Send Orders for Print-Reprints and e-prints to reprints@benthamscience.ae

Current Pharmaceutical Design, 2017, *23*, 1-8 1

REVIEW ARTICLE

Allopurinol: Old Drug, New Indication in Neonates?

Kim V. Annink[1], Axel R. Franz[2], Jan B. Derks[3], Mario Rüdiger[4], Frank van Bel[1] and Manon J.N.L. Benders[1]

[1]*Department of Neonatology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, the Netherlands;* [2]*Department of Neonatology and Centre for Paediatric Clinical Studies, Universitätsklinikum Tübingen, Germany;* [3]*Department of Obstetrics, Wilhelmina Children's Hospital, University Medical Centre Utrecht, The Netherlands;* [4]*Department of Neonatology and Paediatric Intensive Care, Universitätsklinikum Carl Gustav Carus, TU Dresden, Germany*

Abstract: ***Background*:** Hypoxic-ischemic encephalopathy (HIE) is an important cause of neonatal mortality and neurological morbidity, even despite hypothermia treatment. Neuronal damage in these infants is partly caused by the production of superoxides via the xanthine-oxidase pathway and concomitant free radical formation. Allopurinol is a xanthine-oxidase inhibitor and can potentially reduce the formation of these superoxides that lead to brain damage in HIE.

Methods: The aim of this review is to provide an overview of the animal and clinical data about the neuroprotective effect of allopurinol in HIE and the relevant mechanisms leading to brain injury in HIE.

Results: A possible neuroprotective effect of allopurinol has been suggested based on several preclinical studies in rats, piglets and sheep. Allopurinol seemed to inhibit the formation of superoxide and to scavenge free radicals directly, but the effect on brain damage was inconclusive in these preclinical trials. The neuroprotective effect was also investigated in neonates with HIE. In three small studies, in which, allopurinol was administered postnatally and a pilot and one multi-center study, in which, allopurinol was administered antenatally, a possible beneficial effect was found. After combining the data of 2 postnatal allopurinol studies, long-term follow-up was only beneficial in infants with moderate HIE, therefore, large-scale studies are needed. Additionally, safety, pharmacokinetics and the neuroprotective effect of allopurinol in other neonatal populations are discussed in this review.

Conclusion: The available literature is not conclusive whether allopurinol is a neuroprotective add-on therapy in infants with HIE. More research is needed to establish the neuroprotective effect of allopurinol especially in combination with hypothermia.

ARTICLE HISTORY

Received: August 1, 2017
Accepted: September 14, 2017

DOI:
10.2174/1381612823666170918123307

Keywords: Allopurinol, hypoxic-ischemic encephalopathy, perinatal asphyxia, neuroprotection, add-on therapy, reperfusion injury, xanthine-oxidase inhibitor.

1. ALLOPURINOL: FROM GOUT THERAPY TO NEUROPROTECTIVE AGENT

Allopurinol is a xanthine-oxidase inhibitor that inhibits the production of uric acid. The enzyme xanthine-oxidase converts hypoxanthine into uric acid (1). Allopurinol is almost completely metabolized into oxypurinol by aldehyde oxidase in the liver and is eliminated by the kidneys (1). Oxypurinol is the active metabolite of allopurinol and predominantly inhibits xanthine-oxidase because allopurinol is almost completely metabolized into oxypurinol and oxypurinol has a longer half-life than allopurinol (2).

Historically, allopurinol is a well-known therapy for gout in adults by reducing the concentrations of uric acid and thereby the formation of uric acid crystals (1). Furthermore, allopurinol is widely used as therapy for tumour lysis syndrome and kidney stones. Allopurinol also inhibits the production of free radicals. Therefore, new indications have been investigated for this 'old drug'. Currently, research is focusing on possible cardioprotective and neuroprotective effects of allopurinol by inhibiting the formation of the free radical superoxide production in neonates and adults. Free radicals lead to cell damage by causing oxidative stress i.e. the peroxidation of proteins, lipids and DNA, which in turn causes mitochondrial damage and induces apoptotic pathways (3, 4). These free radicals are formed in the presence of oxygen: hypoxanthine and oxygen are converted into uric acid and superoxide by xanthine-oxidase (5). Allopurinol and oxypurinol can both inhibit xanthine-oxidase and therefore, the production of superoxide. An additional working mechanism of allopurinol and oxypurinol is free radical scavenging; in neonates as well non-protein bound iron and the hydroxyl radical seemed to be directly scavenged by allopurinol and oxypurinol (6, 7).

From the 1970's onwards, allopurinol was investigated in animal studies because of its possible cardioprotective effect by inhibiting the formation of oxygen radicals (8-13). Following animal studies, it was shown that allopurinol pre-treatment in adult patients undergoing coronary bypass surgery led to a better recovery (14). Also, the hospital mortality, cardiac performance and postoperative recovery, defined as less inotropic and mechanical support, improved after cardiac bypass surgery in allopurinol treated patients (15). On the contrary, other studies did not find an improvement in cardiac function after surgery in these patients (10). Allopurinol was also investigated as a therapy for chronic heart failure and angina pectoris. The hypothesis was that inhibition of the formation of uric acid and the free radical superoxide might prevent endothelial damage and myocardial oxidative stress (16). However, until this moment, the use of allopurinol as a cardioprotective agent in adults with cardiovascular diseases remains controversial and larger prospective studies are required to determine the cardioprotective effect of allopurinol (17).

The earlier mentioned cardiac studies, which showed that the production of free radicals was leading to hypoxic-ischemic damage of the heart, were the basis of the hypothesis that allopurinol might also be beneficial for the prevention of hypoxic-ischemic damage of the brain. Perinatal asphyxia in the newborn leads to the

*Address correspondence to this author at the Room number: KE 04.123.1 t.a.v. Manon Benders, P.O. Box 85090, 3508 AB Utrecht, The Netherlands; Tel: +31-88-7575400; Fax: +31-88-1555320;
E-mail: m.benders@umcutrecht.nl

production of hypoxanthine and the activation of xanthine-oxidase leading to subsequent brain damage (5). Considering that allopurinol can inhibit the xanthine-oxidase pathway, an allopurinol-induced reduction of superoxide might be neuroprotective in hypoxic-ischemic encephalopathy (HIE).

2. HYPOXIC-ISCHEMIC ENCEPHALOPATHY

One to 8 per 1000 live born neonates experience HIE caused by perinatal asphyxia (18). Perinatal asphyxia is one of the most important causes of death and long-term neurological damage in term born neonates. The current standard of care in perinatal asphyxia is moderate hypothermia for 72 hours, starting within 6 hours after birth. In the TOBY trial, cooled infants were followed up until the age of 6 to 7 years and their outcome was compared to non-cooled infants (19, 20). Despite hypothermia, approximately 45% of these neonates had an adverse outcome at the age of 2 years, defined as severely impaired neurological outcome or death, compared to 53% in the non-cooled group (RR 0.86, 95%CI 0.68-1.07) (19). At the age of 6 to 7 years, 55% of the cooled infants that survived experienced neurologic abnormalities compared to 72% in the non-cooled group (20). Neurologic abnormalities were defined as an IQ score below 85, abnormalities in neurologic examination, hearing or vision. The survival rates did not differ significantly (20). In conclusion, hypothermia is an effective neuroprotective strategy for infants with HIE, however still a sizable amount of these infants dies or has an adverse neurological outcome. Therefore, additional neuroprotective therapies are essential to reduce neurological damage in these infants.

