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Original article

Reversal effect of substituted 1,3-dimethyl-1*H*-quinoxalin-2-ones on multidrug resistance in adriamycin-resistant K562/A02 cells

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Abstract

QA1 and QA3 are the derivatives of substituted 1,3-dimethyl-1*H*-quinoxalin-2-ones that may selectively antagonize P-glycoprotein (P-gp) in multidrug resistance (MDR) cancer cells. Herein, we examined the reversal effect of two compounds on MDR in adriamycin (Adr)-induced resistant K562/A02 cells. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay showed that QA1 and QA3 weakly inhibited the growth of tumor cells. However, the compounds increased Adr-induced cytotoxicity toward K562/A02 cells. The IC_{50} values of Adr toward K562/A02 were decreased in the presence of QA1 or QA3. The maximal reversal fold (RF) of QA1 and QA3 was reached 6.9 and 9.0, respectively. The action of QA1 and QA3 was also confirmed by the increase of intracellular Adr accumulation in K562/A02 cells. In mechanism study, the intracellular accumulation and efflux of Rh123 were measured using multilabel counter with excitation/emission wavelengths of 485/535 nm. An increase of intracellular Rh123 and the decrease of efflux were observed in K562/A02 cells incubation with QA1 or QA3, indicating that the activity of P-gp was blocked. These results suggested that the derivatives of substituted 1,3-dimethyl-1*H*-quinoxalin-2-ones might reverse MDR in K562/A02 cells *via* inhibition activity of P-gp. QA1 and QA3 might be the candidate agents for reversing MDR of cancer.

Keywords: Substituted 1,3-dimethyl-1*H*-quinoxalin-2-ones; K562/A02 cells; P-glycoprotein (P-gp); Multidrug resistance (MDR); Reversal effect

1. Introduction

Multidrug resistance (MDR) is a critical issue in cancer chemotherapy. Over-expression of P-gp is the most frequent cause of MDR. P-gp, a transmembrane glycoprotein, functions as an ATP-dependent drug transporter which unilaterally transports intracellular drugs out of cells to acquire drug resistance. A range of agents that can reverse the MDR phenotype and restore drug sensitivity to cancer cells have been developed [1,2]. However, most of these agents have proven to be intrinsic toxic or the undesired effects on the pharmacokinetics of accompanying anticancer drugs. For instance, initial attempts to develop MDR modulators focused on verapamil and cyclosporin A [3–5], these compounds demonstrated excellent *in vitro* reversal of MDR, but failed to achieve clinical success due to their toxicity and/or their alteration of the pharmacokinetics of the coadministered anticancer drugs [5,6]. Screening 11,000 compounds from commercially available libraries, Smith et al. identified several structural platforms with good potential as MDR antagonists [7]. Among these, the 2-oxoquinoxaline or quinoxalinone scaffold showed promise as a versatile scaffold to study P-gp antagonist [7]. In our lab, we have designed and synthesized a series of substituted 1,3-dimethyl-1*H*-quinoxalin-2-ones through efficient and simple approach [8]. We then found that some of the compounds displayed potential inhibition effect on MDR of cancer. In this study, we examined the reversal effect of the compounds, QA1 and QA2, on MDR in Adr-resistant K562/A02 cells.

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2. Materials and methods

2.1. Chemicals

Compounds QA1 and QA3 (Fig. 1), the derivatives of substituted 1,3-dimethyl-1*H*-quinoxalin-2-ones, were synthesized through an efficient sequence of acylation, nitration, reduction, intramolecular alkylation and oxidation [8]. The compounds were dissolved in dimethylsulfoxide (DMSO, Sigma–Aldrich) for the *in vitro* assay.

2.2. Cell lines and cell culture

The human chronic myeloid leukemia cell line K562, and its MDR counterpart K562/A02 [9], was obtained from the Department of Pharmacology, the Institute of Hematology of Chinese Academy of Medical Sciences (Tianjin, China). K562/A02 cells were maintained in a complete RPMI-1640 medium containing 1 μg/ml adriamycin (Adr, Wanle, Shenzhen, China) at 37 °C in a humidified atmosphere of 5% CO_2. The cells were cultured for 2 weeks in drug-free medium prior to their use in the experiments.

