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Poster Session B

PB - 01
NEUROCHEMICAL AND CEREBROVASCULAR RESPONSES TO CEREBRAL TRAUMA
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Introduction: The purpose of this study was to determine changes in high energy (hypoxanthine, inosine, adenosine) and metabolic (lactate, pyruvate) metabolites as well as amino acids (aspartate, glutamate, GABA) in a fluid percussion model of cerebral trauma. Significant changes occur during the first 2 hours post-injury and little clinical information is available in this time frame. These events may be important determinants of long-term outcome.

Methods: Following induction of anesthesia, Sprague-Dawley rats were intubated and artificially ventilated. Femoral arterial and venous catheters were placed and burr holes were made in the skull for the fluid percussion mount, laser Doppler fibers, and a microdialysis probe. Arterial blood pressure and blood gases were monitored. Microdialysis samples were collected from two hours pre- to two hours post-injury. Cerebral perfusion was monitored continuously. Fluid percussion was effected at 2 atmospheres, a moderate injury. Results: Increases were seen in the following metabolites in response to the injury: Hypoxanthine ($p < 0.01$), Inosine ($p < 0.01$), Adenosine ($p < 0.01$), Lactate ($p < 0.01$), Aspartate ($p < 0.01$), Glutamate ($p < 0.02$), GABA ($p < 0.001$). There was no change in pyruvate concentration. Cerebral perfusion decreased by 40% and remained decreased for the 2 hour post-injury period. Conclusion: Significant early changes occur in the metabolic state and the availability of high energmetabolites. There is a general increase in deleterious excitatory amino acids concomitant with a decrease in cerebral perfusion. These changes may support the formation of free radicals, particularly the highly reactive oxygen species. These alterations and their timing should be considered when devising a treatment protocol.

PB - 03
BRAIN TISSUE OXYGENATION LEVELS AND BARBITURATE COMA THERAPY IN SEVERE TRAUMATIC HEAD INJURY
J Daniel Thorat, I Ng (*Singapore*)

Introduction: Barbiturate coma therapy has been used as a second tier therapy in the management of raised ICP in head injuries based on the AANS-BTF Guidelines. Practically, barbiturate therapy is used as a last ditch effort to control intractable intracranial hypertension. In this study, we sought to analyse the effect of barbiturates on cerebral hemodynamics and tissue oxygenation.

Materials and Methods: This was a prospective observational clinical study of 10 patients with severe traumatic brain injury, carried out at a tertiary level, academic neurosurgical institute. There were 2 females and 8 males. A multi-channel neuromonitoring Licox bolt was inserted in the right frontal region or ipsilateral to the traumatic mass lesions through a frontal pericoronal twist drill. Brain temperature, brain parenchymal ICP, BrPtiO2 (brain tissue oxygen) was measured. The data was captured online by an in- house software system, which recorded the data at 10-second intervals. The patients were monitored for ICP, SBP, DBP, CVP, BrPtiO2, BT, RT, MAP, CPP, and DT. Of these, 5 patients underwent decompressive surgery for the mass lesion with craniectomy. Barbiturate coma was instituted either before or after the decompressive surgery. We compared the differences in cerebral hemodynamics and oxygenation between survivors and non-survivors before institution of barbiturate therapy and during therapy.

Results: 3 patients survived and 7 died. The mean total time of barbiturate therapy in survivors was 40 hrs and in non-survivors was 60 hrs. The pre-barb ICP level was higher (39.0 v/s 29.27) in non-survivors. The overall mean level of on- barb therapy ICP was lower (13.7 v/s 43.89) in survivors ($p < 0.05$) as compared to non-survivors. The decrease in ICP level from the pre-barb therapy to the on- barb therapy was greater amongst survivors (-15.4 v/s 4.8) though not statis-

tically significant. The mean pre-barbiturate therapy level of CPP was above 60 in both groups but there was a fall in CPP by 10 in non-survivors, which was not statistically significant. Although the pre barb PtiO2 was similar in both groups, there was an increase in BrPtio2 when barbiturate was instituted in survivors compared with those who died ($p < 0.05$ 6.0 mmHg: survivors v/s -3.6 mmhg: died).

Conclusion: Barbiturate therapy for intractable intracranial hypertension can favourably impact on the demand-supply relationship of oxygen metabolism and to a lesser extent on cerebral hemodynamics and this is most evident in survivors.

PB - 05

CLINICAL, RADIOLOGICAL AND OUTCOME STUDIES OF MILD HEAD INJURY PATIENTS PRESENTING TO A TERTIARY REFERRAL ACCIDENT AND EMERGENCY CENTER IN MALAYSIA

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Mild head injury (MHI) is a common presentation to many hospitals in both rural and urban settings. Distance, duration of travelling and lack of facilities might result in essential investigations not performed before intervention is carried out. We studied 330 patients presented to Hospital Universiti Sains Malaysia Emergency Department with possible MHI. Standard questionnaire was used to document history and clinical parameters on admission and in the wards. These patients were followed-up for a period of 1 year and their outcome was noted. Skull x-ray and CT scan were ordered from the Emergency Department by the attending doctors. Patients' one-year outcome was classified as discharged well (DW) and discharged with follow-up (DFU) for patients without and with post-traumatic signs and symptoms, respectively. Four patients died and 82 had DFU. Abnormal skull x-ray was associated with older age, presence of vomiting, confusion, bleeding from ear, nose or throat, a normal pupil size on the right side, unequal papillary reflexes, absence of loss of consciousness (LOC), lower Glasgow Coma Scale (GCS) score, multiple clinical presentations and DFU. Abnormal CT scan was associated with older age, multiple clinical presentation and DFU. Similar analysis on outcome revealed older age, vomiting, confusion, headache, bleeding from ear, nose and throat, neurological deficits, absence of LOC and GCS 13 to be associated with DFU. Multivariate analyses however, failed to determine any predictor of cranial or intracranial injuries. In conclusion, there were many variables associated with cranial or intracranial injuries based on skull x-ray and CT scan findings but the study failed to detect predictors among the variables studied.

PB - 07

DOES INDUCED HYPERTENSION REDUCE CEREBRAL ISCHAEMIA WITHIN THE TRAUMATISED HUMAN BRAIN?

JP Coles, LA Steiner, AJ Johnston, TD Fryer, MR Coleman, P Smielewski, DA Chatfield, FI Aigbirihio, GB Williams, S Boniface, K Rice, JC Clark, JD Pickard, DK Menon (*Cambridge, UK*)

We have previously used positron emission tomography (15O PET) to demonstrate evidence of cerebral ischaemia within 24 hours of head injury [1]. In this abstract we addressed whether an increase in cerebral perfusion pressure (CPP) would reduce such ischaemia. 15O PET imaging of cerebral blood flow (CBF), cerebral blood volume (CBV), cerebral metabolism (CMRO2) and oxygen extraction fraction (OEF) was undertaken in 20 patients within 6 days of injury. Using published techniques to quantify pathophysiology and ischaemia within the injured brain [1, 2], we examined the effects of an increase in CPP on 15 regions of interest covering the whole brain and the volume of ischaemic brain (IBV). Increasing CPP from 68 ± 4 to 90 ± 4 mmHg led to a significant increase in CBF and decrease in OEF in all subjects ($p < 0.001$). Changes in CBV and CMRO2 were less consistent but showed clear reductions in CMRO2 and increases in CBV ($p < 0.001$). Although the mean change in IBV was small (15 ± 16 to 5 ± 4 ml; $p < 0.01$), the response was variable. The reduction in IBV was related to baseline IBV ($r^2 = 0.97$; $p < 0.001$), suggesting the greatest benefit of CPP elevation is in patients at high risk of ischaemia. Although we identified a significant reduction in IBV, it was generally small in our patients. Further study is required to identify whether CPP elevation is beneficial within 24 hours of injury. It remains unclear whether the reduction in CMRO2 is clinically significant.

References

[1] J. Cereb, *Blood Flow Metab* **24**(2) (2004), 202–211.
[2] J Cereb, *Blood Flow Metab* **24**(2) (2004), 191–201.

PB - 08

INCREASED PENTOSE PHOSPHATE CYCLE FLUX FOLLOWING SEVERE TRAUMATIC BRAIN INJURY: A ^{13}C-LABELING STUDY

J Dusick, TC Glenn, WNP Lee, PM Vespa, S Bassilian, YC Lee, SJ Lee, SM Lee, NA Martin (*Los Angeles, USA*)

Introduction: Patients with traumatic brain injury (TBI) routinely exhibit cerebral glucose uptake in excess of that expected by the low level of oxygen consumption. However, excess lactate production that should accompany hyperglycolysis is not observed. Utilizing ^{13}C-glucose labeling, studies demonstrate increased flux through the pentose phosphate cycle (PPC) during cellular stress, supplying substrates for macromolecular synthesis, DNA repair, and free radical scavenging. This study assessed both the PPC and the validity of the technique in TBI.

Methods: ^{13}C-glucose was infused for 60 min in 5 TBI patients (adults with severe TBI, GCS < 9) and 1 normal subject. Arterial and jugular bulb blood sampled at multiple time points was analyzed for ^{13}C-labeled isotopomers of glucose and lactate by GC/MS. The product of lactate concentration and fractional abundance of isotopomers determines blood levels. AV differences determine PPC contribution by the brain.

Results: There was good enrichment of labeled glucose in the arterial-venous blood (16.9% average) and incorporation into lactate, demonstrating metabolism of labeled. The average PPC was 23.9% for TBI patients and 13.36% for the normal subject (published norms 5–15%). The PPC correlated with the average oxygen extraction ratio ($p = 0.007$, $r = 0.97$) and $SjvO_2$ ($p = 0.013$, $r = -0.95$).

Conclusion: ^{13}C-glucose labeling allows quantific ation of altered metabolic fluxes. The PPC tends to be up-regulated after TBI relative to normal, correlating with markers of cerebral hypoperfusion. Further studies and confirmation by ^{13}C-MR Spectroscopy are pending. Elucidating the metabolic milieu in the brain following TBI may open doors for novel metabolic support to prevent secondary injury and improve outcomes.

PB - 09

METABOLIC CRISIS WITHOUT BRAIN ISCHEMIA IS COMMON AFTER TRAUMATIC BRAIN INJURY: A COMBINED MICRODIALYSIS AND POSITRON EMISSION TOMOGRAPHY STUDY

P Vespa, D McArthur, J Alger, E Zanier, T Glenn, M Bergsneider, N Martin, D Hovda (*Los Angeles, USA*)

Introduction: Post traumatic seizures are associated with hippocampal hyperglycolysis and eventual tissue loss: A translational study in rodents and humans. Post traumatic seizures are now known to occur in over 20% of traumatic brain injury (TBI) patients and may cause additional damage (Vespa, 1999; Vespa 2002). Hypothesis is that regional tissue damage unrelated to the primary trauma will occur with post traumatic seizures.

Methods: Continous EEG monitoring, brain MRI, microdialysis and positron emission tomography testing were done in severe TBI patients to detect seizures and the metabolic consequences. These results were compared against an experimental model of TBI previously published (Zanier, 2003).

Results: Among 315 TBI patients, 87 (27%) had seizures, of which most occurred within 7 days of injury, were nonconvulsive and were repetitive within subjects. Abnormal diffuse brain activation occurred with seizures as reflected by widespread increase in quantititative EEG parameters alpha trend, total power and alpha/delta ratio suggesting decreased global inhibition ($p < 0.0001$). Seizures elicited increases in glutamate, lactate/pyruvate ratio and glycerol levels compared with interictal states within subjects ($p < 0.01$). Compared with rodents, the extent of microdialysis changes were similar, but more longlasting in total duration ($p < 0.001$). Regional hyperglycolysis occurred in seizure foci and in adjacent regions of brain on PET. Delayed MRI imaging found marked reduction in hippocampal volumes compared with non-seizure TBI patients, similar to the animal model.

