Natural Product Sciences
10(2) : 85-88 (2004)

Evaluation on the P-Glycoprotein Inhibitory Activity of Indonesian Medicinal Plants

Eun Jung Go, Hyang Rim Kim, Soo Yeon Chung, Yeon-Hee Jeong, Na Hyung Kim, Ah-Reum Han, Eun-Kyoung Seo* and Hwa Jeong Lee*

College of Pharmacy, Ewha Womans University, 11-1 Daehyun-dong, Seodaemun-ku, Seoul 120-750, Korea

Abstract – One hundred Indonesian plant extracts were screened to investigate their effects on the P-glycoprotein (P-gp) activity in human uterine sarcoma cells, MES-SA/DX5, for the first time. Among others, four samples, *Alpinia galanga* (BuOH ext.), *Sindora sumatrana* ($CHCl_3$ ext.), *Strychnos ligustrina* ($CHCl_3$ ext.), and *Zingiber cassumunar* Roxb (hexane ext.), exhibited the most potent inhibition on the P-gp activity. They increased cytotoxic activity of daunomycin up to IC_{50} values of less than 1.41 μM, which is a value with a positive control, verapamil. Other 25 samples showed significant P-gp inhibitory activity with IC_{50} values between 1.4 and 4.0 μM. These prospective samples will be subjected to further laboratory phytochemical investigation to find active principles.
Keywords – multidrug resistance, P-glycoprotein, Indonesian plants

Introduction

Recently, multidrug resistance problem has been focused as one of the most serious problems in treating cancers. One of the major mechanisms of multidrug resistance found in tumor cells is the over-expression of P-glycoprotein (P-gp). P-gp is the 170 kDa glycoprotein capable of the ATP-dependent cellular efflux of a wide range of structurally and functionally diverse compounds across the plasma membrane (Endicott *et al.*, 1989; Fardel *et al.*, 1996; Gottesman *et al.*, 1993). Many studies have demonstrated that compounds found in vegetables, fruits, and plant-derived beverages, have not only anticancer activities but may also modulate P-gp activity (Scambia *et al.*, 1994; Chieli *et al*, 1995; Christensen *et al*, 1996; Phang *et al.*, 1993; Critchfield *et al*, 1994; Go *et al*, 2003; Plouzek *et al.*, 1999).

In the present study, one hundred plant extracts that did not exhibit potent cytotoxicity, were tested to evaluate their effects on P-gp activity in a human uterine sarcoma cells, MES-SA/DX5 for the first time.

Experimental

Plant materials and extractions – The Indonesian plants as test samples were collected in Surabaya, Indonesia, in 2001, and were identified by professor Tri Windono (University of Surabaya, Indonesia). The voucher specimens have been deposited at University of Surabaya. 500 g of each dried plant was ground and extracted with methanol by percolation. The filtered methanol extracts were evaporated under vacuum. The aqueous methanol extract was partitioned with *n*-hexane, chloroform, and *n*-butanol, subsequently.

Chemicals – Trichloroacetic acid (TCA), daunomycin (DNM), Hank's balanced salts without sodium bicarbonate (HBSS), verapamil, dimethyl sulphoxide (DMSO) and sulforhodamine B (SRB) were purchased from Sigma-Aldrich (St. Louis, MO). Dulbecco's modified eagle medium/low glucose (DMEM), Trypsin-EDTA (0.25% trypsin-1 mM EDTA) and Penicillin-Streptomycin were from Invitrogen (Calsbad, CA). Fetal bovine serum (FBS) was obtained from Hyclone (South Logan, UT).

