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MODULATION OF MULTIDRUG RESISTANCE BY TAXUSPINE C AND OTHER TAXOIDS FROM JAPANESE YEW

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Abstract: Taxuspine C (**1**), a new taxoid from the Japanese yew *Taxus cuspidata*, increasing the cellular accumulation of vincristine (VCR) in multidrug-resistant tumor cells as potent as verapamil enhanced the chemotherapeutic effect of VCR in P388/VCR-bearing mice. When taxuspine C (**1**) was given i.p. daily at 200 mg/kg with 0.2 mg/kg VCR for 5 days, a treated/control (T/C) value of 138% was obtained. The other new taxoids, taxezopidines G (**8**) and H (**9**), from the yew also increased the VCR accumulation as potent as verapamil. These results suggest that some taxoids may be useful for overcoming multidrug resistance in tumor cells.

When tumor cells acquire resistance to naturally occurring antitumor agents such as *Vinca* alkaloids or anthracyclines, they generally show cross-resistance to other antitumor agents having different structures and different modes of action.[1] A major mechanism for this multidrug resistance (MDR) is attributed to the reduced accumulation of antitumor agents in resistant cells.[2] It has been shown that a particular class of transmembrane glycoprotein (P-glycoprotein) functions as an energy-dependent drug-efflux pump.[3] Since the discovery of a calcium channel blocker, verapamil, as an agent for overcoming MDR, various compounds, including quinidine, tamoxifen, and cyclosporin A, have been reported to overcome MDR.[4] We previously reported that among a number of new and known taxoids isolated from the Japanese yew *Taxus cuspidata*,[5,6] some non-taxol-type taxoids having neither an oxetane ring at C-4 and C-5 nor an *N*-acylphenylisoserine group at C-13 such as taxuspine C (**1**) increased cellular accumulation of vincristine (VCR) in multidrug-resistant tumor cells as potent as verapamil, and inhibited [^{3}H]-azidopine photolabeling of P-glycoprotein efficiently.[5,6] More recently, we found that taxuspine C (**1**) enhanced the chemotherapeutic effect of VCR in P388/VCR-bearing mice, and the other non-taxol-type taxoids, taxezopidines G (**8**) and H (**9**), increased the cellular accumulation of VCR in MDR cells as potent as verapamil. In this paper we describe the modulation of MDR by taxuspine C (**1**) *in vivo* and by taxezopidines G (**8**) and H (**9**) *in vitro*.

Taxoids from *Taxus cuspidata*. The methanolic extract of stems and seeds of the yew *Taxus cuspidata* Sieb. et Zucc. collected at Sapporo was partitioned between toluene and water, and then aqueous layer was partitioned with $CHCl_3$. The toluene and $CHCl_3$ soluble portions were subjected to a silica gel column followed by reversed-phase and silica gel column chromatographies to afford taxuspine C (**1**)[7] and

