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Basic and Applied Clinical Science 0853

Association Between Early Postoperatively Elevated TLR-4 & TLR-5 Gene Expression and Late Nosocomial Infection in Elective High-Risk Hepato-Pancreato-Biliary (HPB) Surgery

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Aims: A key step in the innate immune response is Toll-like receptor (TLR) activation by Pathogen Associated Molecular Patterns (PAMPs) or by Damage Associated Molecular Patterns (DAMPs). The objective of this study was to quantify the expression of TLR-4 and TLR-5 following major Hepato-Pancreato-Biliary (HPB) surgery and to identify if increased TLR activation was associated with a greater incidence in late nosocomial infection.
Methods: Ethical approval was granted to study patients undergoing elective major HPB surgery. Messenger RNA (mRNA) was extracted from whole-blood collected preoperatively, and at 24 and 48 hours postoperatively. TLR-4 and TLR-5 were quantified using a Taqman Polymerase Chain Reaction (qPCR). Postoperative infection and the presence of Systemic Inflammatory Response Syndrome (SIRS) were assessed using predefined criteria. Data were analysed using a Wilcoxon signed-rank test and are presented as median and Inter Quartile Range (IQR). qPCR data are presented as a relative quantification ratio between the candidate and reference genes.
Results: Thirty-one patients were recruited (20 male; median age 65 (IQR 59–75). TLR-4 and TLR-5 expression was increased at 24 hours ($P = 0{\cdot}003$ & $P < 0{\cdot}0001$, respectively) and 48 hours ($P = 0{\cdot}02$ & $P < 0{\cdot}0001$, respectively) postoperatively. An increased TLR-4 and TLR-5 expression was associated with an increased incidence of SIRS at 48 hours (both $P = 0{\cdot}018$). Moreover increased TLR-4 ($P = 0{\cdot}008$) and TLR-5 ($P = 0{\cdot}038$) expression assayed at 48 hours was associated with an increased incidence of late nosocomial infection, median day 6·5 (IQR 2·25–10·75).
Conclusions: We have demonstrated an increase in TLR-4 and TLR-5 expression following major HPB surgery. Furthermore, our study illustrates an association between TLR-4 and TLR-5 upregulation and the development of late post-operative infection and SIRS. This is indicative of an upregulation of the innate immune system, classically via activation from PAMPs or DAMPs following surgery.

Basic and Applied Clinical Science 0997

Stromal Targeting With Phosphodiesterase Type 5 Inhibitors in Oesophageal Adenocarcinoma

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Aims: The UK has the world's highest age-standardised incidence of oesophageal adenocarcinoma (EAC). Deaths from EAC have risen 50% since the 1970's. Conventional anti-cancer therapies are not effective. An activated stroma (defined by Î ± –SMA positive Cancer Associated Fibroblasts (CAF)) is associated with poor prognosis across multiple tumour types. We have recently shown the presence of CAF to be more predictive of survival in EAC than traditional pathological markers. Stromal targeting is a novel area in cancer treatment. No currently licensed drugs are effective in targeting CAF. We describe the repurposing of Phosphodiesterase Type 5 inhibitors (PDE5i) for the treatment of EAC.
Methods: The effects of PDE5i on CAF and EAC was studied with small molecule inhibitors and siRNA knock down in cell culture and functional assays; collagen gel contraction, Transwell® migration, Transwell® invasion and 3D organotypic culture.
Results: PDE5 is ubiquitously expressed in the CAF of EAC patients. PDE5i significantly decrease the expression of Î ± SMA by CAF. Functional effects are seen with significant decrease in collagen gel contraction weight ($p = 0{\cdot}012$) and area ($p = 0{\cdot}037$). Cancer cell migration ($< 0{\cdot}001$) and invasion ($< 0{\cdot}001$) in response to CAF was significantly reduced by PDE5i treatment. siRNA knock down of PDE5 confirmed the effect was PDE5 mediated. PDE5i treatment of EAC cells led to sensitisation to conventional chemotherapy.
Conclusions: Stromal targeting by CAF inactivation is a new potential treatment for hard to treat solid organ malignancies such as EAC. It offers the possibility of increased drug delivery (chemo/immunotherapy), increased host immune cell infiltration, decreased local and distant cancer growth and invasion. PDE5i have been safely used in man for over 20 years. We have compelling evidence that PDE5i treatment of EAC CAF significantly decreases: Î ± SMA expression, contractility and the ability to promote cancer cell invasion. The time is right for the first human trial of PDE5i as an anti-cancer therapy.

