductase) catalyses both the synthesis of bile acids from cholesterol and the inactivation of glucocorticoids. Genetic deficiency of this enzyme is manifest by progressive neonatal liver failure. However, a more subtle impairment in 5-reductase activity may contribute to the altered adrenal function associated with cirrhosis and cholestasis, as a result of impaired cortisol inactivation and enhanced negative feedback on the hypothalamic-pituitary-adrenal axis (HPAA). We have previously reported that bile acids act as competitive inhibitors of AKR1D1, but to date very little is known about transcriptional control of the human *AKR1D1* gene. Bile acids act as transcriptional inhibitors of other enzymes in the bile acid synthesis cascade, notably cholesterol 7-hydroxylase, via their stimulation of the farnesoid X receptor (FXR). The aim of this study was to characterise the promoter region of the human *AKR1D1* gene and establish whether bile acids are amongst the factors which regulate transcription.

Bioinformatic analysis of the 5' untranslated (UTR) region surrounding a previously identified transcription start site (TSS) of *AKR1D1* was performed using MatInspector software. A 1kb 5'UTR region was subsequently cloned and detailed deletion analysis of this region performed by linking sub-regions of the promoter to a luciferase reporter construct. Reporter activity was assessed in a human hepatoblastoma (HepG2) cell line, corrected for transfection efficiency using  $\beta$ -galactosidase, and normalised to a promoter-less control vector. Transfections were performed in triplicate on three occasions, in the absence and presence of chenodeoxycholic acid (physiological FXR agonist) (CDCA; 50 $\mu$ M, 48h). High levels of transcriptional activity were found in the regions -383 to -320 (1445 $\pm$ 48 fold increase) and -175 to -117 (1646 $\pm$ 135 fold increase), suggesting the presence of two proximal promoters. Substantially lower transcriptional activity was observed in region -320 and -175 suggesting a suppressor activity in this region. Additionally, the TSS region (-17 to +20) showed high levels of activity (497 $\pm$ 76 fold increase), suggesting the presence of a basal promoter in this region. Initial bioinformatical screening of the -383/+446 region revealed a putative consensus sequence for FXR (between positions +18 and +30), and addition of CDCA significantly inhibited transcription from the full-length *AKR1D1* promoter (43 $\pm$ 2%). This study represents the first characterisation of the functional promoter areas in the 5'UTR region of the human *AKR1D1* gene. The data suggest a role for bile acids not only in competing with glucocorticoids for metabolism, but also in down-regulating expression of the cortisol metabolising enzyme 5 $\alpha$ -reductase. This may contribute to dysregulation of the HPAA, which associates with poor prognosis in liver disease.

## Altered expression of 3 $\beta$ -hydroxysteroid dehydrogenase between primary human ovarian surface epithelial cells (hOSE) and pre-neoplastic and cancer cell lines in response to inflammatory and anti-inflammatory agents.

*G Papacleovoulou, SG Hillier, JI Mason*

Centre of Reproductive Biology, University of Edinburgh, The Queen's Medical Research Institute, 47 Little France Crescent, Edinburgh EH16 4TJ

**Introduction:** Disorder in inflammatory cascades at the time of ovulation is a prime candidate leading to epithelial ovarian cancer (EOC). Progesterone has been shown to be anti-inflammatory. Local progestogen biosynthesis requires 3 $\beta$ -hydroxysteroid dehydrogenase (3 $\beta$ -HSD). We investigated the intracellular regulation of 3 $\beta$ -HSD isoforms (3 $\beta$ -HSD1 and 3 $\beta$ -HSD2) in primary hOSE cultures and in immortalised (pre-neoplastic) OSE and EOC cancer epithelial cell lines by immune agents. **Patients and Methods:** Local ethical approval and informed consent was obtained from pre-menopausal women who underwent ovarian removal for benign gynaecological disorders. OSE-C2 and hTERT OSE cell lines had been immortalised with the hTERT catalytic subunit. SKOV-3 cell line derived from poorly differentiated adenocarcinoma. Immunohistochemical localisation of 3 $\beta$ -HSD was examined in whole ovarian sections and co-localised with epithelial-specific markers. Following investigation of the isoform mRNA expression pattern (*HSD3B1* and *HSD3B2*) using semi-quantitative and quantitative RT-PCR, hOSE and cell lines were exposed *in vitro* to pro-inflammatory IL-1 or the lymphocyte-associated cytokine, IL-4. Using quantitative RT-PCR, we

