Anti-proliferation activity of pinostrobin from *Boesenbergia pandurata* and its efficacy improvement using cationic liposome on human cancer cell lines

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Abstract

Pinostrobin, a flavanone from *Boesenbergia pandurata*, was previously identified as an inhibitor of Ca^{2+}-signal mediated growth regulation in *Saccharomyces cerevisiae*. This study aimed to investigate the anti-proliferative activity of pinostrobin and the liposome-encapsulated pinostrobin in some human cancer cell lines, as well as determine the effect of pinostrobin on cell cycle progression. Cytotoxicity of free- and liposome encapsulated-pinostrobin against Jurkat, KATOIII, SW620, HepG2, Ca-Ski and BT474 cell lines was assessed. Among the tested cell lines, Jurkat T cells were the most sensitive to pinostrobin. The use of liposomal pinostrobin showed significantly enhance the cytotoxic effect of pinostrobin in all cell lines studied (except for BT474 which showed highly toxic to liposome) with IC_{50} values ranging from 2.6-14.2 µM exhibited >7.0 - 20.9 times higher cytotoxic effect than those of the free form. A flow cytometric analysis revealed that 100 µM pinotrobin treated cells caused significantly increase in sub G1 population at 96 h of Jurkat T cells as well as caused G2/M cell cycle arrest in KATO III cells. The results suggested that pinostrobin should be a potential candidate for various cancer therapies and liposome could be a vehicle of choice to improve the efficacy of pinostrobin.

Keywords: pinostrobin, *Boesenbergia pandurata*, anti-cancer, Jurkat**,** KATOIII, liposome

Introduction

Pinostrobin or 5-hydroxy-7-methoxy flavanone from *Boesenbergia pandurata*, a well known herb used in many Thai dishes and in traditional Thai medicine, has been reported as an inhibitor of Ca^{2+}-signal-mediated cell-cycle regulation in the *Saccharomyces cerevisiae* (Wangkangwan et al., 2009). Many studies were reported on anti-proliferative effect of pinostrobin against various human cancer cell lines (Smolarz et al., 2005 and Ashidi et al., 2007), anti-aromatase activity (Le Bail et al., 2000), anti-oxidant activity (Kong et al., 2009), in *vivo* anti-ulcerogenic activity (Abdelwahab et al., 2011). However, pinostrobin has not yet been approved as a therapeutic agent, and one of the problems arising from its poor solubility.

Cholesterol has been used as a lipid anchor due to its lipid bilayer stabilizing activity (Yeagle, 1985) and cause minimal toxicity to the treated cells. Cationic liposomes have been demonstrated to interact with cells via electrostatic interaction, which could induce endocytosis of the liposomes and also facilitate drug release to the cytosol by endosomal escape (Dass, 2003). Recently, the use of cationic lipid with cholesterol tail to encapsulate and deliver curcumin into cells has been reported (Apiratikul et al., 2013).

In this research, the anti-proliferative effect of pinostrobin and the cationic liposome encapsulated pinostrobin on various human cancer cell lines was examined as well as its role on the cell cycle progression in the affected cancer cell lines were also investigated.

Methodology

Source of pinostrobin

A pure pinostrobin, was isolated and purified from crude extract of *B. pandurata* (Wangkangwan et al., 2009). The stock solution of pinostrobin was prepared in dimethyl sulfoxide (DMSO).

Preparation of liposomal pinostrobin

Cationic liposome was prepared as described in Apirattikul et al., 2013. Liposomal pinostrobin was prepared at ratio of pinostrobin:liposome = 1:20 as described in Apirattikul et al., 2013. The separation of liposomal pinostrobin from free pinostrobin was performed according to (Immordino et al., 2003). The percentage of pinostrobin incorporation into liposome was 83% as determined by HPLC.

Cell lines and cell cultivation

Jurkat T cell (Human acute T cell leukemia), Ca-Ski (Human cervical carcinoma cell line), BT474 (Human breast carcinoma cell line), SW620 (Human colon adenocarcinoma cell line), HepG2 (Human liver hepatocellular carcinoma cell line) and KATO III (Human stomach carcinoma cell line) were obtained from the Institute of Biotechnology and Genetic Engineering, Chulalongkorn University. Cells were grown in RPMI-1640 supplemented with 10% fetal bovine serum, 10^6 U/ml penicillin and 500 mg/ml streptomycin. The cells were cultured at 37°C in 5% CO_2 containing atmosphere.

