Early Decrease of XRCC1, a DNA Base Excision Repair Protein, May Contribute to DNA Fragmentation After Transient Focal Cerebral Ischemia in Mice

Miki Fujimura, MD; Yuiko Morita-Fujimura, MS; Taku Sugawara, MD, PhD; Pak H. Chan, PhD

Background and Purpose—DNA damage and the DNA repair mechanism are known to be involved in ischemia/reperfusion injury in the brain. The x-ray repair cross-complementing group 1 (XRCC1) protein plays a central role in the DNA base excision repair pathway by interacting with DNA ligase III and DNA polymerase β. The present study examined the protein expression of XRCC1 and DNA fragmentation before and after transient focal cerebral ischemia (FCI).

Methods—Adult male CD-1 mice were subjected to 60 minutes of FCI by intraluminal blockade of the middle cerebral artery. XRCC1 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). The spatial relationship between XRCC1 expression and DNA damage was examined by double staining with XRCC1 and TUNEL after FCI.

Results—Immunohistochemistry showed the nuclear expression of XRCC1 in all regions of the control brains and that it was predominant in the hippocampus. The XRCC1 level was markedly reduced in the caudate putamen at 10 minutes, further decreased in the entire middle cerebral artery territory at 1 hour, and remained reduced until 4 and 24 hours after FCI. Western blot analysis of the normal control brain showed a characteristic band of 70 kDa, which decreased after FCI. A significant amount of DNA fragmentation was detected by DNA gel electrophoresis 24 hours but not 4 hours after FCI. Double staining showed that the neurons that lost XRCC1 immunoreactivity became TUNEL positive.

Conclusions—These results suggest that the early decrease of XRCC1 and the failure of the DNA repair mechanism may contribute, at least in part, to DNA fragmentation after FCI. (Stroke. 1999;30:2456-2463.)

Key Words: apoptosis ■ cerebral ischemia, focal ■ DNA fragmentation ■ DNA repair
with the occurrence of DNA fragmentation in hippocampal CA1 neurons after transient global ischemia. These facts suggest that the reduction of DNA BER protein and a failure of the DNA repair mechanism may contribute to DNA damage after ischemia/reperfusion.

To further investigate the relationship between DNA BER protein expression and DNA damage after ischemia, we analyzed the expression of the XRCC1 protein, which is known to play a central role in DNA BER, before and after transient FCI. We further sought to clarify both temporal and anatomic relationships between XRCC1 alteration and the occurrence of DNA fragmentation.

Materials and Methods
Focal Cerebral Ischemia
Adult male CD-1 mice (weight, 35 to 40 g) were subjected to transient focal ischemia by intraluminal middle cerebral artery (MCA) blockade with a nylon suture, as described. 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 cannulation. 10 minutes after occlusion, and 10 minutes after reperfusion. After the midline skin incision, the left external carotid artery was exposed, and its branches were electrocoagulated. An 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.

Immunohistochemistry of XRCC1
Anesthetized animals were perfused with 10 U/mL heparin and subsequently with 4% formaldehyde in 0.1 mol/L PBS (pH 7.4) after 10 minutes, 1 hour, 4 hours, and 24 hours of reperfusion after ischemia. Brains were removed, postfixed for 12 hours in 4% formaldehyde, sectioned at 50 μm on a vibratome, and processed for immunohistochemistry. As a negative control, sections were incubated without primary antibodies. For histological assessment, alternate slices from each brain section were stained with cresyl violet. The sections were first reacted with mouse monoclonal antibody against XRCC1 (Lab Vision) at a dilution of 1:100. To avoid cross-reaction between the secondary antibody and mouse immunoglobulin in the tissue, immunohistochemistry was performed with the use of the DAKO ARK peroxidase kit. After development with diaminobenzidine (DAB), the sections were mounted on slides and counterstained with methyl green solution.

Western Blot Analysis
Whole cell protein extraction was performed. To confirm the early reduction of XRCC1 expression in the ischemic brain, we used the samples obtained from the ischemic core on the ipsilateral side and homologous tissue from the contralateral side. Approximately 30 mg of both ipsilateral stratum 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 Teflon Dounce homogenizer (Wheaton). Equal amounts of the samples (10 μL) were loaded per lane. The primary antibodies were either 1:1000 dilution of monoclonal antibody against XRCC1 (Lab Vision) or 1:10 000 dilution of anti-β-actin monoclonal antibody (Sigma). For XRCC1 detection, Western blots were performed with horseradish peroxidase–conjugated anti-mouse immunoglobulin G with the Boehringer Mannheim chemiluminescent system. As the internal control, Western blot analysis of β-actin was performed with horseradish peroxidase–conjugated anti-mouse immunoglobulin G reagents (Amer- sham International).

