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Reversal effect of a macrocyclic bisbibenzyl plagiochin E on multidrug resistance in adriamycin-resistant K562/A02 cells

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Abstract

Plagiochin E is a new macrocyclic bisbibenzyl compound isolated from *Marchantia polymorpha*. In the previous studies, we reported that when combined with fluconazole, plagiochin E had synergetic effects against the resistant strain of *Candida albicans*. Herein, we examined the reversal effect of plagiochin E on multidrug resistance in adriamycin-induced resistant K562/A02 cells and the parental K562 cells. Its cytotoxicity and reversal effects on multidrug resistance were assessed by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl-tetrazolium bromide) assay. Apoptosis percentage of cells was obtained from Annexin V/fluorescein isothiocyanate (FITC) and propridium iodide (PI) double-staining. The effects of plagiochin E on P-glycoprotein activity were evaluated by measuring rhodamine 123 (Rh123)-associated mean fluorescence intensity and P-glycoprotein expression on the basis of the flow cytometric technology, respectively. The results showed that plagiochin E ranging from 2 to 12 μg/ml had little cytotoxicity against K562/A02 cells. When combined with adriamycin, it significantly promoted the sensitivity of K562/A02 cells toward adriamycin through increasing intracellular accumulation of adriamycin in a dose-dependent manner. Further study demonstrated that the inhibitory effect of plagiochin E on P-glycoprotein activity was the major cause of increased stagnation of adriamycin inside K562/A02 cells, indicating that plagiochin E, as a new class of mutidrug resistance inhibitor, may effectively reverse the multidrug resistance in K562/A02 cells via inhibiting expression and drug-transport function of P-glycoprotein.

Keywords: Plagiochin E; K562/A02 cells; P-glycoprotein; Multidrug resistance; Reversal

1. Introduction

Multidrug resistance, a phenomenon of resistance of cancer cells to diverse structurally and mechanically unrelated anticancer drugs, is a major obstacle to successful cancer chemotherapy. Over-expression of P-glycoprotein is the most frequent event causing multidrug resistance. P-glycoprotein, a transmembrane glycoprotein, functions as an adenosine 5′-triphosphate (ATP)-dependent drug transporter which unilaterally transports intracellular drugs out of the cells to acquire drug resistance (Gottesman et al., 2002; Ushigome et al., 2000; Marbeuf-Gueye et al., 2000). A number of natural and synthetic substances have been tested for their ability to overcome multidrug resistance in vitro and in vivo (Teodori et al., 2006). These substances include calcium channel blockers (e.g. verapamil), calmodulin antagonists (e.g. trifluoperazine) and many polyphenolic compounds, such as curcuminoids, curcumin and eigallocatechin gallate (EGCG) (Di Pietro et al., 2002; Chearwaw et al., 2004; Kitagawa, 2006). However, these compounds failed to be applied in clinic because of their severe side effects and poor pharmacokinetics in vivo (Borowski et al., 2005).

Macrocyclic bisbibenzyls, belonging to the family of phenolic compounds, are a class of characteristic components from liverworts. They are attracting more and more attention for their wide biological activities such as cytotoxicity, antifungi, antivirus, antibacteria and antioxidation (Asakawa et al., 2000).

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Previously, we reported that plagiochin E synergetically inhibited drug-resistant strain *Candida albicans* when combined with fluconazole. The further investigation confirmed that the function of plagiochin E was ascribed to the increased accumulation of fluconazole in the *C. albicans* (Leng et al., 2007). In this study, we examined the reversal effects of plagiochin E on human chronic myeloid leukemia cell line K562/A02, which is known to over-express P-glycoprotein with multidrug resistance phenotype (Qi et al., 2006).

2. Materials and methods

2.1. Plagiochin E

Plagiochin E was first isolated from *Marchantia polymorpha* and its structure was identified as reported previously (Fig. 1) (Niu et al., 2006). The compound was dissolved in dimethylsulfoxide (DMSO, Sigma-Aldrich) for in vitro assays.

2.2. Cell lines and cell culture

The human chronic myeloid leukemia cell line K562, and its multidrug-resistant counterpart K562/A02, were obtained from the Department of Pharmacology, the Institute of Hematology of Chinese Academy of Medical Sciences (Tianjin, China). K562/A02 cells were maintained in a complete RPMI-1640 medium containing 1 μg/ml adriamycin (Wanle, Shenzhen, China) at 37 °C in a humidified atmosphere of 5% CO_2. The cells were cultured for two weeks in drug-free medium prior to their use in the experiments.

