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***RESEARCH ARTICLE***

# EFFECT OF 5,4′-DIHYDROXY-3,6,7,8-TETRAMETHOXYFLAVONE AND 5,3′-DIHYDROXY-3,6,7,8,4′-PENTAMETHOXYFLAVONE ON MITOCHONDRIAL FUNCTION IN LIVING K562 AND K562/*ADR* CANCER CELLS

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**Abstract**

In the previously study we reported speciation of methoxyflavone including 5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone (WP279) and 5,3′-dihydroxy-3,6,7,8,4′-penta methoxyflavone (WP283) in a physiological solution and their anticancer property. The objective of this study was to determine the effect of 5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone and 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone on mitochondrial membrane potential in drug-sensitive,K562 and drug-resistance, K562/*adr* cancer cell lines by using spectrofluorescence technique. The result shows that WP279 and WP283 slightly increased the mitochondrial membrane potential in K562 and K562/*adr* due to the concentration.

**Keywords:** 5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone (WP279), 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone (WP283), mitochondrial membrane potential, spectrofluorescence technique

## Introduction

Flavonoids are important compounds in various natural products such as plant, fruits, vegetables, wine, which can fight against wide range of cancer cells (Fresco *et al.*, 2010; Srivastava and Gupta, 2009; Tayarani-Najaran *et al.*, 2009).

The flavonoids are promising apoptosis inducing agent, property that activates apoptotic program cell death via mitochondrial pathway. Desupha et al. report that flavonoid from Thai traditional plant, Mamao wood (*A. thwaitesianum* Müll. Arg.), induces apoptosis in invasive estrogen-receptor negative MDA-MB 435 cells xenografted in nude mice (Desupha *et al.*, 2007). Koppikar et al. show that the extract compound isolated from the bark of *Cinnamomum cassia* L., family *Lauraceae*, can induce apoptosis in human cervical cancer cell line, SiHa, via alter the mitochondrial membrane potential (Koppikar *et al.*, 2010). Kothan et al. also report that quercetin exhibit anticancer properties and can decrease in mitochondrial membrane potential in drug-sensitive and drug-resistance

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cancer cell line as a concentration dependent manner (Kothan *et al*., 2004).

We have previously studied the speciation of 5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone(WP279) and 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxy-flavone (WP283) in a physiological solution and their cytotoxicity on cancer cell lines. We found that WP279 and WP283 molecules had trend to passive cross the cell membrane and been accumulated inside the cell and induced cancer cell death in micromolar range (Tungjai *et al*. 2008). In this study, we had determined the effect of WP279 and WP283 molecules on mitochondrial membrane potential in living K562 and K562/*adr* cancer cell.

## Materials and methods

### Chemicals

5,4′-dihydroxy-3,6,7,8-tetramethoxyflavone (WP279), and 5,3′-dihydroxy-3,6,7,8,4′-pentamethoxyflavone (WP283) (see chemical structure in figure 1) were kindly provided from Assoc. Prof. Dr. Wilart Poompimon, Department of Chemistry, Faculty of Science, Lampang Rajabhat University, Lampang, Thailand. 3-(4,5-dimethythiazol-2-y)-2,5-diphenyltetrazolium bromide (MTT) was from sigma singapore science park II, singapore. Rhodamine B was from amresco.

