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Research Article

Cytotoxicity of Tetrahydropentagamavunon-0 (THPGV-0) and Tetrahydropentagamavunon-1 (THPGV-1) on Several Cancer Cell Lines

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ABSTRACT

Tetrahydropentagamavunon-0 (THPGV-0) and Tetrahydropentagamavunon-1 (THPGV-1), are analogs of a curcumin metabolite, tetrahydrocurcumin, and a derivate of Pentagamavunon-0 (PGV-0) and Pentagamavunon-1 (PGV-1), respectively. THPGV-0 and THPGV-1 have been successfully synthesized and are investigated for their anticancer potency. Cytotoxic assays were performed toward several cancer cell lines to determine values of IC_{50}. Assessing cytotoxicity on Vero normal cell line showed the selectivity of those compound. THPGV-1 showed highest cytotoxic activity in lymphoma Raji cells, a suspension cell line, with an IC_{50} of 180μM. Both THPGV-0 and THPGV-1 showed similar potencies on T47D breast cancer cell line with IC_{50} values of 250-270μM. Regardless their high selectivity, however, cytotoxic activities of THPGV-0 and THPGV-1 were lower compared to PGV-0 and PGV-1 on HeLa cervical, T47D breast, and WiDr colon cancer cell lines. Further study using different types of cancer cell lines and confirmation of cell viability by another assays and apoptosis detection may give more benefit.

Key words: THPGV-0, THGPV-1, curcumin analog, cytotoxic, anticancer

INTRODUCTION

Curcumin (Figure 1) is a well-known natural compound isolated from the rhizome of turmeric (*Curcuma longa*) and famous for its antioxidant and anti-inflammatory properties (Aggarwal *et al*, 2005). More interestingly, curcumin also possesses anticancer potency as it is able to inhibit the growth of several types of cancer cells (reviewed by Aggarwal *et al.,* 2005). Curcumin as anticancer has been draw the attention of researchers to work in this field aimed to elucidate its mechanism. Recently, curcumin has been confirmed for its antitumorigenic *in vivo* and reported to target reactive oxygen species metabolic pathway to suppress tumor cell growth (Larasati *et al*, 2018).

Inspired by curcumin, Faculty of Pharmacy Universitas Gadjah Mada (UGM) has been challenged to discover and synthesize novel curcumin analogs which possess higher biological activity than curcumin itself. In 2004, at least two compounds namely PGV-0 and PGV-1 (Figure 1) were patented (Reksohadiprodjo *et al.,* 2004). Indeed, PGV-0 and PGV-1 not only show higher antioxidant and anti-inflammatory effects, but also higher anticancer potencies on several cancer cell lines (Table I).

Table I presents the anticancer potencies of curcumin, PGV-0, and PGV-1 indicated by their cytotoxic activities toward cancer cell lines through IC_{50} values. IC_{50} is the inhibitory

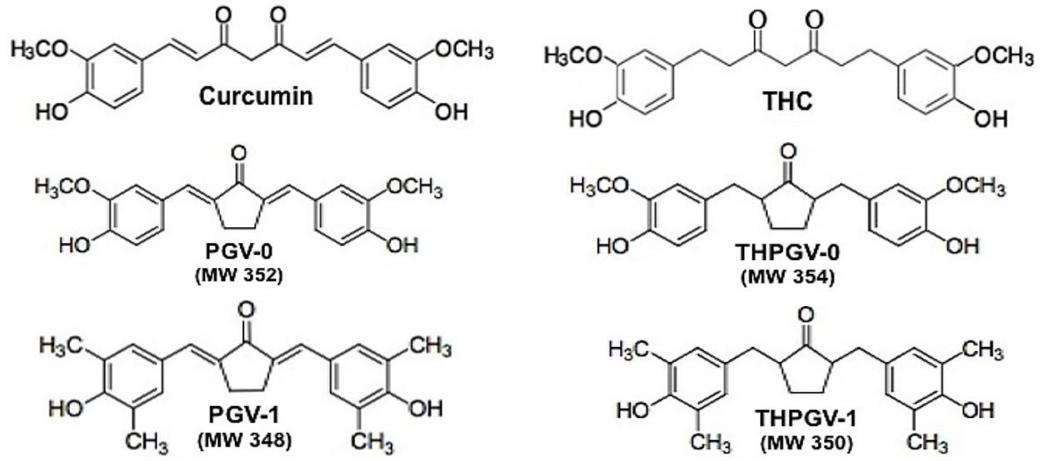

Figure 1. Structure of curcumin, PGV-0, PGV-1, THC, THPGV-0, and THPGV-1

Table I. Cytotoxic activity (IC_{50} μM) of PGV-0 and PGV-1 on several cancer cell lines