2.3. Cytotoxicity and multidrug resistance reversal assay

To determine the reversal effect of plagiochin E on resistant tumor cells, the cytotoxicity of plagiochin E toward K562/A02 cells was first measured by MTT method. Briefly, K562/A02 cells ($1–2\times10^4$ per well) were seeded in 96-well plates. After 24 h incubation, the cells were treated with various concentrations of plagiochin E for 24, 48 and 72 h, respectively. Cell viability was assessed by MTT assay as reported (Qu et al., 2004).

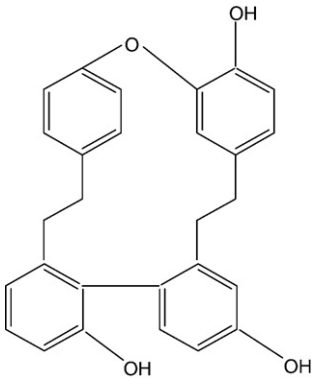

Fig. 1. Structure of plagiochin E.

The reversal effect of plagiochin E was further investigated with the same method. K562/A02 and K562 cells seeded into 96-well plates were treated with varying concentrations of adriamycin in the absence and presence of plagiochin E at doses of minimum inhibitory effect on the cell growth for 48 h, respectively. The inhibitory rate of cells growth was obtained through previously described procedures (Jain et al., 2007), and IC_{50} values for adriamycin (the concentration of drug that is required for 50% inhibition of cell) were calculated from plotted results using untreated cells as reference. The reversal fold (RF) values, as potency parameter of reversal, were calculated from dividing IC_{50} of adriamycin alone by IC_{50} of adriamycin in combination with plagiochin E (Labeed et al., 2003). Triplicate experiments with triplicate samples were performed. Control medium included equivalent amount of DMSO (as solvent control), but the applied dose did not exhibit modulation effects on the cell growth or drug sensitivity in these studies. In all the experiments, verapamil (Wanle, Shenzhen, China) was used as a positive control.

2.4. Apoptosis detection

Surface exposure of phosphatidylserine in apoptotic cells was quantitatively detected using Annexin V/FITC and PI apoptosis detection kit (Becton Dickinson, USA). Briefly, cells (5×10^5 per well) were seeded into 6-well plates and then treated with varying concentrations of plagiochin E as mentioned above. After 48 h, the cells were harvested and washed twice with ice-cold PBS (0.01 M, pH7.2). After 5 min of centrifuging at 200 g, Annexin V/FITC and PI double-staining were performed according to manufacturer's instruction. Cell apoptosis was analyzed on a FACScan flow cytometry (Becton Dickinson, USA). Annexin V-positive, PI-negative cells were scored as apoptotic. Double-stained cells were considered either as necrotic or as late apoptotic (Wang et al., 2006).

2.5. Intracellular adriamycin accumulation

Accumulation of adriamycin was monitored using a standard procedure by incubating tumor cells with adriamycin (3 μg/ml) alone or in combination with plagiochin E (2, 4, 8 μg/ml) for 1 h at 37 °C. Then cells were placed in ice-water to cease the reaction followed by harvesting and washing twice with ice-cold PBS. The intracellular mean fluorescence intensity associated with adriamycin was determined by FACScan flow cytometry (Ji et al., 2005). Data analysis was completed with Cell Quest software.

2.6. Rh123 accumulation and efflux assay

The effect of plagiochin E on P-glycoprotein activity was assessed by measuring intracellular accumulation of Rh123 (Ji and He, 2007). K562/A02 and K562 cells were seeded into 6-well plates at a density of 5×10^5 per well. Cells were pretreated with 0, 2, 4, 8 μg/ml of plagiochin E respectively for 1 h and then incubated with 5 μM of Rh123 in the dark for another 1 h. The cells were washed twice with ice-cold PBS. Mean fluorescence

intensity associated with Rh123 was measured using FACScan caliber equipped with a 488 nm argon laser. The emitted fluorescence was measured by a 530 nm band-pass filter.

In the efflux study, K562/A02 cells were first cultured with medium containing 5 μM of Rh123 at 37 °C for 90 min, washed three times with Rh123-free medium, and then incubated in the absence or presence of 8 μg/ml plagiochin E at 37 °C for 15, 30, 45, 60, 90 min respectively (Liu et al., 2002). Mean fluorescence intensity was measured as described in the accumulation assay.

