Copper-Zinc Superoxide Dismutase Prevents the Early Decrease of Apurinic/Apyrimidinic Endonuclease and Subsequent DNA Fragmentation After Transient Focal Cerebral Ischemia in Mice

Miki Fujimura, MD; Yuiko Morita-Fujimura, MS; Purnima Narasimhan, PhD; Jean-Christophe Copin, PhD; Makoto Kawase, MD; Pak H. Chan, PhD

Background and Purpose—DNA damage and its repair mechanism are thought to be involved in ischemia/reperfusion injury in the brain. We have previously shown that apurinic/apyrimidinic endonuclease (APE/Ref-1), a multifunctional protein in the DNA base excision repair pathway, rapidly decreased after transient focal cerebral ischemia (FCI) before the peak of DNA fragmentation. To further investigate the role of reactive oxygen species in APE/Ref-1 expression in vivo, we examined the expression of APE/Ref-1 and DNA damage after FCI in wild-type and transgenic mice overexpressing copper-zinc superoxide dismutase.

Methods—Transgenic mice overexpressing copper-zinc superoxide dismutase and wild-type littermates were subjected to 60 minutes of transient FCI by intraluminal blockade of the middle cerebral artery. APE/Ref-1 protein expression was analyzed by immunohistochemistry and Western blot analysis. DNA damage was evaluated by gel electrophoresis and terminal deoxynucleotidyl transferase–mediated uridine 5' triphosphate-biotin nick end-labeling (TUNEL).

Results—A similar level of APE/Ref-1 was detected in the control brains from both groups. APE/Ref-1 was significantly reduced 1 hour after transient FCI in both groups, whereas the transgenic mice had less reduction than that seen in wild-type mice 1 and 4 hours after FCI. DNA laddering was detected 24 hours after FCI and was decreased in transgenic mice. Double staining with APE/Ref-1 and TUNEL showed that the neurons that lost APE/Ref-1 immunoreactivity became TUNEL positive.

Conclusions—These results suggest that reactive oxygen species contribute to the early decrease of APE/Ref-1 and thereby exacerbate DNA fragmentation after transient FCI in mice. (Stroke. 1999;30:2408-2415.)

Key Words: cerebral ischemia, focal DNA fragmentation DNA repair endonucleases reactive oxygen species superoxide dismutase mice

The DNA repair enzyme apurinic/apyrimidinic endonuclease (APE/Ref-1) is a multifunctional protein in the DNA base excision repair pathway that is responsible for repairing apurinic/apyrimidinic sites in DNA.1 DNA base excision repair is known to require 2 types of enzymes, such as DNA glycosylases and APEs.2–5 DNA glycosylases remove a damaged base, which could be caused by various kinds of insults, such as oxidative stress in particular, creating an apurinic/apyrimidinic site in the DNA that is then acted on by an APE.3,5 The DNA repair is completed by abasic residue followed by synthesis of a new base by DNA polymerase and ligation. Incomplete repair of apurinic/apyrimidinic sites is reported to cause mutagenesis and genetic instability.5 Recently, DNA damage and repair are drawing more attention in the field of central nervous system injuries, including cerebral ischemia and brain trauma.7 Liu and colleagues8 have suggested that free radicals could attack the nuclear genes and cause genetic instability after mouse forebrain ischemia. As for the relationship of the DNA base excision repair pathway to necrosis and/or apoptosis, recent evidence suggests that downregulation of APE expression is associated with apoptosis in cells of the myeloid lineage.9 Additionally, we have shown in vivo that a decrease of the APE/Ref-1 protein preceded the occurrence of DNA fragmentation in the entire ischemic lesion after transient focal cerebral ischemia (FCI)10 and that loss of APE/Ref-1 was closely associated with the occurrence of DNA fragmentation in hippocampal CA1 neurons after transient global ischemia.11 However, the mechanism by which these early modifications of APE/Ref-1 expression after ischemia/reperfusion injury is regulated in vivo is unknown.

