The role of peroxiredoxin (Prx) II in the kainic acid (KA)-induced neurotoxicity

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Peroxiredoxin (Prx) has been attracted considerable attention in recent years as a new family of thiol-specific antioxidant protein and has 6 isoforms in the mammalian. It has been recognized that Prx II among the Prx isoforms is more specifically expressed in the brain. However little is known about the role of Prx II in the brain. In the present study, it was examined immunodistribution of Prx II in the brain. Prx II-IR was expressed in a region-specific manner in the brain of the Prx II (+/+) mice. Descending order of Prx II-like immunoreactivity (Prx II-IR) is as follows; hippocampal pyramidal cell layer ≒ dentate gyrus > piriform cortex ≒ entorhinal cortex, amygdala ≒ median habenular nucleus > retrosplenial agranular cortex ≒ motor cortex > thalamus ≒ pars compacta of substantia nigra ≒ pars reticulata of substantia nigra > basal nucleus of Meynert ≒ caudate putamen. A very strong Prx II-IR was noted in the pyramidal cell layer and in the granule cells of the dentate gyrus (DG) in the hippocampus.

Accumulating evidence suggests that oxidative stress contribute to the development of the seizures induced by KA. KA has been used as a model for both epilepsy and neurodegenerative disorders. Since hippocampus contains a high density of KA receptor in the pyramidal cell, we used hippocampus for our biochemical study. No significant changes in Prx II-IR were observed in the hippocampus in the early phase after KA insult. However, Prx II-IR was decreased at early period after KA and it was never recovered. In detail, Prx II-IR was significantly disappeared in the CA1 and CA3 regions, while Prx II-immunoreactive-non neuronal populations were mainly proliferated adjacent CA1 and CA3 regions. Western blot analysis supports these findings. Double-labeling immunocytochemistry showed co-localization of the Prx II-positive non-neuronal cells with S-100 or glia fibrillary acidic protein (GFAP)-immunoreactivity, suggesting that the mobilization of

astroglial cells for synthesis of Prx II protein in response to KA insult to minimize peroxidative burdens. Although injection of KA (0.1ug/i.c.v.) produced vigorous seizure behaviors in both Prx II (+/+) and Prx II (-/-) mice, intensity of seizure behaviors of Prx II (-/-) mice were more pronounced than that of Prx II (+/+) mice by KA. Consistently, Prx II (-/-) mice were more fatal than Prx II (+/+) mice. These results highly suggest that Prx II (-/-) mice had more susceptible to KA-induced neurotoxicity than Prx II (+/+) mice. Consistently, KA-induced neuronal degeneration in Prx II (-/-) mice was more accentuated than Prx II (+/+) mice as evaluated by cell body-, Fluoro-Jade B - and TUNEL- stainings.

In addition, KA-induced oxidative stresses (as measured by reactive oxygen species, malondialdehyde, 8-hydroxy 2-deoxyguanosine and protein carbonyl) were increased in the early stage post-KA, and then remained elevated by 1 week later. This increase in oxidative stresses was more pronounced in the Prx II (-/-) mice treated with KA. Advanced glycation endproducts (AGEs), which indicate protein-bound oxidation products of sugars and F4/80-IR, a marker of microglial cell, were proliferated mainly in the striatum radiatum of the CA1 area 1 week after KA in the Prx II (-/-) mice. Further, intensity of protein expression of AGEs is relatively in paralleled with that of oxidative markers. Pretreatment with recombinant Prx II protein significantly attenuated seizure behaviors in a dose-related manner. The neuroprotective effect of recombinant Prx II was reversed by adenosine A_1 receptor antagonist (8-cyclopentyl-theophylline; CPT), but neither adenosine A_{2A} receptor antagonist (8-(3-chlorostyryl) caffeine; CSC) nor adenosine A_{2B} receptor antagonist (alloxazine; ALX). Similarly, this protective effect was significantly counteracted by cannabinoid CB_1 receptor antagonist (N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide; AM 251), but not by cannabinoid CB_2 receptor antagonist (N-[(1S)-endo-1,3,3-trimethylbicyclo[2.2.1]heptan-2-yl]-5-(4-chloro-3-methylpheny l)-1-(4-methylbenzy l) pyrazole-3 carboxamide; SR144528).

