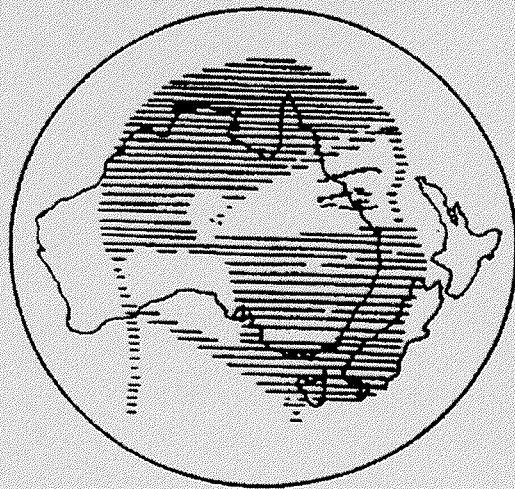

**The Surgical Research Society
of Australasia**



**Annual Scientific Meeting
30-31 August 2001**

**The Royal Hobart Hospital
Tasmania**

**The Surgical Research Society of Australasia
Annual Scientific Meeting
30 – 31 August 2001**

Program

Thursday, 30 August

1300-1315 Official welcome - Professor P Stanton

1315-1500 Opening Symposium - Dr Christopher Newell
Organ snatching or good research: an ethical reflection

1500-1530 Afternoon tea

1530-1700 Free papers

Session 1 Chairman: R Lord

1. A new technique to assess the axilla for breast cancer metastases using cell separation technology: report of a pilot project
Edwards M, Wilkinson S, Twin J
2. Is aortic angiography necessary for accurate planning of endovascular aortic aneurysm stents?
Brown WA, Miller R, Birch SE, Scott A
3. Remodeling of carotid artery following vascular grafting: the role of myofibroblasts and pericytes
Huang P, Hawthorne WJ, Ao P, Angeli GL, Medbury HJ, Fletcher JP
4. Fibrin glue versus suture for repair of intraorbital optic nerve
Waites PB, Cui Q, Knuckey N, Harvey AR
5. Peripheral nerve anatomy is established independently of the vasculature in the upper limb of the embryo
Bates D, Newgreen DF, Taylor GI
6. The angiosomes of the nerves of the lower limb
Suami H, Taylor GI, Pan W-R, Seneviratne S
7. Improvement in respiratory symptoms following laparoscopic nissen fundoplication
Kiroff GK, Brouwer R
8. A study to assess gastric laparoscopic banding and its role in the treatment of morbid obesity
Cehata A, Semple C, Wilkinson S

1700-1800 Executive meeting

Friday, 31 August

800-830 **Annual General Meeting**

830-1030 **Free papers**

Session 2 **Chairman: G Kirov**

9. Improved metabolic control does not prevent raised intra-cranial pressure in a liver biodialysis system tested in a porcine hepatic failure model
Stewart GJ, Mears DC, Bourne R, Sheil AGR
10. Do pancreatic blood vessels matter in acute pancreatitis?
Brooke-Smith ME, Carati C, Toouli J, Saccone G
11. Expression and overexpression of heat shock protein-70 (HSP70) by dendritic cells in the arterial intima and its potential significance in atherogenesis
Bobryshev YV, Lord RSA
12. Excitatory and inhibitory neuromuscular transmission in the colonic circular muscle in chronic idiopathic constipation/slow colonic transit
Stanton M, Hengel P, Bornstein JC, Hutson JM, Chow CW, Cook D, Southwell BR
13. Biomolecular factors in osteoporotic rat fracture healing
Yu Y, Gifford KJ, Low AKW, Walsh WR
14. In-vitro biomechanical comparison of lagged and non-lagged screw fixation of oblique metacarpal fractures
Nicklin S, Ingram S, Ho S, Gianoutsos MP, Walsh WR
15. Cyclic testing of flexor tendon repairs
Nicklin S, Matheson G, Chircop M, Gianoutsos MP, Walsh WR
16. The inhibition of peritendinous adhesions using a collagen synthesis inhibitor
McCombe D, Ruangpanit N, Kubicki M, Guenzler V, Williams J, Thompson EW, Morrison WA
17. A pilot study on the development of intranasal adhesions in sheep
McIntosh D, Wormald PJ
18. The healing of the nasal mucosa in sheep. Does nasal packing make a difference?
McIntosh D, Cowin A, Adams D, Wormald PJ

1030-1100 **Morning tea**

1100-1130 **International Lecturer - Professor Justin Roake**
A surgeon's guide to dendritic cell biology

1130-1300 **Free papers**

Session 3 **Chairman: P Stanton**

19. CD44v6 is a key regulator of CD44v-mediated metastatic behaviour
Barbour A, Reeder J, Antalis T, Walsh M, Fawcett J, Gotley D
20. Vaccination of stage III melanoma and ovarian cancer patients using irradiated and haptenised autologous whole tumour cells
Dyer SL, Berd D
21. Encapsulation of hepatocellular carcinoma: relationship to collagen in tumour stroma and adjacent parenchyma
Lockwood DSR, Yeadon T, Clouston AD, Callaghan S, Gotley DC
22. A comparison of radiofrequency ablation, cryoablation, diathermy and argon beam coagulation for the local treatment of the liver resection edge
Gananadha S, Zhao J, Morris DL
23. Analysis of the breast cancer proteome and protein characterisation of the disease state
Bhatia KD, Lord R, Stanton PD
24. Tumour-associated hepatic stellate cells (HSC) are derived by proliferation – evidence for an autocrine mitogenic loop in hepatocellular cancer (HCC)
Yeadon T, Fawcett J, Crawford D, Lockwood D, Callaghan S, Ooi LLPJ, Gotley D
25. Angiogenesis in haemangiomas is inhibited by mast cell stabilisers in vitro
Donato RR, Peningotn AJ, Keramidaris E, Romeo R, Morrison WA

1300-1400 **Lunch**

1400-1430 **International Lecturer - Professor Justin Roake**
Dendritic cells and cancer vaccines: from laboratory to the clinic

1430-1545 **Free papers**

Session 4 **Chairman: J Hutson**

26. Westran fetal pig pancreas fragment transplants survive and function long term
Hawthorne WJ, Walters S, Patel A, Chapman JR, Allen RDM, O'Connell P
27. Pancreatic islet cell transplants can be hyperacutely rejected
Hawthorne WJ, Horton PJ, Patel AT, Walters SN, Allen RDM
28. Nitric oxide (NO) production following liver transplantation
Tassell B, Liew I, Goto S, Lord R
29. The expression of an immunosuppressive protein (LSF-1) on lymphoid cells correlates with allograft survival following orthotopic liver transplantation
Lord R, Vari F, Goto S
30. Expression of TGF- β 1 mRNA and protein in alveolar macrophages of human lung allografts
Zheng L, Snell GI, Glare E, Williams TJ, Walters EH
31. Red blood cell autoimmunity following cardiopulmonary bypass surgery
Yeo W, Lord R, Stanton P, Dixit A

32. Coronary artery surgery without cardiopulmonary bypass reduces neutrophil activation relative to surgery with bypass
Vallely M, Bannon P, Bayfield M, Hughes C, Wong M, Kritharides L

1545-1615 Afternoon tea

1615-1700 Free papers

Session 5 Chairman: D Gotley

33. Role of insulin 3, testosterone, mullerian inhibiting substance and relaxin in rat gubernacular growth
Temelcos C, Kubota Y, Bathgate RA, Jones KJ, Hutson JM
34. The immunolocalisation and significance of VEGF, VEGF-B and its receptors in the articular cartilage of the femoral condyles of growing rats
Yee G, Walsh WR, Poole MD
35. Glucose-insulin-potassium solution improves left ventricular mechanoenergetics in diabetes
Ramanathan T, Shirota K, Morita S, Nishimura T, Huang Y, Hunyor S
36. Development of a critical size cranial defect in a sheep model: role of different mesh cover and defect size
Ho K, Walsh WR, Gianoutsos MP

1700-1730 Jepson Lecturer - Professor Wayne Morrison
Tissue engineering

1900 for 1930 Annual dinner - Moorilla Estate Vineyard
[Dress: Jacket and tie]

1. A NEW TECHNIQUE TO ASSESS THE AXILLA FOR BREAST CANCER METASTASES USING CELL SEPARATION TECHNOLOGY: REPORT OF A PILOT PROJECT

Edwards M, Wilkinson S, Twin J

Discipline of Surgery, University of Tasmania, Royal Hobart Hospital, Liverpool Street, Tasmania 7000.

Introduction: Accurate staging of the axilla for metastatic disease is critical for deciding optimal management of patients with breast cancer. Lymph node status is the most powerful prognostic factor. Current standard surgical management of breast cancer involves axillary dissection for staging. Pathological staging by routine histology however is known to under-stage the disease extent because only one or two sections are taken from each node, a sample of less than 1% of most nodes. Sentinel node biopsy is currently under trial to determine if thorough pathological staging of the most likely involved node is more accurate than standard pathologic assessment of all nodes.

Method: The present pilot study was undertaken to investigate an alternative method of assessing all axillary nodes for cancer cells. After routine material was taken from lymph nodes for standard pathologic assessment, discarded parts of nodes were used for the study technique. These node parts were mechanically disaggregated, and the cell suspension centrifuged on a density gradient to separate any tumour cells (into the pellet) from lymphocytes (at the top of the gradient). The pellet was then assessed by H&E and immunochemistry.

Results: The results proved highly significant, the technique detecting metastatic cells in three nodes which were negative on routine pathology, in one case changing the status of the patient from node-negative to node-positive.

Conclusion: It is concluded that this technique has the potential to remove sampling error, may offer far more accurate axillary staging than routine histopathology, and should be further evaluated in a controlled trial.

