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Co-treatment with the anti-malarial drugs mefloquine and primaquine highly sensitizes drug-resistant cancer cells by increasing P-gp inhibition

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ABSTRACT

The purpose of this study was to identify conditions that will increase the sensitivity of resistant cancer cells to anti-mitotic drugs. Currently, atovaquine (ATO), chloroquine (CHL), primaquine (PRI), mefloquine (MEF), artesunate (ART), and doxycycline (DOY) are the most commonly used anti-malarial drugs. Herein, we tested whether anti-malarial drugs can sensitize drug-resistant KBV20C cancer cells. None of the six tested anti-malarial drugs was found to better sensitize the drug-resistant cells compared to the sensitive KB cells. With an exception of DOY, all other anti-malarial drugs tested could sensitize both KB and KBV20C cells to a similar extent, suggesting that anti-malarial drugs could be used for sensitive as well as resistant cancer cells.

Furthermore, we examined the effects of anti-malarial drugs in combination with an antimitotic drug, vinblastine (VIN) on the sensitisation of resistant KBV20C cells. Using viability assay, microscopic observation, assessment of cleaved PARP, and Hoechst staining, we identified that two anti-malarial drugs, PRI and MEF, highly sensitized KBV20C-resistant cells to VIN treatment. Moreover, PRI- or MEF-induced sensitisation was not observed in VIN-treated sensitive KB parent cells, suggesting that the observed effect is specific to resistant cancer cells. We demonstrated that the PRI and MEF sensitisation mechanism mainly depends on the inhibition of p-glycoprotein (P-gp). Our findings may contribute to the development of anti-malarial drug-based combination therapies for patients resistant to anti-mitotic drugs.

1. Introduction

Anti-mitotic drugs are widely used to treat numerous types of cancers [1,2]. These compounds inhibit mitosis by targeting microtubules and preventing their polymerization or depolymerization [1–3]. However, patients develop resistance to these drugs [4–7]. Thus, in order to improve the efficacy of treatment, research has focused on increasing anti-mitotic-associated apoptosis.

Various anti-malarial drugs have been developed to effectively combat malaria in humans. Atovaquine (ATO), chloroquine (CHL), primaquine (PRI), mefloquine (MEF), artesunate (ART), and doxycycline (DOY) are the most commonly used anti-malarial drugs [8–10]. These anti-malarial drugs are also shown to be potentially useful in the treatment of cancer [11–21]. These drugs have been investigated in the treatment of numerous types of cancers, sometimes in combination with chemotherapy [22–24]. Since the toxicity of these drugs is already known, these drugs would be readily available for clinical use once their anti-cancer activities are better understood.

Since a better understanding of the mechanism governing the sensitisation effect of anti-malarial drugs in cancer patients could facilitate their therapeutic use, we tried to identify the mechanism involved in the sensitizing effect of anti-malarial drugs on cancer cells. In the present study, we found that co-treatment with either PRI or MEF in combination with vinblastine (VIN) highly sensitized drug-resistant KBV20C cancer cells. We also found that PRI and MEF sensitisation effect involved p-glycoprotein (P-gp) inhibition, which prevented the efflux of VIN. Our results may contribute to the development of PRI- or MEF-based therapy for drug-resistant cancer patients.

2. Materials and methods

2.1. Reagents and Cell culture

Reagents and cell lines [25,26], in this study are provided in the Supporting Information. The cell lines were cultured in RPMI1640

Abbreviations: VIN, vinblasitne; ATO, atovaquone; CHL, chloroquine; PRL, primaquine; MEF, mefloquine; ART, artesunate; DOY, doxycycline; VER, verapamil; P-gp, p-glycoprotein; DMSO, dimethylsulfoxide; C-PARP, cleaved ploy ADP ribose polymerase; FACS, fluorescence-activated cell sorting; FBS, fetal bovine serum; TCA, trichloroacetic acid; PBS, phosphate buffered saline; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; RT, room temperature.

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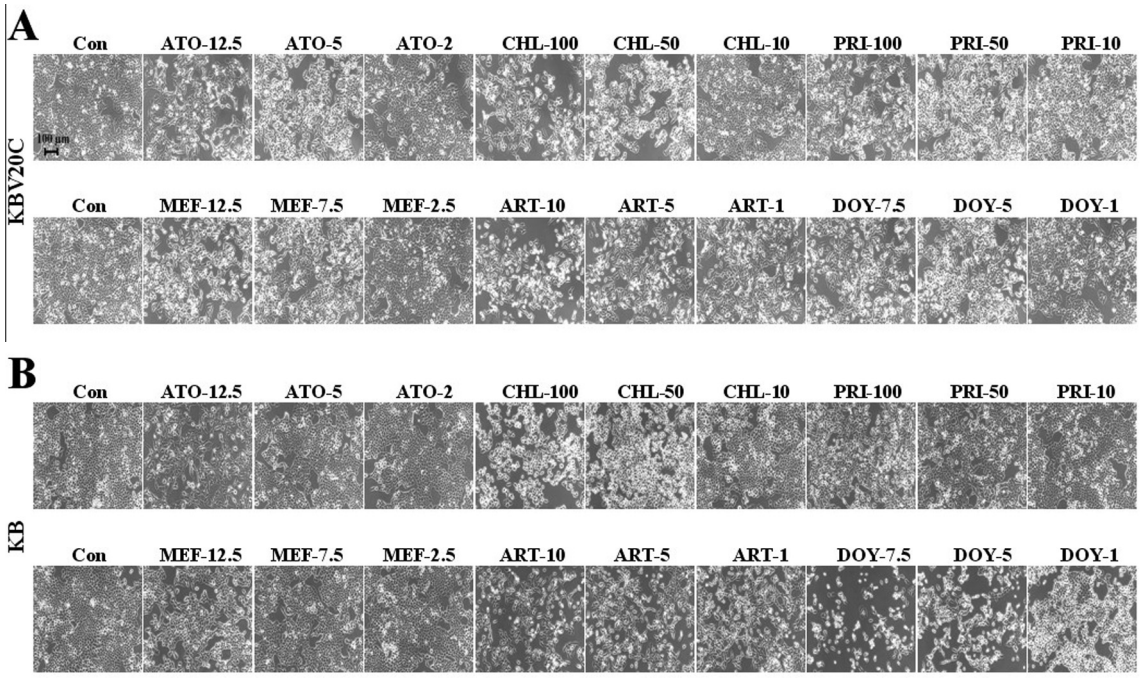

