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Poly(ADP-Ribose) Synthase Inhibition Reduces Ischemic Injury and Inflammation in Neonatal Rat Brain

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Abstract: Poly(ADP-ribose) synthase (PARS), an abundant nuclear protein, has been described as an important candidate for mediation of neurotoxicity by nitric oxide. However, in cerebral ischemia, excessive PARS activation may lead to energy depletion and exacerbation of neuronal damage. We examined the effect of inhibiting PARS on the (a) degree of cerebral injury, (b) process of inflammatory responses, and (c) functional outcomes in a neonatal rat model of focal ischemia. We demonstrate that administration of 3-aminobenzamide, a PARS inhibitor, leads to a significant reduction of infarct volume: 63 ± 2 (untreated) versus 28 ± 4 mm^3 (treated). The neuroprotective effects currently observed 48 h postischemia hold up at 7 and 17 days of survival time and attenuate neurological dysfunction. Inhibition of PARS activity, demonstrated by a reduction in poly(ADP-ribose) polymer formation, also reduces neutrophil recruitment and levels of nitrotyrosine, an indicator of peroxynitrite generation. Taken together, our results demonstrate that PARS inhibition reduces ischemic damage and local inflammation associated with reperfusion and may be of interest for the treatment of neonatal stroke. **Key Words:** Cell death—Neonatal stroke—Neutrophils—Nitrotyrosine.
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The role of NMDA-mediated injury in cerebral ischemia is well documented (Rothman and Olney, 1985; Choi, 1992). After ischemia, energy deficits lead to efflux of various neurotransmitters and neuromodulators, including the excitatory amino acids such as aspartate and glutamate. Abnormal activation of the NMDA-type glutamate receptors can lead to intracellular calcium accumulation followed by a wide spectrum of calcium-related pathologies (Choi, 1995). Among the many proposed downstream pathways of action, the effects of nitric oxide (NO) have received much attention (Dawson, 1994; Iadecola et al., 1994). NO synthase activity is elevated after NMDA receptor stimulation, and the associated free radical cascades involving $NO^{\bullet}$ and peroxynitrite ($ONOO^{\bullet}$) can lead to membrane phospholipid and DNA damage (Beckman, 1994; Dawson, 1994; Chan, 1996).

It has been shown that DNA damage after ischemic/NMDA/NO insults may activate the nuclear enzyme poly(ADP-ribose) synthase (PARS; EC 2.4.2.30) (Zhang et al., 1994, 1995). Also called PARP [for poly(ADP-ribose) polymerase], PARS is a tightly bound chromosomal enzyme located in the nuclei of cells of various organs, including brain (for review, see Pieper et al., 1999). PARS plays a physiologic role in the repair of strand breaks in DNA (Satoh and Lindahl, 1992). Once activated by damaged DNA fragments, PARS catalyzes the attachment of ADP-ribose units to nuclear proteins, including histones and PARS itself. The PARS activation enhances DNA repair by relaxing the chromosomal structure through poly(ADP-ribosyl)ation of histones and other nuclear proteins. The extensive activation of PARS, however, can rapidly lead to cell death through depletion of energy stores because four molecules of ATP are consumed in NAD (the source of ADP-ribose) regeneration (Berger, 1985). Previous data show that a series of PARS inhibitors protects adult rats from cerebral ischemia (Takahashi et al., 1997; Lo et al., 1998) and have the ability to reduce inflammatory responses (Szabo and Dawson, 1998). Furthermore, mice deficient in PARS were resistant to brain injury after transient focal cerebral ischemia (Eliasson et al., 1997; Endres et al., 1997).

The present study was designed to analyze the effects of treatment with 3-aminobenzamide (3-AB), a widely used, nonselective PARS inhibitor, in a model of transient unilateral focal ischemia with reperfusion in the postnatal day 7 neonatal rat. We recently demonstrated, in this model, that NO and peroxynitrite anion [with anti-nitrotyrosine (anti-NT) antibodies as the footprint of

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Abbreviations used: 3-AB, 3-aminobenzamide; CCA, common carotid artery; CCAo, common carotid artery occlusion; MCA, middle cerebral artery; MCAo, middle cerebral artery occlusion; MPO, myeloperoxidase; NO, nitric oxide; NT, nitrotyrosine; PARP, poly(ADP-ribose) polymerase; PARS, poly(ADP-ribose) synthase; PBS, phosphate-buffered saline; PMNL, polymorphonuclear leukocyte.

peroxynitrite] may provide a cytotoxic signal for the induction of cell death (Coeroli et al., 1998).

