



Letter

Positions and numbers of hydroxyl groups in hydroxychalcone derivatives are involved in cytotoxicity against human monoblastic U937 cells

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ABSTRACT — Hydroxychalcone derivatives belonging to polyphenol group, and distributed throughout the plant kingdom, can behave as anti-cancer agents due to their cytotoxic activities. Hydroxychalcone derivatives also have the potential as chemotherapeutic drugs for cancer. In this paper, we revealed that positions and numbers of hydroxyl groups in hydroxychalcones are involved in cytotoxicity against human monoblast U937 cells. Interestingly, 2-hydroxychalcone remarkably reduced viability of U937 cells as compared to chalcone, 2'-hydroxychalcone, 4-hydroxychalcone, and 4'-hydroxychalcone. In addition, 2', 4, 4'-trihydroxychalcone showed stronger cytotoxicity than 2'-hydroxychalcone, 2', 4-dihydroxychalcone, and 2', 4'-dihydroxychalcone. These results demonstrate that positions and numbers of hydroxyl groups in hydroxychalcones are involved in cytotoxicity against U937 cells. Moreover, we showed that *all-trans* retinoic acid-induced differentiation of U937 cells brought about an enhanced resistance against some cytotoxic hydroxychalcone derivatives. These data suggest that hydroxychalcone derivatives in combination with *all-trans* retinoic acid could serve as effective modifiers in therapy for leukemia.

Key words: Chalcone, Hydroxyl group, Cytotoxicity, U937

INTRODUCTION

Polyphenol compounds, which are secondary metabolites in plants, protect them from ultraviolet irradiation and numerous infections. They are contained in usual human diet and show various biological activities. Chalcone (1, 3-diphenyl-2-propen-1-one) and its derivatives, typical polyphenol compounds, are also widely distributed in edible plants, and have multiple interesting biological functions: anti-cancer (Sabzevari *et al.*, 2004; Kim *et al.*, 2013; Pande *et al.*, 2017; Jayasooriya *et al.*, 2018), anti-inflammation (Hsieh *et al.*, 1998), anti-oxida-

tion (Ruby *et al.*, 1994; Haruguchi *et al.*, 1998), anti-angiogenic (Varinska *et al.*, 2012), analgesic and antipyretic (Torigoe *et al.*, 1983), cytotoxicity (Kupcewicz *et al.*, 2014) effects, etc. Hydroxychalcone derivatives are also distributed throughout the plant kingdom and can behave as anti-cancer agents due to their cytotoxic activities (Saydam *et al.*, 2003). Regarding leukemia, some hydroxychalcone derivatives show remarkable cytotoxic effects on several human leukemia cell lines. For example, 4, 4'-dihydroxychalcone, 2'-oxygenated chalcone derivatives, 2', 4'-dihydroxy-6'-methoxy-3', 5'-dimethylchalcone and 4'-hydroxychalcone exert cytotoxicity against

HL60 (Saydam *et al.*, 2003), U937 (Rao *et al.*, 2004), K562 (Ye *et al.*, 2005) and Jurkat (Gul *et al.*, 2009) cells, respectively. Taken together, hydroxychalcone derivatives have great potential for use as anti-leukemia drugs. However, the understanding of relationship between cytotoxicity of hydroxychalcone derivatives and their chemical structure is still poor.

In this paper, we revealed that positions and numbers of hydroxyl groups in hydroxychalcones are involved in cytotoxicity against U937 cells. In addition, we showed that *all-trans* retinoic acid (RA)-induced differentiation of U937 cells brought about enhanced resistance against some cytotoxic hydroxychalcone derivatives.

MATERIALS AND METHODS

Materials

Chalcone, 2-hydroxychalcone, 2'-hydroxychalcone, 4-hydroxychalcone, 4'-hydroxychalcone, 2', 4-dihydroxychalcone, 2', 4'-dihydroxychalcone and 2', 4, 4'-trihydroxychalcone (Tokyo Chemical Industry, Tokyo, Japan), RPMI-1640 culture medium and trypan blue solution (Gibco Laboratories, MD, USA), RA (Sigma, St Louis, MO, USA), fetal bovine serum (FBS) (JRH Biosciences, KS, USA) and plasmocin (InvivoGene, CA, USA) were obtained.

Cell culture and treatment with chalcone derivatives

Human monoblastic leukemia U937 cells (RCB0435) were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan. Cells were grown in RPMI-1640 culture medium containing 10% FBS and 5 µg/mL plasmocin as described (Kikuchi and Imajoh-Ohmi, 1995; Kikuchi *et al.*, 1996; Kikuchi *et al.*, 2011; Kikuchi *et al.*, 2018). Cells (1.0×10^6) in 5 mL of culture medium were incubated in the presence of 20 µM chalcone derivatives for upto 48 hr. Viable cells were counted by the trypan blue dye exclusion method (Kikuchi *et al.*, 2016).