measured relative *HSD3B1* and *HSD3B2* mRNA levels of control and treated samples ( $2^{-\Delta\Delta Ct}$ ). Data-sets were analysed with multiple measures of ANOVA and Newman-Keuls post-hoc testing or student t-tests ( $P < 0.05$  taken as significant). **Results:** Significant  $3\beta$ -HSD was immunodetected in hOSE. Surprisingly, whereas the predominant ovarian isoform is  $3\beta$ -HSD2, *HSD3B1* mRNA transcripts were 10-fold higher than *HSD3B2* mRNA in both hOSE and OSE-C2 cells. No significant difference was observed in the isoform expression in hTERT OSE and SKOV-3 cells. Moreover, hOSE had significantly higher levels of *HSD3B1* and *HSD3B2* mRNA relative to the cell lines. In hOSE, IL-1 down-regulated *HSD3B1* mRNA, whilst it up-regulated *HSD3B2* mRNA. These effects were not observed in any of the cell lines. In hOSE, IL-4 up-regulated *HSD3B1* and *HSD3B2* mRNA as well as  $3\beta$ -HSD activity. This observation was confirmed only in OSE-C2 cells, but only in terms of *HSD3B1* mRNA, since *HSD3B2* mRNA was not constitutively measurable. **Conclusion:** Predominance of  $3\beta$ -HSD1 is noteworthy since it has higher catalytic efficiency for local progesterone formation. Differential effects of the two isoforms in response to immune agents suggest regulation of the two isoforms by distinct signalling molecular pathways. Differential regulation of *HSD3B1* and *HSD3B2* mRNA expression by IL-1 $\alpha$  indicates altered local progesterone production during inflammatory ovulation. IL-4-induced up-regulation of *HSD3B1* and *HSD3B2* expression may be of therapeutic benefit given its inhibitory effects on cell growth, possibly mediated by enhanced progesterone biosynthesis. Loss of these responses to pre-neoplastic and cancer cell lines may have biological significance in the aetiology of EOC, since down-regulation of  $3\beta$ -HSD expression may be a defining feature of neoplastic transformation.

### The effects of substitution of the geminal hydroxyl group of bisphosphonates and phosphonocarboxylates on their target enzyme inhibition.

C Stewart, JE Dunford, Z Xia, R Baron, MS Marma, BA Kashemirov, CE McKenna, FH Ebetino, FP Coxon

Bone Research Group, Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD

Bisphosphonates (BPs) are the most widely used treatment for bone diseases characterised by increased osteoclast-mediated bone resorption such as osteoporosis, Paget's disease and tumour-induced osteolysis. Nitrogen-containing bisphosphonates (NBPs) inhibit bone resorption by inhibiting farnesyl diphosphate synthase (FPPS), thereby preventing the post-translational prenylation of the Ras, Rho and Rab families of proteins in osteoclasts. By contrast, 3-PEHPC (NE10790), a weakly anti-resorptive phosphonocarboxylate analogue (PC) (in which a carboxylate group replaces one of the phosphonate groups of BPs) of the NBP risedronate (RIS), acts by inhibiting Rab geranylgeranyl transferase (Rab GGTase); exclusively preventing the prenylation of Rab proteins. The anti-resorptive potency of NBPs and PCs is determined by both their affinity for bone mineral and their ability to inhibit the target enzyme. In addition to the nitrogen-containing side chain, potent NBPs have an -OH group attached to the central carbon that, in concert with the phosphonate groups, contributes to the bone affinity of N-BPs. However, the role of the -OH group in inhibition of FPPS remains unclear, and its role in PCs is even less well understood. We have therefore studied analogues of these compounds in which the -OH group has been substituted with