Cell line	Curcumin	PGV-0	PGV-1	Reference
T47D breast_	21.6	10.9	-	Meiyanto *et al.*, 2006a
	-	9.39	1.74	Dai *et al.*, 2007
T47D + estradiol	19.8	6.85	-	Nurulita and Meiyanto, 2006
T47D/estrogen	19.1	-	3.16	Meiyanto *et al.*, 2006b
WiDr colon	27	45	8	Septisetyani *et al.*, 2008
MCF-7 breast	-	50	6	Hermawan *et al.*, 2011
MCF-7/HER2	82	80	21	Meiyanto *et al.*, 2014
CT26 colon	93.1	73.4	47.6	Safitri, 2017

concentration for 50% cell population and is used as an early parameter for anticancer screenings (Doyle and Griffiths, 2000). In addition of cancer cell lines (Table I), cytotoxic activity of PGV-0 on lymphoma Raji (Dai, 2003), HeLa cervical cancer (Meiyanto *et al.*, 2003) and myeloma cells (Dai *et al.*, 2004), were also have been identified. Remarkably, these analog compounds are more selective to normal cells compared to curcumin, indicated by higher IC_{50} values toward Vero normal cell line (Marbawati and Sardjiman, 2015; Safitri, 2017).

The most challenging obstacle of curcumin or its analogs for *in vivo* application is due to its poor bioavailability. Tetrahydrocurcumin (THC) (Figure 1) is the major curcumin metabolite after biotransformation process, more stable than its parent compound, and responsible for the biological activities of curcumin in the body (Nugroho, 2006). Addition of four hydroxil species increases the solubility of the compound.

Analogs of THC and derivates of PGV-0 and PGV-1 namely THPGV-0 (Ritmaleni and Simbara, 2010) and THPGV-1 (Ritmaleni *et al.*, 2013a; Ritmaleni *et al.*, 2013b) has been synthesized. Both compounds biologically active as shown by their antihistamine (Nugroho *et al.*, 2010), antibacterial (Ritmaleni *et al.*, 2013b), and antifungal (Ritmaleni *et al.*, 2016) activities. This current study investigated anticancer potency of THPGV-0 and THPGV-1 by determining their IC_{50} values on several cancer cell lines. Equally important, the selectivity toward normal cells was also examined.

MATERIAL AND METHODS

Materials

THPGV-0 and THPGV-1 was synthesized according to the previous published methods (Ritmaleni and Simbara, 2010 and Ritmaleni *et al*, 2013a, respectively). The detail of synthesis can be found in Ritmaleni *et al*, 2013b. Besides being tested for their cytotoxic activity as comparison, PGV-0 and PGV-1 as the starting materials were obtained from Curcumin Research Center, Faculty of Pharmacy Universitas Gadjah Mada (UGM). The compounds were initially dissolved in dimethyl sulfoxide (DMSO, 99.5% pro-GC, Sigma Aldrich) for stock solutions.

Cell culture

HeLa cervical cancer, lymphoma Raji, T47D breast cancer, and Vero normal cell lines were obtained from Integrated Laboratory of Research and Testing (Laboratorium Penelitian dan Pengujian Terpadu, LPPT) UGM. WiDr colon cancer cell line was a collection of Cancer Chemoprevention Research Center, Faculty of Pharmacy UGM. Cells were grown in media, RPMI 1640 (Gibco) for cancer cells or in M199 (Gibco) for Vero cells, supplemented with 10% (v/v) fetal bovine serum (FBS, Gibco), 1% (v/v) penicillin-streptomycin (Gibco), and 1% (v/v) Fungizon (Gibco) and cultured in the CO_2 incubator at 37°C. At around 80% confluence, the attached cell types (HeLa, T47D, WiDr, and Vero) were harvested with trypsinization by using 0.025% trypsin-EDTA (Gibco). Harvested cells were used thereafter for assays. Cell culture and cytotoxicity study were performed in LPPT UGM.

Cell viability assay

Cell viability assays by using MTT method (Itagaki *et al*, 1998) were carried out to determine the cytotoxic activity. The cells were distributed into the 96-well plate, incubated for 24h, and then treated with various concentrations of the compounds for another 24h. The serial concentrations (7.8; 15.6; 31.25; 62.5; 125; 250; 500; and 1,000μg/mL) were prepared from stock solutions and serially diluted in the appropriate culture medium. MTT assays for attached cell lines and MTT assay for Raji suspension cell line were performed according to the previous publications (Septisetyani *et al*, 2008 and Astuti *et al*, 2004, respectively), and carried out in triplicate. The absorbance at 595 nm of diluted formazan after addition of MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) (Sigma Aldrich) reagent and stopper solutions (10% SDS (Merck) in 0.1N HCl (Merck)) were measured by an ELISA reader (Biorad). Untreated cells were served as control, while well without cells was served as blank.

