Pancreatic Society of Great Britain and Ireland

A meeting of the Pancreatic Society of Great Britain and Ireland was held at Freeman Hospital, Newcastle upon Tyne on 18 November 1994. Dr Richard Lendon, President of the Society, chaired the meeting. Sixteen papers and 16 posters were presented. The Rodney Smith Medal and Prize was awarded to Mr S Falconer, and the Society’s Poster Prize was awarded to Mr S Wigmore.

Collagenase, stromelysin, and tissue inhibitor of metalloproteinase expression in pancreatic and ampullary cancer

S Branhall, G Stamps*, N R Lemoine*, J Dunnet, C McConkey, J Neoptolemos (Academic Department of Surgery, City Hospital NHS Trust, Birmingham, *TCRF, Hammersmith Hospital, London, and Clinical Trial Unit, University of Birmingham) Pancreatic cancer is characterised by locally aggressive behaviour and early metastasis. The matrix metalloproteinases (MMP) are a family of nine proteolytic enzymes that are capable of degrading the extracellular matrix, and the functional activity of these enzymes is controlled by one of three specific tissue inhibitors of metalloproteinases (TIMP). There is strong experimental evidence that expression of the MMPs correlate with tumour invasion and that reduced expression of TIMPs correlate with tumour progression and metastasis.

Immunocytochemistry was performed on sections from 50 patients with pancreatic cancer (n=27), ampullary cancer (n=12), low bile duct cancer (n=3), neuroendocrine tumours (n=3), and chronic pancreatitis (n=5) using antibodies raised against the active sites of collagenase (MMP 2), two different antibodies, stromelysin (MMP 3) and tissue inhibitor of metalloproteinase (TIMP 1).

Expression of MMP 2, MMP 3, and TIMP 1 was greater in pancreatic and ampullary cancer than any other pathology (p<0.0001) and expression in the malignant epithelial cells in pancreatic and ampullary cancer was higher than in the stromal tissues (MMP 2: 100% v 37%, MMP 3: 93% v 15%, TIMP 1: 95% v 4%, p<0.00001). There was strong correlation between the expression of the two antibodies for MMP 2 (p<0.0003), between MMP 2 and TIMP 1 (p<0.0009), and between MMP 3 and TIMP 1 (p<0.0007). TIMP 1 expression in lymph node positive patients with ampullary and pancreatic carcinoma was reduced (88% positive, 25% negative and 94% positive, 73% negative). This was significant when the two were combined (p<0.02). Enhanced expression of MMP 2 inversely correlated with tumour differentiation (p<0.05).

In conclusion, MMP 2, MMP 3, and TIMP 1 are strongly implicated in the invasive properties of pancreatic and ampullary cancer.

Isolation of a novel gene rearranged and overexpressed in pancreatic cancer

A Mckee, N R Lemoine (Oncology Unit, Hammersmith Hospital, London) A novel approach has been developed to detect genomic rearrangement or deletion in tumour cells using PCR amplification of DNA with primers to the ubiquitous Alu repeat sequences scattered throughout the human genome. We have isolated a number of fragments that are affected by tumour specific rearrangement and used these as probes to identify clones from robotically gridded high density filters of pancreatic cell CDNA and genomic libraries. These clones in turn have been sequenced and used as probes for fluorescent in situ hybridisation (FISH) mapping of metaphase chromosomes and as probes for in situ hybridisation of tissue sections. One of the CDNAs isolated is a putative novel sequence, which shows frequent and specific upregulation of expression in pancreatic cancer and hence may represent a new oncogene.

Tumour specific targeting of prodrug activation as gene therapy for pancreatic cancer

N R Lemoine, J D Harris, H Hurst, S Sikora (Oncology Unit, Hammersmith Hospital, London) Current treatments available for metastatic malignant disease of the pancreas and gastrointestinal tract are ineffective. One of the most promising of the selective genetic strategies against cancer is VDEPT (virally directed enzyme prodrug therapy), which uses a viral vector to carry a prodrug activating enzyme gene into both tumour and normal cells. By linking the enzyme downstream of tumour specific transcription units, tumour specific prodrug activation is achieved.

