Plasma hypoxanthine: a marker for hypoxic-ischaemic induced periventricular leucomalacia?

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Abstract
Cerebral ischaemia of the immature brain may result in cavitating periventricular leucomalacia (PVL), an important association of cerebral palsy. Hypoxanthine measured by high performance liquid chromatography was used as a marker of peripartum hypoxia and ischaemia in 116 infants at risk of PVL. PVL was detected by ultrasound. The 81 infants who were unaffected had median (range) gestation of 30 weeks (24–32), weight of 1336 g (724–3790), and a plasma hypoxanthine concentration of 7.8 μmol/l (2.4–48.9). The seven infants who had cavitating PVL had a median gestation of 28 weeks (26–30), weight of 1165 g (682–1860), and a hypoxanthine concentration of 31.9 μmol/l (7.1–149). Cavitating PVL was significantly dependent only on hypoxanthine when controlling for the effects of weight and gestation. This suggests that peripartum hypoxia-ischaemia may be one of the aetiological factors in cavitating PVL. Oxidation of hypoxanthine during reperfusion generates free radicals which may contribute to the tissue destruction of PVL. The association of hypoxia-ischaemia with PVL suggests that PVL may be modified by reducing free radical activity.

Cavitating periventricular leucomalacia (PVL) is an important association of cerebral palsy and is found in 7–15% of babies under 1500 g at birth. Between 47% and 80% of infants with parenchymal cavitation develop abnormalities of neuromuscular tone and posture with or without cognitive impairment. PVL is a pathological term which has become synonymous in clinical terminology with high resolution sonographic abnormalities detected in the neonatal period because the parenchymal echodensities and echolucencies detected by ultrasound have been correlated with pathological abnormalities at necropsy. Ultrasound has led to improved understanding of the incidence, timing, and clinical associations of PVL. Risk factors associated with PVL such as prematurity, very low birth weight, antepartum haemorrhage, asphyxia, respiratory distress requiring mechanical ventilation, and sepsis may result in hypoxaemia and hypotension with resultant tissue hypoxia and ischaemia of the vulnerable periventricular ‘watershed zones’ of the immature brain. The purine metabolite hypoxanthine is a marker of tissue hypoxia or ischaemia. Accumulation of hypoxanthine has been detected in cord blood from asphyxiated babies and in perfuse from ischaemic organs. The postischaemic catabolism of accumulated hypoxanthine results in the generation of tissue damaging, oxygen derived, free radicals by the action of an endothelial enzyme, xanthine oxidase, and oxygen. Hypoxic-ischaemic injury to the brain has been reported in animal models and tissue protection demonstrated by the administration of free radical scavengers.

To test the hypothesis that an increase in hypoxanthine (as a marker of hypoxia) is associated with PVL, we have examined an ‘at risk’ population of preterm infants by measuring plasma hypoxanthine concentrations at birth and relating these to the subsequent development of cavitating PVL. We have assumed that cavitation represents the severest degree of ischaemic injury and therefore the severity of the hypoxic insult would be more likely to result in increased circulating hypoxanthine.

Patients and methods
Babies between 24 and 32 weeks’ gestation admitted to the Regional Neonatal Intensive Care Unit were studied. Plasma hypoxanthine was measured in samples obtained from cord blood or as soon as possible after admission to the unit. Care was taken to ensure that samples were taken by large needle puncture of either cord artery or vein or by an indwelling arterial line or clean venesection. Samples were rapidly separated and stored at −30°C to avoid leakage of erythrocyte hypoxanthine. Haemolysed samples were discarded.

Serial cranial ultrasound (ATL Ultramark 4 with a 7.5 MHz transducer) was performed from admission and then at least weekly until discharge. Parenchymal echodensities (of equivalent echodensity to the choroid plexus) seen in both coronal and parasagittal planes were regarded as precavitating PVL. Three groups of echolucencies were identified: porencephaly (lucency widely communicating with the ventricle), cavitating PVL (single or multiple lucencies occurring 2–3 mm from the ventricular wall), and porencephaly with associated PVL (porencephaly with surrounding non-communicating lucencies either single or multiple occurring 2–3 mm from the porencephalic cavity wall).

