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by Artikel 4 Tetraprenyltoluquinone, An Anticancer Compound Fro

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Research Article

Tetraprenyltoluquinone, an Anticancer Compound from *Garcinia cowa* Roxb Induce Cell Cycle Arrest on H460 Non Small Lung Cancer Cell Line

Fatma Sri Wahyuni[*1], Lim Siang Hui[2], Johnson Stanslas[3], Nordin Hj. Lajis[4], Dachriyanus[1,5]
[1]Faculty of Pharmacy, Andalas University, Kampus Limau Manis, Padang, West Sumatra, Indonesia.
[2]CARIF (Cancer Research Institute Foundation) Malaysia.
[3]Faculty of Medicine and Health Sciences, Universiti Putra Malaysia 43400 UPM, Serdang, Selangor, Malaysia.
[4]Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia.
[5]Faculty of Nursing, Andalas University, Kampus Limau Manis, Padang, West Sumatra, Indonesia.
***Corresponding author's E-mail:** fatmasriwahyuni@gmail.com

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ABSTRACT

Tetraprenyltoluquinone (TPTQ), isolated compound from stem bark of *Garcinia cowa* Roxb showed selective cytotoxicity towards H460 lung cancer cell line. We further investigated the ability of this compound in inducing cell cycle arrest by flow cytometry. Cell cycle analysis revealed that TPTQ caused cell cycle arrest in G_0/G_1 phase. Our results indicate that TPTQ induces cell cycle arrest and apoptosis in H-460 cells, suggesting that it might represent a potential new chemotherapeutic agent.

Keywords: Tetraprenyltoluquinone, *Garcinia cowa* Roxb, cell cycle analysis, lung cancer, flowcytometry.

INTRODUCTION

Cancer can be defined as a disease in which disorder occurs in the normal processes of cell division, which are controlled by the genetic material (DNA) of the cell.[1] Several plant-derived compounds are currently successfully employed in cancer treatment such as vincristine (leukemia, lymphoma, breast, lung, solid cancers and others), vinblastine (breast, lymphoma, germ-cell and renal cancer), paclitaxel (ovary, breast, lung, bladder, head and neck cancer), docetaxel (breast and lung cancer).[2] These agents were shown to block the cell cycle at different stages of the cell cycle, for examples; vincristine and paclitaxel inhibit mitosis, methotrexate blocks at the S-phase, doxorubicin blocks at the G_2 phase and topotecan blocks at the S and G_2 phase. Intensive research efforts are now in identifying cell cycle specific inhibitors for the treatment of cancer.[3]

In the previous paper, we have reported the isolation and the structure elucidation of tetraprenyltoluquinone (TPTQ, Fig. 1) from the stem bark of *G. cowa*.[4] This compound was inhibited the small lung cancer cell H460 without no activity towards MCF-7 and HL-60.[5] Since TPTQ showed great selectivity towards NCI-H460, in this study, we further investigated the ability of TPTQ in inducing cell cycle arrest by flow cytometry.

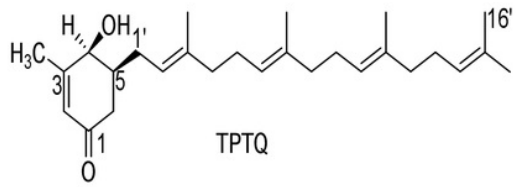

Figure 1: The structure of TPTQ

MATERIALS AND METHODS

Material

TPTQ was isolated from stem bark of *G. cowa*.[5] H460 cell line was purchased from the American Type Culture Collection (Manassas, VA, USA). The cancer cells were cultured in RPMI 1640 medium (Life Technologies, Paisly, UK) with 10%v/v fetal bovine serum (PAA Laboratories, Linz, Austria), 100 IU/mL penicillin and 100 µg/mL streptomycin (Life Technologies, Paisly, UK). Trypsin-EDTA was purchased from GIBCO (Auckland, New Zealand). Phosphate buffered saline (PBS) tablets, propidium iodide, ribonuclease A (RNase A) were obtained from Sigma Chemicals (St. Louis, USA). Tween-20 was purchased from Merck (Hohenbrunn, Germany). Dimethylsulfoxide (DMSO) was purchased from BDH Laboratory (England) and 3-(4,5dimethylthiazol-2yl)-diphenyltetrazolium bromide (MTT) from Phytotechnology Laboratories (Kansas, USA). Culture flask (25 cm^2 and 75 cm^2), 96-well plates and 10 ml serological pipettes were purchased from Becton Dickson (New Jersey, USA).

