STAUROSPORINE DERIVATIVES REVERSE MULTIDRUG RESISTANCE WITHOUT CORRELATION WITH THEIR PROTEIN KINASE INHIBITORY ACTIVITIES

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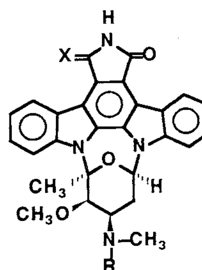
Staurosporine, a potent inhibitor of protein kinases, has been reported to increase the accumulation of vincristine in multidrug-resistant (MDR) tumor cells and to bind P-glycoprotein, which works as an efflux pump in the plasma membrane of MDR cells[1]. We have recently reported that *N*-ethoxycarbonyl-7-oxostaurosporine (NA-382) which has a low but selective inhibitory activity on protein kinase C, potently inhibits the function of P-glycoprotein[2]. Since P-glycoprotein is phosphorylated by protein kinase C[3], it is important to discover whether the drug accumulation by these compounds is concerned with the inhibition of protein kinase C or not. We investigated in this study the effects of several staurosporine derivatives on protein kinase C, protein kinase A, and MDR.

Staurosporine derivatives used in this study are shown in Scheme 1. 7-Oxostaurosporine was synthesized from staurosporine in our laboratory by the known method[4], and the other derivatives NA-381, NA-382, NA-478 and NA-460 were also synthesized from staurosporine by simple methods[5].

At first, the inhibitory activities of these staurosporine derivatives on protein kinase A and protein kinase C were measured by the method described in a previous report[2]. As shown in Table 1, 7-oxostaurosporine was much less inhibitory than staurosporine. Although NA-382 selectively inhibited protein kinase C as reported previously[2], another *N*-ethoxycarbonyl compound (NA-381) had weak inhibitory activity, and *N*-benzoxycarbonyl compounds (NA-478 and NA-460) had negligible inhibitory activity on both kinases. Next, the effects of these derivatives on the intracellular accumulation of vinblastine (VBL) in adriamycin-resistant P388 cells (P388/ADR), which had the MDR phenotype, were studied. The effects were shown by the concentration necessary for a 5-fold increase of intracellular VBL (C_5) in Table 1. The results indicate that the conversion of the lactam moiety of staurosporine to the imide (7-oxostaurosporine) decreased the effect on the drug accumulation, but *N*-substitutions on the tetrahydropyran ring of these

Scheme 1. Chemical structures of staurosporine derivatives.



	R	X
Staurosporine	H	H, H
7-Oxostaurosporine	H	O
NA-381	$COOCH_2CH_3$	H, H
NA-382	$COOCH_2CH_3$	O
NA-478	$COOCH_2C_6H_5$	H, H
NA-460	$COOCH_2C_6H_5$	O

Table 1. Inhibition of protein kinase A and protein kinase C and increase in vinblastine (VBL) accumulation by staurosporine derivatives.

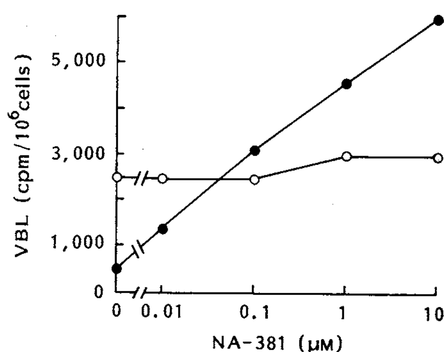
	Kinase inhibition IC_{50} (μM)		C_5[a] (μM)
	Kinase A	Kinase C	P388/ADR
Staurosporine	0.024	0.016	0.96
7-Oxostaurosporine	0.16	0.54	2.0
NA-381	10.2	9.1	0.053
NA-382	1.6	0.14	0.068
NA-478	>30	>30	0.27
NA-460	>30	>30	0.49

[a] Concentration of the compound causing a 5-fold increase of VBL accumulation in P388/ADR cells.

compounds potentiated the effect. Table 1 also shows that the *N*-ethoxycarbonyl compounds, NA-381 and NA-382, were much stronger than the *N*-benzoxycarbonyl compounds, NA-478 and NA-460. Fig. 1 shows the profiles of the dose-dependent effects of NA-381 on VBL accumulation in parental P388 cells (P388/S) and P388/ADR cells.