EXPERIMENTAL PROCEDURES

Neonatal ischemia

Experiments involving animals were approved by the French Ethical Science Committee (statement no. 04223). Ischemia was induced in 7-day-old Wistar rats (weighing 17–21 g) of both sexes, as previously described (Renolleau et al., 1998). Rat pups were anesthetized with an intraperitoneal injection of chloral hydrate (300 mg/kg). After 15 min, each rat was laid on its back, and a median incision was made in the neck to expose the left common carotid artery (CCA). The rat was then placed on the right side, and an oblique skin incision was made between the ear and the eye. After excision of the temporal muscle, the cranial bone was removed from the frontal suture to a level below the zygomatic arch. Then, the left middle cerebral artery (MCA), exposed just after its appearance over the rhinal fissure, was coagulated at the inferior cerebral vein level. After this procedure, a clip was placed to occlude the left CCA and was removed after 1 h. Carotid blood flow restoration was verified with the aid of a microscope. Both neck and cranial skin incisions were then closed. The surgical procedure lasted ~20 min. During surgery, body temperature was maintained at 37–38°C. After awake, rat pups were transferred to their mother for 2, 7, and 17 days of survival.

Therapeutic protocol

To examine the effect of 3-AB (Sigma), a PARS inhibitor, on brain ischemia, 3-AB (5, 10, or 20 mg/kg) dissolved in 0.9% NaCl was administered intraperitoneally at 0, 60, 90, and 120 min after the clip was placed on the left CCA. Eighty-six animals were used in these experiments. Forty-two animals were injected similarly with an equivalent volume of the vehicle in the same schedule.

Tissue preparation

At 48 h after reperfusion, animals were perfused via the ascending aorta under deep anesthesia (chloral hydrate, 300 mg/kg) with warm heparinized saline followed by phosphate buffer (0.12 *M*, pH 7.4) containing 4% paraformaldehyde. Brains were then removed, kept for 2 h in the same fixative solution, and placed in 0.1 *M* phosphate-buffered saline (PBS) containing 10% sucrose for 2 days. Brains were rapidly frozen in isopentane (−40°C) and subsequently stored at −70°C until used.

Measurement of infarct volume

Brains were sectioned with a cryostat in the coronal plane at 1-mm intervals. Sections were collected on gelatin-coated slides and stained with cresyl violet. On each section (interaural 4.7–11.7), cortical area of infarction was measured using an image analyzer (IMSTAR, Paris, France), as previously described (Renolleau et al., 1998). Volumes of infarction were calculated by integrating the necrotic areas corrected for edema according to the method of Golanov and Reis (1995).

Quantification of NT immunoreactivity

Rat pups were killed by decapitation at 48 h of recovery (n = 4). Sample tissues from the ischemic cortex (frontoparietal) and the contralateral intact cortex were freshly excised, homogenized at 4°C in 50 m*M* Tris-HCl (pH 7.4), 1 m*M* EDTA, and 1 m*M* phenylmethylsulfonyl fluoride, and then centrifuged at 15,000 *g* for 15 min to remove insoluble material. The supernatant was retained. Protein concentration was determined by the method of Lowry et al. (1951). Samples in triplicate (10 μg of protein each) were analyzed by dot blots on nitrocellulose membranes as previously described (Coeroli et al., 1998). Membranes were processed with affinity-purified rabbit polyclonal anti-NT antibodies (Beckman et al., 1990; Ye et al., 1996) from Upstate Biotechnology (Euromedex, Souffelweyersheim, France). Membranes were incubated in the first antibody (dilution 1:100) overnight at 4°C, washed, and then incubated in horseradish peroxidase-labeled secondary antibody for 45 min, and specific proteins were detected on x-ray films using enhanced chemiluminescence (ECL; Amersham, France). Quantification was performed with densitometric measurement software for image analysis (Samba 2005/Alcatel TITN Answare, Grenoble, France).

Immunocytochemistry

Antisera. Rabbit antibodies against myeloperoxidase (MPO; Sigma) were used to visualize polymorphonuclear leukocytes (PMNLs). Mouse monoclonal antibodies against rat T lymphocytes (Pan T; Serotec, France) were used to identify infiltrating T lymphocytes. NT was detected with rabbit polyclonal antibodies (see above). Rabbit antibodies against poly(ADP-ribose) polymer (minimum subunit length of 8) were purchased from Biomol Research Laboratory (Tebu, France).

Immunostaining procedure. Sections were incubated with the primary antibody (1:200 dilution) in 0.1 *M* PBS containing 2% bovine serum albumin and 0.3% Triton X-100 overnight at 4°C or in 0.1 *M* PBS containing 0.5% gelatin and 0.3% Triton X-100 overnight at room temperature [for poly(ADP-ribose) polymer]. The secondary (anti-rabbit or anti-mouse IgG) biotinylated antibodies (1:200–1:400 dilution; Dako, France) were visualized by the avidin–biotin peroxidase method (Elite ABC kit; Vector) and diaminobenzidine. Nonspecific peroxidase activity was removed by incubating the sections in 2% hydrogen peroxide in 0.1 *M* PBS at the appropriate stage. As negative controls, alternative sections were incubated in the absence of primary antibody.