Conclusion: Post-traumatic seizures are common and elicit profound changes in microdialysis markers of metabolism and lead to delayed tissue loss in human hippocampal tissue.

PB - 10

POST TRAUMATIC SEIZURES ARE ASSOCIATED WITH HIPPOCAMPAL HYPERGLYCOLYSIS AND EVENTUAL TISSUE LOSS: A TRANSLATIONAL STUDY IN RODENTS AND HUMANS

P Vespa (*Los Angeles, USA*)

Introduction: Post traumatic seizures are now known to occur in over 20% oftraumatic brain injury (TBI) patients and may cause additional damage (Vespa, 1999; Vespa 2002). Hypothesis is that regional tissue damage unrelated to the primary trauma will occur with post traumatic seizures.

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Conclusion: Post-traumatic seizures are common and elicit profound changes in microdialysis markers of metabolism and lead to delayed tissue loss in human hippocampal tissue.

PB - 11

SURGICAL OUTCOME FOLLOWING EVACUATION OF TRAUMATIC INTRACRANIAL HEMATOMAS IN THE ELDERLY

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Objective: The aim of this study is to determine the factors influencing the surgical outcome following craniotomy for head injury and to establish the criteria for surgical intervention in the age of 65 years or older.

Methods: We retrospectively investigated the mechanism of injury, types of CT lesions, Glasgow coma scale (GCS) score at admission, pupillary reactivity, past medical history and surgical outcome following craniotomy in the elderly during 8-year period. Results: There were 35 men and 21 women with a mean age of 70.7 years (range 65–87 years). The mortality rate at discharge was 58.9%. Good outcome was achieved only in 25 percent of the patients. The cause of injury did not affect on the surgical outcome. All of 19 patients with GCS of 5 or less at admission had poor outcome. Outcome was significantly worse in older patients (more than 75 years) compare to younger patients (less than 75 years). Ninety percent of the patients with pupillary abnormality had poor outcome, whereas 57.7 percent of the patients with bilateral reactive pupil had poor outcome. Past medical history did not affect on the surgical outcome following craniotomy.

Conclusion: Surgical outcome is unexceptionally poor in the elderly head-injured patients if the age is 75 years old or older, the GCS is 5 or less and the pupil is bilateral dilated. Craniotomy under those circumstances is not desirable.

PB - 13

EPIDEMIOLOGY OF SEVERE TRAUMATIC BRAIN INJURY PATIENTS ADMITTED TO THE NATIONAL NEUROSCIENCE INSTITUTE(NNI), SINGAPORE

KK Lee, I Ng (*Singapore*)

Introduction: Severe traumatic brain injury (TBI) is a common injury with significant high mortality and morbidity rate. This study aims to review the demographic and mechanism of injury of severe TBI admitted to the National Neuroscience Institute (NNI), Singapore.

Methods: This retrospective study was conducted in NNI between January 1999 and September 2003. Infor-

mation maintained in the Severe Head Injury Database which include demographic data, source of referral, details of injury, presenting Glasgow Coma Score (GCS) and outcome at 6 months in 509 patients were reviewed.

Results: 80% were male and the age ranged from 10–96 years old with the young and active group (21 to 60 years old) constituting 68%. Common mechanisms of injury were motor vehicle (46%) and fall (43%) related accidents. Most TBI victims from motor vehicle accidents were motorcyclists (39%) and pedestrians (34%). 13% were under the influence of alcohol during the time of accident. Traumatic SAH occurred in 36%, multiple injuries in 22% and radiological proven cervical injuries in 6%. Average length of hospital stay was 23 days with a maximum at 223 days. At 6 months post injury, mortality rate was 32%, unfavourable outcome (moderate to severe disability) in 20% and favourable outcome (slight or no disability) in 48%.

Conclusions: Motor vehicle and fall related accidents were the leading causes of moderate and severe TBI, and the patients were predominantly males and young. Half of these patients would be expected to have a favourable outcome.

PB - 14
ANALYSIS OF DATA FROM SCREENING LOGS OF A LARGE MULTI CENTER RANDOMIZED CONTROLLED TRIAL IN TBI

F Slieker, N Kassem, M van Gemerden, D Engel, A Maas, (*Rotterdam, The Netherlands; Iselin, USA*)

Enrollment of patients in a large multi center randomized controlled clinical trial (RCT) evaluating the efficacy and safety of dexanabinol in patients with severe traumatic brain injury has recently been completed. 861 patients were enrolled from 2001 to 2004. In the context of this study screening logs were obtained from 100 participating centers, on which baseline demographic and clinical data were recorded in order to evaluate whether enrollment or exclusion had been appropriate. This database includes anonymous data on over 6.500 patients admitted to the intensive care units of participating centers and contains unique information on demographics and referral policy. On average 9 out every 10 screened patients did not meet the enrollment criteria. The main reasons for exclusion were age and time window. Analysis of data from the screening logs showed a peak incidence below the age of 25 with a strong male predominance. An increasing number of injuries occurred from Monday to Sunday. Referral policy varied considerably between countries with secondary referral rates ranging from 18 to 73%. On average only approximately 50% of patients were admitted to the study hospital within 2 hours after injury. Characteristics of the patient population in countries with a high percentage of secondary referral were clearly different with a larger percentage of patients with mass lesions. We conclude that screening logs should form an important element in RCT's, and that such data are necessary in order to report results of a RCT according to the CONSORT guidelines. We further conclude that characteristics of the patient population in different centers and countries is greatly influenced by aspects of local trauma organization.

PB - 15
PROGNOSTIC MODELS IN TRAUMATIC BRAIN INJURY BASED ON ADMISSION DATA: A SYSTEMIC REVIEW.

N Mushkudiani, D Engel, E Steyerberg, A Maas, (*Rotterdam, The Netherlands*)

Prognostic models for predicting outcome are important for confident clinical decision making, appropriate allocation of resources, and communication with relatives. Models can further be used for purposes of classification and for stratification of patients enrolled in randomized clinical trials. For these purposes prognostic models need to be based on admission data. We aimed to review approaches for creating prognostic models based on admission data in patients with TBI. A systematic literature search, using Medline, Cochrane and other databases from 1970 to 2003, yielded 23 papers reporting prognostic models based on admission characteristics. Nineteen studies reported results on hospital series with a sample size < 500, four were on large multi center studies with $n > 1.000$. The mean number of predictors included in the models was 5.5. The selected predictors varied widely between studies, but most studies included age and GCS or motor score. The most frequently used statistical technique was logistic regression analysis and in these cases predictor selection was done by stepwise methods. Only few papers described methodology for dealing with missing values and validation of models was generally insufficient. We conclude that studies on prognostic modelling in TBI have been limited by small sample sizes and rather crude statistical approaches. We suggest that model development can be improved by using larger samples sizes, more advanced statistical methods for dealing

with missing values, improved variable selection and standardized model validation.

PB - 16
CEREBRAL AUTOREGULATION AND PRESSURE REACTIVITY FOLLOWING DECOMPRESSIVE CRANIECTOMY
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Objective: Analysis of slow waves in arterial blood pressure (ABP) and intracranial pressure (ICP) has been used as an index to describe cerebrovascular pressure-reactivity. It has been previously demonstrated that the pressure-reactivity index (PRx) can be used to reflect global cerebrovascular reactivity with changes in ABP. A positive PRx signifies a positive association between ABP and ICP, indicating a non-reactive vascular bed while a negative PRx is reflective of intact cerebral autoregulation, where ABP waves provoke inversely correlated waves in ICP. To date, there has been no characterization of pressure-reactivity following decompressive craniectomy.

Methods: We conducted a prospective observational study of 33 patients who underwent decompressive craniectomy for intractable intracranial hypertension refractory to medical therapy. The PRx was calculated as a moving correlation coefficient between 30 consecutive samples of values of ICP and ABP averaged for a period of 10 seconds. The time profiles of PRx at 6 hourly intervals were analyzed.

Results: In all patients, the initial PRx 6 hours after surgery was positive, indicative of disturbed pressure-reactivity. With time, the PRx trended towards a more negative value, suggestive of an improving cerebrovascular autoregulatory reserve. The mean PRx at 24 hours was 0.3520 while the mean PRx at 72 hours was 0.2047 ($p < 0.001$).

Conclusion: Surgical decompression, apart from playing a crucial role in management of raised intracranial pressure may have a contribution in the restoration of disturbed cerebrovascular pressure-reactivity.

PB - 18
RISK FACTORS FOR POOR PROGNOSIS IN PATIENTS WITH SEVERE TRAUMATIC BRAIN INJURY
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Background: It has been shown that Glasgow Coma Scale (GCS), age, pupillary light reflex, hypotension, and CT scan features are early indicators of prognosis in severe traumatic brain injury (J Neurotrauma 17:559–627, 2000). The purpose of the present study is to determine the most important indicators of prognosis by multivariate logistic regression.

Methods: The records of 116 patients with severe traumatic brain injury were reviewed searching for arterial blood gas analysis (PaO_2, $PaCO_2$, base deficit), blood glucose level (BS), and body temperature on admission, gender, and intracranial pressure(ICP) on insertion in addition to the early indicators of prognosis described above. Absence of basal cisterns, presence of extensive traumatic SAH (ext-SAH), and degree of midline shift were evaluated on CT within 24 hours of injury. Ext-SAH was defined as presence of CT visible blood both in the basal cisterns and over the convexity. Outcome was measured at discharge and 6 months after injury by Glasgow Outcome Scale. Forward and backward stepwise logistic regression was used to detect an independent risk factor for poor prognosis.

Results: Multivariate logistic regression showed that age ($p = 0.011$), ICP (< 0.001), ext-SAH ($p < 0.001$), and BS on admission ($p = 0.002$) were significantly associated with mortality. All patients ($n = 6$) died with BS more than 300 mg/dl and all patients ($n = 16$) with ICP more than 60 mmHg resulted in unfavorable outcomes such as dead or vegetative state.

Conclusion: BS and ICP were independent prognostic indicators of severe traumatic brain injury in addition to age and ext-SAH.

PB - 19

OPTIMAL TIME TO MEASURE NEUROLOGICAL OUTCOMES DURING RANDOMISED INTERVENTIONAL STUDIES OF PATIENTS WITH TRAUMATIC BRAIN INJURY.

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In traumatic brain injury patients, neurological outcomes continue to improve for more than 2 years after injury. However, interventional studies in TBI patients frequently measure differences between groups for major primary outcomes at 6 months after injury to balance the competing interests of optimal recovery of neurological function and minimal loss from patient dropouts. Whether 6 months is the optimal time to assess these outcomes is uncertain. We recently completed a prospective randomised controlled trial testing pre-hospital hypertonic saline resuscitation in 229 patients with traumatic coma (GCS < 9) and hypotension in Victoria Australia. Neurological outcomes in patients were measured using the Extended Glasgow Outcomes Score (GOSE) at 3 months, 6 months and 24 months after the injury. "Favourable outcomes" were defined as GOSE grades 5–8 (good, and moderate disability). Neurological assessments were done at personal interview by a single trained assessor.

Between 3 months and 6 months after injury, favourable neurological outcomes in all patients tended to increase (32% to 37%). Six months after injury, favourable outcomes were equivalent in both groups (37% in both) and only 1% of patients had been lost. From 6 months to 2 years after injury, overall favourable outcomes changed little (37% to 39%), were still equivalent in both study groups (37% and 40%) while patients lost to follow-up had increased to 4%. Six months after injury is an optimal time point to measure and compare long term neurological outcomes during randomised interventional studies in patients with severe traumatic brain injury.

