ORIGINAL ARTICLE

Early postnatal allopurinol does not improve short term outcome after severe birth asphyxia

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Objective: To investigate whether postnatal allopurinol would reduce free radical induced reperfusion/reoxygenation injury of the brain in severely asphyxiated neonates.
Method: In an interim analysis of a randomised, double blind, placebo controlled study, 32 severely asphyxiated infants were given allopurinol or a vehicle within four hours of birth.
Results: The analysis showed an unaltered (high) mortality and morbidity in the infants treated with allopurinol.
Conclusion: Allopurinol treatment started postnatally was too late to reduce the early reperfusion induced free radical surge. Allopurinol administration to the fetus with (imminent) hypoxia via the mother during labour may be more effective in reducing free radical induced post-asphyxial brain damage.

Reperfusion and reoxygenation after severe birth asphyxia gives rise to excess free radical formation leading to hypoxic-ischaemic encephalopathy and myocardial dysfunction and is an important cause of neonatal mortality and morbidity in the western world.[1] In earlier preliminary studies, allopurinol treatment of the asphyxiated baby showed encouraging results, reducing free radical formation and reperfusion injury to brain and heart.[2 3] Allopurinol reduces free radical production by xanthine oxidase inhibition and probably by scavenging the very toxic hydroxyl free radical.[4] We therefore started a randomised, double blind, multicentre study to investigate if high dose postnatal allopurinol had a reducing effect on reperfusion injury after severe birth asphyxia. However, as mortality remained high (72%) in the population included until now (n = 32), we performed an interim analysis to have an indication if allopurinol had any reperfusion injury reducing effect at all (mortality and short term outcome), which would justify continuation of the study.

PATIENTS AND METHODS

Thirty two severely asphyxiated infants admitted to the three participating neonatal intensive care units were included after written informed parental consent, when the interim analysis was performed. Initially, we planned to include 100 infants based on a significant positive effect on outcomes of 10%, given a variation coefficient of 15%. Inclusion criteria were: a gestational age of at least 37 completed weeks without chromosomal abnormalities; signs of fetal distress such as late decelerations on fetal monitoring or meconium staining; the need for resuscitation for at least five minutes; cord or first pH<7; multi-organ failure and burst suppression pattern or worse (see also below) on an amplitude integrated electroencephalogram (aEEG).[5] Moreover, vehicle or allopurinol had to be administered within 4 hours of age. This randomised, double blind, placebo controlled study was approved by the scientific boards and ethical committees of the participating hospitals.

After randomisation, the infant received intravenously a total dose of 40 mg/kg allopurinol (Apurin; Multipharma, Copenhagen, Denmark) or vehicle taken from identical numbered ampoules in 30 minutes in two doses: as soon as possible after birth and 12 hours later. Plasma concentrations of allopurinol and its metabolite oxypurinol were determined 30 minutes and two hours after the first dose and just before and 30 minutes and two hours after the second dose. Adverse effects of allopurinol on white blood count, skin, and liver enzymes were monitored. Blood samples were collected for determination of lactate and the S100B protein before treatment, at 12 hours of age, and again on days 2 and 3. Liver enzymes and plasma creatinine and urea were measured on day 2 as indicators of multiple organ failure. Hypoxic-ischaemic encephalopathy staging following the Sarnat score[5] was performed before treatment and then daily or more frequently if important alterations occurred in the patient's condition. A neurological examination was performed at discharge in the surviving infants.

After admission, electrical brain activity was monitored by aEEG (CFM, Lectromed; Oxford Instruments, Oxford, UK). aEEG has been proven to be of value for evaluating background and seizure activity. The type of background pattern predicts long term outcome[5]; continuous normal voltage, discontinuous normal voltage, burst suppression, continuous extremely low voltage, and flat trace are recognised patterns. The last three patterns, which are abnormal, are induced by hypoxic-ischaemic encephalopathy. Cranial ultrasonography was performed immediately after admission, 24 hours later, and at least daily thereafter. A two dimensional, pulsed Doppler ultrasound machine (Ultramark 4; Advanced Technology Laboratories, Inc, Bothell, Washington, USA) with a transducer with a multifrequency of 7.5 and 10 MHz (for detection of subcortical lesions) was used. Basal ganglia and (sub)cortical hyperechogenicity were considered to be major abnormalities when present on at least two sequential investigations.[6 7] Magnetic resonance imaging was performed at 24–120 hours. T1, T2, and diffusion weighted images were studied.

