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Potentiative effects of standardized extract of *Centella asiatica* on vinblastine-induced cell death

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Abstract

The purpose of this study was to investigate the influence of a standardized extract from *Centella asiatica* (ECa-233) and asiaticoside (ATS) on vinblastine (VBL)-induced cytotoxicity in porcine renal epithelial cell line (LLC-PK_1) and its *MDR_1*-gene-transfected epithelial cell line (LLC-MDR_1). The cells were co-treated with VBL (at various concentrations) and either ECa-233 or ATS for 72 hr prior to determination of cell viability with the MTT assay. Our results showed that the noncytotoxic ECa-233 and ATS could enhance the cytotoxicity of VBL in both cell types. It was likely that both ECa-233 and ATS were able to inhibit the function of P-glycoprotein, a known membrane efflux pump of VBL and might be useful in cancer chemotherapy.

Keywords: Extract of *Centella asiatica*, asiaticoside, vinblastine, LLC-PK_1, LLC-MDR_1.

Introduction

Centella asiatica is a pan-tropical plant in Thailand. This herbal plant has been used to relieve symptoms in various conditions including mental disorders, inflammation, circulatory problems and immune system deficiencies. In addition, this plant contained the pharmacological activities in wound healing antitumor and cognition enhancement in experimental rats (1). As known, the major components in *Centella asiatica* included triterpene saponins, in particular, asiaticoside and madecassoside, and their aglycone (asiatic acid and madecassic acid, respectively) (2). Asiaticoside at sub-cytotoxic concentration could induce apoptosis, and enhance the cytotoxic effect of vincristine in cancer cells (3).

This study was to examine the potentiative effects of our standardized extract of *Centella asiatica* (ECa-233), in comparison with asiaticoside, on vinblastine-induced cytotoxicity in porcine renal epithelial cell line (LLC-PK_1) and its *MDR_1*-gene-transfected epithelial cell line (LLC-MDR_1).

Methods

Cell culture: LLC-PK_1 cells [ATTC no. CL-101] and LLC-MDR_1 cells [kindly provided by Dr. A. H. Schinkel (Netherlands Cancer Institute, Amsterdam, the Netherlands)] were cultured in M199 supplemented with 10% FBS and 100 unit/ml penicillin-streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C.

Test materials: Standardized extract of *Centella asiatica* (ECa-233) and asiaticoside (ATS) were kindly provided by Assoc. Prof. Dr. Ekarin Saifah and Assist. Prof. Dr. Chamnan Patarapanish, Faculty of the Pharmaceutical Sciences, Chulalongkorn University, respectively. ECa-233 contained triterpenoid glycosides at least 80% with the ratio between madecassoside and asiaticoside of 1.50 ± 0.50.

Cytotoxicity study: Cells were cultured in 96-well plates at a seeding density of $2x10^3$ cells/ well for 24 h prior to experiment. VBL-induced cytotoxicity in presence or absence of our test materials was determined after 72 hr-incubation by the MTT assay (4). Briefly, medium containing MTT (0.4 mg/ml) was added to each well and incubated for 4 hours. The formazan crystal were dissolved in DMSO and the absorbance was read at an excitation wavelength of 570 nm (reference wavelength 620 nm) using a microplate reader (Anthos Labtec HT2 version 1.21E, Australia). Percent cell viability was calculated based on the absorbance measured relative to the absorbance of cells exposed to the control vehicle.

Statistical analysis: All values were presented as mean ± SEM. One-way ANOVA followed by the Dunnett's test were performed for statistical comparisons. *P* value ≤ 0.05 was considered significant.

Results

Our preliminary results indicated that ECa-233 (up to 1000 µg/ml) and ATS (up to 100 µg/ml) had no effect on viability of LLC-PK_1 and LLC-MDR_1 cells, as determined by the MTT assay. In this study, both ECa-233 and ATS were able to significantly shift the concentration response curve of VBL-induced cytotoxicity leftward in both LLC-PK_1 and LLC-MDR_1 cells (Fig.1). The effects of our test materials were comparable with those of verapamil, a known P-gp inhibitor, which was used as positive control in this study. As expected, the degree of VBL resistance in LLC-MDR_1 was 5.1-fold higher than the resistance in LLC-PK_1, suggesting the higher level of *MDR_1* expression in LLC-MDR_1 cell type (Table1). In addition, our test materials and verapamil elicited the greater influence on VBL-induced cytotoxicity in LLC-MDR_1 than in LLC-PK_1.