MTT Proliferation assay

The cytotoxic effect of the pinostrobin and it incorporated with liposome on human cancer cell lines was analyzed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Mosmann, 1983). Briefly, the test cells were seeded at a final concentration of 2.5×10^4 cells/well in a 96-well plate and then treated with various concentrations of pinostrobin or liposomal pinostrobin (at ratio of pinostrobin:liposome = 1:20) or liposome only (at the amount equal to that in the liposomal pinostrobin). For a control experiment, the cells were treated with DMSO or PBS. After 1 and 4 days of incubation at 37°C, 5% CO_2, 10 µl of MTT solution (5 mg/ml) (Bio Basic Inc, Canada) was added and incubated further for 4 h. Then, 100 µl of isopropanol was added to dissolve the

colored formazan crystal produced from MTT. Viability of cells was measured at 540 nm by a microplate reader (ELx800 Bio-tek instrument, USA).

Flow cytometric analysis

A total of 1.25×10^5 cells/well of Jurkat T cells or KATOIII were plated onto 24-well plates. The pinostrobin was added into the wells to obtain final concentration at 2X IC_{50} value and the plates were incubated at 37°C, 5% CO_2 for 24, 48, 72 and 96 h. After fixing, the cells were then stained with 1 mg/ml propidium iodide (Sigma, USA) for 30 min at 37°C in the dark. The samples were analyzed by a flow cytometer (Beckman Coulter, USA).

Statistical analysis

The unpaired t-test (two-tailed) was performed to determine statistical significance of treated cells and the control cells (GraphPad Prism 5).

Results

The effect of pinostrobin and liposomal pinostrobin on the anti-proliferation activity against various human cancer cell lines

The cytotoxic effect of pinostrobin and the liposomal pinostrobin on Jurkat, Ca-Ski, BT474, SW620, HepG2, and KATO III cell lines was analyzed by the MTT assay. Each of the cancer cell lines was treated with varying doses (from 0.1-100 µM) of pinostrobin, liposomal pinostrobin or liposome and the treated cells were cultivated for 4 days. We found that pinostrobin showed cytotoxic effects against Jurkat T-cell, BT474 and KATO III with the IC_{50} values of 51.2, 61.9 and 75.7 µM, respectively. However, the IC_{50} values on Ca-Ski, SW620 and HepG2 were >100 µM, respectively, after 4 day cultivation with pinostrobin (Table 1). All of the liposomal pinostrobin treated cell lines showed dramatically sensitive to the treatments with the IC_{50} values ranging from 2.6 – 14.2 µM (excluding the results from the BT474) suggesting that the efficacy of liposomal pinostrobin was improved > 7.0 – 20.9 times compared to those of the free pinostrobin treatments. However, liposome itself exhibited toxicity to the tested cells in varying extents (% cell viability at IC_{50} dose was in the range of 60-96%) and the most pronounced toxic effect was observed in BT474 cell line (Table 1). These results indicated that the cationic liposome could be used as a basic component of delivery system capable to enhance stability, cellular uptake and cytotoxicity of pinostrobin in most of the tested cell lines except in BT474.

Table 1 IC_{50} of pinostrobin, liposomal pinostrobin and empty liposome

Tested preparation		Cell lines					
		Jurkat	Ca-Ski	BT474	SW620	HepG2	KATO III
IC_{50} (μM)	Free pinostrobin	51.2 ± 1.61	>100	61.9 ± 1.15	>100	>100	75.7 ± 6.03
	Liposomal pinostrobin	2.6 ± 0.17	7.7 ± 0.36	1.96 ± 0.03	14.2 ± 2.10	4.8 ± 0.26	5.3 ± 1.02
	Liposome	10.6 ± 0.55	17.2 ± 0.65	1.6 ± 0.03	21.8 ± 1.66	28.4 ± 1.50	12.4 ± 2.16
Efficacy of liposomal pinostrobin to free form (times)		19.5	>13.1	31.6*	>7.0	>20.9	14.3

*Mainly cytotoxic effect was from liposome.