Data analysis

The percentage of cell viability was defined as (absorbance of treated cells - absorbance of blank)/(absorbance of control - absorbance of blank) x 100%, and were used to calculate IC_{50} values by a linear regression analysis: cell viability (%, y axis) vs log concentration (μg/mL, x axis) (Doyle and Griffiths, 2000). IC_{50} values were converted into molar.

RESULT AND DISCUSSION

Cytotoxicity of THPGV-0 and THPGV-1 on several cancer cell lines

Treatment of THPGV-0 and THPGV-1 decreased percentage of cancer cell viability in a dose-dependent manner (Figure 2). Both THPGV-0 and THPGV-1 showed similar cytotoxic activity in attached type of cancer cell lines (HeLa, T47D, and WiDr), but THPGV-1 decreased the viability of Raji cells, a suspension type of cell cancer, more efficiently than THPGV-0 (Figure 2). To further measure the anticancer potency of THPGV-0 and THPGV-1, IC_{50} values were calculated by using a linear regression analysis. The linear regression is presented in table II and IC_{50} values are listed in table III.

THPGV-0 and THPGV-1 possessed equal IC_{50} values for HeLa and T47D cells and quite close values for WiDr cells. Somehow, on Raji cells, THPGV-1 showed much more potent cytotoxicity, almost six times higher, compared to THPGV-0.

To confirm previous findings and also to minimalize the false negative conclusion due to difference of several variables (i.e. compound source, laboratory facilities, cell culture system, methodology, etc), we also re-investigated cytotoxicity of PGV-0 and PGV-1 at the same time (Table II and Table III).

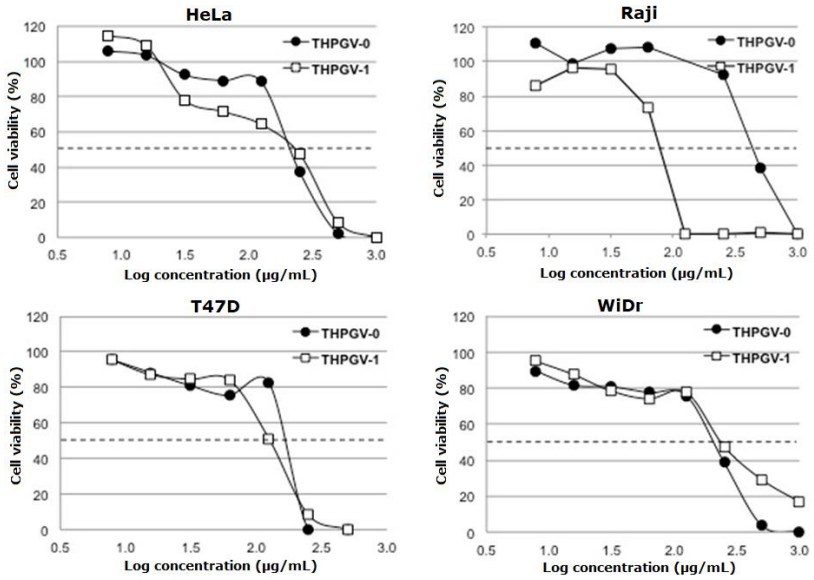

Figure 2. Cytotoxicity of THPGV-0 and THPGV-1 on several cancer cell lines. Cells were treated with a serial concentration of THPGV-0 (*black circle*) or THPGV-1 (*white square*) for 24h, and then were assayed by MTT method in triplicate. Graphics of logarithm (log) of concentration versus percentage of cell viability for each cancer cell lines are presented as indicated. Dashed line marked 50% of cell viability. The graphics demonstrated the phenomenon of dose-dependent cytotoxicity.

Table II. Linear regressions for cell viability assays of THPGV-0 and THPGV-1 on several cancer cell lines.

