Xanthine Oxidase–derived Hydrogen Peroxide Contributes to Ischemia Reperfusion–induced Edema in Gerbil Brains

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Abstract

The contribution of toxic O_2 metabolites to cerebral ischemia reperfusion injury has not been determined. We found that gerbils subjected to temporary unilateral carotid artery occlusion (ischemia) consistently developed neurologic deficits during ischemia with severities that correlated with increasing degrees of brain edema and brain H_2O_2 levels after reperfusion. In contrast, gerbils treated just before reperfusion (after ischemia) with dimethylthiourea (DMTU), but not urea, had decreased brain edema and brain H_2O_2 levels. In addition, gerbils fed a tungsten-rich diet for 4, 5, or 6 wk developed progressive decreases in brain xanthine oxidase (XO) and brain XO + xanthine dehydrogenase (XD) activities, brain edema, and brain H_2O_2 levels after temporary unilateral carotid artery occlusion and reperfusion. In contrast to tungsten-treated gerbils, allopurinol-treated gerbils did not have statistically significant decreases in brain XO or XO + XD levels, and reduced brain edema and brain H_2O_2 levels occurred only in gerbils developing mild but not severe neurologic deficits during ischemia. Finally, gerbils treated with DMTU or tungsten all survived, while > 60% of gerbils treated with urea, allopurinol, or saline died by 48 h after temporary unilateral carotid artery occlusion and reperfusion. Our findings indicate that H_2O_2 from XO contributes to reperfusion-induced edema in brains subjected to temporary ischemia.

Introduction

It has been proposed that toxic O_2 metabolites from xanthine oxidase (XO)[1] contribute to the development of injury seen during reperfusion of a variety of ischemic tissues (1, 2). This impression is based primarily on observations that XO appears located within endothelial cells (3–6), that XO generates O_2 metabolites in vitro (7), that endothelial cells make O_2 metabolites in vitro (8–10), and that treatment with SOD or allopurinol, an XO inhibitor, reduces reperfusion injury in some models of intestinal and cardiac ischemia (11–14). However, there are several problems with making this assumption and using these approaches to determine if XO-derived O_2 metabolites contribute to ischemia reperfusion injury in the brain. First, XO activity after ischemia varies from organ to organ. For example, conversion of xanthine dehydrogenase (XD) to XO during ischemia in rats is faster in liver than in kidney, faster in kidney than in heart, and faster in heart than in lung (15, 16). Second, allopurinol and large, highly charged antioxidants, such as SOD and catalase, may not effectively penetrate the blood brain barrier and optimally inhibit processes involving intracellularly generated short-lived O_2 metabolites (17, 18). Finally, the contribution of O_2 metabolites to reperfusion injury needs to be more convincingly established using corroborative measurements of O_2 metabolites in biological systems (19).

In the present study, we tested the hypothesis that XO-derived H_2O_2 contributes to cerebral reperfusion injury. Brain H_2O_2 was measured using aminotriazole inactivation of brain catalase activities (20–22). Brain XO activity was measured using high pressure liquid chromatography (HPLC) (6). We found that administering a single dose of dimethylthiourea (DMTU), a permeant H_2O_2 scavenger in vitro (23–25), just before reperfusion, decreased brain edema and brain H_2O_2 levels and improved survival in symptomatic gerbils undergoing temporary unilateral carotid occlusion and reperfusion. In parallel, inhibiting brain XO activity using sodium tungstate, which inactivates XO by preventing the biosynthesis of functionally intact XO in a process that involves molybdenum (26, 27), also decreased brain edema and brain H_2O_2 levels and improved survival in symptomatic gerbils undergoing temporary unilateral carotid artery occlusion and reperfusion. The results indicate that H_2O_2 from XO participates in edematous injury during reperfusion of cerebral tissues that have been previously subjected to ischemia.

Methods

Internal carotid artery ligation and reperfusion. Healthy adult male and female gerbils (50–90 g, Tumblebrook Farms, West Brookfield, MA) were housed at constant temperature under simulated day and night conditions. Gerbils were anesthetized with ketamine (80 mg/kg, Parke, Davis & Co., Morris Plains, NJ) and one drop of Dopram (20 mg/ml, Robins Co., Richmond, VA) sublingually. Using an operating microscope, the left common carotid artery was isolated through a ventral midline incision and clamped with a 5 × 1-mm Kleinhurt-Kutz microvessel clip (0.38-in straight blade, Edward Weck, Inc., Research Triangle Park, NC). Interruption of blood flow was verified visually and the incision was closed with 3-0 silk suture. Gerbils were observed for 3 h and then grouped according to neurologic deficit severity (28):

1. *Abbreviations used in this paper:* DMTU, dimethylthiourea; XD, xanthine dehydrogenase; XO, xanthine oxidase.

