calculated versus the value of slot blot for GAPDH to serve as the internal control for quantification of mRNA.

Western Blot Analysis

The SOD enzymes were identified in cells by immunoblotting using Western blot analysis. Polyclonal antibodies from Biodesign (Kennebunk, Maine, USA) were used. The cells were lysed in buffer containing 1% Triton X-100. The proteins were fractionated by gel electrophoresis run at 40 and 100 V and 4 °C during the stacking and separation steps, respectively. The separated proteins were blotted onto nitrocellulose.[14] After reaction with anti-CuZnSOD antibodies (43 μg/ml) or anti-Mn-SOD antibodies (33 μg/ml), immunostaining was performed by incubation in Tris-buffer (10 mmol/l; pH 7.4) for peroxidase activity using the enhanced chemiluminescence (ECL) development system (Amersham). This antibody is purified from human liver and was shown to be specific by the supplier using 2D-IEP and double diffusion. Reactions were observed at 25 kD for Mn-SOD and at 32 kD for CuZnSOD. The bands were then quantified with a laser densitometer.

Statistics

All values are presented as the mean standard error of the mean (SEM) 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 statistically significant.

RESULTS

Effect of Vitamin C on Cell Number of Cultured PC-12 Cells

In the present study, the number of surviving cells was assessed under a light microscope with a counter. The cultured PC-12 cells were divided into 4 groups: the control group incubated with culture medium containing 0.5 % EtOH (vehicle) and treatment groups (vitamin C) incubated with culture medium containing vitamin C at 3 concentrations. The cell numbers of cultured PC-12 cells for 7 days were $(18.3 \pm 1.6) \times 10^6$ cells (vehicle), $(19.2 \pm 1.4) \times 10^6$ cells at 50 μM vitamin C, $(20.1 \pm 1.8) \times 10^6$ cells (at 100 μM vitamin C), and $(19.6 \pm 1.5) \times 10^6$ cells (at 200 μM vitamin C). There were no differences in cell number among all experiment groups.

Effect of Vitamin C on Protein Amount and Activity of Superoxide Dismutase (SOD) in Cultured PC-12 Cells

Fig. 1 shows the Western blot analysis which demonstrates an obvious protein amount of MnSOD after 2 days of vitamin C incubation.

The activity of SOD showed divergent effects after incubation with different concentrations of vitamin C, i.e., the activity of copper-zinc SOD (CuZnSOD) did not change after vitamin C incubation (Fig. 2A, B); but the activity of MnSOD showed a dose-dependent decrease when the dosage of vitamin C increased after a 2-day (short-term) incubation (Fig. 2A). This phenomenon was even more remarkable after a 7-day (long-term) incubation (Fig. 2B), showing the obvious phenomenon of down-regulation.

Effect of Vitamin C on mRNA of SOD in Cultured PC-12 Cells

Similar results were also found with SOD mRNA after incubation with vitamin C in PC-12 cells. The level

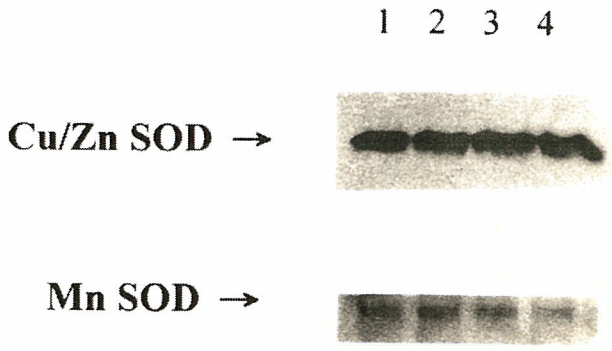

Fig. 1. Western blot of protein from control and vitamin C-incubated samples probed for CuZnSOD and MnSOD. Lane 1 shows the control, and lanes 2-4 indicate the response to a 2-day incubation with vitamin C at 50, 100 and 200 μmol/L, respectively.