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EFFECT OF PERFLUOROCARBON ON PULMONARY SURFACTANT. AN ELECTRON MICROSCOPICAL AND STEREOLOGICAL STUDY

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Background: Partial liquid ventilation (PLV) represents an alternative therapy of severe respiratory insufficiency, caused by disturbances of the pulmonary surfactant. To wean patients from PLV an intact surfactant system is required. Data concerning the interaction of perfluorocarbons (PFC) with surfactant metabolism are controversial. According to in vitro data we hypothesized that intracellular surfactant pool is reduced in PLV treated animals.

Methods: Prospective, randomized animal study on male wistar rats. Surfactant depleted rats were treated with either PLV (Lavaged-PFC, n=5) or conventional mechanical ventilation (Lavaged-Air, n=5) for 1 hour. For control, 10 healthy animals with air (Healthy-Air, n=5) or PFC filled lungs (Healthy-PFC, n=5) were studied. A design-based stereological approach was used for quantification of lung parenchyma and the intracellular and intraalveolar surfactant pool at the light and electron microscopic level.

Results: Compared to Healthy-lungs, Lavaged-animals had more type II cells with lamellar bodies in the process of secretion and freshly secreted lamellar body like surfactant in the alveoli. Fraction of surfactant covered alveolar epithelial surface area and total intraalveolar surfactant content were significantly smaller in Lavaged-animals. Compared with Air-filled lungs, both PFC-groups had a significantly higher total lung volume, but no other differences.

Conclusion: In contrast to the hypothesis, short term PLV in surfactant depleted animals neither affects the intracellular and intraalveolar surfactant composition nor the surfactant content.

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HUMAN MILK AS A NATURAL SOURCE OF ANTI-ANGIOGENIC COMPOUNDS

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Background: Human milk feedings are thought to reduce certain health risks such as the development of retinopathy due to hyperoxia and neovasularization in the neonatal period. As a new approach for the prevention and therapy of diseases involving the formation of new blood vessels, synthetic oligosaccharide ligands for cell adhesion molecules revealed anti-angiogenic effects *in vitro* . Human milk, however, is a natural reservoir of oligosaccharides structurally resembling selectin ligands such as the tetrasaccharide sialyl-Lewis x. Here, we assessed the properties of human milk oligosaccharides to modulate angiogenesis *in vitro* and *in vivo* .

Methods: *In vitro* tube formation assays were performed using bovine retinal endothelial cells (BREC) on collagen-coated dextrane beads in fibrin gels containing isolated neutral (nHMO) or sialylated human milk oligosaccharides (sHMO) at concentrations of 10, 25, and 100ig/mL. After 48h incubation tubular structures radiating from the beads' surface and protruding into the gel were counted. To confirm the anti-angiogenic capacity of HMO *in vivo* , Matrigel™ plugs (containing heparin, VEGF and bFGF) supplemented with 100ig/mL of nHMO or sHMO were subcutaneously injected in mice. The gel plugs were recovered five days after implantation and inspected for the formation of blood filled microvessels.

Results: In contrast to nHMO, sHMO showed anti-antigenic potency in a concentration dependent manner with a maximum effect of about 40% at 25ig/mL. The differences of the effects of nHMO and sHMO were significant at 25ig/mL ($p<0.005$) and 100ig/mL ($p<0.05$). While the nHMO containing plug showed high cellularity and was partially congested with blood, the plug spiked with sHMO was transparent indicating the inhibition of neovascularization.

Conclusion: Human milk oligosaccharides containing sialic acid revealed anti-angiogenic properties *in vitro* and *in vivo* . These potentially beneficial effects may explain why diseases associated with angiogenesis such as retinopathy were reported to be less prevalent in breastfed infants.

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A RARE CAUSE OF SEVERE PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN: THE AGENESIS OF THE DUCTUS VENOSUS

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Background: Persistent pulmonary hypertension of the newborn (PPHN) can be primitive or a consequence of many lung diseases. We report the occurrence of PPHN after a rare disease: agenesis of the ductus venosus (ADV). Ductus venosus (DV) is a fetal vessel draining 20–30% of the oxygenated umbilical vein blood into the inferior vena cava bypassing the liver. Absent DV can be associated with a normal or abnormal umbilical vein connection to the portal vein. In the latter situation, venous umbilical blood bypasses completely the liver and drains 'unrestricted' into the inferior vena cava or into the right atrium. ADV can be associated with hydrops, chromosomal anomalies, atrial septal defects, facial clefts, kidney anomalies (1).

Methods: Between 2000 and 2003, 6 cases of ADV (GA 31–37 weeks) with umbilical vein drainage into the right atrium were referred to our NICU.

