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OXYGEN INDUCES NEURODEGENERATION TRIGGERED BY PRODUCTION OF CASPASE-1 PROCESSED INTERLEUKINS IL-1 AND IL-18

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Backgound/Aims: A proportion of infants born preterm suffer from neurodevelopmental deficits with undefined pathogenesis. Oxygen has been shown to cause widespread neurodegeneration in the immature rat and mouse brain. We hypothesized that the two caspase-1-processed cytokines, interleukin(IL)-1â and IL-18, are involved in oxygen-induced neuronal cell death.

Methods: Six day-old Wistar rats or C57/BL6 mice were exposed to 80% oxygen for various time periods (2, 6, 12, 24, 48 hours). Brain tissue was processed for either histology or RT-PCR and Western Blotting.

Results: Neuronal cell death, as assessed by fluoro jade or silver staining, peaked at 12 - 24 h, preceded by a marked mRNA and protein upregulation of caspase-1, IL-1â, IL-18 and IL-18 receptor-alpha (Il-18R alpha). When rats were intraperitoneally injected with recombinant human IL-18-binding protein (IL-18BP), a specific inhibitor of IL-18, brain injury in response to increased oxygen was attenuated. Mice deficient in interleukin-1 receptor (IL-1R)-associated kinase-4 (IRAK-4), which is pivotal for both IL-1â and IL-18 intracelluar signal transduction, were largely protected against oxygen-mediated neurotoxicity.

Conclusion: These findings suggest involvement of IL-1â and IL-18 in the pathogenesis leading to hyperoxia-induced neuronal cell death in the immature brain. IL-1â and IL-18 could be useful targets for therapeutic approaches aimed at preserving neuronal function following oxygen-induced injury to the developing brain. (supported by BMBF grant Z 0101)

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PILOTSTUDY: COMBINATION OF DELAYED CORD CLAMPING AND RECOMBINANT ERYTHROPOIETIN (RHEPO) FOR THE PREVENTION OF ANAEMIA OF PREMATURITY

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Background/Aim: The optimal management of anaemia of prematurity is still unknown. Therapeutic approaches include delayed cord clamping and early treatment with protein and iron supplements and prophylactic rh Epo. We investigated the possible benefit of delayed cord clamping (45 s) combined with rh Epo 750 IU/kg/week from day 8 -10 with early cord clamping and rh Epo.

Methods: Prospective randomised pilot study in preterm infants <33 weeks gestation. Exclusion criteria: Rh-incompatibility, fetal hydrops, congenital malformations and chromosomal anomalies. In the control group I cord clamping time was „T 20 s and standard protein and iron supplements (2 mg/kg/d) were started on day 28. In group II cord clamping time and protein supplements were the same, but additionally rh Epo 750 IU/kg /week from day 8 °V 10 and Fe-II (6 °V 12 mg/kg/d) from day 14 were given. In group III delayed cord clamping time was 45 s, rh Epo and nutritional supplements were given as in group II. Packed red cell (PRC) transfusions were given according to a strict protocol. Gestational age, birth weight, haemoglobin (Hb), iatrogenic blood loss and frequency and volume of PRC transfusions until day 42 were recorded. The study was approved by the ethics committee of the WWU Münster. Parental consent was obtained prior to delivery. Statistical analysis was performed with a standard ANOVA package.

Results: The table gives the mean haemoglobin values for all groups until the first PRC transfusion was given (Hb until first transfusion, p <0.05 at day 14). Gestational age (group I 30 weeks, group II 29 weeks, group III 30 weeks), birth weight (group I 1152 g, group II 1216 g, group III 1461 g) and iatrogenic blood loss did not differ significantly. No PRC transfusions were given in group II and III, whereas 9/14 (64 %) in group I were transfused (p <0.01, ANOVA). After 14 days the Hb was significantly higher in group III than in group I and II (p <0.05, ANOVA).

Conclusions: To our knowledge this is the first report on the combination of delayed cord clamping of 45 s and rh Epo. In comparison to the control group transfusion requirements were significantly reduced. Delayed cord clamping has helped effectively to sustain the Hb for two weeks in order to cover the well-known delayed onset of rh Epo therapy.

