sion of ischemic tissues.[1,2] OFRs, which can cause the peroxidation of membrane phospholipids, cellular proteins, and nucleic acids, attack both neuronal and glial cell membranes as well as the cerebrovasculature.[3] The brain appears to be particularly vulnerable to such oxidative injury, in that it contains high concentrations of readily peroxidizable fatty acids. Peroxidation of membrane lipids, including those in the plasma membrane, results in changes in their fluidity and permeability, which can subsequently affect the functioning of phospholipid-dependent proteins, including the critical membrane ion pumps and other transport systems.[4,5]

Enzymes protecting against free radicals include superoxide dismutase (SOD), catalase, and glutathione peroxidase. SOD catalyzes the conversion of the superoxide anion radical into H_2O_2. H_2O_2 is removed either by glutathione peroxidase which catalyzes its reduction to H_2O, while converting the reduced glutathione (GSH) into oxidized glutathione (GSSG), or by catalase which decomposes H_2O_2 to oxygen and water. The brain appears to be relatively poorly endowed with glutathione peroxidase and catalase;[6] and this, in conjunction with its relatively high concentrations of polyunsaturated fatty acids, may account for its susceptibility to ischemia/reperfusion injury during stroke attack.

Vitamin E (α-tocopherol) is a lipophilic compound which is present in relatively high concentrations in both plasma and mitochondrial membranes. It reacts with lipid peroxyl radicals to form lipid hydroperoxides which can then be removed by the phospholipase-glutathione peroxidase system.

Administration of α-tocopherol (vitamin E) prior to the onset of ischemia attenuates lipid peroxidation during reperfusion.[7] Conversely, rats raised on diets deficient in α-tocopherol have accentuated cerebral lipid peroxidation in response to ischemia/reperfusion compared to rats raised on a diet with excess α-tocopherol.[8] Dogs given α-tocopherol in combination with mannitol and phenytoin prior to ischemia showed a significant improvement in the recovery of electrical activity and decreased brain edema in comparison with dogs treated with phenytoin alone.[9] α-Tocopherol had a protective effect on ischemic hippocampal neuronal damage in the gerbil hippocampus.[10] Vitamin E protected primary cultures of rat cerebellar neurons exposed to toxic concentrations of L-glutamate by as much as 50% or greater. Because brain and adrenal glands are important organs associated with cardiovascular disease, this study is designed to evaluate the effect of α-tocopherol (vitamin E) on changes of superoxide dismutase (SOD) in rat astrocytes (RBA-1) and pheo- chromocytoma cells (PC-12).

MATERIALS AND METHODS

Preparation of RBA-1 and PC-12

RBA-1 and PC-12 were incubated in Dulbecco's modified Eagle's medium (DMEM) which contained 10% fetal bovine serum. Cells were washed in phosphate-buffered saline (PBS) containing 0.1% EDTA and 0.5% trypsin and centrifuged at 1000 rpm for 5 min. The supernatant was discarded, and a cell suspension was obtained after adding culture medium. Cell suspension (100 μL) was mixed thoroughly with 100 μL PBS containing 0.04% trypan blue. The number of surviving cells was counted using a light microscope and Coulter counter. Surviving cells were divided into 4 groups: a control group incubated with culture medium containing 0.5% EtOH (without vitamin E) and treatment groups (vitamin E) incubated with culture medium containing vitamin E at 50 μM, 100 μM, and 200 μM, respectively.

Assay of SOD Activity

The activity of SOD was measured using a commercial assay kit (Wako, Japan). Cells from 1 dish were harvested and homogenized in 1 mL of 0.9% NaCl.[11] The crude homogenate was centrifugated at 10,000 ×g for 1 h to get the supernatant (cytosolic) and pellet (particulate). For assay of Mn-SOD activity in the pellet, 1 mM potassium cyanide was added to the incubation mixture to inhibit CuZn-SOD activity. The activity of CuZn-SOD was derived by the subtraction of Mn-SOD from total SOD activity in the pellet. The units of SOD activity were derived from a standard curve constructed using purified SOD from bovine erythrocytes (S-2515; Sigma). Results are expressed as unit/mg of protein determined.[12]

Northern Blotting Analysis

Cells for RNA isolation were frozen immediately