3. PATHOPHYSIOLOGY OF BRAIN DAMAGE AFTER PERINATAL ASPHYXIA

Brain damage after perinatal asphyxia is caused by hypoxia leading to neuronal cell damage. In neonates with perinatal asphyxia, there are two moments of neuronal cell damage: a first peak caused by primary energy failure during the hypoxic event at birth and a second peak caused by the reoxygenation and reperfusion after birth, called reperfusion injury (21, 22).

Figure **1** shows the pathophysiologic processes of brain damage after perinatal asphyxia.

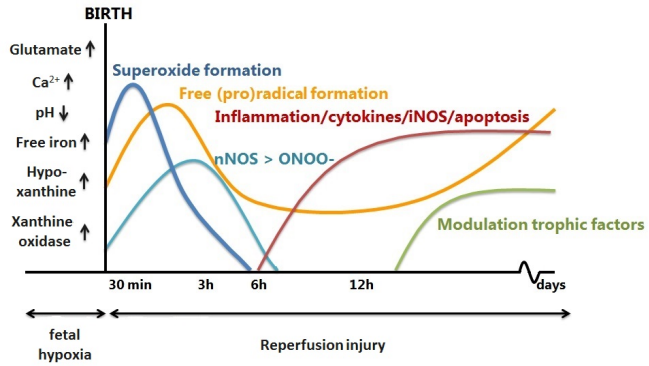

Fig. (1). Activated pathways leading to brain damage in HIE (22). (adapted from van Bel and Groenendaal, 2016).

The acute moment of hypoxia during birth (which is caused by deficient oxygen supply e.g. due to placental abruption or other sentinel events) results in primary energy failure and, consequently, the degradation of ATP and eventually necrotic cell death (21, 22). This leads to the release of excitatory neurotransmitters as glutamate, which results in the over-activation of the NMDA receptors and failure of the Na-K-ATPase pump (22-24). Both generate a calcium influx into the cells, leading to the activation of enzymes, such as proteases, that initiate predominantly apoptotic cell death (21, 25). The acute moment of fetal hypoxia also initiates the production of pro-radicals, such as non-protein bound iron, that later on can form free radicals after reoxygenation upon birth (22, 26-28). ATP degradation also increases the level of adenosine, which is converted into hypoxanthine via inosine and cumulates during fetal hypoxia (5, 29). These elevated hypoxanthine levels can also lead to production of superoxide after birth because xanthine-oxidase levels also rise during hypoxia (29).

After birth, several pathways are activated because of reoxygenation leading to an excessive production of free radicals and superoxide in HIE. The increased levels of hypoxanthine and xanthine-oxidase in combination with the extra supply of oxygen during reoxygenation lead to the activation of the xanthine-oxidase pathway (22, 30-32). Xanthine-oxidase converts hypoxanthine and oxygen into uric acid and superoxide (5), see *Figure 2*. This inappropriate superoxide production reaches its peak within 30 minutes after birth and plays a central role in the activation of destructive molecular pathways (30-32). The superoxide-derived hydrogen peroxide interacts with pro-radicals such as NBPI (resulting in the formation of the very toxic hydroxyl free radical (22). Also nitric oxide (NO), derived from an increased production of endothelial and neuronal nitric oxide synthase, reacts with superoxide to form the toxic compound peroxynitrite ($ONOO^-$) (22, 27, 33, 34). These free radicals and toxic compounds will cause additional neuronal cell damage, but they also activate an inflammatory response leading to the formation of (pro)inflammatory cytokines from about 6-12 hours after birth onwards (22, 35). The subsequent apoptotic activity and eventually down regulation of trophic factors also contribute to the neuronal cell injury and start about 12 to 24 hours after birth and can last for days and even weeks (22, 36, 37). Given this potential pivotal role of superoxide, acute reduction of superoxide formation on top of moderate hypothermia by allopurinol might lead to a reduction of brain damage after perinatal asphyxia.

4. PRECLINICAL STUDIES – NEONATAL ADMINISTRATION FOR HIE

The first animal studies investigating the neuroprotective effect of allopurinol in HIE were performed by Palmer and colleagues in 7-day old rat pups (38-40). In the first studies allopurinol was administered 30 minutes before inducing hypoxia and this resulted in a reduction of cerebral oedema and a lower incidence of infarction (39). Furthermore, ATP increased and the Pi/PCr ratio decreased at 31P-NMR-spectroscopy in rats, which represents a preserved cerebral metabolism (38). In a subsequent study by this group using 7-day old rat pups, allopurinol was given 15 minutes after inducing hypoxia. This led to reduced atrophy and cerebral oedema, as well as less overall brain injury based on a histology scoring system (40). In the studies in which allopurinol was administered before inducing hypoxia core temperature was not measured, so it cannot be excluded that these rats were hypothermic and that this partly has influenced the neuroprotective effect (38, 39). In the study of Palmer *et al* in 1993, the core body temperature was measured before and after allopurinol administration. In the placebo group the temperature varied 0.16±0.18 degrees of Celsius before and after placebo administration and in the allopurinol 0.09±0.32 degrees of Celsius, so these are only minor variations in temperature (40). Hypothermia is therefore an unlikely explanation for the beneficial effect of allopurinol in these rat pups.

The effect of allopurinol in asphyxiated piglets was investigated with phosphorous magnetic resonance spectroscopy, Near Infrared Spectroscopy (NIRS), electroencephalography (EEG) and histology by Peeters-Scholte *et al* (41, 42). After one hour of hypoxia, allopurinol was administered directly during start of reperfusion and 12 hours after reperfusion. On magnetic resonance spectroscopy, a preservation of the cerebral energy status was found and based on T2-weighted MRI less oedema in the cortex, striatum and thalami were found in allopurinol treated animals. However, there

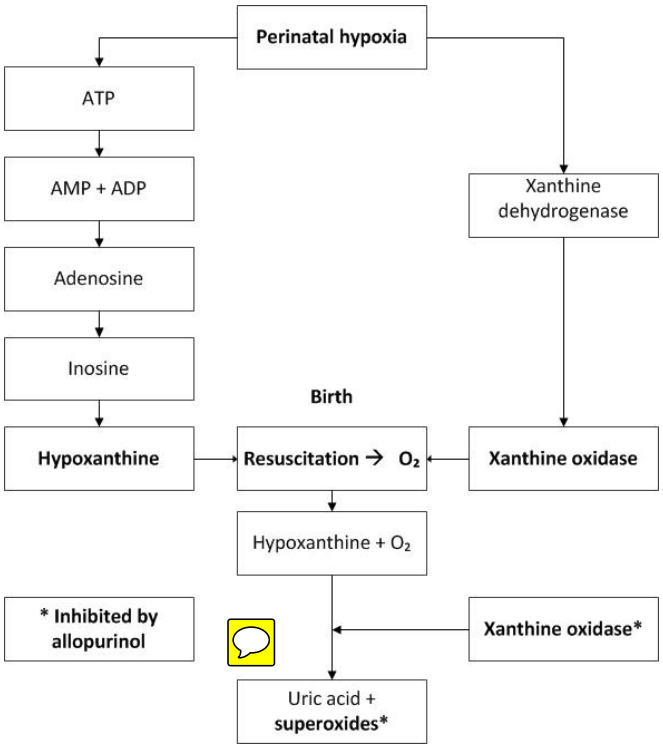

Fig. (2). Before birth, during perinatal hypoxia, several pathways are activated, including the production of hypoxanthine and xanthine-oxidase. Because of hypoxia, ATP is converted to AMP and ADP, AMP leads to the production of adenosine and consequently to inosine, which increases the concentration of hypoxanthine in the blood. Also, xanthine-oxidase concentrations rise because of hypoxia by the production of xanthine dehydrogenase. After birth, the supply of extra oxygen during reperfusion leads to the production of superoxide. Xanthine-oxidase causes a reaction in which hypoxanthine and oxygen are converted to uric acid and superoxide. Allopurinol inhibits xanthine-oxidase and thereby reduces the formation of superoxide. Further, allopurinol is thought to have direct hydroxyl radical scavenger effect and is a non-protein-bound iron chelator.