Abbreviations: aEEG, amplitude integrated electroencephalogram; MRI, magnetic resonance imaging

Data are summarised as mean (SD) or median (range) where appropriate. Differences between treatment groups were compared by unpaired Student's *t* test, Mann-Whitney U test, or χ^2 test, as appropriate. Differences over postnatal time within groups were assessed using analysis of variance for repeated measurements, followed by Scheffe's procedure (analysis of variance) if a significant difference was found. Statistical significance was assumed for $p<0.05$.

RESULTS

Of the 32 infants, 17 received allopurinol and 15 received an equivalent dose of the vehicle. Birth weight and gestational age were 2700–4960 g and 37–41.4 weeks respectively for the allopurinol treated infants and 2390–4720 g and 37–42 weeks for the vehicle treated infants. Cord pH, Apgar scores at five minutes, and maximal Sarnat score (ranging from 0 (normal) to 3 (severely abnormal)) did not differ between the groups (allopurinol group: pH 6.49–7.00; Apgar score 3–6; Sarnat score 3 (n = 15) and 2 (n = 2). Vehicle group: pH 6.64–7.00; Apgar score 1–8; Sarnat score 3 (n = 14) and 2 (n = 1)). All but two infants needed assisted ventilation beyond the resuscitation period. Ten and eight infants from the allopurinol and vehicle groups respectively needed positive inotropic support to maintain blood pressure within normal limits (mean blood pressure ⩾40 mm Hg). Maximal plasma lactate concentration was 17.8 (4.5) and 13.5 (3.9) mmol/l for the allopurinol and vehicle groups respectively. The highest plasma creatinine and urea concentrations, liver enzymes, and S100B were all raised and did not differ between the groups (values not shown). Maximal allopurinol concentrations were mostly well above the therapeutic limits for adults (>13 mg/l) and ranged between 9.8 and 59 mg/ml and between 20.0 and 63 mg/ml during the first and second dose respectively. Oxypurinol concentrations were 2.7–7.1 and 2.4–8.1 mg/ml respectively. Despite the high concentrations, no adverse effects were detected.

Table 1 provides aEEG related data, and the results of ultrasound and magnetic resonance imaging (MRI) investigations of the brain. Twenty infants had no MRI investigation because they died before 72 hours of age. In this group, 12 infants died within 24 hours from cardiac failure. The remainder died within 72 hours because of neurological deterioration. These children all had a flat trace on aEEG with repeated isoelectric EEGs and major abnormalities on ultrasound examination. MRI was not expected to be of additional value in the decision making.

All infants except one allopurinol treated infant needed anticonvulsive therapy ranging from one drug (phenobarbital) to four drugs (phenobarbital, lidocaine, midazolam, and clonazepam). In seven infants from both groups, the aEEG improved from a burst suppression pattern or worse to a normal pattern within 24 hours of life. The ultrasound studies showed a substantial amount of damage in basal ganglia and/or cortical regions in both groups, with no significant differences between the groups. Mortality was high, always due to neurological deterioration. All survivors had a MRI scan. Three of the four allopurinol treated survivors and two of the five vehicle treated survivors had abnormal MRI findings. Neurological examination at discharge was normal in two allopurinol treated and three vehicle treated infants.