Mean	Group I (n=14)	Group II (n=8)	Group III (n=5)
Hb day 3 (g/l)	15.96	15.79	17.52
Hb day 7 (g/l)	15.06	13.47	16.42
Hb day 14 (g/l)	13.38	13.08	15.4
Hb day 21 (g/l)	11.62	13.53	13.98
Hb day 28 (g/l)	10.69	12.35	12.04

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PREMATURITY IN INFANTS BORN TO HIV-SEROPOSITIVE MOTHERS

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Background: Premature birth occurs in approximately 10% of normal infants. There are limited data regarding the risk of premature delivery and other adverse outcomes of pregnancy in HIV-seropositive women receiving antiretroviral therapy.This study assessed the rate of prematurity in infants born to HIV-seropositive mothers and evaluated whether any relationship may exist between maternal HIV RNA copy number and prematurity.

Methods: Maternal viral load, vertical transmission rate and neonatal outcome were prospectively evaluated in a consecutive (1/1/2001- 31/12/2003) series of 156 mother-infant pairs followed at the same Institution up to eighteen months from delivery. Gestational age was based on the last menstruation date and confirmed by an ultrasound examination performed within the 20th week. Maternal viral load was determined during the last trimester of pregnancy, using branched DNA and/or NASBA technique. Deliveries at less than 37 weeks were defined as premature. The diagnosis of HIV-infection in infants was based on a positive PCR for HIV in blood obtained at one and three months of life. Variables assessed included ethnicity, drug use antiretroviral therapy during pregnancy and mode of delivery.

Results: Out of 156 women, 86% were White, 9% Black, and 5% Hispanic. Eighteen (11.5%) women actively used drugs. Combined therapy during pregnancy was administered in 95% of mothers; six mothers refused any therapy. Most women (98%) underwent caesarean section. Maternal viral load (copy/ml) was: <1000, 70%; >=1000, 30%; median (range), 217 (0–370000). Birth weight (g) was: <1000, 3.8%; 1001–1499, 2.0%; 1500–2499, 21.1%; >=2500, 73.1%. Premature birth occurred in 29% of infants (95% confidence interval [CI], 26–32%). Only one infant, born at term, whose mother did not receive antiretroviral therapy during pregnancy, was vertically HIV-infected. No significant difference in rate of premature birth was found between infants born to mother having or not viral load >=1000 copy/ml (75% vs. 68%, odds ratio [95%CI], 1.4 [0.6–3.4]).

Conclusion: Within the population studied, the rate of premature birth was markedly high as compared to normal infants. No evident association was found between maternal HIV RNA copy number and prematurity. Supported by AISTMAR

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INTESTINAL MICROCIRCULATION IN NEONATAL PIGLETS WITH LPS-INDUCED SEPTIC SHOCK: INFLUENCE OF HUMAN PROTEIN C CONCENTRATE (CEPROTIN®)

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Background: Neonatal sepsis is a disease with a high impact on endothelium dysfunction, resulting in an impairment of microcirculation. The first clinical signs may be feeding intolerance and a prominent abdomen with palpable bowl, suggesting that the intestinal microcirculation is a location, where early endothelium response of the neonatal organism can be observed. As of today evaluation of the neonatal microcirculation ist still a challenge, intravital microscopy (Orthogonal Polarization Spectral-Imaging) seems to be a promising new approach. Protein C, the zymogen of vitamin K dependent serine protease activated Protein C plays a pivotal role in regulation of microvascular coagulation and inflammation. Mortality in septic patients can be decreased by the use of Protein C. However, the direct impact of Protein C on the microvascular blood flow has not been investigated, yet.

Methods: Endotoxin shock was induced in 10 piglets under general anesthesia by intravenous application of 500 ig/kg E. coli lipopolysaccarids. 5 piglets received human Protein C concentrate (Ceprotin® ,Fa. Baxter) with an initial bolus of 50 ig/kg followed by continuous infusion of 200 ig/kg/d. 5 piglets served as control group. Monitoring of hemodynamic and coagulation parameters, WBC,Hct and PLT was performed. After laparotomy, intestinal microcirculation was assessed by Cytoscan Intravital Microscopy in a defined time schedule (prae,30,60,90,120,150,180 min after LPS-exposure).

Results: Early impairment of the microcirculation was observed 1 h after LPS-exposure, when macroscopic evaluation of the gut and clincial hemodynamic parameters were still normal. Obvious reduction of microvascular bloodflow (reduction of capillary density, capillary red blood cell velocity) was observed in the control group, whereas microvascular impairment in the PC-group was attenuated and capillary blood flow could be maintained. Microvascular impairment was accompanied by a decrease of platelets and leukocytes with less decrease in the PC-Group compared to the control group (all values in mean).