was no improvement on NIRS, aEEG or histology (41, 42). The authors' explanation was that the moderately asphyxiated piglets might have experienced a positive effect on outcome, but that treatment with allopurinol had no effect when the brain is too severely damaged. Although the cerebral energy status might have been preserved, severe brain damage might already have occurred and did not improve after allopurinol. However, no sub-analysis was performed to confirm this hypothesis (41, 42). Rectal temperature remained stable in both groups during the study. Only short-term outcomes until 24 hours after birth were measured (41, 42).

Several animal studies have been performed to investigate the neuroprotective mechanisms of allopurinol in HIE. Marro *et al* showed that uric acid levels were reduced in allopurinol treated piglets with HIE (43). This implicated that the xanthine-oxidase pathway was indeed inhibited after allopurinol administration in piglets, since uric acid is the final product in this cascade. The same group also showed that the Na-K-ATPase pump, that often fails in HIE, was more active in newborn animals suffering HIE following allopurinol administration compared to controls (43, 44). As discussed earlier, failure of the Na-K-ATPase pump leads to a calcium influx into the cells which results in cell damage and (pro)radical formation. A decreased failure of the Na-K-ATPase pump might result in less neuronal cell damage. More recently, Marro *et al* showed that adenosine and inosine levels were higher in allopurinol treated piglets than in controls (45). This suggested that allopurinol also reduced the conversion of adenosine and inosine into hypoxanthine. Lower hypoxanthine levels might result in less superoxide formation. A high adenosine concentration is also thought to be neuroprotective itself, because adenosine can inhibit the production of excitatory neurotransmitters such as glutamate and increases cerebral blood flow during hypoxia (45). Additionally, high levels of allopurinol and oxypurinol also seem to be direct non-protein-bound iron (NPBI) chelators and hydroxyl radical scavengers (6, 7). NPBI levels in cortical tissues were decreased in allopurinol treated lambs with HIE compared to placebo, but plasma NPBI levels did not differ (7). This suggested that allopurinol and oxypurinol were able to cross the blood brain barrier. However, allopurinol in the dose of 20mg/kg did only partly chelate NPBI levels in lambs (7). Both allopurinol and oxypurinol have a hydroxyl radical scavenging effect. The scavenging effect of oxypurinol was stronger than of allopurinol (6).

Whether the possible neuroprotective effect of allopurinol is mainly caused by xanthine-oxidase inhibition or free radical scavenging has not yet been elucidated.

All above mentioned preclinical studies only measured short-term outcome. The measurement of long-term outcomes such as long-term neurobehavioral studies are essential to establish a possible effect of allopurinol in HIE. Further, the effect of postnatal allopurinol has not been investigated as an addition to hypothermia treatment in preclinical research.

5. POSTNATAL STUDIES IN NEONATES IN HIE

Because of the promising results of allopurinol therapy in animal studies, it was decided to start an open label study in the newborn infant with perinatal asphyxia. The first study in neonates was an unblinded randomized controlled trial (RCT) in which two dosages of 20mg/kg allopurinol or placebo were given after birth to 22 neonates, the first dose up to 4 hours after birth and a second dose 12 hours later (Table **1**) (46). The short-term effect of allopurinol was assessed with chemical biomarkers (lipid peroxidation and antioxidative parameters), the pattern of the cerebral blood flow was measured with Near Infrared Spectroscopy (NIRS) and electrical brain function with amplitude-integrated EEG (aEEG). NPBI levels were significantly lower two days postpartum and uric acid levels were decreased from 16 hours postpartum onwards in the allopurinol group compared to the controls. However, lipid peroxidation and antioxidative parameters were the same in the allopurinol group and in the controls. Moreover, allopurinol induced a relative preservation of cerebral blood flow and electrical brain activity was higher in the allopurinol group suggesting less brain damage. In the allopurinol group 2 of the 11 infants died, whereas in the control group 5 out of 11 died. In conclusion, this study suggested a beneficial effect of allopurinol, without toxic side effects. However, the sample size of this study was small and the study was unblinded (46).

A subsequent multicentre, double-blinded RCT was performed by Benders *et al* in 2006. Allopurinol was administered to 17 infants in the same dosages at the same time points as in the previous study and a placebo was administered to 15 infants. However, only infants with perinatal asphyxia, multi-organ failure and an abnormal aEEG were included. These strict inclusion criteria led to the inclusion of only severely affected neonates. Therefore, mortality was rather high in this study: 76% in the allopurinol group and 67% in the placebo group, which was not statistically different. Short-term outcomes, namely MRI and cranial ultrasound abnormalities, seizures and S100ß concentrations, were similar in both groups (47). This suggested that allopurinol might be more effective in moderately affected neonates than in severely affected neonates.

All participants of the two above mentioned studies were seen for neurocognitive follow-up at the age of 4 to 8 years (mean of 5 years and 5 months). Children were tested with a neurological examination and the Wechsler Preschool and Primary Scale of Intelligence or the Wechsler Intelligence Scale for Children. There was

Table 1. Overview of clinical studies with allopurinol in HIE.

Study	Population	Moment of Administration and Dosages	Results
Van Bel *et al*, 1998	Allopurinol (n=11) vs. controls (n=11)	20mg/kg i.v. within 4 hours after birth, 2nd dose of 20mg/kg i.v. 12 hours later (median allopurinol administration 170 min; range 68-210)	Positive effect on free radical formation, electrical brain activity and a relative preservation of cerebral blood volume. No adverse events.
Benders *et al*, 2006	Allopurinol (n=17) vs. placebo (n=15)	20mg/kg i.v. within 4 hours after birth, 2nd dose of 20mg/kg i.v. 12 hours later (median and range not shown)	No effect on short term outcome in severely affected infants. No adverse events.
Gunes *et al*, 2007	Allopurinol (n=30) vs. placebo (n=30)	20mg/kg i.v. within 2 hours after birth, second dose 12 hours later, then every 12 hours a dose for 3 days (median and range not shown)	Better neurological outcome at 1 year of age. Serum NO decreased after allopurinol. No adverse events.
Kaandorp *et al*, 2012	Allopurinol (n=28) vs. placebo (n=26)	Follow-up study of Benders *et al* and van Bel *et al.* at four to eight years of age.	Improved neurological outcome in moderately affected infants. No adverse events.
Torrance *et al*, 2009	Allopurinol (n=27) vs. placebo (n=27)	500mg allopurinol i.v. antenatally (median allopurinol administration 56min before delivery; range 18-190)	Reduction of S100ß levels in therapeutic allopurinol group. No adverse events.
Kaandorp *et al*, 2015	Allopurinol (n=113) vs. placebo (n=111)	500mg allopurinol i.v. antenatally (median allopurinol administration14min before delivery; IQR=7.3-26.9)	In girls S100ß levels and oxidative stress markers are reduced in the allopurinol group compared to controls No adverse events.

