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Abstract : Quercetin Induce ROS_i Depletion, Impairment the Mitochondrial Function and Apoptosis in K562 and K562/adr Cells

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Natural products from plants such as flavonoids are the potential drugs to overcome multidrug resistance (MDR) in cancer treatments. However, its modes of action are still unclear. In this study, effects of quercetin on mitochondrial membrane potential ($\Delta\Psi m$) change and deplete the intracellular reactive oxygen species (ROS_i) which leading to an increase in the $\Delta\Psi m$, stimulate mitochondrial ROS generation and trigger cellular apoptosis in K562, K562/adr. Quercetin exhibits cytotoxicity against erythroleukemic cells: IC_{50} are 11.0 $\pm$ 2.0 μM and 5.0 $\pm$ 0.4 μM for K562 and K562/adr, respectively. Quercetin induces an increase followed by a decrease in $|\Delta\Psi m|$ value depending on its concentration. Decreasing in $|\Delta\Psi m|$ value is associated with an increase in the percentage of early apoptotic cells. Therefore quercetin is potential apoptotic inducing agent by which the molecule reacts at the mitochondrial level. **Bull Chiang Mai Assoc Med Sci 2006; 39: 113-117.**

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1. Introduction

The occurrence of cellular drug resistance at a clinical level to cytotoxic drugs called multidrug resistance (MDR) can result from a variety of mechanisms that are not fully understood[1]. It is frequently associated with an over expression of drug transporters proteins, 170 kDa P-glycoprotein (P-gp), the *MDR1* gene product[2] and 190 kDa multidrug resistance-associated protein (MRP), the *MRP1* gene product. It is well documented that various molecules can reverse these MDR phenomena via impairment of mitochondrial function including artemisinin, artesunate and dihydroartemisinin, within the antimalarial qinghousu family[3].

In this study, we report herein that quercetin is a potent cytotoxic agent, with greater efficacy in K562/adr than K562 cells. Quercetin induces cellular apoptosis relative to mitochondrial membrane potential ($\Delta\Psi m$) change and deplete the intracellular reactive oxygen species (ROS_i) which leading to an increase in the $\Delta\Psi m$, stimulate mitochondrial ROS generation and trigger cellular apoptosis

2. Materials and methods

2.1 Drug preparation

Quercetin (Extrasynth?se) was prepared in ethanol at 10^{-3} M and aliquots were frozen. We used a new aliquot for each experiment. All series of experiments were performed using Hepes/Na^+ isotonic buffer solution, pH 7.25 at 37^0C, containing 20 mM Hepes plus 132 mM NaCl, 3.5 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 5 mM glucose.

2.2 Cell Culture and cytotoxicity assay

The erythromyelogenous leukemic K562 and its Pgp-overexpression K562/adr cells were cultured in RPMI 1640 medium containing L-glutamine and supplemented with 10% fetal calf serum and 1% penicillin /streptomycin (BioMedia) at 37 ^{0}C in 5% CO_2.

Cytotoxicity assay was performed as followed; cells were plated in a 6-well plate at initial density of $1x10^5$ cells per ml, with 4.0 mL of medium per well and incubated in the presence of various quercetin concentrations. Number of cells was measured by coulter counter. IC_{50} is the quercetin concentration that inhibits cell growth by 50% when measured at 72h of K562 cell lines.

2.3 Staining of the Cells

For detection of apoptosis, the treated cells were centrifuged for 5 min, 1000xg at room temperature (18-24 ^{0}C), resuspended and washed once with 5 mL phosphate-buffered saline prior to staining with Annexin V (apoptosis detection kit (R&D Systems)). Flow cytometry analysis was performed in a Coulter Epics XL-MCL (Coultronics France SA).

2.4 Measurement of mitochondrial membrane potential ($\Delta\Psi m$)

The mitochondrial membrane potential ($\Delta\Psi m$) was measured using a non-invasive functional spectrofluorometric method which can be used to determine and to monitor a spontaneous change in mitochondrial function in drug-sensitive and drug-resistant cells as prevoiusly described by Reungpatthanaphong et al.[(4)].

$$\Delta\Psi m = - 61.51 \log Vi - 258.46 \qquad (1)$$

2.8 Determination of the intracellular reactive oxygen species (ROS_i) by using the initial rate (Vi) method.

Cells (10^5 cells/mL) were suspended in 2 mL buffer solution in a 1 cm quartz cuvette containing 2 mL of HEPES-Na^+ at 37^0C under vigorous stirring for 10 min performed in spectrofluorometer (PerkinElmer LS 50). The fluorescence intensity of DCF was monitored at 523 nm when excited at 502 nm as a function of time. Successive additions of 20 mM $CoCl_2$, and the precise concentration of DCHF-DA, resulted in an increased fluorescence intensity of DCF.