Gel Electrophoresis
Animals were killed 4 and 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 contralateral 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 with To-Pro-1 dye (Molecular Probes). Gel electrophoresis for detecting DNA laddering was performed according to the manufacturer’s instructions ( Trevigen). 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 with 5% powdered milk (BioRad) in PBS for 30 minutes and incubated with streptavidin–horseradish peroxidase conjugate (Trevigen) 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.

In Situ Labeling of DNA Fragmentation
Frozen brain sections at the level of the caudate putamen that showed typical infarction were stained by an in situ technique (mammalian deoxyribonucleotidyl transferase–mediated uridine 5′-triphosphate-biotin nick end-labeling [TUNEL] reaction) to detect the DNA free 3′-OH ends, as described. Briefly, frozen brain sections were fixed for 30 minutes in 3.7% formaldehyde in 0.1 mol/L PBS, pH 7.4. The slides were then washed 2× in SSC (150 mmol/L sodium chloride, 15 mmol/L sodium citrate, pH 7.4) for 5 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, then the slides were washed for 15 minutes with 0.175 mol/L sodium acetate. Staining was visualized with the use of 0.025% DAB and 0.075% H2O2 in PBS with 0.4 mg/mL nickel sulfate. The slides were rinsed with water, stained with methyl green for 10 minutes, dehydrated, and mounted.

Double Labeling With XRCC1 and DNA Fragmentation
To clarify the spatial relationship between XRCC1 expression and DNA damage, we performed double staining of XRCC1 antibody and TUNEL, as previously described. After transcardiac perfusion, fixed sections were immunostained with XRCC1 antibody as described above, the sections were mounted on glass slides, 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 staining as described above. Staining was visualized with the use of 0.025% DAB and 0.075% H2O2 with nickel sulfate. The slides were rinsed with water, stained with methyl green for 10 minutes, dehydrated, and mounted.

Quantification and Statistical Analysis
The number of XRCC1-immunoreactive cells and methyl green–positive cells was counted in a high-power field (×400) and expressed by the percentage of XRCC1-positive cells. The number
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 time point (Table). The time-dependent increase of infarction in the mouse brains with the use of intraluminal suture blockade is consistent with previous reports that employed the same focal stroke model in mice. \(^\text{14,15}\)

Physiological Variables During and After Ischemia

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Occlusion</th>
<th>10 Minutes After Occlusion</th>
<th>10 Minutes After Reperfusion</th>
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<tbody>
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<td>MABP, mm Hg</td>
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<td>70.5±4.4</td>
<td>66.8±0.5</td>
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<td>$P_{aO_2}$, mm Hg</td>
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<td>136.4±3.7</td>
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<td>$P_{aCO_2}$, mm Hg</td>
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<tr>
<td>pH</td>
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</tbody>
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MABP indicates mean arterial blood pressure.

Immunohistochemistry Showed the Constitutive Expression of XRCC1 in Normal Adult Mouse Brain, Which Decreases After Ischemia

The XRCC1 protein was constitutively expressed in the entire region of the normal mouse brain (Figure 1A through 1D). It was mainly expressed in the nucleus and had a regional predominance in the hippocampus, including the CA1 (Figure 1C) and CA3 regions and the dentate gyrus (Figure 1D), compared with the MCA territory cortex (Figure 1A) and caudate putamen (Figure 1B). After 10 minutes of reperfusion that followed 1 hour of FCI, reduction of XRCC1 was observed in the lateral caudate putamen (Figure 1E) but not in the cortex (data not shown). After 1 hour of reperfusion, XRCC1 expression was reduced in the entire MCA territory, including the caudate putamen (Figure 1F) and cortex (data not shown). This reduction was sustained at 4 hours (Figure 1G) and 24 hours (Figure 1H) in the caudate putamen, as well as in the MCA territory cortex. In contrast, the contralateral brain including the caudate putamen (Figure 1I) showed no significant alteration of XRCC1 expression until 24 hours after FCI. There was no immunoreactivity in the control specimens, which were treated without a primary antibody (data not shown).