Table 1 Short term outcome of studied infants

	Allopurinol (n = 17)	Vehicle (n = 15)
Recovery aEEG to normal pattern within 24 h	7 (3†)	7 (2†)
No recovery of aEEG to normal pattern	10 (10†)	8 (8†)
Seizure activity		
None	1 (6%)	1 (7%)
Single or repetitive seizures	8 (47%)	9 (60%)
Status epilepticus	8 (47%)	5 (33%)
Cranial ultrasonography (n = 31)*		
Normal	2 (12%)	3 (20%)
Increased echogenicity of basal ganglia	6 (35%)	7 (47%)
Increased echogenicity of (sub)cortical region	4 (24%)	0 (0%)
Increased echogenicity in both regions	5 (29%)	4 (27%)
Cranial MRI (n = 12)		
Normal	1 (6%)	1 (7%)
Basal ganglia abnormalities	2 (12%)	1 (7%)
(Sub)cortical abnormalities	2 (12%)	2 (13%)
Abnormalities in both regions	1 (6%)	2 (13%)
Mortality	13 (76%)	10 (67%)

*One missing in vehicle group but postmortem MRI.
†Died.
aEEG, Amplitude integrated electroencephalogram; MRI, magnetic resonance imaging.

DISCUSSION

This interim analysis showed that mortality was high and not different between allopurinol and vehicle treated infants. Short term outcome of the surviving infants was not at all favourable and not different between the groups. Although we cannot exclude beneficial effects of early postnatal allopurinol treatment because of the small sample size, the results presented here make it unlikely that clinically relevant positive effects on survival and outcome can be expected in these severely asphyxiated babies.

We can only postulate why this is the case. Perhaps the selection of only extremely severe asphyxiated babies makes it unrealistic to expect beneficial effects from postnatal interventions, because these infants already had substantial neuronal damage. This seems to be supported by the results of a recently published study on selective head cooling after neonatal encephalopathy showing no protection in asphyxiated infants with substantial aEEG abnormalities.[8] Another explanation may be that allopurinol and its metabolite oxypurinol do not reach brain tissue in therapeutic concentrations. However, animal studies have proven that allopurinol and oxypurinol pass through the blood/brain barrier and cause therapeutic concentrations in brain perfusate and cerebrospinal fluid.[9 10] Also, further experimental research in several species shows brain protection when allopurinol is given upon reperfusion and reoxygenation.[4] In addition, we feel that the relatively late start of the treatment, three to four hours after reperfusion, may have played an important role here. It is well documented that

What is already known on this topic

- Allopurinol reduces free radical production in experimental research
- Allopurinol preserves the cerebral energy state, reduces brain oedema, and improves the electrocortical activity in various animal studies

What this study adds

- Allopurinol treatment after perinatal asphyxia (started about four hours after birth) is too late to exert beneficial effects
- If birth asphyxia is too severe, postnatal allopurinol does not influence survival or neurodevelopmental outcome

hypoxia related xanthine oxidase and the free iron driven Fenton reaction with consequent excess formation of the superoxide and hydroxyl free radicals occur upon and immediately after reoxygenation. This implies that two of the free radical reducing effects of allopurinol should optimally occur before or upon reperfusion. In this respect, the study of Boda *et al*[11] is interesting, in which antenatal allopurinol treatment of the mother just before delivery showed therapeutic allopurinol and measurable oxypurinol concentrations in cord blood of the newborn baby. A pilot study of our group in which mothers in labour and with signs of fetal hypoxia (ST elevations on the fetal electrocardiogram, late decelerations on the cardiotocogram, pH<7.20 measured by fetal blood sampling) were treated with allopurinol (500 mg intravenously). We found therapeutic concentrations of allopurinol in cord blood that were somewhat reduced but still within the range of effective inhibition of xanthine oxidase (allopurinol: 3.0–4.1 mg/ml; oxypurinol: 0.8–4 mg/ml). We therefore hypothesise that pharmacological intervention during fetal hypoxic distress and before reperfusion with an immediate postnatal booster of allopurinol (and preferably combined with moderate hypothermia) reduces birth asphyxia related brain damage even in the most severely affected neonate. On the basis of the data provided here we prematurely terminated the postnatal allopurinol trial and have started a randomised controlled trial with allopurinol treatment of the mother when there are signs of fetal hypoxia.

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