Group 1, no deficits (asymptomatic); group 2, mild deficits (circling, ptosis, hemiparesis); and group 3, severe deficits (hemiplegia, rolling fits, stupor).

Gerbils with severe neurologic deficits often died if occlusion was allowed to continue beyond 3 h, and therefore were reanesthetized with metaphane (118 ml, Pitman-Moore, Inc., Washington Crossing, NJ) and vascular clips were removed after 3 h. Gerbils that died spontaneously during carotid artery occlusion (< 5%) were eliminated from study. By comparison, gerbils exhibiting no or mild neurologic deficits were reanesthetized and the clip was removed after 6 h. When the clip was removed, resumption of blood flow was verified visually. In separate randomized experiments, gerbils were treated 30 min before reperfusion with normal saline (0.9% i.p.), DMTU (750 mg/kg, Alfa Products, Danvers, MA), or urea (750 mg/kg, Fisher Scientific Co., Fairlawn, NJ). Gerbils were also treated intraperitoneally with allopurinol (50 mg/kg per d times three, Sigma Chemical Co., St. Louis, MO), which had been dissolved in normal saline using heating and mixing with sodium hydroxide (1 M, pH 10) to increase solubility or fed a normal protein diet with 0.7 g/kg sodium tungstate (ICN Biochemicals, Cleveland, OH) and given H_2O with 10 parts per million tungsten as sodium tungstate (Sigma Chemical Co.) for 4, 5, or 6 wk before carotid artery occlusion. Gerbils fed tungsten had the same weights, hematocrits, and alertness as gerbils fed control diets.

Measurement of brain edema. After reperfusion, gerbils were killed by cervical dislocation and brains were removed. Right (control) and left (experimental) hemispheres were subsequently weighed and dried in an oven at 60°C for 48 h. After heating, dry residues were reweighed. Percentages of dry to wet weights (P) were calculated as follows: (dry weight/wet weight) × 100 = percent dry to wet weight (*P*). Percentages of brain edema were then calculated by the formula: (*P* right − *P* left)/*P* left × 100 = percent brain edema.

Measurement of brain and liver XO and XO+XD activities. Livers and brains were immediately removed from pentobarbital (10 mg)-anesthetized gerbils and placed in preweighed containers with KPO_4 (50 mM), Na_2CO_3 homogenizing buffer (0.2 M, pH 10), PMSF (0.01 M), dithioerythritol (0.01 M), and EDTA (1 mM) (29). Brain tissues were then homogenized in an ultra-turrex homogenizer (Heat Systems Ultrasonics, Farmingdale, NY) at speed 4 for 60 s. After centrifugation at 30,000 g for 30 min, supernatants were collected from each tube, placed on ice, and then quickly passed at 10°C over Sephadex G25 columns previously equilibrated with homogenizing buffer. XO and total enzyme (XO+XD) activities were measured using HPLC by assay of uric acid produced from xanthine as a function of time. XO activity was measured by preparing sample mixtures of phosphate buffer (50 mM) and xanthine (1 mM). XO+XD activity was measured by preparing sample mixtures of phosphate buffer (50 mM), xanthine (1 mM), and NAD (6 mM). Standards were also prepared in parallel which contained uric acid (1 mM), xanthine (1 mM), or purified XO. All samples or standards were incubated for 3 h at 37°C in air. After 3 h, TCA (10%) was added to each tube to achieve a pH 6 to 6.5. Samples were then vortexed and centrifuged at 4,000 g for 15 min. Aliquots of supernatants (10 μl) were injected into the HPLC column (501 pump, 481 detector, and 740 integrator, and C18 Resolve column, Waters Assoc., Div. of Millipore Corp., Milford, MA, KH_2PO_4 buffer (20 mM), pH 5.7, 1 μl/min flow rate) and the uric acid peaks were detected at 290 nM. Standards and samples were run in triplicate. Differences between results of two injections from the same sample did not exceed 5%.