no significant improvement of adverse long-term neurodevelopmental outcome (allopurinol 8% vs. controls 11%, p=1.000) or mortality (allopurinol 54% vs. controls 62%, p=0.376) in the overall analysis. However, when only the moderately asphyxiated infants were analysed, a significant improvement of long-term outcome was found in the allopurinol treated infants compared to the controls. In moderately affected infants, 65% of controls had a severe adverse outcome, defined as death or severe neurodevelopmental disabilities, compared to 25% of the allopurinol treated infants (p=0.047) (48). This follow-up study suggested a positive effect of allopurinol in moderately asphyxiated infants on neurological outcome, but not in severely asphyxiated infants. This is in line with a previous study on neonatal head cooling after acute perinatal asphyxia in term infants (49) and the previously discussed animal studies (41, 42). However, the numbers of this follow-up study were very small, therefore the results should be interpreted with caution (48).

The most recent RCT investigating the benefit of postnatal allopurinol in neonates with HIE was performed by Gunes *et al* in Turkey. Thirty neonates were treated for three days with 2 dosages of 20 mg/kg allopurinol per day, with the first dose being administered within two hours after birth. The control group of thirty neonates received a placebo. This study was not blinded in order to enable the investigators to monitor side effects. Free radical production, measured by NO concentrations in the serum, was decreased after the administration of allopurinol compared to controls. NO concentrations in the cerebrospinal fluid were comparable between the groups. Adverse outcome at one year of age, was reduced in the allopurinol group compared to placebo (39.3% vs. 53.6%, p<0.05). Adverse outcome was defined as cerebral palsy, Bayley score < -2SD, blindness and/or deafness. Mortality did not differ between the groups. No adverse side effects of allopurinol were seen (50). Although this study is larger than the other two, the sample size is still not large enough to draw conclusions about the neuroprotective effect of allopurinol. Furthermore, a longer neurological follow-up is essential to determine the actual effect of allopurinol on the long-term in HIE. Additionally, pharmacokinetic analysis would have been valuable since this study used other dosing protocols than the previous studies.

It can be concluded from these clinical studies that allopurinol might be neuroprotective in moderately affected infants, but is not effective when the brain damage is too severe. Considering that the onset of free radical production is immediately after the onset of reperfusion, administration of allopurinol within four hours after birth might be too late to reduce the peak of these toxic metabolites. Importantly, in none of the studies adverse side effects were seen.

As expected, the conclusion of the Cochrane review including the studies of van Bel *et al,* Benders *et al* and Gunes *et al* stated, that there is not enough evidence to draw conclusions and that larger trials are needed (51). In combination with the idea that allopurinol should be given as early as possible, the hypothesis arose that antenatal administration of allopurinol in case of suspected fetal hypoxia might be more effective.

6. PRECLINICAL STUDIES – ANTENATAL ADMINISTRATION FOR HIE

Pharmacokinetics of allopurinol in mother and fetus were investigated in case of antenatal allopurinol administration in several experimental studies in fetal sheep. Allopurinol crossed the placenta rapidly in a number of animal studies (52-56). After 20mg/kg allopurinol was administered intravenously the maternal allopurinol

and oxypurinol concentrations reached their maximum within 20 minutes after birth in sheep. The oxypurinol concentrations in all fetuses were in the therapeutic range (53).

The neuroprotective effect of antenatal allopurinol (20mg/kg weight of the mother) was tested in five sheep and compared to six controls. Allopurinol was administered to the mother during the induction of hypoxia. Brain damage was studied based on histology 48 hours after birth. In this experiment, there was less hippocampal damage in the allopurinol group compared to the placebo group. The dentate gyrus and thalami also appeared to be less damaged based on histological confirmed neuronal necrosis, although this was not statistically significant (57). Moreover, the cardioprotective effect of 20mg/kg allopurinol compared to placebo was also investigated in these 11 sheep after inducing hypoxia with a cord clamp model (53). The allopurinol treated sheep had less cardiac oxidative stress based on fetal blood pressure, heart rate, T/QRS ratio of the fetal ECG and troponin levels. Furthermore, umbilical blood flow was preserved in the allopurinol treated animals, while this was not the case in the control group. These findings suggested a cardioprotective effect of allopurinol in sheep (53). Importantly, core body temperature was not measured, so the influence of hypothermia is unclear in this study. Another study group found a reduced oxygen radical production in 3 fetal sheep after the administration of 400 mg allopurinol to the dam (55). Superoxide production was determined with chemiluminescence. The concentrations of superoxide rose until the administration of allopurinol, afterwards it declined until normal values within 90 minutes. Based on these results, antenatal allopurinol might reduce superoxide production in sheep with HIE (55).

Again, no follow-up has been performed to assess long-term outcome in these animals.

7. HUMAN ANTENATAL STUDIES IN HIE

To investigate the neuroprotective effect of antenatal allopurinol therapy in neonates with suspected fetal hypoxia, a double blinded randomized pilot study was performed including 54 infants based on an abnormal CTG or abnormal fetal scalp sampling, indicating that fetal hypoxia was imminent. The time between allopurinol administration and delivery of the baby varied between 18 and 190 minutes, which is relatively short. Allopurinol rapidly crossed the placenta in these pregnant women, but only in 15 out of 27 allopurinol treated infants therapeutic allopurinol or oxypurinol levels were reached. Apparently, the time between administration of allopurinol and actual delivery was too short for allopurinol to cross the placenta and to reach therapeutic plasma levels in some foetuses. Therefore, the allopurinol treated group was split into two subgroups: infants with therapeutic allopurinol or oxypurinol levels (n=15) and infants with sub-therapeutic allopurinol or oxypurinol levels (n=12). The sum of the allopurinol and oxypurinol concentrations in the umbilical cord were negatively correlated with S100ß concentrations, a biomarker for brain tissue damage ($r=0.59$, $p<0.01$). Furthermore, the S100ß levels were significantly reduced in the therapeutic allopurinol group compared to the other groups. Limitations of this study were the small sample size and the relatively high rate of sub-therapeutic allopurinol or oxypurinol levels (58).