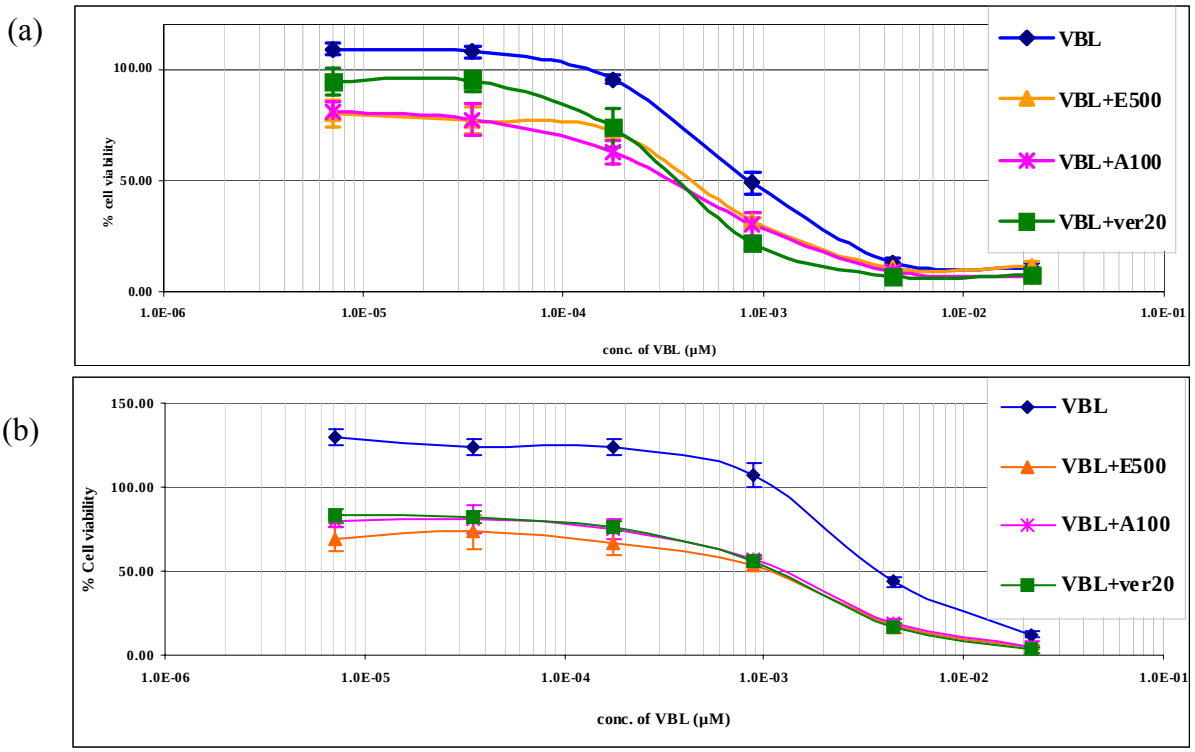

Figure 1 Concentration response curve of VBL-induced cytotoxicity in LLC-PK_1 cells (a) and LLC-MDR_1 cells (b), as determined by the MTT assay. The cells were cultured with a full range of concentrations of VBL in the presence or absence verapamil 20 µM or ECa-233 500 µg/ml or ATS 100 µg/ml for 72 hours. The results were presented as the mean ± S.E of at least triplicate determination.

Table 1 The apparent IC_{50} values of VBL in LLC-PK_1 and LLC- MDR_1 cells. The values were determined in the co-treatment with ECa-233 or ATS or verapamil. Each value represented the mean and S.E of at least three independent experiments.

Groups	LLC-PK_1		LLC-MDR_1	
	IC_{50} of VBL (mean ± S.E, nM)	Fold reversal of MDR[a]	IC_{50} of VBL (mean ± S.E, nM)	Fold reversal of MDR[a]
Vinblastine (VBL)	1.01 ± 0.09		5.19 ± 0.63	
VBL + ECa-233 500 µg/ml	0.48 ± 0.10*	2.45	0.82 ± 0.20*	5.97
VBL + ATS 100 µg/ml	0.44 ± 0.06*	2.10	1.04 ± 0.02*	4.85
VBL + verapamil 20 µM	0.41 ± 0.11*	2.28	0.96 ± 0.15*	5.11

*$P < 0.05$ vs vinblastine group

[a] The fold reversal of MDR was defined as the ratio of the IC_{50} value for VBL to that for VBL with the modulating agent.

Discussion & Conclusion

This study demonstrated that ECa-233 and asiaticoside (ATS) could potentiate the VBL-induced cytotoxicity in LLC-PK_1 and LLC-MDR_1 cells. Cellular accumulation of VBL resulted in cytotoxicity and cell death. Because VBL was a subsurate of P-gp efflux pump, cells with the MDR phenotype over-expression became resistance to VBL treatment . Generally, the presence of P-gp inhibitors such as verapamil could enhance the effectiveness of VBL through increase VBL accumulation in the cells (5). The enhancing effect of P-gp inhibitors on VBL-cytotoxicity was stronger in LLC-MDR_1 cells than in LLC-PK_1 cells. Hence, it was very likely that both ECa-233 and ATS increased VBL toxicity in LLC-PK_1 and LLC-MDR_1 cells through inhibition of P-gp function. These findings suggested that ECa-233 and ATS could be modulators of P-gp functions and might be useful in cancer chemotherapy.

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