Measurement of brain H_2O_2 levels. Gerbils were injected intraperitoneally with aminotriazole (1,000 mg/kg) just before reperfusion (20–22). After reperfusion, gerbils were killed and their brains were removed, rinsed with cold NaCl (5°C, 0.15 M), and stripped of adherent blood vessels. Brains were then separated into right and left hemispheres, weighed, and homogenized in cold potassium phosphate (50 mM) Triton X-100 (1%) buffer (pH 7.0). Catalase activity (30) of brain homogenate samples (100 ml) was assayed in duplicate tubes containing cold sodium perborate ($NaBO_2H_2O_23H_2O$) and KH_2PO_4 (0.05 M). Reactions between sodium perborate and brain catalase were stopped

after 5 min by adding H_2SO_4 (1 M). Unreacted sodium perborate was then measured by titration with $KMNO_4$ (0.01 M). A blank was also measured using a homogenate sample that had been treated with TCA (1 M) to inactivate catalase. Measurement of aminotriazole levels in brain tissue was performed by combining samples of brain homogenates with TCA. After centrifugation at 2,500 g × 20 at 5°C, supernatants were filtered (0.2-μm aerodisc) and then diluted to a total of 5 cm³ using 5% TCA (31). Sodium nitrite (1 ml, 0.01 N) was added and the solutions were swirled. Subsequently, 4,5-dihydroxyl naphthalene-2,7,disulfonoic acid (1 ml, 0.0025 M chromatographic acid, grade 1, Sigma Chemical Co.) was added and the tubes were mixed by swirling. Test tubes were immediately placed in boiling water for 2.5 min and then in ice water. Aminotriazole content of homogenates was measured colorimetrically at 525 nM. For clarity of presentation, brain H_2O_2 levels were presented as the negative value for the differences in catalase units per milligram of left (affected)-right (control) hemispheres.

Measurement of survival. Survival was assessed at intervals for up to 48 h after reperfusion.

Statistical analyses. Brain edema, brain H_2O_2, brain XO, and brain XO+XD data were compared for saline or treated gerbils by one-way analyses of variance using the Student-Newman-Keuls test for multiple comparisons (32). Brain aminotriazole levels from right or left hemispheres were compared by paired Student's *t* test (32).

Results

Neurologic deficits, brain edema, and brain H_2O_2 levels. Neurologic deficits were assessed during carotid occlusion (ischemia) while brain edema and brain H_2O_2 levels were assessed after reperfusion of previously ischemic brains. During unilateral carotid artery occlusion, gerbils consistently developed either no (~ 50%), mild (~ 25%), or severe (~ 25%) neurologic deficits. Neurologic deficit severity occurring during unilateral carotid artery occlusion (ischemia) correlated with degrees of brain edema (as assessed by differences in percent weight gain of left [affected]-right [control] hemispheres, Fig. 1) and brain H_2O_2 levels (as assessed by differences in H_2O_2-
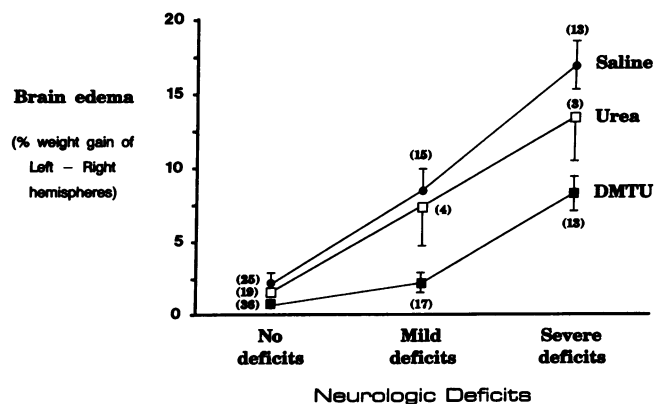
Figure 1. Brain edema (percent weight gain of left-right hemispheres; see Methods) after reperfusion in gerbils developing no, mild, or severe neurologic deficits during temporary unilateral carotid artery occlusion (ischemia). Gerbils that developed mild or severe deficits during ischemia and were then treated with DMTU (but not urea) during reperfusion had less (*P* < 0.05) brain edema after reperfusion than gerbils treated with saline. No differences (*P* > 0.05) were found in brain edema after reperfusion in DMTU-, urea-, or saline-treated gerbils that did not develop neurologic deficits during ischemia. Each value is the mean±1 SE of the number of determinations shown in parentheses.

dependent aminotriazole inactivated–catalase activities for left [affected]-right [control] hemispheres, Fig. 2) after reperfusion. Gerbils without neurologic deficits had negligible levels of brain edema and brain H_2O_2; gerbils with mild deficits had intermediate levels of brain edema and brain H_2O_2; gerbils with severe deficits had the highest levels of brain edema and brain H_2O_2.