2.7. Detection of P-glycoprotein expression

The cell-surface P-glycoprotein levels were measured by immunofluorescence flow cytometry (Kim et al., 2007; Xu et al., 2006). K562/A02 and K562 cells seeded into 12-well plates at a density of 3×10^5 per well were treated with 0, 2, 4 and 8 μg/ml of plagiochin E for 24 h, respectively (Wu et al., 2003). The cells were harvested, washed twice with ice-cold PBS, counted and then labeled with R-phycoerythrin-conjugated mouse anti-human monoclonal antibody against P-glycoprotein according to manufacturer's instruction. The fluorescent intensity was analyzed using FACS Caliber with isotype as control. Duplicate experiments with triplicate samples were performed.

2.8. Statistical analysis

Data was described as the mean±S.D., and analyzed by the Student's *t*-test. *P*-values below 0.05 were considered as statistically significant.

3. Results

3.1. Effects of plagiochin E on the growth of K562/A02 cells

The effect of plagiochin E on K562/A02 cell growth was determined with MTT assay. The viability of the cells was evaluated as described in Materials and methods. Data in Fig. 2 showed that plagiochin E ranging from 2 to10 μg/ml had no significant inhibitory effects on the growth of K562/A02 cells, while the anti-proliferative effect was observed at higher concentrations (12-20 μg/ml). To minimize the effect of plagiochin E itself on the resistant cell growth, we chose lower concentrations of plagiochin E (2, 4, 8 μg/ml) in the reversal experiments.

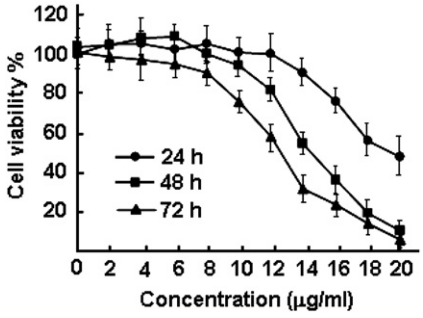

Fig. 2. Effects of plagiochin E on the growth of K562/A02 cells. The cells were treated with various concentrations of plagiochin E for 24, 48, 72 h, respectively. Viable cells were evaluated by MTT assay and denoted as a percentage of untreated controls at the concurrent time point. The bars indicate means±S.D.

Table 1
Effects of plagiochin E on the sensitivity of K562/A02 and K562 cells toward adriamycin

Treatment	K562/A02		K562	
	IC_{50} (μg/ml)	RF	IC_{50} (μg/ml)	RF
Adriamycin	5.78±0.76	–	0.14±0.06	–
Adriamycin+plagiochin E 2 μg/ml	4.03±0.91[a]	1.43	0.14±0.07	1.06
Adriamycin+plagiochin E 4 μg/ml	2.21±0.58[b]	2.62	0.13±0.08	1.12
Adriamycin+plagiochin E 8 μg/ml	0.68±0.09[b]	8.50	0.13±0.08	1.13
Adriamycin+verapamil 8 μg/ml	2.32±0.47[b]	2.50	0.14±0.05	1.03

Effects of plagiochin E on the sensitivity of K562/A02 and K562 cells toward adriamycin was examined by MTT method as described in the cytotoxicity assay and multidrug resistance reversal tests. The cells were treated with varying concentrations of adriamycin in the presence of 0, 2, 4 and 8 μg/ml of plagiochin E. IC_{50} values for adriamycin was calculated and the reversal fold (RF) was evaluated. Data were expressed as means±S.D. of three independent experiments. [a]$P<0.05$, [b]$P<0.01$ *vs.* adriamycin treatment alone.