Cultivation of RA-treated U937 cells with cytotoxic hydroxychalcone derivatives

Cells (1.0×10^6) in 5 mL of culture medium were incubated in the absence or presence of 1 µM RA for 48 hr. Cells (1.0×10^6) were resuspended in 5 mL of fresh culture medium, incubated in the presence of 20 µM 2-hydroxychalcone, 2'-hydroxychalcone or 2', 4, 4'-trihydroxychalcone for upto 24 hr. Viable cells were counted by the trypan blue dye exclusion method (Kikuchi *et al.*, 2016).

Statistical analysis

Data obtained with cell viability assays are presented as averages of four separate experiments. Error bars indicate standard deviation. Statistical differences were calculated with Student's *t* test.

RESULTS AND DISCUSSION

First, in order to study the influence of positions of hydroxyl groups in hydroxychalcone derivatives on cytotoxicity, we examined effects of commercially available monohydroxychalcones on viability of U937 cells. Viability of U937 cells was not changed during cultivation in the absence of chalcone derivatives upto 48 hr (data not shown). After addition of monohydroxychalcone derivatives, viability of U937 cells was measured upto 48 hr. Interestingly, 2-hydroxychalcone remarkably reduced viability of U937 cells (to ~14% at 12 hr, to ~7% at 24 hr and to ~0% at 48 hr) as compared to chalcone (to ~85% at 12 hr, to ~71% at 24 hr and to ~61% at 48 hr) (Fig. 1A). 2'-hydroxychalcone (viability: to ~74% at 12 hr, to ~55% at 24 hr and to ~41% at 48 hr) and 4-hydroxychalcone (viability: to ~75% at 12 hr, to ~70% at 24 hr and to ~49% at 48 hr) also showed stronger cytotoxicity against U937 cells than chalcone (Fig. 1A). In contrast, 4'-hydroxychalcone indicated weaker cytotoxicity (viability: to ~94% at 12 hr, to ~89% at 24 hr and to ~69% at 48 hr) than chalcone (Fig. 1A). These results suggested that positions of hydroxyl groups in monohydroxychalcones may contribute to their cytotoxic effect against U937 cells.

Second, to investigate the influence of numbers of hydroxyl groups in hydroxychalcone derivatives on cytotoxicity, we examined effects of commercially available di or trihydroxychalcone derivatives on viability of U937 cells. Viability of U937 cells was not changed during cultivation in the absence of chalcone derivatives upto 48 hr (data not shown). After addition of several di or trihydroxychalcone derivatives, viability of U937 cells was measured upto 48 hr. Unexpectedly, cytotoxicity of dihydroxychalcones tested did not show stronger than that of 2'-hydroxychalcone. In particular, cytotoxicity of 2', 4-dihydroxychalcone was weaker than that of 2'-hydroxychalcone (Fig. 1B). On the other hand, 2', 4, 4'-trihydroxychalcone caused remarkable decrease in viability of U937 cells (to ~52% at 12 hr, to ~25% at 24 hr and to ~7% at 48 hr) as compared to 2'-hydroxychalcone (to ~73% at 12 hr, to ~47% at 24 hr and to ~41% at 48 hr) (Fig. 1B). Thus, 2', 4, 4'-trihydroxychalcone showed stronger cytotoxicity than 2'-hydroxychalcone, 2', 4-dihydroxychalcone and 2', 4'-dihydroxychalcone. These results suggested that not only positions but also