Time	MAD (mmHg) PC (Control)	Capdensity = % Vascularization PC(Control)	RBCvelocity (im/sec) PC (Control)	Platelets (/nl) PC (Control)	WBC (/nl) PC (Control)
Prae LPS	45(66)	21,4(21,4)	1046(1030)	573(781)	6,8(6,5)
60' after LPS	31(44)	20,3(17,8)	940 (820)	517(724)	2,2(3,9)
180' after LPS	33(35)	20,5(13,7)	890 (750)	890(750)	3,7(3,2)

Conclusion: Early impairment of microcirculation of septic organisms can be observed via OPS-Imaging, when macroscopic examination and standart hemodynamic monitoring remain unchanged. In this small collective, human Protein C seems to attenuate microvascular impairment in septic organisms.

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COMPARISON BETWEEN CEREBRAL TISSUE OXYGENATION INDEX MEASURED BY NEAR- INFRARED SPECTROSCOPY AND VENOUS JUGULAR BULB SATURATION IN CHILDREN

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Background: Near-infrared spectroscopy (NIRS) provides a continuous, non-invasive method to measure regional changes in tissue oxygenation. Spatially resolved spectroscopy as an algorithm allows the calculation of the cerebral tissue oxygenation index (TOI) which expresses the ratio between oxygenated haemoglobin and total haemoglobin in the observed tissue. Objective: Comparison of the TOI measured by NIRS and venous oxygen saturation in the jugular bulb (SjO2) during cardiac catheterization. Additionally we investigated the influence of body weight on the validity of NIRS measuring.

Methods: Patients: Fifty-three children (median age: 3.5 years, range: 0.1 to 16 years) admitted for cardiac catheterization of cyanotic and non-cyanotic congenital heart defects. Cerebral TOI was compared to SjO2 taken from the jugular bulb during cardiac catheterization. First, Pearson's correlation coefficients and p-values were calculated for all patients and than recalculated respectively for the Patients divided into two groups of over and under 10 kg body weight.

Results: Simultaneously measured values for SjO2 (68.5 ± 9.8 %, 40- 84.1 %) and cerebral TOI (66.4 ± 7.2 %, 39-80 %) showed a significant correlation (r=0.6, p<0.001). Correlation in the group of children under 10 kg (N= 22) was even stronger (r= 0.8, p<0.001) whereas correlation in children over 10 kg body weight was only significant on the level p<0.05 (r= 0.44).

Conclusion: Cerebral tissue oxygenation measured by near- infrared spectroscopy shows a significant correlation with venous saturation of the jugular bulb. This correlation is stronger in children under 10 kg body weight which may be due to the higher transparency and convexity of the infantile skull.

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SILDENAFIL INFLUENCE ON CEREBRAL OXYGENATION MEASURED BY NEAR- INFRARED SPECTROSCOPY IN INFANTS AFTER CARDIAC SURGERY

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Background: Sildenafil (Viagra®) has been shown to be a specific and effective pulmonary vasodilator and is increasingly used in patients with pulmonary hypertension. We investigated the effect of sildenafil medication on cerebral oxygenation using near-infrared spectroscopy (NIRS) in children with elevated pulmonary vascular resistance after cardiac surgery.

Methods: Sildenafil was applied in three steps of 15 minutes each with cumulative doses of 0.1, 0.3 and 0.6 mg/kg. We examined the changes of oxygenated haemoglobin (HbO2), deoxygenated haemoglobin (HHb), total haemoglobin (tHb) concentration and cytochrome oxidase (CytOx) oxygenation and cerebral tissue oxygenation index (TOI) in 13 children.

Results: A significant increase was observed in cerebral O2Hb (Ä 2.3 ± 0.6 imol/L; p= 0.02) and tHb (Ä 0.9± 0.2 imol/; p= 0.005) at the beginning of intravenous sildenafil administration with a decrease in HHb (Ä -1.3 imol/l ± 0.4; p = 0.02). These changes lead to a significant elevation in cerebral TOI from 63.4 ± 2.5 % to 65.7 ± 2.8% (p=0.01), while mean systemic arterial pressure and arterial oxygen partial pressure tended to decrease.

Conclusion: We conclude that infusion of sildenafil was associated with an increased cerebral haemoglobin and oxygen supply in children after cardiopulmonary bypass surgery. These observations may indicate an increased cerebral blood flow after sildenafil administration.