The modulation of drug efflux transporter by curcumin in MCF7 breast cancer cells after repeated exposure of endoxifen and estradiol

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INTRODUCTION

Breast cancer (BC) is the most common type of cancer in women. Treatments for BC include surgery, radiation therapy, endocrine therapy, and chemotherapy. For endocrine therapy, tamoxifen (TMX) is the most common estrogen receptor (ER) modulator prescribed for ER-positive BC [1].

TMX is metabolized by CYP3A4 and CYP2D6 into several metabolites, which include 4-hydroxy-TMX, N-desmethyl-TMX, and 4-hydroxy-N-desmethyl-TMX, which is also known as endoxifen (E) [2]. E is an active metabolite of TMX that has recently been developed as a new endocrine therapeutic agent for BC patients unresponsive to antiestrogen treatment [3]. E exerts its anticancer effects by degrading ER alpha, which leads to the inhibition of estrogen-induced BC cell proliferation [4]. However, BC cell resistance to endocrine treatment, including E, is likely to develop over time [5-7].

Numerous mechanisms are responsible for the development of endocrine resistance, including downregulation of components in the ER signaling pathway, alterations to the cell cycle, crosstalk with growth factor signaling pathways, BC stem cells, and modulation of drug transporters [6,8]. A known mechanism of BC cell resistance to TMX is the overexpression of the drug efflux transporters P-glycoprotein, BCRP, MRP1, and MRP2 [9,10]. E is also known as a P-glycoprotein substrate in vitro and in vivo [11]. Thus, BC cell resistance to E might also be due to the overexpression of various drug efflux transporters.

To overcome the emergence of the resistance phenomenon, many groups have attempted to generate inhibitors of drug efflux transporters [12]. Curcumin (CM), a natural diphenolic compound from Curcuma longa, exerts antiproliferative effects and modulates the activities of drug efflux transporters [12,13]. Therefore, the aim of the present study was to determine whether CM can prevent the decreased sensitivity of BC cells to E, when administered together with estradiol (β-E2) and whether the underlying mechanism involves modulation of drug efflux transporters.

MATERIALS AND METHODS

Materials and reagents
CM was purchased from Plamed Green Science Limited (Xi’an, China), E was purchased from Tocris Bioscience (Ellisville, MO, USA), and β-E2 was purchased from Sigma-Aldrich Pte. Ltd. (Singapore). Dimethyl sulfoxide (DMSO) was purchased from Vivantis Technologies Sdn Bhd (Malaysia). High-glucose Dulbecco’s modified Eagle’s medium (DMEM), heat-inactivated fetal bovine serum (FBS), penicillin/streptomycin, and fungizone were purchased from Biowest LLC (Riverside, MO, USA). The High Pure RNA Isolation Kit, Transcripter First Strand cDNA Synthesis Kit, and FastStart DNA Master SYBR Green I were purchased from Roche Molecular Systems, Inc. (Pleasanton, CA, USA). Primers for P-glycoprotein, BCRP, MRP1, and α-actin were purchased from 1st BASE Pte Ltd. (Singapore).

Cell culture
MCF-7 BC cells were kindly provided by Makmal Terpadu Laboratory, Faculty of Medicine, Universitas Indonesia. The cells were maintained in DMEM high glucose supplemented with 10% FBS, 10 U/mL penicillin, 100 μg/mL streptomycin, and 2.5 μg/mL fungizone at 37°C in a humidified atmosphere of 5% CO₂.

Drug preparation
E, β-E2, and CM were diluted in DMSO to the desired concentrations. A DMSO concentration of <0.001% was used as a negative control throughout this study.

Drug treatment
MCF7 cells were seeded into the wells of a 6-well plate at 10,000 cells per well and further treated with control (DMSO) or E + β-E2.

RESULTS

Decreased sensitivity of BC cells was shown by the increased cell viability of MCF7 cells after 8 weeks. This condition was accompanied with increased mRNA expression of P-glycoprotein, BCRP, and MRP1 in cells treated with E+β-E2, as compared with the vehicle only. CM, administered in combination with E+β-E2, resulted in decreased cell viability versus E and β-E2 and also decreased in mRNA expression of P-glycoprotein, BCRP, and MRP1.

Conclusion: CM partially reversed the sensitivity loss of BC cells to E in the presence of β-E2 by modulating drug efflux transporters.

Keywords: Curcumin, Efflux transporters, Endoxifen, Estradiol.

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E is a new drug under development for the treatment of advanced BC; the efficacy of E on cell viability was lost after treatment for 8 weeks. Hence, the aim of the present study was to determine the efficacy of CM administered for the prevention of decreased sensitivity of BC cells to E by coadministration with β-E2.

The efficacy of E on cell viability was lost after treatment for 8 weeks. E is a new drug under development for the treatment of advanced BC; thus, few studies have investigated the mechanism of E resistance.

The results are expressed as the percentage relative to the control cells.

Cell viability assay
Cell viability was determined using the trypan blue dye exclusion assay. The number of viable cells was counted using a hemocytometer.

