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### EFFECT OF EGF ON THE INTESTINAL TIGHT JUNCTION BARRIER IN EXPERIMENTAL NECROTIZING ENTEROCOLITIS

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**Background**: Necrotizing enterocolitis (NEC) is the most common intestinal disease predominately of formula fed, premature babies. Epidermal growth factor (EGF) reduces the incidence of disease in a neonatal rat model of NEC. It has been suggested that increased mucosal permeability may play an important role in the pathogenesis of NEC. Claudin and occludin are the major structural and functional components of the tight junction barrier. These proteins may be altered in disease states. However, the role of tight junctions in NEC pathogenesis and EGF treatment is currently unknown.

**Objective**: The aim of this study was to determine if EGF affects expression of tight junction genes and proteins in the ileum during the development of NEC.

**Methods**: Neonatal rats, either dam fed (DF), milk formula fed (NEC), or fed with formula plus 500 ng/ml EGF (NEC+EGF) were exposed to asphyxia and cold stress to develop NEC. After 96 hours, ileal expression of claudin-3 and occludin were evaluated using Realtime-PCR, Western blot and immunohistochemistry.

**Results**: Claudin-3 and occludin mRNA levels in the ileum were significantly increased 2–4 fold in NEC animals compared to DF animals ($p<0.0001$). Supplementation with EGF normalized mRNA expression to DF levels. Phosphorylated occludin was significantly decreased in NEC animals compared to DF animals ($p<0.01$) and EGF treatment increased expression to DF levels. Non-phosphorylated occludin was only present in NEC and NEC+EGF animals. Histological localization of claudin-3 and occludin proteins was disturbed in animals with NEC compared to DF animals. NEC animals had upregulated expression and disorganization of the tight junction proteins. EGF treatment resulted in localization of the proteins at the tight junction complex.

**Conclusion**: The ability of EGF to normalize expression and localization of tight junction proteins may be one mechanism by which integrity of the mucosal barrier is maintained, thereby protecting the intestinal mucosa against NEC. The increased expression of mRNA and disorganization of protein in animals with NEC may represent a compensation for the inability to form functional tight junctions at the mucosal barrier.

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### EFFECT OF ANTI-TNF-A TREATMENT ON THE INCIDENCE OF EXPERIMENTAL NECROTIZING ENTEROCOLITIS

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**Background**: Necrotizing Enterocolitis (NEC) is the most common gastrointestinal disease predominately affecting premature infants. Pro-inflammatory cytokines, such as TNF-a, have been implicated in intestinal inflammatory diseases. Previously, we have shown that Kupffer cells in the liver are the major producer of TNF-a found in the intestinal luminal contents during experimental NEC. We speculate that TNF-a may play a role in the development of NEC by being an active member of an inflammatory cascade leading to the pathology associated with the disease.

**Objective**: The aim of this study was to determine whether administration of anti-TNF-a reduces the incidence and severity of NEC.

**Methods**: Newborn neonatal rats were fed milk formula and exposed to asphyxia and cold stress twice daily to develop NEC. The pups were divided into two experimental groups: those injected i.p. with 5 mg/kg anti-mouse monoclonal TNF-a once per day for three days (NEC+anti-TNF-a) and those sham injected with saline (NEC). After 96 hours, animals were sacrificed and the number of TNF-a positive cells in the liver and the histologic NEC score were evaluated in the anti-TNF-a injected animals compared to the sham injected animals.

**Results**: The administration of 5 mg/kg/day of anti-mouse monoclonal TNF-a significantly reduced the amount of TNF-a positive Kupffer cells in the liver of neonatal rats. This specific neutralization of hepatic TNF-a production significantly decreased the incidence and severity of experimental NEC ($p<0.01$). Conclusions: Neutralization of hepatic TNF-a production reduces the incidence and severity of NEC. This finding suggests a possible therapy for the treatment of this disease.

