PGV-1 sebagai agen antimitosis poten

PGV-1 is a potent antimitotic agent

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Abstrak

Karsinogenesis melibatkan terganggunya fungsi normal dari kematian sel yang terprogram (apoptosis). Sebagian besar agen antikanker yang digunakan saat ini bekerja dengan menginduksi apoptosis pada sel target. Faktanya, agen antikanker tersebut berinteraksi pada berbagai target yang berbeda yang menginduksi kematian sel melalui beberapa mekanisme, sehingga mengindikasikan bahwa aktivitas antikanker ditentukan dari kemampuannya dalam menghambat pertumbuhan sel.

Pentagamavunon-1 (PGV-1 (2,5-*bis*-(4'-hydroxy,3',5'-dimethyl)-benzilidine-cyclopentanone)) adalah salah satu senyawa analog kurkumin yang menunjukkan potensi dalam menghambat proliferasi sel kanker payudara T47D. Senyawa PGV-1 pada konsentrasi 2,5 mM menghambat pertumbuhan sel T47D dengan menyebabkan cell *cycle arrest* pada fase G2/M yang diikuti dengan hiperploid. Data dari uji poimerisasi menunjukkan bahwa PGV-1 berinteraksi dengan tubulin dengan mekanisme yang berbeda dengan taxol. PGV-1 menghambat polimerisasi tubulin pada kultur sel, sedangkan taxol menstabilisasi polimerisasi tubulin tersebut. Data pengecatan imunositokimia pada sel yang diberi perlakuan PGV-1 menunjukkan sedikit kondensasi tubulin, sedangkan sel yang diberi perlakuan taxol menunjukkan kondensasi tubulin yang kuat pada menit ke-12 setelah agen depolimerisasi diberikan.

Dari penelitian ini dapat disimpulkan bahwa PGV-1 merupakan senyawa inhibitor mikrotubulin yang baru dan berpotensi untuk dikembangkan sebagai agen antikanker.
Kata kunci: Pentagamavunon-1, T47D, tubulin, antimitosis

Abstract

Carcinogenesis can be involved in the malfunctioning of programmed cell death Most of the anticancer drug in current use induce apoptosis in susceptible cells. The fact that disparate agent interacting with different targets seem to induce cell death through some common mechanism suggest that anticancer activity is determined by the ability of inhibiting cell growth.

Pentagamavunon-1 (PGV-1) is one of the curcumin analogue which showed to have potency in inhibiting proliferation of human breast carcinoma cell T47D. The effect on T47D growth is associated with cell cycle arrest in G2/M phase at the concentration of 2.5 mM, followed by hyperploidy. Our data on polymerization assay, indicate PGV-1 interact with tubulin in different manner from taxol. PGV-1 inhibit tubulin polymerization on cell culture while taxol stabilized tubulin polymerization. Immunostainning data on cell treated with PGV-1 showed slightly tubulin condensation, while cell treated with taxol showed tubulin condensation distinctly at 12 minutes after releasing from depolymerization agent.

In conclusion, PGV-1 represent a new microtubule inhibitor and has the potential to be developed for anticancer drug
Key words: Pentagamavunon-1, T47D, tubulin, antimitotik

Introduction

Curcumin is one of the major component of turmeric, a phytochemical agent which shown chemopreventive properties against tumor. Many medicinal properties of curcumin are well recognized. Curcumin has been shown to inhibit proliferation of a wide variety of tumour cells. Incubation of breast cancer cells T47D with 10 μM curcumin for 24 hours induced G1 acummulation cell population (Da'i *et al.,* 2007). Curcumin also was found to induce G0/G1 and/or G2/M arrest, upregulated CDKIs, p21, p27, p53 and slightly down regulate cyclin B1 in umbilical vein endothelial cells (park *et al.,* 2002). Curcumin Research center has found some candidate s of curcumin analogue which has the potency as anticancer.

Pentagamavunon-1 (PGV-1) is one of the curcumin analogue which showed to have potency in inhibiting proliferation of human breast carcinoma cell T47D. the previous research found that PGV-1 at the concentration of 2.5 μM modulates cell cycle progression through G2/M arrest, followed by hyperploidy of the cell and *mitotic catarstrophe* on T47D cells (Dai *et al.,* 2007). In this effect, PGV-1 induces p21 expression and CDK-1 dephosphorilation. PGV-1 may interact with tubulin leading to the inhibition of microtubule polymerization as indicated by occuring of tubulin condensation. However the cells may undergo mitosis but not followed by cytokinesis result in the hyperploidy. This finding suggests that PGV-1 act as microtubule inhibitor and can be categorized as an anti-microtubule agent.