Antioxidant enzymes and DNA repair proteins are thought to be 2 major mechanisms by which cells counteract the
deleterious effects of reactive oxygen species (ROS); however, little is known about the interaction between them. Moreover, no report indicates the direct correlation between ROS and the expression of DNA repair enzymes, including APE/Ref-1, in vivo. To address this critical issue in vivo, in the present study we examined APE/Ref-1 expression after transient FCI in both wild-type and transgenic (Tg) mice that overexpress superoxide dismutase (SOD1) and that have been reported to show smaller infarction volume after transient FCI.12,13 The Tg mice showed less reduction of APE/Ref-1 expression 1 and 4 hours after transient FCI and showed a lesser amount of nucleosomal DNA fragmentation by gel electrophoresis 24 hours after FCI. Furthermore, the relationship between the loss of APE/Ref-1 expression and DNA fragmentation was confirmed by double staining with APE/Ref-1 and terminal deoxynucleotidyl transferase–mediated uridine 5′-triphosphate-biotin nick end-labeling (TUNEL). Taken together, these results suggest that overexpression of SOD1 in Tg mice prevents the early decrease of APE/Ref-1, thereby reducing apoptosis after transient FCI in mice.

**Materials and Methods**

**SOD1 Tg Mice**

Heterozygous SOD1 Tg mice of the SOD1 TGH/SF-218-3 strain, carrying human SOD1 genes with a 3-fold increase in copper-zinc SOD, were derived from the founder stock previously described.14 They were bred on a CD-1 background for at least 8 generations. The SOD1 Tg mice were identified by quantitative demonstration of SOD1 with the use of non-denaturing gel electrophoresis followed by nitroblue tetrazolium staining.14 There were no phenotypic differences between the SOD1 Tg mice and their wild-type littersmates.

**Focal Cerebral Ischemia**

Adult male Tg mice and non-Tg littersmates (35 to 40 g) were subjected to transient focal ischemia by intraluminal middle cerebral artery (MCA) blockade with a nylon suture, as described.13 The mice were anesthetized with 2.0% isoflurane in 30% oxygen and 70% nitrous oxide with the use of a face mask. The rectal temperature was controlled at 37°C with a homeothermic blanket. Cannulation of a femoral artery allowed the monitoring of blood pressure and arterial blood gases, with samples for analysis taken immediately after carotid occlusion, 10 minutes after occlusion, and 10 minutes after reperfusion. Blood gas was analyzed by a pH/blood gas analyzer (Chiron Diagnostics Ltd). After the midline skin incision, the left external carotid artery was exposed, and its branches were electrocoagulated. A 11.0-mm 5-0 surgical monofilament nylon suture, blunted at the end, was introduced into the left internal carotid artery through the external carotid artery stump. After 60 minutes of MCA occlusion, blood flow was restored by the withdrawal of the nylon suture.

**Histological Assessment**

The experimental animals were killed 1, 4, and 24 hours after 60 minutes of MCA occlusion. The brains were removed, rapidly frozen in −20°C 2-methylbutane, and stored at −80°C. They were sectioned with a cryostat to a thickness of 20 μm from the anterior side to the posterior side and stained with cresyl violet.

