N-Acetylcysteine Reduces Lipopolysaccharide-Sensitized Hypoxic-Ischemic Brain Injury

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Objective: Maternal inflammation/infection alone or in combination with birth asphyxia increases the risk for perinatal brain injury. Free radicals are implicated as major mediators of inflammation and hypoxia-ischemia (HI)–induced perinatal brain injury. This study evaluated the neuroprotective efficacy of a scavenging agent, *N*-acetylcysteine (NAC), in a clinically relevant model.

Methods: Lipopolysaccharide (LPS)-sensitized HI brain injury was induced in 8-day-old neonatal rats. NAC was administered in multiple doses, and brain injury was evaluated at 7 days after HI.

Results: NAC (200mg/kg) provided marked neuroprotection with up to 78% reduction of brain injury in the pre+post-HI treatment group and 41% in the early (0 hour) post-HI treatment group, which was much more pronounced protection than another free radical scavenger, melatonin. Protection by NAC was associated with the following factors: (1) reduced isoprostane activation and nitrotyrosine formation; (2) increased levels of the antioxidants glutathione, thioredoxin-2, and (3) inhibition of caspase-3, calpain, and caspase-1 activation.

Interpretation: NAC provides substantial neuroprotection against brain injury in a model that combines infection/inflammation and HI. Protection by NAC was associated with improvement of the redox state and inhibition of apoptosis, suggesting that these events play critical roles in the development of lipopolysaccharide-sensitized HI brain injury.

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Over the past decades, information about perinatal brain injury mechanisms has increased and experimental studies have shown that development of brain injury occurs in different phases, making it possible to commence effective treatments after the insult. Recently, two randomized controlled trials showed that hypothermic treatment of postasphyxiated newborns was accompanied by decreased mortality[1] and attenuation of neurological deficits.[1,2] These studies are critical because they imply that treatment, applied after the hypoxic-ischemic challenge, is clinically possible in newborn humans.

It has been suggested that intrauterine infections and fetal inflammatory response syndrome either cause central nervous system injury directly or increase brain vulnerability in response to secondary perinatal insults such as hypoxia, hyperoxia, mechanical ventilation, or other infections.[3–5] Antenatal infection increases the risk for cerebral palsy,[5] but the combination of infection and asphyxiating conditions increases the risk synergistically.[6] This concept is supported by experimental studies showing that preexposure to bacterial lipopolysaccharide (LPS) sensitizes the immature brain to hypoxia-ischemia (HI).[7,8] Therefore, we believe that it is pertinent that potential neuroprotective agents are evaluated not only in models of HI, but also in combined inflammation/HI models.

Free radicals including reactive oxygen species (ROS), reactive nitrogen species, or both are produced in excess during inflammation, as well as during ischemia/reperfusion, and have been implicated as major mediators of perinatal brain injury.[9] The free radical scavenging agent *N*-acetylcysteine (NAC) is able to cross the placenta,[10] is considered safe during pregnancy[11,12] and, therefore, of potential therapeutic value in humans, and has been shown to reduce oxidative stress and inflammation.[13] In

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this study, we aimed to determine: (1) whether NAC can provide neuroprotection against immature brain injury caused by LPS-sensitized HI, and compared the effect with another free radical scavenging agent, melatonin; (2) the therapeutic window of NAC; and (3) the tentative mechanisms of the protective action by NAC. The overall goal is that these results will contribute to the development of therapeutic strategies for pregnant women or newborn babies suffering from infectious or HI exposures.

Materials and Methods

Induction of Lipopolysaccharide-Sensitized Hypoxia-Ischemia in Neonatal Rats and Drug Administration

LPS (0.1mg/kg; Sigma LPS O55:B5; St. Louis, MO) was given intraperitoneally to 8-day-old Wistar rats (brain maturity corresponding to that of third trimester human fetuses[14]; Charles River, Sulzfeld, Germany) of either sex 3 days before HI. At postnatal day 11, unilateral HI (hypoxia duration, 40 minutes) was induced using the Rice–Vannucci model with some modifications.[15–17] After hypoxic exposure, the pups were returned to their dam until killed. Multiple injections of NAC (25 or 200mg/kg; Sigma, Steinheim, Germany) or vehicle (saline) was given intraperitoneally at 1 hour after LPS, 2 hours before HI, and 0 and 24 hours after HI. For posttreatment, NAC was given intraperitoneally at 0 and 24 hours after HI (posttreatment I) or 2 and 24 hours after HI (posttreatment II), respectively (see Supplementary Figure for the timeline of the experiment and NAC treatment). For the melatonin protection study, multiple injections of melatonin (5 or 20mg/kg; Sigma, Schnelldorf, Germany) or vehicle (10% ethanol + saline) were administered subcutaneously at 1 hour after LPS, 2 hours before HI, and 0 and 24 hours after HI. Control pups were neither subjected to LPS injection nor artery ligation and hypoxia. Rats were housed in a 12-hour light/dark cycle. Free access to a standard laboratory chow diet (B&K, Solna, Sweden) and deionized drinking water was provided. Animals were killed at different time points to evaluate the brain injury and possible mechanisms (see Supplementary Materials and Methods). All rat experimentation was approved by the Ethical Committee of Göteborg (no. 226-2004).

