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

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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-G_1 peak area and the ratio of the CH_2/CH_3 peak area of the cell membrane in BC-M1 cells by flow cytometry and ^{1}H-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 IC_{50} = 0.51 μM in BC-M1 cells and with IC_{50} = 0.57 μM in MCF-7 cells, and showed less cytotoxicity to normal fibroblast cells (skin fibroblasts) with IC_{50} = 5.6 μM. There was a 10-fold difference between two breast cancer cell lines and normal fibroblasts. Of the two anti-breast cancer drugs, tamoxifen showed IC_{50} = 0.12 μM 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 μM AZ-1 and decreased at 2.0 μM AZ-1. The p21 protein increased from 0.5 μM AZ-1, with the highest at 2 μM AZ-1. Regarding the AZ-1 compound-induced BC-M1 cells mediating the apoptosis pathway, the apoptotic body formation, the sub-G_1 peak area, the ratio of CH_2/CH_3 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.

Keywords: apoptosis, bioreductive compound, bis-type aziridinylnaphthoquinone, breast cancer cells (BC-M1 and MCF-7)

Introduction

The bioreductive drugs, aziridinylbenzoquinones, are a class of compounds designed to exploit one of the features of solid tumor biology caused by an inadequate blood supply to the solid tumor; namely, tumor hypoxia. Such regions generally are resistant to radiation and other oxygen-requiring treatment [1-4]. The ideal bioreductive drug should be administered as an inactive prodrug that is only activated

AZ-1 = 2-aziridin-1-yl-3-[(2-{2-[(3-aziridin-1-yl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)thio]ethoxy}ethyl)thio]naphthoquinone; cdk = cyclin-dependent kinase; DMEM = Dulbecco's modified Eagle's medium; IC_{50} = inhibition concentration of 50% cell growth; NMR = nuclear magnetic resonance; PBS = phosphate-buffered saline; TIAR = T-cell restricted intracellular antigen-related protein.