SHORT COMMUNICATION

Non-alkaloids extract from *Stemona sessilifolia* enhances the activity of chemotherapeutic agents through P-glycoprotein-mediated multidrug-resistant cancer cells

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One of the major impediments to the successful treatment of cancer is the development of resistant cancer cells, which could cause multidrug resistance (MDR), and overexpression of ABCB1/P-glycoprotein (P-gp) is one of the most common causes of MDR in cancer cells. Recently, natural products or plant-derived chemicals have been investigated more and more widely as potential multidrug-resistant (MDR) reversing agents. The current study demonstrated for the first time that non-alkaloids extract from *S. sessilifolia* significantly reversed the resistance of chemotherapeutic agents, adriamycin, paclitaxel and vincristine to MCF-7/ADR cells compared with MCF-7/S cells in a dose-dependent manner. The results obtained from these studies indicated that the non-alkaloids extract from *S. sessilifolia* plays an important role in reversing MDR of cancer as a P-gp modulator *in vitro* and may be effective in the treatment of multidrug-resistant cancers.

**Keywords:** *Stemona sessilifolia*; non-alkaloids extract; multidrug resistance; P-gp; MCF-7/ADR cells; cancer

1. Introduction

One of the primary obstacles to the successful treatment of cancer is the development of multidrug-resistant (MDR) cancer cells variants. Overexpression of ABCB1/P-glycoprotein (P-gp) is one of the most common causes of MDR in cancer cells. Regulation of P-gp may resensitise MDR cancer cells to an effective MDR cancer treatment with usually used chemotherapeutic agents (Zhu et al. 2012).

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The Stemona species, belonging to Family Stemonaceae, has been used extensively as a traditional medicine in China and other countries of Southeast Asia for various medicinal and biological properties (Chanmahasathien et al. 2011), especially *Stemona sessilifolia*. The roots of *S. sessilifolia* have been used to treat respiratory diseases, antifungal, insecticides and anticancer (Pilli & Ferreira de Oliveira 2002). Many active compounds have been found from Stemona species. Plants in this family contain an interesting group of alkaloids, the so-called Stemona alkaloids. In addition, it was reported that the root extract from *S. curtisii* and the Stemona alkaloids plays an important role in inhibition of P-gp (Limtrakul et al. 2007; Chanmahasathien et al. 2011). However, the activity of root extract was better than Stemona alkaloids, which indicates that non-alkaloid components may also play an important role as a P-gp modulator. Besides Stemona alkaloids, the other constituent from *S. sessilifolia* is called stilbenoids (Pacher et al. 2002), a group of resveratrol analogues, which may be the major constituent of non-alkaloid components from *S. sessilifolia*. It was reported that resveratrol affects P-glycoprotein overexpression of MDR cells (Fang et al. 2008). From the present findings, we proposed that non-alkaloid components may play an important role as a P-gp modulator (Limtrakul et al. 2007; Chanmahasathien et al. 2011).

This study was an attempt to screen the non-alkaloids extract (NAE) from *S. sessilifolia* for its reversing effect on P-gp-mediated multidrug resistance. In this report, the effect of NAE from *S. sessilifolia* on increasing drug sensitivity and P-gp expression was determined by MTT, Rhodamine123 accumulation assay and western blot assay.

2. Results and discussion

Chemotherapy serves as one of the important treatments for cancer. Resistance to chemotherapy drugs is a major problem in the management of cancer patients and is caused by various molecular mechanisms. One of these mechanisms is the overexpression of MDR1/P-gp, which is the major cause of MDR of human cancers. The process of chemosensitisation involves the co-administration of an MDR modulator with anticancer drugs in order to cause enhanced intracellular anticancer drugs accumulation via impairing the P-gp function. Many P-gp inhibitors, such as verapamil, a calcium channel blocker, have limited clinical use. Many current studies are focused on dietary herbs due to the fact that these have been used for centuries with producing few harmful side effects (Limtrakul et al. 2007; Pitchakarn et al. 2010). Therefore, MDR reversing agents from natural products or plant-derived chemicals which have lower toxicological effects are being investigated widely over the last few years. The present study was an attempt to screen the NAE from *S. sessilifolia* for its increasing effect on P-gp-mediated multidrug resistance. The extract was standardised by the determination of little alkaloids components content using Dragendorff reagent (Figure S1).