1. Pre-hospital hypertonic saline resuscitation of patients with hypotension and severe traumatic brain injury: a randomised controlled trial, *JAMA* **291** (2004), 1350–1357.

PB - 20

THE INFRAORBITAL NERVE DISTURBANCE FOLLOWING ZYGOMATIC COMPLEX FRACTURES

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Zygomatic complex fractures are one of the most common injuries in the middle facial bone. The infraorbital nerve is frequently involved in trauma to the zygomatic complex at the site of the infraorbital fissure, infraorbital canal, or foramen. Traumatic injury to the infraorbital nerve may be due to compression, edema, ischemia or /and laceration. This pathophysiology results in hypesthesia or dysesthesia to the skin of the lower eyelid, cheek, lateral side of the nose and upper lip and to the labial mucosa, teeth and gingivae. The aim of the present study is to document infraorbital nerve injury following zygomatic complex fractures based on Knight and North classification. Twenty consecutive patients with unilateral zygomatic complex fractures were evaluated with regard to the infraorbital nerve sensory disturbance. All patients were treated surgically within 21 days after injury. Our surgical method is open reduction via intraoral, lower eyelid and lateral blow approach with miniplate osteosynthesis. The complete sensory recovery of the infraorbital nerve was 63%. Return to normal sensation occurred average in 23 weeks. Recovery of sensation correlated with the types of Knight and North classification. The persistent part of sensory disturbance is upper lip and gingival as area of superior alveolar nerve. Open reduction and fixation using miniplate offer a better prognosis for complete recovery of the infraorbital nerve function following zygomatic complex fractures.

PB - 21

INTRACRANIAL PRESSURE MONITORING: CLINICAL COMPARISON OF THE VENTRIX PARENCHYMAL FIBRE-OPTIC MONITOR WITH AN EXTERNAL VENTRICULAR CATHETER.

A Adamides, DJ Cooper, P Lewis, CD Winter, N Pratt, T Kossmann, JV Rosenfeld (*Melbourne, Australia*)

The Ventrix parenchymal Intracranial Pressure (ICP) monitor was recently introduced at our institution, providing an opportunity to re-evaluate this device in a clinical setting. Ventricular ICP is widely accepted as

the reference standard to which measurements from monitors in other intracranial compartments should be compared. Hourly ICP readings from a Ventrix monitor were compared with those from a ventricular drain in eight severely head injured patients. Data were recorded for periods ranging from 61 to 132 hours and a total of 759 paired readings were taken. Sixty five percent of the paired readings were within 5 mmHg and 85% were within 10 mmHg. The mean difference ($\pm$ SD) between the paired readings was 4.1 ($\pm$ 5.5) mmHg (parenchymal – ventricular). In six of eight patients the parenchymal and ventricular monitors had a mean difference of only 0.3 ($\pm$ 2.9) mmHg but, as indicated by the high SD, there was significant inter-individual variation with the mean difference in individual patients varying by up to $\pm$ 4.3 mmHg. In the other two patients the Ventrix ICP readings were higher than the ventricular with a mean difference of 10.4 ($\pm$ 1.2) mmHg. Both these patients were found to have expanding haematomas ipsilateral to the parenchymal monitor. Neither of the haematomas required surgical intervention. We conclude that large differences between ICP readings from the two systems ($>$ 5 mmHg) may indicate evolving intracranial pathology and that in this situation the two techniques may be complementary.

PB - 22

IMPLICATIONS OF FULL COMPLIANCE WITH THE CT SCANNING RECOMMENDATIONS OF THE NICE AND SIGN GUIDELINES FOR THE MANAGEMENT OF HEAD INJURY

LT Dunn, J Kerr, D Beard, R Smith, CE Robertson (*Edinburgh, UK*)

Objective: To estimate the additional CT scanning resources needed to comply fully with published guidelines for the early management of patients with head injury.

Methods: Detailed information on the presentation and early management of patients with head injury was collected from 24 Scottish Accident and Emergency Departments in 2 one month periods in 1999 and 2001 on custom-designed proformas. Information was available on all patients admitted to hospital and in addition on all patients discharged from A&E in 4 teaching hospitals. The clinical features and imaging investigations of these patients were compared with the recommendations in contained in the SIGN(2) and NICE(1) guidelines.

Results: Information was available on 2827 adult patients (1300 admitted and 1527 discharged or taking irregular discharge from A&E). 232 patients satisfied one or more of the SIGN criteria for CT scanning and 166 of these were scanned (72% compliance). It would have been necessary to scan an additional 66 patients to achieve full compliance (2.3% of patients). 478 patients fulfilled one of more NICE criteria for scanning. 166 were scanned (35% compliance). A further 312 patients would have needed scans to achieve full compliance (11% of patients). None of the patients with SIGN/NICE indications for CT scanning who weren't scanned subsequently required neurosurgical treatment for a complication related to their injury.

Conclusion: Full compliance with the scanning recommendations in the SIGN and NICE guidelines would require a significant increase in scanning resource and is unlikely to lead to the identification of a significant additional number of patients with intracranial lesions requiring neurosurgical intervention.

PB - 23

CHANGE OF PROGNOSTIC CLINICAL INDICATORS OF SEVERE BRAIN INJURY TREATED BY BRAIN HYPOTHERMIA

Y Takasato, H Masaoka, Y Ohta, T Hayakawa, S Imae, T Sugawara, T Kino, T Yamamoto (*Tokyo, Japan*)

Objectives: We examined the role and problem of the mild brain hypothermia in neuro-intensive care of the severe brain injury. Especially, factors that correlated to the poor outcome were analyzed.

Methods: Object were severe brain injury patients who's Glasgow Coma Scale (GCS) on admission were 8 or less. Twenty-one early period patients (A group) were treated by neuro-intensive care other than the brain hypothermia. Forty-three later period patients (B group) were treated with additional brain hypothermia. The cooling was carried out with the whole body water cooling by the blankets. The temperature was controlled with jugular bulb blood temperature. The target temperature of the cooling was made at 32-35 degree. After this temperature setting for 3 days, the patient was rewarmed gradually to 37 degree during next 4 days. The prognosis was evaluated by Glasgow Outcome Scale (GOS) on discharge.

Results: The comparatively better prognosis was admitted with the B group. As for the good prognosis (GR + MD) rate, the former is 9% and the latter was 21%. The former mortality is 81% and the latter is

37%. GCS on admission, pupil size, blood pressure (shock level), light reflexes and effacement of the basal cistern in CT on admission showed the strong correlation with prognosis(GOS) in the former and did not show the correlation much in the latter.

Conclusion: The most severe brain injury patients was not possible to rescue in the conventional treatment without brain hypothermia. The recovery and neuroprotective mechanism by brain hypothermia is different from the conventional one.

PB - 27

SEX DIFFERENCES IN ACUTE INJURY SEVERITY AND OUTCOME VARIABLES FOLLOWING TRAUMATIC BRAIN INJURY: PILOT DATA.

SS-Younan, R Heriseanu, I Baguley, CD Rae (*Sydney, Australia*)

Objectives: The well-known dimorphisms between male and female brains suggest that sex may play an important role in the brain's response to injury. Emerging evidence has demonstrated that males and females display different patterns on clinical measures following Traumatic Brain Injury (TBI). This study aimed to investigate the effect of a patient's sex on various measures of injury severity and outcome following admission to an Intensive Care Unit for Acute TBI.

Methods: Up to the 30th April 2004, 5 female subjects that met the project's inclusion and exclusion criteria were admitted to the study. Data on 5 age-matched males with acute TBI will also be examined.

Results: Due to the envisaged small sample size, descriptive data will be reported.

Conclusions: Investigations of the role of sex on measures of injury severity and outcome following a TBI remain lacking. Even more rare are prospective investigations of sex differences following TBI in an acute setting. This study attempts to report the first Australian based data of this kind. Findings will be discussed in relation to their clinical significance.

PB - 29

PENETRATING BRAIN INJURY IN THE RAT: VII. PROTEOMICS-BASED IDENTIFICATION OF PROTEIN EXPRESSION

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Protein changes induced by traumatic brain injury can serve as diagnostic markers as well as therapeutic targets for neuroprotection. The objective of the present study is to implement proteomics analysis to detect neurochemical biomarkers following experimental penetrating brain injury (PBI; see Williams et al., part I). The dynamics of proteomic analysis is a multi-step process comprising sample preparation, separation, quantification and identification of proteins. One approach is to separate proteins first by two-dimensional gel electrophoresis (2-DE) according to charge and molecular weight. Proteins are then fragmented and analyzed using matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS). Identification of proteins can be achieved by comparing the mass/charge-ratios of these peptides to those in respective databases. Initial studies are underway, evaluating protein changes in normal and injured brain tissue from rats. Brain tissue was collected 24 h following a frontal PBI (10% severity). Tissue was dissected out from the ipsilateral hemisphere and pooled from two injured animals (1 to 5 mm anterior to bregma), which incorporated the predominant region of cell death and hemorrhage to the frontal cortex and striatum. Results to date have identified more than 100 proteins from a single sample. The mass spectrometric analyses are currently in progress to quantitatively and qualitatively assess the changes in protein expression following PBI injury.

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LATERAL FLUID PERCUSSION INJURY IN THE JUVENILE RAT TRIGGERS EXPRESSION CHANGES IN MULTIPLE FAMILIES OF CELL CYCLE, DEVELOPMENTAL AND CYTOSKELETAL GENES

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Traumatic brain injury induces a complex cascade of molecular and cellular processes, and has been shown to alter experience-dependent neuroplasticity in the immature brain. We hypothesized that genes associated

with cell growth, development and cell structure would show distinct patterns of altered expression following lateral fluid percussion (LFP) in the juvenile (postnatal day 26) rat. Immature rats ($N = 24$) were divided into sham, mild-LFP and severe-LFP (unresponsiveness to toe pinch 75.3 ± 7.8 and 252.3 ± 41.6 s, respectively) groups. At post-injury times of 4 h and 24 h ipsilateral hippocampus and parietal cortex regions were dissected and RNA collected ($N = 4$ per injury group per time point). RNA was labeled and hybridized to rat genome arrays (Affymetrix), with one rat per array. Comparisons were made between sham and injured groups using t-test statistics and a quality setting of 0.75 (www.genesifter.net), and changes from baseline greater than 1.8-fold are reported. In hippocampus, 186 genes were up-regulated and 55 down-regulated, while in parietal cortex, 122 genes increased and 22 decreased. The number of genes altered generally increased from 4 h to 24 h, and with increasing injury severity. Multiple genes directly associated with cell growth (44, including BDNF, DNA topoisomerase), development and differentiation (31, including CEB/P, BMP-4) and cytoskeletal proteins (16, including alpha-tubulin, annexin, vimentin) were affected by LFP. These molecular responses indicate that traumatic injury to the immature brain can affect pathways involving normal brain development and plasticity.

