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Allopurinol Administered after Inducing Hypoxia-Ischemia Reduces Brain Injury in 7-Day-Old Rats

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ABSTRACT. We determined that treatment of immature rats with allopurinol at 15 min after cerebral hypoxia-ischemia reduces brain damage. Seven-d postnatal rats were subjected to right common carotid artery ligation followed by 2.25 h of hypoxia (8% O_2). At 15 min of recovery in room air, the rat pups received either allopurinol (135 mg/kg s.c.) or saline. Some of the rats ($n = 65$) were killed at 42 h of recovery for measurement of cerebral hemispheric water content. Other animals ($n = 63$) were killed at 30 d for morphologic assessment of the severity of damage. In separate rats, we measured the levels of allopurinol and its metabolites in serum and in the brain around the time of peak serum levels. We also determined the effect of allopurinol on rat pup body temperature. Allopurinol reduced the increase in right hemisphere water content and markedly reduced atrophy. No cavitary lesions were seen in the 31 allopurinol-treated rats, whereas 15 of 32 saline-treated rats had cavitary cerebral lesions. Histologic examination confirmed that the allopurinol-treated rats had less brain injury. Serum allopurinol and oxypurinol peaked between 0.5 and 1 h after allopurinol injection. Their peak serum concentrations at 0.75 h postinjection combined was between 360 and 510 μM. Allopurinol did not lower rectal temperature more than 0.04°C. In conclusion, high-dose allopurinol administered at 15 min of recovery from cerebral hypoxia-ischemia markedly reduces both acute brain edema and long-term cerebral injury in immature rats. (*Pediatr Res* 33: 405–411, 1993)

Abbreviations

GFAP, glial fibrillary acidic protein
R/L, right to left

Reperfusion of the previously ischemic brain leads to formation of cytotoxic free radicals (1–3). Endothelial cells lining the cerebral blood vessels can generate superoxide via xanthine oxidase activity (4), prostaglandin metabolism (5), and damaged mitochondria (6). Allopurinol, via its active metabolite oxypurinol, inhibits xanthine oxidase (7). In animal models of cerebral ischemia, pretreatment with allopurinol or oxypurinol successfully reduces ischemic brain injury (8–15). However, the ability of allopurinol pretreatment to reduce hypoxic-ischemic brain injury appears to require doses in excess of that needed to inhibit xanthine oxidase (13, 14, 16).

In vitro studies have shown that allopurinol and oxypurinol can scavenge hydroxyl radicals and chelate transition metals in proportion to their concentration (17–19). Because free radicals may contribute to microvascular dysfunction and subsequent brain injury after cerebral ischemia (1–3), we hypothesized that high-dose allopurinol administered after inducing cerebral hypoxia-ischemia would reduce brain injury.

We have previously reported that allopurinol administered before cerebral hypoxia-ischemia reduces brain injury in the immature rat (11). The present study, using the same model, shows that allopurinol administered 15 min after cerebral hypoxia-ischemia is also neuroprotective. The serum and brain levels of allopurinol and its metabolites are described.

MATERIALS AND METHODS

We induced a hypoxic-ischemic insult to the right cerebral hemisphere of 7-d postnatal rat pups. To determine early injury, we measured right hemispheric water content at 42 h of recovery. The extent of permanent injury was evaluated in pups that were allowed to recover to 30 d of age. The levels of allopurinol and its metabolites as well as temperature alterations after allopurinol injection were determined in normal 7-d-old rat pups.

Animal model. Seven-d-old Wistar (Charles River, Wilmington, MA) rat pups of either sex were taken at random from their litters and anesthetized with a mixture of halothane (4% halothane for induction, 1–1.5% for maintenance), 30% oxygen, and the balance nitrous oxide. The right common carotid artery of each pup was permanently ligated with 4–0 surgical silk through a midline neck incision. The wound was sutured and the animals allowed to recover with their dams for 3 h. Duration of anesthesia was less than 5 min. After carotid ligation, the pups were numbered sequentially.

Rat pups were placed in airtight jars and exposed to a continuous flow of 8% oxygen–92% nitrogen gas mixture as described previously (11). The jars were partially submerged in a circulating water bath maintained at 36.8°C to provide a stable thermal environment. Air temperature in the middle of the jar was maintained between 33° and 34 °C. After 2.25 h of hypoxia, the jars were opened to room air and the pups returned to their dams. At 15 min of recovery, pups were treated with a single s.c. injection of allopurinol 135 mg/kg, pH 11.2 (Zyloprim sodium, Burroughs Wellcome, Research Triangle Park, NC) or an equal volume (0.01 mL/g animal weight) of saline, pH 11.2. The treatment was alternated between allopurinol and saline according to the sequential numbers. We chose the dose based on previous studies in which pretreatment with 135 mg/kg prevented brain injury from a similar insult (11).