Evaluation of inhibitory effects against the P-gp activity – Approximately 5000 MES-SA/DX5 cells/well were seeded in 96 well tissue culture plates and allowed to attach for 24 hours at 37°C. Then, additional medium was added to each well containing the desired final concentration of daunomycin (1.8×10^{-9} M 1.8×10^{-5} M) with or without plant extracts (50 μg/ml). Verapamil (50 μg/ml), a P-gp inhibitor, was used in the study as a positive control. After a two hour exposure to the drug±plant extracts, the cells were washed twice with HBSS and fresh medium was added to each well. The cells were allowed to grow for 72 hours (3 days) following which the total protein was measured using a SRB staining assay (Skehan *et al*, 1990). Briefly, cells were fixed with 10% TCA for an hour, then

*Author for correspondence
Fax: +82-3277-3051, E-mail: Yuny@ewha.ac.kr; Hwalee@ewha.ac.kr

Table 1. IC_{50} values of daunomycin in MES-SA/DX5 cells after 2 hour incubation with plant extracts

Plant name and Authority	Family	Sample code[a]	Part used	IC_{50} (μM)
Acalypha indica L.	Euphorbiaceae	EA215H	Aerial parts	7.90
		EA215C		18.52
		EA215B		8.74
		EA215Aq		6.82
Ageratum conyzoides L.	Asteraceae	EA223H	Whole plants	4.28
		EA223C		–
		EA223B		8.88
		EA223Aq		7.50
Alpinia galanga (L.) Swartz.	Zingiberaceae	EA205H	Rhizome	–
		EA205C		–
		EA205B		0.64
		EA205Aq		20.14
Alstonia scholaris (L.) R. Br.	Apocynaceae	EA210H	Cortex	5.16
		EA210C		3.73
		EA210B		12.20
		EA210Aq		12.22
Amorphophallus campanulatus (Roxb) Bl.Ex Decne	Araceae	EA218H	Tubera	1.88
		EA218C		8.93
		EA218B		10.70
		EA218Aq		5.52
Artocarpus communis Forst.	Moraceae	EA201H	Heart wood	3.59
		EA201C	(Lignum)	–
		EA201B		5.90
		EA201Aq		9.73
Azadirachta indica A. Juss.	Meliaceae	EA200H	Leaves	3.62
		EA200C		–
		EA200B		9.54
		EA200Aq		6.26
Calotropis gigantea (Wild.)Dryand. Ex W.T.Ait.	Asclepiadaceae	EA219H	Underground	8.57
		EA219C	parts(Root)	3.68
		EA219B		>30
		EA219Aq		13.69
Cassia siamea Lamk.	Caesalpiniaceae	EA206H	Leaves	3.88
		EA206C		–
		EA206B		–
		EA206Aq		14.57
Colocasia esculenta (L.) Schott.	Araceae	EA199H	Corm	3.91
		EA199C		4.89
		EA199B		10.80
		EA199Aq		12.73
Curcuma aerusinosa Roxb	Zingiberaceae	EA195H	Rhizome	4.45
		EA195C		1.74
		EA195B		3.43
		EA195Aq		19.43
Curcuma heyneana Val. & v.Zijp	Zingiberaceae	EA196H	Rhizome	3.24
		EA196C		1.51
		EA196B		4.28
		EA196Aq		12.79
Dioscorea hispida Dennst.	Dioscoreaceae	EA220H	Tubera	4.84
		EA220C		4.13
		EA220B		9.13
		EA220Aq		5.71
Eclipta alba (L.) Hassk.	Asteraceae	EA214H	Aerial parts	4.75
		EA214C		11.02
		EA214B		13.91
		EA214Aq		9.57