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REGIONAL NEURONAL SENSITIVITY FOLLOWING TRAUMATIC BRAIN INJURY

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Utilizing a well-characterized rodent controlled cortical contusion model, we studied the pathologic processes that occur following traumatic brain injury (TBI). We were interested in determining the sensitivity of neurons in various regions of the hippocampus and dentate gyrus following TBI. Neuronal sensitivity was evaluated with Fluoro-Jade B (FJB), an anionic fluorescein derivative that has been reported to stain degenerating neurons. Adult male rats were subjected to a mild or moderate cortical deformation injury. Animals were killed at 15 m, 1 h, 3 h, 6 h, 1 d, 2 d or 7 d post injury by perfusion with 4% paraformaldehyde. Systematic random sections throughout the rostro-caudal extent of the hippocampal formation were stained with both FJ B and DAPI histochemistry. Faint FJB positive neurons could be detected as early as 15 m following a moderate injury. The dentate gyrus granule cells and pyramidal neurons in the CA1 region showed the earliest signs of injury. Quantitative analysis showed the greatest number of FJB positive cells in the dentate gyrus. By 1 h post injury CA3 neurons are also involved in the degenerative process. There was a time dependent reduction in FJB positive cells following the TBI possibly reflecting a clearing of cells by phagocytosis. The greatest number of cells could be detected between 3–24 h post trauma and the least at 7 d. The very early cellular involvement in this experimental model of TBI may explain the failure of significant cell sparing following delayed experimental therapeutic intervention. Caution is warranted when employing FJB staining as a measure of histopathology. NIH AG21981, NS39828

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STIMULATION OF NMDA RECEPTORS 1-2 DAYS AFTER TRAUMA IMPROVES OUTCOME IN A MOUSE MODEL OF CLOSED HEAD INJURY

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Traumatic brain injury (TBI) triggers glutamate efflux, presumably causing hyperactivation of NMDA receptors (NMDAR) and neuronal death. Our goal was to investigate dynamic changes in NMDAR function over time (5 min – 30 days) after closed head injury (CHI) in male mice ($n = 4$–9/group). Using quantitative autoradiography of radioactive MK801 under conditions preserving the post traumatic brain milieu (no washing and no addition of glutamate and glycine) we found a bilateral, transient (60%, < 60 min) increase in NMDAR followed by a profound (30–70%) long lasting (> 7 days) loss of binding in cortical and hippocampal regions. These results led us to hypothesize that cognitive deficits following CHI are caused by NMDAR hypofuncion and may be overcome by receptor stimulation with an agonist. Indeed, administration of NMDA (20 mg/Kg i.p. 1 and 2 days after CHI) resulted in a progressive and statistically significant amelioration of neurological deficits assessed 7 and 14 days post injury (neurological severity improvements score $2.0 +/- 0.4$ in vehicle Vs $3.4 +/- 0.3$ in NMDA treated mice, $p < 0.02$, Mann Whitney test) as well as prevention of

specific cognitive deficits assessed by the object recognition test 14 days post injury (%time with new object $55.8+/-$ 1.2 in vehicle Vs 70.7 $+/-$ 2.1 in NMDA treated mice, $p < 0.00005$, Student t-test). These results may explain the very short (< 60 min) therapeutic window of NMDA antagonists in animal models of TBI and the failure of these drugs in clinical trials. They also suggest that TBI victims may benefit from delayed pharmacological stimulation of NMDAR.

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THE EFFECTS OF COMBINED LACTATE AND OXYGEN THERAPY ON CEREBRAL BIOENERGETICS AFTER MODERATE TRAUMATIC BRAIN INJURY

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It is widely accepted that a reduction in brain ATP level occurs after injury. This has been substantiated in several models of experimental brain injury (Vink et al., 1988; Signoretti et al., 2001). Both compromised cerebral oxygenation and metabolism exacerbate secondary insult effects, and reduce ATP production. We have previously shown that intravenous lactate, alone improves behavioural outcome, and brain oxygen consumption. Hyperoxia alone (100% O_2) both in patients, and FPI models, also improved the neurochemical picture, after TBI. In this study, therefore we have tested the effects of combined lactate and 100% O_2, on ATP generation, after FPI. Sprague-Dawley rats were used for this study. The animals were divided into the following groups: sham surgery, injury and 100% O_2, injury (100% O_2) and 100 mM Lactate, injury and 100 mM lactate, injury and 21% O_2. All animals received 100 mM lactate or vehicle at a rate of 0.65 ml/h, for 1 hr, and hyperoxia (100% O_2) was also given for 1 hour, initiated 15 mins after TBI. After the experiment, the brain was removed, immediately frozen in liquid nitrogen, homogenised, and acid extracted, for HPLC. HPLC revealed decreased ATP content ipsilateral to the injury in comparison with the contralateral side, and in injured versus shams. The combination of both lactate and 100% O_2 resulted in the highest ATP levels, only surpassed by the sham animals. Behavioural evaluation, of the same combination is therefore ongoing, in the Morris Water maze.

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BLOCKADE OF N-TYPE VOLTAGE-GATED CALCIUM CHANNELS IS NEUROPROTECTIVE FOLLOWING STRETCH-INJURY OF NEURONAL-GLIAL CELL CULTURES

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Injury to neurons and astrocytes causes a significant, sustained rise in intracellular calcium that leads to cell dysfunction and death. Sources of free intracellular calcium include release from intracellular stores and influx from the extracellular space through various channels. It remains unknown, however to what extent voltage-gated calcium channels (VGCCs) participate in this rise of cytosolic calcium after injury, and if blockade of these channels can reduce levels of intracellular calcium and thereby increase cell survival. We used an *in vitro* stretch-injury model of traumatic cell injury. Mixed neuronal-glial cell cultures were prepared from P1-P2 rat pups that yielded neurons with mature morphological characteristics. SNX-185 (0, 10, 100, 1000 nM) was used to block N-type VGCCs during stain injury. Intracellular calcium concentrations were monitored using Fura-2 and cell viability quantified. Intracellular calcium levels were stable prior to injury. Stretch-injury resulted in a large increase in free intracellular calcium that was maintained for up to 30 minutes. SNX-185 significantly decreased intracellular calcium in a concentration-dependent manner. Neuronal and glial cell death at 24, 48, and 72 hours following stretch-injury was significantly reduced with the 100 nM SNX-185 concentration only. Treatment at 10 or 1000 nM SNX-185, although effective in reducing levels of intracellular calcium, did not increase cell survival. These findings suggest that N-type VGCCs contribute significantly to injury-induced elevations of intracellular calcium in neurons and glia. N-type calcium channel blockers may have the potential to be useful therapeutic agents. Supported by NIH 39090 and UCLA Brain Injury Research Center.

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LONG LASTING PROTECTIVE EFFECTS INDUCED BY THE IMMUNE MODULATOR PN277 GIVEN 24 HOURS POST PERMANENT MIDDLE CEREBRAL ARTERY OCCLUSION IN RATS.

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Stroke is the most common cause of long-term disability in adulthood. In addition to a wide range of motor and sensory deficits, stroke often results in cognitive and behavioural abnormalities. As in other neurodegenerative disorders, the immune response evoked by the injury mediates both degenerative and repair processes. PN277 is a synthetic copolymer that was shown to augment the protective immune response in a controlled way. In this study we examined the protective effect of PN277 in a rat model of permanent middle cerebral artery occlusion (MCAO). A single injection of PN277, administered 24 hours post MCAO, significantly improved neurological outcome measured during 6 weeks post occlusion in compare to the PBS treated group. Behavioural evaluation using the Morris Water Maze showed significant deficits in spatial learning abilities of the rats, as measured 4 weeks post MCAO, with no effect of the treatment. However, in the visible platform task, the control group displayed significantly abnormal behaviour whereas the PN277 treated group behaved similarly to the sham operation group. Administration of PN277 had no effect on infarct volume, but relative to PBS treatment, enhanced survival of hippocampal neurons and increased proliferation in the sub ventricular zone (SVZ) of the brain, the area that contains neuronal progenitor cells. The SVZ of the PN277-treated group also showed increased Nestin-immunoreactivity, a cellular marker for neuronal progenitor cells. PN277 may be used as a unique immune-based therapy that offers long-lasting functional and behavioural recovery, even when given as late as 24 hours after insult.

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GK11, A NON COMPETITIVE NMDA RECEPTOR (NMDAR) ANTAGONIST, IS NOT NEUROTOXIC IN RODENTS AND PREVENTS MK-801-INDUCED NEUROTOXICITY

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After an initial CNS injury, over-activation of NMDARs, due to massive glutamate release, plays a key role in the extension of the secondary lesion. As a result, NMDARs antagonists are potential therapeutic compounds. However, by affecting the physiological functioning, NMDAR antagonists are also neurotoxic by themselves. So far these deleterious side effects have always prevented their clinical use. In this study, we have compared the intrinsic neurotoxicity of two high-affinity non-competitive NMDAR antagonists, MK801 and GK11, either in normal and injured rodent brain (i.e. photothrombotic cortical lesion). We used complementary techniques (behavioral tests, immunohistochemistry, light microscopy examination of semi-thin slices) to measure the changes observed in the cingulate cortex (all animals) and around the lesion (injured animals). Our results showed that under normal condition, the cingulate cortex from GK11-treated animals was undistinguishable from that of controls. On the contrary, MK801-treated rats displayed profound morphological changes: HSP70 immunoreactivity 24 h after the treatment, vacuolation and later on necrosis. Animals treated with MK801 also displayed delayed spatial memory acquisition compared to control and GK11-treated animals. In lesionned animals, although the injury was performed in the frontal cortex, cellular damage was observed in the cingulate cortex of all groups. Interestingly however, cellular alterations were weaker in GK11-treated animals. Thus, typical high affinity NMDAR antagonists, such as MK801, are neurotoxic even in pathological conditions. Conversely, GK11 displays an interesting profile both in terms of neuroprotection and neurotoxicity and appears as a potential drug for the treatment of CNS injuries.

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CHARACTERISTICS OF A RAT FETAL TRANSPLANTATION MODEL FOR CHRONIC CORTICAL GLIOSIS.

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Chronic activation of astrocytes and microglia can result in release of pro-inflammatory cytokines and reactive oxygen species. Gliosis is a common feature of many neuropathological states including neurodegenerative diseases (e.g. Alzheimer's disease), trauma and stroke. Although the neurotoxic consequences of gliosis are recognized, the mechanisms behind this process are poorly understood. We have previously demonstrated that chronic gliosis in an *in vivo* fetal neural transplantation model results in altered processing of many proteins implicated in Alzheimer's disease pathogenesis, namely amyloid precursor protein, presenilin-1 and apolipoprotein E [1,2]. Interestingly, this gliosis is dependent upon the site of transplantation suggesting that investigation into the mechanisms behind these observed reactive events may provide clues as to the triggers of gliosis in neurodegenerative disorders. Fetal cortical tissue was transplanted from embryonic day 15–16 animals to the midbrain and cortex of neonatal rats. The development of gliosis was examined from 2 weeks to 10 months post transplantation. The integrity of the blood brain barrier and vascular supply to grafts was studied using Evan's blue dye injection. Using immunohistochemical and histological techniques, the onset of reactive gliosis was observed 4 weeks post transplantation and continued for up to 10 months. This gliosis is accompanied by vascular and neurochemical abnormalities, protein extravasation at the graft-host interface and lipid peroxidation. This transplantation model is highly applicable to the study of the nature of oxidative stress and the proteins involved in reactive gliosis, and to test the efficacy of new therapeutic strategies for the prevention and treatment of neurodegenerative disorders.

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E NOVO PROLIFERATION FOLLOWING DIFFUSE TRAUMATIC AXONAL INJURY IN RATS

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It is generally accepted that injured adult brain has the potential for repair through the generation of neurons and/or glia from precursors localized in the subventricular zone (SVZ) and hippocampus. Recent studies have described the proliferative response and fate of precursor cells following focal brain injury, however, these processes have yet to be investigated after diffuse brain injury. Therefore, the purpose of the present study was to use the model of traumatic axonal injury (TAI) in rats (Marmarou et al, 1994) to study the proliferative response of cells in the SVZ and hippocampus. Adult rats were sacrificed at multiple time points (3 day, and 1,2, and 4 w; $n = 6$) following TAI or sham operation. To identify proliferating cells, immunohistochemistry using Ki-67 monoclonal antibody was performed on brain cryosections. Qualitative analysis showed that in the SVZ, the amount of Ki-67 positive cells increased approximately 2.5-ford compared to sham controls from 3 days following TAI, and remained elevated at 1, 2 and 4 weeks to 2.5, 1.6 and 1.8 fold of sham levels, respectively. In the hippocampus, the basal amount of Ki-67 positive cells was very low, but increased slightly following TAI. In conclusion, we show the first time that TAI induced marked cells proliferation in the SVZ, whereas in the hippocampus proliferation was very low. This may reflect the lack of vulnerability of the hippocampus to damage following TAI. Current study will determine the fate of the the proliferating cells in both regions using various glial and neuronal cell markers.