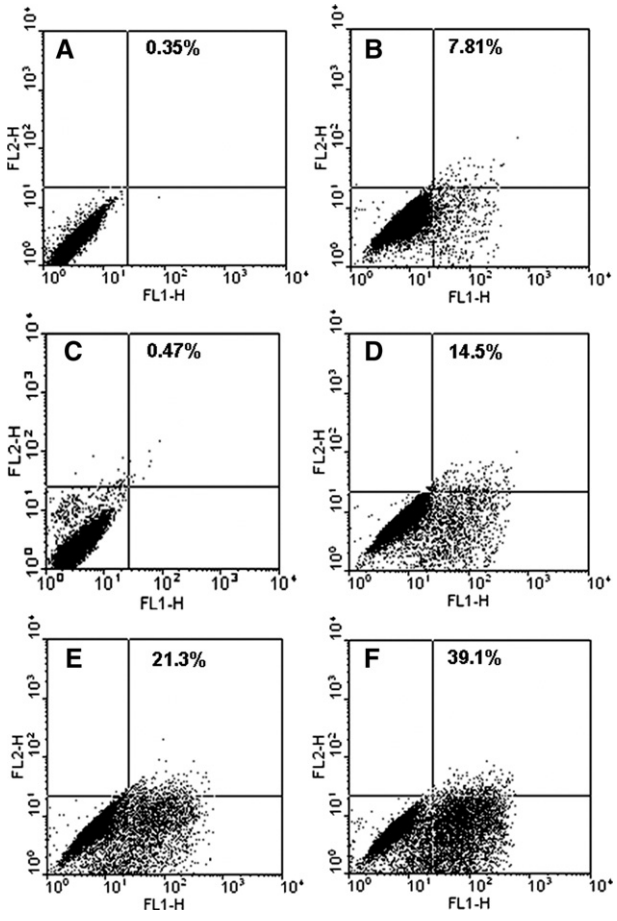

Fig. 3. Flow cytometric analysis of adriamycin-induced apoptosis of K562/A02 cells. K562/A02 cells were incubated with 3 μg/ml of adriamycin in the presence of various concentrations of plagiochin E for 48 h. The rate of apoptosis was measured using flow cytometry as described in Materials and methods. A, control; B, the treatment of 8 μg/ml of plagiochin E; C, the treatment of 3 μg/ml of adriamycin; D–F, the treatments of 3 μg/ml of adriamycin in the presence of 2, 4, 8 μg/ml of plagiochin E, respectively.

3.2. Plagiochin E strengthens the potency of adriamycin

The modulation of plagiochin E on the sensitivity of adriamycin against K562/A02 and K562 cells was shown in Table 1. The treatment of plagiochin E at non-toxic concentrations induced a significant decrease of the IC_{50}s of adriamycin against K562/A02 cells in a concertration-dependent manner. The RF of 8 μg/ml plagiochin E (8.5) was comparable to that of verapamil (2.5). However, no such activity was found in K562 cells. Therefore, significant differences of the IC_{50}s were seen in cell responses (K562 and K562/A02) and in dose responses (2, 4 and 8 μg/ml) to adriamycin treatment. These findings indicated that plagiochin E enhanced the potency of adriamycin against K562/A02 cells, whereas, had little effects on K562 cells, supporting the notion that plagiochin E could reverse P-glycoprotein-mediated resistance of K562/A02 cells.

3.3. Enhancement of adriamycin-induced apoptosis

The regulation of plagiochin E on the cytotoxicity of adriamycin toward K562/A02 cells was also evaluated by quantification of apoptotic cells. As shown in Fig. 3, 8 μg/ml of plagiochin E treatment resulted in 0.47% of apoptosis of K562/A02 cells, and the percentage of apoptosis was 7.81% when exposed to 3 μg/ml of adriamycin. When adriamycin was combined with 2, 4 and 8 μg/ml of plagiochin E, the mean apoptotic population of K562/A02 cells was increased by 1.86, 2.73 and 5.01 times, respectively, compared with 3 μg/ml of adriamycin treatment alone. Plagiochin E raised the adriamycin-induced apoptosis percentage in a dose-dependent manner in K562/A02 cells. The result suggested that the increased inhibitory effect on K562/A02 cells from the combination of plagiochin E with adriamycin was achieved through the action of plagiochin E, which enhanced the adriamycin-induced apoptosis.

3.4. Increased accumulation of adriamycin

The ability of plagiochin E to increase the adriamycin-induced cytotoxicity toward K562/A02 cells was further evaluated by the intracellular adriamycin-associated mean fluorescence intensity. As shown in Fig. 4, the accumulation of adriamycin in K562/A02 cells was significantly lower than that in K562 cells, which is an essential cause of decreased inhibitoy effect of adriamycin on K562/A02 cells. After the treatment with 4 and 8 μg/ml of plagiochin E for 1 h respectively, intracellular accumulation of adriamycin was completely restored in K562/A02 cells, while this was not the fact in K562 cells. The accumulation levels of adriamycin in K562/A02 cells were fitted well with the cytotoxicity in each data point, indicating that plagiochin E elevated the sensitivity of K562/A02 cells toward adriamycin through increasing adriamycin accumulation inside K562/A02 cells, and simultaneously providing a strong evidence that plagiochin E could effectively reverse P-glycoprotein-mediated multidrug resistance.

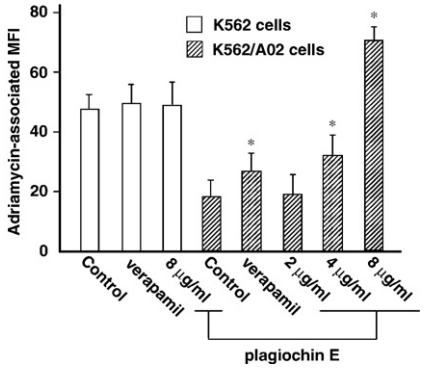

Fig. 4. Effect of plagiochin E on adriamycin accumulation in K562 and K562/A02 cells. Cells were treated with 3 μg/ml adriamycin in the absence and presence of plagiochin E for 1 h. Intracellular accumulation of adriamycin was evaluated by measuring the mean fluorescence intensity, expressed as MFI, using flow cytometry. Data were expressed as means ± S.D. of three independent experiments. *$P<0.05$ *vs.* untreated K562/A02 cells.