**Immunohistochemistry of APE/Ref-1**

Anesthetized animals were perfused with 10 U/mL heparin and subsequently with 4% formaldehyde in 0.1 mol/L PBS (pH 7.4) after 1, 4, and 24 hours of reperfusion following ischemia. Brains were removed, postfixed for 12 hours in 4% formaldehyde, sectioned at 50 μm on a vibratome, and processed for immunohistochemistry. The sections were incubated with blocking solution as described13 and reacted with anti–apurinic/apyrimidinic endonuclease polyclonal antibody (Novus Biologicals) at a dilution of 1:100. Immunohistochemistry was performed with the avidin-biotin technique as described,13 and then the nuclei were counterstained with methyl green solution for 10 minutes. As a negative control, sections were incubated without primary antibodies. For histological assessment, alternate slices from each brain section were stained with cresyl violet. To assess the level of APE/Ref-1 expression, we counted APE/Ref-1–immunoreactive cells, as previously described.13 To evaluate the subpopulation of APE/Ref-1–immunoreactive cells, we performed double staining of APE/Ref-1 and the nuclear neuronal marker NeuN in normal brains. Fixed brain samples was sectioned at 50 μm on a vibratome as described above, and sections were first reacted with mouse monoclonal antibody against NeuN (Chemicon International Inc) at a dilution of 1:100. To avoid the cross-reaction between the secondary antibody and mouse immunoglobulin in the tissue, immunohistochemistry was performed with the Dako ARK peroxidase kit. After development with nickel diaminobenzidine (Ni-DAB), sections were processed to APE/Ref-1 immunohistochemistry as described above. After development with DAB, sections were mounted on slides and counterstained with methyl green solution.

**Western Blot Analysis**

Whole cell protein extraction was performed as previously described.10 Approximately 20 mg of both the ischemic lesion and homologous tissue from the contralateral side was cut into pieces after 4 hours of reperfusion and put into 10× volume of Tris-glycine SDS sample buffer (Novex). Samples were then gently homogenized 20× in a glass Dounce homogenizer (Wheaton). Equal amounts of the samples (10 μL) were loaded per lane. The primary antibodies were either 1:1000 dilution of a polyclonal antibody against APE/Ref-1 (Novus Biologicals) or 1:10 000 dilution of an anti–β-actin monoclonal antibody (Sigma). For APE/Ref-1 detection, Western blots were performed with horseradish peroxidase–conjugated anti-rabbit immunoglobulin G with the Boehringer Mannheim chemiluminescent system. Recombinant human APE/Ref-1 was a generous gift from Novus Biologicals. As the internal control, Western blot analysis of β-actin was performed with horseradish peroxidase–conjugated anti-mouse immunoglobulin G reagents (Amersham International).

**Double Labeling with APE/Ref-1**

**Immunohistochemistry and TUNEL**

To clarify the spatial relationship between APE/Ref-1 expression and DNA damage, we performed double staining of APE/Ref-1 antibody and TUNEL as previously described.13 After transcardiac perfusion, fixed sections were immunostained with APE/Ref-1 antibody as described above, the sections were mounted on glass slides (Superfrost, Fisher Scientific), passed through ethanol (70%, 95%, and 100%), and then immersed in chloroform for 5 minutes. The sections were rehydrated by passage through a decreasing ethanol series, rinsed with water, and processed to TUNEL. The slides were placed in 1× terminal deoxynucleotidyl transferase (TdT) buffer (Life Technologies) for 15 minutes, followed by reaction with TdT enzyme (Life Technologies) and biotinylated 16-dUTP (Boehringer Mannheim) at 37°C for 60 minutes. The slides were then washed in 2× SSC (150 mmol/L sodium chloride, 15 mmol/L sodium citrate, pH 7.4) for 15 minutes, followed by a washing in PBS 2× for 15 minutes. Avidin-biotin horseradish peroxidase solution (ABC kit, Vector Laboratories) was applied to the sections for 30 minutes. Staining was visualized with the use of 0.025% diaminobenzidine and 0.075% H2O2 with nickel sulfate. The slides were rinsed with water, stained with methyl green for 10 minutes, dehydrated, and mounted.