Statistics

Student's unpaired t test was used for comparing tissue loss, cell counts, and quantification of myelin basic protein (MBP) immunoactivity. Analysis of variance followed by Fischer's protected least significant difference post hoc test was used when comparing the results from infarct volume/area, activity assays, and immunoblot quantification. Mann–Whitney U test was used to compare the gross morphology and neuropathology scores. Simple linear regression analysis was used for the comparison of infarct area and infarct volume measurement. p less than 0.05 was considered statistically significant. All data are expressed as mean ± standard error of the mean. Additional methodological information is provided in Supplementary Materials and Methods.

Results

N-*acetylcysteine Conferred Marked Neuroprotection after Lipopolysaccharide/Hypoxia-Ischemia*

Based on previous experimental studies on ischemia and infection, we chose two different doses of NAC.[18–21] After pre- and post-HI administration of NAC (200mg/kg), the infarct volume in the ipsilateral hemisphere was reduced by 78.3% compared with vehicle treatment ($p = 0.0001$; Figs 1A, E), the area of tissue loss was significantly reduced in 6 of 7 brain levels (see Fig 1B), and the neuropathological score was significantly less in all regions evaluated (see Figs 1C, D), whereas no significant changes were found between the 25mg/kg NAC and vehicle groups (see Fig 1A).

We also compared the protective effect of NAC with another free radical scavenging agent, melatonin, which is a promising neuroprotectant because of its lack of toxicity, ability to cross the blood–brain barrier, and efficacy to reduce white matter injury in immature mice.[20] Melatonin (5mg/kg) administration resulted in a moderate reduction (38.1%) of gross morphological brain injury (brain injury score: vehicle vs melatonin, 2.1 ± 1.0 [n = 29] vs 1.3 ± 1.3 [n = 31]; $p = 0.038$), but a higher dose of melatonin (20mg/kg) was not protective (1.5 ± 1.3, n = 31; $p = 0.11$). The total pathological score of the brain also showed reduced brain damage in the melatonin (5mg/kg)-treated rats (6.5 ± 1.5) compared with vehicle-treated rats (11.9 ± 1.3; $p = 0.0007$), a reduction of 45.3% compared with 72.0% in NAC (200mg/kg)-treated rats.

To investigate the therapeutic window of NAC, we next examined the effect of post-HI NAC (200mg/kg) treatment. When NAC was administered immediately after (0 hour) and 24 hours after HI, infarct area in the ipsilateral hemisphere was reduced by 41.2% ($p = 0.028$; Fig 2A) and the total tissue area loss by 28.2% ($p = 0.039$) compared with vehicle treatment. The neuropathological total score ($p = 0.01$; see Fig 2B) and the neuropathological score in different brain regions was significantly lower in NAC-treated compared with vehicle-treated rats for all regions evaluated, except striatum (see Fig 2C), but to a lesser degree compared with 200mg/kg NAC before and after treatment.

When NAC was administered at a more delayed time point starting at 2 hours after LPS/HI, no significant brain protection was observed (see Figs 2D–F).

N-*Acetylcysteine Treatment Reduced White Matter Injury and Microglia Activation*

It has been shown previously that acute white matter injury can be quantified in a sensitive manner by measurements of MBP immunostaining.[22–24] In the ipsilateral hemisphere, a loss of MBP in subcortical white matter was seen compared with the contralateral hemisphere 7 days after HI in all rats examined (Fig 3A).

