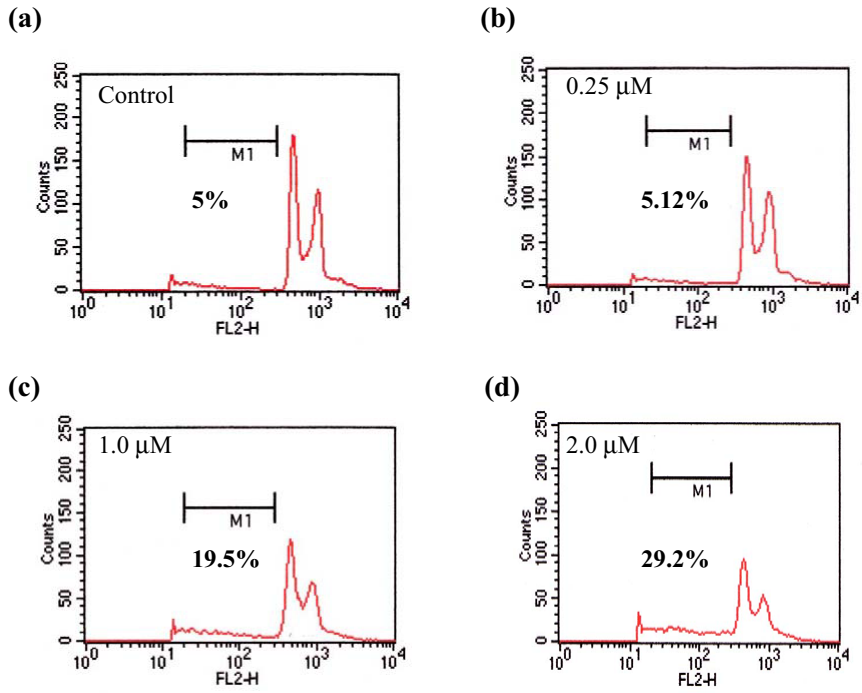
Figure 4



Apoptosis induced by bis-aziridinylnapthoquinone (AZ-1). BC-M1 cells were treated in 24-hour culture with **(a)** dimethylsulfoxide control, **(b)** 0.2 μM AZ-1, **(c)** 1.0 μM AZ-1, or **(d)** 2.0 μM AZ-1 before propidium iodide staining and analysis of the DNA content. Apoptosis is apparent from the large population of cells with increased DNA content in the sub-G_1 area (M1 area). FL2-H, fluorescence two-peak high.

formation of apoptotic bodies was observed obviously at concentrations as low as 0.5 μM AZ-1 in MCF-7 cells (Fig. 6).

Tumor cell apoptosis associated with protein expression and caspase-3 activity

We next set out to determine whether the induction of BC-M1 cell apoptosis by AZ-1 was associated with expression of apoptosis-related proteins. We found that AZ-1 induced changes in BC-M1 cell expression of the checkpoint protein p53 and the cell arrest protein p21 in a dose-related manner (Fig. 7). The p53 protein showed increasing expression from 11% to 43% at 0.5 μM and 1 μM AZ-1, and then decreased to 31% at the 2 μM concentration of AZ-1, and p21 protein increased from 6% to 22% from the 0.5 μM to 2 μM concentrations of AZ-1 added to BC-M1 cells for 24 hours compared with control, respectively. The other proteins including TIAR, pro-caspase protein and cell cdk2 also showed a dose-dependent decreasing manner (Fig. 8). From the western blot results and the values in the relative protein expression-quantifying table (Figs 7 and 8) it was revealed that the expression of proteins was affected by various concentrations of AZ-1 in BC-M1 cells after 24 hours of treatment, and these relative protein expressions were compared with the control. The cdk2, pro-caspase protein and TIAR were reduced to about 62%, 85% and 65%, and to 40%, 67% and 57% when BC-M1 cells treated with 1 μM AZ-1 and 2 μM AZ-1 for 24 hours compared with control, respectively. Based on the results of western blot analysis in the expression of pro-caspase protein, we determined the enzyme activity of caspase-3 in BC-M1 cells after challenge by various concentrations of AZ-1 from 0.5 μM to 4 μM for 24 hours. The enzyme activity of caspase-3 dose-dependently increased with concentrations of AZ-1. The activity was more than twofold over the control in BC-M1 cells after 3 μM AZ-1 treatment for 24 hours (Fig. 9).

Assay of the apoptosis signal by ^{1}H-NMR

According to some previous reports, the ratio of the CH_2 and CH_3 peak area on the cell membrane was directly in proportion with the signal of apoptosis. From our results we also observed the same phenomena that the ratio of the CH_2 and CH_3 peak area was increasing according to the concentration of AZ-1 (Fig. 10). It was about 1.7-fold higher than the control at 2 μM AZ-1 treatment in BC-M1 cells.

Discussion

Breast cancer is the most common malignancy in women, and it is highly curable if diagnosed at early stage. It is now well established that adjuvant systemic therapy improves