The objective of this research was observed the possible mechanisms of PGV-1 as antimicrotubule drug which may interact with tubulin either in polymerizing or depolymerizing of tubulin .

Methodology

Cells and PGV-1

T47D cell line was kindly provided by Division of molecular oncology laboratory, NAIST, Japan. Cells were maintained in DMEM (Sigma) plus 10% fetal bovine serum (Thermo Sci) and 1% penicillin/streptomycin. PGV-1 was obtained from Curcumin Research Center (CCRC) Faculty of Pharmacy Gadjah Mada University.

Flowcytometric analysis

T47D cells were seeded at 5 x 10^5 cells/well on six wells tissue culture plate. After 24 hours incubation cells were treated with 2.5 μM PGV-1 and 1 μM Taxol at 6, 12 and 24 hours incubation time. After treatment cells were collected. Cells were trypsinized, spin at 1000 rpm for 3 minutes and washed twice with cold PBS. Cells were resuspended in propidium iodide solution (50 μg/mL in PBS contained 1% triton X-100) and then treated with RNAse DNAse free (20 μg/mL) for 10 minutes at 37^0C. The treated cells then subjected to Facscalybour flowcytometry. Cell cycle profiles were analysed using cell quest acquisition program.

Immunofluorescence microscopy

T47D cells were grown on glass coverslip at 2.5 X 10^4 cells/well. After 24 hours incubation cells on coverslip were treated with 2.5 μM PGV-1 and 1 μM taxol for 12 hours and 24 hours incubation time. Cells on coverslip were fixed with 4% paraformaldehyde in PBS for 10 minutes at room temperature and permeabilized with 0.1% triton X-100 in PBS for 5 minutes. After blocking in 1% bovine serum albumine in PBS for 60 minutes, cells were incubated in primary antibody against α-tubulin (1:200) for 1 hour and washed with PBS to remove unbound antibody. Then cells were incubated with anti mouse IgG FITC conjugated secondary antibody (1:100) for 1 hour. Nuclear morphology were observed by staining with DAPI (0.1 μg/mL). cells on coverslip were mounted on permafluor mounting and observed on confocal mycroscope.

Microtubule polymerization assay

T47D cells were seeded on 60 mm dish at 8 x 10^5 cells/dish. After 24 hours incubation cells were treated with 2 μg/mL of nocodazol for 2 hours to depolymerize tubulin. Cells then released from nocodazol and lysed at 0, 4, 8 and 12 minutes in 400 μl of microtubule stabilizing lysis buffer containing 4 μM paclitaxel or 4 μM PGV-1, 0.1 M PIPES pH 6.9, 2 M glycerol, 5mM $MgCl_2$, 2 mM EGTA, 0.5% triton x-100, 5 μg/mL leupeptin. Cell lysate were centrifugated at 25.000 rpm for 30 minutes at 22^0C for sedimentation of polymeric tubulin. The sediment (contained polymeric tubulin) was subjected to 150 μl solubilization buffer containing 25 mM Tris-HCl pH 7.5, 0.4 M NaCl, 1% NP-40, 0.5% SDS, 0.1% deoxycholate,5 μg/mL leupeptin. The resulting sample were analyzed on western blot using α-tubulin antibody.

Results and Discussions

Effect PGV-1 on cell cycle progression of cancer cell

Flowcytometric analysis was used to observed the distribution of cell population on cell cycle. As shown in figure 1, there was an accumulation at G2/M phase of T47D cells treated with 2.5 μM PGV-1 and 1 μM taxol after 12 hours incubation. After 24 hours incubation, cells treated with PGV-1 showed decreasing cell population at G2/M phase followed by increasing in polyploidy (M5) and decreasing in sub G1 phase. Cells treated with 1 μM taxol showed a increasing in cells with polyploidy DNA content and decreasing in sub G1.