Quantification of neutrophils. This was performed using a ×40 objective and a camera lucida attached to the microscope. At 48 h postischemia, in ischemic (n = 5) and 3-AB-treated (n = 4) animals, intraparenchymal MPO-positive neutrophils were counted in a 0.3-mm^2 circular area on three sections containing the infarct.

Functional outcome assessment

Sensorimotor neurological deficits were assessed in a blinded fashion by the neurological scoring system in rats at 24 days postnatally. The following parameters were scored for the right paw in the three groups of animals (nonoperated, vehicle-treated, and 3-AB-treated rats, n = 6, 6, and 8, respectively): grasping reflex (Wahl et al., 1992), postural reflex, and limb-placing test (Bona et al., 1997). The sum of scores constitutes the global neurological score, which is 5 in normal nonischemic rats. To assess the neurological outcome, the area of the cavity that was more visible 2 weeks postischemia (Renolleau et al., 1998) was measured at posterior levels (interaural sections 4.7–6.7) in vehicle-treated and 3-AB-treated animals.

Statistical analysis

Statistical comparisons between two groups were made by two-tailed Student's *t* test (infarct size, NT quantification, and number of PMNLs). The significance of differences between groups (3-AB therapeutic window) was determined using an ANOVA followed by probing for the source of the difference

using a Student's *t* test. The neurological scores were analyzed with a nonparametric Mann–Whitney *U* test. All data are expressed as the group mean ± SEM values. A $p < 0.05$ value was considered statistically significant.

RESULTS

Infarction volume in 3-AB-treated animals

Permanent left MCA occlusion (MCAo) in association with a 1-h occlusion of the left CCA produced a reproducible, well-delineated cortical lesion at 48 h of reperfusion, as previously reported (Renolleau et al., 1998). The infarct volume in vehicle-injected ischemic pups was 63 ± 2 mm^3 (mean ± SEM, n = 10). 3-AB (10 mg/kg, n = 11) administration before transient left carotid occlusion led to a significant decrease in the damaged volume by ~56% (28 ± 4 mm^3, $p < 0.0001$) and area (interaural sections 4.7–11.7) as compared with untreated animals, 48 h after reperfusion (Fig. 1). In contrast, there was no significant difference between the control group and 5 mg/kg-treated group (62 ± 7 mm^3, n = 9; Fig. 1A). To ensure that the neuroprotective effects currently observed at 48 h postischemia hold up at 7 days of survival time, we measured contra- and ipsilateral hemisphere volumes in both control and 3-AB-treated animals. The contralateral hemisphere volume of ischemic pups was not significantly different from that found in sham-operated pups [346 ± 17 (n = 6) and 369 ± 6 mm^3 (n = 3), respectively]. In contrast, the ipsilateral hemisphere volume was significantly decreased in ischemic animals (270 ± 7 mm^3, $p < 0.005$, compared with the contralateral hemisphere), as shown in Fig. 2. 3-AB administration significantly reduced this decrease (307 ± 1 mm^3, $p < 0.05$, compared with ischemic animals), although the ipsilateral hemisphere volume still remained smaller than the contralateral hemisphere (307 ± 10 vs. 347 ± 15 mm^3, $p < 0.05$).

Therapeutic window of a single injection of 3-AB

The cortical infarcted volume in pups injected with 3-AB (10 mg/kg), at 60, 90, and 120 min after CCA occlusion (CCAo) onset, did not induce a significant reduction [52 ± 7 (n = 11) vs. 63 ± 3 mm^3 (n = 5)] at 48 h of reperfusion, as shown in Fig. 3. In contrast, a single injection of 3-AB (20 mg/kg) at 60 min after CCAo onset reduced the cortical infarction by 41% [38 ± 5 (n = 11) vs. 64 ± 4 mm^3 (n = 5), $p < 0.01$]. Treatment with 3-AB (20 mg/kg) at 90 and 120 min after CCAo onset had no neuroprotective effect [infarct volume, respectively, 48 ± 7 (n = 11) and 56 ± 7 mm^3 (n = 10)], as shown in Fig. 3.

Effect of 3-AB treatment on NT production

As previously reported (Coeroli et al., 1998), relative dense deposits of NT were observed in a perivascular location, namely, in cuffs of lymphocytes at 48 h of reperfusion (Fig. 4A). Treatment with 3-AB (10 mg/kg) before left CCAo reduced the number of NT-positive cells and cuffs of lymphocytes (Fig. 4B). We also found a significant decrease in the quantity of NT labeling at 48 h of reperfusion between the control and 3-AB-treated group [72 ± 10 (n = 4) and 39 ± 8 OD units (n = 4), respectively, $p < 0.05$] (Fig. 4, right panel).