Therefore, a Dutch multicentre, double-blinded, randomized controlled trial was performed in 222 pregnant women with suspected fetal hypoxia during labour, half of which received 500mg allopurinol intravenously and half a placebo. Primary outcomes were the concentrations of S100ß in umbilical cord blood and the concentrations of oxidative stress markers, e.g. neuroketal and 8-isoprostane. In the total group, there was no significant difference in S100ß or oxidative stress marker levels between allopurinol treated infants and controls. In a post-hoc analysis, S100ß and neuroketal levels were significantly reduced in girls in the allopurinol group compared to the controls, but the oxidative stress markers were similar for boys (59). This gender difference is possibly explained by different pathways for programmed cell death and is also seen in other neuroprotective strategies and other neurological childhood diseases (60, 61). Though this study was not designed to study gender differences, gender differences should be taken into account in future research (59). No adverse events occurred in the allopurinol group (59). A limitation of this study was that most infants had no or only mild asphyxia, which resulted in underpowered results (59). None of the infants were diagnosed with HIE after inclusion. The reason for the relatively high amount of mildly asphyxiated infants is that antenatal monitoring has a poor predictive value for actual perinatal asphyxia with a lot of false positive cases (59). Taking this into account, a very large RCT would be needed to estimate the actual effect of antenatal allopurinol administration.

Although antenatal administration of allopurinol in girls with HIE might be promising, it is not optimal either because of the difficulties to predict which infants will be born with perinatal asphyxia in the daily practice: actual asphyxiated babies might be missed and there will be a lot of overtreatment of fetuses without relevant hypoxia. Therefore early, neonatal allopurinol administration might be a more suitable design for future studies.

8. OTHER INDICATIONS

Allopurinol has also been tested in other neonatal populations in which free radical formation leads to cell damage.

The study of Derks *et al* that was discussed earlier, already suggested a beneficial cardioprotective effect of antenatal allopurinol in animals. Infants undergoing cardiac surgery experience periods of hypoxia, which is known to lead to brain damage and especially to white matter injury (62, 63). Allopurinol might also have neuroprotective effects next to the possible cardioprotective effects in neonates with congenital heart diseases. Serum uric acid levels after allopurinol administration were decreased in infants with a hypoplastic left heart syndrome (HLHS) compared to baseline. This suggests that the xanthine-oxidase pathway is activated in HLHS and that allopurinol can inhibit this pathway (64). In a RCT including infants with a congenital heart disease, allopurinol or placebo was administered before, during and after heart surgery with deep hypothermic circulating arrest (65). Infants with HLHS (n=131) as well as infants with other congenital heart diseases (n=187) were treated in this trial. In the infants with HLHS death, seizures, coma and/or cardiac events occurred in 38% of the infants in the allopurinol group and in 60% of infants in the placebo group ($p=0.01$) (65). This suggested a neuroprotective and cardioprotective effect of allopurinol in infants with HLHS. However, when the outcome parameters were analysed separately, there were no significant differences. Allopurinol was not effective in other congenital heart diseases (65). The authors hypothesized that HLHS infants had a worse cerebral oxygenation status before surgery than infants with other congenital heart diseases, but this has not been confirmed (65).

Marro *et al* investigated the effect of allopurinol in neonates undergoing extracorporeal membrane oxygenation (ECMO) in a RCT. Allopurinol was given to 11 infants in a dose of 10mg/kg before surgery, 20mg/kg was added to the ECMO circulation and afterwards 5mg/kg was given every 8 hours for 72 hours. Fourteen infants received placebo. This study confirmed that uric acid levels are decreased after allopurinol administration and hypoxanthine levels are increased, suggesting that the xanthine-oxidase pathway was inhibited and consequently the formation of free radicals was possibly reduced. The neuroprotective effect of allopurinol could not be verified, because free radicals and neurological outcome were not determined (66).

In premature born babies, the antioxidant system is not fully mature yet, thereby increasing the risk of free radical formation during stress. Therefore, idiopathic respiratory distress syndrome (IRDS) was believed to be caused by free radical formation (67). A

study in 1984 suggested a positive effect on mortality in preterm babies with IRDS (67). Later, allopurinol was also tested in premature born babies with a gestational age of 27 to 31 weeks (oral allopurinol 20mg/kg: n=16, placebo: n=17). In this insufficiently powered study, allopurinol had no effect on the incidence of periventricular leukomalacia, periventricular haemorrhage, porencephaly, necrotic enterocolitis, retinopathy of prematurity or bronchopulmonary dysplasia (68).

9. PHARMACOKINETICS OF ALLOPURINOL IN NEONATES

A combined pharmacokinetic study was performed for the cohorts of van Bel *et al* and Benders *et al.* Almost all neonates reached the target levels (2-13.6μg/ml) after two dosages of 20mg/kg, most of them even reached supra-therapeutic levels. Oxypurinol concentrations could be measured within 1 hour after the first dose of allopurinol. Oxypurinol levels were high for at least 14 hours after a first dose. The half-life of allopurinol was estimated around 7 hours (69). In infants with HLHS the half-life of allopurinol was shorter, around 2.5 hours (64). This difference might be explained by the younger age of the infants with HIE (69).

Despite the supra-therapeutic allopurinol and oxypurinol concentrations in neonates with HIE, no adverse events were seen (46, 47).

In the allopurinol treated infants undergoing ECMO, peak serum levels of allopurinol and oxypurinol were 28.4±3.5μg/ml and 15±4μg/ml respectively (70). The allopurinol and oxypurinol levels in ECMO were even higher than in the HIE studies. This is probably caused by the higher dose and frequency of allopurinol administration compared to the previously mentioned cohorts (69, 70)

Pharmacokinetics were also studied in 24 mothers delivering full-term neonates and 44 mothers delivering preterm neonates. Allopurinol (500mg) was orally administered to all mothers during early labour. All mothers reached therapeutic allopurinol levels and the levels of allopurinol in the cord blood and in the newborn at 24 hours after birth were therapeutic. Therapeutic levels in cord blood were reached as soon as 23 minutes after allopurinol administration, suggesting a quick placental transfer of oral allopurinol (52).

In the antenatal pharmacokinetic study of Kaandorp *et al*, 95% of the infants had an allopurinol concentration above the target concentrations in the cord blood (allopurinol $\geq$ 2 μg/μl and oxypurinol $\geq$ 4 μg/μl). The target concentrations were reached within 5 minutes after the end of maternal allopurinol infusion (71). In the ALLO-trial, 72% of the pregnant women reached the target blood levels of allopurinol (59).

10. SAFETY

In adults, a potentially fatal side effect of allopurinol is the allopurinol hypersensitivity reaction (AHR). This AHR is thought to be caused by potentially immunogenic complexes of oxypurinol and certain human leukocyte antigen (HLA) proteins, particularly HLA-B*58:01 (which is more common in Asians than in Caucasians, similar to the frequency distribution of AHR). AHR predominantly affects the skin and may present as a generalized rash, but can also present as severe exfoliative skin reaction, i.e., as Stevens-Johnson syndrome or toxic epidermal necrolysis. AHR might also present with eosinophilia, leucocytosis, fever, acute hepatocellular injury and/or progressive kidney failure (1).

Most AHR have been diagnosed after daily administration of allopurinol for a duration of more than 2 to 3 weeks, but single cases have been reported after a single day of allopurinol in adults (72). High doses allopurinol, pre-existing renal failure and comedication with ampicillin or amoxicillin seem to increase the risk for AHR.

The rate of occurrence for AHR is frequently cited to be approximately 0.1% in adults treated with allopurinol and this number origins from a report in which 3 out of 1835 hospitalized adults treated with allopurinol had probable AHR (73). More appropriately and more recently, a population-based study reported an annual incidence rate of 4.68 per 1000 new allopurinol users in Taiwan (74). Taking into account that the odds-ratio for AHR in Chinese compared to Caucasian is 70 (75), the incidence is probably less than 1 in 10,000 new Caucasian users. In all antenatal (n=138) and postnatal (n=58) treated infants with HIE, as in other term (n=178) and preterm (n=348) infants, no serious adverse reactions to allopurinol have been reported (46, 47, 50, 58, 59, 65-68, 76).

