Overexpression of Copper-Zinc Superoxide Dismutase Attenuates Acute Activation of Activator Protein-1 After Transient Focal Cerebral Ischemia in Mice

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Background and Purpose—Reactive oxygen species (ROS) have been implicated in reperfusion injury after focal cerebral ischemia (FCI). ROS are known to regulate the activity of transcription factors such as activator protein-1 (AP-1), which is a dimer consisting of members of the Jun and Fos families. We investigated the role of ROS in AP-1 activity after FCI using transgenic mice that overexpressed copper-zinc superoxide dismutase (SOD1) and that had reduced infarction volume after FCI.

Methods—The SOD1 transgenic mice and their wild-type littermates were subjected to middle cerebral artery occlusion and reperfusion by intraluminal suture blockade. After 60 minutes of middle cerebral artery occlusion, mice were allowed to recover for 1, 2, and 4 hours before euthanasia. Protein expression of c-Jun and c-Fos was examined by immunohistochemistry and Western blotting. AP-1 DNA-protein binding activity was assessed by electrophoretic mobility shift assays.

Results—In wild-type mice, immunohistochemistry demonstrated acute c-Jun and c-Fos activation in ischemic cortex and its outer boundary. Expression of both was reduced in SOD1 transgenic mice. Western blotting confirmed that SOD1 overexpression was associated with reduced c-Jun and c-Fos protein levels in ischemic brain. Electrophoretic mobility shift assays revealed that the ischemia-enhanced DNA binding activity observed in wild-type mice was reduced in SOD1 transgenic mice. Supershift assays indicated that c-Jun participated in the bound AP-1 complex.

Conclusions—SOD1 overexpression prevents early activation of AP-1 after transient FCI in mice. This may block the expression of downstream target genes that are injurious, thereby reducing the infarction volume after transient FCI in mice. (Stroke. 2001;32:741-747.)

Key Words: cerebral ischemia, focal & free radicals & superoxide dismutase & transcription factor AP-1 & transcription factors & mice

Reactive oxygen species (ROS) have been implicated in the pathogenesis of ischemic brain injury. Overproduction of ROS during postischemic reperfusion often exceeds the capacity of antioxidant enzymes such as superoxide dismutases (SODs), resulting in oxidative stress and cell damage. ROS are involved in reperfusion after cerebral ischemia. Overexpression of copper-zinc SOD (SOD1) in transgenic (Tg) mice or rats is neuroprotective in brain injuries such as traumatic brain injury, global ischemia, and transient focal cerebral ischemia (FCI). After transient FCI, brain edema, infarct volume, and DNA fragmentation are significantly reduced in SOD1 Tg mice, whereas they are markedly increased in SOD1 knockout mutant mice, suggesting that superoxide radicals are involved in the pathogenesis of neuronal cell death after transient FCI. However, the molecular mechanism underlying regulation of neuronal protection in SOD1 Tg animals is unclear.

ROS play a role in cellular signaling processes, including regulation of transcription factor activity. Activator protein-1 (AP-1) responds to oxidative stress induced by ROS. The immediate early genes, c-jun and c-fos, encode for AP-1, which activates target genes that are involved in the regulation of neuronal function and apoptotic cell death. AP-1 activity is controlled at different levels. The abundance, type, and posttranslational modifications of AP-1 proteins may change its DNA binding and transactivation activity. FCI has been shown to induce the expression of the c-fos and c-jun families in the nervous system. However, the mechanism of this induction and whether the induction of c-fos and c-jun is neuroprotective or deleterious to the brain after ischemia is presently unclear. AP-1 activity may be induced by a wide array of factors, including cytokines, growth factors, bacterial endotoxin, and hypoxia. Since AP-1 may be activated by stimuli that also
induce ROS, it may play a role in the pathogenesis of oxidative stress–associated acute injuries and neurodegenerative disorders, including focal ischemia. However, the signaling role of ROS in activating AP-1 after focal ischemia remains unclear. Because of the apparent link between ROS and AP-1, we were prompted to determine whether alteration of AP-1 activity would contribute to SOD1-associated neuronal protection after transient ischemia. Our aim was to investigate the role of ROS in AP-1 activation after transient FCI, using SOD1 Tg and wild-type (Wt) mice that were subjected to ischemia and reperfusion.