The procedures used in this study were approved by our Committee on Animal Investigation. The investigators were blinded to treatment during all measurements of brain injury described below.

Water content. After 42 h of recovery with their dams, the pups ($n = 65$) were decapitated and their brains removed. A

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portion (150–200 mg) from the posterolateral section of each cerebral hemisphere was placed in a tared 5-mL glass vial and immediately weighed on a microanalytical balance. The posterior half of the right hemisphere (ipsilateral to common carotid artery ligation) represented the area most severely injured, whereas the corresponding area in the left hemisphere served as the control. Subsequently, the specimen was desiccated at 70°C for 72 h. Reweighing ascertained the dry weight of the tissue, and by subtracting dry weight from the total weight the water content of the hemisphere was obtained. Water content was determined as a percentage of the total weight (11).

Neuropathologic procedures. To examine long-term neuropathologic outcome, 64 rat pups were subjected to the combined hypoxic-ischemic insult described above. All the pups survived the 2.25-h hypoxic exposure. Pups were treated with saline or allopurinol at 15 min of recovery and randomized as described above. Treatment groups were identified by clipping the tip of the tail or cutting a small notch in the ear. These procedures incurred minimal bleeding.

At 30 d (23 d of recovery), the animals were killed with a lethal intraperitoneal dose of pentobarbital (150 mg/kg). A single saline-treated pup died during recovery. The brains were carefully removed from the skull and immersion-fixed in a mixture of formaldehyde, 1% acetic acid, and methanol, (1:1:8 vol/vol).

Gross neuropathologic grading. A pair of examiners together allocated the whole brains into normal brains and brains with mild, moderate, or severe categories of damage. The choice of category was based on inspection of the right cerebral hemisphere size (ipsilateral to ligation) compared with the left (contralateral to ligation). Normal referred to no difference in size; mild referred to some discrepancy in size, the right being smaller than the left, without any visible cavitary lesion; moderate indicated discrepancy in size due to a cavitary lesion in the right hemisphere; and severe related to extensive cystic infarction of the right hemisphere with almost total destruction of its posterolateral aspect. These categories of damage have been previously illustrated (11).

Brain morphometry (interhemispheric diameter ratio determination). To assess the degree of right hemisphere atrophy, we used the interhemispheric diameter ratio. Accordingly, a 2-mm coronal slice was cut at the mid–mammillary body level and the diameter of each cerebral hemisphere was measured from the midpoint of a straight line connecting the interhemispheric fissure to the midmammillary region under a dissecting microscope. This coronal level was chosen because it approximated the region of maximal injury. Then the ratio of the R/L hemisphere diameter was calculated and expressed as a percentage. We also determined the R/L hemisphere diameter ratio on eight normal 30-d-old rats that had not been exposed to hypoxia or carotid artery ligation to determine the range of normal interhemisphere assymetry.

Histopathologic scoring of damage. To accurately differentiate between normal (undamaged) brains and mildly damaged brains, we developed a method for histologic scoring of damage. For this study, brains obviously damaged with cavitary lesions on gross inspection were excluded from histologic scoring. This left all 31 allopurinol-treated and 17 saline-treated brains for histologic examination. Accordingly, the coronal slices used for determination of the interhemisphere diameter ratio were processed for paraffin embedding, and 6-μm sections at the level of the infundibulum were obtained. The slides were stained with hematoxylin and eosin, and for GFAP they were stained using an immunoperoxidase technique and counterstained with hematoxylin. The polyclonal GFAP antibody was produced in rabbits against cow spinal cord (Dako Corp., Carpenteria, CA). Reactive astrocytosis is a sensitive indicator of long-standing brain damage and is readily identified with this technique (20).

To determine the normal appearance of astrocyte staining, we examined sections from eight normal (nonligated and nonhypoxic) rats. With this technique, fixed normal brains showed only weak astrocyte staining predominantly in the corpus callosum and internal capsule. Strong staining was observed only in reactive astrocytes within damaged areas. Reactive gliosis was defined as a focal aggregation of cells that stained strongly for GFAP. In contrast to the normal brains, astrocytes in regions of reactive gliosis were increased in number and staining intensity.

