Localization of Manganese Superoxide Dismutase in the Cerebral Cortex and Hippocampus of Alzheimer-type Senile Dementia

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Summary

Manganese-superoxide dismutase (Mn-SOD) was localized in the cerebral cortex and hippocampus of patients with Alzheimer-type senile dementia (ATD) by immunocytochemistry and the relationship of Mn-SOD with two major pathological features of ATD, i.e., senile plaques and neurofibrillar tangles (NFTs), was examined. Many astrocytes in senile plaques exhibited strong immunoreactivity for both Mn-SOD and glial fibrillar acidic protein (GFAP) on serial section analysis. This suggests that Mn-SOD scavenger system is associated with the formation of senile plaques. On the other hand, Mn-SOD immunoreactivity was not significant in NFT-loaded neurons.

Introduction

It has been suggested that free radicals are involved in the pathogenesis of Alzheimer's type disease (ATD) (1, 2). Copper/zinc (Cu/Zn)-superoxide dismutase (SOD), an isozyme of scavenging enzymes of superoxide radical, may participate
In this process, since abundant Cu/Zn-SOD transcripts are present in neurofibrillary tangle (NFT)-loaded neurons in the hippocampus (3) and this enzyme increases in activity in platelets from patients with ATD (4). Manganese (Mn)-SOD, the second major isozyme, has not yet been well studied in ATD brain. We, therefore, attempted to immunocytochemically localize Mn-SOD structures in the cerebral cortex and hippocampus from ATD patients and to determine their morphological relationship to senile plaques and NTFs.

Materials and methods

Brains from 7 ATD patients (69-76 years old) and 1 control subject (68 years old) were examined. Two to six h after death, the brain was dissected and fixed in 10 % formalin for several days and blocks were embedded in paraffin. Frontal sections containing the cerebral cortex (temporal, parietal and frontal lobes) and hippocampus were serially cut at a thickness of 4 μm and mounted on glass slides. The sections were incubated in Mn-SOD antiserum (dilution: 1:500) (given by Dr. N. Taniguchi) overnight at 4 °C and stained by the avidin-biotin-complex (ABC) method (5). Polyclonal antibody against Mn-SOD purified from the human brain was produced in a rabbit. After reaction with 3,3′-diaminobenzidine tetra HCl (DAB), the sections were lightly counterstained with hematoxyline and dehydrated. The consecutive sections were subjected to ABC method to detect glial fibrillary acidic protein (GFAP) (Dako) or stained by Bielschowsky method to detect senile plaques and NFTs.

Results

Mn-SOD was visualized in both normal and ATD subjects as granular or rod-shape immuno-precipitates (Fig. 1A), possibly corresponding to mitochondria as shown in the rat brain (6). Cells with very strong Mn-SOD immunoreactivity were frequently found in the peripheral portion of senile plaques in the cerebral cortex (all lobes examined) (Fig. 1A) and hippocampus in ATD, whereas such strongly labeled cells were not seen in both brain areas of the normal subject. Serial section analysis demonstrated that many astrocytes had strong immunoreactivity for both Mn-SOD and GFAP (Fig. 1A, B). This coexistence was found in many astrocytes not only in classical plaques (Fig. 1C) but also in primitive plaques by Bielschowsky staining of the adjacent sections. Mn-SOD-immunoreactivity was not significant in NFT-loaded neurons (Fig. 2).
Mn-SOD in cerebral cortex and hippocampus in Alzheimer's disease

Fig. 1 Light micrographs of consecutive sections stained with Mn-SOD antibody (A), with GFAP antibody (B) or by the Bielschowsky method (C) in the ATD temporal cortex. Arrows indicate neurons exhibiting strong immunoreactivity for both Mn-SOD and GFAP. Scale: A-C, 20 μm.

Fig. 2 Light micrographs of consecutive sections stained with Mn-SOD antibody (A) or by the Bielschowsky method (B) in the ATD temporal cortex. A large cell containing NFT (arrow) shows no significant immunoreactivity for Mn-SOD compared to the other cells. Scale: A, B, 20 μm.
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Discussion

Little information is so far available on immunocytochemical localization of Mn- and Cu/Zn-SOD in glial cells (7). Very strong immunoreactivity for Mn-SOD in GFAP positive astrocytes in senile plaques suggests that the generation of superoxide radical is facilitated in these astrocytes during plaque formation. Although it remains unclear whether astrocytes are primarily or secondarily involved in the formation of senile plaques (8,9), astrocytic plaque-related reaction with strong GFAP-immunoreactivity is suggested to develop very early during plaque formation; this may precede dystrophic neuritic change and relate to sub-minimal amyloid deposits (10). Therefore, Mn-SOD synthesis may be induced in the astrocytes from a relatively early stage of plaque formation. However, the lack of significant increase in Mn-SOD immunoreactivity in NFT-loaded neurons suggests that Mn-SOD is not closely associated with NFT formation, compared with Cu/Zn-SOD which showed an increased immunoreactivity and thus is speculated to play a role in NFT formation (3). Differential regulation of synthesis between Mn-SOD and Cu/Zn-SOD was also seen in the axotomized motoneuronal reaction (11).

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