Table 1. Continued

Plant name and Authority	Family	Sample code[a]	Part used	IC_{50} (μM)
Elephantopus scaber L.	Asteraceae	EA202H	Aerial part	–
		EA202C		–
		EA202B		11.23
		EA202Aq		5.79
Euphorbia prostata W. Ait.	Euphorbiaceae	EA213H	Whole plants	3.17
		EA213C		9.26
		EA213B		19.97
		EA213Aq		6.26
Excoecaria cochinchinensis Lour.	Euphorbiaceae	EA216H	Leaves	1.86
		EA216C		–
		EA216B		22.24
		EA216Aq		4.45
Justicia gendarussa Burm. F.	Acanthaceae	EA207H	Leaves	7.00
		EA207C		6.30
		EA207B		7.97
		EA207Aq		12.56
Kaempferia rotunda L.	Zingiberaceae	EA209H	Rhizome	2.63
		EA209C		2.54
		EA209B		21.37
		EA209Aq		12.30
Merremia mammosa (Lour.) Hallier F.	Convolvulaceae	EA211H	Tubera	1.66
		EA211C		–
		EA211B		13.95
		EA211Aq		14.57
Parameria laevigata (Juss.) Moldenke	Apocynaceae	EA224H	Cortex	3.59
		EA224C		5.50
		EA224B		6.51
		EA224Aq		5.15
Ruellia tuberosa L.	Acanthaceae	EA222H	Aerial parts	3.21
		EA222C		3.27
		EA222B		10.62
		EA222Aq		5.47
Sindora sumatrana Miq.	Caesalpiniaceae	EA221H	Fructus	1.83
		EA221C		0.63
		EA221B		6.10
		EA221Aq		8.76
Strychnos ligustrina Bl.	Loganiaceae	EA208H	Lignum	2.82
		EA208C		1.34
		EA208B		9.17
		EA208Aq		12.77
Tinospora tuberculata Beumee	Menispermaceae	EA203H	Caulis	6.15
		EA203C		3.58
		EA203B		7.41
		EA203Aq		17.94
Vernonia cinerea (L.) Less.	Asteraceae	EA212H	Aerial parts	2.15
		EA212C		8.54
		EA212B		11.99
		EA212Aq		11.96
Zingiber cassumunar	Zingiberaceae	EA204H	Rhizome	0.93
		EA204C		3.03
		EA204B		8.63
		EA204Aq		17.57
Zingiber zerumbet(L.) J.E.Smith.	Zingiberaceae	EA198H	Rhizome	4.43
		EA198C		1.53
		EA198B		3.01
		EA198Aq		9.43

[a]Sample code : H (hexane), C (chloroform), B (butanol), Aq (aqueous).

Daunomycin 10.87±4.36 (n = 15)

Daunomycin + Verapamil (P-gp inhibitor) 1.41±0.48 (n = 15)

* – : Marked cytotoxicity was found at 50 μg/ml of each plant extract.

washed with water 5 times and air-dried. SRB (0.4% w/v in 1% acetic acid) was added to each well for 30 minutes, followed by 4 washes with 1% acetic acid. After drying the plates, protein bound dye was solubilized in 10 mM Tris base (pH 10.0) and quantitated by measuring the absorbance at 515 nm using an ELISA plate reader. IC_{50} values were calculated using non-linear regression analyses (percent survival vs. DNM concentration).

Results and Discussion

One hundred Indonesian plant extracts were screened to evaluate their effects on P-gp activity in a human uterine sarcoma cells, MES-SA/DX5. MES-SA/DX5 cells are doxorubicin-resistant subline of the human sarcoma cell line, MES-SA and showed marked multidrug resistance (Harker and Sikic, 1985), which was due to, at least in part, over-expression of P-gp. Daunomycin accumulation in the MES-SA/DX5 cells was remarkably reduced by approximately 10% compared to the sensitive MES-SA cells, confirming over-expression of P-gp (Go *et al.*, 2003). As judged in the criteria of P-gp inhibitory activity with IC_{50}<4 μM (cytotoxicity of daunomycin), twenty nine extracts were evaluated as active samples. Among others, four samples, *Alpinia galanga* (BuOH ext.), *Sindora sumatrana* ($CHCl_3$ ext.), *Strychnos ligustrina* ($CHCl_3$ ext.), and *Zingiber cassumunar* (hexane ext.), exhibited their inhibitory effects on the P-gp activity with daunomycin IC_{50} values of 0.64, 0.63, 1.34, and 0.93 μM, respectively. When daunomycin was treated with the positive control, verapamil, it showed cytotoxicity with IC_{50} value of 1.4±0.48 μM, therefore, the four extracts were evaluated to be more potent than the positive control. Moreover, these samples were plant extracts, which were mixtures of various compounds, thus, there will be high possibility to isolate a lead compound from these extracts in our future phytochemical study.

