fective against the superoxide radical anion, hydrogen peroxide, the hydroxyl radical, peroxynitrite, and singlet oxygen.[9,10]

Carcinogenesis is a complex, multistep process including initiation, promotion, and progression. The generation of reactive oxygen species is thought to be linked to tumorigenesis at different levels.[11] Oxidative damage to DNA has been demonstrated in vitro and in vivo, leading to single- or double-strand breaks in DNA and DNA-protein cross-linking, as well as to chromosomal aberrations such as breakage or rearrangement.[12] Modified DNA bases have been identified after exposure of cells to oxidative stress.[13]

The phenomenon of a remarkable decrease in the amount of manganese superoxide dismutase (MnSOD) has been reported in many human and experimental tumors.[14-17] This study was undertaken to evaluate the effect of vitamin C supplementation on the levels of MnSOD in pheochromocytoma cells.

MATERIALS AND METHODS

Preparation of PC-12 Cells

PC-12 cells were incubated in Dulbecco modified Eagle medium (DMEM) which contained 10% fetal bovine serum. Cells were washed and trypsinized by 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 C) and treatment groups (vitamin C) incubated with culture medium containing vitamin C at 50, 100, and 200 μM, respectively.

Assay of SOD Activity

The activity of SOD was measured using a commercial assay kit (Wako, Tokyo, Japan). Cells from 1 dish were harvested and homogenized in 1 ml of 0.9% NaCl.[18] The crude homogenate was centrifugated at 10,000 ×g for 1 h to obtain the supernatant (cytosolic) and pellet (particulate fraction). For assay of MnSOD activity in the pellet, 1 mM potassium cyanide was added to the incubation mixture to inhibit CuZnSOD activity. The activity of CuZnSOD was derived by subtracting of MnSOD activity 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 per milligram of protein determined.[19]

Northern Blot Analysis

Cells for RNA isolation were frozen immediately after removal from liquid nitrogen and then stored at -80 °C. Total RNA was isolated as described previously.[20] RNA was then transferred to Hybond N nylon membranes (Amersham, Manchester, UK) overnight in 2 volumes of saline-sodium citrate (SSC). The transfer was controlled on an UV transilluminator and additionally by staining the blot membrane with 0.05% methylene blue.[21] The filters were rapidly prehybridized at 65 °C in hybridization solution (Quikhyb®, Stratagene, La Jolla, CA, USA). The cDNA probes were also prepared. Plasmids containing cDNA of SOD were supplied by Dr. Y.S. Ho, and plasmids containing cDNAs of catalase and glutathione peroxidase (GPX) were obtained from Dr. T.S. Chiou. Transformation in *Escherichia coli,* plasmid preparation, and cDNA purification were performed according to standard methods.[22] Radioactive probes (^{32}P) were prepared using the multiprime DNA labeling system (Amersham). The prepared cDNA inserts and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes were added directly into the prehybridization solution (Quikhyb®, Stratagene) at a radioactivity of 1×10^6 ct/(min l). Hybridization was performed at 68 °C for 70 min. After washing, the wet blot membranes were sealed in plastic foil and exposed to medium-sensitive medical x-ray film (Fuji, Tokyo, Japan) at 0 °C using intensifier screens. Exposure times were 2-3 days for Northern blots. Hybridization intensity of autoradiographic signals was measured using 2-dimensional densitometry. The obtained density (optical units) was