The neuronal cells from Prx II (-/-) mice were more vulnerable to that of Prx II (+/+) mice against KA (25 uM) insult. In the presence of recombinant Prx II (100ng/ml) protein, the neuronal cells from Prx II (+/+) mice were more effective in preventing KA insuts than those from Prx II (-/-) mice. *In vivo* results are in line with those of primary hippocampal neuronal cell culture. Our results suggest that Prx II is an important neuroprotectant in response to KA toxicity *in vivo* and *in vitro*, and that its neuroprotective action may be related to antiperoxidative mechanism via activations of adenosine A_1 and cannabinoid CB_1 receptors. [This research was supported by a grant (# M103KV01000 803K2201 00820) from Brain Research Center of the 21st Centry Frontier Research program funded by the ministry of Science and Technology of Republic of Korea]

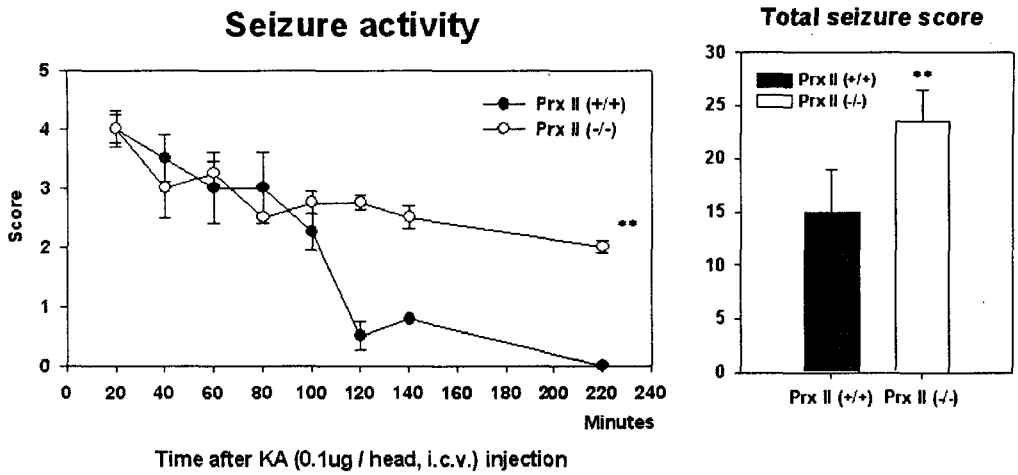
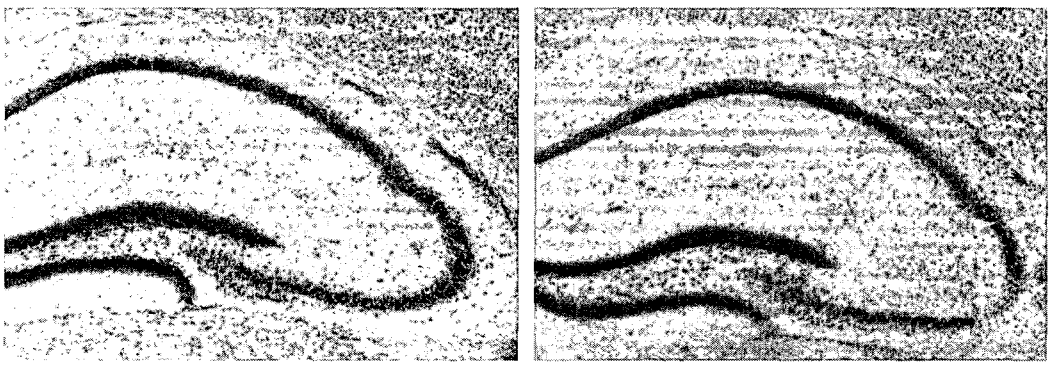


Fig. 1. KA-induced seizures in the Prx II (+/+) and Prx II (-/-). Epileptic seizures were more pronounced in the Prx II (-/-) mice. Each value is the mean ± S.E.M. of 8 animals. $^{**}P$ <0.001, Prx II (+/+) + KA vs Prx II (-/-) + KA (Wilcoxon signed-rank test).



Prx II(+/+) **Prx II(-/-)**

Fig. 2. Representative photomicrographs of Cresyl violet stained sections of the hippocampus 3day after KA in the Prx II (+/+) and Prx II (-/-). The neuronal loss of the CA3 area was noted in the Prx II (-/-) mice.