Twenty five samples displayed significant P-gp inhibitory activity with the IC_{50} values between 1.4 and 4.0 μM. Although they showed less activity than the positive control, they could be evaluated as quite strong samples because the positive control was a single compound whereas the tested samples were plant extracts, which were mixtures of natural compounds. They were including extracts of *Alstonia scholaris*, *Amorphophallus campanulatus*, *Artocarpus communis*, *Curcuma* species, and *Zingiber* species etc. as shown in the Table 1.

On the basis of these results, further phytochemical study will be performed to isolate a potent P-gp modulator from the active plant extracts.

Acknowledgements

This study was supported by the Korea Science and Engineering Foundation for the National R&D Program for Enhancing R&D Infrastructure of Womens University [R06-2002-011-01002-0(2002)]. We are grateful to Prof. Tri Windono and Dr. Gwang-Ho Jeohn at Surabaya University, Surabaya, Indonesia for plant collection.

References

Chieli, E., Romiti, N., Cervelli, F. and Tongiani, R. Effects of flavonols on p-glycoprotein activity in cultured rat hepatocytes. *Life Sci.*, **57**, 1741-1751 (1995).

Christensen, J. G. and LeBlanc, G. A. Reversal of multidrug resistance *in vivo* by dietary administration of the phytochemical indole-3-carbinol. *Cancer Res.* **56**, 574-581 (1996).

Endicott, J. A. and Ling, V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu. Rev. Biochem.*, **58**, 137-171 (1989).

Fardel, O., Lecurer, V. and Guillouzo, A. The P-glycoprotein multidrig transporter. *Gen. Pharmac.*, **27**, 1283-1291 (1996).

Go, E. J., Chung, S. Y., Kim, N. H. and Lee, H. J. Modulation of P-glycoprotein activity by flavonoids in human uterine sarcoma cells. *J. Kor. Pharm. Sci.*, **33**, 305-310 (2003).

Gottesman, M. M. and Pastan, I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.*, **62**, 385-427 (1993).

Harker, G. and Sikic, B. I. Multidrug (pleiotropic) resistance in doxorubicin-selected variants of the human sarcoma cell line MES-SA. *Cancer Res.*, **45**, 4091-4096 (1985).

Phang, J. M., Poore, C. M., Lopaczynska J. and Yeh, G. C. Flavonol-stimulated efflux of 7,12-dimethylbenz(α)anthracene in multidrug-resistant breast cancer cells. *Cancer Res.*, **53**, 5977-5981 (1993).

Plouzek, C. A., Ciolino, H. P., Clarke, R. and Yeh, G. C. Inhibirion of P-glycoprotein activiry and reversal of multidrug resistance *in vitro* by rosemary extract. *Eur. J. Cancer*, **35**, 1541-1545 (1999).

Scambia, G., Ranelleti, F. O., Panici, P. B., DiVincenzo, R. Bonanno, G., Ferrandina, G., Piantelli, M., Bussa, S., Rumi, C., Cianfriglia, M. and Mancuso, S. Quercetin potentiates the effect of adriamycin in a multidrug-resistant MCF-7 human breast cancer cell line: P-glycoprotein as a possible target. *Cancer Chemother. Pharmacol.*, **34**, 459-464 (1994).

Skehan, P. Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren J. T., Bokesch, H., Kenney, S. and Boyd, M. R. New colorimetric assay for anticancer-drug screening. *J. Natl. Cancer Inst.*, **82**, 1107-1112 (1990).

(Accepted April 15, 2004)