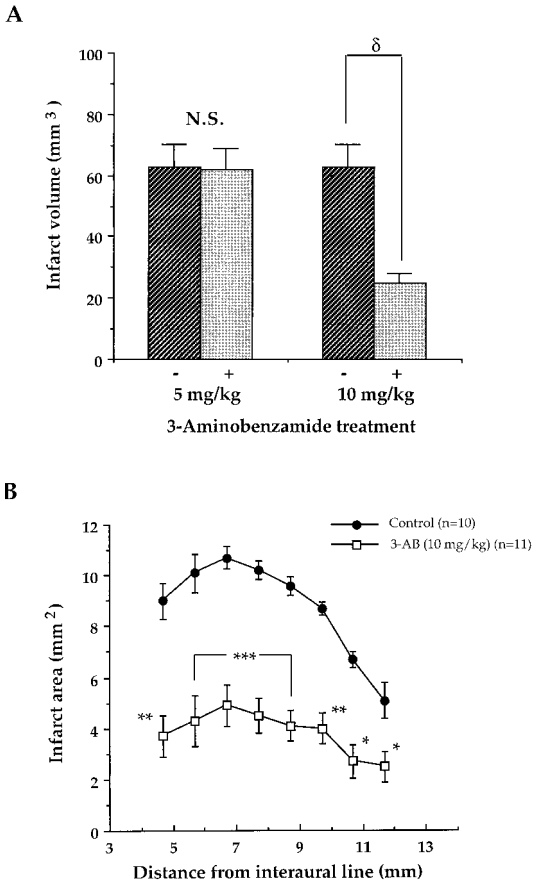

FIG. 1. Effect of 3-AB on infarcted volume measured 48 h after ischemia. Drug was given 30 s before transient left CCAo and consisted of a single intraperitoneal injection with 5 (n = 7) or 10 (n = 11) mg/kg 3-AB or vehicle (control, n = 10). Data are mean ± SEM (bars) values. **A:** Note a significant reduction of the infarct volume with 10 mg/kg treatment. N.S., not significant. $^{\delta}p < 0.001$. **B:** Rostrocaudal distribution of infarct areas plotted as a function of anterior distance from the interaural line in untreated (control) and 3-AB-treated rat pups. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ versus control animals.

Effect of 3-AB treatment on PMNL infiltration

As previously reported, PMNLs invaded the upper layers of infarcted cortex from 48 to 96 h of recovery (Benjelloun et al., 1999). As shown in Fig. 5, a significant reduction in the number of intraparenchymal neutrophils was observed between the control and 3-AB-treated groups at 48 h of reperfusion [35 ± 2 (n = 5) and 18 ± 2 neutrophils/0.3 mm^2 (n = 4) respectively, $p < 0.005$].

Effect of 3-AB treatment on poly(ADP-ribose) polymer formation

To analyze PARS activation after neonatal ischemia, poly(ADP-ribose) immunohistochemistry was performed. A significant number of poly(ADP-ribose)-positive neu-

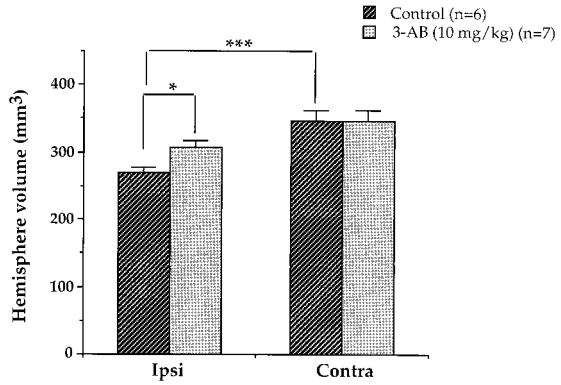

FIG. 2. Effect of 3-AB (10 mg/kg) on lesion evolution indicated by ipsilateral hemisphere volume measured 7 days postischemia. Note that ischemia induced a significant reduction in ipsilateral hemisphere volume compared with contralateral. This reduction in volume was reversed by 3-AB treatment. Data are mean ± SEM (bars) values. $^{*}p < 0.05$, $^{***}p < 0.001$ versus contralateral hemisphere volume.

rons could be identified by strong immunoreactivity in both the nuclei and cytoplasm in the ipsilateral cortex compared with the contralateral side at 48 h of reperfusion (Fig. 6A and B). These cells exhibited swelling and nuclear disruption (arrow in Fig. 6C) as early signs of cell damage. Furthermore, positive cells in the ischemic core frequently displayed a more conspicuous staining than those located at the periphery, in the penumbra (Fig. 6B). The number of poly(ADP-ribose)-positive cells after ischemia was reduced by 3-AB (Fig. 6D).