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TRANSPLANTATION OF HUMAN BONE MARROW STROMAL CELLS INTO CONTUSED ADULT RAT SPINAL CORD

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Background: It has been previously reported that bone marrow stromal cells (BMSCs) can promote axonal regeneration of lesioned adult rat spinal cord. For clinical application, it is necessary to prove whether BMSCs derived from human bone marrow (hBMSCs) have potential to restore injured spinal cord. Here we

show that transplantation of hBMSCs promotes axonal regeneration and functional recovery in lesioned adult rat spinal cord.

Materials and Methods: Tissue samples were obtained from 8-weeks old male Wistar rats. We performed laminectomy at T7 and T8 levels and provided contusion on spinal cord with NYU impactor (10 g, 25 mm). Nine days after contusion, mixture of Matrigel and hBMSCs (hBMSCs group) or Matrigel alone (MG group) was injected into the cavity in the lesion epicenter. In both groups, we assessed recovery of hind limb function using BBB locomotor scale. Five weeks after transplantation, cryosections of spinal cords including the lesion epicenter were made. Macroscopic examination and immunohistochemistry were performed in both groups.

Results and Discussion: In the hBMSCs group, hind limb function showed a tendency to recover better compared with that of the MG group. Macroscopic examination revealed that transplantation of hBMSCs reduced the cavity size. Immunohistochemstry revealed that nerve fibers extended into the cavity filled with hBMSCs. These evidences show that transplantation of hBMSCs promotes axonal regeneration of lesioned spinal cord, resulting in recovery of hind limb function. In conclusion, transplantation of hBMSCs is potentially useful treatment for spinal cord injury.

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SCHWANN CELLS TRANSDUCED WITH CNTF ENHANCE RETINAL GANGLION CELL SURVIVAL AND AXONAL REGENERATION THROUGH CHIMERIC PERIPHERAL NERVE GRAFTS IN ADULT RATS

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Ciliary neurotrophic factor (CNTF) promotes the regeneration of adult retinal ganglion cell (RGC) axons into peripheral nerve (PN) grafts [1]. We recently developed a method for the cellular reconstitution of freeze-thawed PN segments, grafts of which also support regeneration [2]. In the present study we used lentiviral (LV) vectors encoding CNTF (LV-CNTF) to transduce adult purified Schwann cells (SCs) *ex vivo*. These cells were used to repopulate freeze-thawed PN segments which were then transplanted onto the transected optic nerve of adult Fischer rats. Animals survived for 4 weeks before fluorogold (FG) was injected into the distal end of the PN grafts. Counts of FG retrogradely labeled RGCs revealed that the number of regenerated RGCs was 7-fold higher compared to control PN containing SCs transduced with LV-green fluorescent protein (LV-GFP) (LV-GFP mean $=$ 393/retina $\pm$ 227, $n = 7$; LV-CNTF mean $=$ 2636/retina $\pm$ 1071, $n = 12$). Staining with an antibody to III-tubulin (TUJ1) revealed a 2-fold increase in RGC survival (LV-GFP mean $=$ 5586/retina $\pm$ 1070, $n = 7$; LV-CNTF mean $=$ 11862/retina $\pm$ 1263, $n = 8$). Immunostaining of cryosections of PN grafts with pan-neurofilament antibodies showed higher numbers of regrowing axons in nerves reconstituted with LV-CNTF transduced SCs compared with controls. The use of genetically engineered, reconstituted PN grafts to bridge tissue defects could provide a clinical alternative to using multiple PN autografts to promote regrowth in injured CNS.

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EFFECTS OF TRANSPLANTED OLFACTORY ENSHEATHING GLIA OR SCHWANN CELLS IN RAT SPINAL CORD CONTUSION INJURY

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Numerous cell transplantation strategies have been investigated in the attempt to encourage axonal regeneration following spinal cord injury. Olfactory ensheathing glia (OEG) are thought to facilitate regrowth of axons into the CNS during the turnover of olfactory neurons that occurs throughout adult life. A number of studies have associated OEG transplantation with improved recovery following SCI in rats. A previous study examined the effects of OEG transplantation following a delay of seven days after injury. In the present study the delay was fourteen days between contusion injury and the transplantation of either OEG or Schwann cells. Control groups received an injection of medium or no treatment. In order to maintain the clinical relevance of the methods used, adult rats were used as both the source of donor cells and as hosts. Cells were permanently labelled before transplantation, using a lentiviral vector to deliver the gene for DsRed, a red fluorescent protein. Permanent la-

belling allowed the cells to be easily visualised in tissue sections, in association with immunohistochemically detected astrocytes, axons and macrophages. Cell transplantation was associated with a 35% ($p < 0.04$) greater volume of tissue at the lesion site, compared to medium-injected controls. However, there was no difference in the degree of behavioural improvement achieved by each experimental group, measured using a horizontal ladder walking test. Retrograde fluorogold labelling of several descending systems was employed to examine the effect of cell transplantation on axonal regeneration/sparing following spinal cord contusion injury.

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CHARACTERIZATION OF SIGNALING PATHWAYS IN CAMP/CILIARY NEUROTROPHIC FACTOR- INDUCED RETINAL GANGLION CELL SURVIVAL AND AXON REGROWTH

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Neurons of the peripheral nervous system (PNS) regenerate following injury to their axons, whereas central nervous system (CNS) neurons do not. One model in which to study CNS repair involves the transplantation of peripheral nerve (PN) bridges to an injury site. Axotomized retinal ganglion cells (RGCs) regenerate their axons into PN grafts sutured onto the cut optic nerve. Recent studies from our laboratory revealed enhanced regeneration of RGC axons into PN grafts after multiple intraocular injections of ciliary neurotrophic factor (CNTF) and a membrane-permeable analogue of cAMP, CPT-cAMP. The exact mechanisms underlying this observed increase in regeneration are not known. This study examined the effects of blocking major signaling pathways downstream of cytokines (JAK/STAT, PI3-kinase, MAP kinase) and cAMP (cAMP-dependent protein kinase, PKA) on enhanced RGC survival and regrowth. The left optic nerve was transected 1.5 mm behind the optic disk and a 1.5 cm piece of peroneal nerve was autografted onto the cut nerve. In each experimental group, CPT-cAMP, CNTF and the particular pathway inhibitor were intraocularly injected 3, 10, 17 days after the optic nerve-PN graft procedure. Activity assay and immunoblots showed that inhibitors effectively inhibited the activity of designated pathways in injected eyes. Pharmacological inhibition of either PKA, JAK/STAT, PI3-kinase or MAP kinase (ERK) reduced CNTF and CPT-cAMP-induced survival of axotomized RGCs (average reduction $\sim$ 30%). However the effects on CNTF/cAMP-mediated axonal regeneration were even more striking; all inhibitors significantly reduced RGC axonal regrowth into PN grafts by as much as 60–70%.

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A NOVEL ROLE FOR OLFACTORY ENSHEATHING CELLS IN NEUROIMMUNOLOGICAL DEFENCE IN THE RODENT OLFACTORY SYSTEM.

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The dendrites of olfactory receptor cells are exposed to the external environment whilst their axons project directly into the olfactory bulb in the CNS. This provides a route for pathogens into the brain that bypasses the blood-brain barrier, yet infection via this route appears to be limited by local defences within the olfactory system. Given the close apposition of olfactory ensheathing cells (OECs) to olfactory receptor axons along their entire projection, these glial cells could constitute an important component of the olfactory immune defence. We recently detected enriched transcripts for several immune factors in cultured neonatal OECs by microarray analysis, including lysozyme (Lyz), the chemokines Gro1 (CXCL1) and MCP1 (Ccl_2), and the interleukin 6-responsive transcription factor CCAAT/enhancer binding protein beta (Cebpb). Lyz, MCP1 and Gro1 expression were confirmed by real-time RT-PCR (Cebpb not tested). We detected all gene products in cultured OECs by immunostaining, with intense staining for Lyz and Cebpb and weak staining for the chemokines. OECs in the normal adult olfactory system are strongly immunopositive for Lyz and Cebpb, weakly immunopositive for MCP1 and negative for Gro. Lyz mRNA is inducible in OECs when cultured in the presence of Staphylococcus aureus but not Escherichea coli, whereas both chemokines are strongly induced by the latter. We are currently testing the function of these immune factors in OECs in culture and in the olfactory system.

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OLFACTORY ENSHEATHING CELLS EXHIBIT DIFFERENT RATES OF PROLIFERATION AND APOPTOSIS IN THE PRESENCE OF UNINJURED AND INJURED SPINAL CORD TISSUE

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Olfactory ensheathing cells (OECs) have been investigated as a means of repairing the injured spinal cord (SC). However, little is known about OEC phenotype and behaviour within the injured SC. To gain some insight into OEC physiology in the SC environment OECs were co-cultured with injured and uninjured rat SC explants *in vitro*. Explants were obtained from 4 groups: SC that had been subjected to (1) acute needle stab injury, 6 hours post-injury, (2) chronic needle stab injury, 4–5 weeks post-injury, (3) chronic contusion, 4–5 weeks post-injury and (4) uninjured SC. The proliferation rates of OECs co-cultured with explants from these four groups were 23.4%, 37.9%, 23.2% and 28.1% respectively. In the absence of any explants, OECs showed a proliferation rate of 13.7%. The proliferation rate of the p75 negative cell population (possibly fibroblasts) was significantly higher than that of the OECs in the presence of each type of SC explant. Although apoptotic cells stained with active caspase-3 were infrequently observed in all experimental groups, significantly more OECs were apoptotic in the presence of acutely stabbed SC explants (2.9%) than in the presence of both chronically stabbed (1.8%) and chronically contused (1.1%) SC explants. We conclude that the proliferation and apoptosis of OECs differ significantly in the presence of acutely or chronically injured SC tissue and may be influenced by the severity of the injury, and the inflammatory and reparative processes occurring in the injured tissue at the time.

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ADULT OLFACTORY ENSHEATHING GLIA PROMOTE THE REGROWTH OF ADULT RETINAL GANGLION CELL AXONS IN VITRO.

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In vivo, transplanted adult olfactory ensheathing glia (AEG) and adult Schwann cells (SC) support the regrowth of at least some transected axons within adult CNS neuropil. However, the beneficial influence of SCs may be limited by interaction with host astrocytes. We used an *in vitro* adult rat retinal ganglion cell (RGC) explant model to explore the influence of purified adult SCs and AEG on retinal neurite outgrowth in the presence of retinally-derived astrocytes. Quadrants of retina obtained from euthanized adult Fischer rats were plated RGC side down onto type 1 collagen beds, collagen coated with AEG, or collagen coated with SCs. Regrowing neurites extended onto the pure collagen substrate, the growth mostly associated with astrocytes migrating from the retinal explants. The additional presence of AEG, but not SCs, resulted in a significantly greater number of regrowing neurites compared to the control (collagen alone) treatment. Furthermore, AEG-enhanced regrowth was over significantly greater distances, with more than 50% of neurites extending > 1 mm from the explant. The limited extension of neurites over SCs may in part be explained by the frequent number of neurites observed growing onto, but not leaving, clusters of SC. RGC explants fed with media conditioned by purified AEG or SCs, showed no detectable difference in the regenerative abilities of RGCs compared to control treatments. The differences between the observed abilities of AEG and SCs to support the regrowth of RGC neurites may be attributed to the AEG producing an environment more favourable for CNS regeneration.

