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CHOLESTEROL OXIDATION IN INTRAVENOUS LIPID EMULSIONS: SAFETY OF PREPARATIONS BEFORE AND AFTER EXPERIMENTAL HYPEROXIA

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Background: In this study the possible presence of cholesterol oxidation products in two intravenous lipidic emulsions (ILEs) with different fatty acid compositions (LCT, MCT-LCT) has been investigated. These emulsions are currently employed in neonatal parenteral nutrition and their direct venous introduction might be potentially dangerous because of the possible atherogenic role of cholesterol oxidation products (COPs).

Aims: We aimed the present study to onvestigate the possible presence of COPs in both commonly employed intravenous lipidic emulsions.

Methods: The emulsions were analyzed when bottles were opened, i.e. under normal condition of administration, and after a 12 hours direct experimental exposure to air and high (90%) oxygen concentrations. 7-ketocholesterol and 5ƒÑ-epoxycholesterol were chosen as markers of direct and indirect cholesterol oxidation, respectively, and detected by Gas Chromatography-Mass Spectrometry of their trimethysilyl ethers.

Results: The detected amounts of cholesterol oxidation markers were always very low and in some cases below the detection limit of the analytical method for the two COPs (0.1 and 0.3 ƒÝg/g) of extracted lipids. When the bottles were opened (°§basic°ï conditions), in both emulsions the concentrations of 5ƒÑ-epoxycholesterol were higher than the concentrations of 7-ketocholesterol. The concentrations of the detected COPs were lower in LCT than in the MCT/LCT ILEs. The differences between air and oxygen exposure were not particularly significant although the content of the detected COPs was higher after oxygen exposure than after air exposure in both MCT/LCT and in LCT ILEs. Nevertheless, in this experimental environment (air or oxygen exposure) the concentration of 5ƒÑ-epoxycholesterol again proved to be higher than the 7-ketocholesterol concentration.

Conclusion: In agreement with other authors with regards to the presence and possible intake of preformed amounts of cholesterol oxides in currently used ILEs, the results of the present study are reassuring for the safety of neonates. Samples of intravenous preparations seem to be minimally affected upon opening by the possible oxidative stress derived from industrial manufacturing.

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NON-INVASIVE CEREBRAL TEMPERATURE MAPPING BY PROTON SPECTROSCOPIC IMAGING

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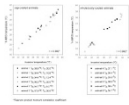
Background: Cerebral hypothermia shows promise as a neuroprotective strategy following perinatal hypoxia-ischaemia. A non-invasive technique for the quantification of regional brain temperature is urgently required to assess the cerebral effects of different cooling strategies. We aimed to test the hypothesis that thermometry by proton magnetic resonance spectroscopic imaging (^{1}H-MRSI) is as accurate as invasive monitoring during whole body hypothermia and selective head cooling.

Methods: Cerebral magnetic resonance (MR) data was acquired from 6 newborn piglets using a 7 Tesla Bruker Biospec MR system. Mild whole body hypothermia was induced in 3 piglets using a thermally regulated water mattress. For each animal measurements were collected at rectal temperatures (T_R) of 38±1°C and 34±1°C. Mild hypothermia was induced in 3 piglets using a water-filled plastic "cooling cap" applied to the animals' scalp. In these animals measurements were obtained at (i) T_R 38±1°C; cap temperature (T_C) 20°C (ii) T_R 34±1°C; T_C 10±2°C. ^{1}H-MRSI data were acquired from a coronal slice (thickness 4mm; TE 45ms; TR 3000ms, 16x16 voxels each 3.75 x 3.75mm^2) and processed using in-house software: for each voxel, separate water and metabolite spectra were obtained from a single non-water suppressed spectrum. The chemical shift difference between the water peak and n-acetylaspartate (NAA) was obtained by a cross-correlation method. This chemical shift difference was converted to absolute temperature using a previously validated calibration curve. A two dimensional cerebral temperature map of the selected slice was obtained in a single measurement. Invasive brain temperature measurements were obtained concurrently using a surgically inserted fibre-optic thermometer, which yielded 4 temperature values at successive 5mm intervals from the probe tip.

Results: The graphs demonstrate 4 data points for each subject at each T_R.

Conclusion: ^{1}H-MRSI is an accurate and robust non-invasive method of cerebral temperature measurement in both total body and selective head cooling models. The determination of regional brain temperature distributions in infants undergoing hypothermia treatment may provide new insights into the local effects of brain cooling and enable its neuroprotective potential to be optimised.