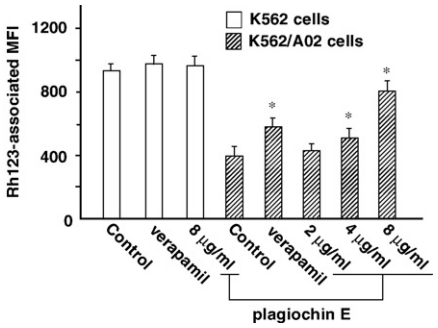

Fig. 5. Effect of plagiochin E on Rh123 accumulation in K562 and K562/A02 cells. Cells were pretreated with 0, 2, 4, 8 μg/ml of plagiochin E for 1 h and then exposed to 2 μg/ml of Rh123 for another 1 h. Rh123-associated mean fluorescence intensity (MFI) was evaluated by flow cytometry. Data were expressed as means ± S.D. of three independent experiments. *$P<0.05$ *vs.* untreated K562/A02 cells.

3.5. Effect on intracellular Rh123 accumulation and efflux inhibition

The aforementioned results proved that plagiochin E had the capability to increase adriamycin accumulation in K562/A02

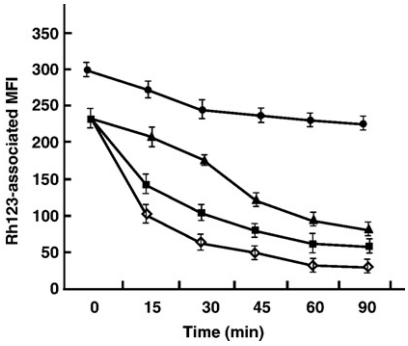

Fig. 6. Effect of plagiochin E on Rh123 efflux in K562/A02 cells. Cells were exposed to 2 μg/ml of Rh123 in the presence of plagiochin E (8 μg/ml). The cells were then washed three times in ice-cold medium and resuspended in 1 ml of medium. Following 15, 30, 45, 60, 90 min of efflux at 37 °C in the medium, the cells were washed twice in ice-cold PBS. The mean fluorescence intensity (MFI) of retained intracellular Rh123 was then estimated by flow cytometry. ● K562 cell control; ▲ K562/A02 cells treated with 8 μg/ml of plagiochin E; ■ K562/A02 cells treated with 8 μg/ml of verapamil; ◇ K562/A02 cells without treatment. Data were expressed as means ± S.D. of three independent experiments.

cells. In this assay, we further investigated the effect of plagiochin E on the P-glycoprotein activity by detecting accumulation of Rh123 in cancer cells. As shown in Fig. 5, K562/A02 cells in the absence of plagiochin E exhibited a significant decrease of Rh123 compared to K562 cells, while a notable increase was seen in K562/A02 cells in the presence of plagiochin E (4 and 8 μg/ml). The short time (60 min) treatment with plagiochin E induced the increase of Rh123 in K562/A02 cells, suggesting that plagiochin E had the ability to inhibit the drug-transport activity of P-glycoprotein, which is a major reason for inducing elevation of intracellular accumulation of adriamycin in K562/A02 cells.

The role of plagiochin E to block outward transporting function of P-glycoprotein was also determined in the Rh123 efflux experiment. Fig. 6 demonstrated that without plagiochin E, a rapid decrease of intracellular Rh123 level was observed in K562/A02 cells after incubation in Rh123-free medium for 90 min. The average percentage of Rh123 retention in individual time point (15, 30, 45, 60, 90 min) was 38.2, 24.0, 18.6, 12.2 and 9.4%, respectively, compared to K562 cells (100%). In the presence of plagiochin E, Rh123 efflux was drastically suppressed in K562/A02 cells, suggesting that P-glycoprotein's active outward transport was inhibited.