**Materials and Methods**

**Focal Cerebral Ischemia**

Experiments were performed in accordance with National Institutes of Health guidelines and were approved by Stanford University’s Administrative Panel on Laboratory Animal Care. Tg mice of the TgHS/SF-218 strain, which carry the human copper-zinc SOD gene, were derived from the founder stock.18 There were no observable phenotypic differences between the Tg mice and their Wt normal littermates. Tg mice (3-month-old males; weight, 35 to 40 g) with a negative control, sections were incubated without primary catalyzed peroxidase-reaction product. Nuclei were counterstained with diaminobenzidine substrate was used for visualization of the Sections were incubated with c-Jun or c-Fos polyclonal antibodies for 60 minutes at 4°C. After 60 minutes of MCA occlusion, mice were killed after 1 hour of ischemia and 4 hours after ischemia. Brains were removed, postfixed for 12 hours in 4% formaldehyde in 0.1 mol/L PBS, pH 7.4, and 1, 2, and 4 hours after ischemia. The left common carotid artery was exposed, and the left external carotid artery and its branches were electrocoagulated. An 11.0-mm 5-0 surgical monofilament nylon suture, blunted at the tip, was introduced into the left internal carotid artery through the external carotid artery stump. After 60 minutes of proximal MCA occlusion, blood flow was restored by removing the suture. Control animals, both Wt and Tg, did not undergo surgery. After 60 minutes of MCA occlusion, mice were allowed to recover for 1, 2, and 4 hours. Physiological parameters were monitored throughout the studies, and the values were the same as previously reported.20

**Immunohistochemistry**

Anesthetized animals were perfused with 10 U/mL heparinized saline followed by 4% formaldehyde in 0.1 mol/L PBS, pH 7.4, 1, 2, and 4 hours after ischemia. Brains were removed, postfixed for 12 hours in 4% formaldehyde, sectioned at 50 μm on a vibratome, and processed for immunohistochemistry. The endogenous peroxidase activity was quenched by immersing the sections in 3% H2O2 for 10 minutes. A nonspecific blocking procedure, using 1% bovine serum albumin, was performed before application of primary antibodies. Sections were incubated with c-Jun or c-Fos polyclonal antibodies (Santa Cruz Biotechnology) at a dilution of 1:200 overnight at 4°C. The sections were incubated with avidin-biotin-horseradish peroxidase (ABC kit, Vector Laboratories) to localize the primary antibodies. A diaminobenzidine substrate was used for visualization of the catalyzed peroxidase-reaction product. Nuclei were counterstained with methyl green solution for 10 minutes and mounted. As a negative control, sections were incubated without primary antibodies.

**Isolation of Nuclear Extract From Brain Tissues**

After 60 minutes of MCA occlusion, mice were killed after 1 hour of recovery. Brains were cut coronally, and the ipsilateral cortex was separately dissected. Nuclear extracts were isolated from 250 mg of brain tissue pooled from 3 mice by the method of Deryckere and Gannon.21 Briefly, frozen cortex was homogenized in 5 mL of solution A (0.6% Nonidet P-40, 150 mmol/L NaCl, 10 mmol/L HEPES, pH 7.9, 1 mmol/L EDTA, 0.5 mmol/L phenylmethylsulfonyl fluoride [PMSF]) with a 10-mL tissue glass homogenizer (Wheaton). Samples were centrifuged for 2 minutes at 760g. The supernatant was centrifuged for 10 minutes at 3000g. The pellets nuclei were resuspended in 4 volumes of solution B (25% glycerol, 20 mmol/L HEPES, pH 7.9, 420 mmol/L NaCl, 1.2 mmol/L MgCl2, 0.2 mmol/L EDTA, 0.5 mmol/L dithiothreitol, 0.5 mmol/L PMSF, 2 mol/L benzamidine, plus 5 μg/mL each of these 3 protease inhibitors: pepstatin, leupeptin, and aprotinin) and then incubated on ice for 20 minutes for high-salt extraction. After a 10-minute centrifugation, the supernatant containing the DNA-binding proteins was stored at −70°C. Total nuclear extract protein content was quantitated by the Bradford assay.