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### SERIAL MAGNETIC RESONANCE IMAGING OF THE PRETERM BRAIN: THE NATURAL HISTORY OF EARLY LESIONS, ABNORMALITIES AT TERM AGE AND RELATION TO OUTCOME

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**Background**. The neural substrate for adverse neurodevelopmental outcome in extremely preterm infants remains incompletely determined. **Aims**. To determine the spectrum of brain abnormalities in the preterm population using serial magnetic resonance (MR) imaging from birth to term age and make preliminary correlations with neurodevelopmental outcome.

**Methods**. Serial MR brain scans were acquired prospectively from birth until term age from a consecutive cohort of preterm infants. Neurodevelopmental assessments were made after 18 months of age using Griffiths Mental Development Scales with calculation of a developmental quotient.

**Results**. Three hundred and twenty seven MR studies were obtained from 119 surviving infants born at 23 to 29 weeks gestation. Initial MR scan acquisition was at a median of 2 days after delivery. Four infants had major destructive brain lesions (haemorrhagic parenchymal infarction, n=2; cystic periventricular leucomalacia, n=2); tissue loss was seen at term in the two survivors. Fifty one infants had haemorrhage (germinal layer, n=47; intraventricular (IVH), n=29; extracerebral, n=6) with 42% of survivors having ventricular dilatation at term. Twenty six infants had punctate white matter lesions, 40% of which persisted to term. Cerebellar haemorrhage occurred in 8 infants and basal ganglia lesions occurred in 17 infants. At term 53% of infants without prior haemorrhage had ventricular dilatation and 80% of infants had diffuse excessive high signal intensity (DEHSI) within the white matter. Complete follow-up is available in 67% of infants. Adverse outcome, characterized by low developmental quotient, is associated with major destructive lesions, cerebellar haemorrhage, DEHSI and ventricular dilatation after IVH, but not with punctate white matter lesions, haemorrhage or ventricular dilatation without IVH.

**Conclusions**. Diffuse white matter abnormalities and post-haemorrhagic ventricular dilatation are common at term and correlate with reduced developmental quotient. Early lesions, except for cerebellar haemorrhage and major destructive lesions, do not show clear relationships with outcome.

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### PROTECTIVE EFFECT OF 17ß-ESTRADIOL AGAINST OXYGEN INDUCED NEURODEGENERATION IN THE DEVELOPING RAT BRAIN

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Neurologic morbidity often occurs in survivors of premature birth. Clinical studies have identified hyperoxia as a risk factor for cerebral palsy. Short exposure to high oxygen levels can trigger neurodegeneration in the developing rat brain. Toxicity of oxygen is associated with inactivation of intracellular proteins that promote survival and increased expression of the proapoptotic Fas-receptor. In this animal model of neonatal brain damage we tested Estradiol against the detrimental effect of oxygen. This hormone has neuroprotective properties that result from activation of estrogen receptors and cross-talk with signaling pathways that are activated by neurotrophins. 6-day old rats were subjected to 80% oxygen and Estradiol was co-administered (600 $\mu$g/kg i.p.) at beginning of exposure. Pups were sacrificed at defined time points and brains were either examined histologically to visualize degenerating cells or were snap frozen for molecular studies. Estradiol conferred significant protection against oxygen-induced neurodegeneration. To explore further possible molecular mechanisms, we investigated the impact of Estradiol on the expression of the death receptor Fas and Fas-Ligand. Following hyperoxia for 12 and 24h increased levels of Fas and Fas-Ligand mRNA were found. In the presence of Estradiol mRNA levels of the Fas-system were reduced. Immunoblot analysis on two signaling pathways (MAPK, PI3-kinase) that control neuronal survival, revealed that co-administration of Estradiol counteracts inactivation of the two key players ERK1/2 and AKT. The downregulation in the expression of Fas and Fas-Ligand and increased levels of active forms of ERK1/2 and AKT may represent important mechanisms that lead to survival of neuronal cells. Our results suggest that Estradiol might be suitable as a neuroprotective agent in preterm and term infants who need to be exposed to oxygen. Especially in preterm infants, who are prematurely deprived of maternal intrauterine estrogen, maintaining physiological plasma levels of 17ß-Estradiol may help to protect from potentially neurotoxic triggers.