Consequently, a recent Cochrane review on postnatal allopurinol for HIE concluded that there are no major concerns about safety based on the available data (51).

Nevertheless, because of the high pH of an allopurinol preparation suitable for intravenous administration, perivascular or intra-arterial infusion of allopurinol must be strictly avoided. 4.5% of the allopurinol treated mothers had irritation of the perivascular tissue (71).

CONCLUSION AND RECOMMENDATIONS

In conclusion, the neuroprotective effect of allopurinol has been tested by postnatal administration in neonates with HIE and by antenatal administration in mothers with imminent fetal hypoxia. In newborn animals as well as in neonates, allopurinol seems to inhibit the formation of superoxide and scavenge free radicals that both can lead to brain damage. Based on available literature about antenatal and postnatal administration of allopurinol in animals and human neonates, allopurinol might reduce brain damage caused by perinatal asphyxia in term born neonates, but only in those with moderate HIE. Moreover, allopurinol in neonates appears to be safe: even when supra-therapeutic concentrations are reached no adverse events have been seen, but rare side effects may not yet have become apparent.

In summary, the literature is inconclusive whether allopurinol is a suitable neuroprotective therapy in infants with HIE. Antenatal administration may result in over-treatment, since it is difficult to select the mothers with imminent fetal hypoxia. In contrast, postnatal allopurinol within 4 hours after birth might be too late because the oxygen radicals are formed shortly after birth. Consequently, very early postnatal allopurinol administration, i.e. immediately after resuscitation, might be the most effective mode of application. However, the efficacy of early allopurinol administration has not been investigated yet, neither has the combination of allopurinol and hypothermia been investigated previously in clinical or preclinical studies.

Currently, a European double blinded, placebo-controlled, randomized controlled trial is designed in which 20mg/kg allopurinol intravenously will be administered immediately after or during resuscitation in asphyxiated babies. Neonates undergoing moderate hypothermia will receive a second dose of 10 mg/kg, 12 hours after birth. The primary endpoint will be death or impaired neurodevelopmental outcome at 2 years of age. Secondary endpoints are surrogate markers of brain injury on MRI, cerebral ultrasound, aEEG, multichannel EEG, plasma biomarkers and oxidative stress markers. This trial is essential to determine whether early allopurinol administration is a possible add-on neuroprotective therapy after perinatal asphyxia.

LIST OF ABBREVIATIONS

aEEG	=	Amplitude integrated electroencephalography
AHR	=	Allopurinol hypersensitivity reaction
CTG	=	Cardiotocography
ECG	=	Electrocardiography
ECMO	=	Extracorporeal membrane oxygenation
EEG	=	Electroencephalography

HIE = Hypoxic-ischemic encephalopathy
HLA = Human leukocyte antigen
HLHS = Hypoplastic left heart syndrome
IRDS = Idiopathic respiratory distress syndrome
MRI = Magnetic resonance imaging
NIRS = Near-infrared spectroscopy
NO = Nitric oxide
NPBI = Non-protein-bound iron
RCT = Randomized controlled trial

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

We thank dr. Floris Groenendaal, neonatologist in the Wilhelmina Children's Hospital, and Raymond Stegeman, PhD student in the Wilhelmina Children's Hospital for carefully reviewing this paper.

We are grateful for a Horizon 2020 grant (HC2020-PHC18-2015-667224) that funds a new randomized controlled clinical trial investigating the effect of early allopurinol administration in HIE and fully funds the PhD position of Kim V. Annink.