**Western Blot Analysis**

Equal amounts (10 μg) of nuclear protein extracts were denatured at 100°C for 5 minutes in Laemmli sample buffer/5% 2-mercaptoethanol. Samples were electrophoresed on 10% polyacrylamide gels and electroblotted onto a membrane (Novex). The membrane was incubated overnight at 4°C with primary rabbit polyclonal antibodies against c-Jun (1:200) or c-Fos (1:200) (Santa Cruz Biotechnology) diluted in TBST/0.5% nonfat milk buffer (10 mmol/L Tris, pH 8.0, 150 mmol/L NaCl, 0.1% Tween-20). The membrane was incubated with horseradish peroxidase–conjugated secondary anti-rabbit IgG (Boehringer Mannheim) at a 1:10 000 dilution in TBST/0.5% milk buffer for 30 minutes at room temperature. The immunoreactive bands were visualized by the chemiluminescence detection system (ECL Plus Kit, Amersham). The film was scanned with a GS-700 imaging densitometer (Bio-Rad), and the results were quantified with the use of Multi-Analyt software (Bio-Rad). After the first reaction, a 1:500 dilution of rabbit polyclonal antibodies against TFIIID basal transcription factor (Santa Cruz Biotechnology) was used to check that the protein was loaded equally in each lane. The phosphorylated c-Jun cell extract (New England BioLabs) and the whole cell lysate of NIH-3T3-phorbol from a normal embryo fibroblast (Santa Cruz Biotechnology) were used as a positive antigen control of c-Jun and c-Fos, respectively (data not shown).

**Electrophoretic Mobility Shift Assays**

Double-stranded oligonucleotide probes used for protein binding in electrophoretic mobility shift assays (EMASs) were as follows (only the upper strand is indicated): AP-1, 5′-ctggagatcggggcggggcagc-3′; Sp-1, 5′-atcgcagctgggacccggccggcggcggcgggaccg-3′; Sp-1, 5′-attgcagcgggccccgaccg-3′ (Promega Corp.). Oligonucleotide probes were radiolabeled with [32P]dATP by T4 polynucleotide kinase (USB) to produce double-stranded DNA probes. EMASs were performed according to the method of Singh et al.22 Binding reactions were conducted in a total volume of 20 μL containing equal amounts of nuclear protein (3 or 5 μg), 0.1 to 1.0 ng of DNA probe (~50 000 cpm), 20 mmol/L HEPES, pH 7.9, 1.5 mmol/L MgCl2, 100 mmol/L NaCl, 1 mmol/L EDTA, 11% glycerol, 1 mmol/L dithiothreitol, 35 mmol/L PMSF, 140 mmol/L benzamidine, and 350 ng/mL of peptatin A, leupeptin, and aprotinin for 20 minutes at 25°C. After incubation, bound and free probes were separated by 5% polyacrylamide gel electrophoresis and visualized by autoradiography. For competition experiments, radiolabeled DNA probe and nuclear proteins were incubated with a 100-fold molar excess of the unlabeled DNA oligonucleotide or an unrelated unlabeled oligonucleotide. Supershift assays were performed as recommended by Santa Cruz Biotechnology by preincubating nuclear extracts with polyclonal antibodies for 60 minutes at 4°C before the addition of labeled probes. The polyclonal antibodies directed against c-Jun were obtained from Santa Cruz Biotechnology. The hybridization signal on the x-ray film was scanned with a GS-700 imaging densitometer (Bio-Rad), and the results were quantified with the use of Multi-Analyt software (Bio-Rad).
Results