2. IS AORTIC ANGIOGRAPHY NECESSARY FOR ACCURATE PLANNING OF ENDOVASCULAR AORTIC ANEURYSM STENTS?

Brown WA, Miller R, Birch SE, Scott A

Department of Surgery, Launceston General Hospital, Charles Street, Launceston, Tasmania

Introduction: Endovascular management of Abdominal Aortic Aneurysms (AAA) relies upon accurate stent design. Typically, the length of the stent is derived from measuring an aortic angiogram, whilst lumen size is determined from an abdominal vessel Computed Tomogram (CT). Using CT, 3D surface rendered images of the abdominal vessels can be attained. We hypothesize that the length of the stent could be measured from these images eliminating the need for aortic angiography with its concomitant morbidity and cost.

Methods: The CT scans from a consecutive series of 50 patients who underwent endovascular AAA repair were reviewed with the assessor blinded to details of the patient and their subsequent management. Measurements of length were assessed from 3D surface rendered images whilst diameter was assessed from axial images. A trifurcate Zenith (Cook) endovascular aortic stent was then designed. These stents were compared to those originally designed for the patient using measurements from both angiogram and CT (Spearman's test of correlation ($p < 0.05$)).

Results: The stent body size and short limb extension (SLE) of stents in each group was similar (correlation coefficient 0.67 ($p < 0.01$) and 0.45 ($p = 0.014$) respectively). However, there was discrepancy in the measurements for the stents' long limb extensions (LLE) (correlation coefficient 0.043), with CT alone significantly underestimating the length required (37 vs 54mm).

Conclusion: In our institution the aortic angiogram calibration catheter is passed through the right iliac vessels, the vessels from which LLE is determined. This suggests that CT underestimates the tortuosity of iliac vessels. We conclude that aortic angiography is necessary for accurate AAA endovascular stent design.

3. REMODELING OF CAROTID ARTERY FOLLOWING VASCULAR GRAFTING: THE ROLE OF MYOFIBROBLASTS AND PERICYTES

Huang P, Hawthorne WJ, Ao P, Angeli GL, Medbury HJ, Fletcher JP*

Department of Surgery, University of Sydney, Westmead Hospital, Westmead, NSW, 2145, Australia.

Introduction: Nonmuscle cells have been shown to be involved in vessel repair. The aim of this study was to investigate the role of myofibroblasts and pericytes in vascular remodeling following carotid patch grafting.

Methods: Twenty female Merino sheep were randomised equally into two groups and implanted with a patch of gelatin sealed Dacron graft into the left common carotid artery. At one and six months, grafted vessels were harvested, processed and assessed for histological morphometry. Myofibroblasts and pericytes were identified by immunohistochemical labelling α -actin and desmin. Cell proliferation and cell phenotype were detected using double immunohistochemical staining with anti-proliferating cell nuclear antigen (PCNA) and anti- α -actin or anti-macrophage antibodies (HAM56). Apoptosis was detected by using in situ terminal deoxynucleotidyl transferase-mediated dUTP-fluorescence nick end labelling.

Results: The intimal area was significantly reduced at six months compared to one month ($P < 0.05$). The carotid artery lumen size at six months was significantly larger than at one month ($P < 0.05$). Myofibroblasts and pericytes were identified in the perigraft adventitia, graft matrix and intima by expression of α -actin but devoiding of desmin. The number of proliferating cells in the intima was significantly higher at one month than at six months ($P = 0.007$). The major proliferating cells were myofibroblasts and pericytes. TUNEL positive cells were significantly greater in the intima at one month than at six months ($p < 0.05$), but were significantly greater in perigraft adventitia at 6 months ($P < 0.05$). HAM 56 positive cells in intima at one month were significantly increased compared to 6 months ($P < 0.05$).

Conclusion: Myofibroblast and pericyte formation around and within the graft matrix is associated with perigraft adventitia and intima remodeling. The balance between myofibroblast and pericyte proliferation and apoptosis may result in the reduction in intimal area at six months.

4. FIBRIN GLUE VERSUS SUTURE FOR REPAIR OF INTRAORBITAL OPTIC NERVE

Waites PB¹, Cui Q¹, Knuckey N², Harvey AR^{1,3},

¹Department of Anatomy and Human Biology. ²Centre for Neuromuscular and Neurological Disorders. ³CTEC, The University of Western Australia, Crawley, WA 6009.

Introduction: Regrowth of damaged axons in the peripheral nervous system (PNS) occurs, but there is little or no spontaneous repair of fibre pathways in the injured adult central nervous system (CNS). Many of the factors that underlie the difference in regenerative potential between PNS and CNS are known and relate to the balance of growth-promoting versus growth-inhibiting influences (1). Experimentally induced regrowth of some adult CNS axons has been achieved using grafts of peripheral nerve (PN) (REFS). However, it has proved very difficult to induce the regrowth of myelinated CNS axons if the injury is more than a few millimetres away from the parent cell bodies. In PN-optic nerve (ON) studies, successful regrowth of retinal ganglion cell (RGC) axons is only seen if the PN is attached intraorbitally, close to the back of the eye. Transplant the PN intracranially, more than 5-6mm from the retina, and growth is dramatically reduced (2). These observations support the idea that the distance of the injury from the cell body is a critical factor in whether or not damaged neurons remain viable and axon regeneration can occur. However, it is also possible that technical factors in previous models of intracranial versus intraorbital ON regeneration contributed to the observed differences in RGC axon response to PN grafts. Suture was used to appose the PN and severed ON intraorbitally, but intracranially the PN was not physically tethered to the ON and therefore may not have been as stable or as well positioned.

Method: As first step to address this issue, we have compared fibrin glue versus suture in intraorbital PN-ON repair. In the adult rat the ON was divided intraorbitally, then using autologous peroneal nerve grafts we either sutured the ON or used fibrin glue to appose the nerve to the PN graft. 3-4 weeks after surgery, the retrograde fluorescent tracer fluorogold was injected into the distal end of the PN grafts (at least 10mm distal to the PN-ON apposition site). Two days later rats were perfused (4% paraformaldehyde), the retinas removed, flatmounted on glass slides and the number of regenerating RGCs was counted.

Result: There was no significant difference in number of cells regenerated with suture apposition median 3639 (2871-4266) compared to glue 3704 (3217-5160) although there was a trend to higher numbers in the fibrin glue group.

Conclusion: Apposition of optic and peripheral nerve ends using fibrin glue in the intraorbital ON paradigm gives equivalent results to suture. Thus in the next series of studies fibrin glue apposition can be used to compare RGC axon regeneration after intracranial versus intraorbital PN transplantation.

Refs: (1) Hirsch and Barr, *Adv Exp Med Biol* (1999), 468:199.. (2) Cui et al (1999) *Invest Ophthalmol Vis Sci* 40:760

5. PERIPHERAL NERVE ANATOMY IS ESTABLISHED INDEPENDENTLY OF THE VASCULATURE IN THE UPPER LIMB OF THE EMBRYO

Bates D, Newgreen DF, Taylor GI

Embryology Lab, MCRI, Royal Children's Hospital, Melbourne VIC, 3000. Jack Brockhoff Plastic & Reconstructive Surgery Research Unit, Melbourne VIC, 3000

Introduction: During embryogenesis, blood vessels appear in the upper limb before peripheral nerves¹. An observation that nerves co-distribute with the vasculature is consistent with the vascular elements forming a scaffold along which the nerves track². Our aim was to determine whether normal vascular anatomy was a prerequisite for the development of normal nerve anatomy in the upper limb.

Methods: Cibacron beads were soaked in PBS, hVEGF₁₆₅ or Flt-1/Fc protein. A single bead was then surgically implanted into the right upper limb of a four-day-old (E4) quail embryo. Embryos were allowed to develop for a further 2 days at which time the peripheral nerve and vascular pattern was analysed.

Results: 7 of 13 embryos receiving beads soaked in hVEGF₁₆₅ survived to E6. Five of these were abnormal showing supernumerary blood vessels surrounding the bead. No peripheral nerves deviated from their normal trajectory to travel with the ectopic blood vessels. 7 of 10 embryos receiving beads soaked in Flt-1/Fc survived to E6. Three of these showed focal regions of avascularity in areas where peripheral nerves normally track. Nerve distribution was normal despite the absence of a normal vascular scaffold. 11 of 20 control embryos receiving beads soaked in PBS survived to E6. All eleven embryos demonstrated normal neurovascular anatomy.

Conclusions: Despite changes to the normal vascular anatomy, peripheral nerve anatomy was normal. It appears that in the upper limb, blood vessels do not provide peripheral nerves with guidance cues and are not an essential substrate for nerve outgrowth.

1. Bates, D., Newgreen, D. & Taylor, G. Neurovascular Development in the Forelimb of the Avian Embryo. in *Proceedings of the Australian Neuroscience Society* Vol. 11 (Melbourne Convention Centre, 2000). 2. Spence, S. & Poole, T. Developing blood vessels and associated extracellular matrix as substrates for neural crest migration in Japanese quail, *Coturnix coturnix japonica*. *Int. J. Dev. Biol.* 38, 85-98 (1994).

6. THE ANGIOSOMES OF THE NERVES OF THE LOWER LIMB

Suami H, Taylor GI, Pan WR, Seneviratne S.

Jack Brockhoff Reconstructive Plastic Surgery Research Unit of the Royal Melbourne Hospital, Department of Anatomy and Cell Biology, University of Melbourne

Introduction: The angiosome concept was applied to the nerves of the lower limb in order to delineate the vascular supply of the sciatic and femoral nerves and their branches. The aim of the study is to confirm the nerve vascularization with the clinical relevance to harvesting lower limb nerves as vascularized nerve transfers and to identify nerves at risk of devascularization in compression and trauma.