Each hemisphere was divided into 11 anatomical regions (Fig. 1) that were individually examined and a point was assigned for the presence of reactive gliosis. The histopathologic score ranged from 0 to 11 and represented the sum of the damaged regions per hemisphere. Normal brains and hemispheres always scored 0 because a point was only allotted for a region that had a focus of reactive gliosis.

Measurement of allopurinol and metabolite levels. Seven-d-old rat pups were injected s.c. with 135 mg/kg of allopurinol and decapitated at intervals ranging from 15 min to 6 h. Blood was collected from the severed neck vessels and the serum deproteinated with 30% perchloric acid in a 10% vol/vol ratio. The head was immediately frozen in liquid nitrogen and stored at −70°C. The brain was dissected from the skull in a cold box at −20°C. A sample of tissue from the cerebral hemisphere (±100 mg) was powdered and weighed in the cold box, then extracted into perchloric acid as previously described (21). The brain extracts were analyzed using HPLC as modified from Wung *et al.* (22). We used a Waters-Bondapak C-18 reverse phase column (Waters Associates, Milford, MA) fitted with a C-18 precolumn filter module. The mobile phase consisted of 50 mM potassium phosphate, pH 6.0, with 1% methanol vol/vol, at a flow rate of 1.4 mL/min. Peaks were detected at 254 nm and their identity verified by retention time, coelution with known standards, and by matching the UV absorbance spectra of each peak with authentic standards for allopurinol, oxypurinol, and allopurinol

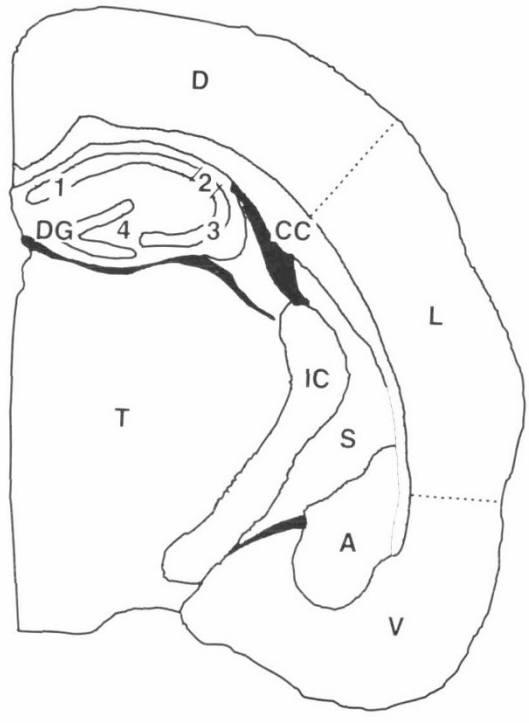

Fig. 1. Coronal section of right hemisphere. The drawing of the right hemisphere from a representative coronal brain section used to determine the histopathology score is subdivided into the following regions: *D*, dorsal cortex; *L*, lateral cortex; *V*, ventral cortex; *1*, *2*, *3*, and *4*, CA1, CA2, CA3, and CA4 regions of the hippocampus, respectively; *DG*, dentate gyrus; *T*, thalamus; *S*, striatum; *A*, amygdaloid nucleus; *CC*, corpus callosum; and *IC*, internal capsule. The CC and IC were not part of the 11 regions composing the score. The ventral cortex was the cortex below the rhinal fissure; the remaining cortex above the rhinal fissure was divided equally into lateral and dorsal portions.

riboside using a Hewlett-Packard 1090 photodiode-array detector (Hewlett-Packard Co., Palo Alto, CA).

Effect of allopurinol on rat pup body temperature. Rat pups (nonligated and nonhypoxic) that were feeding from their dams were removed from the nest and fitted with a rectal temperature probe (511, Yellow Springs Instrument Co., Yellow Springs, OH). They were positioned into plastic syringe barrels housed within a neonatal incubator set at 33.5°C. This temperature was chosen because it resembled the average nesting temperature. Ninety min were required for the rectal temperature to stop fluctuating by more than 0.2°C in 5 min. Then, three temperature recordings for each pup were obtained over 30 min and the average of the three temperatures represented the "predrug" temperature. The pups were then injected with the experimental dose of saline or allopurinol and given another 90-min equilibration period. Three temperature measurements were obtained over the next 30 min and averaged to form the "postdrug" temperature. The effect of the drug was determined by the difference between postdrug and predrug temperatures.

Statistical methods. We used the two-tailed t test, Cochran trend test, and Mann-Whitney U test where appropriate; $p < 0.05$ was considered significant.