![Figure 1](http://stroke.ahajournals.org/)

**Figure 1.** Immunohistochemistry of XRCC1 (counterstained by methyl green). Nuclear distribution of XRCC1 is shown in the entire region of the normal brain, including the MCA territory cortex (A), caudate putamen (B), CA1 region of the hippocampus (C), and dentate gyrus (D) of the hippocampus. After 10 minutes of reperfusion following FCI, reduction of XRCC1 was observed in the lateral caudate putamen (E) but not in the cortex (data not shown). After 1 hour of reperfusion, XRCC1 expression was reduced in the entire MCA territory, including the caudate putamen (F) and cortex. This reduction was sustained at 4 (G) and 24 hours (H) in the entire MCA territory, including the caudate putamen (G, H). The contralateral caudate putamen showed no significant alteration of XRCC1 expression until 24 hours after FCI (I) compared with nonischemic caudate putamen (B). Bar=0.02 mm.
Western Blot Analysis of XRCC1 Protein Expression After Transient MCA Occlusion
XRCC1 immunoreactivity was evident as a band of molecular mass 70 kDa of the whole cell fraction from the nonischemic brain (lane 1, top panel) and was significantly decreased 4 hours after 60 minutes of MCA occlusion (lane 2, top panel). In contrast, a consistent amount of β-actin immunoreactivity is shown in the bottom panel. C indicates nonischemic control; I, ischemic sample.

DNA Laddering Was Detected by Genomic DNA Gel Electrophoresis
To detect the occurrence of apoptosis as characterized by intranucleosomal DNA fragmentation, we analyzed DNA from both the ischemic brain and the homologous sample on the contralateral side. DNA laddering was absent in both the control tissue and ischemic tissue 4 hours after ischemia (Figure 3, lanes C and 4). A significant amount of DNA laddering was detected 24 hours after ischemia (Figure 3, lane 24).

Early Decrease of XRCC1 Preceded the Occurrence of DNA Fragmentation Detected by TUNEL Staining After FCI
To elucidate the spatial and temporal profile of DNA fragmentation, we examined in situ labeling of DNA breaks in the infarcted brain sections (Figure 4), as described. TUNEL staining did not label normal neuronal cells in the noninfarcted area (Figure 4A) or the cells in the infarcted area 4 hours after ischemia (Figure 4B). In contrast, 2 different patterns of staining were observed in the neuronal cells in the infarcted area 24 hours after ischemia (Figure 4C). Some neuronal cells in the infarcted area were densely labeled in their nuclei, accompanied by small particles around the nuclei that resembled apoptotic bodies (Figure 4C, arrows). These cells are compatible with those in the apoptotic cell death process, as previously described. Besides these typical apoptotic neuronal cells, slightly TUNEL-stained cells were also observed (Figure 4C, arrowheads). These cells showed diffuse nuclear and cytoplasmic TUNEL staining, which is consistent with necrotic cells. To quantify the apoptotic neurons, the number of TUNEL-positive cells was counted 4 and 24 hours after FCI. As illustrated in Figure 5, only a small number of TUNEL-positive cells were detected 4 hours after ischemia. They were significantly increased 24 hours after ischemia both in the caudate putamen (Figure 5A; P<0.0001) and the cortex (Figure 5B; P<0.01). As shown in Figure 5, the reduction of XRCC1 expression preceded the occurrence of DNA fragmentation, which was detected by gel electrophoresis and TUNEL at 24 hours but not at 4 hours after FCI. In addition, there was no significant difference in the number of XRCC1-positive cells between nonischemic controls and the contralateral side after FCI (data not shown).

Double Staining With XRCC1 and TUNEL Showed the Spatial Relationship Between XRCC1 Loss and DNA Damage
Double staining with XRCC1 and TUNEL 24 hours after ischemia showed a significant amount of TUNEL-positive cells with the characteristic features of apoptosis (densely labeled in their nuclei, accompanied by apoptotic bodies) in the entire MCA territory on the ipsilateral side, including the cortex (Figure 6A, arrowheads). None of these cells showed XRCC1 immunoreactivity. On the other hand, some cells had a faint expression of XRCC1 (Figure 6A, asterisks), but none were TUNEL positive. No TUNEL-positive cells were seen in the contralateral side at this time point (Figure 6B). There was no immunoreactivity in the control specimens, which were treated without a primary antibody (data not shown).