Material and methods: Two lower limb cadaver specimens injected with a lead oxide and gelatine mixture were meticulously dissected. The vascular supply of the sciatic and femoral nerves from the lumbar and sacral plexus to the terminal nerve branches was documented with reference to the source vessels. The nerves were then tagged with fine electrical wire prior to radiological examination of the specimen in order to delineate both the diameter and the distribution of the nerves. A montage was then constructed of the nerves in their entirety from the spinal cord to the terminal nerves with respect to their vascular supply.

Results: The nerves were supplied segmentally by source vessels which were colour coded in accordance with the angiosome concept. Then each segment was classified into five categories whether both the nerves and the source vessels were branched or unbranched. This work has identified several options for free vascularized nerve transfer.

Conclusion: The study is in progress and further work is under way to confirm these findings and to delineate the nerve supply of the obturator nerve in order to complete the picture of the lower limb nerve vascularization.

7. IMPROVEMENT IN RESPIRATORY SYMPTOMS FOLLOWING LAPAROSCOPIC NISSEN FUNDOPLICATION

Kiroff GK, Brouwer R

Dept of Clinical and Biomedical Science, The University of Melbourne, The Geelong Hospital, PO Box 281, Geelong, Victoria

Introduction: Cough, wheeze and nocturnal aspiration are common and distressing symptoms for patients with gastro-oesophageal reflux disease (GORD). There is now accumulating evidence that these symptoms can be improved with surgical treatment for GORD.

Methods: We report a prospective series of 29 patients (out of a total antireflux surgery group of 102) who presented with predominantly respiratory symptoms. Typical and respiratory symptoms were graded according to a standard scale. All patients had preoperative investigations confirming GORD. These patients were treated by a standard laparoscopic fundoplication and followed up for a minimum 14 months (range 14 to 48 months). Patients were contacted and interviewed by an independent observer.

Results: Conversion to open surgery was necessary in 3 patients. There were 4 significant complications. Ultimately complete control of typical reflux symptoms was achieved in 88%. Cough was completely relieved in 81% and improved in a further 13%. Wheeze and nocturnal bronchospasm was completely relieved in 50% and improved in the balance. No patients developed new respiratory symptoms. Dysphagia remains a significant problem, with only 42% of patients completely free of trouble.

Conclusions: Overall respiratory symptoms were improved in the majority of patients with cough responding somewhat better than wheeze. Careful evaluation of patients remains essential and an algorithm for patient selection will be discussed.

8. A STUDY TO ASSESS GASTRIC LAPAROSCOPIC BANDING AND ITS ROLE IN THE TREATMENT OF MORBID OBESITY

Chehata A, Semple C, Wilkinson S

Department of Surgery, Royal Hobart Hospital, Liverpool Street, Hobart, Tasmania 7000

Introduction: Obesity is the commonest chronic health problem in Australia with approximately 15% of males and females being obese. It is an increasing problem in the Western world. We have reviewed our initial experience in the treatment of this condition by laparoscopic gastric banding

Methods: From September 1998 to March 2001, 110 patients underwent Laparoscopic gastric banding for severe obesity; 100 female, 10 male, with a mean age of 41 years (range 16-61). The mean preoperative weight was 122kgs (range 83-170) and the mean body mass index (BMI) was 45.5 kg/m² (range 34.3 - 65.8). 108 patients from 110 were able to be contacted by phone and questioned using a standard questionnaire to obtain information regarding previous weight loss techniques and patient satisfaction 2 patients were not in Australia at the time of the study.

Results: There were no postoperative deaths. Subjective patient satisfaction was rated as 9.2 out of 10 with 105 patients happy with their post operative results. 1 patient was unhappy and 2 had no response

Months post op	Weight	BMI
0	122	45.5
6	106	39.8
12	97	36.4
18	88	34.0
24	87	32.4

Post operative complications included: vomiting 58%, dysphagia 35%, nausea 27%, infection 4%, oral antibiotics 3%, IV antibiotics 1%, pulmonary embolus 1%, DVT 1%. Re-operation occurred in 23 patients 21%, 13 slipped gastric band 14%, 5 port change 5%, 4 reversals 4%.

Conclusions: Laparoscopic gastric banding has an excellent safety profile with tolerable side effects and with significant weight reduction (20.5% at one year). This represents the least invasive surgical treatment of morbid obesity.

9. IMPROVED METABOLIC CONTROL DOES NOT PREVENT RAISED INTRACRANIAL PRESSURE IN A LIVER BIODIALYSIS SYSTEM TESTED IN A PORCINE HEPATIC FAILURE MODEL

Stewart GJ¹, Mears DC¹, Bourne R², Sheil AGR¹

¹Department of Transplantation Surgery, ²Institute for Magnetic Resonance Research Blackburn Building, University of Sydney, 2006, NSW

A practical liver support system for fulminant hepatic failure (FHF) remains a needed therapeutic modality. An extracorporeal liver support system comprising a hepatocyte bioreactor containing porcine hepatocytes and a dialysis circuit was studied in a porcine ischaemic hepatic failure model. Liver devascularisation was achieved by means of an end-to-side porto-caval shunt and ligation of the arterial inflow (n=11). Blood pressure, heart rate and intra-cranial pressure (ICP), were recorded for 12 hours from devascularisation. Serial blood samples were collected for determination of the animal's progressing metabolic status. In some animals, Magnetic Resonance Spectroscopy (MRS) has been performed on plasma, CSF and brain biopsies to identify factors responsible for hepatic encephalopathy. Plasma circulated through the biodialysis system containing hepatocytes resulted in significantly less metabolic disturbance than in a group of historical controls that dialysed with no hepatocytes (n=8). The accumulation of ammonia (\pm SD) was (238 \pm 100) to (582 \pm 283) over 8 hours of treatment compared with (438 \pm 168) increasing to (910 \pm 583) for the control. Lactate also increased from (5.0 \pm 1.8) to (6.7 \pm 4.8) compared with (7.7 \pm 5.7) to (11.7 \pm 7.8).

However, all animals developed raised ICP (24 \pm 6.3 to 40 \pm 9 mmHg) and a decrease in calculated cerebral perfusion pressure (51.5 \pm 21.5 to 12 \pm 12 mmHg). This occurred regardless of the less severe progression of metabolic disturbance in the biodialysed group. This mirrors the often-fatal clinical development of hepatic encephalopathy in FHF patients. MRS results suggest tissue components that may correspond to this outcome.

10. DO PANCREATIC BLOOD VESSELS MATTER IN ACUTE PANCREATITIS?

Brooke-Smith ME, Carati C, Toouli J, Saccone G

Department of General and Digestive Surgery, Flinders Medical Centre, Adelaide. South Australia

Introduction: Decreased perfusion of the pancreas is important in the progression from mild to necrotising pancreatitis. The location of necrosis is limited to one part of the human pancreas seems to occur most commonly in the tail. The pattern of vascular anatomy is one factor that influences perfusion and the sites of selective pancreatic damage.

Aim: To provide an anatomical basis for perfusion measurement in a possum model of acute pancreatitis.

Methods: Initial dissections localised the major arteries to the pancreas in anaesthetised Australian Brush-tailed possums. For the vascular casting studies of the pancreas microfil[®] (a siliconised rubber) was perfused into the possum pancreatic circulation. After processing, a dissecting microscope was used to examine the specimens.

Results: From dissection of three animals and the vascular casts of another three animals, the arterial supply and venous drainage of the pancreas was found to be fundamentally similar to that of the human. Additionally the casts showed that in the proximal tail and body there were lobes with end arteries. The lobes were divided into lobules with each lobule appearing to be supplied by one arteriole and drained by one vein. Some of the arterioles traversed a long straight course in close proximity.

Conclusions: Necrosis in acute pancreatitis is more likely to develop in areas of the pancreas containing end arterioles than areas with extensive anastomotic connections. Therefore when designing experiments to examine alterations in perfusion in pancreatitis, these are the areas which deserve the greatest attention.

11. EXPRESSION AND OVEREXPRESSION OF HEAT SHOCK PROTEIN-70 (HSP70) BY DENDRITIC CELLS IN THE ARTERIAL INTIMA AND ITS POTENTIAL SIGNIFICANCE IN ATHEROGENESIS

Bobryshev YV, Lord RSA

Surgical Professorial Unit, St. Vincent's Hospital, Darlinghurst NSW 2010

Introduction: Cellular overexpression of heat shock protein-70 (HSP70) is one of the early physiological events indicating the destabilisation of the tissue microenvironment. We examined the characteristics of HSP70 expression in early and advanced atherosclerotic lesions.

Methods: Twenty-six carotid artery and 16 aortic specimens obtained at endarterectomy and aortic reconstruction were examined using immunohistochemical techniques. The nature of cells expressing HSP70 was studied in consecutive sections double stained with antibodies to HSP70 and cell type specific markers including CD3 (T-cells), CD68 (macrophages), CD1a and fascin (dendritic cells), von Willibrand factor (endothelial cells) and α -smooth muscle actin (smooth muscle cells). Staining with HLA-DR and CD1d was used to identify cells involved in antigen presentation.

Results: In advanced atherosclerotic lesions, several cell types including macrophages, dendritic cells and smooth muscle cells overexpressed HSP70. In contrast, in early atherosclerotic lesions only dendritic cells overexpressed HSP70. Dendritic cells expressing HSP70 frequently contacted T-cells and co-expressed HLA-DR. Furthermore, dendritic cells clustering with T-cells expressed CD1d, a unique molecule responsible for presenting lipid antigens.