Early Activation of c-Jun After Transient FCI
To investigate the expression of activated c-Jun after transient FCI, the temporal expression of activated c-Jun was examined with the use of the anti–c-Jun antibody in immunohistochemical studies. The brains of the control mice had low levels of constitutive c-Jun activity (Figure 1D), which was consistent with previous findings from another group.23 One hour after MCA occlusion, an acute increase in c-Jun immunoreactivity (IR) was observed in the part of ischemic cortex and mainly in the outer boundary of the ischemic cortex (Figure 1A). Two hours after ischemia, c-Jun IR was induced throughout the ischemic cortex (Figure 1B) and its outer boundary. There was little induction of c-Jun IR in the ischemic core (data not shown). At 4 hours, an increase in c-Jun IR was observed in the outer boundary of the ischemic cortex (Figure 1C), whereas a decrease of c-Jun IR was seen in ischemic core (data not shown). These data suggest that c-Jun IR in the ischemic cortex and its outer boundary was enhanced by ischemia/reperfusion injury (Figure 1A through 1C).

Effect of Overexpression of SOD1 on c-Jun and c-Fos Expression After FCI
To clarify the role of oxygen free radicals in the expression of c-Jun and c-Fos, regional c-Jun and c-Fos protein expression was investigated in SOD1 Tg mice and Wt littermates after FCI. In Tg mice, overexpression of SOD1 was associated with reduced levels of c-Fos IR in the ischemic cortex and its outer boundary 2 hours after FCI (Figure 2D) compared with Wt littermates (Figure 2C). No significant difference in c-Jun IR was observed between Wt and Tg control animals (Figure 2A and 2B). These data suggest that c-Jun IR in the ischemic cortex and its outer boundary was enhanced by ischemia/reperfusion injury (Figure 1A through 1C).

Effect of Overexpression of SOD1 on c-Jun and c-Fos Protein Levels After FCI
To determine whether the abundance of c-Jun and c-Fos protein was increased 1 hour after FCI, Western blot analysis of c-Jun and c-Fos was performed. Nuclear extracts were isolated from brain tissue pooled from 3 mice. Our data were reproducible, and the representative data were shown in Figure 3. Anti–c-Jun and anti–c-Fos antibodies detected a single band with apparent molecular weights of 39 kDa (top panel) and 62 kDa (middle panel), respectively. No apparent difference in c-Jun and c-Fos protein levels was observed between Wt and Tg control animals (Figure 3A). These observations were consistent with the previous findings using c-fos and c-jun mRNA expression.24,25 Compared with Wt controls (Figure 3B, lane 1), 1.2-fold and 1.1-fold increases in activated c-Jun and c-Fos, respectively, were detected in the Wt mice (Figure 3B, lane 2). In contrast, ischemia in SOD1 Tg mice resulted in a 1.1-fold increase and no change in activated c-Jun and c-Fos, respectively (Figure 3B, lane 3). TFIIID basal transcription factor protein was used as an internal control for equal protein amount (Figure 3, bottom panel). The results, as depicted in Figure 3, demonstrate that...

Figure 1. High-power views of immunohistochemical experiments to detect the temporal expression of c-Jun after FCI (A through D). c-Jun immunoreactivity was investigated in the ischemic cortex of Wt mice 1, 2, and 4 hours after FCI (A, B, C). The control brain section revealed basal immunoreactivity of c-Jun (D). Bar=0.02 mm.

Figure 2. Overexpression of SOD1 decreased activated c-Jun and c-Fos immunoreactivity 2 hours after FCI. The c-Jun immunoreactivity was investigated in the ischemic cortex of Wt mice (A) and SOD1 Tg mice (B). The c-Fos immunoreactivity was investigated in the ischemic cortex of Wt mice (C) and SOD1 Tg mice (D). Bar=0.02 mm.