Results: In 4 cases PPHN, as shown by echocardiography, developed after birth; cardiomegaly was present in uterus but no hydrops. In two newborns PPHN was severe and required inhaled nitric oxide (NO); in the remaining 2 newborns mechanical ventilation and vasoactive amines were able to treat PPHN. Two newborn showed no cardiorespiratory diseases: one showed policytemia and the other mild hypoglicemia. Outcome was good in 5 newborns; in one severe encephalomalacia followed profound hypoxemia during PPHN. To our knowledge this is the first report of severe PPHN in newborns with ADV. Two main pathogenetic factors can explain this association: 1) liver bypassed by the oxygenated umbilical vein flow can result in liver hypoxia and/or in absent liver metabolism of vasoactive substances. It has been demonstrated that hypoxia enhances endothelin-1 (ET-1) gene expression in the liver (2); moreover, PPHN was found in a child with congenital porto-caval shunt suggesting a pathogenetic role of toxic metabolites reaching the pulmonary vascular bed (3); 2) in fetuses with ADV increased right cardiac output has been reported (1). Increased pulmonary blood flow can decrease NO production and increase ET-1 level in the lung as well as ET-1 receptors mediating vasoconstriction (4).

Conclusion: Severe PPHN can complicate ADV. Timely and aggressive treatment of PPHN in such cases can lead to normal outcome. Understanding of the pathogenesis can help to optimise therapy.

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TREATMENT OF CHRONIC LUNG DISEASE ASSOCIATED PULMONARY HYPERTENSION WITH INHALED NITRIC OXIDE ADMINISTERED VIA NASAL CPAP

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Background: Pulmonary hypertension (PH) is a major complication of chronic lung disease (CLD), and its treatment is based on the use of oxygen. Inhaled nitric oxide (iNO) is a highly selective pulmonary vasodilator and it has been used in the therapy of this condition via endotrachal tube during mechanical ventilation. Its use in CLD associated PH with non-invasive delivering methods has not yet been documented to our knowledge.

Methods: We report the case of a newborn born prematurely (GA 27 wks BW 475 gms) who developed CLD. At 43 wks post conceptional age oxygen need increased to FiO2=0.7 in nasal CPAP (nCPAP) to maintain O2 saturation (SaO2) within the normal range. Chest X-ray showed cardiomegaly (CTI=0.66). Echocardiography showed indirect signs of PH (hypertrophy of the right ventricle, systolic "D" shaped interventricular septum, moderate pulmonary valve insufficiency) and the value of the mean pulmonary arterial pressure (mPAP) measured through pulmonary insufficiency (PI) was 40–45 mm Hg immediately before starting iNO . INO (INOmax-INO Therapeutics) was administered via nCPAP (Infant Flow System-EME) in conjunction with a iNO delivery system (INOvent-Datex Ohmeda) at a starting dose of 10 ppm.

Results: 5 minutes after starting iNO at 10 ppm via nCPAP, echocardiography showed a decrease of mPAP to 14–15 mm Hg and a significant reduction of PI. After 30 min the oxygen need decreased to FiO2=0.5 to keep SaO2 normal. Subsequently the dose of iNO was progressively decreased with no rebounds in mPAP and stopped 13 days from the start. At the end of treatment echocardiography showed a stable mPAP of 14–15 mm Hg. Met Hb on blood and NO2 in the inspiratory line of nCPAP circuit were never beyond safety levels. On the fifth day of iNO treatment, therapy with dexamethasone was started at the dose of 0.25 mg/kg/day in two doses. Dexamethasone dose was progressively decreased and stopped the day after iNO suspension. At the end of treatment the patient was off nCPAP and oxygen was needed only during feeding. 50 days after the start of iNO treatment echocardiographic PH was absent, chest X-ray showed a reduction of cardiomegaly (CTI=0.52) and the clinical status was stable with a minimal oxygen need.

Conclusion: INO administered via nCPAP has been shown to be effective and safe in reducing CLD associated PH in our patient. On the basis of this experience non-invasive iNO administration can be suggested as an alternative therapy associated with CLD.

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FAECAL CALPROTECTIN LEVELS AT TWO MONTHS OF AGE IN HEALTHY INFANTS AND IN INFANTS WITH ATOPIC AND GASTROINTESTINAL DISORDERS

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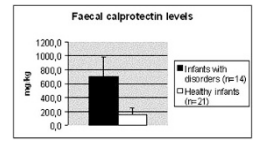
Background: Recently the mielomonocytic calcium-binding protein calprotectin has been proposed as sensible marker in faeces for gastrointestinal inflammation, but there are only a few studies about this topic in infancy. The aim of the study was determining normal faecal calprotectin levels in healthy infants during the first 3 months of life and comparing differences between healthy infants and those with atopic diseases, gastroesophageal reflux or severe colic.