the electronegative halogens fluorine, chlorine or bromine (halo), or with hydrogen (desoxy-). We found that all the halo- and desoxy- analogues displayed reduced mineral affinity compared to the parent compounds. Desoxy-RIS was approximately 4-fold less potent than RIS at inhibiting FPPS in vitro, while the halo- analogues also exhibited reduced potency for inhibition of FPPS, with decreasing potency as atomic size increases (i.e.  $F > Cl > Br$ ). These trends correlated with the potency of the desoxy- and halo- compounds for inhibiting Rap1A prenylation in J774 macrophages, and reducing viability of these cells. By contrast, desoxy-3-PEHPC and 3-PEHPC were equipotent at inhibiting Rab GGTase, inhibiting Rab prenylation and reducing cell viability. Interestingly, although the halo-3-PEHPC analogues and 3-PEHPC were equipotent for inhibition of Rab GGTase in vitro, these compounds showed a similar potency trend to the halo-RIS analogues in cell-based assays ( $F > Cl > Br$ ), with the fluorinated analogue more potent than 3-PEHPC and the others less potent. This data indicates that the -OH group plays a role in the interaction of NBPs with FPPS, an effect that cannot be explained simply by electronegativity, since substitution with halogens of similar electronegativity also reduces potency. By contrast, the -OH group is not crucial for the interaction of 3-PEHPC with Rab GGTase and substitution with the halogen fluorine actually increases potency for inhibition of Rab prenylation.

### Breath carbon monoxide as an indication of smoking during pregnancy.

Z Usmani, D Tappin, P Craig, D Shipton

PEACH Unit, 8th Floor Tower Block, The Queen Mother's Hospital, Yorkhill, Glasgow G3 8SJ

**Aims:** Health care services often use a carbon monoxide (CO) breath test to validate self reported smoking. A cut-off level of 8 parts per million (p.p.m.) or higher is used to identify smoking though this has not been re-examined for pregnant women.

**Design:** Data on self reported and breath CO validated smoking status was analysed for 2548 women who attended the antenatal clinic. **Setting:** Southern General Hospital, Glasgow. **Findings:** 546/2584 (21%) women self reported as current smokers. A cut off of 8ppm would have identified only 325/546 self reported smokers (sensitivity: 59%). 27/2002 self reported non-smokers had levels greater than 8ppm (specificity: 98.7%). Receiver operating characteristic (ROC) analysis revealed that CO cutoff levels of 2 or 3 ppm resulted in the best sensitivity and specificity. A cut-off of 2 ppm would have identified 468/546 of self reported smokers (sensitivity: 86%). 206/2002 self reported non-smokers had levels less than 2ppm (specificity: 90%). If all these women were truly smokers, the real level of smoking in pregnancy was 26.5% (752/2548) and 27% of true smokers provided false answers to the self reported question at maternity booking. The positive predictive value was 69% (95% CI: 66.2-72.4). The negative predictive value was 96% (95% CI: 94.9-97.6). **Conclusions:** A cut-off level for the breath CO test well below 8ppm and as low as 2 or 3ppm may be more appropriate in the antenatal clinic setting. At 8ppm there may be gross underestimating of levels of smoking in a pregnant population. Even at 2ppm, the low positive predictive value reflects the CO test's inability to detect light smokers, those most likely to quit. Antenatal services continue to use biochemical markers to validate responses to self reported smoking. However, the use of exhaled CO measurements as a method of assessing the validity of self-reported smoking status may not be appropriate.