Conclusion: Dendritic cells are involved in the very early phases of atherogenesis. Direct contacts between activating dendritic cells expressing HSP70 and T-cells might be important in T-cell activation and might facilitate the presentation of lipid antigens to T-cells directly within the arterial wall.

12. EXCITATORY AND INHIBITORY NEUROMUSCULAR TRANSMISSION IN THE COLONIC CIRCULAR MUSCLE IN CHRONIC IDIOPATHIC CONSTIPATION/SLOW COLONIC TRANSIT

Stanton MP¹, Hengel P¹, Bornstein JC⁵, Hutson JM¹, Chow CW², Cook D³, Southwell BR⁴

Departments of Surgical Research¹, Pathology² Radiology³ and Gastroenterology⁴, Murdoch Children's Research Institute, Royal Children's Hospital, Parkville 3052. Department of Enteric Neuroscience⁵, University of Melbourne, Parkville, Victoria, Australia.

Introduction: Electrophysiological responses were measured in transverse colonic circular muscle (CCM) from severely constipated children, to analyse neurotransmission.

Method: Children with scintigraphic slow colonic transit (SCT) underwent laparoscopic seromuscular colonic biopsy (ethics approved). Previously we found deficient substance P (tachykinin) immunoreactivity (SP-IR) in approximately 100/200 idiopathic constipation children. 'Control' transverse colon came from adult colectomies. Isotonic contractile responses to electrical field stimulation (EFS) +/- hyoscine (muscarinic antagonist), SR48968 (tachykinin NK2-receptor antagonist) and NOLA (nitric oxide synthase inhibitor) were recorded. EFS was re-tested in tetrodotoxin to ensure neurogenic responses.

Results: EFS responses were similar in 29 SCT children and 9 controls (25% vs 28% of maximal cholinomimetic response), and reduced in hyoscine (to 16%, $p < 0.0001$ vs 13%, $p < 0.05$). 11 children with reduced SP-IR, had larger responses to EFS alone, than 18 normal SP-IR children (39% vs 23%, $p < 0.02$). In hyoscine, reduced SP-IR children had lower responses (by 15% vs 7%, $p < 0.02$). In SR48968, EFS responses were 0.5% lower in 21 children, and 1.7% greater in 4 adults. Reduced and normal SP-IR children were similar. NOLA increased EFS contractile responses equally in adults and SCT children (by 11%).

Conclusions: Cholinergic excitation is maintained in SCT children CCM. In reduced SP-IR, a larger component of EFS-induced contraction is acetylcholine-mediated. NK2-receptor blockade produces only small changes in SCT and control EFS responses. Blocking nitric oxide inhibition increases EFS-induced contraction equally in adults and SCT children. These results suggest altered neuronal stimulation, with increased cholinergic excitation/contraction in SP-deficient SCT children, while nitric oxide inhibition/relaxation appears unaffected.

13. BIOMOLECULAR FACTORS IN OSTEOPOROTIC RAT FRACTURE HEALING

Yu Y; Gifford KJ; Low AKW; Walsh WR

Orthopaedic Research Laboratories Level 2, South Wing, Edmund Blackett Building, Prince of Wales Hospital, University of New South Wales, High St, Randwick. NSW. 2031

Introduction: Fracture healing is known to be delayed in osteoporosis. Bone morphogenetic proteins (BMPs), transforming growth factor beta (TGF- β) and their signal transducers, Smads, insulin-like growth factor I (IGF-I) and matrix metalloproteinases (MMPs) play important roles in normal rat fracture healing. However, their function in the oestrogen deficient rat fracture healing has not been reported.

Methods: Forty-eight 3-month old female Sprague-Dawley rats were used. Half underwent ovariectomy (OVX) and the other half sham surgery (Sham). A closed fracture of the right femur was created 10 weeks later. Animals were killed in groups of 6 at 1, 2, 4 and 6 weeks post-fracture. Bone mineral density (BMD) and the expression and distribution of BMP-2, 7, TGF- β , Smad1-7, IGF-I, MMP-1 (collagenase) and 3 (stromelysin-1) was evaluated.

Results: No differences in BMD within the callus of the two groups was noted. TGF- β , BMPs and Smads showed elevated but similar patterns of expression in the Sham and OVX groups. IGF-I expression however, was down regulated in the OVX groups throughout. MMP-3 expression was up regulated in the OVX groups at early time points (1 and 2 weeks).

Discussion: Fracture healing is impaired in the estrogen deficient state though the mechanism(s) have yet to be reported. The data from the current study suggests that the down-regulation of IGF-I in the estrogen deficient state might influence MMP-3 expression. Over expression of MMP-3 may favour degradation of the matrix over formation of new bone and enhance bone resorption⁽¹⁾. The results provide a possible molecular basis for the impaired or delayed healing observed in the estrogen deficient state.

Reference: (1) Breckon JJW, et al, JBMR (1999) 14(11):1880-1890.

14. IN-VITRO BIOMECHANICAL COMPARISON OF LAGGED AND NON-LAGGED SCREW FIXATION OF OBLIQUE METACARPAL FRACTURES

Nicklin S, Ingram S, Ho S, Gianoutsos MP, Walsh WR

Orthopaedic Research Lab, UNSW, Prince of Wales Hospital, Randwick, NSW, 2031

Introduction: Although a variety of fixation techniques have been reported for fixation of oblique or spiral metacarpal fractures, lag screw fixation has been reported to be the most biomechanically stable construct. Lag screws are inserted following over-drilling of the proximal cortex, which provides compression at the fracture site. We believe the compression provided by the Leibinger Bow system makes over-drilling unnecessary.

Methods: Twenty fresh frozen cadaveric metacarpal bones (index, ring and middle) were utilized. Bones were cleared of soft tissue and the proximal ends embedded in Woods metal using a Teflon mould. Long oblique osteotomies were performed with a fine oscillating saw. Bones were randomly allocated to lagged and non-lagged groups. All bones were held in the Leibinger Bow and fixed with 2 screws at right angles across the fracture site. The proximal cortex of the lagged specimens was over-drilled and the non-lagged specimens were not. Bones underwent cantilevered bending to failure in a mechanical testing machine. Axial stress was calculated from load to failure and the moment of inertia for each specimen.

Results: All specimens failed through the proximal screw. Although there was a trend for the non-lagged specimens to be stronger ANOVA statistical analysis revealed no significant difference in axial stress between groups.

Discussion: Minute errors during over-drilling of the proximal cortex can easily lead to inadequate fixation. This data suggests that use of the Leibinger Bow System may eliminate the need for this over-drilling. This not only shortens the procedure, but also reduces the chance of error leading to poor fixation.

15. CYCLIC TESTING OF FLEXOR TENDON REPAIRS

Nicklin S, Matheson G, Chircop M, Gianoutsos MP, Walsh WR

Orthopaedic Research Lab, UNSW, Prince of Wales Hospital, Randwick, NSW

Introduction. New flexor tendon repair techniques have been proposed to withstand the increased loads of active mobilisation. Most reports on biomechanics of tendon repair are based on static testing. Cyclic testing more closely replicates the clinical situation and leads to gap formation at lower loads than static testing. This study examines 3 types of tendon repair using a new cyclic testing protocol.

Methods: 30 fresh frozen cadaveric tendons were randomly assigned to 3 groups, Kessler repair with simple or cross-stitch epitendinous suture or Savage repair with simple epitendinous suture. All repairs were performed in-situ in Verdan's zone 2. Samples underwent tensile cyclic testing in a saline bath at a rate of 0.1Hz. Each specimen was subjected to 2 phases of testing replicating passive and active motion. Gap formation, stiffness and mode of failure were recorded.

Results: The Savage repairs were stiffer and more resistant to gap formation than Kessler repairs. The simple epitendinous suture seemed to be more resistant to gap formation than the cross-stitch suture although there was no significant difference in ultimate strength.

Discussion: Cyclic testing is a more rigorous testing protocol that more closely replicates the clinical situation. This study shows that some repairs form significant gaps at lower loads than the reported ultimate load to failure seen with static testing. Although cyclic testing has its limitations, we believe it is essential to fully assess tendon repair techniques, especially those considered for active mobilisation post-operatively. This study suggests the Savage repair may be a better option for active mobilisation protocols.

16. THE INHIBITION OF PERITENDINOUS ADHESIONS USING A COLLAGEN SYNTHESIS INHIBITOR

McCombe D¹, Ruangpanit N², Kubicki M³, Guenzler V⁴, Williams J³,
Thompson EW², Morrison WA¹

¹Bernard O'Brien Institute of Microsurgery, St. Vincent's Hospital, Melbourne
²St. Vincent's Institute of Medical Research, St. Vincent's Hospital, Melbourne
³Department of Mechanical and Manufacturing Engineering, University of Melbourne, Melbourne
⁴Fibrogen Inc., South San Francisco, United States of America

Introduction: The inhibition of the formation of peritendinous adhesions has been attempted with numerous pharmaceutical and physical means with little success either because of limited efficacy, toxicity or the lack of specificity and the consequent impairment of wound and tendon healing. The aim of this study is to demonstrate a specific inhibitor of collagen synthesis is effective in minimising the biomechanical effect of adhesions in an animal model of tendon adhesion formation.

Method: In vitro analysis of the collagen synthesis inhibitor's effect on the individual fibroblast cell populations of the flexor tendon, synovial sheath and dermis was performed.

In vivo, a model of Zone II flexor tendon adhesion was established in a rat hind foot with analysis of the work of flexion used to indicate the effect of the drug. Treatment groups received the drug by oral gavage, twice daily for 2-6 weeks with analysis at either 2 or 6 weeks.

Assessments of dermal collagen formation and cutaneous wound healing were also made in the rats by biochemical, biomechanical and histological assays.