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REDUCING THE CMV INFECTIVITY OF MOTHER'S OWN BREAST MILK

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Background: Infants can acquire primary postnatal CMV infection from the breast milk of their CMV infected mothers(1). About 60% of Australian mothers are CMV positive. Most of these will eventually excrete CMV into their breast milk(1). CMV transmission has been documented in 37% of preterm infants of CMV infected mothers(1) - symptomatic infection such as neutropenia, thrombocytopenia, hepatopathy, sepsis-like deterioration occurred in about half of these (1). The infants most at risk of symptomatic infection are the extremely low birth weight infants and those who acquire CMV early(2). An increased risk of adverse neurodevelopmental outcome in low-birth weight infants who acquired CMV early has been suggested (3) . Preventing CMV transmission in extremely preterm infants from maternal milk is a clinical problem. Methods that reliably remove CMV such as heating also remove beneficial properties of breast milk. Freezing is not harmful to the protective effects of breast milk. Previous small studies of freezing (to -20°C) breast milk with naturally acquired CMV showed a reduction in CMV titres, or elimination of CMV. These studies used a culture method with a low sensitivity.

Aim: A pilot study using a sensitive culture method, to determine the length of freezing at -20°C required to eliminate CMV from breast milk. Methods: Breast milk was collected from CMV seropositive women. The breast milk was frozen in 1 ml aliquots at -20°C in a quality-controlled freezer. CMV culture was performed at day 0,1,3,5,7,10 and 14 after freezing. CMV was cultured in human embryonic fibroblasts in tube cultures and monitored for the characteristic CMV cytopathic effect. Immunofluorescence with CMV-specific monoclonal antibodies gave a sensitive measure of the presence of CMV. CMV culture was chosen as the endpoint as it is the marker of CMV infectivity. PCR, while more sensitive, does not necessarily indicate infectivity.

Results: Breast milk was collected on one occasion from 19 women, PCR detected CMV in 12, CMV was cultured in 5 of these samples. After 7 days of freezing at -20°C CMV could not be cultured in any of the samples.

Conclusion: We recommend freezing breast milk to -20°C for 7 days is a relatively simple method to substantially reduce the CMV infectivity of breast milk for extremely preterm infants. 1. Hamprecht K, et al Lancet 2001;357(9255):513–8. 2. Maschmann J et al Clinical Infectious Diseases 2001;33:1998–2003. 3. Paryani SG et al J Pediatr 1985;107(3):451–6.

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THE ROLES OF OXYGEN AND VASCULAR GROWTH FACTORS IN PATHOGENESIS OF RETINOPATHY OF PREMATURITY IN A MURINE MODEL

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Purpose: To investigate the changes of VEGF°¢FGF-2°¢IGF-1 and ER mRNA and protein levels in mice retinae of normal retinal vascular development and retinopathy of prematurity to elucidate risk factors and pathogenesis of retinopathy of prematurity (ROP).

Methods: Four hundred and seventy-four 7-day-old (P7) C57BL/6J mice, half female and half male, were assigned to four groups according to oxygen therapy and the gender. Total RNA was extracted from 2 mice for one sample. VEGF, FGF-2 and ER mRNA expression were determined by reverse transcription-polymerase chain reaction (RT-PCR). The protein levels of VEGF, FGF-2, IGF-1 and ER were determined by immunohistochemistry.

Results: Gender and oxygen therapy couldn't affect the expression of VEGF, bFGF, IGF-1 and ER (P0.05). However, the age was the independent factor which could affect their expression ($p<0.05$). While hyperoxia and age were integrated, they obviously affected these factors expression ($p<0.0001$). The level of VEGF mRNA increased since P7, peaked at P9, and declined since P11 to a low level and maintained to P17 in nomoxic groups. In hyperoxic groups, it declined since P8 and remained declining during oxygen exposure; while it increased slowly since mice were taken back to room air and increased rapidly since P15 which is significant compared to the controls, indicating a close relationship with changes of O2 concentration. The level of FGF-2 mRNA maintained low in normoxia; while in hyperoxic groups it had no change during the hyperoxia exposure and increased since 3 days after back to room air and maintained to P21. The level of ER mRNA increased since P7, peaked at P9, declined since P11 and maintained to P17 in nomoxic group. In hyperoxic groups, it had no change during the hyperoxic period and rised since 5 days after back to room air and maintained to P21. The changes of protein levels of these three factors were later than that of their mRNA, but had the same trend. The protein of IGF-1 declined since P7 and maintained low since P11, while in hyperoxic group, it had no change during the hyperoxic exposure, increased since mice were back to room air and declined since P16 to a low level.

Conclusion: Our study suggested that hyperoxia followed by hypoxia was very important in the pathogenesis of ROP. VEGF, FGF-2, IGF-1 and ER played important roles in the development of normal retina vascularization and the pathogenesis of ROP. VEGF may be the most important factor.