Methods: Between September 2003 and January 2004 stools samples of 21 healthy infants (mean age 58±24 days) and 14 infants with atopic diseases (atopic dermatitis, cow's milk intolerance) and/or severe infantile colic and/or gastroesophageal reflux (mean age 59±19.1 days) diagnosed by clinical examination and laboratoristic analysis were collected at our Department. Exclusion criteria were infections and intake of anti-inflammatory drugs. Stool samples were stored at –20°C until they were analysed. Faecal calprotectin levels were detected using a quantitative ELISA (Calprest, Eurospital SpA, Trieste, Italy). The study protocol was approved by the hospital's ethic commette and parents gave written consent to inclusion of their infants in the study. Statistical analysis was performed using Student's t-test. A value of $p<0.05$ was used for statistical significance.

Results: Calprotectin levels in healthy infants were significantly lower than those in infants with the detected diseases (157.9±86.9 mg/kg vs 770.1±287.4 mg/kg, p=0.000). No differences were found in relation to the kind of feeding (breast-fed: 408.4±327.2, formula-fed: 349.3±338.4; p=0.627). The two groups were equivalent for age (healthy infants: 58.3±23.6 days, infants with disorders: 58.7±19.1 days; p=0.958) and for gender (healthy infants: M/F=12/9, infants with disorders: M/F=6/8).

Conclusion: It is well known that faecal calprotectin levels in infants during the first months of life are higher than in healthy adults and show a wide interindividual and age-dipendent variation. Surprisingly, contrary to previous published data, we have not found any difference between breast-fed and formula-fed infants. Anyway, our finding suggests that this marker might be useful not only to detect bowel inflammation, but also to identify gut involvement related to atopy. Alterations in calprotectin levels may be associated with specific disorders in infancy such as atopic dermatitis, cow's milk intolerance, severe infantile colic and gastroesophageal reflux. Fund of scientific research: MURST 60%.



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SURGICAL CLOSURE OF A PATIENT DUCTUS ARTERIOSUS (PDA) IS ASSOCIATED WITH INCREASED NEUROSENSORY IMPAIRMENT IN EXTREMELY LOW BIRTH WEIGHT (ELBW) INFANTS: RESULTS FROM THE TRIAL OF INDOMETHACIN PROPHYLAXIS IN PRETERMS

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Background: The Victorian Infant Collaborative Study Group has reported that surgery with general anesthesia during the initial hospitalization increases the risk of adverse sensorineural outcome in ELBW infants. PDA ligation was the single most frequent type of surgery in this study. **Objective:** To determine whether surgical PDA closure was a risk factor for neurosensory impairment in ELBW infants who participated in the international Trial of Indomethacin Prophylaxis in Preterms (TIPP).

Methods: Using the TIPP database, we studied 1180 children who survived their first day of birth. Infants were divided into 3 groups according to their PDA status in the NICU: 'No PDA', 'Non-surgical PDA', and 'Surgical PDA'. As in TIPP, the primary outcome was a composite of death or neurosensory impairment at a corrected age of 18 months (cerebral palsy, cognitive delay, deafness, and blindness). Deaths and impairments were also examined separately. Odds ratios and 95% confidence intervals were calculated to estimate the differences in prognostic risk for the 'No PDA' and the 'Surgical PDA' groups in comparison with the 'Non-surgical PDA' group. The analysis was adjusted for gestational age, gender, multiple birth, antenatal steroids, mother's education, and moderate to severe pulmonary hemorrhage. We also examined the relationship between the rate of surgical PDA closure in individual study centres and the 18-month outcome.

Results: The results are summarized in the table.

Outcome	PDA Subgroup	Event rate	Unadjusted Odds ratio		Adjusted Odds ratio	
			OR	P value	OR (95% CI)	p value
Death or neurosensory impairment	No PDA Non-surgical PDA Surgical PDA	307 / 708 (43%) 154 / 315 (49%) 66 / 111 (59%	0.8 -1.5	0.06 -0.07	1.0 (0.8–1.4) -1.4 (0.9–2.2)	0.88 -0.17
Death	No PDA Non-surgical PDA Surgical PDA	141 / 737 (19%) 70 / 329 (21%) 16 / 114 (14%)	0.9 -0.6	0.45 -0.10	1.3 (0.9–1.8) -0.5 (0.3–0.9)	0.18 -0.02
Neurosensory impairment	No PDA Non-surgical PDA Surgical PDA	162 / 567 (29%) 84 /245 (34%) 50 / 95 (53%)	0.8 -2.1	0.10 -0.002	0.9 (0.6–1.3) -1.9 (1.2–3.1)	0.52 -0.01

There was a significant direct correlation between the rates of surgical PDA closure in individual study centres and the prevalence of neurosensory impairments in survivors ($p=0.032$).

Conclusion: Surgical PDA closure was associated with reduced mortality but increased neurosensory impairment in ELBW infants. It remains uncertain whether PDA ligation is a cause or a marker of adverse long-term outcome in this population.