To examine whether NAE affects the viability of cells, MCF-7/S and MCF-7/ADR cell lines were exposed to various concentrations of NAE for 24 h and cytotoxicity was estimated by tetrazolium-based colorimetric MTT assay as previously described. The maximum un-cytotoxic concentration (IC$_{50}$) of NAE in MCF-7/S and MCF-7/ADR cells were higher than 200 µg/mL (MCF-7/ADR: 254 ± 2.58 µg/mL and MCF-7/S: 189 ± 2.49 µg/mL). This result suggested that NAE from *S. sessilifolia* had less toxicity on MCF-7/S and MCF-7/ADR cells, so we adopted 100, 150 and 200 µg/mL of NAE as low, moderate and high concentration in the following experiments.

To investigate whether the resistance caused by different anticancer drugs was reversed with the treatment of NAE, we detected the sensitivity of these anticancer drugs on MCF-7/ADR cells. MCF-7/S and MCF-7/ADR cells were exposed to different concentrations of widely used anticancer drugs respectively. As shown in Table S1, MCF-7/ADR cells, compared to MCF-7/S cells, exhibited a significant resistance to adriamycin (55.71-fold), paclitaxel (43.09-fold) and
vincristine (40.52-fold), indicating that MCF-7/ADR cells exhibited a MDR phenotype, especially towards adriamycin. In Table S2, the NAE significantly enhanced the sensitivity of adriamycin to MCF-7/ADR cells in a dose-dependent manner, but to MCF-7/S cells, the IC\textsubscript{50} values with or without NAE showed no significant difference. The effect of NAE at 150 μg/mL or 200 μg/mL was moderately stronger than the P-gp inhibitor verapamil at 10 μM. These findings indicated that non-alkaloids extract increased the sensitivity of anticancer drugs to MCF-7/ADR cells, whereas it had no effect on MCF-7/S cells, supporting the notion that non-alkaloids extract (NAE) from \textit{S. sessilifolia} may reverse the drug resistance of MCF-7/ADR cells.

The overexpression of membrane pump protein P-gp in cancer cells is the major mechanism associated with MDR. The fluorescent substrates of P-gp were usually chosen to determine whether the inhibitory effects were substrate specific or not. Accumulation of fluorescent substrates Rh123 was determined by flow cytometry. When P-gp is overexpressed, the accumulation of Rh123 is decreased, same if vice versa. As shown in Supplementary Figure S2, treated with 100 and 200 μg/mL of NAE, respectively, the accumulation of Rh123 was significantly increased (\(p < 0.01\)) compared with the untreated control, and reflected a dose-dependent manner, which indicated that the function of P-gp in MCF-7/ADR cells was reduced with the treatment of NAE.

Reversal of P-gp-mediated MDR can be achieved by either decreasing P-gp expression or by inhibiting P-gp function. MCF-7/ADR cells were treated with NAE at 100 and 200 μg/mL for 12 and 24 h, and the results showed (Figure S3) that NAE decreased the expression of P-gp and it obviously altered P-gp expression levels in MCF-7/ADR cells at 200 μg/mL concentration for 24 h.

In conclusion, this is for the first time that the effect of non-alkaloids extract from \textit{S. sessilifolia} in affecting the activity of the multidrug transporter P-gp/ABCB1 is being discovered. Additional purification studies are imperative to identify the active compound(s) in the extracts. Once the individual components of NAE are identified, our future studies will focus on determining the mechanism of P-gp regulations. The results reported here also open the possibility for investigations of the effect of non-alkaloids extract in animal experiments to determine if this component has potential as an effective chemosensitiser to be used in combination with conventional chemotherapeutic agents \textit{in vivo}.

3. Conclusion

In summary, non-alkaloids extract from \textit{S. sessilifolia} significantly reversed the resistance of chemotherapeutic agents to MCF-7/ADR cells, reduced P-gp function and decreased the level of P-gp expression, but the mechanism of P-gp regulations should be explored in deep.

Supplementary material

Experimental details relating to this paper are available online, alongside Tables S1–S2 and Figures S1–S3.

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Disclosure statement

No potential conflict of interest was reported by the authors.
Note
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