## ST2 gene knockout attenuates airway eosinophilia but has no effect on airway hyper-responsiveness

P Kewin, D Xu, M Patel, B Choo-Kang, N Pitman, NC Thomson, F-Y Liew

Division of Immunology, Infection & Inflammation, University of Glasgow, Glasgow

**Introduction:** Asthma is a common allergic airways disease, the cardinal features of which are eosinophilic inflammation and airway hyper-responsiveness (AHR). There is currently considerable debate as to the causative relationship between eosinophilic inflammation and AHR. Both these processes are promoted by T helper 2 (Th2) cell effector functions. The membrane receptor ST2L is found exclusively on Th2, not Th1, cells (Xu D et al, J Exp Med, 1998) and is important in both Th2 differentiation and effector responses. Therefore, we hypothesised that ST2 gene knockout (ST2KO) would result in attenuation of eosinophilic airway inflammation and AHR. **Methods:** Wildtype (WT) and ST2KO BALB/c mice were sensitised with ovalbumin (OVA) and alum intraperitoneally on days 1 and 14. Allergic airways disease was induced by intranasal challenge with OVA on days 14, 25, 26 and 27. AHR (measured as Penh) was assessed by unrestrained whole body plethysmography response to methacholine on day 28, and the mice culled. Bronchoalveolar fluid (BALF) cell counts were performed on cytospins. Peribronchial and perivascular eosinophil accumulation was assessed on H&E stained lung sections. **Results:** When compared to WT mice, ST2KO mice had significantly reduced total BALF cell and eosinophil counts, and a lower percentage of eosinophils in the BALF. Reduced tissue eosinophils were also seen (results not shown). In contrast, there was no difference in AHR between the two groups. **Conclusion:** ST2KO attenuates allergic airways eosinophilic inflammation, but has no effect on AHR, adding further weight to the argument that these processes are differentially regulated.

## Right ventricular pacing is associated with impaired endothelial function.

H Su, CC Lang, AY Noman, AD Struthers, AM Choy

Division of Medicine & Therapeutics Division of Cardiology, Ninewells Hospital, Dundee DD1 9SY

**Purpose:** Several pacemaker and ICD trials have suggested that conventional right ventricular pacing (RVP) is associated with increased mortality and hospitalisation especially in those with impaired cardiac function. The pathophysiology underlying these deleterious effects is not clearly understood. The purpose of this study was to investigate the effects of RVP on: (1) endothelial function (2) resting and exercise cardiac output; (3) and plasma brain natriuretic peptide (BNP), a plasma index of ventricular wall stress. **Methods:** 13 patients (age = 72.0 ± 6.2, male = 9) with dual chamber pacemakers implanted for sinus node disease were studied after chronic pacing in 3 randomly assigned pacing modes: dual chamber with long atrioventricular delay (DDD-L), dual chamber with short atrioventricular delay (DDD-S) and ventricular pacing (VVI). Three patients had left ventricular systolic dysfunction with an ejection fraction <40%, five had diastolic dysfunction and one was normal. Cardiac output was determined by the inert gas rebreathing method and endothelial function was measured as the reactive hyperaemia – peripheral arterial tone (RH-PAT index). BNP was analysed

using immuno-fluorescence. **Results:** The mean percentage (± SD) of RVP in the 3 modes was 21.2±30.3% in DDD-L, 88.4±19.6% in DDD-S and 46.9±29.7% in VVI (p = 0.001). Endothelial function was significantly decreased when patients were in modes that had more RVP: 2.03±0.40 in DDD-L, 1.71±0.37 in DDD-S and 1.69±0.28 in VVI (p<0.05). Mean BNP increased nearly 2 fold when patients changed from DDD modes to VVI: 73.8±111.6pg/mL in DDD-L and 78.6±85.9pg/mL in DDD-S vs 146.4±152.1pg/mL in VVI. Resting and exercise cardiac output was similar in all modes 4.03±1.02L/min and 5.53±2.67/min in DDD-L vs 3.68±0.71/min and 5.31±1.75L/min in DDD-S vs 3.95±1.47L/min and 6.30±3.54L/min in VVI respectively. **Conclusion:** These novel findings suggest that RVP is associated with significant deterioration of endothelial function and increased ventricular wall stress. These data may provide the pathophysiological mechanism for the worse outcomes associated with right ventricular pacing.

## Insulin resistance is highly prevalent and is associated with reduced exercise tolerance in non-diabetic patients with heart failure.