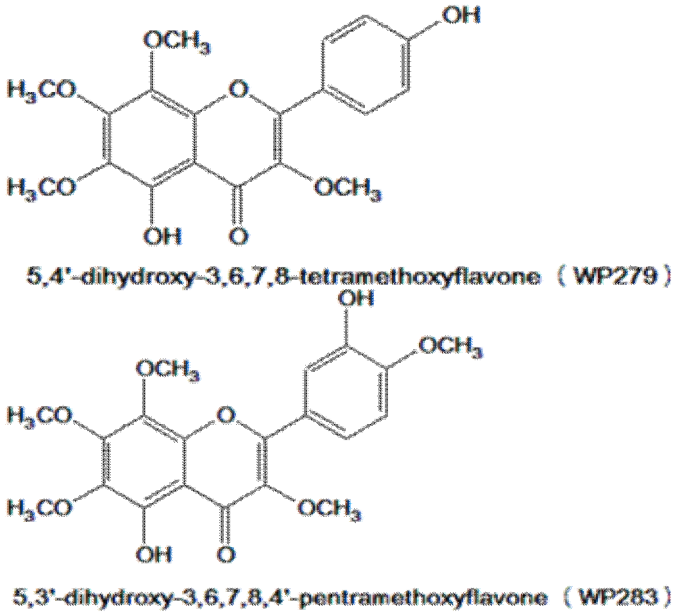


**Figure 1.** *Chemical structure of WP279 and WP283*

### Cell lines and Culture conditions

The human adriamycin-sensitive erythroleukemia cells (K562) and human adriamycin-resistant erythroleukemia cells (K562/*adr*, overexpressing P-gp) (Mankhetkorn *et al.*, 1996 ; Meesungneon *et al.*, 2000) were grown in RPMI–1640 medium containing L-glutamine and supplemented with 1% penicillin-streptomycin (Sigma Chemical Co.) and 10% fetal bovine serum (Gibco Biocult Ltd.), at 37$^{O}$C, 95% humidity, 5% $CO_2$. In culture condition, $10^5$cells/mL grew exponentially to about 8-10 x $10^5$cells/mL in 3 days. To have cells in the exponential growth phase for the experiments, density of cell were initiated at 5 × $10^5$ cells/mL and grew to density about 8-10 × $10^5$ cells/mL after 24 hours. The number of cells was counted by a haemocytometer. For the resistant K562/*adr* cells, they were treated with 100 nM doxorubicin for 2 weeks before experiments.

**Measurement of mitochondrial membrane potential (ΔΨm)**

A non-invasive functional spectrofluorometric method used to spontaneous monitor mitochondrial membrane potential change in cancer cells have been previously described by Reungpatthanaphong et al. (Reungpatthanaphong *et al.*, 2003). Briefly, cells ($1 \times 10^6$ cells/mL) were incubated with 40 nM rhodamine B ($C_T$) in 1 cm quartz cuvettes containing HEPES–$Na^+$ buffer, pH 7.3 and vigorously stirred at 37 °C. The rhodamine B fluorescence spectrum (F0) at 582 nm (excited at 553 nm) was recorded depending on time. MTT (200 μM) was added to the solution after 20 minutes, yield of rhodamine B fluorescence progressive decreasing. ΔΨm could be calculated by an equation as follow:

$$\Delta\Psi m = -61.51 \log Vi - 258.46,$$

(where $V_i = dF/dt \times C_T/F_0$ )

$V_i$ was the initial rate of decrease in rhodamine B fluorescence. The slope of the tangent to the curve $F = f(t)$ after the addition of MTT was $-dF/dt$.

**Results and Discussions**

**Modulation of mitochondrial membrane potential in K562 and K562/*adr* cells by WP279 and WP283**

The impaired cellular energetic state could be directly studied as a function of mitochondria. The mitochondrial function could be studied by measuring the change in mitochondrial membrane potential (ΔΨm). As we already mentioned in Materials and Methods, the mitochondrial membrane potential of drug-sensitive and drug-resistant cancer cells could be determined using rhodamine B. A typical experiment was shown in figure 2.