Effect of 3-AB on motor deficits and cavity area

In vehicle-treated rats (n = 6), ischemia caused at 24 days a significant neurological deficit on the right side ($p < 0.001$) compared with nonoperated naive rats (Fig. 7A) without affecting the left side (data not shown). This neurological deficit was associated with tissue damage and the formation of a cavity during the 3 weeks after ischemia (Fig. 7B). Treatment with 3-AB (10 mg/kg, n = 8) markedly reduced the neurological deficit (Fig. 7A) and the volume of the cavity (Fig. 7B), whereas the ipsilateral hemisphere volume was not significantly different (data not shown) in comparison with vehicle. Furthermore, of the eight brains studied, two animals did not exhibit a cavity.

DISCUSSION

In the current study, we demonstrate for the first time that administration of 3-AB, a PARS inhibitor, reduces the infarct size, ameliorates the neurological score, and attenuates inflammatory responses in rat pups subjected to focal cerebral ischemia. These effects correlate with a decrease in poly(ADP-ribose) polymers. These suggest that the activation of PARS may play an important role in the pathogenesis of brain damage in neonatal cerebral ischemia.

Involvement of PARS in neuronal damage was initially studied by Zhang et al. (1994): These authors observed that the inhibition of PARS rescued cortical neurons in culture from NO and NMDA-mediated toxicity. Cosi et al. (1994) showed by immunohistology that glutamate induced poly(ADP-ribosyl)ation in cerebellar granule cells. They also showed that inhibitors of PARS reduced glutamate neurotoxicity. More recently, data were reported on neuroprotective effects of inhibiting PARS on focal cerebral ischemia in adult rats (Takahashi et al., 1997; Lo et al., 1998) with a percentage of decreased infarct volume (~50%) similar to what we found in this study. In our neonatal stroke model, a single injection of 3-AB at a concentration of 10 mg/kg was efficient when given before transient CCAo but not after. However, 3-AB at a concentration of 20 mg/kg was able to salvage ischemic tissue (by 41%) even when administered with the onset of reperfusion and not after. A similar 3-AB protocol was previously reported to be protective in a model of ischemia–reperfusion of the heart or skeletal muscle in rabbits (Thiemermann et al., 1997). Injection of 3,4-dihydro-5-[4-(1-piperidinyl)butoxyl]-1(2*H*)-isoquinone (10 mg/kg), a more potent and selective PARS inhibitor, was shown to attenuate (by 54%) cerebral damage in a rat focal ischemia model when injected 2 h before MCAo and 2 h after onset of MCAo (30 min after reopening of the bilateral CCAo) (Takahashi et al., 1997). 3,4-Dihydro-5-[4-(1-piperidinyl)butoxyl]-1(2*H*)-isoquinone in a single injection (40 mg/kg), in the same ischemic model, also reduced infarct volume by 43% when injected 30 min after MCAo (Takahashi et al., 1999). Therefore, PARS inhibitors are neuroprotective, even when the treatment is initiated after the onset of ischemia, but demonstrated a short therapeutic window (Takahashi and Greenberg, 1999), as we found following neonatal cerebral ischemia. These results strengthened the proposal that the activation of

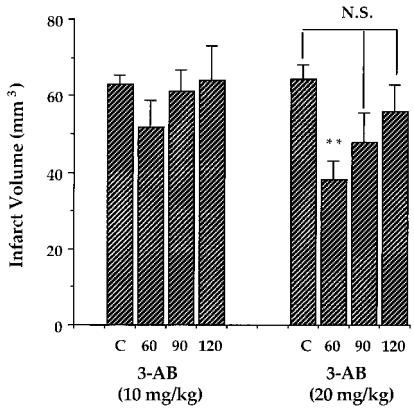

FIG. 3. Effect of a single injection of 3-AB (10 and 20 mg/kg) at various times after onset of CCAo on infarct volume measured at 48 h of reperfusion. Injection of 3-AB at 10 mg/kg did not induce neuroprotection when given 60, 90, and 120 min after onset of CCAo. In contrast, 3-AB (20 mg/kg) injected 60 min after onset of CCAo was still able to reduce the infarct volume. 3-AB (20 mg/kg) given 90 and 120 min after CCAo onset was devoid of any effect. N.S., not significant; $^{**}p < 0.01$.