Discussion
The present study provides the first evidence that the early decrease of the DNA BER protein XRCC1, which is constitutively expressed in normal mouse brain, may play a role, at least in part, in the DNA-damaged cell death pathway in transient FCI. The immunohistochemistry of the normal mouse brain showed for the first time that the XRCC1 protein was constitutively expressed in the entire mouse brain and has a nuclear distribution and a regional predominance in the hippocampus. As early as 10 minutes after reperfusion, a significant reduction of the XRCC1 expression was seen in the lateral caudate putamen (Figure 1E), extended to the entire MCA territory after 1 hour of reperfusion (Figure 1F), and remained reduced until 4 and 24 hours after transient FCI. This reduction was confirmed by Western blot analysis of
whole cell extract (Figure 2). DNA fragmentation was not detected 4 hours after FCI, while it markedly appeared 24 hours after FCI, as shown by DNA gel electrophoresis (Figure 3) and TUNEL staining (Figure 4). Finally, the spatial relationship between the loss of XRCC1 expression and DNA fragmentation was shown by the double staining with XRCC1 and TUNEL (Figure 6A). These results indicate that early reduction of XRCC1 precedes the occurrence of DNA fragmentation in the ischemic MCA territory that is destined to show necrosis and/or apoptosis and that the reduction of XRCC1 may contribute to DNA-damaged cell death after FCI. Although XRCC1 has no known catalytic activity for undergoing DNA repair, it is believed to play a central role in the DNA BER pathway by interacting with DNA polymerase β and DNA ligase III.1-6 Furthermore, using the same FCI model, we previously reported that the DNA BER enzyme APE/Ref-1 was reduced in the entire MCA territory on the ischemic side before the peak of DNA fragmentation.12 Thus, it is suggested that the tissue in the ischemic MCA is deficient in at least 3 major BER enzymes, such as APE/Ref-1, DNA polymerase β, and DNA ligase III, which can be extremely detrimental for the cells in overcoming DNA damage caused by ischemia/reperfusion injury.

Programmed cell death and the DNA-repairing mechanism are both assumed to play important roles in cerebral ischemia.10,13,15,16,19 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).20 The interleukin-1β-converting enzyme family caspases are the human homologues of the nematode Caenorhabditis elegans, Ced-3,21 and are considered to play a critical role in programmed cell death.20 One substrate for caspase is PARP, whose proteolytic cleavage results in a dysfunctional PARP that is unable to contribute to repair or genomic maintenance.20 Furthermore, the Ca2+/Mg2+-dependent endonuclease that generates internucleosomal DNA cleavage characteristic of apoptosis is negatively regulated by poly(ADP)-ribosylation.

Figure 4. End-labeling of DNA fragmentation in brain sections at the level of the caudate putamen (3 mm from the anterior tip) by TUNEL staining. Photomicrographs of the caudate putamen of the nonischemic hemisphere (A), caudate putamen of the ischemic hemisphere 4 hours after ischemia (B), and caudate putamen of the ischemic hemisphere 24 hours after ischemia (C) are shown. Two patterns of staining are shown 24 hours after ischemia. TUNEL-stained cells that show cell shrinkage, chromatin condensation, and small apoptotic bodies are considered apoptotic cells (C, arrows). Necrotic neurons with diffuse light labeling of DNA fragmentation are also shown (C, arrowheads). Bar=0.02 mm.

Figure 5. Temporal profiles of the percentage of XRCC1 immunoreactive cells and TUNEL-positive cells in the caudate putamen (A) and MCA territory cortex (B). The percentage of XRCC1-positive cells was significantly reduced as early as 1 hour after transient ischemia in both the caudate putamen (P<0.0001) and the cortex (P<0.005) compared with the nonischemic controls. The reduction was sustained in the caudate putamen 4 and 24 hours after ischemia. XRCC1 was further decreased in the cortex 4 and 24 hours after ischemia. No increase in the number of TUNEL-positive cells was seen in either territory. A significant increase in TUNEL-positive cells was detected in the caudate putamen (P<0.0001) and the cortex (P<0.01) 24 hours after ischemia, much later than the reduction in XRCC1. #P<0.05; *P<0.01; **P<0.001; ***P<0.0001.
mice after transient cerebral ischemia, suggesting that PARP knockout suggested its role in DNA damage, they clearly show the XRCC1 interacts with PARP by its central region containing after ischemia. Interestingly, a recent study showed that PARP activation plays a detrimental role in neuronal damage.

