Transient Increase of Manganese–Superoxide Dismutase in Remote Brain Areas After Focal Photothrombotic Cortical Lesion

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Background and Purpose—Free radicals including superoxide are responsible for postlesional cytotoxicity. In contrast to the constitutive CuZn–superoxide dismutases (SODs), manganese–superoxide dismutase (Mn–SOD) is inducible and has the potential to protect neurons by its superoxide dismutating activity. Therefore, we studied the presence and the regional changes in Mn–SOD within the brain after focal cortical ischemia.

Methods—Focal cortical photothrombotic lesions were produced in the hindlimb region of rat brains. Animals were anesthetized and transcardially perfused with Zamboni’s fixative. Mn–SOD was immunohistochemically localized using an antisera against rat-Mn–SOD. Changes in Mn–SOD immunoreactivity were quantified by image analysis.

Results—Focal photothrombosis caused a perilesional increase in Mn–SOD after 24 hours, followed by a further significant increase at 48 hours in perilesional cortex, ipsilateral corpus callosum, hippocampus, and thalamus, as well as in a homotopic cortical area within the nonlesioned hemisphere. Up to day 2, Mn–SOD was present in neurons and astrocytes. Up to day 7, Mn–SOD increased in the entire ipsilateral and contralateral cortex but remained higher elevated in the ipsilateral hippocampus and thalamus. Thereafter, Mn–SOD decreased globally but remained elevated in some cortical neurons up to day 60.

Conclusions—The early transient increase of Mn–SOD in distinct brain regions, which are functionally connected via afferents and efferents, suggests that these regions are affected by the injury. It suggests that Mn–SOD protects the cells in these regions from superoxide-induced damage and therefore may limit the retrograde and anterograde spread of neurotoxicity. (Stroke. 1998;29:203-211.)

Key Words: astrocytes ■ brain ■ diaschisis ■ homotopic cortical area ■ manganese–superoxide dismutase

Superoxide radicals react rapidly with NO to form highly cytotoxic peroxynitrite,1,2 which acts through lipid peroxidation.3-5 Superoxide dismutases are known to be the most effective endogenous scavengers of superoxide radicals,6,7 and changes in both CuZn-SOD and Mn–SOD affect processes of aging and learning.8 Furthermore, mutations within the CuZn-SOD gene leading to alterations in SOD activity contribute to several neuropathological conditions,9 whereas SODs coupled to polyethylene glycol,10,11 lecithinized SOD,12 and SOD entrapped in liposomes13 act neuroprotectively.

In ischemia, a reduction of SODs within the ischemic brain areas has been observed.13 Mice that express the human CuZn-SOD gene are even partially resistant to cerebral ischemia.9 Superoxide radicals are of special importance after transient ischemia in reperfused brain regions in which such radicals are massively generated and contribute to the so-called reperfusion injury.1,4,6,15,16

Most of the studies cited above focused on CuZn-SOD. Recently it has been shown that mice deficient of mitochondrial Mn–SOD suffer from early neurodegeneration.17 The induction of Mn–SOD under pathological conditions is variable and related mainly to the type of injury. It has also been found that Mn–SOD expression is directly correlated with the grade of brain tumors in man.18

Rats are often used in studies on postlesional changes and regeneration after experimentally induced infarcts.19 All studies on postlesional changes in Mn–SOD used, thus far, models of global ischemia or MCAO.14,20,21 MCAO results in massive ischemic lesions, including damage to subcortical regions and the choroid plexus.22,23 We were interested in changes induced by small clearly defined cortical lesions. We therefore used the photothrombosis model of closed head injury developed by Watson et al.24 This widely used model25 results in lesions of defined size within the frontal motor cortex and hindlimb area, causing minor deficits in grip-strength within 24 hours. These deficits are restored after 18 days.26 Therefore, this model

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allows to differentiate those specific effects that are a direct consequence of the ischemic focus. In the vicinity of the lesion, within the so-called penumbra, neurons and glial cells are at risk of becoming damaged by spreading depressions or apoptosis. Additionally in remote brain regions, e.g., contralateral to the lesion, functional alterations that follow an initial description of von Monakow and are called diaschisis may be observed.

Diaschisis effects are usually regarded as functional changes caused by the interruption of input from the lesioned brain region, and after the initial description they should be transient. Here we describe that there is an induction of Mn–SOD in widespread remote brain areas after focal ischemia. This finding indicates that protection against superoxide radicals takes place not only around the lesioned area but may contribute to the widespread remote diaschisis effects.