RESULTS

Water content. Two rat pups received allopurinol ($n = 41$) for every one that was treated with saline ($n = 24$). No pups died during the 42-h recovery period of either treatment group. The left hemisphere (contralateral to ligation) water content for the saline-treated pups was 87.92 ± 0.22%, and for the allopurinol-treated pups it was 87.80 ± 0.30% (mean ± SD). These results are not statistically different and fall within the normal range as previously determined in 12 untreated, normal, 7-d-old rat pups (11). The left hemisphere water contents were combined to form a normal (mean ± 2 SD) reference range against which the distribution of water content in the edematous right hemisphere is illustrated (Fig. 2). Twenty-six of 41 allopurinol-treated brains had a normal right hemisphere water content. The distribution of the elevated water contents in the remaining 15 can be seen in Figure 2. In contrast, the right hemisphere water content of the 24 saline-treated pups was 91.70 ± 0.95% (mean ± SD); only one of the 24 saline-treated pups had a right hemisphere water content within the normal range. The right hemisphere water content of the allopurinol-treated rat pups was significantly less than that of the saline-treated pups [$p < 0.0001$ (Mann-Whitney U test)].

Gross neuropathology. In those pups allowed to recover until 30 postnatal d, brain abnormalities ranged from atrophy of the right cerebral hemisphere to cystic destruction (cavitary lesions) of that hemisphere. The results are illustrated in Table 1.

Significantly less damage was seen in the allopurinol-treated pups. Twenty-four of 32 saline-treated rats developed gross brain injury. Nine were considered mild, eight moderate, and seven severe. In contrast, only 15 of the 31 allopurinol-treated appeared damaged, all of which were graded as mild. None had cavitary lesions. Statistical analysis showed that allopurinol significantly reduced the amount of atrophy and prevented cystic infarction [$p < 0.001$ (Cochran trend test)].

Brain morphometry (interhemisphere diameter ratio). The ratio of the R/L hemisphere diameter for eight normal 30-d-old rat pups (not subjected to carotid ligation or hypoxia) was 97 ± 2.6% (mean ± SD). Therefore, the normal range for hemisphere asymmetry that would include roughly 95% of normal brains is 91.8 to 102.2% (mean ± 2 SD). Accordingly, a ratio less than 91.8% can be regarded as abnormal and indicative of atrophy or infarction. The normal range (mean ± 2 SD) is illustrated by the striped area in Figure 3, which shows the distribution of atrophy for the experimental animals.

Damage in the 32 saline-treated brains was biphasic. Seventeen brains had an R/L hemispheric diameter ratio greater than 70%. These brains did not have cavitary lesions. Of the remaining 15 brains, all had cavitary lesions and the diameter of the right hemisphere was less than 65% of the left (R/L <65%). In contrast, all 31 allopurinol-treated brains had an R/L hemisphere ratio greater than 70% and none had cavitary lesions. Atrophy was markedly less in the allopurinol-treated rats [$p < 0.001$ (Mann-Whitney U test)].

Histopathology. All the brains without cavitary lesions were examined histologically. No abnormalities were seen in the left cerebral hemispheres. Because none of the allopurinol-treated brains had cavitary lesions, all 31 were examined histopathologically for the presence of reactive gliosis and histologic scoring of damage. One allopurinol-treated brain section, with an R/L hemisphere diameter ratio of 99.32%, was excluded from examination due to the suboptimal quality of the histologic sections. The brains of 19 (63%) of the 31 allopurinol-treated rats were undamaged. In contrast, only eight of 32 (25%) saline-treated brains were undamaged, because 15 had cavitary lesions and nine had reactive gliosis. Thus, gross and microscopic neuropathologic evaluation confirmed that damage was less in the allopurinol-treated brains [$p < 0.001$ (Cochran trend test)] (Table 2).

Twenty brains (11 allopurinol-treated and nine saline-treated) had between one and 11 regions of reactive gliosis. Allopurinol did not alter the regional susceptibility to injury from that seen in the saline-treated pups. The lateral CA3 zone of the hippocampus was the most consistently involved region (20 of 20) in both treatment groups and in two animals was the only region damaged. The CA2 zone was damaged in 18 of 20, whereas the CA1 region was damaged in only 14 of 20 animals. The lateral cortex and thalamus was damaged in 15 of 20 animals. The white matter, including the corpus callosum and internal capsule, was consistently spared (in brains without cavitary lesions).