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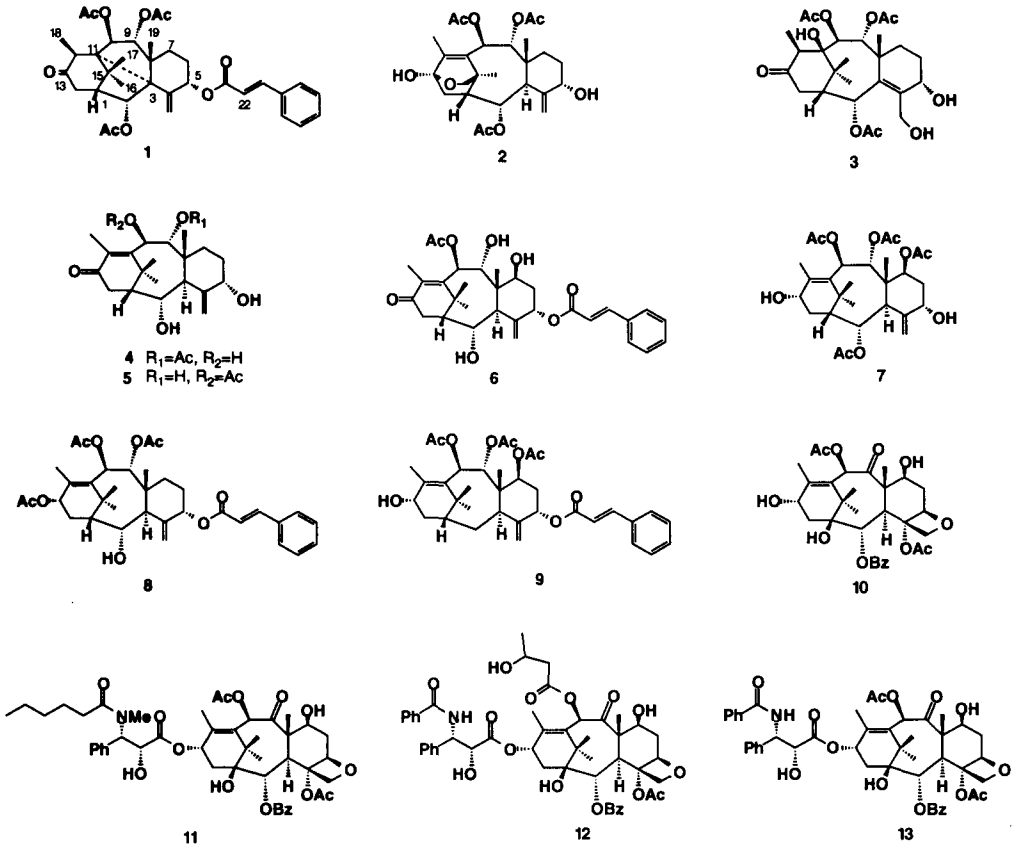

taxezopidines A ~ H (**2** ~ **9**)[8,9] together with known taxoids (**10** ~ **13**)[9]. Since the yield of taxuspine C (**1**) from the yew was very low, **1** was derived from taxinine, a major taxoid from the yew, by the following photochemical reaction[7]; irradiation of taxinine in dioxane with a mercury lamp (500 W) gave rise to compound

Table 1. Effect of Taxuspine C (1) on Antitumor Activity of Vincristine (VCR) in P388/VCR-bearing Mice

Treatment[a]	n	Median[b] (days)	Range (days)	T/C (%)
Control	5	11.4	10-15	100
VCR (0.2 mg/kg)	5	12.6	10-15	110
VCR (0.1 mg/kg)	5	10.6	10-11	92
Taxuspine C (**1**) (200 mg/kg) + VCR (0.2 mg/kg)	5	15.8	12-20	138
Taxuspine C (**1**) (200 mg/kg) + VCR (0.1 mg/kg)	5	12.0	10-15	105

[a] CDF_1 mice were given i.p. implants of 10^6 P388/VCR leukemia cells on day 0. Taxuspine C (**1**) together with VCR was given i.p. daily for 5 days. [b] T/C value: median survival time of treated mice divided by that of control mice.

1 (50%) and its 22-Z-isomer (33%), which were separated.

Combined Chemotherapeutic Effect of VCR and Taxuspine C (1) on P388/VCR-bearing Mice. VCR (0.1 or 0.2 mg/kg) alone given i.p. daily for 5 days starting on day 1 had no significant chemotherapeutic effect on P388/VCR-bearing mice (Table 1). When taxuspine C (**1**, 200 mg/kg) together with VCR was given i.p. daily for 5 days, the life span of P388/VCR-bearing mice was increased. The prominent result was observed at a taxuspine C (**1**) dose of 200 mg/kg given daily with 0.2 mg/kg VCR, whereby the T/C value was 138%.

Increased Cellular Accumulation of VCR in Multidrug-Resistant Cells by Taxoids. The effect of taxoids (**1** ~ **13**) on the cellular accumulation of VCR in multidrug-resistant human ovarian cancer 2780AD cells was examined and the results were shown in Table 2. Verapamil at 1 and 10 μg/mL increased the VCR accumulation in a dose dependent manner. Among these taxoids, taxuspine C (**1**)[6] and taxezopidines G (**8**) and H (**9**) increased the VCR accumulation as potent as verapamil. Compound **11** increased moderately the accumulation, while baccatin III (**10**) and taxol (**13**)[6] decreased the VCR accumulation in 2780AD cells. The potent compounds **1**, **8**, and **9** were non-taxol-type taxoids and possessed a cinnamoyl group at C-5, according to the previous studies.[5,6] On the other hand, it is interesting that among taxoids **10** ~ **12** having an oxetane ring, baccatin III (**10**) lacking a phenylisoserine group at C-13 decreased the VCR accumulation like taxol (**13**), whereas compound **11** having a modified phenylisoserine group at C-13 and compound **12** possessing the same phenylisoserine group at C-13 as taxol but a modified acyl group at C-10 did not reduce the VCR accumulation so potent as taxol (**13**).