Compound	Line equation, cell viability vs log concentration (µg/mL) (linearity, r)*			
	HeLa	Raji	T47D	WiDr
THGPV-0	y = -55.85x+ 173.54 (0.903)	y = -45.591x+ 167.55 (0.847)	y = -54.76x+ 142.3 (0.853)	y = -45.23x+ 143.9 (0.909)
THGPV-1	y = -55.556x+ 169.82 (0.979)	y = -64.413x+ 165.89 (0.885)	y = -56.62x+ 143.3 (0.925)	y = -33.02x+ 129.1 (0.922)
PGV-0	y = -55.58x+ 170.40 (0.903)	y = -19.712x+ 102.49 (0.904)	y = -55.27x+ 130.8 (0.923)	y = -35.88x+ 113.6 (0.912)
PGV-1	y = -25.901x+ 96.281 (0.948)	-	y = -31.74x+ 72.76 (0.988)	y = -17.31x+ 78.90 (0.995)

*The cell viability was measured by MTT assay in triplicate for each compounds on each cell lines.

Table III. Cytotoxic activity (IC_{50} µM) of THPGV-0 and THPGV-1 on several cancer cell lines*.

Compound	HeLa	Raji	T47D	WiDr
THGPV-0	460	1.070	270	330
THGPV-1	410	180	250	570
PGV-0	200	1.300	170	170
PGV-1	170	-	30	130

*The cell viability was measured by MTT assay in triplicate for each compounds on each cell lines and IC_{50} values were calculated.

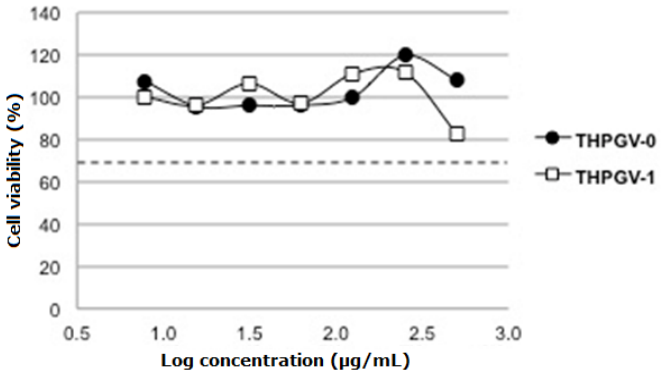

Figure 3. Selectivity of THPGV-0 and THPGV-1 on Vero normal cell line. Cells were treated with a serial concentration of THPGV-0 (*black circle*) or THPGV-1 (*white square*) for 24 h, and then were assayed by MTT method in triplicate. Graphics of logarithm (log) of concentration versus percentage of cell viability is presented. Dashed line marked 50% of cell viability.

Table IV. Selectivity of THPGV-0 and THPGV-1 on Vero normal cell line.

Compound	Line equation, cell viability vs log concentration (µg/mL)	Linearity (r)	Concentration	Cell viability*
THGPV-0	y = 6.36x+91.83	0.461	696µM	>80%
THGPV-1	y = -1.9508x+104.22	0.125	1.404µM	>80%
PGV-0	y = -11.64x+101.00	0.867	1.465µM	>100%

*The cell viability was measured by MTT assay in triplicate for each compounds.

Agreed with previous reports, PGV-1 was always more potent than PGV-0 on HeLa, T47D, and WiDr cells. Unfortunately, due to our limitation, we failed to obtain PGV-1 data in Raji cells. Nevertheless, we can conclude that in general THPGV-0 and THPGV-1 possessed less potent cytotoxicity compared to PGV-0 and PGV-1, at least on above-tested cancer cell lines (Table III).

Selectivity of THPGV-0 and THPGV-1 toward Vero normal cell line

Vero cell line was used as a model for normal cells. The MTT assays revealed that even at the highest concentration of THPGV-0 or THPGV-1 (1.000µg/mL), the cell viability was more than 80% (Figure 3). Since 50% of cell viability was never reached during treatment, the IC_{50} value was not calculated nor extrapolated (Table IV). It can be concluded that THPGV-0 and THPGV-1 were selective on Vero normal cells.

Again, to confirm previous findings, the parent compound was also tested (Table IV). PGV-0 was also selective on Vero cells. As PGV-1 is more potent than PGV-0, further confirmation of PGV-1 selectivity will be valuable.

Discussion

Our current study investigated the anticancer potency of THPGV-0 and THPGV-1, a derivate of PGV-0 and PGV-1, respectively, by determining the IC_{50} values *in vitro*. We showed that THPGV-0 and THPGV-1 are equally selective on Vero normal cells, but THPGV-1 is more potent than THPGV-0 on cancer cells. This corresponds well with previously reported findings of the parent compounds, in which PGV-1 possesses more potent anticancer activity than PGV-0 on T47D and MCF-7 breast cancer cell lines (Dai *et al*, 2007; Hermawan *et al*, 2011), metastatic

breast cancer cells MCF-7/HER2 (Meiyanto *et al*, 2014), and on WiDr and CT26 colon cancer cell lines (Septisetyani *et al*, 2008; Safitri, 2017).