Materials and Methods

Focal cerebral ischemia was induced in the hindlimb region by the Rose bengal technique described by Watson et al. 

Results

In the brains of control rats, Mn–SOD-related immunoreactivity showed no significant differences between the left and right hemispheres at the various fronto-occipital levels (Fig 1a and b). All immunoreactive neurons were nonpyramidal cells representing bi- and multipolar interneurons. We observed two types of interneurons with regard to staining intensity: more than 90% of all neurons exhibited weak immunostaining, whereas 5% to 10% of the neurons showed stronger staining. In controls, Mn–SOD-positive neurons were found in all layers of the retrosplenial cortex, whereas neurons in the adjacent frontal motor cortex were restricted mostly to layer VI. In parietal cortices they were located throughout layers III–V. In the hippocampus, Mn–SOD-positive interneurons were present in

Immunohistochemistry for Mn–SOD

Rehydrated paraffin sections were rinsed twice in PBS (10 mmol/L + 0.9% NaCl, pH 7.4), incubated in PBS with 5% H2O2 to inhibit endogenous peroxidase, and rinsed 3x5 minutes in PBS. Sections were then incubated for 30 minutes in TBS (50 mmol/L + 0.9% NaCl) containing 10% normal goat serum (Vector Laboratories), followed by an overnight incubation at 4°C, in TBS containing anti-rat Mn–SOD antibody at a dilution of 1:500.

Sections were rinsed 3x5 minutes in TBS, incubated for 2 hours in TBS with biotinylated anti-rabbit IgG (1:100; Vector Laboratories), rinsed 3x5 minutes in TBS, and incubated for 90 minutes in TBS with ABC-Complex (Vector Laboratories) according to manufacturer's instructions. After 3 rinses in 0.05 mol/L Tris–HCl buffer (pH 7.6) sections were stained with diaminobenzidine, rinsed in TB, dehydrated in ethanol, cleared in xylene, and coverslipped with DePeX.

Frozen, free-floating sections were rinsed four times in PBS (15 minutes each). Some sections were stored, whereas others sections were rinsed in TBS (pH 8.0) for 5 minutes followed by an incubation at +37°C in the same buffer containing 0.3% Triton–X-100, 1 mg /ml-NADPH/mL (Boehringer, Mannheim) and 0.25 mg/ml Nitroblue tetrazolium (Boehringer, Mannheim). The development of the blue tetrazolium precipitate, which reveals NADPH-d activity, was monitored microscopically. The location of NADPH-d corresponds to that of nitric oxide synthases the enzymes, which produce NO radicals that in turn react to peroxynitrite in the presence of superoxide.

Sections stained for NADPH-d and those stored in PBS were rinsed twice in PBS followed by an incubation in PBS with 3% H2O2 for 30 minutes. After two rinses in PBS and two rinses in TBS, sections were immunohistochemically stained for Mn–SOD as described above except for the following modifications: each rinse was extended to 15 minutes, the rat-Mn-SOD antibody was used at a dilution of 1:1000 and the incubation period was 48 hours at 4°C. Stained, free-floating sections were mounted in chrome alum gelatin onto glass slides, air-dried, cleared in xylene, and coverslipped with DePeX. Control sections were processed similarly, but primary antibody was omitted.

The antibody used against rat-Mn–SOD was produced and characterized by Kurobe and Kato and others and Western blots obtained with this antibody in rat brain extracts are published by Sugaya et al. On frozen free-floating sections from our Wistar rats, the rat-Mn–SOD antibody stained the same cerebral cells as a commercially available monoclonal anti-human Mn–SOD antibody (Bender, Vienna, Austria) at a final dilution of 1:100 (data not shown).

Sections were microscopically evaluated and photographed using a Zeiss Axiomat (Zeiss, Oberkochern) and Agfa APX 25 film. Sections were subsequently processed by a computerized image analyzer IBAS (Kontron KS 400); brain regions were marked using a graphics tablet IBAS (Kontron KS 400); brain regions were marked using a graphics tablet