Overexpression of the oncogene ERBB2, occurs in many pancreatic and gastric tumours and involves transcriptional upregulation of each copy ERBB2. We have constructed chimeric minigenes consisting of the ERBB2 promoter linked to cytosine deaminase or herpes simplex thymidine kinase. We have made viral particles to transduce a panel of ERBB2 positive and negative pancreatic cell lines. Significant cell death was seen for ERBB2 positive cell lines transduced with the ERBB2-CD construct and treated with the prodrug 5-FC. Cytosine deaminase activity was low in ERBB2 negative transduced cell lines but was increased at least 10-fold in ERBB positive transduced cells. This tumour specific cytotoxic system is currently being developed for use in a clinical protocol that could be used against tumours that overexpress ERBB2.

Cytotoxicity of lithium gamma linolenic acid on pancreatic carcinoma cell lines in vitro

D Ravichandran, A J Cooper, C D Johnson (University Surgical Unit, Southampton General Hospital, Southampton) Lithium salt of gamma linolenic acid (LIGLA), an essential fatty acid derivative, has been reported to be effective in prolonging survival in pancreatic cancer without significant side effects. We tested the in vitro susceptibility to LIGLA of two pancreatic ductal carcinoma cell lines (Panc 1 and MIA PaCa2, ECACC, Porton Down).

Cells were grown under standard conditions (37°C, 5% CO2, 100% relative humidity, in culture medium containing 10% FBS), harvested and seeded at 2500 cells in 100 μl of medium per well in 96 well cell culture plates. LIGLA (Scotia, Guildford) in 100 μl of medium was added 24 hours later to achieve final concentration varying from 5 to 140 μg/ml. Control experiments were run with medium alone, lithium chloride (0.06 to 2 mmol/l) to exclude a lithium effect, and palmitic acid (4 to 140 μg/ml) to exclude a non-specific fat overload effect. Plates were kept in triplicate, inspected daily, and removed when the cells in medium only became confluent or on the seventh day. Cell viability was assessed by a microculture tetrazolium assay.

Secretin induces a paradoxical sphincter of oddi (SO) hyperpressure effect in alcoholic patients without pancreatic disease

S Mossi, R Delrez, J Portal, R Laugher (Hospital de la Conception, Marseille, France) Secretin relaxes SO in normal controls but also in chronic pancreatitis patients who present with a basal SO dysfunction. LIGLA had a dose and time dependent cytotoxic effect on both cell lines and may provide a useful therapeutic adjunct in patients with pancreatic cancer.

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A double-blind placebo controlled study of lexpifant (a potent PAF antagonist) in acute pancreatitis

S W GALLOWAY, A N KINGSMORTH et al (Department of Surgery, University of Liverpool) Platelet activating factor is a key inflammatory mediator implicated in the pathophysiology of acute pancreatitis. The aim of this study was to evaluate the effects of three days of intravenous lexpifant on the clinical course and serum markers of inflammation in human AP. Eighty-three patients with AP were randomised to receive either placebo (P) or active (A) medication in a double-blind study. Patients were monitored daily by APACHE II scoring and measurement of serum parameters—polymorphonuclear elastase (PMNE), E-selectin (E-sel), interleukin-6 (IL-6). The two groups were found to be evenly matched at baseline.

There was a consistent trend to a reduction in clinical severity and serum markers of systemic inflammation (except for PMNE) in the group treated with lexpifant. Stratified analysis showed that these effects were most notable in the 26 cases of severe AP (APACHE II score ≥8). A larger study looking at clinical outcome has now started.

Acute lung injury in the microembolic model of acute pancreatitis

S W GALLOWAY, A N KINGSMORTH (Department of Surgery, University of Liverpool) Severe acute pancreatitis (AP) is associated with the development of an acute lung injury. The aim of this study was to discover if pulmonary injury was a feature of the microembolic model of AP in rats and to assess the therapeutically beneficial effect of a platelet activating factor (PAF) antagonist BB-882. AP was induced in 10 male Wistar rats by microembolisation of the pancreatic bed with polystyrene microspheres. After 12 hours tissue capillary permeability was assessed by an Evan’s blue dye (EBD) extravasation technique and compared with control animals. A further group of rats received BB-882 30 minutes after induction of AP.

There was a significant increase in the tissue content of EBD in the pancreas and lungs of the group with AP (Student’s t-test; p<0.05). BB-882 modified the pulmonary changes when given after the induction of the AP, as shown by a significant reduction in EBD content of the lungs (p<0.01). Increased pulmonary capillary permeability was seen as an early feature of the microembolic model of acute pancreatitis and these changes seem to be ameliorated by administration of a PAF antagonist.

