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INFLUENCE OF INTRAUTERINE GROWTH RESTRICTION ON GENE EXPRESSION OF SURFACTANT ASSOCIATED PROTEINS IN PRETERM MICE

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Background: Intra-uterine growth restriction (IUGR) is an important risk factor for perinatal morbidity and mortality in prematurity. Placental insufficiency leading to chronic fetal hypoxemia is a well known cause for IUGR. Therefore, the aim of the study was to establish a mouse model for the experimental induction of IUGR caused by reduced maternal inspiratory oxygen to obtain a chronic fetal hypoxemia. Besides auxological data for evaluation of growth restriction, pulmonary surfactant protein (SP) mRNA expression was investigated, addressing the potential impact of IUGR on lung development.

Methods: Pregnant mice (C57BL/6) were randomized into two groups: dams (n=5) of the first group were held under hypoxic conditions (10% O2) starting at day 14 of gestation. The second group of dams (n=5; control) remained under normoxic conditions. All animals were fed ad libitum. At day 17.5 of gestation (term 19 - 21 days) fetuses of both groups were delivered prematurely by C-section. To estimate the degree of growth restriction the following key characteristics were selected: birthweight, body length (vertex-tail), head length (rostral-occipital). Pulmonary mRNA expression of SP-A, SP-B, SP-C, SP-D was quantified using real time PCR (deltadeltaCT-method; housekeeping gene: beta-actin). Statistical analyses were performed using Mann-Whitney U test.

Results: Compared to controls, hypoxic fetuses showed significantly reduced ($p<0.0001$) birthweight (-28.6%); body length (-13.8%) and head length (-7.7%). Furthermore, pulmonary mRNA expression of SP-A, B and C was significantly decreased in fetuses kept under hypoxic conditions in contrast to controls ($p<0.05$). SP-D mRNA expression was not altered.

Conclusion: Maternal hypoxia revealed to be adequate for the experimental induction of IUGR. Reduced mRNA expression of SP-A, B and C in growth restricted preterm mice (day 17.5 of gestation) may indicate an impaired lung maturation.

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HIGH-FLOW NASAL CANNULA CPAP VERSUS INFANT FLOW NASAL CPAP IN NEWLY-EXTUBATED NEONATES <1250G

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Background: Continuous positive airway pressure (CPAP) has become a preferred method of respiratory support to preterm infants, especially those with birth weights <1250 g. In a study by Sreenan et al, standard nasal cannula with flows up to 2.5 L/min were used in neonates <2.0 kg. This 'high-flow' system was shown to deliver similar positive distending pressures compared to ventilator-generated nasal CPAP, and was effective in reducing apnea of prematurity. Since that publication, 'high-flow' CPAP has become increasingly popular in our neonatal intensive care. Its use has been felt to minimize nasal irritation from conventional nasal prongs. Our usual method of CPAPdelivery is an infant flow system (IFS). The purpose of this study was to determine whether high-flow CPAP was as effective as IFS CPAP in neonates <1250 g.

Methods: Preterm infants <1250 g were randomized following their first extubation to either high-flow or IFS CPAP. Those randomized to the high-flow had flow determined by the following equation: flow (L/min)=0.92+0.68x, where x=weight in kg (Sreenan et al, 2001). The primary outcome was re-intubation within 7 days. Secondary outcomes included post-extubation Fi02 and number of apneas and bradycardias over a 7 day period. Additional secondary outcomes included nasal damage, assessed by digital photography and our own grading system (I – mild, II – moderate, III – severe) at days 1, 7 and 30 post-extubation.

Results: To date 26 neonates have been enrolled, 13 were randomized to conventional IFS CPAP and 13 to high-flow CPAP. The groups were similar in birthweight, gestational age (GA), use of caffeine and age at extubation. Mean GA was 27.5 ±1.8 weeks and mean birthweight was 964 ±162 g. Mean post-extubation age for the high-flow group was 96 ±147 hrs (median 48 hrs, range 7.5–552 hrs) compared to 193 ±360 hrs for the IFS CPAP group (median 24 hrs, range 18–1224 hrs). 8 of 13 infants randomized to high-flow CPAP were re-intubated within 7 days compared to 3 of 13 infants in the IFS CPAP group (p=0.047). There was a trend to higher Fi02 post-extubation and higher number of apneas and bradycardias in the infants randomized to the high-flow CPAP, although the differences were not statistically different. There was no appreciable difference in the degree of nasal damage.