Histological Preparation

Rats were anesthetized with pentobarbital and perfused at various times (4 hours, and 1, 2, 3, 5, 7, and 14 days; n=5 to 7 each) after inactivation with physiological saline containing 2.2 mmol/L CaCl2 (100 mL, ice-cold) followed by 300 mL Zamboni fixative with 0.05% glutaraldehyde. Brains were dissected, post-fixed overnight in the same fixative, and either cut on a vibratome or submerged in 30% sucrose in PBS (pH 7.3) for 48 hours, frozen on dry-ice and sectioned with a cryotome. Additional lesioned rats were decapitated (n=5 to 7) at 4 hours, and 1, 2, 3, 5, 7, 14, 30, and 60 days after lesion. The brains were immersion fixed in Bodian’s fixative, dehydrated in increasing ethanol concentrations, cleared in methylbenzoate, and embedded in paraffin. Ten-micrometer sections were cut, deparaffinized in xylene, rehydrated, and used for immunostaining. The latter procedure resulted in good preservation of the lesion core. The core was frequently lost in frozen free-floating sections.
the stratum pyramidale with highest packing densities in subiculum and CA3. The highest packing density within the hippocampal formation occurred in the polymorphic cell layer of the dentate gyrus. Only faintly Mn–SOD-positive fibers were present in thalamic nuclei. In cortex as well as in the hippocampus, NADPH-d-positive interneurons were intermingled with those positively stained for Mn–SOD.

In lesioned rats, the amount of immunoreactivity for Mn–SOD increased in interneurons and pyramidal neurons during the first 24 hours within the core of the lesion (not shown). A similar increase occurred in the contralateral cortex (c-f). Increased Mn–SOD is present in ipsilateral corpus callosum, hippocampus, and thalamic nuclei. c, core; DG, dentate gyrus; DLG, dorsal lateral geniculate nucleus; Fr2/1, frontal motor cortex 2 + 1; h, hippocampus; HL, hindlimb area; LPT, lateral posterior thalamic nucleus; LDT, lateral dorsal thalamic nucleus; Oc2, occipital area 2 (L, lateral; ML, mediolateral, MM, mediomedial); Par1, parietal cortex 1; rf, rhinal fissure; RS, retrosplenial cortex (A, agranular; G, granular). Scale bar=1 mm.

The active perilesional rim was most pronounced in the medial retrosplenial areas. These areas were characterized by more Mn–SOD-positive cells than other regions even in controls. In the corpus callosum underlying the core, single astrocytes appeared strongly stained. These cells formed a darkly stained line at the transition zone between corpus callosum and cortical layer VI (Figs 1c, 1d, and 4a). Mn–SOD also increased within the ipsilateral hemisphere, the subiculum, the alveus and the stratum pyramidale areas CA1 to 3, and dentate gyrus of the hippocampus, as well as in dorsal thalamic nuclei (Figs 1c, 1d, 3, and 5). A significant increase in immunoreactivity also occurred within the contralateral cortex. The contralateral area, with increased numbers of Mn–SOD-positive cells was, however, larger than the site of the lesion itself and showed clear differences in the fronto–occipital direction. At the most frontal level of the lesion we observed an almost exact mirror focus, but the width of this focus increased toward occipital levels (Figs 1c, 1d, 2, 3a through d, 5). At the most occipital level the contralateral focus spread down to the dorsal aspect of the rhinal fissure. Surprisingly enough, no such dramatic spread was seen medial to the mirror focus. It is noteworthy, that no dramatic upregulation of Mn–SOD oc-

Figure 1. Distribution of Mn–SOD immunoreactivity within whole brain sections cut approximately at bregma levels −2.12 mm (a, c, e) or −3.80 mm (b, d, f); Control animals (a, b) and at days 2 (c, d) and 7 (e, f) after lesion. Mn–SOD is enhanced in the core and the perilesional rim (r) at day 2 (c, d) and in the entire perilesional cortex at day 7 (e, f). A similar increase occurred in the contralateral cortex (c–f). Increased Mn–SOD is present in ipsilateral corpus callosum, hippocampus, and thalamic nuclei. c, core; DG, dentate gyrus; DLG, dorsal lateral geniculate nucleus; Fr2/1, frontal motor cortex 2 + 1; h, hippocampus; HL, hindlimb area; LPT, lateral posterior thalamic nucleus; LDT, lateral dorsal thalamic nucleus; Oc2, occipital area 2 (L, lateral; ML, mediolateral, MM, mediomedial); Par1, parietal cortex 1; rf, rhinal fissure; RS, retrosplenial cortex (A, agranular; G, granular). Scale bar=1 mm.