M Al-Zadjali, AM Choy, AD Struthers, F Khan, CC Lang

Division of Medicine & Therapeutics, University of Dundee, Ninewells Hospital, Dundee DD1 9SY

**Background & Purpose:** There is increasing evidence of a reciprocal interrelationship between chronic heart failure (CHF) and insulin resistance (IR) such that IR may be pathophysiologically linked to the evolution of the disease in CHF. However, the prevalence of IR in the CHF population has not been fully defined. The purpose of this study was to establish the prevalence of IR among non-diabetic CHF patients and to identify potential contributory factors and to assess its relation to disease severity. **Methods:** The homeostatic model of insulin resistance (HOMA-IR) was assessed in a cohort of 92 CHF patients; mean age (69.4 ± 10.3 yrs) [range 36-90 yrs], males 81%, CHF of ischaemic aetiology 84% and BMI (27.4 ± 4.3kg/m<sup>2</sup>). All were on regular CHF medication. Clinical and biochemical parameters including indices of oxidative stress, neurohormones were determined. Additionally, patients underwent cardiopulmonary exercise testing and peripheral endothelial function testing by reactive hyperemia peripheral arterial tonometry (RH-PAT). **Results:** Prevalence of IR as defined by Fasting Insulin Resistance Index >2.7 was 67.4% in CHF and was significantly higher than in the 16 subjects who were coronary artery disease controls (P<0.05). The degree of IR was not related to the aetiology of CHF and to the left ventricular ejection fraction. There was a significant correlation between IR and serum triglyceride (r = 0.333, P<0.01), HDL cholesterol (r = - 0.234, p<0.05), impaired fasting glucose (r = 0.461, P<0.01) and central obesity (r = 0.351, p<0.01). The degree of IR was related to the exercise capacity and peak oxygen consumption (VO<sub>2</sub>). The prevalence of IR increased significantly with worsening functional NYHA Classes I, II, III and IV [50% (7/14), 58.3% (21/36), 78.4% (29/37) and 100% (5/5) p<0.05], (r = 0.257, p<0.05). The IR patients had a significantly lower exercise duration (280 ± 19.5 vs. 785.4 ± 34.6 s, p< 0.01) and peak VO<sub>2</sub> (5.6 ± 2.2 vs. 12.6 ± 1.6 ml/kg per min, p< 0.05). Exercise peak cardiac output determined by the inert gas re-breathing method was lower in patients with IR (5.9 ± 2.3 vs. 7.6 ± 0.89 l/min, p<0.05). However, RH-PAT did not differ between patients with IR and patients with normal insulin sensitivity.

**Conclusion:** These findings suggest that IR is highly prevalent among CHF patients and is associated with decreased exercise effort and capacity in patients with CHF. Targeting IR might represent a new strategy in the treatment of CHF.

### The effect of replicative stress on DNA ligase I-deficient mice.

*J Rodrigues, N Redhead, D Melton*

Sir Alastair Currie CRUK Labs, Western General Hospital, Edinburgh

DNA ligase I is the enzyme that completes lagging strand synthesis during DNA replication. Knockout mice, which would completely lack ligase I, are not viable, though mice homozygous for a function-reducing point mutation in the *Lig1* gene (46BR mice), which thus have limited ligase I function, exhibit a phenotype comparable to other genome instability cancer syndromes. However, whilst such genome instability usually arises from defects of DNA repair, the genome instability in 46BR mice may arise directly from impaired DNA replication, as *Lig1* null cells show no DNA repair defects. This would thus represent a novel mechanism of genome instability. It was hypothesised that increasing demand on DNA replication would exaggerate the genome instability seen in 46BR mice. This was investigated by examining tissue during stages of physiological stress, in the form of rapid growth, and through repeated stimulation of the immune system of the mice. Polyclonal immune stimulation was achieved by repeatedly injecting young mice with lipopolysaccharide and ovalbumin at fixed intervals. Blood, spleens and thymuses were then harvested from mice aged 3-9 weeks old. Additional data were obtained from tissue of newborn 46BR mice and from *Lig1* null foetal tissue harvested before the fetuses perished. 3-week-old 46BR mouse spleens were larger than wild types and showed evidence of stressed haematopoiesis with retarded DNA synthesis, as assessed by the extent of tritiated thymidine incorporation per S-phase cell; this was not exacerbated by immune stimulation. Impaired DNA replication in mutant mice was also suggested by their immunodeficiency: 12-fold reduced concentrations of IgG were elicited in response to the antigens compared to wild types. Micronucleus incidence, a marker of genome instability, was elevated in 3-week-old mutant splenocytes, a difference that disappeared in the older mice suggesting increased instability at the earlier age. Indeed, flow cytometry and comet assays demonstrated that this instability may develop in mutant mice within 24 hours of birth, and is most apparent in actively-proliferating haematopoietic tissues. Evidence of a potential struggle to complete S-phase was seen in *Lig1* null foetal livers, soon before the inability of these livers to take over erythropoiesis causes these fetuses to fail. This work confirmed that the 46BR replication abnormalities are most pronounced during periods of high replicative demand, and at these times indicators of genome instability are also raised.