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis
Total cellular RNA was extracted from cell pellets using the high pure RNA isolation kit in accordance with the manufacturer's protocol. RNA (1 µg in 20 µL) was converted to cDNA using the Transcriptor First Strand cDNA Synthesis kit. qRT-PCR was performed using the FastStart DNA Master SYBR Green I kit, according to the manufacturer's protocol, with β-actin as the housekeeping gene, and qRT-PCR was performed with the following primers: β-actin (F: 5′-TTC GGC TTG CAA CAA CTA TG-3′; R: 5′-TCC AGA CAC ACC AGC GAT AA-3′), P-glycoprotein (F: 5′-TCA GTG CTT ATT TTG GTC-3′; R: 5′-TCT TGC TGG ATC ATG GAA TC-3′), BCRP (F: 5′-TGC TTC GTC CAA CAA CTA TG-3′; R: 5′-TCT AGC AGC ACC ACG GAT AA-3′), and MRP1 (F: 5′-ATG TCA CGT GGC CTA CCA GC-3′; R: 5′-GAA GAC TGA ACT CCC TTC CG-3′).

The total reaction volume was 20 µL. Reactions were performed in a 32-well plate using a LightCycler® Nano Instrument (F. Hoffmann-La Roche Ltd., Basel, Switzerland) with the following cycling conditions: 95°C for 10 min, followed by 45 cycles at 95°C for 20 s and annealing temperature of 55°C for 1 min for β-actin or 53°C for 20 s for P-glycoprotein, MRP1, and BCRP. The raw quantification cycle (Cq) values were analyzed by relative quantification using the Livak method to determine normalized expression ratios of target genes.

Data analysis
Data were analyzed by one-way analysis of variance followed by the Tukey method, with α=0.05, and are presented as the mean ± standard deviation. All statistical analyses were performed with GraphPad Prism 6 software (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS
E in the presence of β-E2 showed control of cell viability in the 1st week of drug treatment. Concomitant treatment with E and CM in the presence of β-E2 showed a synergistic effect, with additional suppression of cell viability (Fig. 1). However, after 8 weeks of treatment with E and β-E2, the efficacy of E was clearly lost, as BC cell viability had surpassed that of the control cells. Concomitant treatment with a low concentration of CM did not improve the sensitivity of BC cells to E, whereas larger concentrations showed good suppression of cell viability after 8 weeks of treatment (Fig. 1).

P-glycoprotein mRNA expression analysis showed that continuous treatment with E + β-E2 resulted in increased expression, whereas concomitant treatment with CM both at low and high concentrations reversed expression levels back to normal (Fig. 2). BCRP mRNA expression was also increased after 8 weeks of treatment with E and β-E2. CM at both concentrations was shown to suppress BCRP mRNA expression but not potent enough to reverse expression back to control values (Fig. 3).

Continuous treatment with E in the presence of β-E2 was also shown to increase MRPI mRNA expression. However, lower concentrations of CM failed to suppress the increased expression of MRPI, whereas CM at higher concentrations tended to suppress MRPI mRNA expression, but the effect was not sufficiently potent (Fig. 4).
estrogen sulfotransferase 1E1 and human phenol sulfotransferase 1A1 isofrom 1 to catalyze the sulfation of β-E2, which plays important roles in the termination of estrogen signaling through the loss of ER activity.

Drug efflux transporters are involved in the decreased sensitivity of BC cells to TMX [9,10]. In the present study, the sensitivity of BC cells to E was diminished, which was followed by increased activities of the drug efflux transporters P-glycoprotein, BCRP, and MRP1. According to Tett et al. [15], E is a substrate of P-glycoprotein (multidrug resistance 1). However, it remains unclear as to whether E induces or inhibits P-glycoprotein expression.

CM, a polyphenol derived from *C. longa*, has a wide range of beneficial properties as a chemotherapeutic agent and modulator of ABC efflux transporters [12,13]. A recent study also showed that CM administered together with TMX increased the antitumor activity, as compared to TMX alone. This effect may occur through the suppression of P-glycoprotein (MDR1) expression [16-18]. Squirewell et al. [14] proposed a mechanism of the interactions of polyphenols, including CM, with ABC transporters and showed that polyphenols counteracted tumor cell chemoresistance by interacting with the ATP-binding domains of P-glycoprotein and inhibits its ATPase activity site and steroid interacting regions of the protein cytosolic domains [13]. However, for a flavonoid to interact with MRP1, it also needs transport stimulation of reduced glutathione [1,19]. This phenomenon might explain the different patterns of CM effects in regard to P-glycoprotein, BCRP, and MRP1.

Low-concentration CM failed to reverse the decreased sensitivity of E, even when mRNA expression levels of P-glycoprotein and BCRP were significantly decreased. This finding suggests that MRP1 expression might be the most consistent match with cell viability. Hence, MRP1 might be the dominant transporter in the development of decreased sensitivity of BC cells to E.

**CONCLUSION**

CM partially reversed the loss of sensitivity of BC cells to E in the presence of β-E2 by modulation of the drug efflux transporters P-glycoprotein, BCRP, and MRP1. CM should be further investigated as a drug candidate for the prevention of E resistance in BC. However, further studies are needed to elucidate the underlying mechanisms.

**CONFLTS OF INTEREST**

All authors have none to declare.

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