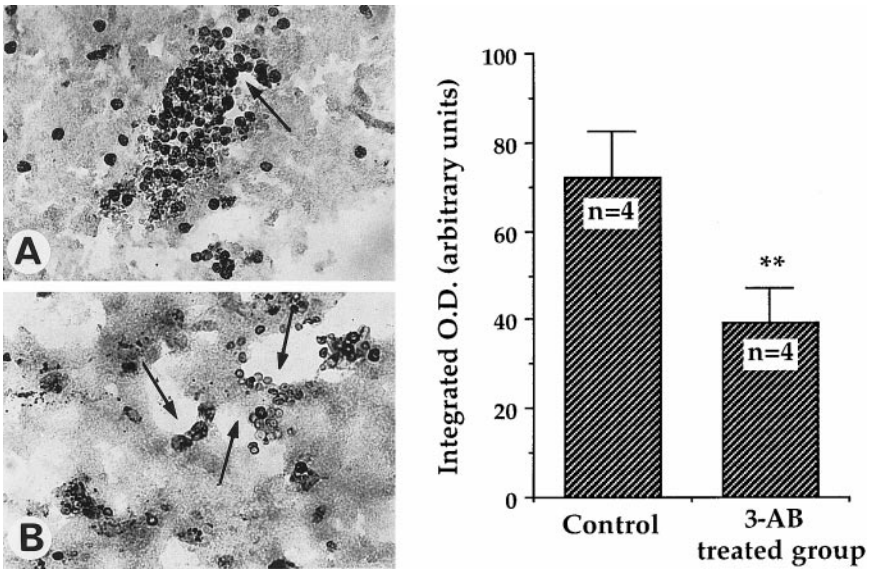

FIG. 4. Immunoreactivity and quantification of the presence of NT in untreated (control) and 3-AB-treated ischemic rat pups at 48 h of reperfusion. **Left panels:** The peroxynitrite-specific nitration product was visualized using a specific affinity-purified NT antibody as primary, biotinylated antibody as secondary, and peroxidase-conjugated antibody as tertiary (**A**). Note that NT staining, in cuffs of T lymphocytes around vessels (arrows), was decreased after 3-AB treatment (**B**). The window area was 0.3 mm^2. **Right panel:** Histogram of densitometric measurements [optical density (O.D.)] of NT proteins on dot blots in control and 3-AB-treated rat pups. Data are mean ± SEM (bars) values. $^{**}p < 0.01$.

PARS may play an important role in the pathogenesis of brain damage in the early part of reperfusion.

Only limited information has been published regarding the time course of PARS activation in ischemic brain injury. In the transient MCAo model, the increase in immunoreactivity of poly(ADP-ribose) polymer was observed in ischemic tissue 5–10 min after reperfusion (Endres et al., 1997; Takahashi et al., 1999) but not after longer periods of reperfusion (3 or 6 h) (Endres et al., 1997). Following permanent intraluminal MCAo, poly-(ADP-ribosyl)ation was widely and markedly detected 2 h after the ischemic insult in the cortex and striatum in which infarction developed 24 h after and was reduced by 3-AB treatment (Tokime et al., 1998). During stroke in wild-type mice, immunohistochemistry demonstrated poly(ADP-ribose) polymer in the ipsilateral cortex after 2 h of MCAo and 2 h of reperfusion. In contrast, there is a complete absence of any ADP-ribose polymer staining following ischemia in the PARP−/− animals (Eliasson et al., 1997). The current study showed that poly(ADP-ribose) polymers could be observed in the ischemic tissue at 48 h of reperfusion, although no temporal profile was investigated. Immunoreactivity, with the same antibody as the one used in a recent study on spinal cord injury (Scott et al., 1999), was mainly located in the nucleus of neurons, where DNA damage and repair should occur. A similar study reported prominent staining in nuclei with occasional faint staining in the cytoplasm, probably because of degeneration of nuclei (Tokime et al., 1998). We recently reported that DNA dou-

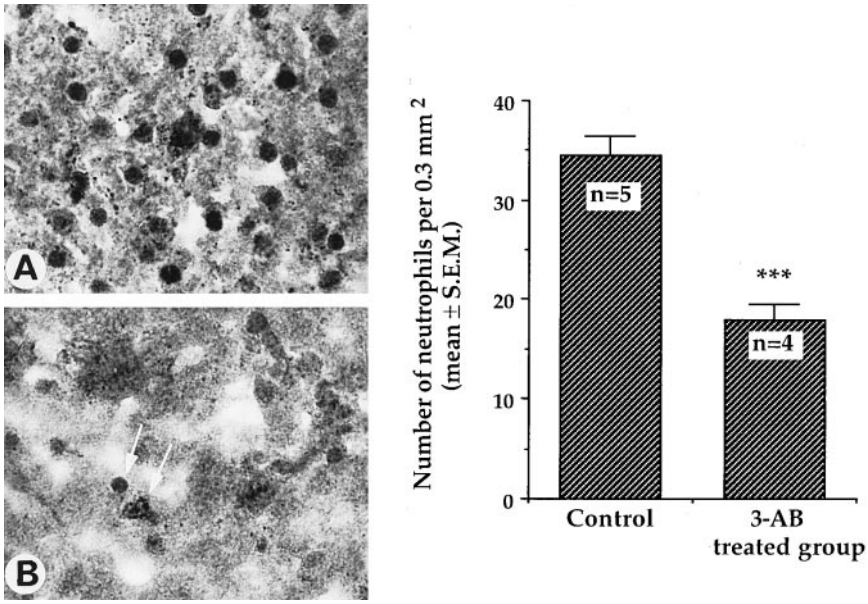

FIG. 5. Immunohistochemistry with anti-MPO antibodies used to stain neutrophils and quantification of the number of neutrophils in untreated (control) and 3-AB-treated ischemic rat pups at 48 h of reperfusion. **Left panels:** Presence of MPO-positive staining in neutrophils in control animals (**A**). Note a reduction of the staining in 3-AB-treated animals (**B**). The window area was 0.25 mm^2. **Right panel:** Histogram of the number of neutrophils per 0.3 mm^2 observed in the infarct. Note a significant reduction of this number in 3-AB-treated animals. Data are mean ± SEM (bars) values. $^{***}p < 0.001$.