PGV-1 inhibit tubulin polymerization

To observe the effect of PGV-1 on microtubule interaction we observed the polymerization mechanism on cells treated with PGV-1. We used tubulin-depolymerizing agent Nocodazole to transiently deploymerize tubulin in cells, followed by release in complete medium without nocodazole. Tubulin staining in the cells were determined by fluorescent microscopy and tubulin polymerization were determined by western blot analysis. Tubulin staining showed that cells without treatment exhibit characteristic staining of individual microtubule as indicated by a fine network mesh of microtubular material and nuclei of these control were intact as visualized by staining with Dapi. Cells treated with PGV-1 showed a slightly fine network mesh and some intact nuclei. Cells treated with taxol showed tubulin condensation distinctly and condensed chromatin (Fig 2). Tubulin polymerization assay displayed that cells treated with taxol indicated an accumulation of polymerized

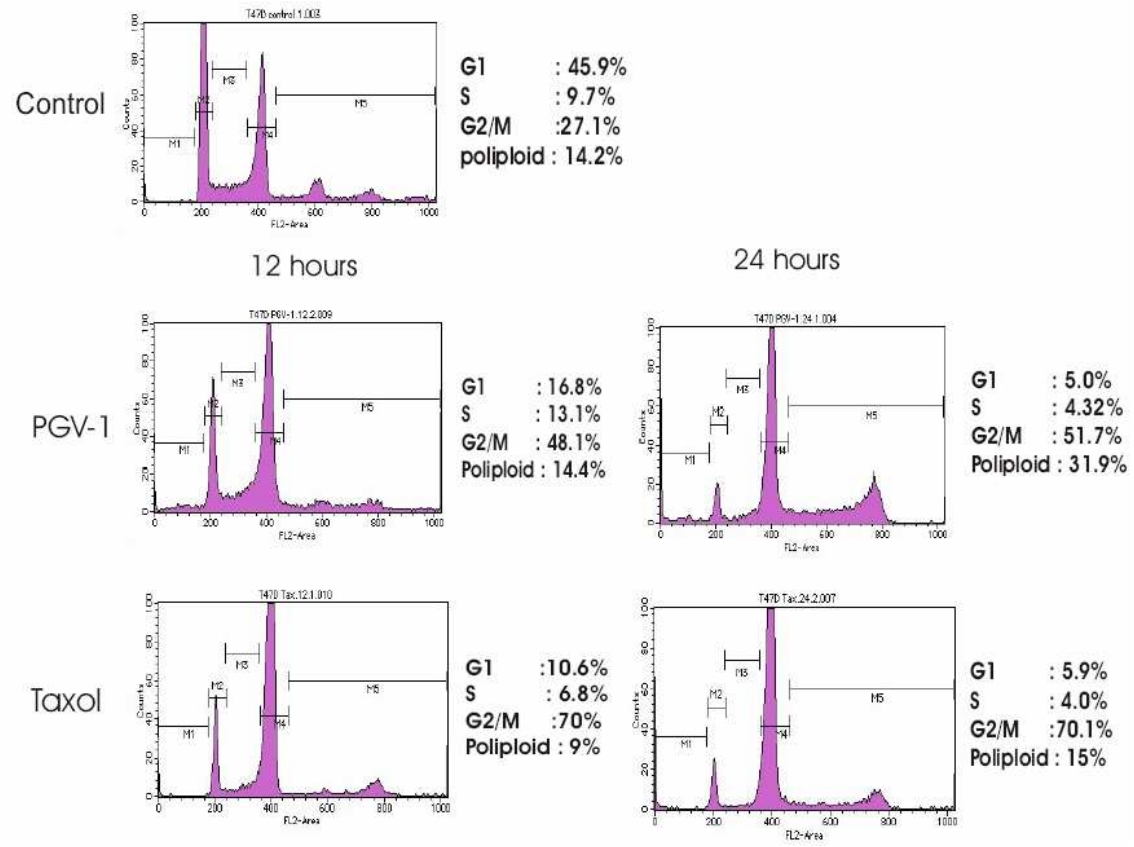

Figure 1. Flowcytometric analysis. Cells were treated with 2.5 μM PGV-1 and 1 μM Taxol, incubated for 12 and 24 hours, and stained with propidium iodide. T47D cells shown an accumulation on G2/M phase.