3.6. Plagiochin E decreases the expression of P-glycoprotein

To comfirmed if plagiochin E down-regulated P-glycoprotein expression, cells were exposed to various concentrations of plagiochin E. The expression level of P-glycoprotein in K562/A02 and K562 cells was analyzed by flow cytometry. K562 cells showed virtually the same fluorescent intensity labeled by anti-P-glycoprotein monoclonal antibody as that in the control (Fig. 7 A). Plagiochin E treated K562 cells expressed the similar amount of P-glycoprotein with untreated one (Fig. 7 B, C, D). In contrast, K562/A02 cells exhibited a strong fluorescent peak corresponding to P-glycoprotein (Fig. 7 E). After 24 h incubation with 2, 4, and 8 μg/ml of plagiochin E, the expression level of P-glycoprotein was decreased by 12.7%, 30.4%, 45.3% respectively (Fig. 7 F, G, H) in comparison to untreated K562/A02 cells. The result indicated that plagiochin E down-regulated expression of P-glycoprotein in a dose-dependent manner.

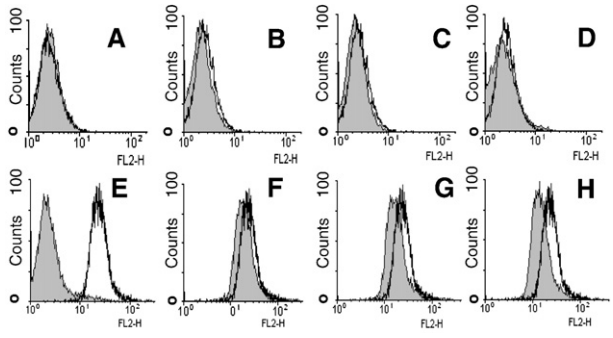

Fig. 7. P-glycoprotein expression in K562 (top panels) and K562/A02 cells (bottom panels). Cells were incubated with various concentrations of plagiochin E for 24 h and subjected to flow cytometry analysis of P-glycoprotein. P-glycoprotein was determined by flow cytometry using R-PE-conjugated mouse anti-human monoclonal antibody against P-glycoprotein and the non-specific fluorescent labeling was corrected by the isotype control. A, K562 cells showed virtually the same curve as that in the isotype control; B–D, K562 cells without (white background) or with 2, 4, 8 μg/ml of plagiochin E (gray background), respectively. E, isotype control (left) and P-glycoprotein expression curve (right) of K562/A02 cells; F–H, K562/A02 cells without (white background) or with 2, 4, 8 μg/ml of plagiochin E (gray background).

4. Discussion

In this study, we evaluated the efficacy of plagiochin E as a potent reversal agent to overcoming multidrug resistance of K562/A02 cells. The results showed that plagiochin E alone ranging from 2 to10 μg/ml did not display a significant anti-proliferative effect on K562/A02 cells, while the compound at these concentrations enhanced the cytotoxicity of adriamycin toward K562/A02 cells through accumulating adriamycin inside cells. Moreover, the increased sensitivity of K562/A02 cells to adriamycin was also confirmed by the enhanced adriamycin-induced apoptosis in the presence of plagiochin E.

Rh123 is a special substrate for P-glycoprotein. The uptake of Rh123 is resulted from passive inward diffusion (Lehnert et al., 1996; Krishna and Mayer, 2000), while the efflux is known to be P-glycoprotein-dependent. Rh123 has been used extensively as an indicator of P-glycoprotein activity in drug-resistant cell lines with P-glycoprotein over-expression (Green et al., 2001, Galski et al., 2006). Herein, Rh123 was used to assess the modulating ability of plagiochin E in drug-transport function of P-glycoprotein. As shown in Fig. 5, the treatment of plagiochin E at 4 and 8 μg/ml resulted in a remarkable increment of the fluorescent intensity from Rh123 in K562/A02 cells, indicating that plagiochin E elevated accumulation of adriamycin in K562/A02 cells by suppressing the drug-transport activity of P-glycoprotein. The accumulation level of Rh123 was hardly varied in K562 cells no matter with or without plagiochin E treatment, supporting the notion that plagiochin E enhanced adriamycin-induced cytotoxicity toward K562/A02 was attributed to its inhibition activity to P-glycoprotein.

In the previous study, we examined the activity of the compound in reversing fluconazole-resistant *C. albicans* (Leng et al., 2007). Plagiochin E might enhance the accumulation of fluconazole *via* inhibiting the activity of Cdr1p, a 170 KD transmembrane glycoprotein with homology to ABC superfamily proteins (Leng et al., 2007). The result is quite similar to the effects which block the function of P-glycoprotein or inhibit the expression of P-glycoprotein. On the one hand, plagiochin E inhibited drug-transport function of P-glycoprotein, this conclusion was confirmed by the fact that short time incubation (60–90 min) of K652/A02 cells with plagiochin E caused an increase of intracellular Rh123 and a inhibition of its efflux (Figs. 5, 6). On the other hand, the reversal effect of plagiochin E on multidrug resistance of K562/A02 cells may be actualized by down-regulating P-glycoprotein (Fig. 7).