The kinetics of DCF production was followed and the initial rate of an increase in DCF fluorescence intensity (Vi) was determined by the tangent to the curve of F = f(t) during a first 50s after addition of DCHF-DA. The Vi is linearly proportional to the number of cell (ROS_i) which can be quantified by linear relation fitting of these data using the equation: $[ROS_i] = a.Vi$. The determined ROS_i is in $M.cell^{-1}$, where “a” is the ratio of ROSi/Vi.

3. Results

3.1 Cytotoxicity of quercetin against K562 and K562/adr cells

Quercetin exhibits cytotoxicity against K562 and K562/adr cell with IC_{50} (means±SD) values equal to 11.0±2.0 μM and 5.0±0.4 μM for K562 and K562/adr cells, respectively. It should be noted that quercetin exhibits more cytotoxicity in MDR cell than its corresponding drug-sensitive cell. The resistance factor value (RF) is equal to 0.45.

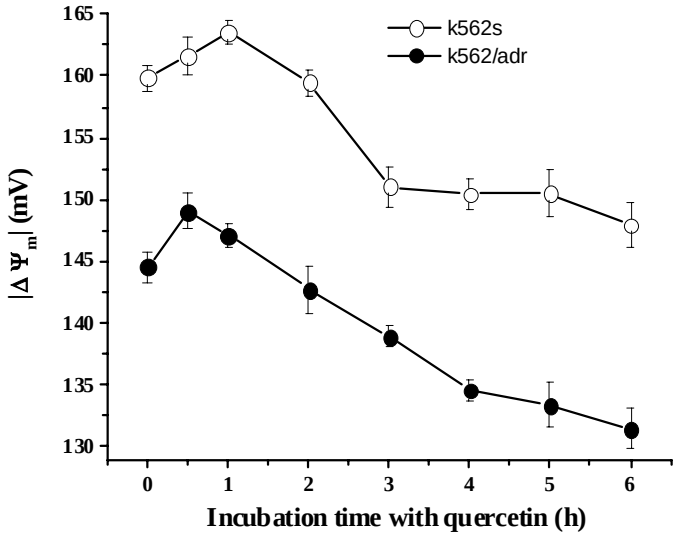

Fig. 1. Absolute value of ΔΨm of (○) K562 and (●) K562/adr in the presence of 10 μM quercetin as a function of time. Data represent mean±SD of three independent experiments

3.2 Effect of Flavonoids on ROS_i depletion

The typical results representing the effect of quercetin, on ROS_i depletion in cells is illustrated in Figure 1B where the rate of DCF production was significantly lower, compared with those of the control series, signifying the lowering of the ROS_i concentration. The ROS_i concentration decreased inversely with the concentration of quercetin added into the cells. Quercetin (50 μM) can deplete ROS_i from the basal level of 55±5 pM.cell^{-1}, 30±4 pM.cell^{-1} for K562, K562/adr, respectively, to 10±pM. cell^{-1} in all cell line.