Conclusion: Neonates <1250 g extubated to high-flow CPAP were re-intubated at a higher rates compared to IFS CPAP. High-flow nasal cannula should not be used as an equivalent form of CPAP at this time.

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A NEW METHOD FOR SCORING BEHAVIOR IN NEWBORN INFANTS WITH CONGENITAL HEART DISEASE

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Introduction: Previous acidosis and hypoxia is common in newborn infants with newly diagnosed congenital heart disease (CHD). These infants often need prostaglandin E_1 (PGE_1) and they may show fatigue and irritability. Very little is known about the behavior of newborn infants with CHD. The aim of the present study was to create a simple method for pre- and postoperative evaluation of behavior in newborn infants with CHD, "Behavior score for Infants with Congenital Heart disease", BICH. **Method:** NICU nurses with experience from care of infants with CHD were asked to describe behavior of infants with CHD. Based on these descriptions, a scale with 41 items divided into 3 categories (general, sleep, wakefulness) was created and applied preoperatively on 34 infants with CHD. The infants' gestational ages were 36–41 weeks and their postnatal ages 1 to 15 (median 3) days. Brazelton Neonatal Behavior Assessment Scale (NBAS) was performed in 15 of the 34 infants. Postoperatively 22 infants were assessed (11 with NBAS).

Results: The overall mean preoperative BICH score correlated (p-value; r_S) with: lowest preoperative pH (0.023; 0.390) and base excess (0.001; 0.562), administered dose of PGE_1 (0.001; -0.548). Furthermore the mean BICH score also correlated significantly with several supplementary items from the NBAS, including quality of alertness (0.022; 0.586), cost of attention (0.008; 0.678), examiner facilitation (0.003; 0.724), general irritability (0.022; 0.605) and robustness (0.015; 0.633). The postoperative BICH score correlated significantly with duration of peroperative perfusion time (0.014; 0.517) and aortic clamping (0.027; 0.472), and with two supplementary NBAS items: general irritability (0.008; 0.812), and state regulation (0.004: 0.851). The preoperative mean overall and subgroup BICH scores were significantly lower than the postoperative scores (p= 0.001, 0.000, 0.002 and 0.002, respectively).

Conclusion: A new method for evaluation of newborn infants with congenital heart disease, BICH, has been developed and shown good correlation with preoperative and postoperative morbidity as well as with several supplementary NBAS items.

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URINARY EXCRETION OF AQUAPORIN 2 IN TERM AND PRETERM INFANTS

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Background: Homeostasis of the body osmolality is partly governed by water reabsorption through aquaporin 2 (AQP2), a vasopressin-regulated water channel of the renal collecting duct. The role of AQP2 in the development of urinary concentrating capacity during postnatal life is unknown. The aim of this study is to determine urinary excretion of AQP2 during early postnatal age at different gestational ages and during different clinical and/or biological conditions in the newborn infants.

Methods: 119 premature and 6 full-term newborn infants were included in the study. Urine sample was collected at 72 hours and 3 weeks of life. Clinical and biological status was known at each sampling. No child received nephrotoxic treatment. Urinary AQP2 levels were determined by dot blot and expressed in terms of AQP/urinary creatinine.

Results: 160 urine samples were analysed. Urine osmolality was low. Urinary AQP2 level increased with gestational age ($p<0.05$). Blood acidosis and renal impairment decreased the urinary excretion of AQP2. Prenatal glucocorticoid administration and aldosteronism (urinary Na/K ratio) increased excretion of AQP2.

Conclusion: Renal concentrating capacity of the newborn is low. Nevertheless, we show that AQP2 excretion undergoes developmental changes with gestational age. In addition, different biological conditions in the neonate seems to have an impact on the level of urinary AQP2 excretion.

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UTILITY OF RAPID B-TYPE NATRIURETIC PEPTIDE ASSAY FOR DIAGNOSIS OF SYMPTOMATIC PATENT DUCTUS ARTERIOSUS IN PRETERM INFANTS

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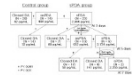
Background/aims: In preterm infants, the rapid and accurate determination of the presence of a hemodynamically significant patent ductus arteriosus (hsPDA) is extremely important, but this is often difficult. Plasma B-type natriuretic peptide (BNP) measurement has been reported to be a helpful aid in the diagnosis of hsPDA in preterm infants. The aim of our study was to investigate the usefulness of rapid BNP assay as a diagnostic marker of symptomatic PDA (sPDA) in preterm infants.