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EXERCISE COUNTERACTS THE GROWTH INHIBITORY ACTION OF MYELIN IN THE ADULT CNS USING A BDNF-MEDIATED MECHANISM.

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To successfully regenerate, neurons need to overcome the effects of hostile environments such as the inhibitory effects of myelin. The capacity of exercise to enhance neural plasticity is getting increasing recognition, but the capacity of exercise to reduce growth inhibitory signals remains a fascinating possibility. Here we show that animals exposed to voluntary exercise for periods between 3 and 28 days have a decrease in myelin-associated glycoprotein (MAG) in the lumbar region of the spinal cord. MAG is a major source of myelin inhibition. Since exercise induces BDNF, a neurotrophin shown to help overcome the inhibitory effects of myelin, we used specific immunoadhesin chimera to selectively block the function of BDNF during exercise. BDNF blockade abrogated the decrease in MAG after exercise. In addition, BDNF blockade suppressed an

exercise-induced increase in the active and total forms of the mitogen activated protein kinase (MAPK-II). The MAPK system is an intermediate step for the action of BDNF on neuronal growth. MAPK seems to signal through the protein kinase A (PKA) system to affect neuronal growth. We measured PKA as increases in PKA have been related to the ability of BDNF to overcome growth inhibition. Exercise increased PKA levels, and this effect was suppressed by BDNF blockade. The overall results indicate that exercise enhances the regenerative capability of neurons in the adult CNS by increasing growth and decreasing inhibition using a BDNF-mediated mechanism. (Supported by NIH award NS39522 and UCLA BIRC).

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REPAIRING THE HUMAN SPINAL CORD WITH NASAL OLFACTORY ENSHEATHING CELLS

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Transplantation of olfactory ensheathing cells promotes behavioural, anatomical and physiological recovery after spinal cord transection in rat. We are undertaking a Phase I clinical trial in humans, in which autologous olfactory ensheathing cells are transplanted into the injured spinal cord of paraplegic patients. The primary aim of this trial is to establish that the procedure is safe. A secondary aim is to assess the patients for any positive outcome. Six participants have been enrolled: three whom have undergone transplantation surgery (the Transplant group), and three whom have had no surgery (the Control group). The participants undergo rigorous examinations including physical, medical and psychosocial assessments, X-ray, MRI and neurological and neurophysiological assessments. On completion of these initial examinations and in-depth instruction on the trial, the participants were selected and gave informed consent to enter the trial. The surgical patients had a biopsy of olfactory mucosa under general anaesthesia. Olfactory ensheathing cells were then isolated from this tissue and purified and expanded in number in the laboratory for about six weeks. The surgical patients then underwent a laminectomy under general anaesthesia and the patient's own olfactory ensheathing cells were implanted into the damaged spinal cord using a specially designed delivery device. All participants were assessed with the same set of protocols outlined above at three-month intervals for two years and again at three years. All assessments are made with the assessors blind to the participant status (Transplant or Control). The outcomes of this trial cannot yet be divulged, as it is ongoing.

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MECHANISMS OF ACUTE CERVICAL CORD INJURY ASSOCIATED WITH OPLL

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Objectives: The patients with ossification of the posterior longitudinal ligament (OPLL) sometimes present with acute spinal cord injury by minor trauma. In the present study, we reviewed our experience of acute cervical cord injury associated with OPLL to understand the pathomechanisms and to provide clinical information for management of this disorder.

Materials: Thirty patients between 1991 to 2002 were retrospectively analyzed. There were 28 men and 2 women, aged from 45 to 78 years (mean 63.3 years). Most patients showed incomplete spinal cord injury (Frankel grade A: 3, B: 1, C: 16, D: 10).

Results: Radiological studies showed continuous or mixed type OPLL in 16 patients and segmental type OPLL in 14 patients. The sagittal diameter of the spinal canal was reduced to 4.1–10 mm at the narrowest level due to OPLL. Developmental size of the spinal canal was significantly smaller in the patient group of segmental OPLL. Magnetic resonance (MR) imaging demonstrated that spinal cord injury occurred predominantly at the caudal edge of continuous type OPLL or at the disc levels. Surgical treatment was performed in 26 patients either by posterior (19 patients) or anterior (7 patients) decompression at various time intervals from trauma. Twenty-two patients (73%) showed improvement of Frankel grade.

Conclusions: The present study demonstrates the pre-existing factors and pathomechanisms of acute spinal cord injury associated with cervical OPLL. MR imaging is useful to understand the level and mechanism of injury. Further investigation will be needed to elucidate the role of surgical decompression.

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DEFINING PROGNOSIS AFTER ACUTE SPINAL CORD INJURY (SCI)
R Marshall, J Clark (*Adelaide, Australia*)

Our research has concentrated upon descriptions of bone loss, with reference to duration of SCI and both severity and degree of injury. An observation of radiodensitometric variability in the bone loss of neurologically diverse SCI groups, led to an examination of surrogate indices of neurological recovery and a postulated role for bone resorption markers. Serial blood and urine were obtained during a prospective study at 3, 6, 12, and 24 wks from SCI onset and, levels ascertained using standard, biochemical assays. Routine physical and behavioural outcomes were evaluated using a battery of standardised tests and biological data then separated according to outcome for group comparisons:- complete motor paralysis (SCIc), motor recovery (SCIr). Concentrations of bone resorption markers reached peak amplitude by 12 weeks (Pyr:Cr, SCIc 618 $+/-$ 339, SCIr 277 $+/-$ 101 nmol.mmol^{-1}). Upon comparison of SCIc and SCIr groups, significance was identified for resorption indices at 3 wks ($p = 0.02$), $p < 0.01$ at 6 wks and $p < 0.001$ by 12 wks. Due to wide inter-individual variability, it is suggested that specificity and precision are refined for wider prognostic purposes. Broadly, in controlled conditions bone resorption markers appear to discriminate prognosis within a neurologically heterogeneous, SCI cohort. Additionally the present observations are consistent with CNS participation in the pathophysiology of bone loss in the SCI group.

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HIGH RESOLUTION MRI AND CORRELATIVE IMMUNOHISTOCHEMISTRY ON ARCHIVED POST MORTEM HUMAN SPINAL CORD FOLLOWING TRAUMATIC INJURY
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The present post-mortem study provides a correlation between high resolution magnetic resonance imaging MRI (9.4 tesla) and immunohistopathology in an archived sample of traumatically injured human spinal cord. The tissue had been removed from the spinal cord of a patient who had died 7 months after a severe macerating injury at level C5. Proton density weighted MRI was performed in 3 orthogonal planes and clearly revealed both white and gray matter. The normally heavily myelinated white matter funiculi demonstrated a low intensity signal. An abnormal, symmetrical hyperintense signal could be detected in the white matter columns and funiculi that were undergoing Wallerian degeneration, including the lateral (crossed) and ventral (uncrossed) corticospinal tracts, the anterior reticulospinal and vestibulospinal tracts as well Schultze's comma tract in the posterior column. These changes correlated closely to the immunohistochemical data which revealed corresponding areas to be almost completely devoid of neurofilament-positive axons, but containing myelin debris that was scattered amongst reactive hypertrophic astrocytes and rounded phagocytic macrophages. Such correlative investigations of high field MRI in spinal cord undergoing Wallerian degeneration are relatively rare but are important for obtaining a better understanding of the images generated by this approach, as well as their relevance to the on-going pathophysiological processes that take place in such conditions.

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THE FIRST EXPERIENCE OF TREATMENT OF ACUTE SPINAL CORD INJURY PATIENTS BY PROTEINASE INHIBITOR.
P Katunyan P, T Klushnik, I Scherbacova, S Pushkin, D Merenkov, N Koulyakina (*Moscow, Russia*)

The previous research shows that the intravenous (i/v) infusions of blood substitute, known as "Perftoran" (Pf) reduced the elastase activity (EA) in blood.

Aim: To clarify the relationship between clinical results and the EA level in serum and cerebrospinal fluid (CSF) of patients with acute SCI after treatment by Pf. The EA was measured before and 3 weeks after treatment. The acute SCI patients classified into 3 groups: macrodex solution or Pf medullar lavage and i/v infusions treatment 19 (1st group) and 22 (2nd group) patients respectively; 3–4 months post-injury only i/v Pf treatment (3rd group, 14 patients). The EA was detected as 150 $-/+$ 10.3 nM/min/ml in normal serum and was no detected in normal CSF. The EA was detected as 344 $-/+$ 62.7 in serum and 14.1 $-/+$ 4.3 nM/min/ml in CSF after SCI. The EA was of no change significantly in mostly patients of 1st group. The serum EA was declined to 189.6 $-/+$ 61.3 nM/min/ml and to 4.7 $-/+$ 1.3 in CSF in cases of the successful Pf treatment in 2nd group. The re-

turn of walking ability was observed 2–4 weeks post-surgery in these cases. A decrease of serum EA till 213 $-/+$ 38.1 nM/min/ml was observed in 3rd group without clinical results. Results: The immediate medullar lavage and i/v infusions by Pf in acute SCI after decompression surgery result recuperation of the leg motor function to 13 of 22 paraplegic patients in contrast of 5 of 19 same patients without Pf treatment.

Conclusion: The high proteinase inhibition capacity of Pf may limit antimyelin and antiendothelial activity of neutrophil elastase and protect medullar tissue against metabolic disintegrations during first hours after the SCI.

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AM36 REDUCES NITRIC OXIDE PRODUCTION IN PRIMARY MICROGLIAL CULTURES

S Feeney, B Jarrott, J Callaway (*Melbourne, Australia*)

The activation of microglia has been implicated in the secondary pathology that accompanies trauma to the brain and spinal cord [1]. On activation microglia undergo a number of structural and phenotypic changes including increased expression of protease enzymes, cytokines and free radicals such as nitric oxide leading to neurotoxic effects [2]. As this secondary injury contributes to neuronal damage it provides a target for pharmacological intervention. The ability to study primary microglia *in vitro* allows the effect of neuroprotective agents to be examined on the activation of microglia to determine if this is one of the mechanisms of neuroprotection. AM36, a novel neuroprotective compound, has been shown to reduce expression of inducible nitric oxide synthase after focal ischemia [3]. To further understand the antioxidant neuroprotective action of AM36 we examined nitric oxide production by Griess Reagent in cultured rat microglia that were activated with the bacterial endotoxin lipopolysaccharide (1 ng/ml – 50 ug/ml) for 16 hours following a 30 minute pre-treatment with AM36 (1 uM – 10 uM). We report that AM36 reduced nitic oxide production at 5 uM at all LPS concentrations used and reduced nitric oxide production at 10uM at all LPS concentrations with significant reductions demonstrated at 0.5 ug/ml – 50 ug/ml. These results indicate that AM36 may contribute to neuroprotection by reducing nitric oxide release from microglia.