Figure 2. Changes in the numbers of Mn–SOD immunoreactive neurons (A) and astrocytes (B) ± SD in the ipsilateral perilesional rim (black columns) and contralateral homotopic region (gray columns) as found in paraffin sections. Positions of measured areas are indicated by arrows in Fig 3. Note the rapid and continuous increase of Mn–SOD in neurons of the rim up to day 7. Ipsilateral immunoreactive astrocytes and contralateral neurons first appear by day 1. Mn–SOD-positive neurons are still more numerous after 60 days compared with controls. Controls are positioned at 0. *Significantly different compared with controls (P<.001) as determined by Dunnett’s test.
curred in the caudolateral direction within the perilesional cortex (Fig 1c and 1d, see schematic Fig 3b through 3d). This slighter increase in the perilesional cortex resulted in significantly lower optical density on day 2 (Fig 5B and 5C), which remained so within the rim up to day 7 (Fig 5B). As seen in other lesion models, astrocytes were also Mn–SOD positive in all affected regions from the third day onward (Figs 2, and 4a through 4c). The first Mn–SOD-positive astrocytes were seen on day 1 in the rim (Fig 2b).

Between days 2 and 7 immunoreactivity increased continuously within the cortex of the ipsilateral hemisphere, including piriform and entorhinal areas. This finding was also true for the corpus callosum, hippocampus, and thalamic nuclei (Figs 1e, 1f, 2A and 2B, 5A through 5E). The increase was most pronounced within the contralateral cortex (Figs 1e, 1f, 5B, and 5C). However, contralateral retrosplenial areas and areas ventral to the rhinal fissure were less affected (Fig 1e and 1f). In contralateral hippocampus and thalamic nuclei immunoreactivity was less enhanced than in the ipsilateral side, but compared with controls a significant increase had occurred (Figs 1e, 1f, 5D, and 5E). Immunoreactivity within the core of the lesion was completely lost between days 5 and 7.

After 7 days, the staining intensity for Mn–SOD dropped continuously in all affected regions, and fewer neurons and glia cells were immunostained (Figs 2A, and 2B, 4a, 4b). Thirty and sixty days after lesion, the number of Mn–SOD-positive neurons in the rim and contralateral cortex was still raised above that of controls. But differences between the numbers of Mn–SOD-positive neurons in the rim and the corresponding contralateral area between 4 hours and 60 days were only small (Figs 2A and 6). At no stage were we able to detected an increase of Mn–SOD in NADPH-d positive neurons, but low
amounts of Mn–SOD in neurons as well as transient colocalization in astrocytes could not be ruled out.

Discussion

General Considerations

Our results show that there is a clear, transient increase in the inducible, mitochondrial Mn–SOD in remote brain regions after focal photothrombotic ischemia. The rapid perilesional increase was not due to nonspecific binding, because it was absent in sections stained without primary antibody. The rapid increase in Mn–SOD in the ipsilateral retrosplenial cortex, which directly borders the lesion, may be related to the greater number of Mn–SOD-positive neurons seen in this area in controls. A larger population of such neurons could also explain why the contralateral retrosplenial area, which possibly receives only a few direct connections from the lesioned area was spared: it may be intrinsically more protected by Mn–SOD from toxic effects than other areas. Retrosplenial areas contain the fewest NOS-I expressing neurons of all cortical areas, which suggests that it has a smaller potential to form cytotoxic peroxynitrite from NO and superoxide. From the data it was obvious that the onset of Mn–SOD upregulation was faster in the core and the ipsilateral perilesional cortex than in remote regions. The first increase in Mn–SOD occurred between 4 hours and 24 hours, suggesting a rapid de novo synthesis involving transcription of the gene and translation of its mRNA. The delayed increases in remote brain regions suggests that the mechanisms or the time points of the induction may be different. Most probably direct injury, as it occurs in the core and the perilesional rim leads to an instant induction of Mn–SOD expression, whereas more time is needed to transfer the signal via afferents or efferents to the remote regions. The instant induction may also occur in response to the hypoperfusion within the lesion, because an interaction between SOD and blood flow has been shown in a study demonstrating that SOD reduces cerebral hypoperfusion after traumatic injury.33

The strong and more widespread increase in cortical Mn–SOD in the contralateral occipital hemisphere at day 2 compared with the laterocaudal ipsilateral areas and rim was unexpected. This increase may be due to ipsilateral-contralateral differences in the degree of edema and in the supply of oxygen and nutrients. Mn–SOD transcription and translation depend on nutrients, oxygen, and metabolic activity. Nutrients may be more effectively supplied to the unlesioned cortex. This hypothesis is supported by the observation that the perilesional cortex becomes hypometabolic during the first week due to the stress imposed on the brain by spreading depression induced in the perilesional cortex. Furthermore, by day 7 differences in Mn–SOD were much smaller between total ipsilateral and contralateral cortex, a time point by which edema becomes reduced in this model.34,35