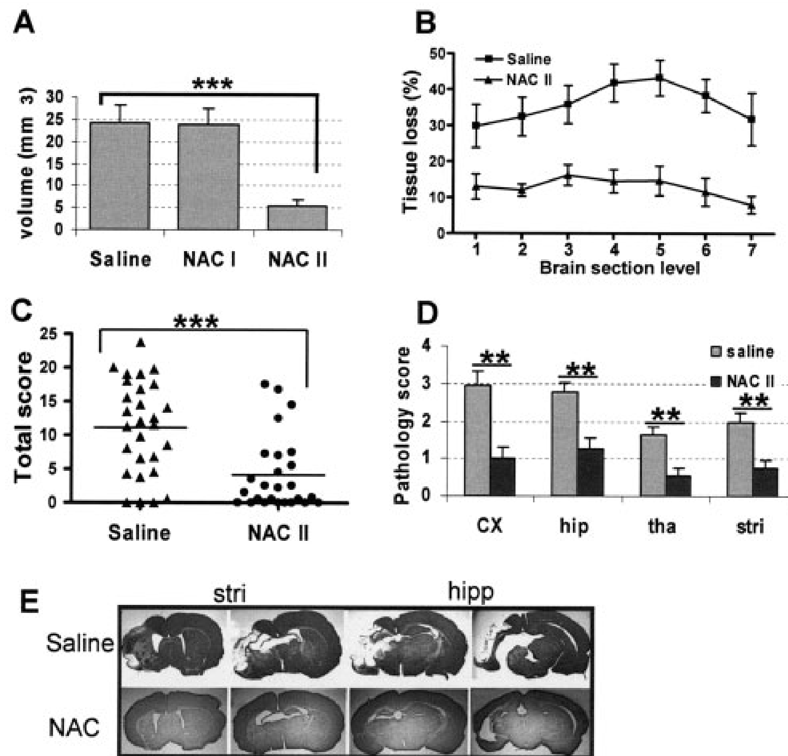

Fig 1. Pretreatment plus posttreatment of N*-acetylcysteine (NAC) conferred marked neuroprotection at a 200mg/kg dose. (A) Brain infarct volume with different dosages of NAC. Brain tissue loss (%) at multiple levels (level 1 is the most anterior part) (B); neuropathological score in total (C) and in multiple brain areas (D) in saline- and NAC-treated (200mg/kg only) rats. (E) Representative pictures of saline- (top) and NAC-treated (200mg/kg; bottom) brain with microtubule-associated protein-2 (MAP2) staining in multiple brain levels.* Horizontal lines *in C represent the median values.* Triangles *and* circles *in C represent scores for individual animals. Veh (saline): n = 29; NAC 25mg/kg (NAC I): n = 31; NAC 200mg/kg (NAC II): n = 25.* *p < 0.05; **p < 0.01; ***p < 0.001. *Cx = cortex; hip = hippocampus; stri = striatum; tha = thalamus.*

However, the level of MBP remaining in the subcortical white matter of the ipsilateral hemisphere was found to be significantly higher in NAC-treated rats than in vehicle-treated rats ($p = 0.005$) (see Figs 3A, C). NAC-treated rats showed a lesser degree of MBP loss in the ipsilateral hemisphere compared with vehicle-treated rats ($p = 0.02$; see Fig 3C).

The activation of microglia was examined by using isolectin immunostaining. Little lectin immunoreactivity could be observed in the normal control brains and contralateral hemispheres, whereas intense lectin staining was found in the ipsilateral hemisphere (cortex, striatum, hippocampus, and thalamus) at 7 days after HI (data not shown). NAC treatment reduced the number of isolectin-positive cells in both the cortex ($p = 0.003$) and thalamus ($p = 0.04$) (see Fig 3B).

Effect of N*-Acetylcysteine on Multiple Redox Signaling*

N-ACETYLCYSTEINE REDUCED PEROXYNITRITE FORMATION AND LIPID PEROXIDATION AFTER LIPOPOLYSACCHARIDE/HYPOXIA-ISCHEMIA. To study the effect of NAC on the ROS/reactive nitrogen species status after LPS/HI-induced brain injury, we examined brain homogenate from 2 and 24 hours after either LPS or LPS/HI with respect to nitrotyrosine and isoprostane production, that is, markers of peroxynitrite formation and lipid peroxidation, respectively. Immunoblots showed that nitrotyrosine immunoreactivity was detected mainly as a band with a molecular weight of 50kDa. NAC treatment significantly decreased the nitrotyrosine formation in the ipsilateral hemisphere at 24 hours after LPS/HI compared with vehicle-treated rats (Figs 4A, B). For lipid peroxidation detection, NAC treatment provided a significant reduction of isoprostane activation in NAC-treated compared with vehicle-treated rats at 2 hours after LPS/HI in the ipsilateral hemisphere (22.4% reduction; $p = 0.04$; Fig 5A).

EFFECT OF *N*-ACETYLCYSTEINE ON ENDOGENOUS ANTIOXIDANT MOLECULES AND ENZYMES AFTER LIPOPOLYSACCHARIDE/HYPOXIA-ISCHEMIA. Next, we examined the effect of NAC on endogenous scavenger molecules. Immunoblotting showed that NAC significantly increased the level of thioredoxin-2 (Trx2), a 12kDa protein with antioxidant effect and redox regulating functions, at 2 hours after LPS/HI in the ipsilateral