In this study, we only used one normal cell line as the model and were not able to calculate the SI (Table IV). However, the selectivity toward other types of normal cell lines as well as normal primary cell cultures is also important to be further evaluated. One parameter that can be used to assess selectivity of tested compounds is selectivity index (SI), as introduced by previous reports (Popiolkiewicz *et al*, 2005; Pena-Moran *et al*, 2016). Pena-Moran *et al* (2016) describe SI as the value of IC_{50} of normal cells divided by IC_{50} of cancer cells, and a SI≥10 is considered belongs to a selective compound.

Those above-mentioned cancer cell lines are attached type. However, THPGV-1 exhibits highest cytotoxic activity in lymphoma Raji cells, a suspension type cell. Regrettably, in this study we did not have an IC_{50} value of PGV-1 on Raji cells, thus we cannot draw a further conclusion to measure the potency of THPGV-1 compared to PGV-1. Nonetheless, further screening of THPGV-1 anticancer properties compared to PGV-1 on suspension cancer cell lines will give more beneficial information.

To confirm the MTT result, another methods to detect cell viability than such colorimetric assay based on the reduction by living cells of tetrazolium salt (Mosmann, 1983) can be carried out. To mention few examples are a manual direct counting, infrared assay using nuclei and cytosol staining or cytoskeletal antibodies, and luminescence assay for ATP (Posimo *et al*, 2014). Nonetheless, the IC_{50} value is only one parameter among several parameters of anticancer potency. Another important parameter to be investigated is the potency of apoptotic induction. Indeed, previous reports have been successfully showed that the parent compound, PGV-0 and PGV-1, induce apoptosis more efficiently than curcumin on several cancer cell line: T47D (Nurulita and Meiyanto, 2006; Meiyanto *et al*, 2006b; Meiyanto *et al*, 2007), WiDr (Septisetyani *et al*, 2008), and MCF-7 (Hermawan *et al*, 2011).

Regarding the structure-activity relationship, the α,β-unsaturated carbonyl is crucial for the cytotoxic activity (Sardjiman, 2018; private communication). Compared to the parent compound PGV-0 or PGV-1, THPGV-0 or THPGV-1 (Figure 1) loss the double bonds in the α,β position of carbonyl, resulting the decrease of electrophilicity of C carbonyl. The positive charge of the above-mentioned C carbonyl is weaker to result cytotoxic activity compared to the α,β-unsaturated carbonyl. On the other hand, antioxidant or antibacterial properties are depends on the hydroxyl group or the presence of electron withdrawing groups in the orto position of the hydroxyl group, respectively (Sardjiman *et al*, 1997; Sardjiman, 2000). Therefore, both THPGV-0 and THPGV-1 possess more potent antioxidant and antibacterial activities, but show less potent cytotoxic activity compared to their parent compounds.

Regardless its low cytotoxicity, THPGV-0 is promising to be developed as pharmaceutical compound accounts on its antioxidant property. In fact, Ritmaleni and Murrukhmihadi have been doing quite extensive studies to develop THPGV-0 as anti-aging agent for topical application (Ariella, 2016; Putri, 2016; Alamsyah, 2016; Faharvia, 2016; Krisdayani, 2016) due to its sun protecting factor (SPF) activity and little skin irritation risk in animal models (Febriana, 2016; Wastuwidya, 2017; Wulandari, 2017).

More importantly, THPGV-1, the compound that possesses more potent cytotoxic than THPGV-0 but less potent than its parent compound, can be further developed as combinatorial agent for chemotherapy (co-chemotherapy). Co-chemotherapy is an approach for cancer therapy by combining clinically approved chemotherapeutic agents with less toxic compounds to enhance its efficacy and to reduce its toxicity to normal cells (Jenie and Meiyanto, 2007). Former *in vitro* studies have been reported the effectiveness of this strategy toward several cancer cell lines, for example: combination of PGV-0 (Ikawati and Septisetyani, 2018) or PGV-1 (Septisetyani *et al*, 2018) with 5-fluorouracil to sensitize WiDr cells, and combination of PGV-0 or PGV-1

with doxorubicin on MCF-7 cells (Hermawan *et al*, 2011) and MCF-7/HER2 cells (Meiyanto *et al*, 2014).

CONCLUSION

In general, THPGV-0 and THPGV-1 exhibit lower cytotoxic activities compared to PGV-0 and PGV-1. Regarding their relatively higher solubility, further investigation by using other suspension cell line types and other methods may give more clear information.

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