Which clinical prognostic score for acute pancreatitis? Results of a prospective multicentre study of 719 episodes

D HEATH, D ALEXANDER, C WILSON, M LARVIN, C W IREY, M J MCMAHON (General Infirmary, Leeds; Royal Infirmary, Glasgow; Guy’s Hospital, London) Acute pancreatitis is the most unpredictable abdominal emergency, and requires prompt recognition and intervention for organ system failure (OSF) and pancreatic collections. Of the focus of prognostic systems, only clinically based scores offer rapid assessment. The aim of the study was to evaluate systematised clinical assessment (mild: severe pain/tender- ness/systemic features absent), Ranson, Glasgow, and APACHE II scores, for the prediction of OSF and collection defined by Atlanta criteria. During a four year period, patients admitted to 25 hospitals in Yorkshire and Glasgow with confirmed acute pancreati- tis were assessed and recorded by research fellows. Of 719 episodes (382 male; 337 female, median 57 years; gall stones 36%; alcohol 24%; other causes 7%), 529 episodes were uncomplicated (74%). Of 190 severe episodes (26%), 133 patients survived (18%) and 57 died (8%).

Dynamic contrast enhanced magnetic resonance imaging is superior to dynamic computed tomography in acute pancreatitis

M LARVIN, J WARD, P J ROBINSON, A G CHALMERS, M J MCMAHON (The General Infirmary and St James’ University Hospital, Leeds) Dynamic, contrast enhanced computed tomography (CT) is the preferred imaging modality for the assessment of acute pancreatitis. However, CT images are often repeated, but require large doses of contrast, while regulatory authorities warn that irradiation risks are underestimated. Improvements in magnetic resonance (MR) imaging, including the use of dynamic, contrast enhanced resolution, and gadopentetate dimeglumine (GdDTPA) contrast enabled us to develop an analogous technique, and to compare dynamic CT and MR imaging prospectively. Axial/coronal T2 weighted spin echo and gradient echo, ‘‘turbo’’ fast low angle shot (FLASH) MR sequence were acquired (Siemens Magnetom, 1-0 Tesla) in one breathhold, before and after 0.1 mmol/kg GdDTPA. MR images were obtained with a plane. Images were scored ‘’blindly’’ for viability, collection content, extrapancreatic signs, and aetiology. In 32 patients (18 male/14 female; median 53 years; gall stones=19; alcohol=5) using intravenous iopamid 370 at 2 mL/s. CT scans were scored ‘’blindly’’ for viability, collection content, extrapancreatic signs, and aetiology. In 32 patients (18 male/14 female; median 53 years; gall stones=19; alcohol=5) CT images were obtained. Images were scored ‘’blindly’’ for viability, collection content, extrapancreatic signs, and aetiology. In 32 patients (18 male/14 female; median 53 years; gall stones=19; alcohol=5) CT images were obtained. Images were scored ‘’blindly’’ for viability, collection content, extrapancreatic signs, and aetiology.
Pancreatic carcinoma: an audit of treatment, survival, and quality of life

C WILSON, M E C VAN WYK, J FUNNELL, J E KRIEG, P C BORMAN, J TERRBLANCHE (Surgical Gastroenterology, Department of Surgery, Great Ormond Street Hospital and University of Cape Town) Since 1989 data has been collected prospectively on all patients presenting with pancreatic carcinoma. There were 213 patients recorded, 108 men: 105 women, median age 64 (range 28-89 years). Jaundice was the presenting complaint in 155 patients (73%). The initial treatment was surgical in 76 patients (37%) (bypass – 63, resection – 10, laparotomy – 3), endoscopic stent placement in 64 (30%), and percutaneous stent placement in 16 (7%). The remaining 57 patients underwent palliative procedure performed. Seventeen patients had additional radiotherapy or chemotherapy, or both.

At the time of analysis 165 (77%) had died, 34 (16%) were alive, and the outcome in 14 patients was unknown. Median survivals were: resection 487 days, bypass 144 days, stent placement 127 days, and no procedure 36 days. The total time spent in hospital was significantly shorter when palliation was by stent placement rather than bypass surgery (median 165 ± 21 days, p=0.009), this difference being most noticeable for those having endoscopic stent placement and survival of up to six months (median 12 ± 23 days, p=0.0002).

Quality of life was assessed by sequential ECQG scores in 70 patients and showed good preservation of performance, usually until the last month of life. Their requirement for narcotic analgesia increased, particularly during the last two months of life.