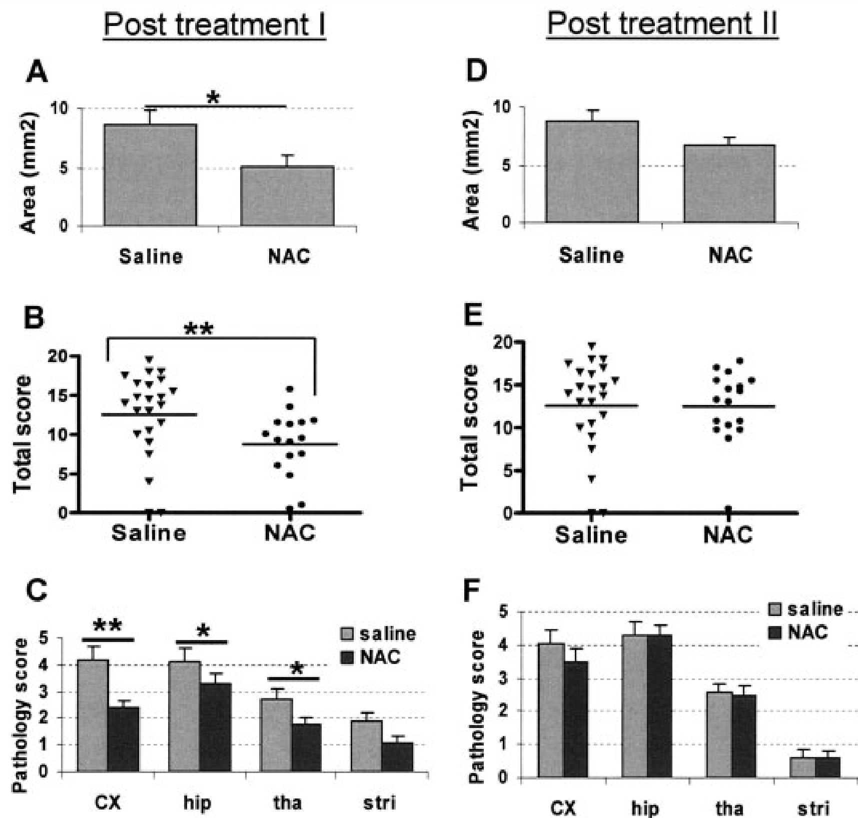

Fig 2. Effect of N*-acetylcysteine (NAC) after treatment on neonatal brain injury at 200mg/kg dosage. Tissue area loss (A, D) and neuropathological score in total (B, E) and in multiple brain areas (C, F). (A–C) NAC was given at 0 and 24 hours after hypoxia-ischemia (HI; posttreatment I). Vehicle (veh): n = 23; NAC: n = 16. (D–F) NAC was given at 2 and 24 hours after HI (posttreatment II). Veh: n = 23; NAC: n = 17.* Horizontal lines *in B and E represent the median values.* Triangles *and* circles *in B and E represent scores for individual animals.* *p $<$ *0.05;* **p $<$ *0.01. Cx = cortex; hip = hippocampus; stri = striatum; tha = thalamus.*

hemisphere ($p = 0.008$; see Figs 4A, C), whereas NAC had no significant effect on the two endogenous antioxidant enzymes superoxide dismutase-1 (SOD1) and catalase, although catalase activation was significantly increased after LPS administration ($p = 0.001$; see Fig 4A). The total glutathione (GSH) concentrations decreased by 2 and 24 hours after LPS/HI ($p = 0.0002$ and $p < 0.0001$, respectively), and NAC administration restored the level of GSH at 2 and 24 hours after LPS/HI (see Fig 5B), which is consistent with a NAC-mediated restoration of total cysteine (see Fig 5C).

Effect of N*-Acetylcysteine on Apoptosis/Caspases after Lipopolysaccharide/Hypoxia-Ischemia*

The cytoskeletal membrane-associated protein α-fodrin is a well-established substrate of both calpains and caspase-3. Calpain cleaves α-fodrin into 145 and 150kDa fragments, whereas caspase-3 cleaves α-fodrin into a 120kDa fragment. The antibody against α-fodrin can detect both intact α-fodrin and the breakdown products of calpain and caspase-3 proteolysis. By studying the distribution of breakdown products, it is possible to assess the contribution of both proteases in one sample (Fig 6A). The quantification of the α-fodrin immunoblotting bands showed that the activity of calpain and caspase-3 in the ipsilateral hemisphere was significantly increased after LPS/HI. Treatment with NAC significantly decreased the calpain- (see Fig 6B) and caspase-3 (see Fig 6C)–mediated accumulation of α-fodrin substrates. The effect was most pronounced at 24 hours after LPS/HI and indirectly suggests that the activation of these enzymes were attenuated by NAC.

The activation of caspase-3 was further confirmed by measuring the Ac-Asp-Glu-Val-Asp-aminomethyl coumarine (DEVD) cleavage of caspase-3 activity (see Fig 6D). After LPS/HI, the activity in the ipsilateral hemisphere was significantly increased already after 2 hours and reached a peak at 24 hours after HI. NAC treatment significantly decreased caspase-3 activation at 24 hours after HI (53.1% reduction; $p < 0.0001$; see Fig 6D).