Instruments

Holten Laminar Airflow microbiological safety cabinet class II was obtained from Heto-Holten (Allerød, Denmark), and Galaxy CO_2 incubator was purchased from RS Biotech (Ayrshire, Scotland). A microplate reader equipped SOFTmax® Prosoftware (Versamax, Molecular Devices, California, USA) was used to measure of the formazan solution. FACSscan flow cytometry (Becton Dickinson, Sunnyale, CA) was used in cell cycle analysis.

Cell Culture

H-460 cell lines were maintained in RPMI 1640 culture medium, supplemented with 10% heat-inactivated FBS,

100 U/ml penicillin and 100 µg/ml streptomycin. Cells were grown in 25 cm2 tissue culture flasks in a humidified atmosphere containing 5% CO2 at 37 ºC. Once the cells reach 80% confluency, 1 ml of trypsin-EDTA solution was added to the flask for 5-10 min to detach the monolayer cells. The cells were occasionally observed under the inverted microscope until the cell layer was dispersed. Then, 3 ml of complete growth medium was added to the flask followed by repeated gentle pipetting to split apart the cell clumps. Approximately 0.5 - 1 × 10^6 cells were subcultured into a new 25 cm^2 flask containing 8 ml of fresh medium.

Cell cycle distribution analysis

Cells were seeded at density of 5 x 10^5 cells/ 5 ml RPMI 1640 medium in six well culture dishes. After leaving them overnight for attachment, the cells were treated with TPTQ at two concentration, 16 µM and 32 µM for 24 72 hours . The cells were trypsinized and collected in cold 1% BSA-PBS buffer and the cell density was determined. Approximately 5x10^5 5x10^6 cells were collected for each determination. The cells were then centrifuged at 1000 rpm for 10 min and washed twice in 1% BSA-PBS buffer. The cell pellet was resuspended in 1 ml of 0.2 µm-filtered PBS and transferred to a polystyrene round bottom tube (12 x 75 mm). Twenty µl of 10 mg/ml RNase A solution and 40 µl of 2.5 mg/ml propidium iodide were added and the tubes were then incubated at 37°C for 30 min.

DNA content (PI bound to DNA) of 10.000 cells for each determination was analyzed by FACScan flow cytometry (Becton Dickinson, Sunnyale, CA) in which an argon ion laser (488 nm) was used to excite PI and emission above 550 nm was collected. The cells forward scatter (FSC, linear scale), side scatter (SSC, linear scale), fluorescence 1 (F11, log scale), fluorescence 2 (F12, linear scale) and fluorescence 3 (F13, log scale) were recorded in Macintosh system. The single cell population was gated and DNA histograms were generated using WinMDI 2.8 flow cytometry software (Joseph Trotter, The Scripps Research Institute; URL http://facs.scripps.edu) and the percentage cell cycle phases was analyzed using the Cyclered ™ developed by Terry Hoy of Wales College of Medicine software .[6] Regions were assigned to each histogram which represented the pre-G_1 (apoptotic population, DNA content < G_1 but >10% G_1, S and G_2/M phases of cell cycle.[7]

RESULTS

Compared with the vehicle-treated controls, TPTQ treatment resulted in an appreciable arrest of H460 cells in G_0/G_1 phase of the cell cycle. It was observed that the G_0/G_1 phase population of the untreated cells was 39% and the percentage of cells in G_0/G_1 phase was significantly increased (47% cells and 57% at 16 µM and 32 µM concentrations of TPTQ, respectively; Fig. 2) after 24 h of treatment. This increase in the G_0/G_1 cell population was accompanied with a concomitant decrease of cell numbers in S phase and G2 M phases. Similar with 48 h incubation, the percentage of cells in G_0/G_1 phase was significantly increased consistently. About 56% and 58% cell of DNA population was shown for each concentrations of TPTQ, compare to 45% of the G_0/G_1 phase population of the untreated cells. The same results were showed at 72 h of incubation. The DNA population from 52% for untreated cell, become 70% and 74%, in 16 µM and 32 µM concentrations, respectively.