Effect of DMTU or urea treatment on brain edema and brain H_2O_2 levels. Gerbils that developed mild or severe neurologic deficits during temporary unilateral carotid artery occlusion (ischemia), which were then treated with a single dose of DMTU (but not urea) just before reperfusion (after ischemia), had less ($P < 0.05$) brain edema (Fig. 1) and brain H_2O_2 levels (Fig. 2) after reperfusion than saline-treated control gerbils. In contrast, brain edema and brain H_2O_2 levels were not different in DMTU-, urea-, or saline-treated gerbils that did not develop neurologic deficits after temporary unilateral carotid artery occlusion and reperfusion.

Effect of treatment with tungsten or allopurinol on brain XO activity, brain edema, and brain H_2O_2 levels. Gerbils fed tungsten for 4, 5, or 6 wk had progressively decreased levels of brain (and liver) XO and XO+XD activities (Table I). Complete inhibition (100%) of brain XO and brain XO+XD activities occurred in gerbils fed tungsten for 6 wk. Treatment of gerbils with allopurinol (50 mg/kg per d times three) did not significantly ($P > 0.05$) decrease brain XO and brain XO+XD activities (decreases of only 22 and 23%, respectively). In contrast, allopurinol treatment decreased liver XO and liver XO+XD activities by 49 and 61%, respectively (Table I). After temporary unilateral carotid artery occlusion, gerbils that had developed mild or severe neurologic deficits during ischemia and had been fed tungsten for 4, 5, or 6 wk had progressively decreased brain edema (Fig. 3) and brain H_2O_2 levels (Fig. 4) during reperfusion when levels were compared with saline-
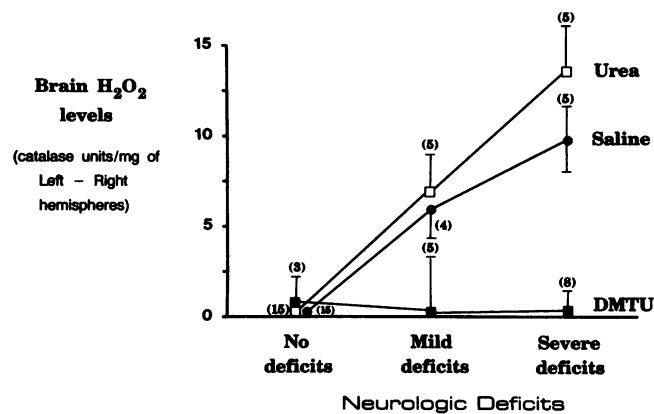

Figure 2. Brain H_2O_2 levels (negative value for catalase units per milligram for left-right hemispheres; see Methods) after reperfusion in gerbils developing no, mild, or severe neurologic deficits after temporary unilateral carotid artery occlusion (ischemia). Gerbils that developed mild or severe neurologic deficits during ischemia and were then treated with DMTU (but not urea) during reperfusion had decreased ($P < 0.05$) brain H_2O_2 levels after reperfusion compared with gerbils treated with saline. No differences ($P > 0.05$) in brain H_2O_2 levels after reperfusion were found in DMTU-, urea-, or saline-treated gerbils that did not develop neurologic deficits during ischemia. Each value is the mean±1 SE of the number of determinations shown in parentheses.

treated gerbils. By comparison, only allopurinol-treated gerbils with mild neurologic deficits had significantly ($P < 0.05$) decreased levels of brain edema (Fig. 5) and brain H_2O_2 (Fig. 6). In separate analyses, correlations were found between brain edema and brain XO+XD activity (Fig. 7), brain edema and brain H_2O_2 levels (Fig. 8), or brain H_2O_2 levels and brain XO+XD activity (Fig. 9). Aminotriazole levels ($\sim 245\pm38$ μg/g brain measured 3 h after aminotriazole injection) were not different ($P > 0.05$) in right and left hemispheres of saline-, DMTU-, urea-, tungsten-, or allopurinol-treated gerbils (data not shown).

Survival. All DMTU- or tungsten-treated symptomatic gerbils survived for 48 h while < 40% of symptomatic gerbils treated with saline, urea, or allopurinol survived for 48 h after temporary unilateral carotid artery occlusion and reperfusion (Fig. 10).