Results: In vitro, collagen synthesis was inhibited in all fibroblast cultures. Inhibition of fibroblast proliferation was also seen albeit at higher concentrations. In vivo, the additional work attributed to adhesion, was reduced by 70% in the treatment groups. The cutaneous wounds healed at an equivalent rate in both the control and treatment groups, however, the dermal collagen appeared reduced in the treatment groups.

Discussion: The collagen synthesis inhibitor studied appears to be effective in vivo in reducing the biomechanical effect of adhesions formed in response to tendon injury. The drug is not however, specific for the fibroblasts of the sheath and hence other methods of localising the effect of the drug to the fibroblasts of the fibroosseous sheath will have to be developed.

17. A PILOT STUDY ON THE DEVELOPMENT OF INTRANASAL ADHESIONS IN SHEEP

McIntosh D, Wormald PJ

Department of Otolaryngology, Queen Elizabeth Hospital, Woodville Road, Woodville, South Australia, 5011.

Introduction: Endoscopic sinus surgery (ESS) is becoming one of the most common operations performed by ENT surgeons, accounting for up to 50% of all procedures performed. Intranasal adhesions are the most common complication following ESS, affecting between 6-30% of cases. To date there has been no investigations into the pathogenesis of intranasal adhesions. We have developed the sheep as a suitable animal model for producing intranasal adhesions. This should allow for further research into the development and prevention of intranasal adhesions.

Method: A review of the literature shows that animals used for sinus surgery includes dogs, rabbits, and sheep. Dogs were considered an inappropriate model due to lack of availability and the rabbit is not suitable for transnasal endoscopic surgery. Hence, the sheep was trialed as the most suitable candidate. A middle turbinectomy was performed. This allows for greater access to the sheep's nasal cavity. Using a sickle knife, apposing mucosal injuries were made. Post-operative endoscopy was then performed.

Results: Post-operative endoscopy demonstrated the development of adhesions between the two mucosal surfaces.

Conclusion: Sheep are a suitable model for endoscopic sinus surgery. The use of sheep for research into pathological processes that commonly affect humans is possible. The development of an animal model of intranasal adhesion formation should allow for greater understanding into the development of adhesions.

18. THE HEALING OF THE NASAL MUCOSA IN SHEEP. DOES NASAL PACKING MAKE A DIFFERENCE?

McIntosh D¹, Cowin A², Adams D², Wormald PJ¹

¹Department of Otolaryngology, Queen Elizabeth Hospital, Woodville Road, Woodville, South Australia, 5011. ²Child Health Research Institute, Women's and Children's Hospital, Adelaide, South Australia 5000.

Introduction: Endoscopic sinus surgery has advanced the management of mucosal disorders of the nose and paranasal sinuses. With the precision that current technology allows, diseased mucosa can be removed with minimal loss of normal tissue. However, despite this accuracy, it is still necessary in some instances to remove the full thickness of the nasal mucosa. The implications for this are that bare areas remain. This may result in mucus stasis and crusting. There are a growing number of products that are claimed to promote tissue healing but these have not been assessed in a scientific manner. This study aims to examine the effect of different nasal packing materials in a controlled manner using a sheep animal model.

Methods: Full thickness injuries were made on both lateral nasal walls in sheep. One side was left unpacked as a control and the other side was packed with either Merocel ® or Merogel ®. Serial biopsies were then taken to chart the progress of tissue healing. These biopsies were studied using both light and electron microscopy.

Results: Preliminary results evaluating epithelial height and cilia return for both types of nasal packing showed improved healing rates compared to controls, with the Merogel ® treatment group healing better than the Merocel ® treated group.

Conclusion: Nasal packing is beneficial in post-operative healing with Merogel ® showing more promise than Merocel ®.

19. CD44v6 IS A KEY REGULATOR OF CD44v-MEDIATED METASTATIC BEHAVIOUR

Barbour A^{1,3*}, Reeder J¹, Antali T^{1,2}, Walsh M², Fawcett J^{1,3}, Gotley D^{1,3}

¹Queensland Institute of Medical Research, 300 Herston Rd, Herston, 4029 ²Department of Pathology, University of Queensland, Herston Rd, Herston, 4029 ³Department of Surgery, Princess Alexandra Hospital, Ipswich Rd, Woolloongabba, 4102

Introduction: CD44 is the principal cellular receptor for hyaluronic acid (HA). Alternative splicing of 10 exons, termed exons v1-v10, generates several variant isoforms, termed CD44v. CD44v promote epithelial tumour progression and metastasis by mechanisms that remain poorly understood. CD44v6 containing isoforms may form molecular aggregates on the cell surface that promote CD44-mediated cell adhesion or heparin-binding growth factor presentation by CD44v3 *in vitro*. We have reported that CD44v2-10-growth factor presentation confers a hyaluronan-binding independent metastatic phenotype on a human hepatocellular carcinoma (HCC) cell-line *in vivo*. However, the molecular mechanisms by which tumour cells utilize CD44v6 to facilitate metastasis remain unclear.

Methods: A mutant CD44v2-10 cDNA, with CD44v6 deleted, was generated (designated CD44Δv6). The CD44v2-10 and CD44Δv6 cDNAs were transfected into SKHep1 cells, a non-metastatic human HCC cell-line that does not express CD44v.

Results: SCID mice inoculated subcutaneously with SKHep1 wild type, vector alone controls and transfectants overexpressing CD44v2-10 all developed large primary tumours after 10 weeks. In addition, CD44v2-10 transfectant-derived primary tumours gave rise to pulmonary metastases in 50% of animals (p<0.05). In contrast, CD44Δv6 transfectants demonstrated a statistically significant reduction in primary tumour growth, compared with control cell lines, that was associated with the loss of metastatic propensity. *In vitro*, CD44Δv6 transfectants showed a reduction in VEGF binding and an increase in apoptosis under confluent tissue culture conditions in the presence of HA.

Conclusions: These findings demonstrate that v6 is vital for the metastatic phenotype conferred by CD44v2-10 and strategies that disrupt CD44v6 function may translate to effective anti-metastatic therapies in the clinic.

20. VACCINATION OF STAGE III MELANOMA AND OVARIAN CANCER PATIENTS USING IRRADIATED AND HAPTENISED AUTOLOGOUS WHOLE TUMOUR CELLS

Dyer SL*, Berd D[†]

*AVAX Australia Pty Ltd, Suite 1, Level 4, 1-5 Railway St, Chatswood NSW 2057

[†] Thomas Jefferson University, 11th & Walnut Streets, Philadelphia, 19107, PA, USA

Introduction: Autologous whole tumor cells irradiated and haptenised with dinitrophenyl (DNP) have been used successfully to improve overall survival and relapse free survival in over 400 Stage III patients with melanoma and a small number of patients with ovarian carcinoma.

Method and Result: In melanoma, a non-randomized, open Phase II trial in the USA enrolled 214 patients with bulky regional lymph node metastases (1). Twenty of these patients had in-transit metastases as well and 40 had clinically evident metastases to two nodal sites. With a median follow up of 4.4 years (1.8-10.4 years) the 5 year overall survival (OS) rate is 47% (one nodal site=51%, two nodal sites=33%). As expected, extent of nodal involvement was a strong predictor of 5 year OS (mass only = 63%, mass plus one or two microscopically (+) nodes=44%, mass plus three or more microscopically (+) nodes =32%, P=0.001). Moreover, the induction of delayed-type hypersensitivity (DTH) to unmodified autologous tumor cells was associated with significantly longer survival (DTH> 5 mm =69%, DTH< 5mm = 33%, P=<0.001). That this effect was tumour-antigen-specific is suggested by the observation that DTH to neither DNP-modified autologous tumour cells nor PPD affected outcome (P=0.920, 0.242, respectively).

Conclusion: This technology has significant benefit to Stage III bulky melanoma patients in the adjuvant setting and is currently available as a treatment option in Australia as M-Vax™. Moreover, this technology is applicable to other cancers. Trials in advanced ovarian cancer patients are planned to commence in Australia shortly.

(1) Berd, D. Vaccine 19 (2001) 2565-2570

21. ENCAPSULATION OF HEPATOCELLULAR CARCINOMA: RELATIONSHIP TO COLLAGEN IN TUMOUR STROMA AND ADJACENT PARENCHYMA

Lockwood DSR, Yeadon T, Clouston AD, Callaghan S, Gotley DC

Department of Surgery, University of Qld, Princess Alexandra Hospital, Ipswich Rd, Woolloongabba, Qld 4102

Introduction: Hepatocellular carcinomas (HCC) vary in their ability to invade host liver. At the less invasive end of this spectrum are encapsulated HCC. This capsule may represent compressed parenchyma, or extracellular matrix (ECM) synthesised under the influence of tumour-secreted factors. The influence of ECM in adjacent liver and tumour on encapsulation is unknown. We chose four subtypes of collagen, a central ECM component, to study ECM near the growth margin, and its influence on tumour invasiveness.

Methods: Encapsulated or invasive tumours in cirrhotic and non-cirrhotic livers were selected, and data collected on Scheuer fibrosis stage and presence of vascular invasion. Immunohistochemistry for collagen types I, III, IV and VI was performed on specimens incorporating peripheral tumour, growth margin, and adjacent liver. Staining intensity was graded in adjacent parenchymal sinusoids and tumour stroma, and analysed against extent and thickness of encapsulation.

Results: Fibrillar collagen predominated in capsule, while types IV and VI were found mostly within tumour. Encapsulation was associated with decreased collagen I staining within tumour and adjacent parenchymal sinusoids, decreased tumour septum formation, and reduced vascular invasion. Capsule thickness was significantly increased in larger tumours, but not cirrhotic parenchyma. Peritumoural sinusoidal collagen did not reflect underlying parenchymal disease.