DISCUSSION

Cell cycle analysis of TPTQ was evaluated by using flow cytometry and this compound increased the number of cells at G_0/G_1 phase and the effect was consistent at different concentrations at various time duration of treatment. H-460 cells that were treated with TPTQ at 16 µM for 24 hr, induced 8% and 6% increase in the number of H-460 cells in the G_0/G_1 and S phase of the cell cycle, respectively, while there was a 14% decrease in the number of cells entering the G_2-M. Similarly, cells treated with 32 µM of TPTQ showed an 18% increase in the number of H-460 cells in the G_0/G_1 phase. However, a 4% decrease was observed in the number of cells entering the S phase of the cell cycle. After 24 h, the percentage of the sub-G1 cell population increased, suggesting that the blockage in G_0/G_1 phase results in the triggering of the apoptotic program.

Treatment for 48 hr caused 11% and 13% increase in the number of cells in the G_0/G_1 phase at 16 µM and 32 µM, respectively. A 12% decrease in the number of cells entering S phase of the cell cycle was observed at both concentrations. The 72 hr treatment at both 16 and 32 µM, increased the number of G_0/G_1 phase cells by 18% and 22%, respectively. These results corresponded with the 18% and 22% decrease in the number of H-460 cells in S phase, respectively, without any change in amount of cells in G_2/M phase. The sub-G_1 cells (apoptotic population) could be observed in the DNA histograms of cells after 72 hr of treatment at 16 µM and 32 µM.

At 72 h time points, 16 µM of this compound also induced sub-G_1 population indicative of apoptotic cells. When the concentration of TPTQ increased to 32 µM, there was a dramatic increase in apoptotic cells throughout the duration of treatment. This strongly suggests that, the apoptotic cells had originated very likely from cells that were arrested at the G_1-phase. The histogram of the cells at two concentrations and three time periods were shown in Figure 2.

Based on the data above, the percentage of DNA in G_0/G_1 phase consistently increased in treated cells when compared to the control cells in a time-dependent manner. This cell cycle analysis indicated TPTQ induced G_0/G_1 phase cell cycle arrest and followed by induction of apoptosis.

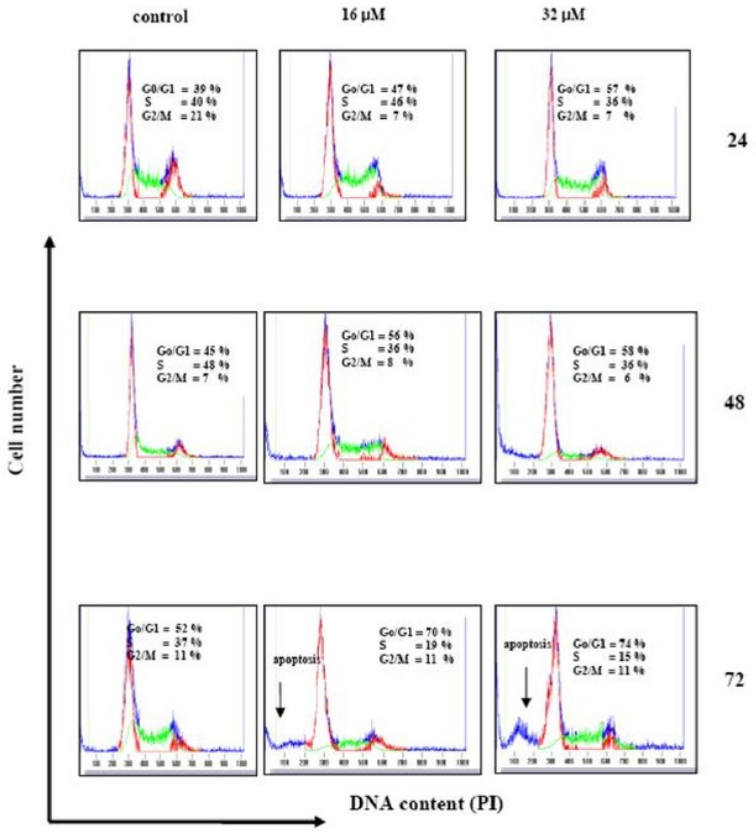

Figure 2: DNA histogram showing the cell cycle phase distribution of control and TPTQ treated H-460 cells. The cells were treated with 16 µM and 32 µM of TPTQ for various periods of time 24, 48 and 72 hr.

CONCLUSION

The mechanism of antitumor activity of TPTQ on H-460 cells was therefore concluded as induction of G_0/G_1 phase cell cycle arrest, which then leads to cells to undergo apoptotic cell death.

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