Figure 5. Relative intensity of Mn–SOD immunoreactivity expressed as optical density (OD±SD) in ipsilateral (black columns) and contralateral (gray columns) retrosplenial cortex (A), rim (B), entire cortex of the hemisphere (C), hippocampus (D), and thalamic nuclei (E) in controls (0 days) and days 2 and 7 after lesion. Note that ipsilateral regions always show higher ODS except in B and in C at day 2 (see also Fig 1). *A value of P<.001 was significant compared with control; †, additional significant difference between day 2 and day 7, P<.005.

Figure 6. Paraffin section showing the core (C), rim, and perilesional cortex 60 days after lesion. Only darkly and lightly stained Mn–SOD positive neurons are present as shown enlarged in the inset. Extracellular granule-like staining is more enhanced in the deeper layers but not in the corpus callosum (cc) or glial scar (g). No astrocytes within the glial scar are Mn–SOD positive. Scale bar=100 μm.
The appearance of injury-related Mn–SOD in pyramidal neurons and astrocytes is seen not only under experimental conditions but has also been observed in ALS and brain tumors. The lack of Mn–SOD in layers I and II in our control rats and at 4 hours corresponds to the situation seen in humans. From the work of Sillevis-Smitt et al and Blauweers et al it can be concluded that the immunoreactive glia in the corpus callosum represent the metallothionein-rich protoplasmic astrocytes, also found in the white matter of patients suffering from ALS.

The gradual spread of the injury-related increase in Mn–SOD to brain regions either directly associated with the lesion core or connected to it via afferents and/or efferents indicates that it is not a general edema-related upregulation. This gradual spread also argues against a contribution of radicals generated in the process of photothermogenesis within the vessels to the induction of Mn–SOD. Indeed, it is highly unlikely that radicals generated within the cerebral vessels during photothermogenesis survive long enough to be transported to the contralateral hemisphere and are able to induce Mn–SOD in distinct areas. Also radical-induced, pathological, intravascular changes are restricted to the ipsilateral hemisphere in this lesion model. Keeping in mind that antibodies bind to proteins that appear only after transcription and translation of mRNA have taken place, induction of Mn–SOD seems to occur very early. The increase in the number of Mn–SOD-positive cells seen between days 2 to 7, as well as the astrocytic responses, may have been induced by further damage due to peroxynitrite, as observed in other studies. Degenerative processes taking place in and around axonal and dendritic fibers that lost their somata in the core may also be effective. Such processes have been revealed by the use of other markers for cortico–thalamic projection neurons.

### Diaschisis and Pathophysiological Considerations

Von Monakow described diaschisis as the alterations occurring within the brain, distant from primary cerebral insults. The distant changes have been further classified as ipsilateral effects or diaschisis associativa, or as crossed cerebellar diaschisis, or as transhemispheric diaschisis. The latter type describes alterations occurring in the hemisphere contralateral to the lesion. Diaschisis is frequently observed by electrophysiological and scanning techniques or metabolic changes in humans and several animal models of stroke, traumatic brain injury, or epilepsy. Histochemically identified associative and transhemispheric diaschisis has been observed in all known animal models of cerebral stroke by the use of different markers. Therefore, it is not clear to what extent certain effects are model dependent, and it seems more likely that the detection of diaschisis depends on the markers or tracers and their concentrations studied (optimal concentrations may be different for the penumbra and the remote regions) as well as on the time after injury, because diaschisis is a transient phenomenon. Noteworthy, Szele et al reported that remote changes in GAP43, synaptophysin, and certain growth factors differ according to the methods used to induce the cortical lesions. Compared with cortical photothermogenesis, subcortical injury and related edema are certainly different in MCAO, and changes of Mn–SOD and CuZn-SOD differ partly in both models.

In the model of unilateral cortical photothermogenesis, however, transhemispheric diaschisis is more pronounced after lesioning deep cortical layers and has been characterized in terms of electrophysiological parameters such as hyperexcitability as a consequence of disinhibition caused by a reduction in GABA receptors. Additionally it has been shown that hyperexcitability results from radical-induced release of several neurotransmitters. The release of these transmitters is, however, prevented by superoxide dismutase and catalase. The latter suggests that our observed increase of Mn–SOD in perilesional regions as well as in regions affected by diaschisis may act protective by scavenging superoxide and reducing radical-induced transmitter release.