Basic and Applied Clinical Science 0090

Exercise Training Following Neoadjuvant Chemoradiotherapy in Rectal Cancer Improves Mitochondrial Function - a Randomized Controlled Trial.

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Aims: Patients with locally advanced rectal cancer routinely undergo neoadjuvant chemoradiotherapy (NACRT). Whilst NARCT improves survival, novel data from our group has shown it to cause deterioration in physical fitness which can be ameliorated by exercise training. We hypothesised that NARCT would impair in vivo muscle mitochondrial function as measured by post-exercise phosphocreatine recovery rate constant (k PCr min −1) and that exercise training post-NARCT would improve both physical fitness and mitochondrial function.
Methods: 12 patients undergoing standardized NACRT were recruited prospectively. Before and after completion of NACRT (baseline and week 0) all patients underwent cardiopulmonary exercise testing (CPET) and phosphorus magnetic resonance spectroscopy (31 P MRS) muscle examination. Patients were then randomized 1:1 to an exercise or control group. The exercise group underwent a tailored structured exercise training programme between week 0 (completion of NACRT) and week 6. CPET and 31 P MRS tests were re-done at week 6. Primary outcome variables were oxygen uptake (Vo 2) at estimated lactate threshold (LT), and k PCr . Measurements were compared between exercise and control groups using ANCOVA (correcting for week 0 variable).
Results: All 12 patients competed the study (mean (SD) age 64 (10) vs. 72 (7) years in exercise vs. control group). Following NACRT there was significant reduction in Vo 2 at LT (−2·36, $p = 0{\cdot}004$), and in k PCr (−0·34 min −1 , $< 0{\cdot}001$). The table compares (mean (95% CI)) changes between variables in the exercise and control groups.
Conclusions: These data show that NACRT is associated with decreased physical fitness and impaired mitochondrial energetics. Further, exercise training following NARCT significantly improved physical fitness with a significant recovery in in vivo muscle mitochondrial function.

Vascular and Transplant 0132

HOT Study (Heme Oxygenase-1 in Renal Transplantation): A Randomised, Placebo-Controlled, Blinded, Phase IIb Trial to Determine if Hemin Can Upregulate Heme-Oxygenase 1 and Protect Renal Transplant Recipients

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Aims: There are few proven therapies that protect against the inevitable ischaemia-reperfusion injury (IRI) that occurs during transplantation. IRI increases the likelihood of delayed graft function (DGF), which negatively impacts on long-term survival of a transplanted kidney. One enzyme of interest, heme oxygenase-1 (HO-1), degrades heme and protects against IRI oxidative stress. Heme arginate (HA), a form of hemin, can safely induce HO-1 in humans. Clinical renal recipients with higher HO-1 levels have improved graft function. The HOT study aimed to evaluate whether HA could safely upregulate HO-1 and protect recipients of deceased donor kidneys.

Methods: 40 recipients were randomised to active (two doses 3 mg kg −1 HA: pre-operatively, day 2) or placebo (NaCl: same schedule). Recipient blood was taken daily for peripheral blood mononuclear cells (PBMC) extraction. Urine was also collected. Graft biopsies were taken pre-op and day 5. DGF was calculated.

Results: HA upregulated PBMC HO-1 protein at 24 hours more than placebo: HA 11·1 ng/ml [1·0–37·0] vs. placebo 0·14 ng/ml [−0·7–0·3](p = <0·0001). PBMC HO-1 mRNA was also increased: HA 2·73 fold [1·8–3·2] vs. placebo 1·41 fold [1·2–2·2] (p = 0·02). HA increased HO-1 protein immunopositivity in day 5 renal tissue compared with placebo: HA 0·21 [−24–0·7] vs. placebo −0·03 [−76–0·15] (p = 0·02) and the percentage of HO-1 positive renal macrophages also increased: HA 50·8 cells per hpf [40·0–59·8] vs. placebo 22·3 [0–34·8] (p = 0·012). DGF rates and urinary biomarkers of injury were reduced after HA but not significantly. However, the study was not powered for this. Adverse events were equivalent between groups.