There was a very good correlation ($R^2 = 0.88$) between the R/L hemisphere diameter ratio and histopathologic score. Brain injury (reactive gliosis) was seen only in those brains with an R/L diameter ratio < 95%. This included three brains considered normal morphometrically.

Allopurinol and metabolite levels. Allopurinol administered at 135 mg/kg s.c. peaked in the serum at 340–480 μM (46.3–64.5 μg/mL) at 0.5–0.75 h postinjection. Thereafter, the levels declined, with a serum half-life of 52 min. Brain levels of allopurinol were approximately one fourth of the peak serum levels. A level of 77 μM (10.5 μg/mL) was attained between 0.5 and 0.75 h postinjection (see Fig. 4).

The serum levels of oxypurinol, the active metabolite of allopurinol, peaked 0.75–3 h after allopurinol injection at 21–27 μM (3.2–4.1 μg/mL) and then declined, with a half-life of 170 min. Brain oxypurinol levels, 15 μM (2.2 μg/mL), were similar to the serum levels at 0.5 h after allopurinol injection. The metabolite of allopurinol present in highest concentration was allopurinol riboside, which reached a maximum serum concentration of 143–175 μM (38.4–47.11 μg/mL) at 1–2 h postinjection.

Temperature effects. To ascertain if allopurinol induced hypothermia, the postdrug temperature measurement was subtracted from the predrug measurement in four allopurinol-treated and four saline-treated pups. In the allopurinol-treated pups, the temperature difference was 0.09 ± 0.32°C, whereas in the saline-treated pups the temperature difference was 0.16 ± 0.18°C (mean ± SD). Thus, only minor temperature fluctuations of a similar degree in response to either treatment were observed.

DISCUSSION

This study shows that allopurinol protects 7-d-old rat pups from hypoxic-ischemic injury even when the drug is administered 15 min after the insult. The effectiveness of treatment during recovery ("rescue therapy") supports the concept that brain damage evolves during resuscitation ("secondary damage").