Figure 3. Overexpression of SOD1 decreases activated c-Jun and c-Fos protein levels 1 hour after FCI. A, Western blot analysis using nuclear extract isolated from the Wt control cortex (lane 1) and Tg control cortex (lane 2) 1 hour after FCI. B, Western blot analysis using nuclear extract isolated from the Wt control cortex (C, lane 1), Wt ischemic cortex (Wt, lane 2), and Tg ischemic cortex (Tg, lane 3) 1 hour after FCI. The c-Jun protein was evident as a single band of molecular mass 39-kDa nuclear protein (top panel). The c-Fos protein was evident as a single band of molecular mass 62-kDa nuclear protein (middle panel). c-Jun and c-Fos protein levels were increased in the Wt mice after FCI and were decreased in the Tg mice. TFIIID basal transcription factor protein was used as an internal control (bottom panel).
cortical protein levels are similar in Wt mice before and after FCI. While a difference in protein levels between Wt and Tg is present, these minor increases in c-Jun and c-Fos are out of accord with the large increases in their mRNA reported after reperfusion.\(^{16,26}\) This result suggests that posttranslational control affecting the stability of protein may override increased transcription of gene.

**Effect of Overexpression of SOD1 on AP-1 DNA Binding Activity After FCI**

Since a significant increase in c-Jun and c-Fos IR was observed in ischemic cortex at 1 hour, EMSAs were used to further investigate the impact of transient FCI on AP-1 DNA binding activity. The same cortical nuclear extracts that were used in the Western blotting were used in the EMSAs. In the present study 1 DNA-protein complex was detected in the mouse brains (Figure 4A), which was consistent with previous studies.\(^{27}\) The specificity of the DNA-protein complex was verified with the use of different unlabeled competitors. This complex was completely abolished by competition with a 100-fold molar excess of unlabeled AP-1 oligonucleotide (Figure 4A, lane 2) but not by competition with an unrelated oligonucleotide, Sp-1 (Figure 4A, lane 3). Classic AP-1 resembles a heterodimeric or homodimeric protein composed of Jun and Fos family. Supershift assays were used to determine whether antibodies against c-Jun would recognize proteins present in the complex formed with the AP-1 probe.

Compared with nuclear extracts without antibodies (Figure 4B, lane 1), the DNA-protein complex was decreased and supershifted by antibodies against c-Jun, suggesting that the complex contains c-Jun (Figure 4B, lane 2). Thus, the supershift assays further confirmed the binding specificity of the DNA-protein complex. Additionally, the EMSA data were consistent with the upregulation of c-Jun observed in the ischemic cortex using immunohistochemistry.

To determine the effect of overexpression of SOD1 on AP-1 DNA binding activity after FCI, EMSAs were performed with the use of nuclear extracts from Wt and Tg mice. In Wt mice, ischemia/reperfusion remarkably increased AP-1 DNA binding activity by 1.6-fold (\(P<0.01\)) (Figure 5, lane 2) compared with the Wt controls (Figure 5, lane 1). In the Tg mice, AP-1 DNA binding activity after ischemia was increased by 1.1-fold (Figure 5, lane 3) compared with the Wt controls (Figure 5, lane 1). No significant difference in AP-1 DNA binding activity was detected between Wt controls (Figure 5, lane 1) and Tg controls (Figure 5, lane 4). These data suggest that AP-1 DNA binding activity was increased...
after ischemia in the Wt mice and that the ischemia-enhanced DNA binding activity was significantly reduced in the Tg mice (P<0.05).