Interestingly, LPS/HI also induced a significantly increased activation of the inflammatory caspase-1 at 2 and 24 hours after LPS and 2 and 24 hours after

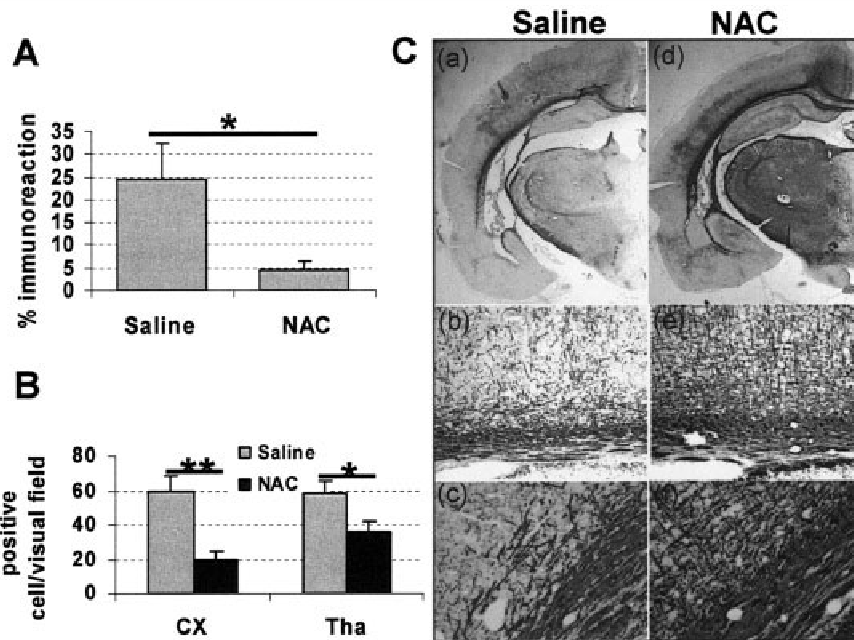

Fig 3. Effect of N*-acetylcysteine (NAC) treatment on myelination and microglia activation after lipopolysaccharide/hypoxia-ischemia (LPS/HI)–induced brain injury. Quantification of myelin basic protein (MBP) staining shows the white matter injury in saline and NAC (200mg/kg) pretreated + posttreated brains at 7 days after HI. Loss of MBP-immunoreactive staining in the subcortical area of the ipsilateral hemisphere (expressed as percentage loss vs contralateral hemisphere) (A), and representative pictures (C) of MBP immunohistochemistry staining at hippocampus level in saline- (a, b, c) and NAC-treated (d, e, f) rats. Lectin-positive (B) cells were counted in the cortex (CX) and thalamus (Tha) in ipsilateral hemisphere in the saline- and NAC-treated groups at 7 days after HI.* *p < 0.05; **p < 0.01.

LPS/HI in the ipsilateral hemisphere. NAC treatment reduced caspase-1 activation by 25.2% ($p = 0.0009$) at 24 hours after LPS and by 18.3% ($p = 0.02$) at 24 hours after LPS/HI in the ipsilateral hemisphere (see Fig 6E).

Discussion

We presently found that the scavenging agent NAC provided marked neuroprotection in a clinically relevant model of combined LPS/HI in neonatal rats. The protective effect of NAC was much more pronounced than another free radical scavenger melatonin when administrated before and after LPS/HI. NAC was also efficient when administered directly after HI (3 days after LPS). Besides the reduction of total tissue loss, NAC reduced white matter injury. The mechanism of NAC neuroprotection appears to be related to the reduced oxidative stress, as indicated by lower levels of isoprostane, nitrotyrosine, and preservation of the scavengers GSH and Trx2, attenuated activation of apoptotic proteases (caspase-3, calpain), and reduced inflammation as indicated by attenuated activation of microglia and caspase-1 (see summary in Fig 7).

NAC, an acetylated sulfur-containing amino acid, is able to cross the placenta[10] and blood–brain barrier,[25] and its profile as a GSH precursor, antioxidant, antiapoptotic,[26] and antiinflammatory[27] agent makes it an interesting substance acting at multiple neuroprotective sites. NAC has recently been demonstrated to attenuate amniotic and placental cytokine responses after maternal infection induced by LPS[12], and to restore the maternal and fetal oxidative balance and reduce fetal death[28] and preterm birth.[29] Based on previous experimental studies on ischemia, meningitis, and LPS exposure where doses of 20 to 380mg/kg have been effective but to a varying degree in the respective studies,[18–21] we chose a low (25mg/kg) and a high (200mg/kg) dose of NAC in this study and showed that NAC at a dose of 200mg/kg given as multiple injections provides significant neuroprotection. This finding is in accordance with previous studies showing that NAC reduced infarction volume and brain injury in the middle cerebral artery occlusion model in adult rats,[21] forebrain ischemia in gerbils,[19] and in meningitis.[18] Furthermore, we demonstrate that NAC administered 0 and 24 hours after HI (3 days after LPS) also reduced brain damage. In contrast, when the initial treatment was delayed until 2 hours after HI, there was no protection, which could mean that either higher or additional doses of NAC are necessary (NAC half-life is about 6 hours), or that the therapeutic window is limited by the transient nature of ROS increase after HI.