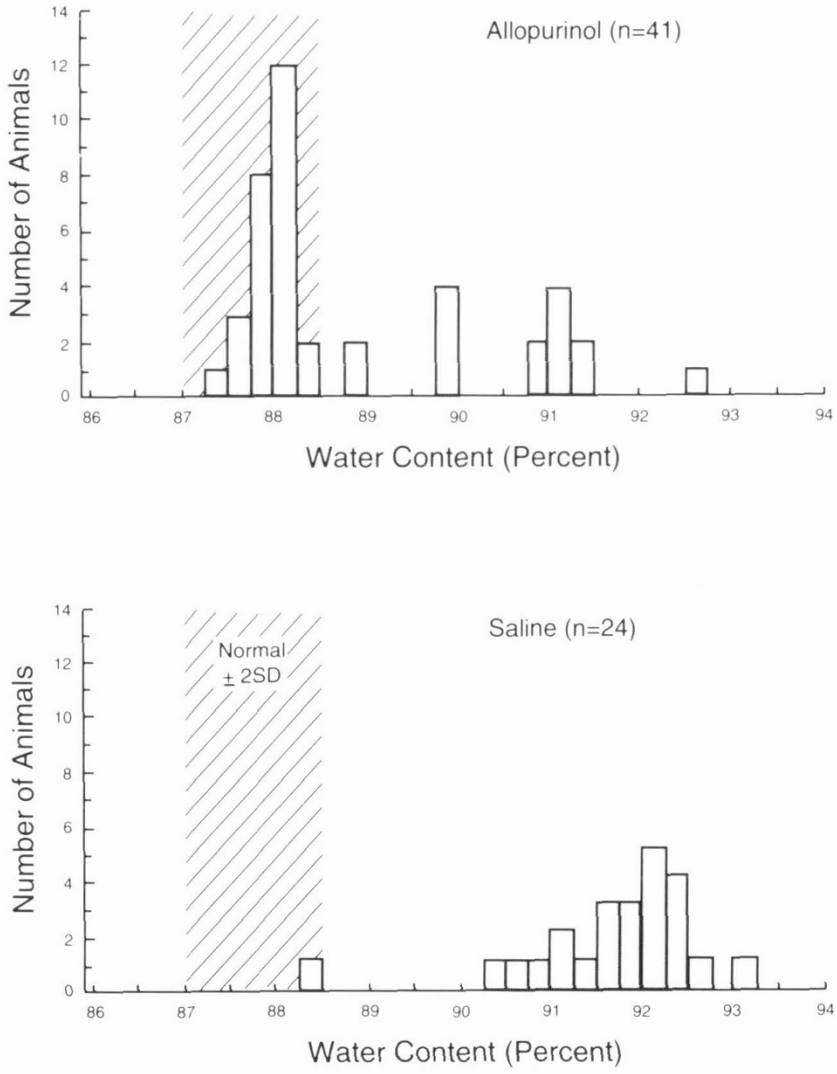

Fig. 2. Right hemisphere water content at 42 h of recovery after hypoxia-ischemia. The normal range (mean ± 2 SD) for cerebral water content is illustrated. Pups were treated at 15 min after hypoxic-ischemic insult with allopurinol 135 mg/kg s.c. or saline. The allopurinol-treated pups had lower right hemisphere water contents than the saline-treated pups. $p < 0.0001$ (Mann Whitney U).

Table 1. *Gross neuropathologic grading**

Gross pathologic category	Treatment group (no. of animals)	
	Allopurinol ($n = 31$)	Saline ($n = 32$)
Normal	16	8
Mild	15	9
Moderate	0	8
Severe	0	7

* $p < 0.0001$ (Cochran trend test). Neuropathologic grading by a team of two examiners.

Because this is the first study to show that allopurinol administered during recovery reduces hypoxic-ischemic brain damage in any age animal, it could be possible that this neuroprotective effect is unique to the immature brain. However, studies in adult gerbils showed that oxypurinol (40 mg/kg intraperitoneally) administered 30 min after transient cerebral ischemia reduced the increased locomotor activity that is associated with hippocampal injury (23). Although our study confirms that high-dose allopurinol rescue therapy is neuroprotective, it does not address mechanisms directly apart from suggesting that this beneficial effect is not mediated through hypothermia. We monitored the effect of allopurinol on core body temperature because core body temperature closely reflects brain temperature in this model (24) and because mild hypothermia (3–6°C), even during recovery from cerebral hypoxia-ischemia, can be protective (25–27).

Allopurinol's neuroprotective mechanism has usually been attributed to its ability to inhibit xanthine oxidase. In the brain, xanthine oxidase is precariously concentrated within endothelial cells (28, 29). This may subject the blood-brain barrier to free radical attack. Patt *et al.* (30) showed that inhibition of xanthine oxidase activity with a tungsten-enriched diet reduced edema and hydrogen peroxide production in the postischemic gerbil brain. They also showed that brain edema and hydrogen peroxide production correlated with xanthine oxidase activity. Liu *et al.* (31) showed that infarct volume in adult rats undergoing bilateral carotid occlusion was reduced by pretreatment with superoxide dismutase and catalase conjugated to polyethelylene glycol. Because these substrate-specific conjugated enzymes do not cross the blood-brain barrier (32), they are thought to scavenge superoxide and hydrogen peroxide from within cerebral blood vessels. This implicates free radical–induced microvascular injury in the pathogenesis of cerebral infarction.

Recently, the role of xanthine oxidase as the major source of endothelial cell–derived superoxide during cerebral reperfusion has been questioned. Terada *et al.* (28) showed that cultured endothelial cells from bovine cerebral microvessels sponta-

