In vivo, vitamin E protects against lipid peroxidation, quenches singlet oxygen ($^1\Delta gO_2$), and also reacts with the superoxide radical. Scavenging of $^1\Delta gO_2$ by tocopherols includes physical quenching in which the excited state of oxygen is deactivated without light emission, as well as chemical quenching which leads to the production of various oxidation products. Physical quenching by energy transfer almost always predominates; the exact rate of the reaction depends on solvent polarity. This has led to the suggestion that a charge-transfer intermediate may also be involved in the quenching process.[2,3]

Oxygen free radicals (OFRs) arise in sequential fashion from molecular oxygen by successive single electron reductions. These radicals, being chemically reactive, remove electrons or hydrogen atoms from neighboring molecules, thus radicalizing these in turn and creating a "cascade" effect. The initial oxygen radical in the sequence is the superoxide anion radical ($O_2^{\cdot-}$). The short half-life of $O_2^{\cdot-}$ limits its ability to diffuse away from its site of generation. Divalent reduction of O_2 yields the nonradical species O_2^-, which protonates at physiological pH level to form hydrogen peroxide (H_2O_2). H_2O_2 has a relatively long half-life, is membrane permeable, and can thus initiate damage at sites distant to its point of origin. In the presence of transition metals such as iron and copper, H_2O_2 is reduced to the hydroxyl radical (•OH) and hydroxyl ion (OH^-). •OH is highly reactive, with a short half-life and a limited diffusion capacity. The addition of a fourth electron ultimately results in the formation of H_2O.

Antioxidants which have evolved during the course of evolution include both antioxidant enzymes and nonenzymatic antioxidants. Maintenance of the balance between the production and elimination of oxidants is critical for continued cell viability. Disturbances of the balance, due to either an excessive production of radicals or a reduced effectiveness of the scavenging systems, can lead to cell deterioration and death. The present study was undertaken to evaluate the effect of the nonenzymatic antioxidant, vitamin E, on the natural antioxidant enzyme, superoxide dismutase (SOD), and to observe whether vitamin E has a beneficial effect on this system.

MATERIALS AND METHODS

Three groups ($n = 8$) of male Wistar rats (250-350 g) at 2 months old were injected intraperitoneally with normal saline (1 ml/kg) or normal saline plus 0.5% ethanol (1 ml/kg) or vitamin E (200 mg/ml/kg) plus saline and 0.5% ethanol once daily for 3 consecutive days. The experimental animals were then sacrificed, and whole organs (brain, liver, and kidneys) were used to carry out assays of SOD activity and SOD-mRNA levels. Brain, liver, and kidney specimens were homogenized with an Ultraspec TM-II (Biotecx, Houston, TX, USA).

SOD activity measurement

The activity of SOD was measured using a commercial assay kit (Wako, Tokyo, Japan). The crude homogenate of brain, liver, and kidneys specimens was centrifugated at 10,000 × g for 1 h to obtain the supernatant (cytosol) and pellet (particulate). For assaying Mn-SOD activity in the pellet, 1 mM potassium cyanide was added to the incubation mixture to inhibit Cu/Zn-SOD activity. The activity of Cu/Zn-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, St. Louis, MO, USA). Results are expressed as units /mg of protein determined by the dye.[4]

RNA preparation

Organs for RNA isolation were frozen in liquid nitrogen immediately after removal and stored at –80 °C. Total RNA was isolated as described previously.[5] RNA was then transferred to Hybond N nylon membranes (Amersham, Buckinghamshire, UK) overnight in saline-sodium citrate (SSC). The transfer was controlled on an UV transilluminator and additionally by staining the blot membrane with 0.05% methylene blue.[6] Filters were rapidly prehybridized at 65 °C in hybridization solution (Quikhyb®, Stratagene, La Jolla, CA, USA). cDNA probes were also prepared. Plasmids with cDNA were supplied by Dr. Ye-Shih Ho (Institute of Chemical Toxicology, Wayne State Univ. Detroit, MI, USA). Transformation in *Escherichia coli,* plasmid preparation, and cDNA purification were