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### MDMA (ECSTASY) CAUSES NEURODEGENERATION IN THE DEVELOPING RAT BRAIN

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MDMA (3,4-methylenedioxymethamphetamine) causes damage to fine serotonergic fibers and apoptotic neurodegeneration in the adult rat brain. But little is known about the toxic effects of MDMA in the developing brain. The incidence of MDMA abuse is particularly high in adolescents. But the prevalence of MDMA use in woman of child-bearing age and the consequences on their offsprings are unknown. For this reason we administered MDMA (20 – 60 mg/kg) i.p. to 7-day-old rats. Rats treated with normal saline alone served as controls. Pups were sacrificed 24 hrs after injection and brain sections were examined histologically by means of De Olmos cupric silver staining to visualize degenerating cells. MDMA doses of 60 mg/kg induced significant apoptotic neurodegeneration. To explore pathogenetic mechanisms, we investigated the impact of MDMA on the neurotrophine BDNF (brain derived neurotrophic factor). Pups were sacrificed at defined time points (2, 6, 12 and 24 hrs) after injection and brains were processed for RT-PCR, real-time PCR and Western-Blot analysis. mRNA levels for BDNF were upregulated at 6 hrs in animals receiving MDMA compared to controls and even increased at 12 and 24 hours. real-time PCR analysis of the thalamus revealed similar pattern of upregulation of BDNF mRNA transcription. Determined by immunoblot protein expression of BDNF also showed an increase at 12 and 24 hours. These preliminary data suggest that a single injection of MDMA causes neurodegeneration in the neonatal rat brain and that higher doses are required for similar toxicity compared to adult rats. The upregulation in the expression of brain derived neurotrophic factor (BDNF) is may be an important mechanism that lead to survival of neuronal cells in the developing brain.

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### CORTICAL GROWTH AND NEUROCOGNITIVE IMPAIRMENT IN PRETERM INFANTS

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**Background**. Neurocognitive impairment after preterm birth is common and directly related to increasing prematurity. The neural substrate for this dysfunction is unknown, but cannot be wholly accounted for by major destructive brain lesions.

**Aims**. To test the hypothesis that the timing of premature delivery is associated with a dose-dependent reduction in the rate of growth of the cerebral cortex which parallels the increase in neurocognitive impairment.

**Methods**. MR images were obtained from a consecutive cohort of extremely preterm infants serially from soon after delivery until term corrected age and neurodevelopment was assessed using the Griffiths Developmental Scales at median 24 months corrected age. Total cerebral volume and cortical surface area were measured semi-interactively, and their rate of growth found to be related by a power law, the scaling exponent of which gives a direct measure of the growth rate of cortical surface area relative to cerebral volume. A generalized least squares random effects regression model was constructed to test the effect of gestational age at birth and potential confounding variables on the scaling exponent, and the relation to neurodevelopmental outcome was tested.

**Results**. 113 infants born at 23–29 weeks gestation were imaged providing 274 images without major destructive lesions for analysis. Complete neurodevelopmental data are available on 63 infants. Increasing prematurity was associated with a reduced rate of cortical growth ($p<0.0001$) which was independent of intrauterine or postnatal somatic growth. There was a significant relation between reduced cortical growth and the Griffiths Developmental Quotient ($p<0.05$), seen with all subscales except the locomotor.

**Conclusions**. Reduced rate of growth of the cerebral cortex parallels the dose-dependent effect of prematurity on neurodevelopmental function. Cortical growth failure may be a neural substrate for the high rate of non-locomotor neurocognitive impairment seen in preterm infants.