### Cortical noradrenaline transporters are not reduced after fatal head injury: a human post-mortem study.

*P Dibdin, D Dewar*

Division of Clinical Neurosciences, Wellcome Surgical Institute, Gartnavel Estate, University of Glasgow

Traumatic brain injury is the most common cause of death and

disability in young people (Ghajar 2000) with survivors reporting the mental sequelae to exceed the physical sequelae as the major cause of disability (Fleming 2005). The abnormalities underlying the cognitive sequelae are not fully understood however previous studies have suggested that abnormalities of the cholinergic system may underlie the deficits seen after head injury (Dewar 1996 and Murdoch 1998). Other neurotransmitter systems also have extensive cortical projections and abnormality of these systems cannot be ruled out. The hypothesis is that the noradrenaline and serotonin systems are also damaged after head injury. The aim is to use radioligand binding assays to determine if serotonin and noradrenaline transporter levels are decreased in head injured patients. Ethical approval was obtained from the South Glasgow Local Research Ethics Committee. Tissue was obtained from 15 patients (11 male, 4 female) who died following a head injury and from 9 control patients (4 male, 5 female) who died from causes other than central nervous system pathology. The patients were matched for age and post-mortem delay. Preliminary studies using 3H-Paroxetine for measurement of the serotonin transporter produced inadequate results so this method was taken no further. 3H-Nisoxetine binding assays were carried out to determine the levels of noradrenaline transporter (NAT) in head-injured patients versus controls. The results showed no statistical difference between the level of NAT in head-injured patients and controls ( $p = 0.2964$ ). No relationship was seen between level of NAT and survival time or between level of NAT and presence of certain neuropathological features (oedema, subdural haematoma, diffuse axonal injury). In conclusion the results from this study suggest that there is no abnormality of the noradrenaline transporter after fatal head injury.

### The relationship between the systemic inflammatory response and VEGF, VEGF-C, IL-6 and IL-10 in patients undergoing potentially curative resection for colorectal cancer.

*F Breckenridge, AM Wallace, JEM Crozier, EF Leitch, RF McKee, DC McMillan*

University Department of Surgery, Royal Infirmary, Glasgow G31 2ER

**Introduction:** The detrimental relationship between the inflammatory response and cancer was first proposed by Virchow in 1863. More recently, the interactions between the host systemic inflammatory response (SIR) and tumour development and progression have increasingly been appreciated. Furthermore, as a marker of the SIR, an elevated C-reactive protein (CRP > 10mg/l) has recently been reported as a stage independent prognostic factor in a variety of primary solid tumours including colorectal cancer. However, the mechanism underlying this relationship remains unclear. A plausible explanation is that the SIR provides a pro-angiogenic environment with an increase in VEGF. Alternatively, the SIR may reflect the production of inflammatory cytokines with an increase IL-6 and IL-10 and the downregulation of T-lymphocytic immunity. The aim of the present study was to examine the inter-relationships between CRP, VEGF A and C, IL-6 and IL-10 in patients undergoing resection for colorectal cancer. **Methods:** Patients undergoing potentially curative resections (n=61) were included in the study. The majority of patients were male (57%), over 65 years (70%), had colonic tumours (67%) and TNM stage I/II disease (62%). Preoperative concentrations of CRP were obtained from routine blood samples. A pre-operative blood

sample was taken and stored at  $-80^{\circ}\text{C}$ . Circulating levels of VEGF, VEGF-C, IL-6 and IL-10 were measured by enzyme linked immunosorbant assay (ELISA). **Results:** In relation to the reference range, VEGF was elevated in 51% of patients, VEGF-C in 87%, IL-6 in 39% and IL-10 in 38%. There was no association between CRP and age, sex, tumour site or stage. When patients were grouped according whether (CRP  $>10\text{mg/l}$ ,  $n=20$ ) or not (CRP  $<10\text{mg/l}$ ,  $n=41$ ) they had a SIR, VEGF ( $P=0.057$ ), IL-6 ( $P<0.001$ ) and IL-10 ( $P=0.001$ ) were elevated in the SIR group. In contrast, VEGF-C concentrations were not