Methods: 66 preterm infants, ranging from 25 to 34 gestational weeks of age, underwent clinical and echocardiographic examinations for PDA every other day from the third day of life until the disappearance of ductal flow. Simultaneously, plasma BNP concentrations were measured using a commercial kit, Triage® BNP test kit (Biosite Diagnositics, San Diego, California, U.S.A.). When two or more clinical significant features of PDA were noted, and a large ductal flow was confirmed by color Doppler echocardiography, sPDA was diagnosed and treated with indomethacin.

Results: On the third day of birth, the BNP concentration in the sPDA group (N=23) was significantly higher than in the control group (N=43) ($P<0.001$). 17 infants (74%) in the sPDA group became asymptomatic after a first course of indomethacin and their BNP levels concomitantly decreased. In the control group, only 14 infants (32.5%) had a ductal shunt but did not develop sPDA (asPDA infants). The BNP concentration of asPDA infants was significantly higher than that of closed PDA infants ($P<0.001$). Moreover, BNP concentrations were significantly correlated with the magnitudes of the ductal shunt, such as the ratio of left atrial to aortic root diameter and the diastolic flow velocity of the left pulmonary artery ($r = 0.686$ and $r = 0.839$, respectively, $P<0.001$). The area under the ROC curve using BNP to diagnose sPDA was 0.997 (95% CI, 0.991 to 1.004, $P < 0.001$). A cutoff BNP value of 1,110 pg/mL had a sensitivity of 92.0%, specificity of 100% and a positive predictive value of 100% for diagnosis of sPDA.

Conclusion: Although not a stand-alone test, the rapid BNP assay clearly adds valuable information for the detection of preterm infants with sPDA that require treatment. Particularly, a cutoff BNP level of 1,110 pg/mL differentiated well between preterm infants with and without sPDA. Moreover, serial plasma BNP measurements may be of value in determining the clinical course of PDA in preterm infants.



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THE HUMAN CATHELICIDIN: ANOTHER ANTIMICROBIAL PEPTIDE OF URINARY TRACT

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Background: Human antibacterial peptides play a crucial role in maintenance of normal bacterial flora and in fighting pathogens as parts of innate immune defence. The human cathelicidin (LL-37) has been studied mainly in blood cells and epithelium of skin, respiratory and gastrointestinal tracts. In the present study, we sought to investigate LL-37 expression in human urinary tract and its role during bacterial urinary tract infection (UTI).

Methods: Urine of 28 healthy children was analysed by LL-37 ELISA, as well as urine of 29 children during acute phase of UTI. Healthy kidney tissue from 11 patients nephrectomised due to renal cancer was examined using ELISA, Taqman real-time PCR and immunohistochemistry. In addition, kidneys from healthy mice and mice transurethraly infected with *E.coli* were analysed immunohistochemicaly for mouse cathelicidin (CRAMP). The expression of LL-37 mRNA was studied in 6 different human renal epithelial and uroepithelial cell lines, both cancer-derived and normal, and also after stimulation with uropathogenic *E.coli*.

Results: Low levels of cathelicidin were found in the urine of healthy children (0.313 ng/ml, range 0.194 to 5.944) as well as in healthy renal tissue (51.590 ng/ig tissue, range 20.725 to 102.355). By immunohistochemistry, cathelicidin could be localised mainly to the cytoplasm of renal tubular cells. Upon urinary tract infection, urinary levels of cathelicidin significantly increased (2.353 ng/ml, range 0.133 to 312.5, $p<0.001$, Mann-Whitney U test) and were correlated with the urine leukocyte levels ($R=0.60$, $p<0.001$, Spearman rank correlation test). Correspondingly, immunohistochemical staining of infected mice showed cathelicidin mainly in the cytoplasm of invading leukocytes. mRNA for LL-37 was present in the pieces of renal cortex and pelvis renalis, and also in renal epithelial and uroepithelial cell lines. After stimulation with uropathogenic *E.coli*, LL-37 mRNA in all studied cell lines significantly decreased by 38 to 64% ($p<0.01$, respectively, Mann-Whitney U test).

Conclusion: Our results indicate that cathelicidin LL-37 is expressed in human renal tubular and uroepithelial cells. During bacterial infection, invading leukocytes produce substantial amounts of LL-37. In renal tubular and uroepithelial cells, on the other hand, the constitutive production of LL-37 seems to be suppressed by *E.coli*, which could be a pathogenic mechanism promoting bacterial establishment within urinary tract.