**Gel Electrophoresis**

Animals were killed 24 hours after 60 minutes of MCA occlusion. Thirty to 50 mg wet weight of ischemic tissue was taken from the third 2-mm section along with homologous tissue from the contralat-
eral side after the brain was cut coronally. Samples were incubated overnight in 0.6 mL lysis buffer (0.5% SDS, 10 mmol/L Tris-HCl, and 0.1 mol/L EDTA) with 0.6 mg proteinase K (Boehringer Mannheim) at 55°C. The DNA was extracted with equal volumes of phenol and phenol-chloroform-isooamyl alcohol (25:24:1) and precipitated overnight in 0.2 mol/L sodium chloride in 100% ethanol at −80°C. The DNA was washed with 75% ethanol 2 times, air dried, and resuspended in DNase-free water (Sigma). The DNA concentration was measured by using To-Pro-1 dye (Molecular Probes). Gel electrophoresis for detecting DNA laddering was performed according to the manufacturer’s instructions (Trevigen), as previously described. Before electrophoresis, 1 μg of DNA was incubated with 50 μg/mL of DNase-free RNase (Boehringer Mannheim) for 30 minutes at 37°C. Then the samples were reacted with Klenow enzyme (Trevigen) and dNTP (Trevigen) in 1× Klenow buffer (Trevigen) for 10 minutes at room temperature. Samples were mixed with loading buffer and subjected to electrophoresis on 1.5% agarose gel. Then the gel was washed with 0.25 mol/L HCl, 0.4 mol/L NaOH/0.8 mol/L NaCl, and 0.5 mol/L Tris buffer (pH 7.5). DNA was transferred to a nylon membrane overnight in 10× SSC. The membrane was first blocked by 5% powdered milk (BioRad) in PBS for 30 minutes and incubated with streptavidin–horseradish peroxidase conjugate for 30 minutes. Finally, the bands were visualized by the chemiluminescence method with the use of PeroxyGlow (Trevigen), and the films were exposed to x-ray film.

**Quantification and Statistical Analysis**

The number of the APE/Ref-1–immunoreactive cells and methyl green–positive cells was counted in a high-power field (×400) by a investigator who was blinded to the studies and expressed by the percentage of APE/Ref-1–positive cells. The number of the TUNEL-positive cells was also counted, as previously described. The quantitative analysis of these cells was evaluated by factorial ANOVA between each group. Significance between groups was assigned at P<0.05.

**Results**

**Physiological Data and Cerebral Infarction**

Physiological parameters showed no significant differences in mean arterial blood pressure and arterial blood gas analysis between each group. The preischemic physiological values were as follows (wild-type/SOD1 Tg): mean arterial blood pressure, 71.50±3.42/72.50±6.61 mm Hg; PaO₂, 157.25±20.13/169.50±7.09 mm Hg; PaCO₂, 33.05±4.67/31.57±3.54 mm Hg; pH, 7.33±0.063/7.364±0.022 (mean±SD; n=4). There was no deviation from these values over the period of assessment. An ischemic lesion of the core of the caudate putamen was visible as a pale, slightly stained area in the ischemic hemisphere as early as 1 hour after reperfusion and extended to the entire MCA territory at 4 hours by cresyl violet staining (data not shown). The time-dependent increase of infarction in mouse brain with the intraluminal suture blockade is consistent with previous reports that employed the same focal stroke model in mice. The APE/Ref-1 protein was constitutively expressed in the hippocampus (Figure 1C and 1I) compared with the cortex (Figure 1B and 1H) and the caudate putamen (Figure 1A and 1G) from wild-type animals as well as from SOD1 Tg mice. This regional predominance was confirmed by Western blot analysis of APE/Ref-1 proteins from each region, showing that more expression was observed in the hippocampus and cerebellum than in the striatum and cortex (data not shown). After 1 hour of reperfusion, following 60 minutes of ischemia, a significant reduction of APE/Ref-1–immunoreactive cells was observed in the caudate putamen from both groups (Figure 1D and 1J). Four hours after ischemia, the number of APE/Ref-1–positive cells was significantly decreased in the entire MCA territory, including the caudate putamen (Figure 1E and 1K) and cortex, and was sustained 24 hours after ischemia (Figure 1F and 1L). As shown in Figure 2, the percentage of APE/Ref-1–positive cells was not significantly different in the nonischemic brain between the wild-type and SOD1 Tg mice; however, the percentage of APE/Ref-1–positive cells was significantly lower in the wild-type animals in the lateral caudate putamen 1 and 4 hours after ischemia and in the MCA territory cortex 4 hours after ischemia. As shown in Figure 1M, double staining of APE/Ref-1 (gray color) and the neuronal marker NeuN (brown color) clearly indicate the concurrent expression of both proteins, suggesting that APE/Ref-1 is mainly expressed in neurons. A single stain with APE/Ref-1 that was developed by Ni-DAB showed nuclear localization of the gray color (Figure 1N). In contrast, NeuN immunohistochemistry is known to stain the nucleus as well as the cytosol in a lesser amount (Chemicon). There was no immunoreactivity in the control specimens, which were treated without a primary antibody (Figure 1O).