Effect of pinostrobin on cell cycle progression of Jurkat T cells and KATO III cells

To address whether pinostrobin caused cell cycle perturbation, DNA contents of Jurkat T and KATO III treated for 96 h with or without 100 μM pinostrobin were analyzed by flow cytometry. The results showed that pinostrobin treatment caused increase in the sub G1population from 2.4% in the control treatment (untreated cells) to 11.3% in the Jurkat T cells treated with 100 μM pinostrobin (Fig 1d). The increase in sub G1 population could be observed since day 2 of treatment (Fig 1).

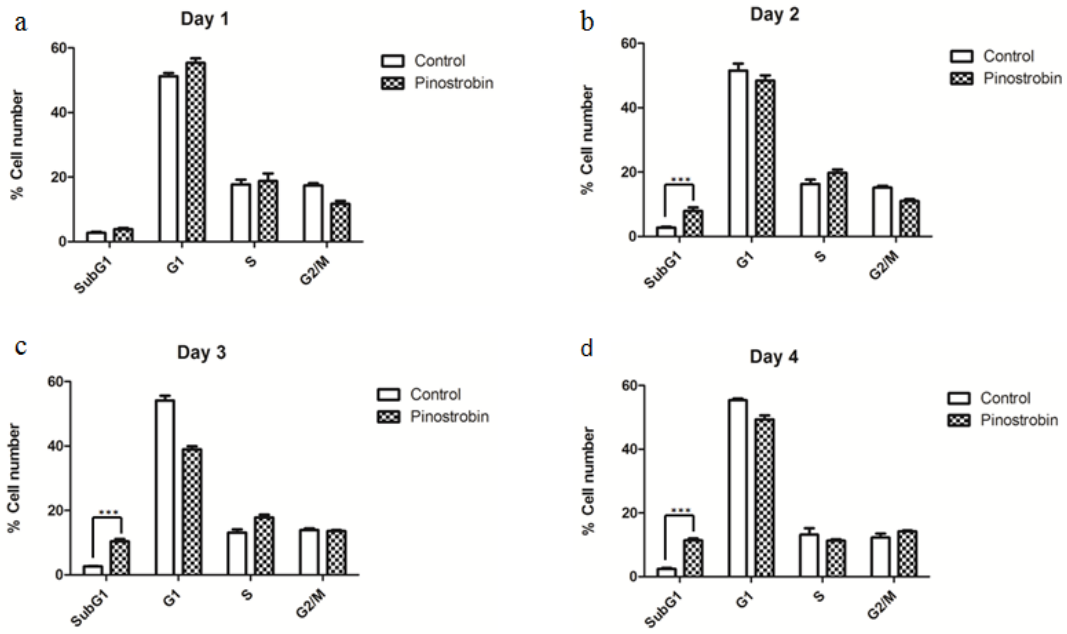

Figure 1: Effect of pinostrobin on cell cycle distribution in Jurkat T cells. The cells were incubated for 24 (a), 48 (b), 72 (c) and 96 h (d) in the absence (control) or presence of 100 μM pinostrobin, after which the DNA content was determined by flow cytometry. The data showed the values of mean ± SD from the triplicate experiments. *** significant differences at *p*-value <0.001.

Pinostrobin treatment caused significantly increase in population of G2/M phase cells from 18.6% to 31.4% in the KATO III treated with 100 µM pinostrobin (Fig 2d).