Discussion

We found that neurologic deficits occurring during temporary unilateral carotid occlusion (ischemia) correlated and predicted degrees of brain edema and brain H_2O_2 levels measured after reperfusion in saline-treated control gerbils. Gerbils without neurologic deficits (during ischemia) had minimal amounts of brain edema and brain H_2O_2 levels (after reperfusion); gerbils with mild neurologic deficits (during ischemia) had intermediate levels of brain edema and brain H_2O_2 (after reperfusion); and gerbils with severe neurologic deficits (during ischemia) had the greatest brain edema and brain H_2O_2 levels (after reperfusion). Gerbils were used for these studies because they commonly lack the posterior communicating artery (incomplete Circle of Willis), and therefore, occlusion of one carotid artery provides a model that is similar to human stroke (33–38). Although the severity of neurologic deficit in each gerbil was not predictable, large groups of gerbils were always tested and the proportions of gerbils that developed either no, mild, or severe neurologic deficits during carotid occlusion (ischemia) were always similar in each test group.

Our study implicates H_2O_2 in the development of cerebral edema during reperfusion after ischemia. In saline-treated gerbils subjected to temporary unilateral carotid occlusion (ischemia) and then reperfusion, correlations occurred between brain edema and brain H_2O_2 levels (as assessed by differences in catalase levels between left (affected) and right (control) hemispheres of aminotriazole-treated gerbils. Irreversible inactivation of catalase by aminotriazole is a highly specific way of measuring H_2O_2 in biological systems (20–22). Differences in catalase inactivation by H_2O_2 were not due to alterations in aminotriazole levels that were comparable in left and right hemispheres of brains from treated and control gerbils. Moreover, in gerbils treated with DMTU after ischemia (just before reperfusion), both brain edema and brain H_2O_2 levels were decreased after temporary unilateral carotid artery occlusion and reperfusion compared with saline-treated gerbils. DMTU is a highly permeant scavenger that decreases H_2O_2 (but not superoxide anion [O_2^-]) in vitro (23, 24). It should be emphasized that since DMTU was given only during reperfusion (after ischemia), it did not decrease neurologic deficits occurring during ischemia, but only increases in brain edema and brain H_2O_2 levels measured after reperfusion. This finding un-

Table I. Effect of Tungsten or Allopurinol Treatment on Gerbil Brain and Liver XO or Total Enzyme (XO+XD) Activities

Treatment	Brain XO activity	Brain XO+XD activity	Liver XO activity	Liver XO+XD activity
		ng/mg		
Saline	9±1 (7)*	48±3.2 (10)	35±3 (3)	552±10 (2)
Tungsten (4 wk)	5±5 (3)‡	17±2.0 (3)‡	5±2 (2)‡	153±20 (2)‡
Tungsten (5 wk)	0 (2)‡	8±2.0 (3)‡	0 (2)‡	2±1 (2)‡
Tungsten (6 wk)	0 (2)‡	0 (2)‡	0 (2)‡	0 (2)‡
Allopurinol	7±5 (5)§	37±4.5 (5)§	18±1 (2)‡	216±26 (3)‡

* Mean±SEM (number of determinations in parentheses). ‡ Value significantly different ($P < 0.05$) from value obtained after treatment with saline. § Value not significantly different ($P > 0.05$) from value obtained after treatment with saline.

derscores that neurologic deficit severity during ischemia does not predict increases in brain edema or brain H_2O_2 in gerbils treated during reperfusion with DMTU. In contrast, gerbils treated with urea, a potent osmotic agent that does not decrease H_2O_2 concentrations in vitro (23, 24), had the same increased levels of brain edema or brain H_2O_2 as saline-treated gerbils. These studies effectively link H_2O_2 or an H_2O_2-derived product with the development of brain edema during reperfusion after ischemia (39).

H_2O_2 generation appears to be from XO. Tungsten-treated gerbils had progressively decreased brain XO and brain XO+XD activities that corresponded with progressively decreased brain edema and brain H_2O_2 levels. Again, note that tungsten treatment did not decrease neurologic deficits occurring during ischemia but only increases in brain edema and brain H_2O_2 levels during reperfusion. Both XO and XO+XD activities were measured because in spite of rapid addition of protease and oxidation inhibitors, conversion of XD to XO may be altered during collection, preparation, and/or assay. However, since brains from gerbils treated with tungsten for 6

