after removal in liquid nitrogen and then stored at -80 °C. Total RNA was isolated as described previously.[13] RNA was then transferred to Hybond N nylon membranes (Amersham, 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.[14] The filters were rapidly prehybridized at 65 °C in hybridization solution (Quikhyb®, Stratagene, CA, USA). The cDNA probes were also prepared. Plasmids containing cDNA of SOD were supplied by Dr. Y.S. Ho and plasmids containing cDNA 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.[18] Radioactive probes (P^{32}) 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. mL). 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, Japan) at -70 °C using intensifier screens. Exposure times were 2-3 days for Northern blots. Hybridization intensity of autoradiographic signals was measured using two-dimensional densitometry. The obtained density (optical unit) was calculated versus the value of slot blot for GAPDH to quantify the mRNA.

Statistics

All values are presented as means ± SE from each group. Statistical significance was evaluated using one-way ANOVA when multiple groups were compared; where only 2 groups were compared, Student's *t-test* was used. A value of $p < 0.05$ was considered to be significant.

RESULTS

Effect of Vitamin E on Cell Number of Cultured RBA-1 and PC-12 Cells

In the present study, the number of surviving cells was counted under a light microscope with a counter. Both the cultured RBA-1 and PC-12 cells were divided into 4 groups: the control group incubated with culture medium containing 0.5% EtOH (vehicle) and treatment groups (vitamin E) incubated with culture medium containing vitamin E at 3 concentrations. After 7 days of culturing, the cell number of RBA-1 in vehicle-treated medium was $(8.4 \pm 0.6) \times 10^6$ and this value did not significantly ($p > 0.05$) differ from the vitamin E-treated group at 50 μM with $(9.2 \pm 0.5) \times 10^6$ cells, at 100 μM with $(8.0 \pm 1.1) \times 10^6$ cells or at 200 μM with $(7.9 \pm 0.6) \times 10^6$ cells, respectively. Similar results were observed in cell number of cultured PC-12 cells for 7 days of culturing with $(19.3 \pm 1.5) \times 10^6$ cells and at 50 μM vitamin E with $(19.8 \pm 1.2) \times 10^6$ cells, at 100 μM vitamin E with $(19.8 \pm 1.3) \times 10^6$ cells and at 200 μM vitamin E with $(19.4 \pm 0.9) \times 10^6$ cells.

Effect of Vitamin E on the Activity of Superoxide Dismutase (SOD) in Cultured RBA-1 and PC-12 Cells

The activities of SOD in RBA-1 and PC-12 were influenced by vitamin E. After 2 days of incubation, vitamin E increased the SOD activity, for both Mn-SOD and CuZn-SOD, at a concentration of 50 μM in RBA-1, but not in PC-12. However, at concentrations from 100 to 200 μM, vitamin E decreased the activity of Mn-SOD but not the activity of CuZn-SOD, whereas the activity of SOD in PC-12 remained unchanged (data not shown). After 7 days of incubation, vitamin E attenuated the activity of CuZn-SOD in RBA-1 in a concentration-dependent manner. Also, the activity of Mn-SOD was lower in RBA-1 incubated with vitamin E at 100 or 200 μM for 7 days (Table 1). However, the activity of SOD in PC-12 after 7 days of incubation with vitamin E showed no change.

Effect of Vitamin E on the mRNA of SOD in Cultured RBA-1 and PC-12 Cells

Representative responses of mRNA levels in RBA-1 cells to the incubation of vitamin E are shown in Fig. 1. The responses of SOD were quantified using GAPDH as an internal standard as indicated in Table 2. Similar to the changes of activity, the mRNA levels of SOD, for both Mn-SOD and CuZn-SOD, were elevated by vitamin E with 2 days of incubation at a concentration of 50 μM (Table 2). Also, the mRNA levels of catalase and glutathione peroxidase (GPX) in these