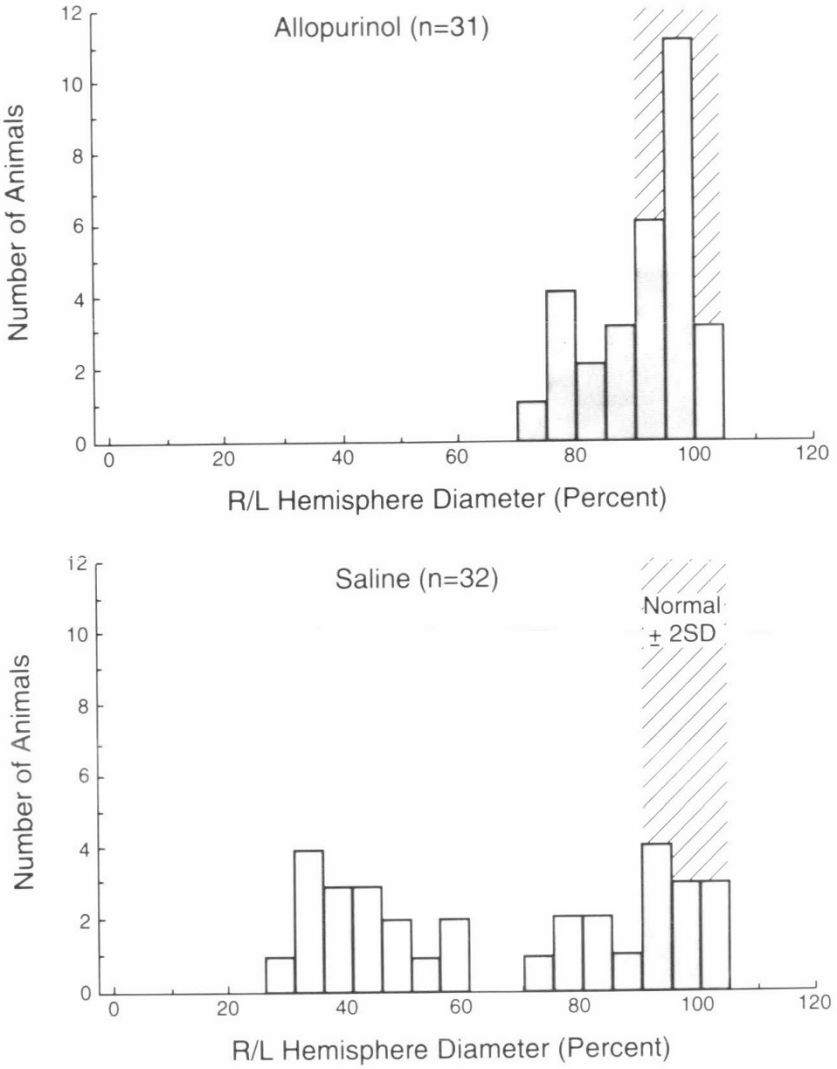

Fig. 3. Right hemisphere atrophy determined morphometrically by the R/L hemisphere diameter ratio. The normal range (mean ± 2 SD) of hemisphere asymmetry is illustrated. Pups were treated at 15 min after hypoxia-ischemia with allopurinol 135 mg/kg s.c. or saline. A ratio less than 65% was associated with cavitary lesions. The allopurinol-treated pups were markedly less damaged than the saline-treated pups and had no cavitary lesions. $p < 0.001$ (Mann Whitney U).

Table 2. *Combined gross and microscopic assessment of established brain damage**

Assessment	Treatment group (no. of animals)	
	Allopurinol ($n = 31$)	Saline ($n = 32$)
Gross damage (cavitary lesions)	0	15
Microscopic damage (reactive gliosis)	11	9
Undamaged	20	8

* $p < 0.0001$ by the Cochran trend test, suggesting that the distribution of damage is shifted toward more severe damage in the saline group relative to the allopurinol group.

neously secrete superoxide. However, superoxide secretion was only partially repressed when xanthine oxidase activity was inhibited. Lindsay *et al.* (13) reduced infarct volume in a rat model of permanent middle cerebral artery occlusion with allopurinol pretreatment but needed doses in excess of that required to inhibit xanthine oxidase. Similar findings were obtained by Betz *et al.* (14). These studies suggest that xanthine oxidase inhibition does not fully explain the neuroprotective mechanism of high-dose allopurinol pretreatment.

We showed recently in the same neonatal rat pup model of cerebral hypoxia-ischemia that brain energy metabolism during the insult is preserved by high-dose allopurinol pretreatment (33). Therefore, pretreatment with high-dose allopurinol may reduce the severity of the hypoxic-ischemic insult. In a preliminary report, Chemtob *et al.* (34) pretreated newborn pigs with 140 mg/kg allopurinol and showed that allopurinol reduced postasphyxial cerebral hypoperfusion. Previous studies have suggested that allopurinol has a vasodilatory action (35, 36). Clearly, the effectiveness of allopurinol in this study cannot be attributed to any attenuation of the primary hypoxic-ischemic insult, inasmuch as it was given 15 min into recovery.

The near 4-fold concentration of allopurinol in the serum compared with brain homogenate suggests that allopurinol exerts dose-dependent effects from within blood vessels. Because the regional pattern of blood flow changes after hypoxia-ischemia has not been completely characterized in this model, it is still possible that allopurinol given at 15 min of recovery in some way prevented microvascular dysfunction and secondary ischemia. Prevention of microvascular injury and secondary infarction could explain why cavitary lesions did not occur in the brains of the allopurinol-treated pups and why, in contrast, 15 of 32 saline-treated animals had cavitary lesions.