wk totally lack both XO and XD+XO activity, the potential contribution of XO is negated regardless of any extrinsic variations in conversion of XD to XO. Similarly, prior findings that tungsten treatment decreases XO activity in rat lungs and that tungsten-treated cultured lung endothelial cells lack XO and fail to generate O_2^- support the premise that tungsten decreases XO activity (6). Sulfite oxidase and aldehyde oxidase are two other mammalian enzymes that are known to contain molybdenum, and are therefore potentially susceptible to inhibition by tungsten. In contrast, allopurinol-treated gerbils did not have statistically significant decreases in brain XO or XO+XD activities. In addition, brain edema and brain H_2O_2 levels were decreased only in allopurinol-treated gerbils that developed mild but not severe neurologic deficits during ischemia. The reasons for the relative lack of protection with allopurinol are not clear. Although allopurinol effectively reduces reperfusion injury in small intestine and heart (11-13), the relatively large dose of allopurinol that was used may have been poorly transported across the blood brain barrier, where its concentration has been estimated to be only one-third to one-half that of the
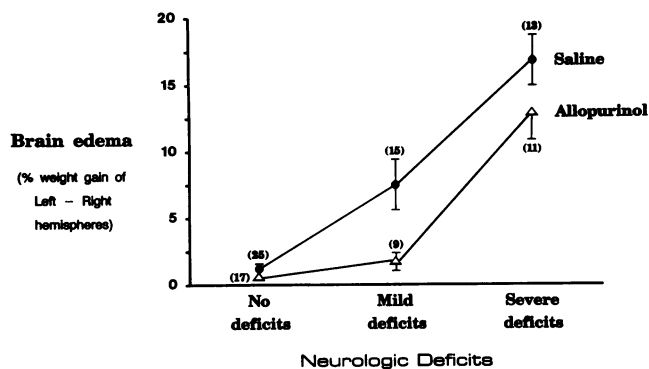

Figure 3. Brain edema (percent weight gain of left-right hemispheres) after reperfusion in gerbils developing no, mild, or severe neurologic deficits during temporary unilateral carotid artery occlusion (ischemia). Gerbils that developed mild or severe neurologic deficits during ischemia and which had been previously fed tungsten-rich diets for 4, 5, or 6 wk had less ($P < 0.05$) brain edema after reperfusion than gerbils fed regular diets. No differences ($P > 0.05$) in brain edema were found after reperfusion in tungsten- or regular diet–fed gerbils that did not develop neurologic deficits during ischemia. Each value is the mean±1 SE of the number of determinations shown in parentheses.
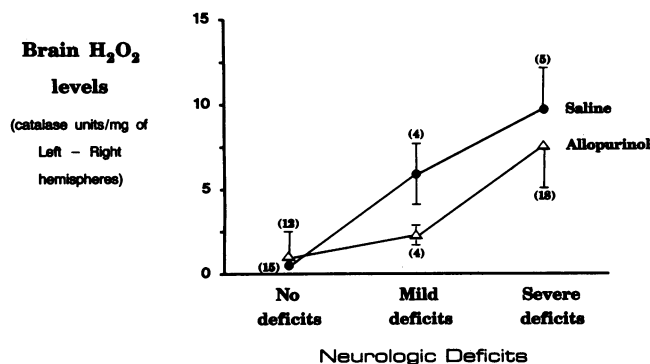

Figure 4. Brain H_2O_2 levels (negative value for catalase units per milligram of left-right hemispheres) after reperfusion in gerbils developing no, mild, or severe neurologic deficits after temporary unilateral carotid artery occlusion (ischemia). Gerbils that developed mild or severe neurologic deficits during ischemia and had previously been fed tungsten-rich diets for 4, 5, or 6 wk had decreased ($P < 0.05$) brain H_2O_2 levels after reperfusion compared with gerbils fed regular diets. No differences ($P > 0.05$) in brain H_2O_2 levels were found after reperfusion in tungsten- or regular diet–fed gerbils that did not develop neurologic deficits during ischemia. Each value is the mean±1 SE of the number of determinations shown in parentheses.