Conclusions: The primary outcome was achieved and demonstrates for the first time that HA safely induces HO-1 in renal transplant recipients and may offer benefits. Larger studies are planned to investigate the effect of HO-1 upregulation on clinical outcomes and evaluate the benefit to patients at risk of IRI.

Basic and Applied Clinical Science 0299

Genetic Signatures Associated with Susceptibility to Cancer Cachexia Vary According to Weight-Loss or Low Muscle Mass Phenotype

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Aims: Cancer cachexia is characterised by loss of weight (WL), muscle and fat. We aimed to (i) replicate SELP rs6136 and other genes previously associated with WL (ii) explore associations with 92 predefined new candidate single nucleotide polymorphisms (SNPs) and (iii) explore our panel of candidate SNPs (i and ii) for association with CT-defined low muscularity (LM) +/− WL. We also explored whether the transcription in muscle (n = 134 cancer patients) of identified genes was altered according to cachexia phenotype.

Methods: The replication study included 545 new cases. Combined analysis of prior and new cohorts (n = 1276) explored associations of new candidate SNPs with WL and LM. Human muscle transcriptome was analysed using Agilent platform.

Results: SNPs rs6136 (SELP) and rs4149570 (TNFRSF1A) shown in our prior study to associate with WL, were replicated (Table 1). New candidate SNPs in the following genes showed association with WL: IFT172, ACVR2B, TLR4, FOXO3, IGF1, LEPR, FOXO1, TOMM40, and CPN1 (Table 2). SNPs in ACE, MT2A, WDR20, PPARG, LPIN2 and LEPR, ACVR2B, TNF, ACE were associated with LM and concurrent LM + WL, respectively (Table 3). There was concordance between muscle-specific expression for ACVR2B, FOXO1 and 3, LEPR, PPARG, TLR4, TNFRSF1A and TOMM40 genes and LM or WL (< 0·05).

Conclusions: rs6136 in the SELP gene is the leading replicated SNP to associate with WL. New SNP associations for cachexia phenotypes that include an index of muscle mass and muscle specific gene expression signatures for WL or LM provide insights into potential risk factors/biomarkers for muscle loss in cancer cachexia.

Basic and Applied Clinical Science 0430

Colorectal Cancer Exosomes Activate AKT Signalling in Fibroblasts: A Functional Role for Secreted Nanoparticles

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Aims: Exosomes are nanoparticles which are secreted and transferred between tumour and stromal cells. Their cargo consists of nucleic acids (DNA, mRNA, microRNA) and proteins which can be shuttled from one cell to another. Exosome transfer influences cellular function but the mechanisms are not well defined, particularly with respect to stromal cells and in colorectal cancer. We aimed to isolate and label exosomes from colorectal cancer cells and demonstrate their transfer to fibroblasts. In addition we investigated the cellular effects of colorectal cancer exosomes on fibroblasts.

Methods: Exosomes were isolated from DLD-1 colorectal cancer cells by selective centrifugation and validated by transmission electron microscopy, western blotting and flow cytometry. The resulting exosome pellet was labelled with a lipophilic (green) fluorescent dye and co-cultured with MRC5 fibroblasts for 24 hours. The fibroblasts were then washed thoroughly with PBS to remove all extracellular particles and visualised by fluorescence microscopy. Cell lysate was analysed by western blotting for activation of key signalling pathways such as Ras (ERK) and AKT. Flow cytometry was used to quantitatively analyse cell cycle and apoptosis.

Results: Microscopy revealed presence of fluorescent labelled exosomes within MRC5 fibroblasts. There was marked increase of AKT pathway activation in MRC5 fibroblasts after co-culture with DLD-1 exosomes. As a result, significant changes to pro-apoptotic stimuli have been observed. There was no change in ERK activation.

Conclusions: We have shown that colorectal cancer exosomes can be transferred to fibroblasts in vitro. This transfer induces selective phosphorylation of AKT and resistance to apoptosis in recipient fibroblasts. Cancer exosomes promote resistance to apoptosis and can create an apoptosis resistant niche in the tumour microenvironment.