Accumulating evidence suggests that free radicals contribute to various diseases that affect oligodendro-

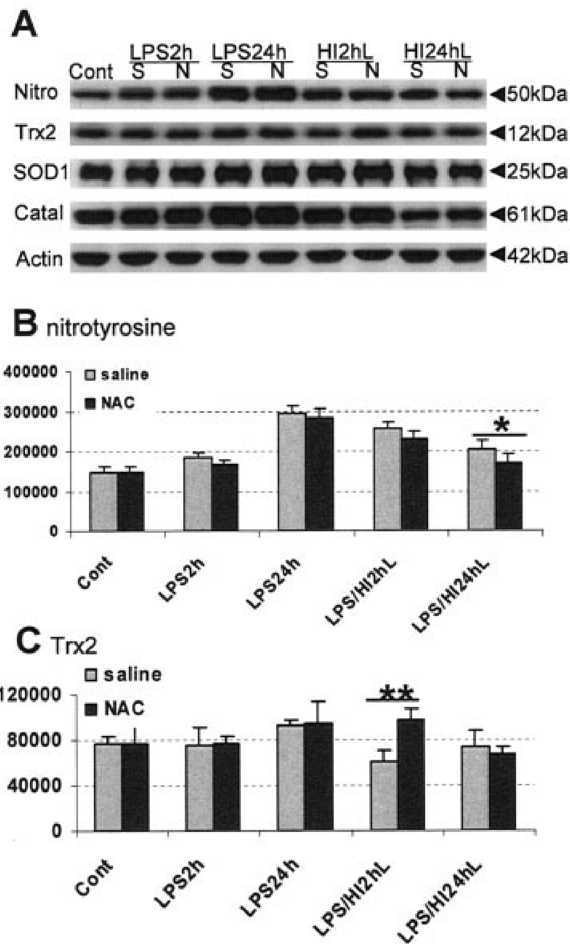

Fig 4. Nitrotyrosine, thioredoxin-2 (Trx2), superoxide dismutase-1 (SOD1), and catalase immunoblots. Nitrotyrosine, Trx2, SOD1, and catalase immunoreactivity in sham-, vehicle- (S), and N*-acetylcysteine (NAC)–treated (N) animals 2 and 24 hours after lipopolysaccharide (LPS) or LPS/hypoxia-ischemia (HI) (A). (B, C) Quantification of nitrotyrosine and Trx2, respectively.* $*p < 0.05$; $**p < 0.01$.

cytes, including multiple sclerosis[30] and cerebral palsy caused by periventricular leukomalacia.[31] Oligodendrocytes are sensitive to oxidative stress in vitro, and especially early differentiating oligodendrocytes appear more vulnerable to ROS injury compared with mature oligodendrocytes.[32–34] In this study, we observed that NAC reduced white matter injury with preservation of MBP expression in the subcortical region. This corresponds with reports from other studies that NAC protects white matter in other models of white matter injury.[20,32,35,36]

We found presently that NAC attenuated induction of microglia, as well as activation of inflammatory caspase-1, both after LPS and after LPS/HI. This agrees with our previous findings that caspase-1 messenger RNA and protein are upregulated after neonatal HI[37] and is interesting because neonatal caspase-1–deficient mice are protected from HI brain injury.[38] The NAC-attenuating effect on the proinflammatory response may or may not be related to neutralization of ROS, although it is supported by the fact that ROS is an early triggering event of inflammation.[39]

Peroxynitrite is one of the most reactive and damaging molecules because it both generates hydroxyl radicals and impairs protein functions through nitrosylation.[40] Detection of protein nitrosylation has been proposed to be a relatively specific means for detection of peroxynitrite[40] and occurs after ischemia in the neonatal brain.[41,42] This study showed that NAC inhibited the nitrotyrosine formation in the immature brain after LPS/HI, results that are consistent with data in the adult gerbil brain after ischemia.[19] Most likely, NAC scavenges superoxide radicals (possibly partly through enhanced synthesis of GSH), leading to a lesser production of peroxynitrate. Such an interpretation is consistent with our finding that NAC reduced lipid peroxidation, as indicated by reduced 8-isoprostane levels. Measurement of F2-isoprostanes, such as 8-isoprostane, have been proved to be valuable in assessing oxidative stress in vivo, because they are specific products of lipid peroxidation and LPS induces isoprostane generation both in vitro[43] and in vivo.[44,45] Furthermore, 8-isoprostanes increase in cerebrospinal fluid and plasma of preterm infants with white matter damage.[46,47]