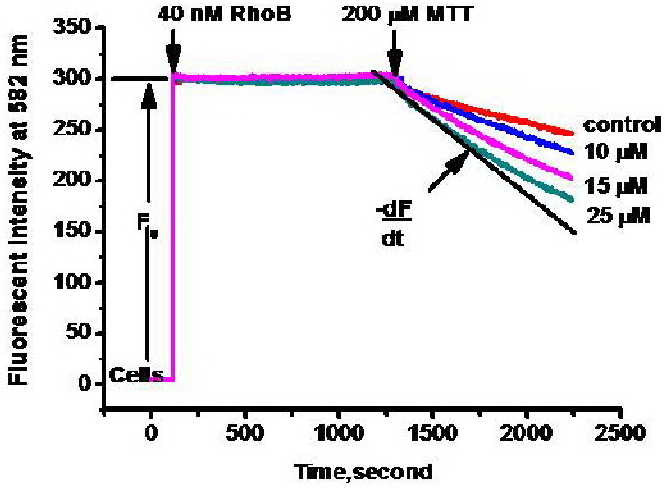


**Figure 2.** *Typical kinetics of the uptake of rhodamine B by cells with and without molecules*

Modulation of the absolute value of ΔΨm in K562 and K562/*adr* cells in the presence of WP279 and WP283 was investigated in relation of concentration. At initially (without molecules), the absolute value of ΔΨm was 157 ± 6.0 mV and 139 ± 10 mV in K562 and K562/*adr* cells, respectively. After incubated with various concentrations of WP279 and WP283 (2-25 □M) for 20 minutes, the absolute value of ΔΨm was slightly increased. Typical results of WP279 and WP283 induced changes in the absolute value of ΔΨm in function of concentration, which were indicated in figure 3. It was well established that flavonoid's slightly alter in the mitochondrial membrane potential, even in a very narrow window, could affect the cellular energetic state, leading to cell death (Kothan *et al.*, 2004). The results lead conclusion that WP279 and WP283 affected mitochondrial membrane potential in drug-sensitive and drug-resistant cancer cells. WP279 and WP283 could induce death of human erythroleukemia cells and small lung carcinoma cancer cells (Tungjai *et al.*, 2008). We considered that WP279 and WP283 induced cancer cell deaths via alter mitochondria membrane potential.