Inhibiting the activity of P-glycoprotein is an ideal means to overcome tumor multidrug resistance. Many natural phenolic compounds, such as curcuminoid and curcumin, have been reported to inhibit the P-glycoprotein activity through blocking

its drug-transport function or down-regulating its expression (Anuchapreeda et al., 2002; Limtrakul et al., 2004). In this study, we added a new member to the class of compounds with P-glycoprotein inhibition activities to the multidrug-resistant cancer cells. Plagiochin E appears to be similar to verapamil and curcuminoids in the mechanism to reverse P-glycoprotein-mediated drug resistance. Therefore, the compound is of dual functions of inhibiting P-glycoprotein's drug-pump and expression.

In conclusion, plagiochin E, as a novel bisbibenzyl compound from liverwort, has potent effects in reversing P-glycoprotein-mediated multidrug resistance. This suggests that plagiochin E may be a potential candidate for reversing drug resistance in cancer chemotherapy. The reversal effect study of plagiochin E on different resistant cancer cell lines and its in vivo pharmacokinetics is in progress.

Acknowledgement

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References

Anuchapreeda, S., Leechanachai, P., Smith, M.M., Ambudkar, S.V., Limtrakul, P.N., 2002. Modulation of P-glycoprotein expression and function by curcumin in multidrug-resistant human KB cells. Biochem. Pharmacol. 64, 573–582.

Asakawa, Y., Toyota, M., Tori, M., Hashimoto, T., 2000. Chemical structures of macrocyclic bis(bibenzyls) isolated from liverworts (Hepaticae). Spectroscopy 14, 149–175.

Borowski, E., Bontemps-Gracz, M.M., Piwkowska, A., 2005. Strategies for overcoming ABC-transporters-mediated multidrug resistance (MDR) of tumor cells. Acta Biochim. Pol. 52, 609–627.

Chearwaw, W., Anuchapreeda, S., Nandigama, K., Ambudkar, S.V., Limtrakul, P., 2004. Biochemical mechanism of modulation of human P-glycoprotein (ABCB1) by curcumin I, II, and III purified from Turmeric powder. Biochem. Pharmacol. 68, 2043–2052.

Di Pietro, A., Conseil, G., Perez-Victoria, J.M., Dayan, G., Baubichon-Cortay, H., Trompier, D., Steinfels, E., Jault, J.M., de Wet, H., Maitrejean, M., Comte, G., Boumendjel, A., Mariotte, A.M., Dumontet, C., McIntosh, D.B., Goffeau, A., Castanys, S., Gamarro, F., Barron, D., 2002. Modulation by flavonoids of cell multidrug resistance mediated by P-glycoprotein and related ABC transporters. Cell. Mol. Life Sci. 59, 307–322.

Galski, H., Sivan, H., Lazarovici, P., Nagler, A., 2006. In vitro and in vivo reversal of MDR1-mediated multidrug resistance by KT-5720: implications on hematological malignancies. Leuk. Res. 30, 1151–1158.

Gottesman, M.M., Fojo, T., Bates, S.E., 2002. Multidrug resistance in cancer: role of ATP-dependent transporters. Nat. Rev., Cancer 2, 48–58.

Green, L.J., Marder, P., Slapak, C.A., 2001. Modulation by LY335979 of P-glycoprotein function in multidrug-resistant cell lines and human natural killer cells. Biochem. Pharmacol. 61, 1393–1399.

Jain, S., Laphookhieo, S., Shi, Z., Fu, L.W., Akiyama, S., Chen, Z.S., Youssef, D.T., van Soest, R.W., El Sayed, K.A., 2007. Reversal of P-glycoprotein-mediated multidrug resistance by sipholane triterpenoids. J. Nat. Prod. 70, 928–931.

Ji, B.S., He, L., 2007. CJY, an isoflavone, reverses P-glycoprotein-mediated multidrug-resistance in doxorubicin-resistant human myelogenous leukaemia (K562/DOX) cells. J. Pharm. Pharmacol. 59, 1011–1015.

Ji, B.S., He, L., Liu, G.Q., 2005. Reversal of p-glycoprotein-mediated multidrug resistance by CJX1, an amlodipine derivative, in doxorubicin-resistant human myelogenous leukemia (K562/DOX) cells. Life Sci. 77, 2221–2232.