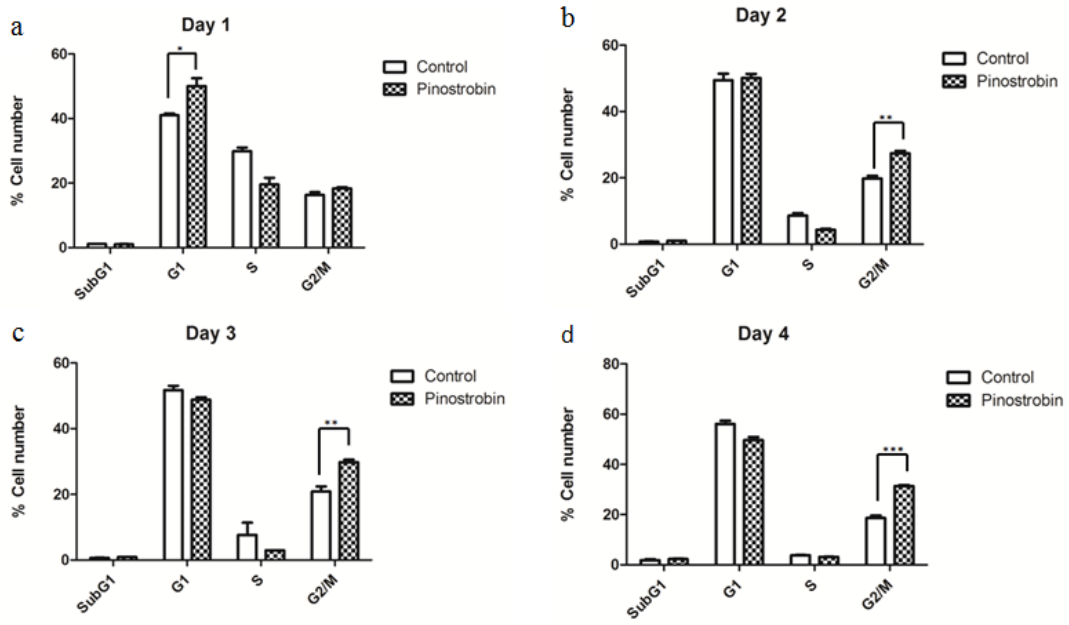

Figure 2: Effect of pinostrobin on cell cycle distribution in KATO III. The cells were incubated for 24 (a), 48 (b), 72 (c) and 96 h (d) in the absence (control) or presence of 100 µM pinostrobin, after which the DNA content was determined by flow cytometry. The data showed the values of mean ± SD from the triplicate experiments. *, **, *** significant differences at *p*-value <0.05, <0.01 and <0.001, respectively.

Discussion

In all cell lines studied, liposomal pinostrobin showed enhance efficacy of pinostrobin on anti-proliferative activity from > 7 – 21 times lower in IC_{50} dose except for the case of BT474 in which liposome itself seemed highly toxic to the cells. Previously, it has been reported that liposomes were toxic to various cells (Mayhew et al., 1987; Campbell, 1983; Smistad et al., 2007). However, in our studies, the liposome showed no or subtle toxicity to HepG2 and KATO III (96% and 81% viable cells, respectively for their IC_{50} doses of liposomal pinostrobin). Therefore, liposome was not suitable for delivery pinostrobin into BT474 cell line (Table 1). Jurkat T cells was the most sensitive to liposomal pinostrobin treatment (Table 1). For the effect on cell cycle progression, pinostrobin caused increase in sub G1 population of Jurkat T cells (Fig.1). This finding was supported by the report of Smolarz et al. (2005) which detected apoptotic cells in pinostrobin treated Jurkat or HL60 cell lines. In addition, we also found pinostrobin caused G2/M cell cycle arrest in KATOIII (Fig. 2). It has been reported that the flavanone (including pinostrobin) from Cajanus cajan leaves caused G1 arrest in CCRF-CEM leukaemia cells (Ashidi et al., (2006)). Effects of flavonoids (including pinostrobin) on cell cycle of many prostate cancer cell lines have been previously reported (Haddad et al. (2006). Flavonoids have been shown to alter a number of key proteins implicated in growth and differentiation (Haddad et al., (2006). Sukardiman et al., (2000) suggested one of the molecular mechanism of pinostrobin from *Temu kunci* (*Kempferia pandurata*) in human mammary carcinoma was DNA topoisomerase I. Since in our studies, different cell lines responses differently to pinostrobin treatment. This might suggests several mechanisms involved in pinostrobin's action on anti-proliferation. Further

studies to explore the other molecular mechanisms of pinostrobin on anti-proliferation are interesting.

Conclusion

Our studies showed that pinostrobin from *B. pandurata* exhibited cytotoxic effect against Jurkat T cells, BT474 and KATTO III out of six cell lines studied with IC_{50} values of 51.2, 61.90 and 75.7 µM, respectively. Liposomal pinostrobin revealed significant enhancement on the cytotoxic effect of pinostrobin compared to those of the free form. Toxicity of liposome was varied depended upon cell lines. The cationic liposome appeared to be highly toxic to BT474 cell line while showed no or subtle toxic effect to HepG2 and KATOIII.

The treatment of pinostrobin at 100 µM caused significant increase in sub G1 population of Jurkat T cells as well as caused G2/M cell cycle arrest in KATO III. The results from our study provided a basis for further studies on activity of pinostrobin for treating and preventing cancer.

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