Discussion
In the present study our results indicate that superoxide radicals may modulate the activation of c-Jun and c-Fos after transient FCI. Ischemic cortex and surrounding regions displayed an increased level of c-Jun immunoreactivity 1, 2, and 4 hours after FCI in Wt mice (Figure 1). The FCI-induced acute activation of c-Jun and c-Fos IR was reduced in SOD1 Tg mice 2 hours after FCI (Figure 2). Western blotting demonstrated that SOD1 overexpression was associated with reduced c-Jun and c-Fos protein levels in ischemic brain (Figure 3). Finally, EMSAs revealed that the ischemia-enhanced DNA binding activity observed in Wt mice was reduced in SOD1 Tg mice (Figure 5). Supershift analysis with anti-Jun antibody indicated that AP-1 binding complexes activated by ischemia contain c-Jun proteins (Figure 4). Together, these results suggest that SOD1 may prevent the early activation of AP-1 after transient FCI.

Increasing evidence indicates the importance of AP-1 in neural homeostasis and dysfunction. Jun and Fos are widely expressed in the central nervous system in both the constitutive and inducible forms.29 FCI has been shown to induce their expression in the nervous system.16,17 Consistent with this observation, we found low basal expression of c-Jun and c-Fos throughout normal brain. Additionally, a significant induction of c-Jun and c-Fos expression was detected as early as 1 hour in ischemic cortex and its outer boundary. Induction increased throughout the ischemic cortex and its outer boundary 2 hours after FCI (Figures 1 and 2). In addition, we have demonstrated previously that c-fos and junB mRNA were induced throughout the ipsilateral cortex in rat brain at 1 and 4 hours after MCA occlusion.26 Although these alterations in gene expression after FCI have been documented, it is not certain presently whether these variations are secondary to changes in infarct size or whether they mediate the changes in infarct size.

The antioxidant enzyme is one of the major mechanisms by which cells counteract the deleterious effects of ROS after FCI. We have shown that SODs play a protective role against FCI.3,9,11 Superoxide may be involved in reperfusion brain injury after cerebral ischemia.1,3 Additionally, ROS regulate the activity of transcription factors, including AP-1 and nuclear factor-kB.12 Our findings suggest that AP-1 is involved in the neuroprotective role of SOD against FCI. After mild ischemia (ie, 10 minutes), we have observed differences between Tg and Wt mice in c-fos mRNA expression in the dorsal hippocampus and thalamus, suggesting that c-fos gene was activated by a number of mechanisms in FCI, including oxidative stress.24 In the present study immunohistochemistry showed a mild reduction in c-Jun and a marked reduction in c-Fos IR in Tg mice compared with Wt mice 2 hours after a longer ischemic duration (ie, 60 minutes) (Figure 2). SOD1 overexpression was associated with reduced c-Jun and c-Fos protein levels in ischemic brain (Figure 3). Moreover, EMSAs demonstrated a significant increase in DNA binding activity after FCI that was dramatically decreased in SOD1 Tg mice (Figures 4 and 5). A smaller quantity of c-Jun and c-Fos proteins may contribute to decreased DNA binding activity in SOD1 Tg mice. Thus, overexpression of SOD1 suppressed ischemia-induced activation of c-Jun and c-Fos through a decrease in protein levels and DNA binding activity, suggesting that superoxide radicals modulate AP-1 activity after focal ischemia in a multistep process. Our observations have been supported by recent studies which showed that the methamphetamine-induced increases in cortical c-fos mRNA were attenuated by overexpression of SOD1. This result indicated that superoxide radicals might play an important role in the activation of c-fos after methamphetamine-induced monoaminergic toxicity.20 In addition, we have shown previously that superoxide radicals modulate both immediate early gene (c-fos and c-jun) and heat shock genes (hsp70) mRNA expression in the hippocampus and cortex after kainic acid–induced seizures.25