General 0517

Efficacy of Combination of 0·2%GTN snd Lignocaine Ointments in Wound Healing and Pain Relief After Milligan Morgan Hemorrhoidectomy- a Comparison With Lignocaine and 0·2% GTN Ointments Separately. A Double Blinded Randomised Control Trial

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Aims: In this study we compared the efficacy of combination of 0·2% Glyceryl Trinitrate (GTN) and 2% Lignocaine ointments with both the constituents (0·2 % GTN and 2% Lignocaine ointment) separately to find a better treatment option which not only decrease post operative pain but also improve healing rates after Milligan Morgan hemorrhoidectomy.

Methods: Patients undergoing Milligan Morgan hemorrhoidectomy were randomized in 3 groups by using computer generated table. Group A received combination of 0·2% Glyceryl Trinitrate and 2 % Lignocaine ointment, group B 2% Lignocaine and group C received 0·2% Glyceryl Trinitrate ointment. These ointments were given on twice daily basis. Pain scores were measured on 100 mm Visual Analogue Scale. Pain scores and number of oral analgesics used were compared on daily basis till 7th post operative day. The time required for

complete healing (in weeks) was also compared. Double blinding was ensured and the data was entered on SPSS and p value was calculated.

Results: Out of 210 patients, 192 (67 in group A, 64 in group B and 61 in group C) completed the study. Demographical data was comparable in all the three groups. **Results** were statistically significant ($p < 0{\cdot}05$ or less) in terms of pain relief and number of analgesics used from 1 st post op day to 4 th post op day in combination Group. Time required for complete healing was also significantly less in the combination group and in the GTN group. The overall efficacy of the combination group was aslo statistically significant as compared to the other two groups. There were no significant side effects in any group.

Conclusions: The combination of 0·2% GTN and 2% Lignocaine showed better pain relief resulting in less use of oral analgesics and faster healing of the wound after Milligan Morgan hemorrhoidectomy as compared to the either active ingredient used alone

Cancer/Surgical Oncology (GI) 0823

Minimally Invasive Sentinel Lymph Node Biopsy in Oesophageal Adenocarcinoma

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Aims: Sentinel lymph nodes are the first nodes draining a primary tumour and the most likely sites of early metastases. A minimally invasive technique of identifying sentinel nodes in oesophageal adenocarcinoma could revolutionise management by determining whether patients with submucosal disease can be treated solely by endoscopic resection and whether other patients are suitable for a less radical lymphadenectomy.

We evaluated a laparoscopic technique of identifying abdominal sentinel nodes in patients with oesophageal adenocarcinoma and assessed whether these nodes could predict overall lymph node status.

Methods: This was a prospective trial, recruiting patients with lower-third oesophageal adenocarcinoma planned for two-stage oesophagectomy with two-field lymphadenectomy. Sentinel node identification was performed immediately before resection, following endoscopic injection of 99 m Technetium-nanocolloid. A laparoscopic gamma probe measured radioactivity from all nodal stations at laparoscopy, from the open abdomen, from the mediastinum following thoracotomy and ex vivo following specimen removal. Sentinel nodes had in vivo radioactivity greater than twice and ex vivo greater than 10 times background. A specialist upper gastrointestinal histopathologist examined specimens using haematoxylin and eosin and immunohistochemistry.

Results: A total of 1297 lymph nodes were examined from 40 patients (median 31 nodes). The median age and BMI were 65·5 years and 26·5 kg/m 2 respectively. The overall sentinel node detection rate was 85% and sensitivity 88%. The laparoscopic abdominal sentinel node detection rate was 58% (23/40). Lymph node metastases were identified in 13 of these 23 patients, in whom laparoscopic abdominal sentinel nodes were positive in 10 but negative in three (sensitivity 77%). Two of these negative patients had mediastinal sentinel node micrometastases. Eleven patients had only mediastinal sentinel nodes. Five patients had no sentinel nodes. Adhesions prevented laparoscopy in one patient.

Conclusions: Laparoscopic identification of abdominal sentinel nodes using 99 m Technetium in patients with oesophageal adenocarcinoma was safe and technically feasible but not sensitive enough to predict overall nodal status.