Conclusions: Increased collagen in peritumoural sinusoids does not favour encapsulation, and may in fact promote invasion. The behaviour of capsular stromal cells does not parallel their counterparts within tumour and nearby parenchyma. This could reflect their contrasting cell-cell and cell-matrix interactions.

22. A COMPARISON OF RADIOFREQUENCY ABLATION, CRYOABLATION, DIATHERMY AND ARGON BEAM COAGULATION FOR THE LOCAL TREATMENT OF THE LIVER RESECTION EDGE

Gananadha S, Zhao J, Morris DL

UNSW, Department of Surgery, St. George Hospital, Gray Street, Sydney NSW 2217, Australia

Introduction: Surgical margin is the only technical variable that has an impact on long term outcome after liver resection. Edge cryotherapy has been used with reduced local recurrence rates. There is currently no data on the use of other agents, in this study we compared radiofrequency (RFA), cryotherapy, diathermy and argon beam for the local treatment of liver resection edge.

Methods: A total of 68 ablations were produced on the surface of ex vivo sheep liver using the various modalities. Cryoablations were produced using both the Cryotech (5mm trocar and paddle probes) and the Erbe (3.5mm trocar and paddle) probes. Radiofrequency ablations were produced using a custom made surface application probe and the RITA 1500 generator. Conmed 7500 system was used to produce diathermy and argon beam coagulation.

Results: Argon beam and the diathermy resulted in ablation to maximum depth of 3.5 mm with end-point and spray modes at various power setting. RF ablation resulted in consistent ablations the diameter of which varied in a linear manner to the time of RF application and the depth of the ablations with the length of electrode deployment. Cryotherapy was as effective as RFA with both the cryotherapy systems but the Erbe trocar probe resulted in a deeper ablation whereas the cryotech paddle probe resulted in a larger ablation.

Conclusions: RF and cryotherapy are equally effective as liver edge ablation device. Diathermy and Argon are considered less effective. Cryotherapy requires expensive complex equipment which at least with liquid nitrogen systems requires to be prepared for use and this may not be available if the need for edge treatment during resection was unplanned.

23. ANALYSIS OF THE BREAST CANCER PROTEOME AND PROTEIN CHARACTERIZATION OF THE DISEASE STATE

Bhatia KD, Lord R, Stanton PD

Discipline of Surgery, University of Tasmania, GPO Box 252-28, Hobart 7001

Introduction: In essence, this project seeks to look at the differences in the levels of known biological markers of breast cancer between different population groups such as Australia, Japan and Taiwan. The latter two nations have a much lower incidence of the disease and it has been suggested that environmental and/or dietary factors may account for this observation but this is unsubstantiated. The study uses a molecular separation method that resolves complex protein mixtures into individual protein molecules. Using this method a biological map of all the protein molecules that make up breast cancer is constructed and compared to similar maps of normal breast tissue. The differences found may represent proteins generated as a result of malignant change.

Method: Patients who have been diagnosed with breast cancer, primary or metastatic are invited to participate in the study. Patients undergoing breast reduction surgery are also invited to participate so as to provide normal breast tissue for comparison. The technique of 2D gel electrophoresis (proteomics) shall then be employed to resolve complex protein mixtures in terms of iso-electric focusing and size. The differences in the biological map including unique proteins or differential expression of known proteins will be identified using mass spectrometry in collaboration with the Australian Proteome Analysis Facility, Macquarie University.

Results: The qualitative and quantitative definition of known markers of breast cancer such as HER-2, Cathepsin G, p53 product, Ki-67, the oestrogen receptor, etc, will be studied. Identification of potential diagnostic markers or treatment targets is also likely due to the large number of proteins analysed. The study is currently ongoing and is hoped to reach completion by December 2001.

24. TUMOUR-ASSOCIATED HEPATIC STELLATE CELLS (HSC) ARE DERIVED BY PROLIFERATION – EVIDENCE FOR AN AUTOCRINE MITOGENIC LOOP IN HEPATOCELLULAR CANCER (HCC)

Yeadon T¹, Fawcett J¹, Crawford D², Lockwood D¹, Callaghan S¹, Ooi LLP³, Gotley D¹

¹University of Queensland Department of Surgery and ²Department of Gastroenterology and Hepatology, Princess Alexandra Hospital, Brisbane. ³National Cancer Centre, Singapore General Hospital, Singapore

Background: Hepatocellular cancer (HCC) is one of the commonest solid organ tumours and has a poor prognosis. In about half of these tumours, a fibrous capsule is present which tends to confer a better prognosis. We have previously demonstrated that activated hepatic stellate cells (HSC) are responsible for capsular collagen. The aim of this study was to determine whether these cells are derived from migration or local proliferation and the likely stimulus for their increased numbers.

Materials and Methods: We have developed an animal model that mimics the morphology of human encapsulated HCC. We undertook a time course experiment using 35 Buffalo rats in which rat HCC cells were inoculated into the liver. Two rats were sacrificed at 2 day intervals and livers harvested. One hour prior to sacrifice the rats were anaesthetised and BromodeoxyUridine (BrdU), a marker for DNA synthesis, was administered. Sections of rat liver were studied by immunohistochemistry for BrdU incorporation, and expression of platelet-derived growth factor (PDGF) and PDGF receptor protein. Parallel sections were stained for activated HSC using an anti- α smooth muscle actin antibody. The cellular source of PDGF mRNA expression was identified by *in situ* hybridisation.

Result: Activated HSC were seen at the growth margin of the HCC inoculum from day 2. Capsular collagen was apparent from day 10, and abundant HSC were associated with this process. Extensive BrdU staining of capsular HSC was seen during capsular development. PDGF protein was expressed in the tumour capsule, and not within the tumour or hepatocytes. PDGF mRNA was co-localised to activated HSC only. PDGF receptor was expressed solely on the cell surface of the HSC.

Conclusion: The abundant capsular HSC are likely derived from HSC proliferation rather than migration. PDGF is likely to be an important mitogen, acting via an autocrine loop in HSC.

25. ANGIOGENESIS IN HAEMANGIOMAS IS INHIBITED BY MAST CELL STABILIZERS IN VITRO

Donato RR, Penington AJ, Keramidaris E, Romeo R, Morrison WA

Bernard O'Brien Institute of Microsurgery, St Vincent's Hospital, Fitzroy

Haemangiomas are localised tumors of blood vessels often appearing in infancy. While their pathogenesis is not completely clear, they are composed of proliferating endothelial cells with the other major cell types including mast cells, macrophages, plasma cells and pericytes. The natural history of haemangiomas is characterised by a period of proliferation lasting around twelve months followed by slow involution over the ensuing years.

Under normal physiological conditions, the microvasculature is held in a quiescent state while in haemangiomas there are factors acting on the endothelial cells to proliferate and initiate angiogenesis. Haemangiomas, therefore, lend themselves as an ideal model to study angiogenesis. Mast cells have been implicated in mediating angiogenesis in various experimental models and their high population in haemangiomas suggests a similar influence. Mast cell stabilizers inhibit degranulation and release of angiogenic factors. To this end, we have studied the effects of mast cell stabilizers on human haemangioma explants.

Using an *in vitro* culture system, human haemangioma biopsies were placed in fibrin gel and exposed to various concentrations of FCS and mast cell stabilizers. We found that the amount of vessel sprouting demonstrated a dose dependent response to 0, 2 and 10% FCS over a fourteen day period. In contrast, there was also a dose dependent decrease in those treated with the mast cell stabilizer Iodoxamide. Similar effects were seen with other mast cell stabilizers.

These preliminary results show that *in vitro* angiogenesis is inhibited by mast cell stabilizers.

26. WESTRAN FETAL PIG PANCREAS FRAGMENT TRANSPLANTS SURVIVE AND FUNCTION LONG TERM

Hawthorne WJ, Walters S, Patel A, Chapman JR, Allen RDM, O'Connell P

The National Pancreas Transplant Unit, University of Sydney, Westmead Hospital, Westmead, NSW 2145.

Introduction: Transplantation of fetal porcine pancreas fragments (FPPF) have not yet been shown to survive & provide function long-term as a means to treat diabetes. Our aims were to evaluate if FPPF from Westran (WTP) and outbred pigs transplanted into Westran pigs could survive and function long-term.

Method: Foetuses were removed at 70-90 days gestation and FPPF were prepared. FPPF were transplanted beneath the splenic and kidney capsule of WTP's. Wedge biopsies were taken on days 3, 7, 10, 14, 21, 28 & 60. At either 90 or 120 days an IVGTT was performed via selective splenic artery and vein sampling both before and after the islet graft had been removed.

Result: 118 foetal donors were used to transplant 31 WTP's. All third party FPPF grafts were rejected within 14 days. Macroscopic examination of WTP grafts in WTP demonstrated an increase in transplanted FPPF tissue volume with a greater increase seen under the kidney capsule. Histological analysis of all 25 WTP with WTP grafts showed that by day 60 FPPF stained strongly positive for keratin, PAS, insulin, somatostatin and chromogranin with discrete endocrine nest formation. Graft function at 120 days was shown by a prompt rise in serum insulin levels following an IVGTT. After removal of the transplanted islet tissue, no insulin was released after IVGTT.

Conclusion: Westran FPPF transplanted into WTP were accepted whilst outbred pig FPPF were rejected. These results suggest we have a model suitable for studying the efficacy of FPPF as a method for correcting diabetes.

27. PANCREATIC ISLET CELL TRANSPLANTS CAN BE HYPERACUTELY REJECTED

Hawthorne WJ, Horton PJ, Patel AT, Walters SN, Allen RDM.