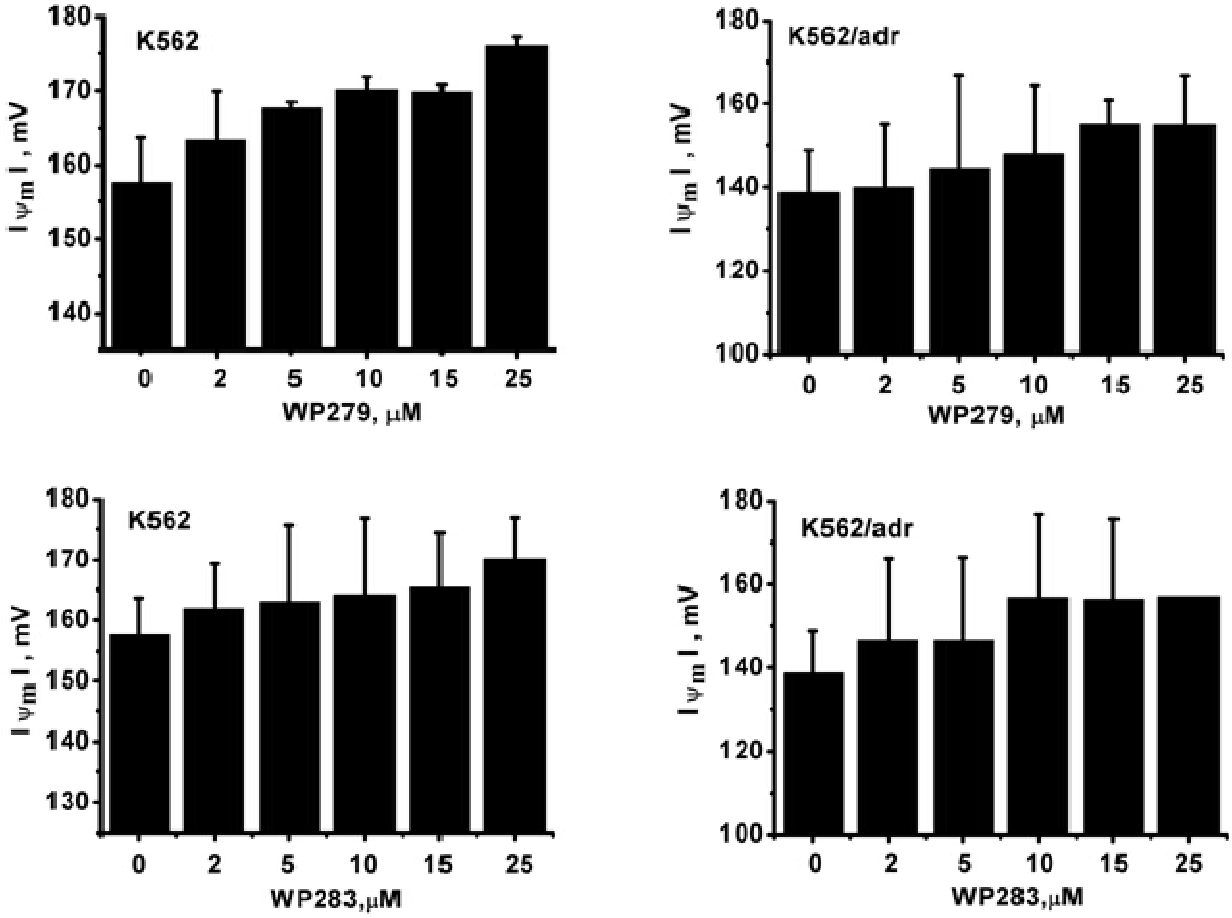


**Figure 3.** *The variation of ΔΨm according molecules' concentration in K562 and K562/adr cell*

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**REFERENCES**

Dechsupa S., Kothan S., Vergote J., Leger G., Martineau A., Beranger S., Kosanlavit R., Moretti J.L., Mankhetkorn S. (2007). Quercetin, siamois 1 and siamois 2 induce apoptosis in human breast cancer MDA-MB-435 cells xenograft in vivo. *Cancer biology & therapy*., 6, 56-66.

Fresco P., Borges F., Marques M.P.M., Diniz C. (2010). The anticancer properties of dietary polyphenols and its relation with apoptosis. *Current pharmaceutical design*., 16, 114-134

Koppikar S.J., Choudhari A.S., Suryavanshi S.A., Kumari S., Chattopadhyay S., Kaul-Ghanekar R. (2010). Aqueous cinnamon extract (ACE-*c*) from the bark of *Cinnamomum cassia* causes apoptosis in human cervical cancer cell line (SiHa) through loss of mitochondrial membrane potential. *BMC Cancer*., 10, 210-222.

Kothan S., Dechsupa S., Leger G., Moretti J.L., Vergote J., Mankhetkorn S. (2004). Spontaneous mitochondrial membrane potential change during apoptotic induction by quercetin in K562 and K562/adr cells. *Canadian journal of physiology and pharmacology*., 82, 1084–1090.

Mankhetkorn S., Dubru F., Hesschenbrouck J., Fiallo M., Garnier-Suillerot A. (1996). Relation

among the resistance factor, kinetics of uptake and kinetics of the P-glycoprotein-mediated efflux of doxorubicin, daunorubicin, 8-(S)-fluoroidarubicin and idarubicin in multidrug-resistant K562 cells. *Molecular pharmacology.*, 49, 532-539

Meesungneon J., Jay-Gerin J., Mankhetkorn S. (2002). Relation between MDR1 mRNA levels, resistance factor and the efficacy of P-glycoprotein-mediated efflux of pirarubicin in multidrug resistant K562 sublines. *Canadian journal of physiology and pharmacology*., 80, 1054-1063

Reungpatthanaphong P., Samlee M. (2002). Modulation of multidrug resistance by artemisinin, artesunate and dihydroartemisinin in K562/*adr* and GLC4/*adr* resistant cell lines. *Biological & pharmaceutical bulletin.*, 25(12), 1555-1561.

Srivastava J.K., Gupta S. (2009). Extraction, Characterization, Stability and Biological Activity of Flavonoids Isolated from Chamomile Flowers. *Molecular and cellular pharmacology*., 1(3), 138-146.

Tungjai M., Poompimon W., Loetchutinat C., Kothan S., Dechsupa N.,Mankhetkorn S. (2008). Spectrophotometric characterization of behavior and the predominant species of flavonoids in physiological buffer: determination of solubility, lipophilicity and anticancer efficacy. *The open drug delivery journal*, 2, 10-19.

Tayarani-Najaran Z., Emami S.A., Asili J., Mirzaei A., Mousavi S.H. (2009). Analyzing cytotoxic and apoptogenic properties of scutellaria litwinowii root extract on cancer cell lines, *eCAM*., 1- 9