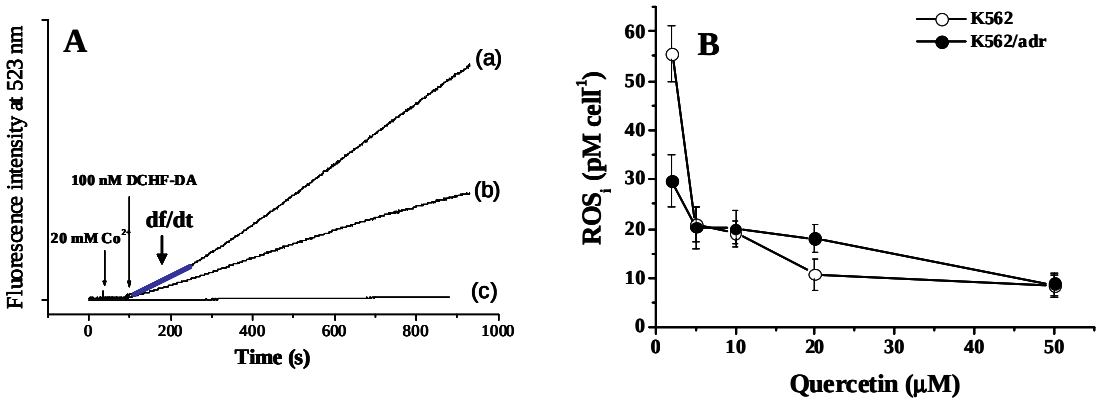

Fig. 2. (a) Kinetics of DHCF-DA oxidation: cells (2 x 10^5) were suspended in 2 mL of HEPES-Na^+ buffer for 10 min before addition of (1) 20 mM $CoCl_2$ and 100 nM DCHF-DA, (2) of 20 mM $CoCl_2$, 100 nM DCHF-DA and 10 μM quercetin and (3) of 20 mM $CoCl_2$, 100 nM DCHF-DA and 10 μM quercetin without cell. The fluorescence intensity at 523 nm (excited 502 nm) were recorded as a function of time and the initial rate of an increase in DCF fluorescence intensity (Vi) was determined by the tangent to the curve of F = f(t) during a first 50s after addition of DCHF-DA. The determined ROSi is in $M.cell^{-1}$ was calculated using the expression [ROSi] = a.Vi., where "a" is the ratio of ROSi/Vi.; (B) Variation of ROSi obtained from the series of experiments (a) as a function of flavonoid concentration used in (○) K562, (●) K562/adr cell. Data are the mean ± SD of three independent experiments.

To determine whether the ROS_i depletion affected the mitochondrial energetic regulation, the $\Delta\Psi m$ of cell lines was measured at varied times in the presence of 10 μM quercetin. At basal level (without quercetin), $|\Delta\Psi m|$ is 160 ± 1.0 mV in K562, K562/adr cells, respectively. After quercetin addition (10 μM), $|\Delta\Psi m|$ was slightly increased to reach a maximal value (+4.4%, + 3.3% of initial value) at 1h and 30 min then progressively decreased (-5.5%, -3.8% of initial value) at 3h for K562 and K562/adr In order to determine the relations among the $|\Delta\Psi m|$, the % early apoptotic cells and the ROS_i concentration, a series of experiments were performed using K562 and K562/adr cells. At 3h after addition of quercetin into cells, the $|\Delta\Psi m|$, the % early apoptotic cells and the ROS_i concentration were determined. The correlation among the $|\Delta\Psi m|$, the % early apoptotic cells and the ROS_i concentration is indicated in Figure. 3 (K562).

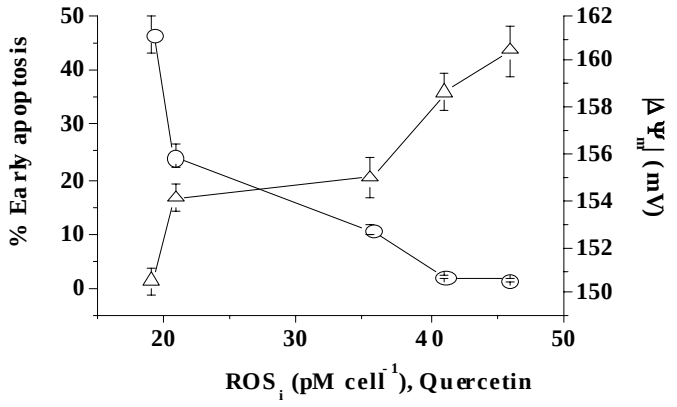

Fig. 3. Variation of Absolute value $\Delta\Psi_m$ (○) and percentage of early apoptotic cell (△) as a function of ROS_i in K562. Cells were treated with 10 μM quercetin for 3h.

4. Discussion

Overall our results demonstrate that as antioxidant molecule, quercetin that normally play important role to protect the cells against oxidative damage, can induces apoptosis in cancer cell lines.

The immediate increase in $\Delta\Psi$m after quercetin addition is due to an increase in mitochondrial matrix rhodamine B concentration on which the mitochondrial energetic state depends. The depletion of ROS_i induced by quercetin should affect the intracellular redox-state where the maintenance processes may require ATP, and thus, the mitochondria may play pivotal role as energy source. We have checked that an increase in the $\Delta\Psi$m accompanied by elevated oxidative phosphorylation, results in increased cellular ATP contents and increased mitochondrial ROS production. These results suggest that these quercetin mediated-action is via two distinct processes, depletion ROS_i and directly interaction of intact molecules on the adenine nucleotide translocator in the inner mitochondrial membrane. A similar finding that mitochondria play a central role on drug-induced cell death is confirmed by recent studies.

The results of this study reveal that quercetin mediated-action at mitochondria level; impaired mitochondrial energetic state followed by an induction of apoptosis and inhibition of caner cell growth. Quercetin could be a new generation of antitumour drug, particularly for overcoming MDR phenomena.

Reference

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