Palliation of jaundiced patients by stent placement was as successful (69%) as end-to-end pancreaticojejunostomy (66%) with cancer and 32 of 35 patients (91%) with chronic pancreatitis. In 44 patients, anastomosis between the pancreatic remnant and jejunum was performed. There were no deaths within 30 days of surgery. However, three patients (with cancer) died at 31, 57, and 69 days of myocardial infarction, stroke, and sepsis, respectively. An uncomplicated course was made by 24 of 36 anastomoses (66%) with cancer and 32 of 35 patients (91%) with chronic pancreatitis. In 44 patients, anastomosis between the pancreatic remnant and jejunum was performed. Three major and one minor pancreatic leaks occurred after end to end pancreaticojejunostomy, and one minor leak occurred after percutaneous pancreaticojejunostomy. Hospital stay was shortest in patients who had a purse string anastomosis; median 11 days (range 6-23) versus 16 days (10-72; p=0.001). The purse string pancreaticojejunostomy is quicker and easier to perform than conventional pancreaticojejunostomy and may be associated with a quicker recovery.

Tissue factor and tissue factor pathway inhibitor in human pancreatic carcinoma

A K KAKAK, N R LEMOINE, R N WILLIAMS (Department of Surgery and ICRF Oncology Unit, Hamersmith Hospital, London) Human pancreatic carcinoma is associated with a high incidence of thrombotic complications, and thrombin generation may facilitate tumor invasion and metastasis. The nature of the procoagulant and, in particular, the role of tissue factor (TF) and its physiological inhibitor tissue factor pathway inhibitor (TFPI) have not been defined. Specimens of normal human pancreas (n=18) and pancreatic adenocarcinoma (n=53) were studied immunohistochemically for TF and TFPI expression using rabbit polyclonal antibody to TF and antihuman TFPI antibodies. Tumour specimens were assessed by two observers for TF expression and were graded independently for histological differentiation. Normal pancreatic did not express TF or TFPI. The nature of TF staining (+/+/+++/+++), was seen in three of 15 (20%) well differentiated, nine of 18 (50%) moderately differentiated, and 17 of 22 (77%) poorly differentiated tumours. The effect of tumour differentiation on TF expression was highly suggestive as analysed by the x2 test for linear trend (x2=6.67; p=0.0098). There was no heavy staining for TFPI but 17 of 24 tumours stained lightly for TFPI. There was no relation with tumour grade.

The strong expression of TF may explain the hypercoaguable state in pancreatic cancer. The weak expression of TFPI may be insufficient to inhibit the strong procoagulant stimulus of TF.

Results of purse string pancreaticojejunostomy after partial pancreaticectomy

J G WILLIAMS, S R BRAMHALL, J P NEOFOTIOMOS (Academic Department of Surgery, City Hospital NHS Trust, Birmingham) Morbidity and mortality after pancreatic resection has fallen steadily. However, leakage from pancreaticojejunostomy in pancreatic carcinomas has been a significant cause of death after partial pancreaticectomy. A novel technique of purse string pancreaticojejunal anastomosis is described. Seventy one patients underwent pancreatic resection between 1987 and 1994. There was no mortality within 30 days of surgery. However, three patients (with cancer) died at 31, 57, and 69 days of myocardial infarction, stroke, and sepsis, respectively. An uncomplicated course was made by 24 of 36 anastomoses (66%) with cancer and 32 of 35 patients (91%) with chronic pancreatitis. In 44 patients, anastomosis between the pancreatic remnant and jejunum was performed. Three major and one minor pancreatic leaks occurred after end to end pancreaticojejunostomy, and one minor leak occurred after purse string pancreaticojejunal anastomosis. Hospital stay was shortest in patients with a purse string anastomosis; median 11 days (range 6-23) versus 16 days (10-72; p=0.001). The purse string pancreaticojejunostomy is quicker and easier to perform than conventional pancreaticojejunostomy and may be associated with a quicker recovery.