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AM-36 AND MINOCYCLINE DECREASE DAMAGE AND RECOVERY TIME IN A REVERSIBLE SPINAL CORD INJURY MODEL IN RATS

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Spinal cord injury (SCI) usually occurs to young individuals leading to permanent paraplegia and quadriplegia, and increased costs to society. Our research focusses on developing drugs to minimise the effects of SCI. Thus we undertook a study of two putative neuroprotectants, AM-36, which acts as both a sodium channel blocker and antioxidant [1], and minocycline which inhibits microglial activation [2]. Long Evans rats were anaesthetized (pentobaritone 100 mg/kg i.p.), and laminectomy performed at spinal level T12. An inflatable balloon catheter was inserted underneath the vertebra and inflated for 5 minutes, causing reversible paraplegia. Rats had almost complete functional recovery by 15 d. AM-36 (6 mg/kg, i.p.) or vehicle were administered at 3 h after the injury and daily thereafter, until sacrificed, whilst minocycline (45 mg/kg i.p.) was administered at 3 h and twice daily until sacrificed. Behavioural tests were conducted daily. At 15 d post-injury, rats were anaesthetised and transcardially perfused, to fix the spinal cords. Sections were processed to examine the size of the cyst, and showed that AM-36 and minocycline decreased cyst size by $\sim$ 35% and $\sim$ 25% compared with untreated rats. AM-36 and minocycline treatment significantly decreased the recovery time following SCI, as seen in the ladder walking test, with differences from untreated rats at 7 d and 8 d. These differences remained significant from untreated rats for the remainder of the study (ANOVA, $P < 0.05$). These data indicate that AM-36 and minocycline have neuroprotective effects in SCI.

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MACROPHAGES AND MICROGLIA: SOMETIMES SAVIOURS, SOMETIMES KILLERS.

PE Batchelor, S Denii, M Katz, T Wills, S Tan, S Lockhart, DW Howells (*Melbourne, Australia*)

CNS trauma results in peri-wound sprouting but axonal regeneration across the lesion site fails. Our studies have demonstrated that activated macrophages and microglia stimulate peri-wound sprouting in the rodent brain. Fibers grow over these cells in the peri-wound area and axonal growth ceases around macrophages at the lesion edge. Macrophage expression of BDNF and GDNF is highest at the wound edge but low in the wound interior. We find that if an ongoing gradient of BDNF and GDNF is supplied, axonal growth continues beyond the lesion edge. BDNF and GDNF were covalently attached to microspheres and these implanted into the striatal injury site 1 week after lesion. A profusion of axons grew out from the lesion edge across the surface of the spheres. This data suggests that sprouting fibers normally grow towards the wound edge along a cellular derived trophic gradient. Growth normally stops at the point of maximal neurotrophic factor expression but can be encouraged beyond this point through provision of an ongoing artificial trophic gradient.

In the rodent spinal cord we have found that the microglial/macrophage response to injury is an order of magnitude greater in the white and grey matter of the spinal cord than the brain. The intensity of this inflammatory response appears to induce greater secondary damage and necrotic cavitation.

Overall, macrophages appear capable of existing in either cytotoxic or growth stimulating phenotypes. In recent *in vitro* studies we have found ways to alter macrophage phenotype and attenuate cytotoxicity.

Supported by the Victorian Trauma Foundation.

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THE PHENOTYPE OF ACTIVATED MACROPHAGES ASSOCIATED WITH SPINAL CORD REPAIR.

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Severe spinal cord injury (SCI) results in primary tissue destruction that leaves the environment hostile to tissue survival and healing, causing secondary damage. Though repair mechanisms, like neurogenesis, axonal sprouting and activation of cellular immunity, have been demonstrated in the injured CNS, they fail to restore function. Recently we showed that controlled tissue-specific immune activity induces functional recovery after SCI. Implantation of rat blood-derived macrophages co-incubated with skin improved functional recovery of severely contused rats. Human skin-coincubated macrophages are now in phase II clinical study in patients with acute, complete SCI. We have compared human skin-co-incubated macrophages to known types of macrophage activations: classical (incubated with lipopolysaccharide) and alternative (incubated with interleukin-4), for their immunological phenotype and biological activity on neuronal survival and neurite outgrowth *in vitro*.

Conditioned media from cultures of lipopolysaccharide-activated macrophages adversely affected the neuronal growth and survival of cultured rat cortical neurons. In contrast, conditioned media from cultures of skin- or IL-4-activated macrophages had no such effect. Secretion of proinflammatory cytokines was high in lipopolysaccharide-activated macrophages but virtually absent in IL-4-activated macrophages. Skin-activated macrophages secreted intermediate levels of interleukin-1 and interleukin-6, but no TNF-alpha. The number of CD80-positive macrophages was elevated in cultures incubated with lipopolysaccharide as compared to macrophages incubated with skin or IL-4. Skin-activated macrophages thus represent a unique cellular phenotype, distinct from “classically” or “alternatively” activated macrophages. We propose that this phenotype supports an immune response that promotes neuronal cell survival and repair, resulting in wound-healing and functional recovery.

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EFFECTS OF GRANULOCYTE COLONY STIMULATING FACTOR (G-CSF) ON SPINAL CORD INJURY IN MICE

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Objective: The aim of the present study is to elucidate the efficacy of granulocyte-colony stimulating factor (G-SCF) to treat spinal cord injury.

Materials and Methods: Bone marrow cells were isolated from male green fluorescent protein transgenic (GFP Tg) mice and transplanted intravenously into tail vein of lethally irradiated female C57BL/6 mice. Four weeks after bone marrow transplantation, spinal cord injury was produced by a static load (20 g, 5 min.) at the T8 level. G-CSF (200 μg/kg/d) was injected subcutaneously for 5 days. Hind limb motor recovery was assessed with Motor Function Scale (Farooque, 2001). Spinal cord section was made for histology. Immunohistochemistry for GFP and cell lineage markers was performed to evaluate mobilization of bone marrow-derived cells into injured spinal cord.

Results and Discussion: G-CSF treated mice showed significant recovery of hind limb function compared to that of the control mice. Immunohistochemistry revealed that GFP-positive cells were detected in the spinal cord sections, indicating that bone marrow-derived cells were mobilized and migrated into injured spinal cord. In conclusion, G-CSF may have beneficial effect for spinal cord repair.

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GLUTAMINE TREATMENT FOLLOWING SPINAL CORD INJURY IN RATS INCREASES FUNCTIONAL OUTCOME AND TISSUE SPARING

ST Rigley, E SchËltke, JD Golding, BHJ Juurlink (*Saskatoon, Canada*)

Secondary damage after spinal cord injury is mediated by oxidative stress and inflammation. Following injury, concentrations of the potent antioxidant glutathione (GSH) are decreased in the spinal cord which potentiates mechanisms of secondary damage. In an attempt to maintain the GSH concentrations, the non-essential amino acid glutamine was tested as it was shown to increase GSH concentrations both *in vivo* and *in vitro*. To examine the therapeutic potential of glutamine, spinal cord trauma was induced in male Wistar rats by a compression injury using a 50 g aneurysm clip at the level of T6. One mmol/Kg glutamine or vehicle was injected intraperitoneally 30 minutes after surgery and every 12 hours after for one week. Following injury blood GSH concentrations decreased, however a single intraperitonal injection of 1 mmol/Kg glutamine caused blood GSH concentrations to recover to basal levels by 2 hours and were maintained for 10 hours. Locomotor function and hind limb strength were assessed weekly for 6 weeks after which animals were sacrificed and tissues collected. Animals that received glutamine had dramatically increased locomotor function, hind limb strength and more tissue spared than the vehicle control. These findings support the ability of glutamine to reduce oxidative stress and increase functional recovery following spinal cord injury. This research is funded by the Christopher Reeve Paralysis Foundation.

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MORPHOLOGICAL REPAIR AND FUNCTIONAL RECOVERY FOLLOWING SPINAL CORD TRANSECTION IN THE DEVELOPING OPOSSUM

MA Lane, KM Dziegielewska, NR Saunders (*Melbourne, Australia*)

Unlike in the adult mammal, the immature spinal cord exhibits a remarkable degree of axonal repair and functional recovery following injury. The present work uses the South American opossum, Monodelphis domestica, to examine the effects of spinal cord injury at postnatal day 7 or 14 (P7 or 14). Animals received a complete mid-thoracic spinal cord transection whilst attached to the mother. Mothers were anesthetized with halothane, and pups with methoxyfluothane as required. All experiments were conducted following NH&MRC guidelines, and with approval of the University Animal Ethics Committee. Animals were collected at intervals within the first two-weeks following injury, or left to recover until adulthood. Changes in cytoarchitecture and axonal growth over time were examined with histological stains and immunohistochemistry. Animals left until adulthood underwent functional testing, and tracing studies. Fluorescently labeled dextran amine was used to retrogradely trace supraspinal projections made through the injury site. Axonal volume passing through the injury site was quantified. Growth across the injury site was observed 5 days post-injury in P7 animals. Growth was delayed

in animals injured at P14. The axonal volume in the injury site of adults injured at P14 was less than seen in P7 injured animals. However, this study shows that supraspinal growth across the injury site, and functional recovery, is comparable between animals injured at either age. Furthermore, functional recovery correlated with measurements of morphological repair.

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RE-EMERGENCE OF 3CB2 ANTIGEN AS A SPECIFIC MARKER OF RADIAL GLIA AFTER SPINAL CORD INJURY IN ADULT RATS

S Shibuya, O Miyamoto, T Itano, S Mori, H Norimatsu (*Kagawa, Japan*)

Recent studies have proved the existence of neural stem cells in the adult CSN including the spinal cord, and demonstrated various changes in response to injury and ischemia. Moreover, radial glia (RG) that emerge at the developmental stage has been shown also to possess the properties of neural stem cells. We used a rat spinal cord injury (SCI) model to investigate the regeneration and repair mechanism by an immunohistochemical study to verify whether RG emerge in spinal cord tissue and to identify its localization and pattern of expression. SD rats ($n = 25$) had a laminectomy at Th11-12, and SCI was created by compression with a 30 g weight for 10 min. In the injury group, rats were examined 24 h and 1, 4 and 12 weeks after injury. Frozen sections 20 μm in thickness were prepared from regions 5 and 10 mm rostral and caudal to the injury epicenter. Immunohistochemical staining was performed using antibodies to 3CB2, nestin and GFAP. 3CB2 expression started at one week after injury and peaked at 4 weeks. Cells showing long arborizing processes were observed below the pial surface and extending along the whole circumference. From 4 weeks after injury, 3CB2 expression was observed in the gray matter around the central canal. In double immunohistochemistry, these 3CB2-positive cells merged completely with nestin and GRAP expression. These results demonstrate that emergence of RG derived from subpial astrocytes after SCI is associated with neural regeneration, and that a phenomenon equivalent to vascular niche occurs in the gray matter.

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INACTIVATION OF THE P75 NEUROTROPHIN RECEPTOR AFTER COMPRESSIVE SPINAL CORD INJURY IN MICE DOES NOT NECESSARILY DECREASE APOPTOSIS OR IMPROVE FUNCTIONAL RECOVERY AND THE EFFECT IS STRAIN DEPENDENT

GKT Chu, WR Yu, MG Fehlings (*Toronto, Canada*)

Introduction: Apoptotic cell death may play an important role in spinal cord injury (SCI). After dorsal hemisection of the spinal cord, p75 knockout mice with the C57/Bl6 strain have decreased apoptosis caudally. However, dorsal hemisections are not common compared to compression injuries, therefore we hypothesize that after compressive SCI, p75 receptor inactivation would decrease apoptosis and improve functional recovery.

Methods: Two strains (C57/Sv129 and C57/Bl6) of mice were used. Each strain had knockout and wild type mice. The mice were injured with an 8.4 g clip (Fejota) at the T6 level. Three and seven days after injury, the lesion site was extracted for immunoblotting of cleaved caspase-9, and caspase-3. For functional recovery, the animals were assessed with a modified Basso, Beattie, and Breshnahan (BBB) locomotor rating scale.