The National Pancreas Transplant Unit, University of Sydney, Westmead Hospital, Westmead, NSW, 2145

Aim: There is dispute over the role of hyperacute rejection (HAR) of pancreatic islet cell transplants (PIC). Our aim was to biopsy PIC grafts at the relevant times and determine the mechanism of HAR in a pre-sensitised canine model.

Methods: Six outbred dogs were sensitised to their donor by intraperitoneal inoculation of homogenised lymph nodes on 3 occasions at fortnightly intervals before PIC allotransplantation into subcapsular sites in the spleen and liver. Recipients underwent hemi-pancreatectomy and PIC autotransplantation as a control. Wedge biopsies were taken from both allo and auto sites of both liver & spleen at 10, 30, 60, 90, 120, 180, 240, 300mins; 12 and 24hrs; 3 and 7 days post transplantation.

Results: All recipients developed high titre (>1:100) anti-donor lymphocytotoxic antibodies prior to PIC transplantation. Histopathology and immunohistochemical staining of both liver and splenic biopsies demonstrated viable PIC autografts, with identifiable islets staining for insulin at all time points. PIC allografts developed a cellular infiltrate within 90 minutes and were difficult to identify beyond 24 hours. Both liver and splenic biopsies contained foci of intense polymorphonuclear leukocyte infiltrate and wide spread islet cell destruction.

In conclusion: Hyperacute rejection may be modified by a lack of vascularisation at the time of transplantation but leads to graft destruction within 24hrs. This study supports the use of routine lymphocytotoxic cross-match tests for recipients of islet cell transplants and implies that particular care is required when transplanting islets after previous grafts or in patients with a positive crossmatch.

28. NITRIC OXIDE (NO) PRODUCTION FOLLOWING LIVER TRANSPLANTATION

Tassell B, Liew I, Goto S, Lord R

Discipline of Surgery, Clinical School University of Tasmania, Hobart, Tasmania 7001

Introduction: Despite an allogeneic barrier between the inbred strains of rats DA and PVG, when orthotopic liver transplant (OLT) is performed self tolerance is induced and the rejection episode appears to be self limiting. The aim of this study was to determine the presence of inducible nitric oxide synthase (iNOS) and Heme oxygenase-1 (HO-1) during the rejection episode following OLT in the DA to PVG model.

Methods: OLT was performed using the allogeneic model DA to PVG, the rejector combination DA to LEW and the syngeneic combination DA to DA. The presence of iNOS and HO-1 in the donor liver and recipient spleen was characterised using H&E staining and immunoblotting.

Results: Following OLT from DA to PVG the presence of iNOS and HO-1 in the donor liver and recipient spleen peaked at days 7 and 14. However, by Day 40 this upregulation resolved. In the syngeneic combination no HO-1 was detected at any time point following OLT in either the donor liver or the recipient spleen. The rejector combination showed high production in the donor liver of HO-1 on days 7 and 10, however in the recipient spleen HO-1 was detected on day 7 but was upregulated more by day 10 following OLT.

Conclusion: Peak production of iNOS on days 7 and 14 is consistent with the rejection episode seen in the rejector combination (DA to LEW). However, unlike the rejector combination, the upregulation of these proteins is not seen on day 40. The presence of iNOS alone does not indicate whether NO is present. The upregulation of HO-1 on these days does show that NO is present at a critical level as its concentration regulates the synthesis of this protein. These results may indicate that the suppression of iNOS in other models may contribute to the survival time of the transplant by decreasing the production of NO.

29. THE EXPRESSION OF AN IMMUNOSUPPRESSIVE PROTEIN (LSF-1) ON LYMPHOID CELLS CORRELATES WITH ALLOGRAFT SURVIVAL FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION

Lord R, Vari F, Goto S

Discipline of Surgery, Clinical School University of Tasmania, Hobart, Tasmania 7001

Introduction: Liver suppressor factor one (LSF-1) is a 40 kDa immunosuppressive protein generated in response to orthotopic liver transplantation (OLT) between the rat strains DA (donors) into PVG (recipients). We have previously shown that the protein is suppressive both *in vitro* and *in vivo* and have generated a polyclonal antibody (pAb) against the available N-terminal sequence. In this study we characterised the cells recognised by the pAb and their number at different times after treatment with LSF-1 in OLT between rejector and non-rejector combinations.

Methods: OLT was performed between DA (donors) into LEW (rejector combination) or PVG (non-rejector combination). Flow cytometry (FC) using dual labelling was performed using FITC labelled LSF-1 cells taken at different times from the spleen in tandem with R-PE antibodies against each of the following CD4, CD8, CD11b, CD3 CD2, TCR and B cell markers. Immunofluorescence assays were also used to examine the localisation of LSF-1 on the cell populations involved.

Results: LSF-1 in the spleen was localised to two cell populations and was maximal at day 14 and then slowly declined to day 36. FC indicated that >95% of the cells expressing LSF-1 were CD4+CD11b+. OLT rejector combinations injected with LSF-1 had prolonged graft survival with a corresponding increase in CD4/CD11b/LSF-1 positive cells.

Conclusions: The presence of a macrophage cell type expressing LSF-1 correlates with observed OLT graft survival. LSF-1 clearly has a role in allograft acceptance in liver transplantation that may serve a role in the clinical setting.

30. EXPRESSION OF TGF- β_1 mRNA AND PROTEIN IN ALVEOLAR MACROPHAGES OF HUMAN LUNG ALLOGRAFTS

Zheng L*, Snell GI, Glare E, Williams TJ, Walters EH*

Dept of Respiratory Medicine, The Alfred Hospital and Monash Medical School.
*Discipline of Medicine, University of Tasmania Medical School, Hobart, Tasmania 7001, Australia

Introduction: Alveolar macrophages (AM) are believed to be involved in orchestrating the fibrotic response in pulmonary fibrosis. We have demonstrated increased TGF- β_1 protein levels in BAL fluid from lung transplant recipients (LTR) and an association between the BAL TGF- β_1 levels with the decline of lung function in LTR with Bronchiolitis Obliterans Syndrome (BOS).

Aim: of this study was to determine whether AM from LTR were implicated in the increased TGF- β_1 production found in their BAL fluid.

Methods: BAL were performed in 14 controls and 14 LTR, 3-6 mon post transplant (Tx), with FEV_{1,0}% best post-Tx of 97 \pm 5%. AM were isolated from BAL cells and cultured for 24 h, at 37°C, 5% CO₂, with and without LPS (1 μ g/ml). TGF- β_1 protein levels in BAL fluid and AM culture supernatants were measured with commercially available ELISA kits, and TGF- β_1 mRNA expression in BAL cells and cultured AM were quantified by competitive RT-PCR.

Results: Compared with controls, and despite the increase in BAL TGF- β_1 protein levels in LTR (p=0.001), no significant difference was found in mRNA expression from BAL cells. Expression of TGF- β_1 mRNA and protein in AM with and without LPS stimulus did not statistically differ from controls *in vitro*.

Conclusion: The paradoxical findings of increased TGF- β_1 protein levels in BAL fluid *in vivo*, but not in AM culture supernatants *in vitro*, nor in TGF- β_1 mRNA levels of cultured AM from LTR, may implicate the airway epithelial cells in causing the increased BAL TGF- β_1 post lung transplant.

31. RED BLOOD CELL AUTOIMMUNITY FOLLOWING CARDIOPULMONARY BYPASS SURGERY

Yeo W, Lord R, Stanton P, Dixit A

Department of Surgery, Royal Hobart Hospital, Liverpool St, Hobart, Tasmania 7000

Introduction: There has been a lot of research performed on the use of cardiopulmonary bypass pump and the systemic inflammatory response generated during the immediate and early postoperative period, also called "post-pump syndrome". This research focuses on a later presentation occurring at about 2 weeks post operation. Patient complains of transient breathlessness and cognitive dysfunction. This is unlikely a result of cellular mediated inflammation because the pro-inflammatory cytokines have since fallen to normal or near normal circulating values. We postulated that cardiopulmonary bypass pump also activates humoral immunity. This damages red blood cells and exposes "cryptic epitopes" previously hidden from the immune surveillance. Autoantibodies against these hidden red cell antigens are formed. Because IgG peaks at day 14, there could be a correlation between this and the delayed clinical presentation of transient breathlessness and cognitive dysfunction.

Method: This is a prospective and randomised study comparing on-pump and off-pump patients undergoing coronary artery bypass surgery. Visual Analogue Scale perception of breathlessness, psychometric tests and collection of two 5 ml blood samples are performed preoperatively, on day 7, day 14 and day 30 postoperatively. One of each 5 ml sample is sent off for routine red cell count, hematocrit and IgG count. The second 5 ml sample is centrifuged and serum stored at -70 degrees Celsius for bench studies. Bench studies include antibody preparation, immunoblotting, localisation and sequencing of samples.

Result: Preliminary results are encouraging. Antibodies against red blood cells are seen in fluorescence localisation studies. Immunoblotting studies comparing proteins between each time point assayed and for different patients show peak at about day 14 postoperatively with a number of proteins concentration being recognised.

Conclusion: Early results confirm the presence of red cell antibodies peaking at about day 14 post surgery.

32. CORONARY ARTERY SURGERY WITHOUT CARDIOPULMONARY BYPASS REDUCES NEUTROPHIL ACTIVATION RELATIVE TO SURGERY WITH BYPASS

Vallely M, Bannon P, Bayfield M, Hughes C, Wong M, Kritharides L

Heart Research Institute, Cardiothoracic Surgical Unit Royal Prince Alfred Hospital, Dept Immunology Children's Hospital Westmead, Dept Cardiology Concord Hospital, Sydney, Australia.