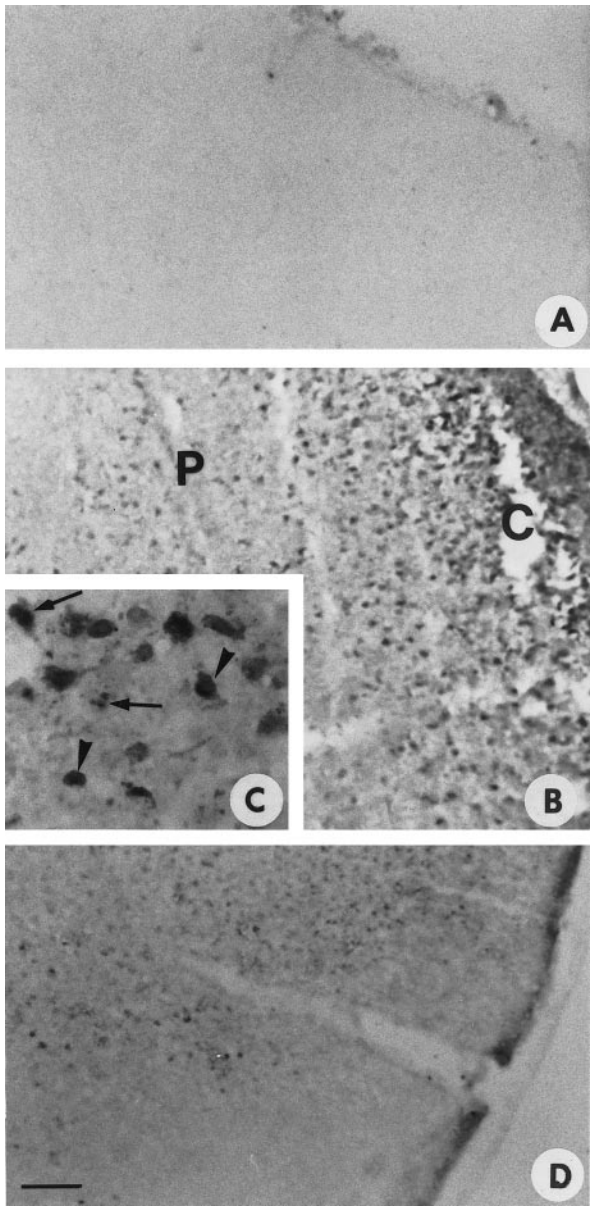

FIG. 6. Representative photomicrographs of poly(ADP-ribose) immunohistochemical study in untreated and 3-AB-treated ischemic rat pups at 48 h of reperfusion. **A:** No immunoreactive cells were observed in the contralateral cortex. **B:** Poly(ADP-ribosyl)-ation was found throughout the ischemic area. Note the fading staining in the penumbra (P) compared with that observed in the core (C). **C:** Immunoreactivity was mainly found in the nucleus (arrowheads at example). Note disrupted nuclei (arrows). **D:** Decreased poly(ADP-ribose) polymer formation in 3-AB-treated pups. Bar = 50 μm in A, B, and D and 15 μm in C.

ble- and single-strand breaks occurred in cortical neurons following neonatal ischemia and 24–48 h of reperfusion; furthermore, cellular response to oxidative stress and DNA damage involves the inducible expression of Gadd45, a DNA damage- and repair-related protein, with the staining mainly found in the cytoplasm, 48 h after reperfusion (Charriaut-Marlangue et al., 1999). Further study is needed to clarify whether activation of PARS and poly(ADP-ribosyl)ation will occur in the cytoplasm, as well as the locus of poly(ADP-ribosyl)ation in the cytoplasm.