Cytotoxicity Studies. Cytotoxic activity of all the taxoids (**1** ~ **13**) against murine lymphoma L1210 cells and human epidermoid carcinoma KB cells was shown in Table 3. Taxol (**13**)[6] and a taxol-type taxoid **12** exhibited very potent cytotoxicity against KB cells (IC_{50}, 0.0088 and 0.0025 μg/mL, respectively), while another taxol-type taxoid **11** possessing an oxetane ring and a modified phenylisoserine group showed relatively weak cytotoxicity against KB cells (IC_{50}, 0.17 μg/mL) as compared with those of **12** and **13**. The

Table 2. Effects of Taxoids (1 ~ 13) on Accumulation of Vincristine (VCR) in Multidrug-Resistant 2780AD Cells.

	VCR accumulation (% of control) with a taxoid concentration of			VCR accumulation (% of control) with a taxoid concentration of	
Compound	1 μg/mL	10 μg/mL	Compound	1 μg/mL	10 μg/mL
1	276[a]	692	**8**	419	873
2	213	190	**9**	312	706
3	219	203	**10**	97	84
4	234	223	**11**	178	423
5	251	246	**12**	143	148
6	118	217	**13 (Taxol)**	93	50
7	112	187	**Verapamil**	285	666

[a]The amounts of VCR accumulated in multidrug-resistant human ovarian cancer 2780AD cells were determined in the presence of 1 and 10 μg/mL of taxoids. The values represent means of triplicate determinations, and expressed as the relative amounts of VCR accumulated in the cells as compared with the control experiment.

Table 3. Cytotoxicity of Taxoids (1 ~ 13) against Murine Leukemia L1210 Cells and Human Epidermoid Carcinoma KB Cells

Compound	L1210 IC_{50} (μg/mL)	KB IC_{50} (μg/mL)	Compound	L1210 IC_{50} (μg/mL)	KB IC_{50} (μg/mL)
1	5.8	>10	**8**	5.8	9.2
2	>10	>10	**9**	6.2	>10
3	>10	2.6	**10**	>10	10
4	>10	>10	**11**	0.48	0.17
5	>10	>10	**12**	0.95	0.0025
6	>10	8.5	**13 (Taxol)**	0.33	0.0088
7	>10	9.2			

non-taxol-type taxoids **1** ~ **9** without an oxetane ring and an *N*-acylphenylisoserine moiety did not show such cytotoxicity. On the other hand, baccatin III (**10**) possessing an oxetane ring but no *N*-acylphenylisoserine group showed weak cytotoxicity.

It was previously reported that among numerous taxoids isolated from the Japanese yew *T. cuspidata*, some non-taxol-type taxoids such as taxuspine C (**1**) reversed resistance against VCR in multidrug-resistant tumor cells *in vitro* as potent as verapamil, and inhibited the binding of azidopine to P-glycoprotein in adriamycin-resistant tumor cells.[6] In this study it was found that taxuspine C (**1**) given i.p. enhanced the chemotherapeutic effect of VCR in P388/VCR-bearing mice. These observations indicate that taxuspine C (**1**) interacts directly with P-glycoprotein and inhibits the active efflux of antitumor agents, thus overcoming multidrug resistance *in vivo*, like verapamil. In addition, the other non-taxol-type taxoids, taxezopidines G (**8**) and H (**9**), having a cinnamoyl group at C-5 were also found to increase the cellular accumulation of VCR in multidrug-resistant 2780AD cells as potent as verapamil, and exhibited weak or no cytotoxicity. These results suggest that some taxoids, natural or designed ones, may be good modifiers of multidrug resistance in cancer chemotherapy.[10]

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