**Western Blot Analysis of APE/Ref-1 Protein Expression After Transient MCA Occlusion**

As shown in Figure 3, APE/Ref-1 immunoreactivity was evident as a single band of molecular mass 37 kDa of a whole cell fraction from the nonischemic striatum in both wild-type mice (lane 1, top panel) and SOD1 Tg mice (lane 2, top panel) and was reduced 4 hours after ischemia in both groups (lanes 3 to 6, top panel). SOD1 Tg mice showed much less reduction of APE/Ref-1 protein levels (lanes 5 and 6, top panel; mean optical density=0.191) than wild-type littermates (lanes 3 and 4, top panel; mean optical density=0.098). On the other hand, a consistent amount of β-actin immunoreactivity between each lane is seen in the bottom panel, suggesting that the amount of the loaded protein was consistent. These data not only confirm the specificity of the antibody for APE/Ref-1 used in this study but also suggest that there was more reduction of APE/Ref-1 after transient focal ischemia in wild-type animals than in SOD1 Tg mice.

**Double Labeling With APE/Ref-1 Expression and DNA Fragmentation Detected by TUNEL Staining After FCI**

As shown in Figure 4, constitutive expression of APE/Ref-1 was detected in the nonischemic caudate putamen, in which there were no TUNEL-positive cells (Figure 4A). TUNEL-positive cells were barely recognized 4 hours after FCI, while a marked reduction of APE/Ref-1–positive cells was seen at this point (Figure 4B). Twenty-four hours after FCI, a
significant number of TUNEL-positive cells with the characteristic features of apoptosis (densely labeled in their nuclei, accompanied by apoptotic bodies) were seen (Figure 4C, arrowheads). None of these cells showed APE/Ref-1 immunoreactivity. On the other hand, none of the cells with APE/Ref-1 immunoreactivity were TUNEL positive 24 hours after FCI (Figure 4C, stars). To quantify the temporal profile of DNA fragmentation after FCI, the number of TUNEL-positive cells in the caudate putamen was counted 4 and 24 hours after FCI as well as in the control specimens, as previously described.15 As illustrated in Figure 4D, only a small number of TUNEL-positive cells were detected in the control specimens and in the brain 4 hours after FCI, while they significantly increased 24 hours after FCI (P<0.0001).
The present study provides the first evidence that ROS contribute to the early decrease of the DNA repair enzyme APE/Ref-1 and subsequent DNA fragmentation after focal ischemia/reperfusion injury. We observed that the early decrease of the DNA repair enzyme APE/Ref-1 has a role in the apoptotic cell death pathway in transient FCI and that this reduction in Tg mice is prevented in part by the overexpression of the antioxidant enzyme SOD1. These observations derive from the following findings. First, a marked reduction of APE/Ref-1 was seen in the entire ischemic area in wild-type mice as early as 1 hour after ischemia (Figure 1) and preceded the occurrence of DNA fragmentation (Figure 4). Second, double staining with APE/Ref-1 and TUNEL clearly showed that the neurons that lost APE/Ref-1 immunoreactivity became TUNEL positive, indicating the spatial relationship between APE/Ref-1 expression and DNA damage after transient FCI. Third, despite the decrease of APE/Ref-1 expression after transient FCI in SOD1 Tg mice, its reduction was significantly less than that of wild-type mice, as shown by both immunohistochemistry (Figures 1 and 2) and Western blot analysis (Figure 3). Finally, nucleosomal DNA fragmentation was seen 24 hours after transient FCI and was reduced in SOD1 Tg mice (Figure 5), suggesting the possibility that a lesser reduction of APE/Ref-1 in Tg mice may contribute to the smaller amount of nucleosomal DNA fragmentation compared with that of the wild-type mice. Taken together, this suggests that overexpression of SOD1 in Tg mice may prevent the early decrease of APE/Ref-1 after transient FCI and could thereby contribute to reducing the amount of DNA fragmentation.