REFERENCES

[1] Stamp LK, Day RO, Yun J. Allopurinol hypersensitivity: investigating the cause and minimizing the risk. Nat Rev Rheumatol. 2016; 12: 235-42.
[2] Day RO, Graham GG, Hicks M, McLachlan AJ, Stocker SL, Williams KM. Clinical pharmacokinetics and pharmacodynamics of allopurinol and oxypurinol. Clin Pharmacokinet. 2007; 46: 623-44.
[3] Kowaltowski AJ, Vercesi AE. Mitochondrial damage induced by conditions of oxidative stress. Free Radic Biol Med. 1999; 26: 463-71.
[4] McCord JM. Oxygen-derived free radicals in postischemic tissue injury. N Engl J Med. 1985; 312: 159-63.
[5] Hagberg H, Andersson P, Lacarewicz J, Jacobson I, Butcher S, Sandberg M. Extracellular adenosine, inosine, hypoxanthine, and xanthine in relation to tissue nucleotides and purines in rat striatum during transient ischemia. J Neurochem. 1987; 49: 227-31.
[6] Moorhouse PC, Grootveld M, Halliwell B, Quinlan JG, Gutteridge JM. Allopurinol and oxypurinol are hydroxyl radical scavengers. FEBS Lett. 1987; 213: 23-8.
[7] Shadid M, Buonocore G, Groenendaal F, Moison R, Ferrali M, Berger HM, *et al*. Effect of deferoxamine and allopurinol on non-protein-bound iron concentrations in plasma and cortical brain tissue of newborn lambs following hypoxia-ischemia. Neurosci Lett. 1998; 248: 5-8.
[8] Lindsay WG, Toledo-Pereyra LH, Foker JE, Varco RL. Metabolic myocardial protection with allopurinol during cardiopulmonary bypass and aortic cross-clamping. Surg Forum. 1975; 26: 259-60.
[9] McCord JM, Roy RS, Schaffer SW. Free radicals and myocardial ischemia. The role of xanthine oxidase. Adv Myocardiol. 1985; 5: 183-9.
[10] Pacher P, Nivorozhkin A, Szabo C. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. Pharmacol Rev. 2006; 58: 87-114.
[11] Parker JC, Smith EE. Effects of xanthine oxidase inhibition in cardiac arrest. Surgery. 1972; 71: 339-44.
[12] Shatney CH, MacCarter DJ, Lillehei RC. Effects of allopurinol, propranolol and methylprednisolone on infarct size in experimental myocardial infarction. Am J Cardiol 1976; 37: 572-80.
[13] Wechter J, Phillips LJ, Toledo AH, Anaya-Prado R, Toledo-Pereyra LH. Allopurinol protection in patients undergoing coronary artery bypass graft surgery. J Invest Surg. 2010; 23: 285-93.
[14] Coghlan JG, Flitter WD, Clutton SM, Panda R, Daly R, Wright G, *et al*. Allopurinol pretreatment improves postoperative recovery and reduces lipid peroxidation in patients undergoing coronary artery bypass grafting. J Thorac Cardiovasc Surg. 1994; 107: 248-56.
[15] Johnson WD, Kayser KL, Brenowitz JB, Saedi SF. A randomized controlled trial of allopurinol in coronary bypass surgery. Am Heart J. 1991; 121: 20-4.
[16] Kelkar A, Kuo A, Frishman WH. Allopurinol as a cardiovascular drug. Cardiol Rev. 2011; 19: 265-71.
[17] Okafor ON, Farrington K, Gorog DA. Allopurinol as a therapeutic option in cardiovascular disease. Pharmacol Ther. 2017; 172: 139-50.
[18] Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. Early Hum Dev. 2010; 86: 329-38.
[19] Azzopardi DV, Strohm B, Edwards AD, Dyet L, Halliday HL, Juszczak E, *et al*. Moderate hypothermia to treat perinatal asphyxial encephalopathy. N Engl J Med. 2009; 361: 1349-58.
[20] Azzopardi D, Strohm B, Marlow N, Brocklehurst P, Deierl A, Eddama O, *et al*. Effects of hypothermia for perinatal asphyxia on childhood outcomes. N Engl J Med. 2014; 371: 140-9.
[21] Inder TE, Volpe JJ. Mechanisms of perinatal brain injury. Semin Neonatol. 2000; 5: 3-16.
[22] van Bel F, Groenendaal F. Drugs for neuroprotection after birth asphyxia: Pharmacologic adjuncts to hypothermia. Semin Perinatol. 2016; 40: 152-9.
[23] Ferriero DM. Neonatal brain injury. N Engl J Med. 2004; 351: 1985-95.
[24] Hilton GD, Nunez JL, Bambrick L, Thompson SM, McCarthy MM. Glutamate-mediated excitotoxicity in neonatal hippocampal neurons is mediated by mGluR-induced release of Ca++ from intracellular stores and is prevented by estradiol. Eur J Neurosci. 2006; 24: 3008-16.
[25] Orrenius S, Burkitt MJ, Kass GE, Dypbukt JM, Nicotera P. Calcium ions and oxidative cell injury. Ann Neurol. 1992; 32 S33-42.
[26] Dorrepaal CA, Berger HM, Benders MJ, van Zoeren-Grobben D, Van de Bor M, Van Bel F. Nonprotein-bound iron in postasphyxial reperfusion injury of the newborn. Pediatrics. 1996; 98: 883-9.
[27] Fellman V RK. Reperfusion Injury as the Mechanism of Brain Damage after Perinatal Asphyxia. Pediatric Research. 1997; 41: 599–606.
[28] Kaandorp JJ BM, Derks JB, *et al*. Fetal hypoxia is an important determinant of birth asphyxia and subsequent adverse outcome: antenatal neuroprotection at term. Paediatrics and Child Health 2010; 20: 356-61.
[29] Saugstad OD. Role of xanthine oxidase and its inhibitor in hypoxia: reoxygenation injury. Pediatrics. 1996; 98: 103-7.
[30] Kjellmer I, Andine P, Hagberg H, Thiringer K. Extracellular increase of hypoxanthine and xanthine in the cortex and basal ganglia of fetal lambs during hypoxia-ischemia. Brain Res. 1989; 478: 241-7.
[31] Ono T, Tsuruta R, Fujita M, Aki HS, Kutsuna S, Kawamura Y, *et al*. Xanthine oxidase is one of the major sources of superoxide anion radicals in blood after reperfusion in rats with forebrain ischemia/reperfusion. Brain Res. 2009; 1305: 158-67.
[32] Pietz J. GN, Gluck L. Hypoxanthine: a marker for asphyxia. Obstet Gyneacol 1988; 72: 762-6.
[33] Beckman JS. The double-edged role of nitric oxide in brain function and superoxide-mediated injury. J Dev Physiol. 1991; 15: 53-9.
[34] van den Tweel ER, Nijboer C, Kavelaars A, Heijnen CJ, Groenendaal F, van Bel F. Expression of nitric oxide synthase isoforms and nitrotyrosine formation after hypoxia-ischemia in the neonatal rat brain. J Neuroimmunol. 2005; 167: 64-71.
[35] Hedtjarn M, Mallard C, Hagberg H. Inflammatory gene profiling in the developing mouse brain after hypoxia-ischemia. J Cereb Blood Flow Metab. 2004; 24: 1333-51.
[36] Scheepens A, Wassink G, Blanco CE. The effect of a global birth asphyxia on the ontogeny of BDNF and NGF protein expression in the juvenile brain. Brain Res Dev Brain Res. 2003; 140: 215-21.
[37] Skoff RP, Bessert D, Barks JD, Silverstein FS. Plasticity of neurons and glia following neonatal hypoxic-ischemic brain injury in rats. Neurochem Res. 2007; 32: 331-42.
[38] Williams GD, Palmer C, Heitjan DF, Smith MB. Allopurinol preserves cerebral energy metabolism during perinatal hypoxia-ischemia: a 31P NMR study in unanesthetized immature rats. Neurosci Lett. 1992; 144: 103-6.

[39] Palmer C, Vannucci RC, Towfighi J. Reduction of perinatal hypoxic-ischemic brain damage with allopurinol. Pediatr Res. 1990; 27: 332-6.

[40] Palmer C, Towfighi J, Roberts RL, Heitjan DF. Allopurinol administered after inducing hypoxia-ischemia reduces brain injury in 7-day-old rats. Pediatr Res. 1993; 33: 405-11.

[41] Peeters-Scholte C, van den Tweel E, Ioroi T, Post I, Braun K, Veldhuis W, *et al*. Pharmacological interventions in the newborn piglet in the first 24 h after hypoxia-ischemia. A hemodynamic and electrophysiological perspective. Exp Brain Res. 2002; 147: 200-8.

[42] Peeters-Scholte C, Braun K, Koster J, Kops N, Blomgren K, Buonocore G, *et al*. Effects of allopurinol and deferoxamine on reperfusion injury of the brain in newborn piglets after neonatal hypoxia-ischemia. Pediatr Res. 2003; 54: 516-22.

[43] Marro PJ, Hoffman D, Schneiderman R, Mishra OP, Delivoria-Papadopoulos M. Effect of allopurinol on NMDA receptor modification following recurrent asphyxia in newborn piglets. Brain Res. 1998; 787: 71-7.

[44] Marro PJ, McGowan JE, Razdan B, Mishra OP, Delivoria-Papadopoulos M. Effect of allopurinol on uric acid levels and brain cell membrane Na+,K(+)-ATPase activity during hypoxia in newborn piglets. Brain Res. 1994; 650: 9-15.

[45] Marro PJ, Mishra OP, Delivoria-Papadopoulos M. Effect of allopurinol on brain adenosine levels during hypoxia in newborn piglets. Brain Res. 2006; 1073-1074: 444-50.

[46] Van Bel F, Shadid M, Moison RM, Dorrepaal CA, Fontijn J, Monteiro L, *et al*. Effect of allopurinol on postasphyxial free radical formation, cerebral hemodynamics, and electrical brain activity. Pediatrics. 1998; 101: 185-93.

[47] Benders MJ, Bos AF, Rademaker CM, Rijken M, Torrance HL, Groenendaal F, *et al*. Early postnatal allopurinol does not improve short term outcome after severe birth asphyxia. Arch Dis Child Fetal Neonatal Ed. 2006; 91: F163-5.

[48] Kaandorp JJ, van Bel F, Veen S, Derks JB, Groenendaal F, Rijken M, *et al*. Long-term neuroprotective effects of allopurinol after moderate perinatal asphyxia: follow-up of two randomised controlled trials. Arch Dis Child Fetal Neonatal Ed. 2012; 97: F162-6.

[49] Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, *et al*. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomised trial. Lancet. 2005; 365: 663-70.

[50] Gunes T, Ozturk MA, Koklu E, Kose K, Gunes I. Effect of allopurinol supplementation on nitric oxide levels in asphyxiated newborns. Pediatr Neurol. 2007; 36: 17-24.

[51] Chaudhari T, McGuire W. Allopurinol for preventing mortality and morbidity in newborn infants with hypoxic-ischaemic encephalopathy. Cochrane Database Syst Rev. 2012(7): DOI: 10.1002/14651858.CD006817.pub3.

[52] Boda D NI, Kiss P, *et al*. . Treatment of mothers with allopurinol to produce therapeutic blood levels in newborns. Prenat Neonat Med 1999; 4: 130-4.

[53] Derks JB, Oudijk MA, Torrance HL, Rademaker CM, Benders MJ, Rosen KG, *et al*. Allopurinol reduces oxidative stress in the ovine fetal cardiovascular system after repeated episodes of ischemia-reperfusion. Pediatr Res. 2010; 68: 374-80.

[54] Kane AD, Camm EJ, Richter HG, Lusby C, Tijsseling D, Kaandorp JJ, *et al*. Maternal-to-fetal allopurinol transfer and xanthine oxidase suppression in the late gestation pregnant rat. Physiol Rep. 2013; 1: e00156.

[55] Masaoka N, Nakajima Y, Hayakawa Y, Ohgame S, Hamano S, Nagaishi M, *et al*. Transplacental effects of allopurinol on suppression of oxygen free radical production in chronically instrumented fetal lamb brains during intermittent umbilical cord occlusion. J Matern Fetal Neonatal Med. 2005; 18: 1-7.

[56] van Dijk AJ, Parvizi N, Taverne MA, Fink-Gremmels J. Placental transfer and pharmacokinetics of allopurinol in late pregnant sows and their fetuses. J Vet Pharmacol Ther. 2008; 31: 489-95.

[57] Kaandorp JJ, Derks JB, Oudijk MA, Torrance HL, Harmsen MG, Nikkels PG, *et al*. Antenatal allopurinol reduces hippocampal brain damage after acute birth asphyxia in late gestation fetal sheep. Reprod Sci. 2014; 21: 251-9.

[58] Torrance HL, Benders MJ, Derks JB, Rademaker CM, Bos AF, Van Den Berg P, *et al*. Maternal allopurinol during fetal hypoxia lowers cord blood levels of the brain injury marker S-100B. Pediatrics. 2009; 124: 350-7.

[59] Kaandorp JJ, Benders MJ, Schuit E, Rademaker CM, Oudijk MA, Porath MM, *et al*. Maternal allopurinol administration during suspected fetal hypoxia: a novel neuroprotective intervention? A multicentre randomised placebo controlled trial. Arch Dis Child Fetal Neonatal Ed. 2015; 100: F216-23.

[60] Hurn PD, Vannucci SJ, Hagberg H. Adult or perinatal brain injury: does sex matter? Stroke. 2005; 36: 193-5.

[61] Nijboer CH, Groenendaal F, Kavelaars A, Hagberg HH, van Bel F, Heijnen CJ. Gender-specific neuroprotection by 2-iminobiotin after hypoxia-ischemia in the neonatal rat via a nitric oxide independent pathway. J Cereb Blood Flow Metab. 2007; 27: 282-92.

[62] Algra SO, Jansen NJ, van der Tweel I, Schouten AN, Groenendaal F, Toet M, *et al*. Neurological injury after neonatal cardiac surgery: a randomized, controlled trial of 2 perfusion techniques. Circulation. 2014; 129: 224-33.

[63] Khalil A, Suff N, Thilaganathan B, Hurrell A, Cooper D, Carvalho JS. Brain abnormalities and neurodevelopmental delay in congenital heart disease: systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2014; 43: 14-24.

[64] McGaurn SP, Davis LE, Krawczeniuk MM, Murphy JD, Jacobs ML, Norwood WI, *et al*. The pharmacokinetics of injectable allopurinol in newborns with the hypoplastic left heart syndrome. Pediatrics. 1994; 94(6 Pt 1): 820-3.

[65] Clancy RR, McGaurn SA, Goin JE, Hirtz DG, Norwood WI, Gaynor JW, *et al*. Allopurinol neurocardiac protection trial in infants undergoing heart surgery using deep hypothermic circulatory arrest. Pediatrics. 2001; 108: 61-70.

[66] Marro PJ, Baumgart S, Delivoria-Papadopoulos M, Zirin S, Corcoran L, McGaurn SP, *et al*. Purine metabolism and inhibition of xanthine oxidase in severely hypoxic neonates going onto extracorporeal membrane oxygenation. Pediatr Res. 1997; 41: 513-20.

[67] Boda D, Nemeth I, Hencz P, Denes K. Effect of allopurinol treatment in premature infants with idiopathic respiratory distress syndrome. Dev Pharmacol Ther. 1984; 7: 357-67.

[68] Russell GA, Cooke RW. Randomised controlled trial of allopurinol prophylaxis in very preterm infants. Arch Dis Child Fetal Neonatal Ed. 1995; 73: F27-31.

[69] van Kesteren C, Benders MJ, Groenendaal F, van Bel F, Ververs FF, Rademaker CM. Population pharmacokinetics of allopurinol in full-term neonates with perinatal asphyxia. Ther Drug Monit. 2006; 28: 339-44.

[70] Marro PJ, Baumgart S, Delivoria-Papadopoulos M, Zirin S, Corcoran L, McGaurn SP, *et al*. Purine metabolism and inhibition of xanthine oxidase in severely hypoxic neonates going onto extracorporeal membrane oxygenation. Pediatr Res. 1997; 41(4 Pt 1): 513-20.

[71] Kaandorp JJ, van den Broek MP, Benders MJ, Oudijk MA, Porath MM, Bambang Oetomo S, *et al*. Rapid targ*et al*lopurinol concentrations in the hypoxic fetus after maternal administration during labour. Arch Dis Child Fetal Neonatal Ed. 2014; 99: F144-8.

[72] Ramasamy SN, Korb-Wells CS, Kannangara DR, Smith MW, Wang N, Roberts DM, *et al*. Allopurinol hypersensitivity: a systematic review of all published cases, 1950-2012. Drug Saf. 2013; 36: 953-80.

[73] McInnes GT, Lawson DH, Jick H. Acute adverse reactions attributed to allopurinol in hospitalised patients. Ann Rheum Dis. 1981; 40: 245-9.

[74] Yang CY, Chen CH, Deng ST, Huang CS, Lin YJ, Chen YJ, *et al*. Allopurinol Use and Risk of Fatal Hypersensitivity Reactions: A Nationwide Population-Based Study in Taiwan. JAMA Intern Med. 2015; 175: 1550-7.

[75] Stamp LK, Taylor WJ, Jones PB, Dockerty JL, Drake J, Frampton C, *et al*. Starting dose is a risk factor for allopurinol hypersensitivity syndrome: a proposed safe starting dose of allopurinol. Arthritis Rheum. 2012; 64: 2529-36.

[76] McGaurn SP, Davis LE, Krawczeniuk MM, Murphy JD, Jacobs ML, Norwood WI, *et al*. The pharmacokinetics of injectable allopurinol in newborns with the hypoplastic left heart syndrome. Pediatrics. 1994; 94: 820-3.