PBMC regulation of the hepatic acute phase response (APPBR in cancer patients occurs via an IL-6 dependent mechanism

J S FALCONER, R S SORRENSON, S M M PAKAIK, S C CARTER, K H PEARSON (University Department of Surgery, Royal Infirmary, Edinburgh) The hepatic APPR is associated with increased energy expenditure in cancer patients and may contribute to cancer cachexia. In this study we investigated the hypothesis that the APPR is regulated by peripheral blood mononuclear cells (PBMC) via an IL-6 dependent mechanism. Cachexia cancer patients (n=32) were subdivided on the basis of serum C-reactive protein as a measure of the presence of an APPR. PBMC were isolated and the supernatants from 24 hour cultures added to culture of isolated human hepatocytes in the presence or absence of neutralising antibody to IL-6. Hepatocyte C reactive protein production was measured by ELISA. In patients with an APPR both serum and PBMC IL-6 were increased. PBMC super- natants induced an APPR in cultured human hepatocytes, which was abolished by antibody to IL-6. Therapeutic strategies that modulate PBMC activation and IL-6 production may control the generation of the APPR and thereby abrogate cancer cachexia.

The acute phase response and survival in pancreatic cancer

J S FALCONER, R K C H PEARSON, J A ROSS, L ELSTON, J S WIGMORE, D C CARTER (University Department of Surgery, Royal Infirmary, Edinburgh) Pancreatic cancer patients have among the highest incidence of cachexia and lowest survival rates of any group of cancer patients. The aim of this study was to find out if certain metabolic and nutritional indices related to the acute phase response (APR) are prognostic factors independent of stage of disease. The variables at the time of diagnosis of 102 patients with unresectable pancreatic cancer were entered into a Cox's proportional hazards model. Included in the analysis were serum C reactive protein (CRP) and albumin, weight loss, age, sex, and stage of disease (USCC). The median survival of those with an APR (CRP >10 mg/l, n=45) was 66 days compared with 222 days in those without an APR (n=57; p=0.0001, Mann-Whitney U). The results of multivariate analysis in which each factor has been adjusted for the influence of other factors are shown in the Table. Factors for inclusion in this model were identified by a preliminary univariate analysis.

Bupropion versus placebo in the treatment of pancreatic cancer cachexia

J S WIGMORE, J S FALCONER, J A ROSS, C E PLESTER, J A MALIGN, D C CARTER, K H PEARSON (University Department of Surgery, Royal Infirmary, Edinburgh) Profound weight loss is commonly associated with pancreatic cancer. Cachexia has been linked with increased resting energy expenditure (REE) and activation of the hepatic acute phase response (APPR). These effects are thought to be mediated by IL-6 through direct or proinflammatory mediators. Sixteen patients with histologically confirmed carcinoma of the pancreas under-
went nutritional assessment and serum sampling before and after treatment with 1200 mg per day for seven days of the cyclooxygenase inhibitor ibuprofen. REE was measured by indirect calorimetry (Deltatrac Analyser).

Measurement of REE was higher than predicted values in all patients but was reduced after treatment with ibuprofen (n=10) compared with placebo (n=6). Reduction in REE was paralleled by reduction in serum C reactive protein concentration in the ibuprofen group.

These results show that ibuprofen may have a significant benefit in reducing REE and acute phase protein production in patients with pancreatic cancer. Further studies are required to discover if these changes correlate with reduction in weight loss in patients with cachexia.

**Pancreatic cancer cell derived IL-8 stimulates hepatic acute phase protein production in vitro and serum IL-8 correlates with serum CRP in pancreatic cancer patients in vivo**

S J WIGMORE, J A ROSS, K C H FEARON, J S FALCONER, J P MALNGAY, D C CARTER
(Universtity Department of Surgery, Royal Infirmary, Edinburgh) The hepatic acute phase protein response is an important component of the catabolic processes that contribute to weight loss in pancreatic cancer. We show that both recombinant human IL-8 and tumour cell derived IL-8 stimulated acute phase protein production in isolated human hepatocytes. Addition of recombinant human IL-8 or pancreatic cancer cell supernatants resulted in the following changes in CRP production.

Stimulation of CRP production by human pancreatic cancer cell supernatants could be inhibited by the addition of neutralising antibodies as follows:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>MiaPa Ca-2</th>
<th>CFPAC</th>
<th>PANC1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-h IL-8</td>
<td>113</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>Anti-h IL-6</td>
<td>7</td>
<td>78</td>
<td>0</td>
</tr>
</tbody>
</table>

Furthermore, we show that serum IL-8 correlates with the presence of an acute phase protein response in 63 patients with pancreatic cancer (r=0.68, p=0.0001). Tumour cell derived IL-8 may have a role in the aetiology of the acute phase response in cancer cachexia.