Curcumin Increases Anti-Cancer Activity of Tamoxifen in MCF-7 Breast Cancer Cells Through the Suppression of MDR1 mRNA Expression

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Introduction: A major limitation in cancer treatment is the development of multidrug resistance (MDR). Tamoxifen is a substrate of multidrug resistance protein 1 (MDR1) or P-glycoprotein, while curcumin is known as MDR1 inhibitor. The objective of this study was to analyze cell viability and MDR1 mRNA expressions in breast cancer cells, treated with tamoxifen alone or in combination with curcumin.

Method: MCF-7 breast cancer cells lines were treated with tamoxifen 1 µM alone or in combination with curcumin at 10, 20 and 35 µM, while tamoxifen-verapamil (50 µM) was used as positive control for MDR1 inhibitor. The cells were harvested and counted for their viability and extracted for total RNA. MDR1 mRNA expressions were quantified using quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Results: MCF-7 cells viability were decreased in concomitant treatment of tamoxifen + curcumin compared with tamoxifen alone, and appeared to be dose-dependent. The expressions of MDR1 mRNA were shown to be suppressed in MCF-7 cells co-treated with tamoxifen and curcumin.

Conclusion: Concomitant administration of tamoxifen and curcumin resulted in an increased anticancer activity compared with tamoxifen alone. This effect is thought to be via the suppression of MDR1 mRNA expressions.

Keywords: MDR1, Tamoxifen, Curcumin, MCF-7 Breast Cancer Cells, P-Glycoprotein.
cancer cells resistance development to tamoxifen through the inhibition of MDR1 expressions.

2. METHODS

2.1. Materials and Reagents

This is an in vitro study in MCF7 breast cancer cells. Breast cancer cell lines MCF-7 was a kind gift from Makmal Laboratory (FKUI, Indonesia). Tamoxifen was purchased from Santa Cruz (USA). Curcumin, verapamil and dimethylsulfoxide (DMSO) were obtained from Sigma-Aldrich Ltd. (Singapore). Dulbecco minimal essential medium (DMEM), Triple Express and Fetal bovine serum (FBS) was obtained from Gibco Ltd. (Singapore). Total RNA Mini Kit obtained from Geneaid (Taiwan). Primer pairs were purchased from First BASE Ltd. (Singapore). KAPA SYBR fast one-step RT-PCR kit Bio-Rad iCycler obtained from Kapa Biosystems (USA).

2.2. Cell Culture

Cell were cultured on DMEM supplemented with fetal bovine serum (FBS) 10%, fungizone 2.5 µg/mL, penicillin 100 IU/mL and streptomycin 100 µg/mL at 37 °C and 5% CO₂. Cells were sub-cultured at about 90% confluency and placed to a 6-well plates for further study.

2.3. Preparation of Drugs

Tamoxifen, verapamil and curcumin were dissolved in DMSO and then diluted with DMEM into desired concentrations. Final concentrations of DMSO were less than 0.001%.

2.4. Tamoxifen Dose Determination

We determine the optimum dose tamoxifen that can reduce the viability of MCF-7 cells, a 1 × 10⁵ cells were placed on a 6-well plates and treated with tamoxifen at concentrations of 0.25 µM, 1 µM and 4 µM. Medium were replaced every two days and tamoxifen were reapplied. Tamoxifen were dissolved in DMSO (less than 0.001%). Every two days, cells were trypsinized and counted using trypan blue exclusion method.

2.5. Drug Treatment

MCF-7 cells at 1 × 10⁵ were cultured on a 6-well plate and incubated for 24 prior to drug treatment. Tamoxifen and curcumin (10, 20 and 35 µM) were applied to the cells and were changed at day 1, 4, 6, 9, 12 days. DMSO was used as negative control and verapamil 50 µM was used as positive control for MDR1 inhibitor. Cells were harvested using Triple Express and counted, then were subjected for total RNA extraction using a total RNA mini kit reagent (Geneaid) using procedure provided by the manufacturer.

2.6. Quantitative Reverse Transcriptase-Polymerase Chain Reactions (qRT-PCR)

Quantitative RT-PCR were done on Bio-Rad iCycler machine, using KAPA SYBR Fast one-step qRT-PCR kit (KAPA, USA). Primer sequences used in qRT-PCR were: MDR1: Fwd-3′-AATA GATGCTTTCTGTGGCAAG-5′; Rev-5′-AAAGCGACTGAATTCAACA-3′; GAPDH: Fwd 3′-GGTGGTCTCCTCTGACTTCAACA-3′.