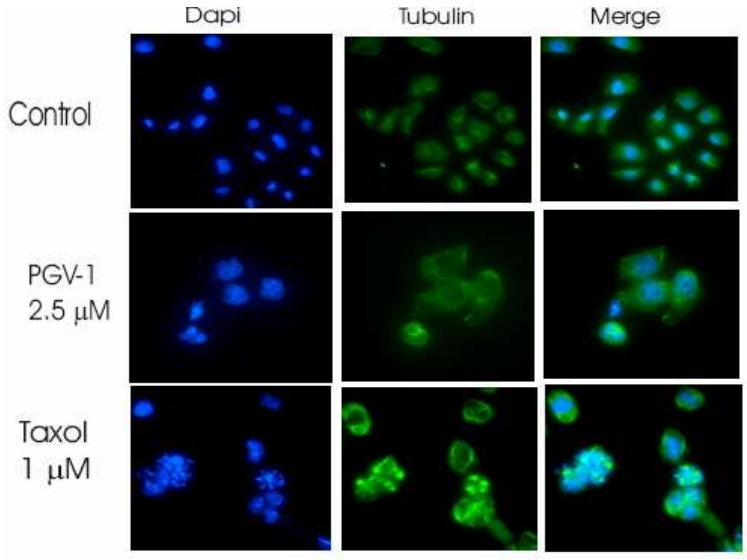

Figure 2. Effect of PGV-1 on tubulin polymerization. microtubule were stained with green-FITC conjugated α-tubulin antibody, DNA were stained with DAPI and observed on fluorescent microscope.

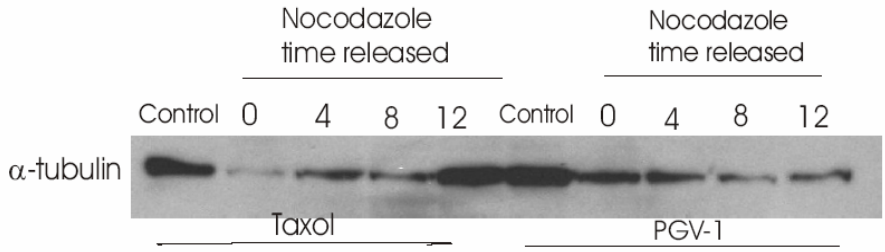

Figure 3. Efect PGV-1 on tubulin polymerization. T47D cells were treated with 2.5 μM PGV-1 and 1 μM taxol

tubulin start at 4 minutes after releasing nocodazole and then striking accumulation at 12 minutes after releasing nocodazole. In contrast cells treated with PGV-1 showed a decreasing accumulation of polymerized tubulin at 0, 4, 8 and 12 respectively after releasing nocodazole (Fig 3). This finding suggest that PGV-1 and taxol interact with tubulin in different mechanism.

Discussion

Pentagamavunon-1 (PGV-1) is the one of curcumin analogue which has the potency of inhibiting cell proliferation. Its IC 50 on T47D cells is 1.5 mM and 2.5 μM on MCF-7 cells lower than curcumin (20 μM) (Da'i et al, 2007).

Our data found that PGV-1 induce cell cycle arrest in G2/M phase significantly at 2.5 mM concentration followed by hyperploidy of the cells and seems to induce mitotic catarstrophe. Our previous study shown that PGV-1 induced p21 expression and cdc-2 activation (Da'i, 2007). Activation of cdc-2 caused cell accumulation at hiperploidi area (M5) in flowcytometric analysis. A compound which has the potency of inducing G2/M arrest followed by hiperploidi, cdc-2 and p21 activation indicate the characteristic of antimicrotubule (Okada and Mak, 2004, Wang *et al.,* 2000).

Microtubule are a major component of the mitotic spindle, which pulls the chromosome apart at mitosis and then splits

the dividing cell into two. The majority of antimitotic agents induce mitotic arrest by interacting with tubulin (Zhang et.al, 2006). Several antimitotic agent have been reported to induce P53 and inhibit cyclin-dependent kinase, p21 and activate/inactivate several protein kinase including raf/Ras, mitogen activated protein kinase and p34cdc2. The previous study on PGV-1 found to induce apoptosis by p53-independent apoptosis in T47D cells (Da'i *et al,* 2007).

Our data on mycroscopic analysis found that PGV-1 disrupt microtubule assembly. Cell treated with taxol found a distinct microtubule condensation and condensed chromatin. This finding indicated that PGV-1 has a different mechanism from taxol in interacting with tubulin. Taxol stabilized tubulin polymerization while PGV-1 inhibit tubulin polymerization. However, the antimitotic effect of PGV-1 should be confirm further by the fact that treatment of cells with this compound triggered several molecular event involved in mitotic signaling cascade. This include of some important mitotic regulatory protein such as Bcl2, cdc25C and cyclin B accumulation.

Conclusion

PGV-1 modulates cell cycle progression through G2/M arrest and inhibit tubulin polymerization.

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