The p53-dependent apoptotic pathway of breast cancer cells(BC-M1) induced by bis-type bioreductive compound aziridinylnaphthoquinone

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摘要.

Abstract

INTRODUCTION: Several aziridinylbenzoquinone drugs have undergone clinical trials as potential antitumor drugs. These bioreductive compounds are designed to kill cells preferentially within the hypoxia tumor microenvironment. The bioreductive compound of bis-type naphthoquinone synthesized in our laboratory, 2-aziridin-1-yl-3-[(2-{2-[(3-aziridin-1-yl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)thio]ethoxy }ethyl)thio]naphthoquinone (AZ-1), had the most potent death effect on the breast cancer cells BC-M1 in our previous screening. In the present study, we determined that the mechanism of the death effect of BC-M1 cells induced by AZ-1 was mediated by the apoptosis pathway. METHODS: We evaluated the cytotoxicity of AZ-1 and the anti-breast cancer drugs tamoxifen and paclitaxel to BC-M1 cells and MCF-7 cells by the MTT assay and measured the apoptosis phenomena by Hoechst 33258 staining for apoptotic bodies. We also quantified the sub-G1 peak area and the ratio of the CH2/CH3 peak area of the cell membrane in BC-M1 cells by flow cytometry and 1H-NMR spectra, respectively. The apoptosis-related protein expressions, including p53, p21, the RNA-relating protein T-cell restricted intracellular antigen-related protein, cyclin-dependent kinase 2 (cell cycle regulating kinase) and pro-caspase 3, were detected by western blot, and the caspase-3 enzyme activity was also quantified by an assay kit. RESULTS: AZ-1 induced two of the breast cancer cell lines, with IC50 = 0.51 microM in BC-M1 cells and with IC50= 0.57 microM in MCF-7 cells, and showed less cytotoxicity to normal fibroblast cells (skin fibroblasts) with IC50= 5.6 microM. There was a 10-fold difference between two breast cancer cell lines and normal fibroblasts. Of the two anti-breast cancer drugs, tamoxifen showed IC50= 0.12 microM to BC-M1 cells and paclitaxel had much less sensitivity than AZ-1. The expression of p53 protein increased from 0.5 to 1.0 microM AZ-1 and decreased at 2.0 microM AZ-1. The p21 protein increased from 0.5 microM AZ-1, with the

highest at 2 microM AZ-1. Regarding the AZ-1 compound-induced BC-M1 cells mediating the apoptosis pathway, the apoptotic body formation, the sub-G1 peak area, the ratio of CH2/CH3 of phospholipids in the cell membrane and the enzyme activity of caspase-3 were all in direct proportion with the dose-dependent increase of the concentration of AZ-1. The death effect-related proteins, including T-cell restricted intracellular antigen-related protein, cyclin-dependent kinase 2, and pro-caspase-3, all dose-dependently decreased with AZ-1 concentration. CONCLUSIONS: The AZ-1-induced cell death of BC-M1 cells mediating the apoptosis pathway might be associated with p53 protein expression, and AZ-1 could have the chance to be a candidate drug for anti-breast cancer following more experimental evidence, such as animal models.