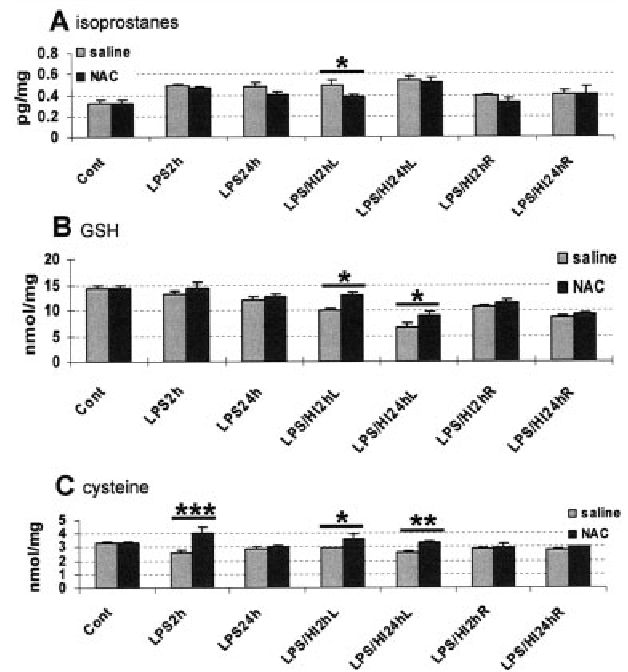

Fig 5. Effect of N*-acetylcysteine (NAC) treatment on isoprostanes (A), glutathione (GSH) (B), and cysteine (C) 2 and 24 hours after lipopolysaccharide (LPS) or LPS/hypoxia-ischemia (HI). Values are normalized to protein concentration.* $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

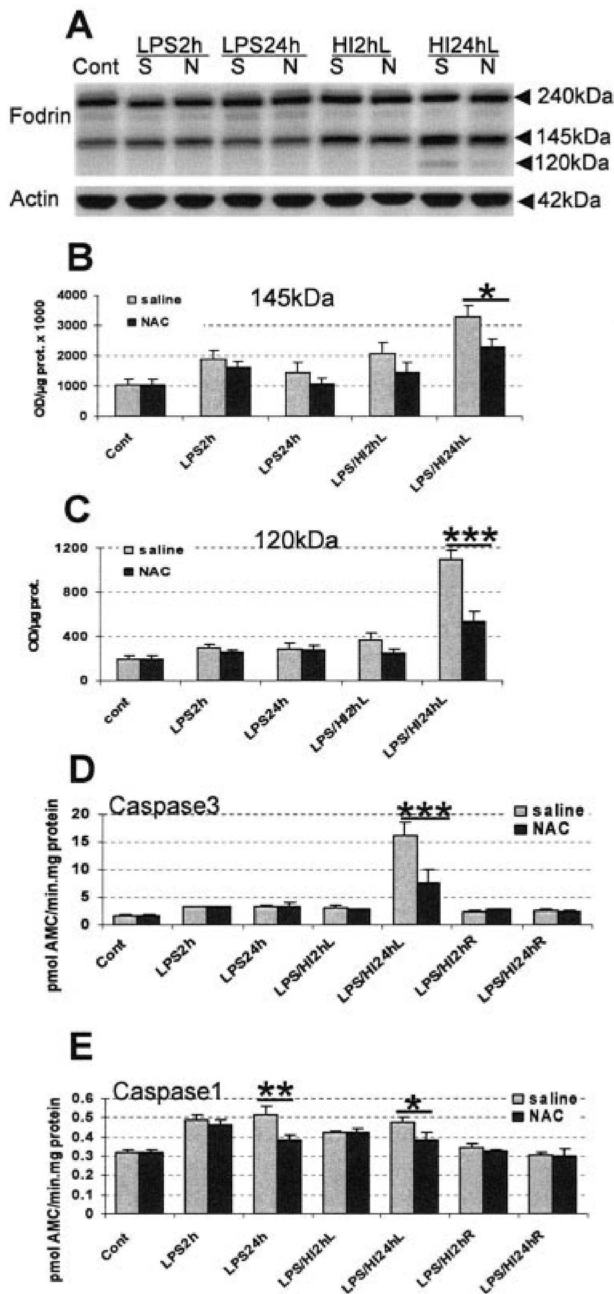

Fig 6. Effect of N*-acetylcysteine (NAC) treatment on calpain, caspase-3, and caspase-1 activity after lipopolysaccharide (LPS) administration or LPS/hypoxia-ischemia (HI). Fodrin immunoblots (A) show effect of NAC treatment on calpain (fodrin 145kDa cleavage product) and caspase-3 (fodrin 120kDa cleavage product) activation at 2 and 24 hours after LPS administration or LPS/HI. (A) Representative picture of the immunoblotting. (B, C) Quantification of 145 and 120kDa binds. (D, E) Caspase-3 and caspase-1 activity after LPS administration or LPS/HI.* *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