Figure 5. Brain edema (percent weight gain of left-right hemispheres) after reperfusion in gerbils developing no, mild, or severe neurologic deficits after temporary unilateral carotid artery occlusion (ischemia). Gerbils that developed mild neurologic deficits during ischemia and had been previously treated with allopurinol had less ($P < 0.05$) brain edema after reperfusion than gerbils treated with saline. No differences ($P > 0.05$) in brain edema were found after reperfusion in gerbils that developed no or severe neurologic deficits and had been previously treated with allopurinol or saline before temporary unilateral carotid artery occlusion. Each value is the mean±1 SE of the number of determinations shown in parentheses.
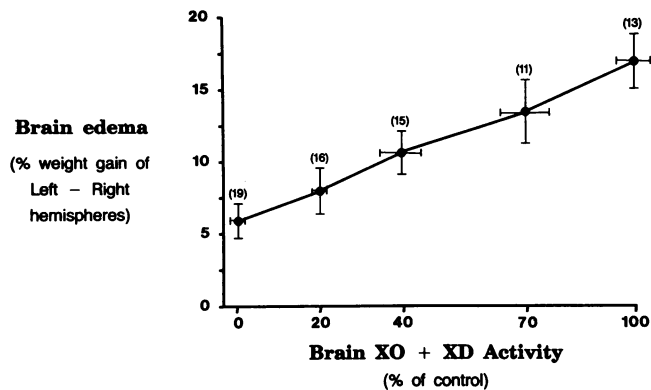

Figure 7. Brain edema (percent weight gain of left-right hemispheres) increased as a function of increases in brain XO+XD activity in gerbils undergoing temporary unilateral carotid artery occlusion (ischemia) and reperfusion. Each value is the mean±1 SE of the number of determinations shown in parentheses.

rest of the body (5, 40). This possibility was suggested when allopurinol treatment appreciably inhibited liver XO and liver XO+XD activities while decreasing brain XO and brain XO+XD activities only slightly. It is also possible that some XO inhibition has reversed during preparation and removal of allopurinol by Sephadex filtering. However, this possibility did not occur in livers from gerbils treated with allopurinol or in gerbils treated with tungsten. Allopurinol is a competitive inhibitor that is relatively specific for XO, and by comparison to tungsten does not inhibit sulfite or aldehyde oxidase.

The specific mechanism by which H_2O_2 contributes to acute edematous brain damage is unclear. Molecular O_2 is a cofactor in XO-dependent conversion of hypoxanthine to xanthine and xanthine to uric acid. This reaction produces O_2^- by one-electron reduction or H_2O_2 by two-electron reduction of O_2 (7, 41, 42). The relative contribution of these two pathways is dependent on both pH and O_2 tension. Higher O_2 tensions favor single electron reduction and O_2^- formation (7). Either spontaneously or through the action of SOD, O_2^- is converted to H_2O_2, and then to the more toxic hydroxyl radical ($^{\cdot}OH$) by metal catalyzed Fenton or Haber-Weiss reactions (43). H_2O_2 or H_2O_2-derived products can directly alter key cell proteins, lipids, and nucleic acid molecules in vitro (44). Exogenously generated H_2O_2 and related O_2 metabolites can also cause reversible abnormalities in contractile mechanisms and leaks in endothelial cells in vitro (45), perfusion pressure increases and increases in thromboxane in isolated lungs (46), and inactivate antiproteases (39, 47).

The contribution of intracellular XO-derived H_2O_2 to ischemia and reperfusion-induced injury is significant. Gerbils

Figure 6. Brain H_2O_2 levels (negative value for catalase units per milligram of left-right hemispheres) after reperfusion in gerbils developing no, mild, or severe neurologic deficits after temporary unilateral carotid artery occlusion (ischemia). Gerbils that developed mild neurologic symptoms during ischemia and had been previously treated with allopurinol had decreased ($P < 0.05$) brain H_2O_2 levels after reperfusion compared with gerbils treated with saline. No differences ($P > 0.05$) in brain H_2O_2 levels after reperfusion were found in gerbils that developed no or severe neurologic deficits during ischemia and had been previously treated with allopurinol or saline before temporary unilateral carotid artery occlusion. Each value is the mean±1 SE of the number of determinations shown in parentheses.
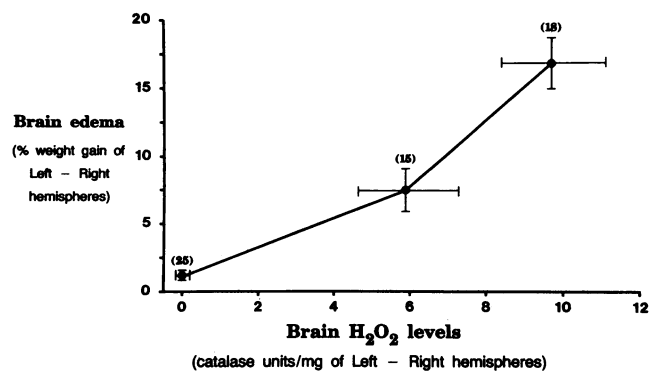

Figure 8. Brain edema (percent weight gain of left-right hemispheres) increased as a function of increases in brain H_2O_2 levels (negative value for catalase units per milligram of left-right hemispheres) in gerbils undergoing temporary unilateral carotid artery occlusion (ischemia) and reperfusion. Each value is the mean±1 SE of the number of determinations shown in parentheses.