In our study we found two types of diaschisis: (1) changes in the expression of Mn–SOD in the ipsilateral cortex, hippocampus, and thalamic nuclei (diaschisis associativa); and (2) changes within the contralateral hemisphere (transhemispheric diaschisis). Transcortical changes involving even the contralateral thalamus have been shown during regenerative processes after experimental stroke. The latter authors found a highly upregulated expression of synaptophysin and formation of new synapses in the contralateral cortex and thalamus 14, 30, and 60 days after lesion, which is most probably related to synaptogenesis within the contralateral thalamo–cortical circuitries.

Transhemispheric diaschisis involving neurochemical changes have also been described in man, where nonphosphorylated neurofilaments become phosphorylated in regions contralateral and homotopic to the insult. Similar, regionally limited changes have been seen in rodents for the β-amyloid precursor protein, CuZn-SOD, glial fibrillary acidic protein, isoleucin B-positive microglia, c-fos expression, and 2-deoxyglucose uptake.

Chou et al found CuZn-SOD and NADPH–d colocalized in motor neurons of ALS patients. Mn–SOD was not increased in NADPH–d positive neurons. However, due to the methods used it cannot be excluded that low levels of Mn–SOD may be present in NADPH–d positive neurons. Because NADPH–d is a marker for nitric oxide synthases, this enzyme identifies cells that produce NO radicals. Superoxide radicals react more efficiently with NO than with SODs. Furthermore, the resulting peroxynitrite is known to nitrate SODs, inhibiting their activity. Therefore, upregulation of Mn–SOD does not seem very advantageous. We observed that NADPH–d and Mn–SOD–positive cells are found close together, which suggests that the SOD-containing cells protect themselves against peroxynitrite formation by scavenging their own oxygen radicals within the mitochondria before they escape into the cytoplasm and extracellular space where they can react with freely diffusing NO. Thus Mn–SOD may protect tissues connected with the lesion during the period when cytotoxic and degenerative processes are effective, so that they remain intact until recovery and new axonal sprouting and new synaptogenesis can take place. This hypothesis is further validated by the localization of Mn–SOD in the lesions and the upregulation of Mn–SOD in subcortical regions connected with the lesion during the period when interrupting of specific astrocyte functions results in increased release of glutamate and other excitatory transmitters.
supported by the finding that Mn–SOD decreases between days 7 and 14. Degenerative processes may have come to a hold by day 14 and regeneration has probably begun, as seen by the onset of synaptophysin expression.18 In the photothrombosis model regeneration leads to complete functional and behavioral recovery after day 18 as seen in grip-strength tests.20 The increase in Mn–SOD immunoreactivity found in neurons by days 30 and 60 is still significant and suggests that superoxide production continues to be above normal. Increased metabolic activity, however, leads to enhanced superoxide production within the respiratory chain of mitochondria.

Superoxide radical-induced brain injury has been described to be the main mechanism of injury caused by brain reperfusion after transient ischemia.46 The CuZn-SOD and possibly also the Mn–SOD are thought to represent the inducible and neuroprotective Mn–SOD.

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Ischemia-Induced Increase of Mn–SOD in Remote Brain Areas


In this very interesting article, Bidmon et al demonstrate a transient increase of MnSOD in remote brain areas after focal photothermobic cortical lesions. This increase in MnSOD in the ipsilateral cortex bordering the lesion may be related to the greater number of MnSOD-positive neurons seen in this area. The authors show that MnSOD may be involved in limiting tissue damage in remote brain areas that were not ischemic by scavenging radicals formed in response to differentiation. It has been described that superoxide radical–induced brain injury may be one of the important mechanisms of injury caused by brain reperfusion following brain ischemia. The MnSOD, as well as the CuZn-SOD, may represent the main endogenous protective systems against ischemic reperfusion injury. Bidmon and colleagues demonstrated that MnSOD may limit the damage in remote brain areas that were not ischemic by scavenging superoxide anion formed in response to differentiation. The authors also point out that their data indicate that manganese ions may be important during these conditions. The precise mechanism by which this endogenous protection occurs is unclear at this time. However, manganese may inhibit calcium channels, and these calcium channels are essential for the activation of inducible and neuroprotective MnSOD.

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