It is possible that posttreatment (rescue therapy) with allopu-

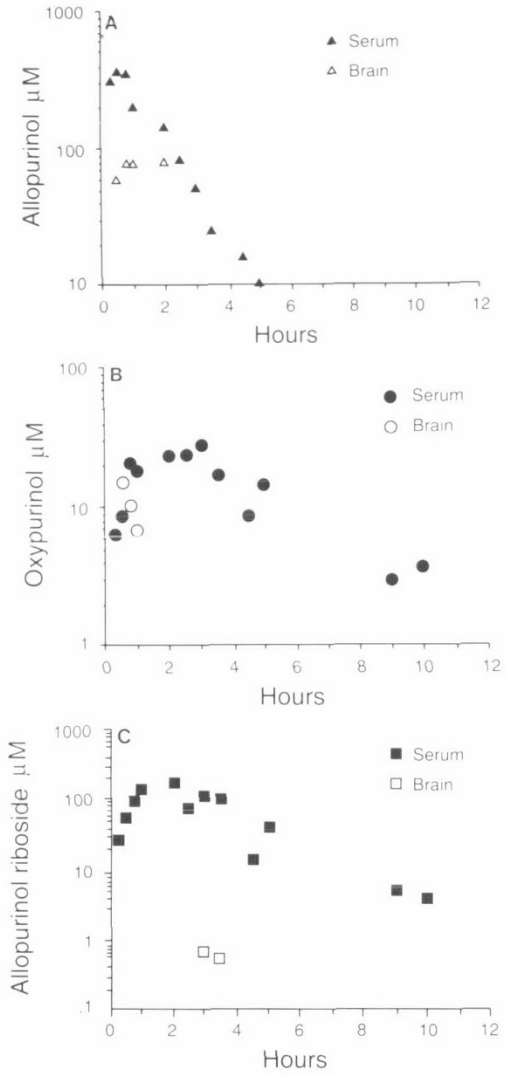

Fig. 4. Drug concentrations. Pups were injected with allopurinol 135 mg/kg s.c. at time 0. Serum (*filled symbols*) and brain (*open symbols*) concentrations of allopurinol (*A*) and its metabolites oxypurinol (*B*) and allopurinol riboside (*C*) were later measured at intervals. The points represent the average of two to six animals.

rinol may have a different mechanism of action compared with pretreatment. Allopurinol and oxypurinol both scavenge hydroxyl radicals and chelate transition metals in a dose-dependent manner. However, concentrations in the 0.5 to 1-mM range are required (17–19). Serum levels of allopurinol and its active metabolite oxypurinol peaked between 30 min and 1 h after injection. The serum allopurinol levels were nearly four times higher than brain levels. By combining the peak serum levels of allopurinol and oxypurinol, a concentration of approximately 0.5 mM was achieved in the first hour after injection. This level may be sufficient to exert a direct antioxidant effect, although the ability of allopurinol to increase plasma antioxidant capacity *in vivo* still needs to be demonstrated. At the serum levels attained in this study, allopurinol can facilitate mitochondrial electron transport in the respiratory chain (37). In addition, allopurinol can inhibit neutrophil lysosomal enzyme release at concentrations from 0.01 to 1 mM (38). The high serum levels of allopurinol and oxypurinol suggest that their site of action is within blood vessels. They would also have acted in the parenchyma, if a damaged blood-brain barrier allowed extravasation of intravascular contents.

In our study, more allopurinol was converted to allopurinol riboside than to oxypurinol. This is consistent with a report by Nelson and Elion (39), who showed that animals given large doses of allopurinol metabolize relatively little to oxypurinol in comparison to allopurinol riboside. We have not found that allopurinol riboside shares allopurinol's neuroprotective effect (unpublished data). Oxypurinol, however, administered at the same time postinsult and at the same dose as allopurinol was administered in this study, also prevents brain injury (40).

Oxypurinol may have advantages over allopurinol as a rescue therapy. Oxypurinol is a better free radical scavenger than allopurinol (41); it scavenges hypochlorous acid, whereas allopurinol does not (42). It has a longer half-life and it noncompetitively inhibits xanthine oxidase. Its activity is not inhibited by the high levels of hypoxanthine and xanthine that accumulate with ischemia (41). In addition, oxypurinol does not release a superoxide radical during its metabolism as allopurinol does (41, 43).

In conclusion, this study shows that allopurinol rescue therapy administered 15 min after cerebral hypoxia-ischemia in neonatal rats reduces brain edema, selective neuronal necrosis, and cystic infarction. This study has important clinical implications for the management of hypoxic-ischemic (asphyxiated) newborn infants. Additional studies are needed to define the neuroprotective mechanisms and therapeutic time-dose window of allopurinol rescue therapy.

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