Kim, Y.K., Song, Y.J., Seo, D.W., Kang, D.W., Lee, H.Y., Rhee, D.K., Han, J.W., Ahn, C.M., Lee, S., Kim, S.N., 2007. Reversal of multidrug resistance by 4-chloro-*N*-(3-((*E*)-3-(4-hydroxy-3-methoxyphenyl)acryloyl)phenyl)benzamide through the reversible inhibition of P-glycoprotein. Biochem. Biophys. Res. Commun. 355, 136–142.

Kitagawa, S., 2006. Inhibitory effects of polyphenols on P-glycoprotein-mediated transport. Biol. Pharm. Bull. 29, 1–6.

Krishna, R., Mayer, L.D., 2000. Multidrug resistance (MDR) in cancer. Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. Eur. J. Pharm. Sci. 11, 265–283.

Labeed, F.H., Coley, H.M., Thomas, H., Hughes, M.P., 2003. Assessment of multidrug resistance reversal using dielectrophoresis and flow cytometry. Biophys. J. 85, 2028–2034.

Lehnert, M., de Giuli, R., Twentyman, P.R., 1996. Sensitive and rapid bioassay for analysis of P-glycoprotein-inhibiting activity of chemosensitizers in patient serum. Clin. Cancer Res. 2, 403–410.

Leng, P., Guo, X.L., Yang, Y., Lou, H.X., 2007. Primary study of antifungal activities and reversal of fluconazole resistance of Plagiochin E. Chin. Pharm. J. 3, 314–317.

Limtrakul, P., Anuchapreeda, S., Buddhasukh, D., 2004. Modulation of human multidrug-resistance MDR-1 gene by natural curcuminoids, Biomed. Chromatogr. Cancer 4, 13.

Liu, Z.L., Onda, K., Tanaka, S., Toma, T., Hirano, T., Oka, K., 2002. Induction of multidrug resistance in MOLT-4 cells by anticancer agents is closely related to increased expression of functional P-glycoprotein and MDR1 mRNA. Cancer Chemother. Pharmacol. 49, 391–397.

Marbeuf-Gueye, C., Salerno, M., Quidu, P., Garnier-Suillerot, A., 2000. Inhibition of the P-glycoprotein- and multidrug resistance protein-mediated efflux of anthracyclines and calceina cetoxymethyl ester by PAK-104P. Eur. J. Pharmacol. 391, 207–216.

Niu, C., Qu, J.B., Lou, H.X., 2006. Antifungal bis[bibenzyls] from the Chinese liverwort *Marchantia polymorpha* L. Chem. Biodivers 3, 34–40.

Qi, J., Liu, N., Zhou, Y., Tan, Y., Cheng, Y., Yang, C., Zhu, Z., Xiong, D., 2006. Overexpression of sorcin in multidrug resistant human leukemia cells and its role in regulating cell apoptosis. Biochem. Biophys. Res. Commun. 349, 303–309.

Qu, X.J., Yang, J.L., Russell, P.J., Goldstein, D., 2004. Changes in epidermal growth factor receptor expression in human bladder cancer cell lines following interferon-alpha treatment. J. Urol. 172, 733–738.

Teodori, E., Dei, S., Martelli, C., Scapecchi, S., Gualtieri, F., 2006. The functions and structure of ABC transporters: implications for the design of new inhibitors of P-glycoprotein and MRP1 to control multidrug resistance (MDR). Curr. Drug Targets 7, 893–909.

Ushigome, F., Takanaga, H., Matsuo, H., Yanai, S., Tsukimori, K., Nakano, H., Uchiumi, T., Nakamura, T., Kuwano, M., Ohtani, H., Sawada, Y., 2000. Human placental transport of vinblastine, vincristine, digoxin and progesterone: contribution of P-glycoprotein. Eur. J. Pharmacol. 408, 1–10.

Wang, G.H., Chen, H.F., Huang, M.H., Wang, N.L., Zhang, J.C., Zhang, Y., Bai, G.R., Fong, W.F., Yang, M.S., Yao, X.S., 2006. Methyl protodioscin induces G2/M cell cycle arrest and apoptosis in HepG2 liver cancer cells. Cancer Lett. 241, 102–109.

Wu, J.Y., Fong, W.F., Zhang, J.X., Leung, C.H., Kwong, H.L., Yang, M.S., Li, D., Cheung, H.Y., 2003. Reversal of multidrug resistance in cancer cells by pyranocoumarins isolated from Radix Peucedani. Eur. J. Pharmacol. 473, 9–17.

Xu, D., Lu, Q., Hu, X., 2006. Down-regulation of P-glycoprotein expression in MDR breast cancer cell MCF-7/ADR by honokiol. Cancer Lett. 243, 274–280.