AP-1 has been widely viewed as an oxidative stress response factor. AP-1 binding activity was remarkably increased after ischemia/reperfusion. This finding is in accordance with previous reports in rat brain.16,30 In the present study we demonstrated that SOD1 overexpression significantly attenuated FCI-induced AP-1 binding activity. This result suggested that ROS could induce changes in AP-1 binding activity. AP-1 acts as a redox-sensitive transcription factor in several cell types and is activated by treatment with agents such as superoxide and H2O2.31,32 Our results would support the notion that free radicals are associated with FCI-induced AP-1 activation as part of ischemia pathophysiology. However, little is known regarding the precise mechanisms underlying ROS-mediated AP-1 activation. Protein kinase C, arachidonic acid, and phospholipase A2 are suspected to be involved in H2O2 induction of c-jun33 and c-fos expression in vascular smooth muscle cells. The possibility of involvement of protein kinase C, arachidonic acid, and/or phospholipase A2 in the FCI-induced c-Jun and c-Fos expression remains to be determined. Paradoxically, in addition to oxidative stress, a number of antioxidants, including dithiocarbamates and N-acetylcysteine, have been shown to stimulate the DNA binding and transcriptional activity of AP-1 in some cell types.35 Clearly, additional studies are needed to clarify the role of AP-1 in ROS-mediated gene expression after FCI.

The activity and function of AP-1 transcriptional complexes may be regulated at different levels, including the following: (1) transcription activation of AP-1 genes; (2) the overall combination of the different subunits at a given time; (3) posttranslational modifications; and (4) interactions with other non–AP-1 proteins.36 The abundance of AP-1 proteins is thought to be regulated at the transcriptional level of the respective genes. Recent findings have also suggested that the stability of c-Jun and c-Fos proteins may be modulated by phosphorylation.37,38 Our data suggest that AP-1 binding activity is upregulated after FCI and that c-Jun plays a significant role in this transcription factor complex. The changes in the protein levels are too small to explain the increase in DNA binding activity after FCI. Therefore, posttranslational modifications may likely be involved. The ability of c-Jun to activate gene transcription is strongly
increased by phosphorylation at 2 serines\textsuperscript{39} by c-Jun N-terminal kinases (JNKs).\textsuperscript{40} Phosphorylation by JNK leads to increased transcriptional activity of c-Jun in the absence of increased abundance of c-Jun.\textsuperscript{41} Phospho-c-Jun appears to be more important than the total amount of c-Jun.\textsuperscript{42,43} Since JNK may be activated by free radicals, it is possible that ROS induce AP-1 activity via the JNK pathway after ischemia/reperfusion. Oxidative stress has been shown to activate the JNK pathway, which may lead to phosphorylation of c-Jun. This posttranslational modification of c-Jun can alter the transactivation activity of AP-1 complexes, which in turn can stimulate downstream target genes. Many of these target genes are involved in the control of the cell cycle and/or apoptosis.\textsuperscript{36} In addition to posttranslational modifications, ROS may regulate the DNA-binding activity of AP-1 by modulating the redox status of a critical set of cysteines in the DNA-binding domains of both Fos and Jun.\textsuperscript{44} Further investigations will be necessary to clarify whether early AP-1 activity is induced through phosphorylation of specific subunits by selectively activated kinase pathways.

We have demonstrated that c-Jun is an important component of increased AP-1 DNA binding after FCI (Figure 4B). Although c-fos and c-jun expression may be neuroprotective in selected experimental paradigms,\textsuperscript{45,46} activation of AP-1 in the neurons has been linked to apoptosis in nervous system. In particular, c-Jun has been thought to initiate degeneration via de novo protein synthesis of apoptotic effectors.\textsuperscript{47} Whether c-Jun serves a role in the FCI-activated apoptosis described previously\textsuperscript{48} remains to be elucidated. Taken together, these data suggest that c-Jun was the major constituent of the AP-1 DNA complex after FCI. However, AP-1 is a dimeric complex consisting of members of the Jun and Fos families. The possibility of involvement of other members of Jun and Fos families remains to be elucidated.

In conclusion, we have shown that overexpression of SOD1 attenuates early activation of AP-1 after transient FCI in mice. This event may block the expression of downstream target genes that are deleterious and thereby reduce the infarction volume. This work provides the foundation for redox-mediated gene therapies directed at amelioration of ischemia/reperfusion injury. Further studies will be directed at the elucidation of the genes activated by this transcription factor.

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