Antioxidant enzymes and DNA repair proteins are thought to be 2 major mechanisms by which cells counteract the deleterious effects of ROS, and we have shown evidence that antioxidant enzymes such as SOD play a protective role in ischemia/reperfusion injury in the mouse brain. Therefore, we believe that the prevention of the early release of APE/Ref-1 in SOD1 Tg mice may contribute to the smaller amount of nucleosomal DNA fragmentation compared with that of the wild-type mice. Taken together, this suggests that overexpression of SOD1 in Tg mice may prevent the early decrease of APE/Ref-1 after transient FCI and could thereby contribute to reducing the amount of DNA fragmentation.

Discussion

The present study provides the first evidence that ROS contribute to the early decrease of the DNA repair enzyme APE/Ref-1 and subsequent DNA fragmentation after focal ischemia/reperfusion injury. We observed that the early decrease of the DNA repair enzyme APE/Ref-1 has a role in the apoptotic cell death pathway in transient FCI and that this reduction in Tg mice is prevented in part by the overexpression of the antioxidant enzyme SOD1. These observations derive from the following findings. First, a marked reduction of APE/Ref-1 was seen in the entire ischemic area in wild-type mice as early as 1 hour after ischemia (Figure 1) and preceded the occurrence of DNA fragmentation (Figure 4). Second, double staining with APE/Ref-1 and TUNEL clearly showed that the neurons that lost APE/Ref-1 immunoreactivity became TUNEL positive, indicating the spatial relationship between APE/Ref-1 expression and DNA damage after transient FCI. Third, despite the decrease of APE/Ref-1 expression after transient FCI in SOD1 Tg mice, its reduction was significantly less than that of wild-type mice, as shown by both immunohistochemistry (Figures 1 and 2) and Western blot analysis (Figure 3). Finally, nucleosomal DNA fragmentation was seen 24 hours after transient FCI and was reduced in SOD1 Tg mice (Figure 5), suggesting the possibility that a lesser reduction of APE/Ref-1 in Tg mice may contribute to the smaller amount of nucleosomal DNA fragmentation compared with that of the wild-type mice. Taken together, this suggests that overexpression of SOD1 in Tg mice may prevent the early decrease of APE/Ref-1 after transient FCI and could thereby contribute to reducing the amount of DNA fragmentation.