Effects of hydroxyl positions and numbers on cytotoxicity of chalcones

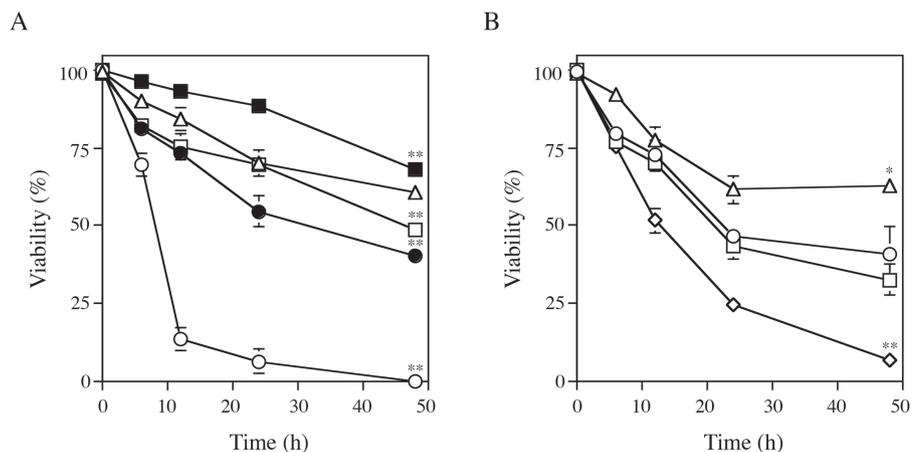


Fig. 1. Cytotoxicity of hydroxychalcone derivatives. (A) Calcone and monohydroxychalcones. Cells (1.0×10^6) in 5 mL of culture medium were incubated with $20 \mu\text{M}$ chalcone (open triangles), 2-hydroxychalcone (open circles), 2'-hydroxychalcone (closed circles), 4-hydroxychalcone (open squares) or 4'-hydroxychalcone (closed squares) for upto 48 hr. Viable cells were counted by the trypan blue dye exclusion method. Data represent the average of three separate experiments. Statistical differences were calculated using Student's *t* test. **, $p < 0.01$ compared with the data for chalcone. (B) Di and trihydroxychalcones. Cells (1.0×10^6) in 5 mL of culture medium were incubated with $20 \mu\text{M}$ 2'-hydroxychalcone (open circles), 2', 4'-dihydroxychalcone (open triangles), 2', 4'-dihydroxychalcone (open squares) or 2', 4', 4'-trihydroxychalcone (open rhombuses) for upto 48 hr. Viable cells were counted by the trypan blue dye exclusion method. Data represent the average of three separate experiments. Statistical differences were calculated using Student's *t* test. *, $p < 0.05$; **, $p < 0.01$ compared with the data for 2'-hydroxychalcone.

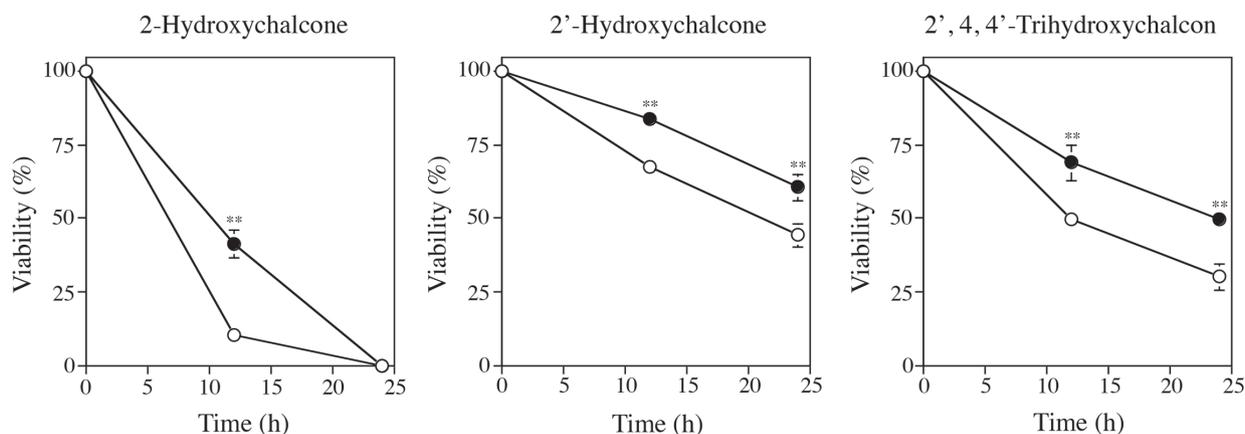


Fig. 2. Influences of RA-induced monocytic differentiation on sensitivity against cytotoxic hydroxychalcones. Cells (1.0×10^6) in 5 mL of culture medium were incubated in the absence or presence of $1 \mu\text{M}$ RA for 48 hr. Undifferentiated (open circles) and differentiated (closed circles) cells (1.0×10^6) were resuspended in 5 ml of fresh culture medium, incubated with $20 \mu\text{M}$ 2-hydroxychalcone, 2'-hydroxychalcone or 2', 4', 4'-trihydroxychalcone for upto 24 hr. Viable cells were counted by the trypan blue dye exclusion method. Error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test. **, $p < 0.01$ compared with the data for undifferentiated cells.

numbers of hydroxyl groups in hydroxychalcones may be involved to a certain degree, in the cytotoxicity against U937 cells.

Upon stimulation by RA, U937 cells can be differentiated to macrophage-like cells (Kikuchi and Imajoh-

Ohmi, 1995; Kikuchi *et al.*, 1996; Kikuchi *et al.*, 2011; Kikuchi *et al.*, 2018). To study influences of the RA-induced monocytic differentiation on sensitivity of U937 cells against several cytotoxic hydroxychalcones, we measured viability of RA-untreated or -treated U937 cells

cultured in the presence of cytotoxic hydroxychalcones (2-hydroxychalcone, 2'-hydroxychalcone or 2', 4, 4'-tri-hydroxychalcone). Viability of undifferentiated or differentiated U937 cells was not affected during culture in the absence of chalcone derivatives for upto 24 hr (data not shown). As expected, RA-treated U937 cells showed distinct resistance against the three cytotoxic hydroxychalcones as compared to RA-untreated U937 cells (Fig. 2). These results suggested that cell differentiation may bring about resistance against cytotoxicity of hydroxychalcone derivatives.

Our findings in this study reveal that positions and numbers of hydroxyl groups in hydroxychalcone derivatives are involved in cytotoxicity, and that RA-induced differentiation brought about an enhanced resistance against the cytotoxic hydroxychalcone derivatives. Our results propose that hydroxychalcone derivatives in combination with RA have the potential to serve as effective modifiers in therapy for leukemia. However, further studies are warranted before practical application as therapy for leukemia. Studies on cytotoxicity of hydroxychalcone derivatives will become more important for development of leukemia treatment.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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