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MILDLY ELEVATED LEVELS OF TSH IN PRETERM NEONATES AT FIRST MONTH OF LIFE ARE OFTEN TRANSIENT

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Background: Preterm infants may present mildly elevated levels of TSH (MELTSH) that is levels higher than the '"normal" ones (5 mU/L) but lower than the cut off level of congenital hypothyroidism (20 mU/L). It has been suggested that these MELTSH may indicate hypothyroidism requiring treatment even with normal free thyroxine levels. The aim of this study was to determine the rates of MELTSH in preterm neonates at the first six weeks of life and whether this condition is a transient one.

Methods: The study population consisted of 146 healthy preterm neonates (28–36 weeks of gestation) with MELTSH and FT4 serum levels >6 pg/ml. TSH levels were measured by chemiluminescence immunoassay and FT4 levels by RIA method at 2, 4 and 6 weeks of life. Among them 45 (31%) were small for gestational age (SGA) with a birthweight <10th percentile. The rates of MELTSH between SGA and appropriate for gestational age (AGA) neonates were compared by chi-square test. A multivariate logistic regression analysis was applied to evaluate the influence of possible risk factors (gestational age, birth weight, fetal distress, maternal chorioamnionitis, etc) on the incidence of MELTSH.

Results: MELTSH was found in 79/146 neonates (54%) in 44/146 (30%) and in 24/146 (16.5%) at the 2nd, 4th and 6th week respectively. The incidence of neonates with MELTSH did not differ between SGA and AGA at the 2nd and the 6th week of life. However at 4 weeks a higher percentage of SGA neonates (42%) had MELTSH compared to AGA ones (24.7%, $p<0.05$). In the multivariate analysis it was found that, among the factors studied, being SGA is the only independent variable related with the presence of MELTSH (RR 2.8, CI 1.1–7.3).

Conclusion: A high percentage of preterm neonates, especially in SGA, presents MELTSH at the first month of life but this finding is transient in most cases. Our results suggest that in preterm neonates with MELTSH and normal FT4, monitoring of TSH levels up to the second month of life is needed before the institution of replacement therapy.

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DETERMINANTS OF VITAMIN B12 STATUS IN INFANTS

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Background: Vitamin B_{12} deficiency is associated with increased plasma methylmalonic acid (MMA) and/or homocysteine. The principal cause of vitamin B_{12} deficiency in infants is impaired maternal vitamin B_{12} status. Recent studies suggest increased plasma MMA and homocysteine during infancy is common although the cause and significance are unclear. **Aims**: To determine the normal range of plasma MMA in infants during the first months of life and to correlate this with other biochemical and dietary markers of vitamin B_{12} status. We also aimed to determine if breast-fed infants had higher plasma MMA than formula-fed infants.

Methods: A prospective cohort study was conducted to measure biochemical markers of vitamin B_{12} status in normal infants and their mothers. One hundred and one infant-mother pairs had blood samples taken when the infants were 4 and 10 weeks of age for measurement of plasma vitamin B_{12}, red cell folate, plasma MMA and homocysteine. Maternal dietary intake of vitamin B_{12} was calculated using a validated food frequency questionnaire. Statistical analyses were conducted using SPSS statistical software.

Results: The range of plasma MMA for all infants was 0.09–18.43 μmol/L (median 0.40, interquartile range 0.23–0.89 μmol/L). At 10 weeks of age, 46% of breast-fed and 21% of formula-fed infants had plasma MMA greater than 0.50 μmol/L ($p=0.016$). Breast-fed infants had lower plasma vitamin B12 ($p<0.01$) and red cell folate ($p<0.01$), and higher plasma homocysteine ($p=0.02$) than formula-fed infants. In breast-fed infants, plasma MMA and vitamin B_{12} strongly correlated with maternal plasma MMA and vitamin B_{12}. This correlation was not seen in formula-fed infants. Breast-fed infants also showed a strong relationship between plasma homocysteine and vitamin B_{12} and MMA. The relationship between infant vitamin B_{12} status and maternal dietary intake will be presented.

Conclusion: The normal range for plasma MMA in young infants is considerably higher than for older children and adults. Breast-fed infants have higher plasma MMA and homocysteine, and lower vitamin B_{12} and red cell folate than formula-fed infants. Due to the strong correlation between infant and maternal vitamin B_{12} status in breast-fed infants, these findings may indicate sub-optimal vitamin B_{12} status in breastfeeding mothers. Further analysis of dietary data will help to elucidate the cause of these findings. The clinical significance remains to be established in future studies.