Antioxidant enzymes and DNA repair proteins are thought to be 2 major mechanisms by which cells counteract the deleterious effects of ROS, and we have shown evidence that antioxidant enzymes such as SOD play a protective role in ischemia/reperfusion injury in the mouse brain. Therefore, we believe that the prevention of the early release of APE/Ref-1 in SOD1 Tg mice may contribute to the smaller amount of nucleosomal DNA fragmentation compared with that of the wild-type mice. Taken together, this suggests that overexpression of SOD1 in Tg mice may prevent the early decrease of APE/Ref-1 after transient FCI and could thereby contribute to reducing the amount of DNA fragmentation.
DNA repair enzyme APE/Ref-1, which has been implicated in apoptosis after ischemia/reperfusion. The results showed less reduction of APE/Ref-1 in SOD1 Tg mice in the caudate putamen at 1 and 4 hours and in the MCA territory cortex 4 hours after FCI (Figure 2). There was no reduction of APE/Ref-1 in the MCA territory cortex 1 hour after FCI in either group (Figure 2). These results indicate that SOD1 partly mediates the early decrease of APE/Ref-1 after transient FCI. Furthermore, it may further contribute to the reduction of DNA fragmentation, as shown in Figure 5, since double staining with APE/Ref-1 and TUNEL clearly shows a spatial relationship between the loss of APE/Ref-1 expression and DNA fragmentation in the lateral caudate putamen. The temporal profile of both the percentage of APE/Ref-1–positive cells and the number of TUNEL-positive cells is shown in D. *P<0.0001 compared with nonischemic caudate putamen; #P<0.001 compared with the samples 1 hour after FCI.

APE/Ref-1 in the MCA territory cortex 1 hour after FCI in either group (Figure 2). These results indicate that SOD1 partly mediates the early decrease of APE/Ref-1 after transient FCI. Furthermore, it may further contribute to the reduction of DNA fragmentation, as shown in Figure 5, since double staining with APE/Ref-1 and TUNEL clearly shows a spatial relationship between the loss of APE/Ref-1 expression and DNA fragmentation (Figure 4).

APE/Ref-1 is known to play a pivotal role in repairing DNA damage caused by ROS. A recent in vitro study demonstrated that APE/Ref-1 is activated selectively by sublethal levels of ROS and that cells with activated APE/Ref-1 develop resistance to genotoxic ROS and ROS generators. It is also reported that APE/Ref-1 depression by the transfection of antisense APE/Ref-1 mRNA in glioma cells resulted in much higher sensitivity to ROS than in control cells. These results suggest that the level of APE/Ref-1 expression, which can be modified by ROS insults, is a critical factor for the cells to counteract the deleterious effects of ROS. Also in vivo, we have previously reported that APE/Ref-1 rapidly decreased after transient FCI in the entire...
ischemic territory that was destined to show necrosis and apoptosis,10,11 suggesting that this reduction could be extremely detrimental to neurons in overcoming oxidative stress during ischemia/reperfusion injury. On the basis of these observations, the lesser degree of reduction of APE/Ref-1 in SOD1 Tg mice after transient FCI (Figures 1 and 2) may provide more resistance against oxidative stress during reperfusion and could thereby reduce infarction volume, as observed previously in SOD1 Tg mice after FCI.12,13 A future study using Tg and/or knockout mutant mice that overexpress or are deficient in APE/Ref-1 should provide important tools to address this issue.

Programmed cell death and the DNA repair mechanism are both assumed to play important roles in cerebral ischemia.26–30 However, little is known about the interaction of these processes, except the data suggesting a link between apoptosis and the cleavage of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP).31 The interleukin-1β–converting enzyme family caspases are the human homologues of the nematode Caenorhabditis elegans, Ced-3,32 and are considered to play a critical role in programmed cell death.31 Mitochondrial cytochrome c is reported to be released from mitochondria to the cytosol,33 where it interacts with the protein Apaf-1 and activates caspase-9, which then activates other caspases, including caspase-3.34 One substrate for caspase-3 is PARP, whose proteolytic cleavage results in a dysfunctional PARP that is unable to contribute to repair or genomic maintenance.31 Furthermore, the Ca2+/Mg2+–dependent endonuclease that generates internucleosomal DNA cleavage characteristic of apoptosis is negatively regulated by poly(ADP-ribose)–ribosylation. Therefore, inactivation of PARP could increase DNA cleavage and contribute to programmed cell death. As for APE/Ref-1, there is no evidence suggesting its link to this mitochondrial pathway, and it is more likely that its expression is directly regulated by free radical production during reperfusion.