The current study also demonstrates for the first time that PARS inhibition by 3-AB reduces PMNL recruitment and accumulation into the infarct area. Extravasated PMNLs following neonatal stroke were recently reported (Benjelloun et al., 1999) and become activated once in the inflammatory site, secreting various substances such as chemokines and cytokines, complement components, proteases, NO, reactive oxygen metabolites, and peroxynitrite, all important mediators of tissue injury. Inhibition of PARS by 3-AB was reported to reduce neutrophil recruitment and the extent of edema in zymosan- and carrageenan-triggered models of local in-

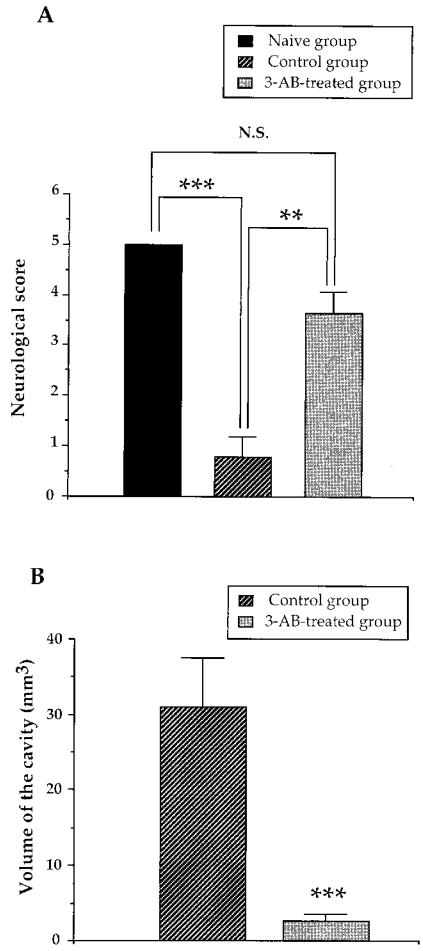

FIG. 7. Effect of 3-AB on neurological deficits and area of the ipsilateral cavity measured 24 days postnatally (17 days after ischemic onset). **A:** Neurological deficits for the right paw were assessed by three neurological tests as described in Experimental Procedures. Nonoperated rats were used as naive. Note that 3-AB treatment improves neurological score. **B:** The area of the ipsilateral cavity was measured in ischemic rats treated with either 3-AB or vehicle (control). Data are mean ± SEM (bars) values. N.S., not significant; $^{**}p < 0.005$, $^{***}p < 0.001$ versus vehicle-treated rats.

flammation (Szabo et al., 1997; Cuzzocrea et al., 1998). In addition, genetic disruption of PARS provides protection against myocardial ischemia and reperfusion injury by inhibiting the expression of P-selectin and intracellular adhesion molecule-1 and consequently by inhibiting the recruitment of neutrophils into the jeopardized tissue (Zingarelli et al., 1998). Also, peroxynitrite levels present in a perivascular and vascular location in T lymphocytes and neutrophils (Coeroli et al., 1998) were reduced in 3-AB-treated animals. Recent data demonstrating that PARS inhibition by 3-AB or the novel potent inhibitor 5-iodo-6-amino-1,2-benzopyrone attenuated peroxynitrite-induced mitochondrial perturbations, which are, to a significant degree, related to PARS activation (Endres et al., 1998; Virag et al., 1998), may underlie the observed effects. Taken together, these data suggested that PARS regulates the recruitment of inflammatory cells into the inflamed tissues via several distinct mechanisms. Therefore, PARP-1 appears to promote inflammation at two levels, and through its effects on nuclear factor κB (Javier Oliver et al., 1999) and by mediating the cytotoxicity of NO derivatives (see above), one or both of these mechanisms might also explain the resistance of PARP-1$-/-$ mice to brain ischemia.

The behavioral consequences of perinatal asphyxia are of great importance because hypoxia–ischemia encephalopathy in children often causes functional and intellectual deficits (Levene, 1991). In the neonatal rat, moderate to severe ischemic neuronal changes were seen in the ipsilateral cerebral cortex (Towfighi et al., 1995; Renolleau et al., 1998) and striatum, thalamus, and hippocampus (Towfighi et al., 1995). By 3 weeks of recovery, the infarcted area appeared as a smooth-walled cavity (Renolleau et al., 1998) that often communicated with the lateral ventricle (Towfighi et al., 1995). In this study, sensorimotor tests were evaluated following neonatal ischemia and related to the loss of brain tissue. PARS inhibition by 3-AB induced a significant reduction of the cavity volume and was accompanied by improved neurological scores, suggesting that reduced PARS activity preserves neurological function, which holds up at long-term. A few studies on functional outcome following PARS inhibition reported a reduction of cognitive and motor deficits after ischemia in the adult rat (Sun and Cheng, 1998) and traumatic brain injury in mice deficient in PARS (Whalen et al., 1999).

In conclusion, we have demonstrated that administration of the PARS inhibitor 3-AB reduces ischemia-induced cerebral infarction, inflammatory responses, and functional deficits and correlates with a reduction in poly(ADP-ribose) polymers. These beneficial effects are observed 48 h after ischemia and sustained 7 and 17 days after. Further studies are needed to confirm these results and to define PARS inhibition-based strategies to produce a more wide window of therapeutic opportunity in neonatal stroke. Pharmacological inhibition of PARS may represent a therapeutic strategy for reducing neurological injury after cerebral ischemia.

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