Samples were prepared by mixing 200 nM of each primer, 7.5 µL KAPA SYBR Fast qPCR master mix Green, 0.4 mL KAPA RT Mix (50×), 0.4 mL dUTP, 275 ng RNA template, and RNase-free water to 15 mL. Samples were then incubated in the qRT-PCR machine with the following conditions: 5 min at 42 °C for cDNA synthesis, 5 min at 95 °C for reverse transcriptase inactivation, and 35 cycles consisting of 3 seconds at 95 °C for denaturation stage and 1 min at 64 °C for annealing stage, 30 seconds for the extension phase. After that final elongation for 5 minutes and 30 seconds to the cycle 81 times for melting time. Threshold value (Ct) was calculated automatically by the software. The data is then processed according to the Livak method. Gene target in this study were MDR1/P-glycoprotein, whereas the reference gene used was GAPDH.

3. RESULT

To determine optimum dose of tamoxifen that induced cancer cells resistance to drugs, we exposed MCF-7 cells to several concentrations of tamoxifen. We found that 1 µM of tamoxifen was potent enough to suppress cell growth and after five days of culture resulted in an increase in cell growth (Fig. 1).

We used tamoxifen 1 µM for further experiment and applied them to MCF-7 with and without curcumin (10 µM, 20 µM and 35 µM) or verapamil 50 µM. We observed that addition of curcumin or verapamil (as positive control of MDR1 inhibitor)
Our result showed that initially tamoxifen reduced the expression of MDR1 expressions (normalized to GAPDH as reference gene) compared to control (DMSO), but after five days of treatment, there was increased in expressions (Fig. 3). Addition of curcumin 10, 20 and 35 μM or verapamil 50 μM were shown to decreased MDR1 expressions induced by continuous treatment of tamoxifen. The decreased in MDR1 mRNA expressions was shown to be dose-dependent. Curcumin at 20 and 35 μM was able to decrease MDR1 expressions as potent as verapamil (as positive control of MDR1 inhibitor).

4. DISCUSSION

Our study was aimed to determine the role of curcumin in inhibiting MDR1. We started our study by determining the most appropriate dose to induce drug resistance in MCF-7 cancer cells and we found that 1 μM is the most appropriate dose. Our result is in accordance with the result by Tehrani10 which showed that tamoxifen 1 μM reduced about 40% of MCF7 cell viability. This result is also consistent with previous studies by Motahari20 and Lykkefseldt21 in T47D breast cancer cells that confirmed that 1 μM of tamoxifen is adequate to induce tamoxifen resistance. Therefore, we used tamoxifen 1 μM for further experiments.

We showed that cell viability in MCF7 cancer cell induced by tamoxifen began to increase from Day 6. Whether this effect is reversible or not, is still undetermined. Study by Motahari20 indicates that stable resistance phenotype of tamoxifen can be achieved after three months of continuous culture. Curcumin was able to suppress the cell viability in tamoxifen-induced MCF-7 cells, and the effect was shown to be dose dependent.

In our study, tamoxifen was initially reduced MDR1 mRNA expression and began to increase on Day 6. Study by Teft10 and Sane22 proved that tamoxifen can induce MDR1 by activating pregnane X receptor (PXR), a transcription factor. Active form of PXR will form heterodimer with Retinoïd X Receptor (RXR), then bind to PXR responsive elements that stimulates MDR1 gene. Harmsen et al.23 also showed the effect of tamoxifen binding to PXR in colon cancer of adenocarcinoma type. This binding resulted in the decreased of doxorubicin concentrations in cancer cells.23

Our result showed that curcumin in combination reduced cell viability along with decreased MDR1 expression. This phenomenon was also showed in other types of cancer. Study by Tang21 showed that curcumin reduced MDR1 expressions in human gastric carcinoma cell line along with increased vincristine concentration. Mechanism on how curcumin inhibits MDR1 were shown by Labbozzetta25 and Anuchapreeda. Results by Labbozzetta25 revealed that curcumin inhibits MDR1 in tumor cells by occupying MDR1 substrate binding site. While study by Anuchapreeda26 showed that curcumin inhibits MDR1 by inhibiting ATP hydrolysis by MDR1-ATPase so that there were no energy for efflux.

5. CONCLUSION

We conclude that concomitant administration of tamoxifen and curcumin resulted in an increased anticancer activity compared with tamoxifen alone. The magnitude of viability reduction was proportional to dose and the time of exposure. This effect is thought to be via the suppression of MDR1 mRNA expressions.

References and Notes