2.3. Determination of P-gp

K562/A02 and K562 cells were seeded into six-well plates at a density of 5×10^5/well in the presence of Adr at a concentration of 1 μg/ml. Cells were washed twice with ice-cold PBS and labeled with R-phycoerythrin-conjugated mouse anti-human monoclonal antibody against P-gp according to manufacturer's instruction (Becton Dickinson, USA), and the nonspecific labeling was corrected by its isotype control. The fluorescent intensity on cells was analyzed using FACScan flow cytometry (Becton Dickinson, USA) [10].

2.4. Assay of cytotoxicity and reversal of MDR

To test for reversal activity of substituted 1,3-dimethyl-1*H*-quinoxalin-2-ones to P-gp-mediated MDR, cytotoxicity of compounds QA1 and QA3 toward K562/A02 cells was first measured by MTT assay [11]. Briefly, K562/A02 cells ($1-2 \times 10^4$/well) were seeded in 96-well plates. After 12 h incubation, the cells were treated with various concentrations of QA1 or QA3 for 24, 48, 72, 96 and 120 h, respectively. Cell viability was assessed by adding 20 μl of MTT reagent (5 mg/ml, 3-[4, 5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide; Sigma, USA) for 4 h-incubation. Light absorbance of the solution was measured at 570 nm on the plate reader (TECAN, Austria).

The reversal effect of QA1 and QA3 was then investigated with the same method. The cells seeded in 96-well plates were treated with varying concentrations of Adr in the absence or presence of various concentrations of QA1 or QA3 (2–10 μg/ml) for 72 h. IC_{50} values for Adr (concentration resulting in 50% inhibition of cell growth) were calculated from plotted results using untreated cells as 100%. The reversal fold (RF) values, as potency of reversal, were calculated from fitting the data to RF = IC_{50} of Adr alone/IC_{50} of Adr in the presence of QA1 or QA3 [12]. Triplicate experiments with triplicate samples were performed. Control cultures included equivalent amount of DMSO (as the solvent control), which does not modulate the growth or drug sensitivity of these cells at the concentrations used in these studies. To assess the toxicity of the compounds toward drug sensitive cells, the effects of QA1 and QA3 on the growth of K562 cells were determined by the same methods. In all the experiments, verapamil (Wanle, Shenzhen, China) was used as a positive control agent.

2.5. Intracellular Adr accumulation

Accumulation of Adr was measured using a standard procedure by incubating K562/A02 cells for 1 h at 37 °C in the presence of Adr (3 μg/ml) alone or in combination with QA1 and QA3 (4, 8 μg/ml). Cells were then harvested and washed twice by ice-cold PBS. Cells were placed in ice-water to block the reaction until analysis. The intracellular mean fluorescence intensity (MFI) associated with Adr was determined by multilabel counter (PerkinElmer, USA) with excitation/emission wavelengths of 485/585 nm 30 min later [13].

2.6. Rh123 accumulation

The effect of QA1 and QA3 on P-gp activity was assessed by measuring intracellular accumulation of Rh123 [10]. K562/A02 and K562 cells were seeded into 96-well plates at a density of 1×10^4/well. Cells were pretreated with QA1 or QA3 (4, 8 μg/ml), respectively, for 90 min and then were incubated with 2 μg/ml of Rh123 in culture medium in dark at 37 °C in 5% CO_2 for another 90 min. The cells were washed twice with ice-cold PBS. The MFI associated with Rh123 was measured using multilabel counter with excitation/emission wavelengths of 485/535 nm [10].

2.7. Efflux experiment

K562/A02 and K562 cells were first cultured with medium containing 2 μg/ml of Rh123 at 37 °C for 90 min, washed 3

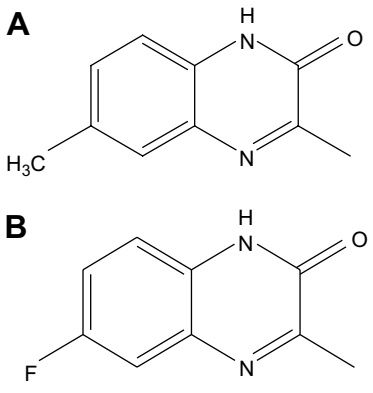

Fig. 1. Structures of substituted 1,3-dimethyl-1*H*-quinoxalin-2-ones compounds QA1 (A) and QA3 (B).