GSH represents one of the most important intracellular defenses against damage by ROS. Depletion of total GSH is a marker for oxidative stress in ischemic brain,[48] and ischemic outcome is worsened by pharmacological depletion of GSH.[49] NAC increases cysteine levels and thereby maintains the intracellular stores of GSH,[50] especially when the demand for GSH is increased.[51] Here, we report the NAC restored cysteine and GSH in brain homogenates at some time points after LPS/HI brain injury. This indicates that NAC is deacetylated to cysteine, which increases the concentrations of GSH. However, according to some data, the GSH pathway is less important for scavenging ROS in the immature brain because the activity of GSH peroxidase is low,[9,52] a deficit that may not be easily overcome by providing additional amounts of a GSH precursor.

In addition to GSH, the thioredoxin system functions to maintain the cellular environment in a reduced state and protects against ROS. Trx2 is abundant in mitochondria and widely distributed in rat brain.[53] Trx2 appears to be involved in neuronal responses to hypobaric hypoxia,[54] HI injury in neonatal rats,[55] and the development of endotoxin tolerance in adult mice.[56] In this study, we found that NAC treatment significantly reduced the decrease in the level of Trx2, which may indicate that it plays a role in NAC-mediated protection in this model.

It has been shown previously that LPS increased SOD1 activity in the brain at 72 hours (but not at 12 hours), whereas brain catalase activity remained unchanged after LPS administration.[57] In this study, LPS increased catalase, but not SOD1. Treatment with NAC, however, did not influence SOD1 or catalase

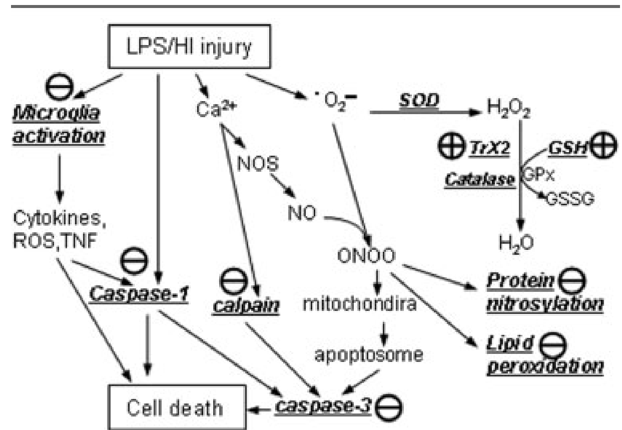

Fig 7. Signaling pathways trigged by lipopolysaccharide/hypoxia-ischemia (LPS/HI) and the effects of N*-acetylcysteine (NAC) treatment.* Bold, italic, *and* underlined *letters indicate molecules/cells that have been examined in this study.* Minus sign *indicates inhibition by NAC, and* plus sign *indicates activation by NAC. GPx = glutathione peroxidase; GSH = glutathione; GSSG = glutathione disulfide. NO = nitric oxide; NOS = nitric oxide synthase; ROS = reactive oxygen species; SOD = superoxide dismutase; TNF = tumor necrosis factor; Trx2 = thioredoxin.*

levels after either LPS or LPS/HI, suggesting that NAC-mediated protection may not depend on the levels of these enzymes.

A number of studies suggest that activation of caspase-3 plays an important role in immature brain injury.[58,59] This activation appears, at least in part, to be due to activation of calpains.[59] NAC reduced the activation of both calpain (as indicated by a decreased accumulation of the 150kDa fragment of α-fodrin) and caspase-3 (reduced 120kDa α-fodrin and enzyme activity) after LPS/HI, which agree with findings showing that NAC inhibits caspase-3 and apoptosis in oligodendroglial cells in vitro.[60] ROS exert mitochondrial stress that may trigger mitochondrial outer membrane permeabilization, release of proapoptotic proteins, and cell death.[61] We propose that one important role of NAC is to attenuate this sequence of events through reduction of mitochondrial accumulation of ROS.

Taken together, NAC protected the brain from LPS-sensitized HI. Multiple injections of NAC, started after LPS but before HI, provided remarkable neuroprotection (78% reduction of brain injury) compared with 41% reduction when the first NAC injection was given immediately after HI. The protection in both gray and white matter was probably through the following mechanisms: (1) enhancement of antioxidative responses and attenuation of ROS production, (2) inhibition of calpain-caspase activation, and (3) attenuation of inflammation. NAC, in clinical use for more than 40 years as a mucolytic agent, has been given to pregnant women in high doses as an antidote for paracetamol intoxication.[10,62] To our knowledge, there are no clinically approved therapeutic agents that target free radical production in the fetus/mother or neonates. These results suggest that NAC is a candidate agent that may be of therapeutic value in situations of antenatal/postnatal infection in combination with HI events, provided that the situation allows treatment early in the process.

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