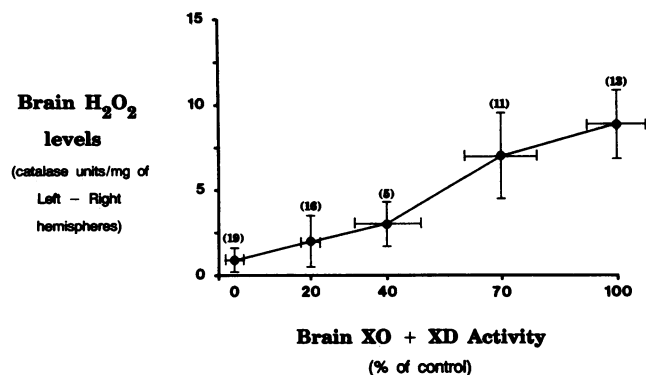**Brain H₂O₂ levels**
(catalase units/mg of Left − Right hemispheres)

Brain XO + XD Activity
(% of control)

Figure 9. Brain H$_2$O$_2$ levels (negative value for catalase units per milligram of left-right hemispheres) increased as a function of increases in brain XO+XD activity in gerbils undergoing temporary unilateral carotid artery occlusion (ischemia) and reperfusion. Each value is the mean±1 SE of the number of determinations shown in parentheses.

depleted of XO by treatment with tungsten or gerbils with reduced brain H$_2$O$_2$ levels after treatment with DMTU all survived for 48 h while, in contrast, > 60% of saline-, urea-, or allopurinol-treated gerbils died after temporary unilateral carotid artery occlusion and reperfusion. It has been shown that intracerebral injection of exogenous XO and hypoxanthine causes cerebral edema and breakdown of the blood brain barrier, and that topical application of XO causes brain endothelial cell permeability that is partially inhibited by SOD and catalase (48–50), but the contribution of intrinsic intracellular XO has not been defined previously.

While our results suggest that H$_2$O$_2$ from XO may contribute to acute edematous cerebral edema after ischemia reperfusion injury, a contribution from neutrophils is also possible. Neutrophils accumulate along damaged endothelial cells and appear to contribute to injury in other models of ischemia reperfusion damage (51–55). In addition, O$_2$ metabolites from XO might also alter endothelial cell surfaces and/or cause release of factors that attract and activate neutrophils (53, 56, 57). In other situations, stimulated neutrophils or other factors, such as endotoxin, may initiate injury by activating XO-dependent mechanisms that cause a "self-inflicted" damage to endothelial cells (10, 54).

Survival at 48 h
(survivors/total, % surviving)

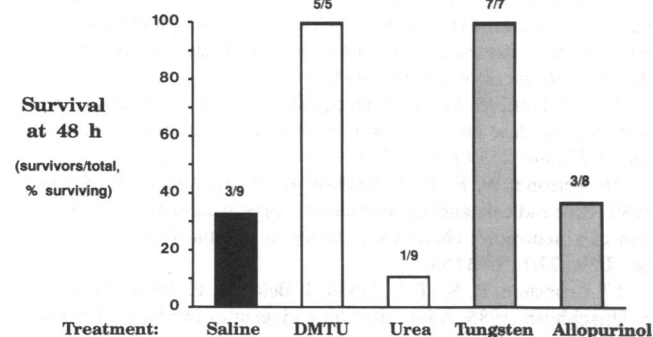Treatment: Saline, DMTU, Urea, Tungsten, Allopurinol

Figure 10. Survival (percent) at 48 h (number of gerbils surviving/number tested) after reperfusion of gerbils undergoing temporary unilateral carotid occlusion (ischemia). Gerbils treated with DMTU during reperfusion or fed tungsten for 6 wk had increased (P < 0.05) survivals compared with gerbils treated with saline, urea, or allopurinol.

Note added in proof. Since submission, another paper has been published which shows that both allopurinol and tungsten treatment decrease reperfusion injury in ischemic isolated rat hearts (58).

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