Hypoxanthine was measured by reverse phase high performance liquid chromatography using a Waters model 481 LC spectrophotometer. The column (8×100 mm inside diameter) was packed with a Nova-Pak C18 4 μm cartridge (Waters), and a Waters Guard-Pak μBondapak
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The assay had a recovery of >95% of known concentrations of hypoxanthine from 5 µmol/l to 100 µmol/l and the coefficient of variation was 5.6%. Results of hypoxanthine were not available until 6–8 weeks after birth and this avoided observer bias in the diagnosis of PVL. The reference plasma hypoxanthine concentration in our unit was 4.7 µmol/l (95% confidence interval (CI) 3.7–5.7) with the upper limit (2 SD) of 9.5 µmol/l determined from cord arterial blood obtained from 22 unstrained term infants delivered at elective caesarean section (figure).

Statistical analysis was by Mann-Whitney U test for comparison of non-parametric data, and multiple logistic regression analysis was used to control for confounding variables when dichotomous outcome variables were evaluated. The SPSS-X 3.1 statistical package on an IBM 3081 mainframe computer was used.

Informed parental consent was obtained for all patients studied and ethical approval was granted by the hospital ethical committee for the study.

Results

Of the 116 babies studied, 15 died within 14 days without developing cavitating PVL (four had echodensities). Of the 101 survivors, 81 did not develop parenchymal cavitation but four of them had parenchymal echodensities which resolved. The remaining 20 (17.2%) babies developed parenchymal cavitation: seven of these had cavitating PVL alone, eight had porencephaly alone, and five had porencephaly with associated PVL.

The two groups with porencephaly, with or without PVL, had higher median hypoxanthine concentrations than the unaffected group but the differences were not significant (table 1). The group with PVL alone had a median hypoxanthine which was significantly higher at 31.9 µmol/l (p<0.005). This difference persisted after adjusting for gestation and weight (p<0.05) by multiple logistic regression, with PVL as the dependent variable, and weight, gestation, and hypoxanthine as the independent variables. A measure of the risk of developing PVL, the odds ratio (OR), was calculated from the regression model. The OR (with 95% CI) for developing PVL was 1.89 (1.01 to 3.53) for every 10 µmol/l increase of hypoxanthine (p<0.05). The unit change of the independent variable is arbitrarily selected for convenience, such as one week for gestation or 10 µmol/l for hypoxanthine.

No significant dependence on hypoxanthine (while controlling for weight and gestation) could be demonstrated for porencephaly alone (OR 0.75; 95% CI 0.43 to 1.85) or porencephaly with associated PVL (OR 1.52; 95% CI 0.97 to 2.38).

The details of babies who developed parenchymal cavitation, including the time delay in sampling of blood for hypoxanthine, are shown in table 2 and the figure demonstrates the plot of hypoxanthine concentrations for each group. An increased concentration of hypoxanthine within two hours of birth, thought to indicate antenatal hypoxia,16 27 was found in all three groups of parenchymal cavitation: 4/7 of the group with PVL, 4/8 of the porencephaly group, and 3/5 of the porencephaly and associ-
Porencephaly and PVL alone

<table>
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Discussion

Events such as antepartum haemorrhage and clinical perinatal asphyxia have been associated with subsequent development of PVL, and paraxial hypodense echodensities detected by ultrasound may appear within three days of birth, suggesting that many cases are related to peripartum events. PVL is a manifestation of cerebral hypoxia-ischaemia, and an increased concentration of plasma hypoxanthine is a biochemical marker of peripartum hypoxia. The association we have found between increased early plasma hypoxanthine and the development of PVL gives indirect evidence to support the hypothesis that cerebral hypoxia-ischaemia may induce PVL in preterm infants.

There is conflicting evidence for an association between increased concentrations of plasma hypoxanthine and adverse clinical outcome in term babies. Increased cord hypoxanthine or hypoxia-ischaemic excitation in asphyxiated babies with a reduced 'optimality score' or abnormal neurodevelopmental signs during the first week of life but there is considerable overlap with unaffected babies. Neurological assessment at 2 years could not be correlated with cord hypoxanthine in term babies but we are not aware of any evidence for preterm babies. The fetal cerebral oxygen uptake rapidly decreases with falling arterial oxygen saturation, especially in combination with acidemia, and so the effect of global hypoxoxemia on the vulnerable periventricular watershed zones could affect the fetal and preterm brain more than the term infant with more developed periventricular anastomoses.