Therefore, inactivation of PARP could increase DNA cleavage and contribute to programmed cell death. On the other hand, excessive activation of PARP is believed to also be deleterious because it might cause energy depletion and ultimate cell death. In fact, most recent studies with PARP knockout mice implicate the deleterious role of PARP in FCI. Although these studies did not provide the data that suggested its role in DNA damage, they clearly show the marked reduction of the infarct volume in PARP knockout mice after transient cerebral ischemia suggesting that PARP activation plays a detrimental role in neuronal damage after ischemia. Interestingly, a recent study showed that XRCC1 interacts with PARP by its central region containing a BRCT module. In this study, overexpression of XRCC1 in Cos-7 and HeLa cells resulted in the marked decrease of PARP activity in vitro, which suggests that XRCC1 may negatively regulate the activity of PARP. On the basis of these findings, the early reduction of XRCC1 during reperfusion in the present study could contribute to the early activation of PARP and subsequent energy depletion and then could be extremely detrimental for the cells in overcoming ischemia/reperfusion injury.

The selective reduction of XRCC1 after FCI was implied by our following findings. Western blot analysis showed the significant loss of XRCC1 4 hours after FCI (Figure 2, top panel), while there was no alteration of β-actin after FCI (Figure 2, bottom panel). Furthermore, immunohistochemistry showed the marked reduction of XRCC1 in the entire ischemic area 1 hour after FCI (Figure 1), when the immunoreactivity of the nuclear neuronal marker NeuN showed much less alteration in the ischemic area (data not shown). The exact mechanism by which the selective reduction of XRCC1 occurs after FCI is unclear. It is conceivable that XRCC1 reduction is caused by a selective decrease of XRCC1 protein synthesis, an increase of protein degradation, a difference in the half-life of the protein, or a difference in posttranslational regulation. Further examination is warranted to clarify this issue. Nevertheless, we have previously reported that APE/Ref-1, another DNA BER protein, decreased rapidly after focal cerebral ischemia/reperfusion in mice, and our preliminary study using the same stroke model demonstrates that mice overexpressing the endogenous antioxidant enzyme superoxide dismutase-1 (SOD1) show less reduction of APE/Ref-1 after ischemia/reperfusion compared with wild-type mice. Therefore, it is conceivable that the mechanism of the selective loss of XRCC1 could also be linked to oxidative stress. In fact, our transient FCI model is known to be associated with reactive oxygen species, as shown by our previous study using SOD1 transgenic mice and knockout mice deficient in mitochondrial manganese SOD could be extremely detrimental for the cells in overcoming ischemia/reperfusion injury.

In conclusion, we have shown that XRCC1 rapidly decreased after transient FCI in mice and that this reduction...
preceded the occurrence of ischemic apoptosis and infarction. Furthermore, a spatial relationship between XRCC1 loss and DNA damage was shown by double staining with XRCC1 immunohistochemistry and TUNEL. These results indicate that the early decrease of XRCC1 and the failure of the DNA repair mechanism may contribute, at least in part, to DNA-damaged cell death after transient FCI.

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References

Normal cellular metabolism, particularly mitochondrial respiration, produces reactive oxygen species (ROS) (e.g., hydrogen peroxide and superoxide anion and hydroxyl radicals) as end products. Under physiological conditions, excessive ROS are neutralized by endogenous antioxidants (e.g., ascorbate, α-tocopherol, β-carotene, and glutathione) and antioxidant enzymes (e.g., superoxide dismutase, catalase, and glutathione peroxidase). 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 crosslinking 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.

Individual bases in DNA may be oxidized to form adducts such as 8-hydroxyguanine (8-OH-Gua), 8-hydroxyadenine (8-OH-Ade), 4,6-diamino-5-formamidopyrimidine (FapyAde), 2-hydroxyadenine (2-OH-Ade), 5-hydroxycytosine (5-OH-Cyt), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua), 5-hydroxy-5-methylhydantoin (5-OH-5-MeHyd), and 5-hydroxyhydratoin (5-OH-Hyd). These oxidative DNA adducts are potentially mutagenic or lethal. The XRCC1 protein is involved in base excision repair and therefore, may contribute to enhance genome integrity and stability after ischemic insult. In the preceding article by Fujimura et al, the expression of the XRCC1 protein was reduced in the ischemic region in a mouse MCAO model. Suppressed XRCC1 expression preceded DNA fragmentation and cell death. This finding raises the possibility that a defective DNA repair machinery caused by reduced expression of DNA repair proteins such as XRCC1 may contribute to irreversible DNA damage and ultimate cell demise after focal cerebral ischemia-reperfusion.

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