altered. VEGF concentration were directly correlated with IL-10 ( $r_s=0.27$ ,  $P=0.038$ ), but not IL-6 ( $P=0.436$ ). IL-6 concentrations were correlated with IL-10 ( $r_s=0.32$ ,  $P=0.014$ ). **Conclusions:** Whilst VEGF and VEGF-C are abnormal in many patients, they do not appear to be strongly related to CRP concentrations. In contrast, IL-6 and IL-10 are more closely related to CRP. The results of this study suggest the mechanism by which the SIR influences cancer specific survival involves a cytokine driven downregulation of T-lymphocytic immunity.

## POSTER PRESENTATIONS

### **The effect of intracoronary GTN on stent size used during angioplasty**

*J Rodrigues, I Starkey, J Spratt, A Sengupta, T Shaw*

Department of Cardiology, Western General Hospital, Edinburgh

### **The effect on monocyte CD40 expression by statin treatment in Acute Coronary Syndrome Patients: Findings from the Global Registry of Acute Coronary Events (GRACE)**

*JCL Rodrigues, J Sarma, K Lyall, K Carruthers, KAA Fox*

The Centre for Cardiovascular Research, The Queen's Medical Research Institute, The University of Edinburgh, Little France, Edinburgh

### **The Role of Tumour-Associated Macrophages in Small-Cell Lung Cancer**

*JCL Rodrigues, P Hodgkinson, F Maclaren, S Howie, T Sethi*

The Centre of Inflammatory Research, The Queen's Medical Research Institute, The University of Edinburgh, Little France, Edinburgh

### **Effect of statins on gamma-delta T cell populations in simulated sepsis**

*ND Wilson, DA Lowes, NR Webster, MJ Rogers, HF Galley*

Academic Unit of Anaesthesia & Intensive Care & Bone Research Group, School of Medicine, University of Aberdeen, Aberdeen

### **Comparison of von Frey hairs and pressure gauge in determining Quantitative Sensory Test (QST) changes**

*GM McGrory, MG Serpell, J Asbury*

### **Multicentre study of intensive adsorptive granulocyte and monocyte apheresis versus intravenous prednisolone in patients with severe ulcerative colitis**

*H Hanai, AR Saniabadi, T Iida, F Watanabe, M Yamada, K Takeuchi*

### **Investigating the relationship between microvascular rarefaction and systemic hypertension: A mathematical and computational model of the circulation**

*GDA Vaughan, PB Mark, J McConnell, MK Johnson, MS Olufsen, NA Hill, CAR Sainsbury*

### **Long term effects of use of financial incentives in treatments for obesity**

*V Paul-Ebhohimhen, A Avenell*

### **The relationship between the systemic inflammatory response, carotenoid concentrations and lipid peroxidation in patients undergoing potentially curative resection for colorectal cancer**

*EY Leung, D Talwar, JEM Crozier, EF Leitch, RF McKee, D StJ O'Reilly, DC McMillan*

### **Isolation of oxytocin receptor signalling via inhibition of myosin light chain kinase – A study in myometrial smooth muscle**

*CA Higgins, L Anderson, J Banks, A Blanks, S Thornton, W Martin, SM Nelson*

### **Docosahexaenoic acid (DHA) enhances the effect of docetaxel in prostate cancer cells**

*I Shaikh, I Brown, A Schofield, KWJ Wahle, A Schofield, SD Heys*

### **The directed selection of Recombinant Human Anti-Testosterone Antibodies and Their Use in immuno-Modulating Androgen Receptor Function**

*AR Kenyon<sup>1,2</sup>, K Charlton<sup>2</sup>, AJR. Porter<sup>1</sup> and IJ McEwan<sup>1</sup>*

<sup>1</sup>School of Medical Sciences, College of Life Sciences and Medicine

<sup>2</sup>Haptogen Ltd Polwarth Building, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD