Treatment of mothers with allopurinol to produce therapeutic blood levels in newborns

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ABSTRACT

Objectives A number of experimental and clinical studies have shown that allopurinol, a xanthine oxidase inhibitor, can reduce hypoxic–ischemic reperfusion injury, but only by preventive or early treatment, which is rarely possible in clinical circumstances. The aim of the present study was to evaluate whether allopurinol administered to the mothers before the onset of labor in animal experiments, or after the onset of labor in human studies, would cross the placenta so as to establish a therapeutic level of allopurinol in newborns without affecting the process of delivery.

Material and methods In randomized investigations, brood sows were treated with allopurinol prior to farrowing. In human studies, mothers with full-term or preterm pregnancies received a single dose of allopurinol orally immediately after the onset of labor.

Results In both animals and humans, the process of delivery and the postnatal events in the newborns were similar in the treated and the control groups. The free placental transfer of allopurinol and its metabolite oxypurinol led to the attainment of therapeutic blood levels in the resulting newborn piglets and human infants.

Conclusions The placental transfer of allopurinol administered to parturient mothers may be effective in starting the early treatment of newborns with a high risk of hypoxic-reperfusion cerebral damage.

INTRODUCTION

Hypoxic–ischemic reperfusion cell damage caused by free radicals has been recognized as a highly significant pathological factor in numerous types of acute and chronic diseases[1,2]. The enzyme xanthine oxidase is attracting increasing interest because of its probable relationship to the development of various diseases, including pre-eclampsia of pregnancy[3–6] and 'free radical diseases' of newborns, such as idiopathic respiratory distress syndrome (IRDS)[7] and cerebral hypoxic–ischemic reperfusion damage[8]. A number of experimental and clinical studies have shown that allopurinol, a xanthine oxidase inhibitor and free radical scavenger, can reduce hypoxic–ischemic reperfusion injury[9–13]. However, this is achieved mainly by preventive or early treatment, which is rarely possible in clinical circumstances[14]. In the present animal experiments and human clinical studies aimed at achieving potentially more effective 'early' treatment, an evaluation was made to determine whether allopurinol given to the mothers before, or immediately after, the onset of labor would cross the placenta so as to establish a therapeutic level of allopurinol in the newborns without any effect on the process of delivery.

MATERIALS AND METHODS

Animal investigations

Thirty randomly selected brood sows were treated with a single daily dose of 30 mg/kg allopurinol (Milurit®Egis) for 4–8 days before the expected date of farrowing, and compared with a similarly selected group of untreated controls. The number and birth

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weights of the newborn piglets born to the treated and control sows are shown in Table 1. Their death rate within 1 week of life was also recorded, and the allopurinol levels in the blood of the treated sows and their newborns were monitored.

Human studies

A total of 24 mothers with full-term normal pregnancies and 44 with preterm pregnancies were included in the study. The full-term and preterm cases were separately randomized in equal treated and control groups. The treated parturient mothers received a single dose of 600 mg allopurinol orally at the onset of labor.

The clinical characteristics of the newborn infants are shown in Table 2. The birth-weight distributions of the full-term and premature infants born to allopurinol-treated mothers were nearly identical with those of the respective groups born to the control mothers. After delivery, the occurrence and severity of respiratory morbidity, and the blood plasma uric acid level, were recorded in both groups and the allopurinol levels in the mothers and their infants were monitored.

Respiratory morbidity of newborns was defined as any sign of respiratory embarrassment that required greater than 15 min of oxygen supplementation after nursery admission.

The diagnosis of IRDS was based on the following: the presence of the classical triad of inspiratory sternal retraction, expiratory grunting and reticulogranularity as shown by the chest X-ray; if paO_2 values between 6.5 and 13.0 kPa could be attained only at inspired oxygen concentrations exceeding 0.6 FiO_2, and if application of artificial respiration therapy with a BEAR CUB BP 200 respirator was necessary to maintain the desired paO_2 values.

Transient tachypnea (wet-lung, IRDS II) of the newborn was defined by the same clinical criteria as IRDS: bronchograms, cyanosis in room air, grunting and chest retractions, but was only diagnosed if a significant improvement occurred by 24 h of age on the application of oxygen therapy alone or with continuous positive airway pressure breathing.

Table 1 Animal experiments on brood sows during farrowing and their newborns

	Controls	Allopurinol-treated group
Number of sows	29	29
Number of newborn piglets	277	261
Weight of newborn piglets (g)		
at birth (mean ± SD)		
at 1 week of age (mean ± SD)	1510 ± 310	1520 ± 350
Number of piglets that died	2490 ± 550	2590 ± 660
(between 1 and 7 days of age)	18	16

Blood samples

In the course of the animal experiments, blood samples were withdrawn from the jugular vein of 15 brooding sows treated with allopurinol and from 20 of their newborn piglets on the day of farrowing, and again from the newborn piglets after 24 h.

In clinical studies, blood was taken from the mothers, and from the umbilical vein immediately after delivery and again from the newborn infants 24 h after delivery. Blood samples anticoagulated with heparin

Table 2 Clinical characteristics of newborn infants of parturient mothers included in the study

	Infants delivered by control mothers (n = 33)	Infants delivered by allopurinol-treated mothers (n = 35)
Birth weight of all newborns (g) (mean ± SD)	2335 ± 695	2328 ± 787
Birth-weight distribution (n)		
> 2500 g	12	12
2001–2500 g	12	14
1501–2000 g	3	4
< 1500 g	6	5
Idiopathic respiratory distress syndrome (n)	5	6
Transient tachypnea (n)	4	2
Died at 1–7 days of age (n)	2	2

were centrifuged immediately and the separated blood serum was stored at –20°C until further analysis.

Laboratory tests

The concentration of uric acid in the plasma of blood samples was determined by Morin's method as modified in our department[15]. The concentrations of allopurinol and its active metabolite, oxypurinol, were determined on the basis of the total xanthine oxidase-inhibiting activity measured by a previously published method[16].

Statistical analysis

Data registered in the course of the study are reported as means ± standard deviation. Accordingly, variance analysis followed by Student's *t* test was used in the statistical analysis. Correlations between biochemical parameters were characterized by calculations of linear regression and correlation. The significance level for all tests was taken as $\alpha = 0.05$.

Ethical aspects

In human studies, informed consent was obtained on all occasions and the study protocol was evaluated and found to be ethically acceptable by the Scientific and Ethical Committee of Albert Szent-Györgyi University Medical School.

RESULTS

Animal experiments

No abnormalities were observed in the course of the farrowing of allopurinol-treated brood sows as compared to the control animals; farrowing proceeded normally in both groups.

The control and treated sows farrowed nearly identical numbers of piglets. The death rate and weight gain during the first week of life in the allopurinol-treated group were practically identical with those in the control group (Table 1). The causes of death were weakness, malnutrition and occasionally diarrhea, but this was not of the epidemic type. Four and five cases of diarrhea resulting in death occurred for the farrowing of one sow in each of the allopurinol-treated and the control group, respectively. The data on these two brood sows and their newborn piglets were not included in the evaluation.

Therapeutic allopurinol concentrations were detectable in the blood of every allopurinol-treated sow (Figure 1). The allopurinol levels in the blood of the newborn piglets, collected with a few hours' delay on the day of farrowing, were somewhat lower than the corresponding maternal values, but still high enough to ensure an effective inhibition of xanthine oxidase activity. These values had decreased by the age of 24 h but, with the exception of four of the 20 measured cases, therapeutic allopurinol blood levels still persisted by this time.

Clinical experience and analytical results

Table 2 shows that there was no significant difference between the two groups in the frequency of IRDS or transient tachypnea.

The results of blood tests are shown in Figures 2 and 3. Serum allopurinol concentrations corresponding to a therapeutic blood level were measured in all delivering mothers, even when the time elapsing between administration of the drug and the actual childbirth was as short as 23 min. Efficient placental transfer of allopurinol and its rapid equilibration between mother and fetus are demonstrated by the very close correlation between the allopurinol values measured in the maternal blood and in the umbilical blood of the infant (0.748, $p < 0.01$; $n = 37$). A similarly close correlation of the uric acid levels was also observed in the maternal and umbilical blood samples (0.875, $p < 0.01$; $n = 66$).

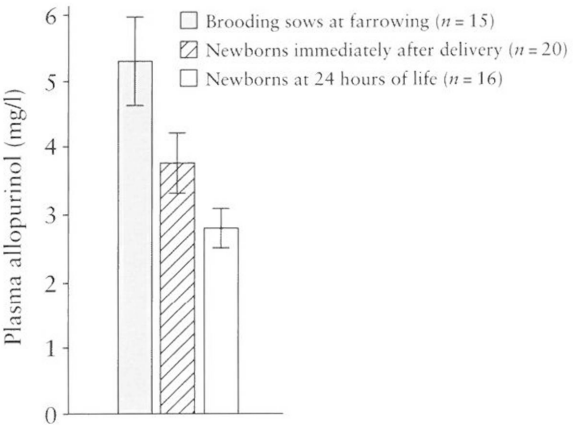

Figure 1 Plasma allopurinol levels in brood sows at farrowing, and in their newborn piglets immediately after delivery and at 24 h of age