Coronary artery surgery without cardiopulmonary bypass (OPCAB) may reduce bypass-related (CABG) inflammation. However, detailed comparison of neutrophil (PMN) activation after CABG and OPCAB has not been undertaken. The relative importance of PMN integrins CD11b and CD18, essential for endothelial transmigration, and PMN degranulation (detected by release of lactoferrin), in CABG- and OPCAB-related inflammation are unknown.

Patients undergoing first time CABG (n=10) or OPCAB (n=10) had 6 blood samples taken before surgery (preop), before and during ischaemia, during reperfusion, 3h and 24h post-operatively (postop). PMN were isolated and analyzed by flow cytometry for expression of cell-surface CD11b, CD18 and lactoferrin. Total PMN counts were also determined. Data are expressed as mean±SEM of n subjects, and two-way ANOVA was used for comparisons between groups.

OPCAB and CABG demonstrated an identical 3.5-fold increase in circulating PMN post-op and similar steady expression of lactoferrin. However, OPCAB reduced CD11b expression in all samples relative to preop, at 3h postop being only 52±6% of pre-op value. In contrast, during CABG ischaemia and reperfusion CD11b expression was 130±22% and 137±23%, respectively of pre-op, and in other samples CD11b expression remained unchanged relative to preop (P<0.001). OPCAB reduced CD18 expression in all samples relative to preop. In contrast, CABG CD18 expression remained unchanged relative to pre-op during bypass and was reduced relative to preop in postop samples, but not to the same extent as OPCAB postop samples (3h postop; CABG 62±5% OPCAB 45±4%) (P<0.001).

PMN integrin expression, and not PMN degranulation distinguish the post CABG and post OPCAB inflammatory state. OPCAB induces a physiological post-surgical recruitment of PMN expressing less CD11b and CD18 than occurs after CABG, and thus may reduce end-organ injury.

33. ROLE OF INSULIN 3, TESTOSTERONE, MULLERIAN INHIBITING SUBSTANCE AND RELAXIN IN RAT GUBERNACULAR GROWTH

Temelcos C¹, Kubota Y¹, Bathgate RA², Jones KJ², Hutson JM¹

¹F. Douglas Stephens Surgical Research Laboratory, Murdoch Children's Research Institute, Melbourne, Victoria ²Howard Florey Institute, University Of Melbourne, Melbourne, Victoria.

Introduction: Testicular descent is a multistaged process, influenced by various anatomical and hormonal factors. Transabdominal testicular descent is mediated by gubernacular enlargement and regression of the cranial suspensory ligament, but its mechanism remains controversial. The aim of this study was to determine which hormones have a direct effect on the proliferation of cells in the gubernaculum *in vitro*, using an organ culture system.

Methods: Gubernacula were harvested from 17-day-old rat fetuses, placed in organ culture dishes and incubated for 2 days. The culture medium contained no additives (control), or was mixed with synthetic rat insulin 3 (InsI3), inactive InsI3, dihydrotestosterone (DHT), Mullerian inhibiting substance (MIS), human gene 2 relaxin, DHT+InsI3 or MIS+InsI3. A co-culture with testes was also performed. Cell proliferation was assessed using a bromodeoxyuridine (BrdU) labelling index. Statistical significance was assessed with the Student's t-test, with p<0.01 and p<0.05 defined as significant and mild difference, respectively.

Results: The BrdU labelling index of controls was 27.2±0.62% and testis co-culture 35.6±0.65%, which was significant (p<0.001). MIS and relaxin had a mild effect on gubernacular growth, whilst InsI3 and DHT had a more marked effect. Testis co-culture specimens had more DNA synthesis than those of InsI3, DHT, MIS and relaxin alone (p<0.01). The combination of MIS+InsI3 showed an effect close to that of co-culture with testis. However the most pronounced effect was that of InsI3+DHT.

Conclusion: Several hormones influence growth of the gubernaculum *in vitro*, including the recently reported hormone InsI3. Further studies are needed to determine the exact interrelationship between these hormones as well as that of estrogen.

34. THE IMMUNOLocalISATION AND SIGNIFICANCE OF VEGF, VEGF-B AND ITS RECEPTORS IN THE ARTICULAR CARTILAGE OF THE FEMORAL CONDYLES OF GROWING RATS

G Yee^{1,2}, W R Walsh¹, M D Poole²

¹Orthopaedic Research Laboratories, UNSW, Prince of Wales Hospital, Randwick, N S W

²Dept of Plastic and Reconstructive Surgery, UNSW, St George Hospital, Kogarah, N S W

Vascular endothelial growth factor (VEGF) has been shown to be essential for hypertrophic chondrocyte apoptosis and angiogenesis at the growth plate of long bones, suggesting a central role in endochondral ossification.¹ VEGF is also expressed in articular cartilage chondrocytes in human osteoarthritic and rheumatoarthritic joints but not normal adult joints.² The results of our study show VEGF, VEGF-B and Flt-1 (fms-like tyrosine kinase 1), one of the VEGF receptors, to be localised to the superficial layer of chondrocytes in the articular cartilage of the femoral condyles of growing rats. Significantly, the other VEGF receptor primarily responsible for endothelial cell mitogenesis and chemotaxis, KDR/Flk-1 (kinase domain region/fetal liver kinase 1), is not localised to the articular cartilage. Articular cartilage is an avascular and alymphatic tissue. As such, the localisation of VEGF, VEGF-B and the receptor, Flt-1, in the articular cartilage of the femoral condyles of the growing rat suggests a role for VEGF other than angiogenesis.

References: 1. Gerber HP, Vu TH, Ryan AM, Kowalski J, Werb Z, Ferrara N. VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. *Nat Med* 1999; 5:623-8. 2. Pufe T, Petersen W, Tillmann B, Mentlein R. The splice variants VEGF121 and VEGF189 of the angiogenic peptide vascular endothelial growth factor are expressed in osteoarthritic cartilage. *Arthritis Rheum* 2001; 44:1082-8.

35. GLUCOSE-INSULIN-POTASSIUM SOLUTION IMPROVES LEFT VENTRICULAR MECHANOENERGETICS IN DIABETES

Ramanathan T, Shiota K, Morita S, Nishimura T, Huang Y, Hunyor S

Cardiac Technology Centre, Royal North Shore Hospital, Sydney, Australia

Background: The mechanism by which glucose-insulin-potassium solutions (GIK) enhance recovery of left ventricular function in diabetic patients following coronary artery surgery is not well understood. We evaluated the effect of GIK on left ventricular function, ventriculoarterial coupling and left ventricular mechanoenergetics in a chronic ovine model of diabetes.

Methods: Diabetes was induced in six sheep with streptozotocin. Following six months diabetes, the response of the left ventricular pressure-volume relationship to 60 minutes intravenous GIK (1000ml D₅W, 100IU regular insulin, 90mmol KCl at 1.5ml/kg/h) was determined. Instantaneous left ventricular pressure and volume were measured with a micromanometer-tipped catheter and a conductance catheter during steady-state conditions and after transient inferior vena cava occlusion.

Results: GIK increased end-systolic elastance, an index of left ventricular contractility by 68% (P=0.01). This led to a 20% decrease in total mechanical work (P=0.01) and a 35% decrease in energy wasted as heat (P=0.01). Stroke work did not change significantly. Consequently stroke work efficiency (stroke work/total mechanical work) increased from 50.1 ± 3.5% to 60.2 ± 5.1% (P=0.01). This was consistent with the improvement in the ventriculoarterial coupling ratio (1.7 ± 0.3 to 1.0 ± 0.1; P<0.01) which varies inversely with stroke work efficiency.

Conclusions: In an ovine model of diabetes GIK improves contractility and enhances coupling between the left ventricle and the arterial system. This improves left ventricular mechanoenergetics by decreasing total mechanical work without significantly effecting stroke work, resulting in improved stroke work efficiency. Improved efficiency facilitates understanding of the preservation of left ventricular function afforded by GIK.

36. DEVELOPMENT OF A CRITICAL SIZE CRANIAL DEFECT IN A SHEEP MODEL: ROLE OF DIFFERENT MESH COVER AND DEFECT SIZE

Ho K, Walsh WR, Gianoutsos MP

The University of NSW, Orthopaedic Research Laboratories, Prince of Wales Hospital, Randwick, NSW 2031

Introduction: The critical size limits of cranial defects remain poorly understood. This study aims to develop and characterize the radiological and histological aspects of a critical size cranial defect in a sheep model.

Method: Three different sizes (10, 20 and 25mm diameter) and two time points (8 and 16 weeks) were evaluated with two animals in each group. Each animal had identical size defect created on both sides of parietal skull. Two pieces of mesh were used to protect each defect – the inner layer prevented brain herniation and the outer layer prevented soft tissue interposition. Polylactic acid co-polymer mesh was used on one side and Titanium mesh on the other. Specimens were harvested at sacrifice. Analysis included a quantitative assessment of the quantity of bone within the defect using computer digital analysis as well as non-invasive assessments of bone mineral density (DEXA), CT scanning, and radiographic appearance (Faxitron). Cellular features and histology were performed along with immunohistochemical staining to assess growth factor expressions.

Result: All animals underwent surgery uneventfully. Faxitron, CT scanning and DEXA scanning at 8 weeks revealed new bone present at the margin of the defect. Islands of new bone were observed to flow radially towards the centre of the defect. Healing in the 20 and 25mm defects were inferior to the 10mm defect. No adverse effect of the Polylactic acid co-polymer was evident compared to Titanium mesh.

Conclusion: This data suggests that the 10mm defect would heal spontaneously and is unsuitable as a critical size defect model. Preliminary conclusions from the 8 weeks harvest group suggest that 20mm diameter defect is adequate as a critical defect for the study of bone graft substitute in a sheep cranial defect model. In addition, this work will be utilised for examining the role of a new hydroxyapatite based bone graft substitute in combination with autologous growth factors compared to autologous bone graft.