In conclusion, we have shown that overexpression of SOD1 in Tg mice partially prevents the early decrease of APE/Ref-1 during reperfusion that follows FCI and reduces the amount of DNA fragmentation at the later time point. These results indicate that antioxidant treatment provides therapeutic value against cerebral ischemia and reperfusion injury by regulating the expression of DNA repair enzymes such as APE/Ref-1, thereby blocking DNA-damaged cell death after ischemia/reperfusion.

Acknowledgments

This study was supported by National Institutes of Health grants NS14543, NS25372, NS36147, NS38653, and N01NS82386. Dr Chan is a recipient of the Jacob Javits Neuroscience Investigator Award. We are grateful to Dr Charles J. Epstein, PhD, Department of Pediatrics, University of California, San Francisco, School of Medicine for continuous collaboration by providing breeding pairs of SOD1 Tg mice. We thank C. Christensen for editorial assistance and L. Reola, B. Calagui, and J.O. Kim for technical assistance.

References


24. Ramana CV, Boldogh I, Inazu T, Mitra S. Activation of apurinic/apyrimidinic endonuclease in human cells by reactive oxygen species and...


**Editorial Comment**

Normal cellular metabolism, particularly mitochondrial respiration, produces reactive oxygen species (ROS) (eg, hydrogen peroxide and superoxide anion and hydroxyl radicals) as end products. Under physiological conditions, excessive ROS are neutralized by endogenous antioxidants (eg, ascorbate, α-tocopherol, β-carotene, and glutathione) and antioxidant enzymes (eg, superoxide dismutase, catalase, and glutathione peroxidases). Severe oxidative stress may overwhelm the antioxidant mechanisms and lead to oxidative DNA damage. Among the DNA lesions caused by ROS are base modifications, single-strand breaks, double-strand breaks, strand scissions, and the cross-linking of bases. The second line of defense against oxidative DNA damage is the DNA repair machinery. Oxidative DNA damage can be rapidly and efficiently repaired. However, this second line of defense against oxidative damage of DNA may be compromised in the setting of ischemia-reperfusion.

After cerebral ischemia, individual bases in DNA may be oxidized to form various oxidative DNA adducts, including 8-hydroxyguanine (8-OH-Gua), 8-hydroxyadenine (8-OH-Aden), 4,6-diamino-5-formamidopyrimidine (FapyAde), and others. Base excision repair is a major mechanism to reduce DNA damage, although other pathways, such as nucleotide excision repair, photoreactivation, recombination, and gene conversion, are also important in maintaining DNA integrity. Base excision repair involves the resynthesis of 1–3 nucleotides. Base excision repair represents the removal of oxidative base damages by specific glycosylases followed by the action of apurinic/apyrimidinic (AP) endonucleases that cleave the phosphodiester backbone, resulting in the loss of the abasic sugar. The nucleotide gap is then filled predominantly by mammalian DNA polymerase-β using correct dNTPs as directed from the sequence in the template, followed by ligation with DNA ligase.

In the preceding article by Fujimura et al, AP endonuclease expression was reduced after transient focal cerebral ischemia in wild-type mice but significantly less in transgenic mice overexpressing SOD1. These findings suggest that oxidative damages to proteins, including DNA repair enzymes, may compromise postischemic DNA repair. Thus, excessive ROS production may jeopardize both the first and second lines of defense against oxidative DNA damage.

**References**

Copper-Zinc Superoxide Dismutase Prevents the Early Decrease of Apurinic/Apyrimidinic Endonuclease and Subsequent DNA Fragmentation After Transient Focal Cerebral Ischemia in Mice

Miki Fujimura, Yuiko Morita-Fujimura, Purnima Narasimhan, Jean-Christophe Copin, Makoto Kawase and Pak H. Chan

Stroke. 1999;30:2408-2415
doi: 10.1161/01.STR.30.11.2408

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1999 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/30/11/2408

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/