Fig. 1. Anti-malaria drugs have similar sensitisation effect on drug-sensitive KB and drug-resistant KBV20C cells, except DOY. (A) KBV20C cells and (B) KB cells were grown on 6-well plates and treated with 2 μM ATO (ATO-2), 5 μM ATO (ATO-5), 12.5 μM ATO (ATO-12.5), 10 μM CHL (CHL-10), 50 μM CHL (CHL-50), 100 μM CHL (CHL-100), 10 μM PRI (PRI-10), 50 μM PRI (PRI-50), 100 μM PRI (PRI-100), 2.5 μM MEF (MEF-2.5), 7.5 μM MEF (MEF-7.5), 12.5 μM MEF (MEF-12.5), 1 μg/ml ART (ART-1), 5 μg/ml ART (ART-5), 10 μg/ml ART (ART-10), 1 μg/ml DOY (DOY-1), 5 μg/ml DOY (DOY-5), 7.5 μg/ml DOY (DOY-7.5), or DMSO (Con). After 48 h, all cells were observed using an inverted microscope with a 10X objective lens.

containing 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin (WelGENE, Daegu, South Korea).

2.2. Cellular viability assay

The detailed method is described in the Supporting Information.

2.3. Calcein-AM uptake tests

The detailed method [27–29] is described in the Supporting Information.

2.4. Western blot analysis

The detailed method [27–31] is described in the Supporting Information.

2.5. Hoechst staining

The detailed method is described in the Supporting Information.

3. Results

3.1. Anti-malarial drugs have similar sensitisation effect on drug-sensitive KB and drug-resistant KBV20C cancer cells, except DOY

To identify anti-malarial drugs that can sensitize cancer cells resistant to anti-mitotic drugs, ATO, CHL, PRI, MEF, ART, and DOY were tested. The oral squamous cancer cell line KB and its sub-line KBV20C, which show multi-drug resistance to the microtubule-targeting drugs [25,26], were used to test the sensitisation effects of anti-malarial drugs. Drug concentration ranges previously used by other groups for in vitro studies were chosen. We assume that positive results obtained with the selected drug concentrations may be easily applied in clinical settings.

Microscopic observations for cellular growth were performed after treatment with anti-malarial drugs. KB and KBV20C cells were treated for 48 h in the presence of increasing concentrations of anti-malarial drugs, and their cellular growth was compared. Three different concentrations of each drug were used. As shown in Fig. 1A–B, cellular growth was similar between KB and KBV20C cells treated with ATO, CHL, PRI, MEF, or ART. KBV20C cells offered resistance to DOY. In fact, treatment with high concentrations of DOY only reduced growth in drug-sensitive KB cells. While none of the anti-malarial drugs alone had a stronger effect on KBV20C cells compared to KB cells, our results indicate that anti-mitotic drug-resistant cancer cells can be sensitized by most of the anti-malarial drugs to a similar level as the sensitive cells.

3.2. Co-treatment with either PRI or MEF sensitizes VIN-treated KBV20C resistant cancer cells

We next tested whether any anti-malarial drug can sensitize the KBV20C drug-resistant cells to an anti-mitotic drug, VIN. We performed a cellular viability assay to determine whether ATO, CHL, PRI, MEF, ART, or DOY sensitizes VIN-treated KBV20C cells. As shown in Fig. 2A–F, we found that co-treatment with PRI or MEF and VIN reduced viability at the three different concentrations tested. However, the other drugs tested did not have any sensitizing effects. Comparison of sensitisation levels between PRI and MEF showed that MEF reduced viability at lower concentrations than PRI, suggesting a higher sensitizing effect of MEF on VIN-treated KBV20C cells. Microscopic observation for cellular growth after co-treatment with anti-malarial drugs and VIN were performed. Fig. 3A shows that cellular growth was highly reduced in VIN-treated KBV20C cells co-treated with PRI or MEF. In addition, microscopic observation showed that co-treatment with PRI or MEF