Our study may have underestimated any association between increased plasma hypoxanthine and PVL because delay in sampling (including outborn babies) was unavoidable in many babies and also because subsequent postnatal hypoxic-ischaemic insults after the admission sampling would not have been detected by hypoxanthine measurements: any increase due to birth asphyxia might have returned to normal giving a false negative association with PVL. On the other hand normal concentrations detected in babies without PVL might have been due to delayed sampling. We found no correlation between sample time and hypoxanthine concentration and this suggests that in the non-cavitating group the normal value of hypoxanthine was not due to delayed sampling. The half life of plasma hypoxanthine is three minutes in rabbits and 40 minutes in pigs after a hypoxic-ischaemic episode; in term babies increased concentrations may be detected for two hours after asphyxia and in preterm babies increased values have persisted for 24 hours after an hypoxic episode. Plasma hypoxanthine is likely to be trapped in tissues because of inadequate tissue perfusion and circulation; increased values may therefore be detected for some time after the hypoxic insult, until adequate circulation is re-established. This may explain the marked increase in hypoxanthine (142-9 μmol/l) at seven hours in one baby in the PVL group, despite establishing adequate oxygenation and blood pressure after an emergency caesarean section for foetal breech presentation (table 2, case 5).

The degree and timing of the hypoxic episode would influence the magnitude and duration of hypoxanthine increases. An insult occurring in the peripartum period may not therefore have been detected by admission sampling of outborn babies at six to eight hours. Two of the lowest values of hypoxanthine in the PVL group were recorded from outborn infants. About half of the babies from our study who developed any form of parenchymal cavitation had evidence of birth asphyxia, evidenced by increased concentrations of hypoxanthine (greater than 2 SD
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Plasma hypoxanthine has been detected after postnatal events such as pneumothorax, mechanical ventilation for severe respiratory distress syndrome, shock, and necrotising enterocolitis (own observations). Global hypoxia increases plasma hypoxanthine (probably arising from organs with a lower threshold for purine degradation, such as the liver and gut) whereas focal ischaemia within an organ, especially the brain with a higher threshold for purine degradation, may not increase systemic plasma hypoxanthine sufficiently to be detectable by remote sampling. Plasma hypoxanthine measured in peripheral plasma is therefore a marker of global hypoxia but this is likely to be associated with global cerebral hypoxia-ischaemia. Cerebral hypoxia and ischaemia may therefore be a mechanism of parenchymal injury in pneumothorax, severe respiratory distress requiring mechanical ventilation and shock.

In our study, plasma hypoxanthine was only slightly increased in the two groups in which porencephaly was found. This could be because early sampling did not detect a later postnatal event or because porencephaly may result from different pathogenic mechanisms. First, localised parenchymal infarction secondary to the presence of a contiguous large blood clot and release of vasoactive substances such as thromboxane, prostacyclin, or potassium may lead to porencephaly rather than PVL. Second, parenchymal venous infarction due to obstruction of veins of the periventricular white matter by detension of the germinal matrix and vessels after periventricular haemorrhage, could also cause porencephaly and possibly PVL. PVL associated with porencephaly is likely to be secondary to the pathogenic mechanisms responsible for porencephaly. Not all cases of PVL occur in association with periventricular haemorrhage and it is therefore likely that PVL is due to local or generalised cerebral ischaemic infarction, and porencephaly due to local ischaemia or venous infarction. Unfortunately sampling of plasma hypoxanthine from the cerebral venous drainage is not possible in premature babies, and therefore it is difficult to detect direct evidence of local or even global cerebral ischaemia based on hypoxanthine concentrations. Cerebral arteriovenous hypoxanthine difference increases significantly during severe asphyxia in the fetal lamb brain, even though there is a high threshold for degrading energy rich intracellular purines.

Hypoxanthine is also a free radical generator and this recognition has resulted in renewed interest in the hypoxia-ischaemic reactive oxygen species hypothesis. Hypoxia ischaemia and free radical generation are also pathogenic factors in ischaemic disorders of the infant brain, this suggests possible strategies for intervention. Prophylactic inhibition of xanthine oxidase in preterm babies at risk of PVL may reduce the free radical generation after reperfusion/reoxygenation and may therefore limit the extent of tissue destruction. Allopurinol and its major active metabolite oxypurinol are specific inhibitors of xanthine oxidase that have been shown to limit tissue damage due to free radical activity. Other free radical scavengers may also have a role in ameliorating damage. Difficulties would be encountered in antenatal hypoxia, but peripartum hypoxic-ischaemic insults may be more amenable to therapeutic intervention.