Results: The C57/Sv129 knockout mice had decreased cleaved caspase-3 levels at seven days compared to wild type. The knockout mice had an increased BBB score (3.78) after 6 weeks compared to wild type (1.07). The C57/Bl6 knockout mice had decreased levels of cleaved caspase-9, at three days. However, there was no difference in cleaved caspase-3 levels between wild type and knockout mice at seven days for this strain. After eight weeks, the wild types had greater BBB scores (7.11) than the knockouts (3.82).

Conclusions: Unlike dorsal hemisections of the spinal cord, the inactivation of the p75 receptor does not decrease apoptosis after compression and may worsen functional recovery. Furthermore, background strain differences can modify the response to SCI so murine injury studies should be interpreted carefully.

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METHYLPREDNISOLONE REDUCES SPINAL CORD INJURY IN RATS WITHOUT AFFECTING TUMOR NECROSIS FACTOR-A PRODUCTION.

Y Taoka, T Shimakawa, K Yagi, T Chikawa, K Okajima (*Tokushima, Japan*)

Methylprednisolone (MP) is the only therapeutic agent currently available for traumatic spinal cord injury (SCI). However, little is known about its therapeutic mechanism(s). We have demonstrated that tumor necrosis factor-a (TNF- a) plays a critical role in post-traumatic SCI in rats. Since MP has been shown to inhibit TNF- a production *in vitro*, it is possible that MP can reduce SCI by inhibiting TNF- a production. To examine this possibility, we investigated the effect of MP in rat SCI. Leukocytopenia and high-dose intravenous administration of MP markedly reduced the motor disturbances and histolohical damage observed following spinal cord trauma. Leukocytopenia significantly reduced tissue levels of both TNF- a m-RNA and TNF- a, and the accumulation of leukocytes in the injured segments, while MP had no effects. Lipid peroxidation and vascular permeability at the site of spinal cord lesion were significantly reduced in animals with leukocytopenia and in those given anti-P selectin monoclonal antibody, and in those given MP compared to sham-operated animals. These findings suggested that MP reduces the severity of SCI not by inhibiting the production of TNF- a at the injured site, but by inhibiting activated leukocyte induced lipid peroxidation of the endothelial cell membrane. We have recently reported that some therapeutic agents that inhibit TNF- a production attenuate the motor disturbances induced by compression trauma of spinal cord in rats (Taoka et al., J. Neurotrauma, 2000). These observations raise the possibility that MP and some pharmacological agents that inhibit leukocyte activation may have synergistic activities when used in the treatment of SCI.

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TREATMENT OF SPINAL CORD INJURY VIA TOPICAL PERFUSION WITH AN ATP SOLUTION

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ATP depletion damages ATP sensitive ion channels following spinal cord injury (SCI), leading to membrane failure and cell death. These processes may be reversed by the application of exogenous ATP. ATP solution, stabilized in a lecithin suspension, has been shown to protect hepatic tissue and skin following injury (unpublished data). Besides supporting metabolism, ATP may be trophic in neurogenesis and cell survival by acting on its P2X and P2X receptor subunits. We suggest that exogenous administration of ATP solution to the spinal cord following SCI may prevent irreversible damage and improve functional recovery. Sixteen Sprague-Dawley rats received a 25 gm-cm SCI at T10 and were assigned into one of two groups: 1) injury/vehicle (control: $n = 8$) and 2) injury/ATP enhanced solution containing 3000 μmol ATP/ml ($n = 8$). A perfusion well was designed to irrigate ATP solution to the injured spinal cord. Flow rate was 5 ml/hour for 24 hours. Locomotion was tested weekly for 6 weeks after SCI using the BBB score. At week 6, rats were fixed, and their spinal cords were assessed for lesion size and tissue preservation. BBB scores were higher in rats irrigated with the ATP enhanced solution compared to the control group. Locomotor function was significantly better in the ATP-treated groups at 2, 4, and 5 weeks ($p \leqslant 0.05$). Tissue sparing and lesion size of the spinal cords will be analyzed after 6 weeks. We conclude that topical application of exogenous ATP provides functional and anatomical neuroprotection following moderate SCI.

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NEUROPATHIC PAIN IN SPINAL CORD INJURY (SCI): IS PREMORBID BONE MINERAL DENSITY (BMD) A RISK FACTOR?

J Clark, M Jelbart, J Strayer, H Rischbieth, R Marshall (*Adelaide, Australia; Cincinnati, USA*)

Background: Pain is a universal experience in early SCI due to the prevalence of nerve, bone and soft tissue trauma. However, the participation of deeper structures in the spectrum of risk for chronic pain does not appear to have been reported. The present authors previously described SCI bone loss from acute admission. These radiological and biochemical data offered a window of opportunity to examine variables that may predispose to chronic pain.

Subjects and Methods: Thirty (30) patients, all men, 16–52, 27 $\pm$ 8 years, ASIA A-D, C4-T12 participated. BMD (DEXA) and bone biochemistry were determined 3, 6, 12 and 24 weeks from injury. Pain status was identified at rehabilitation discharge. Group data were

analysed en masse; and after extraction of heterotopic ossification (HO) cases. All data are reported as means, SD, t-tests 5% probability unless otherwise stated.

Results: Thirty six %, 11 patients, 30 $\pm$ 10 years, ASIA A-D, 8 tetraplegia, experienced chronic pain. Nineteen were pain free at discharge (26 $\pm$ 6 years, ASIA A-D, 6 tetra). Admission hip BMD for the chronic pain group was 1.01 $\pm$ 0.1 g.cm^{-2} (3 wks), and 1.16 $\pm$ 0.1 for the asymptomatic group (p=0.01*). Similar trends were seen at 6 wks (1.03 $\pm$ 0.1 g.cm^{-2}, pain) and (1.13 $\pm$ 0.1, $p = 0.02$*; asymptomatic group). Extraction of hip BMD data for HO patients (5, all asymptomatic) did not influence statistical trends (SCI 3 weeks, $p = 0.01$). Bone resorption was apparent in both groups (consistently peaked at SCI 16 wks, $p > 0.05$). Global parameters (total BMD, bone biochemistry) did not show early sensitivity to discriminate potential for chronic pain.

Conclusion: A cluster of patients with relatively low admission hip BMD experienced chronic pain. The present data, although preliminary, suggest that pre-injury skeletal status and in particular bone quality and/or quantity, may contribute to the spectrum of risk for chronic pain in patients with SCI.

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THE EFFECT OF TRAUMATIC SPINAL CORD INJURY ON mRNA EXPRESSION OF FERRITIN AND THE TRANSFERRIN RECEPTOR IN THE RAT

B Koszyca, T Naeve, C van den Heuvel, L Qin Yang, P Blumbergs (*Adelaide, Australia*)

Normally Fe^{2+} is strictly controlled within the central nervous system (CNS) because of its potential to react with oxygen and form free radicals [1,2]. Traumatic spinal cord injury (TSCI) leads to cell damage and haemorrhage, both of which may increase the pool of free iron [3]. The aim of this study was to examine the response of the iron storage protein ferritin (Ft) and the iron transport protein transferrin receptor (TfR) in a previously described rat model of TSCI. Animals were killed at 1 hour, 3 hours, 6 hours, 1 day or 3 days and either their spinal cords were removed and snap frozen in liquid nitrogen for PCR analysis or fixed in paraformaldehyde for immunohistochemical assessment. Results from the study demonstrated TfR and Ft immunoreactivity within endothelial, glial and macrophage cells. Increased staining of neurones for TfR was also seen, in association with an increased in Ft mRNA expression at 3 days in severe TSCI. These findings support the hypothesis of an alteration of normal iron metabolism following neurotrauma, with possible implications for free radical mediated secondary damage. The findings suggest that this alteration involves mechanisms other than the previously described iron responsive element (IRE) – iron regulatory protein (IRP) pathway.

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INCREASED CEREBRAL DOPAMINERGIC ACTIVITY AFTER SEVERE TRAUMATIC BRAIN INJURY IN NEWBORN PIGLETS - 18FDOPA POSITRON EMISSION TOMOGRAPHY STUDIES

R Bauer, P Brust: B Walter, F Füchtner, R Hinz, H Kuwabara (*Jena, Leipzig, Rossendorf, Germany; Baltimore USA*)

There is evidence that the dopaminergic system is sensitive to traumatic brain injury (TBI). However, the age-dependency of this sensitivity has not been studied together with brain oxidative metabolism. We postulate that the acute effects of severe TBI on brain dopamine turnover are age-dependent. Therefore 18F-labelled 6-fluoro-L-3,4-dihydroxyphenylalanine (FDOPA) together with Positron-Emission-Tomography (PET) was used to estimate the activity of the aromatic amino acid decarboxylase (AADC) in the brain of eleven newborn piglets (7–10 days old) and nine juvenile pigs (6–7 weeks old). Six newborn and five juvenile animals were subjected to a severe fluid-percussion (FP) induced TBI. The remaining animals were used as sham operated untreated control groups. Simultaneously, the regional cerebral blood flow (CBF) was measured with colored microspheres and the cerebral metabolic rates of oxygen and glucose were determined. One-hour after FP-TBI, [^{18}F]FDOPA was infused and PET scanning was performed for two hours. Two hours after FP-TBI administration a second series of measurements of physiological values including CBF and brain oxidative metabolism data had been obtained. Severe FP-TBI elicited a marked increase in the rate constant for fluorodopamine production (k_3^{FDOPA}) in all brain regions of newborn piglets studied by between 97% (mesencephalon) and 143% (frontal cortex) ($P < 0.05$). In contrast, brain hemodynamics and cerebral oxidative metabolism remained unaltered after TBI. Furthermore, the permeability-surface area product of FDOPA (PSFDOPA) was unchanged. In addition, re-

gional blood flow differences between corresponding ipsi- and contralateral brain regions did not occur after TBI.

Thus, it is suggested that severe FP-TBI induces an upregulation of AADC activity of newborn piglets which is not related to alterations in brain oxidative metabolism.

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A PIG MODEL WITH SECONDARY INCREASE OF INTRACRANIAL PRESSURE AFTER SEVERE TRAUMATIC BRAIN INJURY AND TEMPORARY BLOOD LOSS

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There is a lack of animal models of traumatic brain injury (TBI) which adequately simulate the long term changes in intracranial pressure (ICP) increase following clinical TBI. We therefore reproduced the clinical scenario in an animal model of TBI and studied long term postinjury changes in ICP and indices of brain injury. After induction of anesthesia, juvenile piglets were randomly traumatized using fluid-percussion injury (FPI) to induce either moderate (mTBI $=$ 6 pigs: 3.2 $\pm$ 0.6 atm) or severe TBI (sTBI $=$ 7 pigs: 4.1 $\pm$ 1.0 atm). Injury was followed by a 30% withdrawal of blood volume. ICP, and systemic hemodynamic were monitored continuously. Repeated measurements of global cerebral blood flow (CBF) and cerebral metabolic rate of oxygen ($CMRO_2$) were performed at baseline, at the end of blood withdrawal, after volume replacement, and at 8 and 24 hours postinjury. ICP peaked immediately following FPI (mTBI: 33 $\pm$ 16 mmHg; sTBI: 47 $\pm$ 14 mmHg, $P < 0.05$) in both groups. In the sTBI group we noted a second peak at 5 $\pm$ 1.5 hours postinjury. This second ICP peak was accompanied by a 50 % reduction in CBF (44 $\pm$ 31ml·min·100 g^{-1}) and CMRO2 (2.5 $\pm$ 2.0 ml·min·100 g^{-1}). We thus describe an animal model of severe TBI with a reproducible secondary ICP increase accompanied by patterns of diffuse brain damage. This model may helpful to verify new therapeutic approaches in severe TBI.