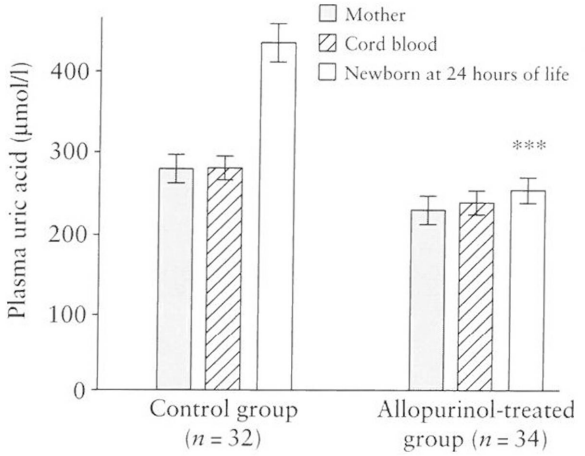

Figure 2 Plasma uric acid levels of delivering mothers and their newborns in the allopurinol-treated group and the controls. ***$p < 0.001$

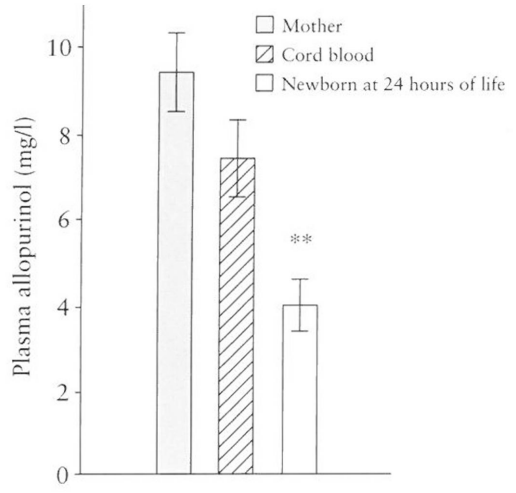

Figure 3 Plasma allopurinol levels of delivering mothers and their newborns. **$p < 0.002$

The blood allopurinol levels measured in the newborn infants at the age of 24 h were somewhat reduced relative to those in the umbilical blood samples, but were still within the effectively inhibitory range in the majority of the cases (Figure 3).

The appropriate effect of allopurinol administered to delivering mothers was clearly indicated by the concentrations of uric acid in the plasma of the tested patients. The uric acid levels in the sera of both allopurinol-treated mothers and their newborn infants were consistently lower at all times than those in the control group (Figure 2). It is important to note that, while the uric acid level in the sera of the 24-hour-old control infants was regularly (and occasionally quite significantly) increased, the value of this parameter in infants born to allopurinol-treated mothers was typically lower and only minimally exceeded that measured at the time of birth. Thus, the difference between the uric acid levels in the sera of control and allopurinol-treated infants is highly significant ($p < 0.001$). The opposite is observed when the uric acid levels in the plasma of the infants are compared with the allopurinol levels in the maternal blood (-0.473, $p < 0.05$; $n = 66$) (Figures 2 and 3).

DISCUSSION

The experimental results described above demonstrate that allopurinol passes through the placenta rapidly and without hindrance. The usual therapeutic doses of allopurinol are harmless and do not interfere with the process of delivery. This was first verified in animal tests. In human mothers to whom an appropriate dose is administered during parturition, an effective allopurinol concentration may be built up in the blood of the infant by the time of delivery. As a result, in newborn infants born to allopurinol-treated mothers (in contrast with the control group), the uric acid level in the serum is not elevated, or only slightly, on the first day after delivery, and 24 h later the uric acid concentration of the umbilical blood is typically unchanged relative to the originally low value, or is further decreased. The increase in uric acid level by 24 h as a premonitory sign of prognostic value signalling intracranial bleeding has recently been recognized[17]. Accordingly, treatment of the mothers with a single dose of allopurinol in cases when the fetus is at high risk is equivalent to the very early, quasi-preventive administration of allopurinol to the infants. These results do not yet allow a decision as to whether allopurinol treatment is effective for endangered fetuses; neither the number of tests nor the duration of treatment was sufficient for this. In order to treat disturbances of adaptation to extrauterine life effectively, it might be necessary to continue allopurinol treatment for at least 3 days. A therapeutic blood level of allopurinol at the time of the delivery could be followed by further maintenance treatment, which would be easily manageable by oral medication of the infants. In order to form a judgment as to the efficiency of allopurinol treatment in the envisaged indications, randomized or even multicentric studies should be carried out. In addition, the question of whether allopurinol

influences the maturation of the lungs in the period preceding full term should be addressed in suitably planned animal tests. Only if these investigations yield favorable results should further efforts be made to clarify all the remaining open questions.

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