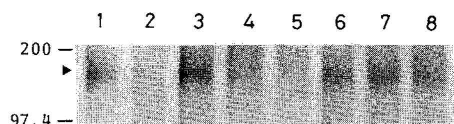
These results indicated that there was no correlation between the protein kinase inhibitory activities and the effect on VBL accumulation in MDR cells. Then we investigated the mechanism of VBL accumulation using a photolabeling agent of P-glycoprotein[6)], azidopine, which is useful for research on MDR modifiers. As shown in Fig. 2, NA-381 markedly and dose-dependently inhibited the [^{3}H]azidopine photolabeling of P-glycoprotein in the plasma membrane of P388/ADR cells, although 7-oxostaurosporine, which had a minor effect on VBL accumulation, barely inhibited the photolabeling even at 10 μM. These results suggested that conversion of the lactam moiety to the imide did not increase the affinity to P-glycoprotein, and

Fig. 1. Effects of NA-381 on vinblastine (VBL) accumulation in P388/S (○) and P388/ADR (●) cells.



Cells were suspended in 20 mM Hepes buffered RPMI-1640 medium supplemented with 10% fetal calf serum and incubated with 3.7 kBq of [^{3}H]VBL (370 MBq/mmol, Amersham Japan) in the absence or presence of NA-381 for 30 minutes at 37°C.

Fig. 2. Effects of staurosporine derivatives on the [^{3}H]azidopine photolabeling of P-glycoprotein (indicated by an arrow) in P388/ADR cell membrane.



Plasma membrane fraction (100 μg protein) isolated by the Percoll density gradient method[7)] was incubated with 37 kBq of [^{3}H]azidopine (2.07 TBq/mmol, Amersham Japan) in the absence or presence of varying concentrations of a compound for 20 minutes at 25°C and irradiated UV light for 15 minutes. Membrane proteins were separated on SDS-polyacrylamide gel electrophoresis (7.5% gel) and fluorographed. Lane 1: control, lane 2: vinblastine 10 μM, lanes 3~5: NA-381 (1, 3, 10 μM), lanes 6~8: 7-oxostaurosporine (1, 3, 10 μM). Molecular weight markers are shown at the left side of gel.

Table 2. Cytotoxicities and combined effects with vinblastine (VBL) of staurosporine derivatives in P388/S and P388/ADR cells.

	Cytotoxicity[a] IC$_{50}$ (μM)		Combined effect with VBL[b] (fold)	
	P388/S	P388/ADR	P388/S	P388/ADR
Staurosporine	0.0011	0.00079	1.0 (0.2 nM)	1.2 (0.2 nM)
7-Oxostaurosporine	0.045	0.027	1.0 (5 nM)	1.0 (5 nM)
NA-381	1.9	3.4	2.4 (0.5 μM)	15.1 (0.5 μM)
NA-382	4.4	4.6	2.8 (0.5 μM)	21.7 (0.5 μM)
NA-478	15	>30	—	10.5 (0.5 μM)
			—	18.8 (1.0 μM)
			2.3 (2.0 μM)	21.3 (2.0 μM)
NA-460	7.2	15.2	—	5.7 (0.5 μM)
			—	16.2 (1.0 μM)
			3.1 (2.0 μM)	18.0 (2.0 μM)

[a] Cells were suspended in RPMI-1640 medium supplemented with 10% fetal calf serum and incubated for 3 days in the absence or presence of a compound.

[b] Cells were incubated with VBL and a staurosporine derivative at concentrations indicated in parenthesis. Results were represented by the increase (fold) of VBL cytotoxicity; the IC$_{50}$ value of VBL obtained by combined experiment/the IC$_{50}$ value of VBL *per se*. Fifty percent growth-inhibitory concentration of vinblastine in P388/S and P388/ADR cells were 2.1 ± 0.2 and 60 ± 3 μM, respectively.

that the accumulation of VBL by staurosporine derivatives was due to the direct inhibition of the function of P-glycoprotein.

We furthermore investigated the effects of these derivatives on the cell growth of MDR cells and the results are shown in Table 2. The *N*-ethoxycarbonyl (NA-381 and NA-382) and *N*-benzoxycarbonyl (NA-478 and NA-460) compounds were far less cytotoxic than staurosporine or 7-oxostaurosporine. Although non-toxic concentrations of staurosporine or 7-oxostaurosporine did not influence the growth-inhibitory effect of VBL on P388/ADR cells, NA-381, NA-382, NA-478, and NA-460 significantly potentiated the effects of VBL at the non-toxic concentration *per se*. The combined effects of these compounds in P388/S cells were slight. Thus, these compounds selectively reversed the VBL resistance in P388/ADR cells *in vitro*.

Taking account of chemical structures, this study indicated that substitutions of the amino group of tetrahydropyran ring rather than conversion of lactam to imide might be effective to increase the affinity to P-glycoprotein, while both chemical modifications decreased the inhibitory activities on protein kinase A and protein kinase C. Consequently, this study suggested that the inhibition of protein kinases by staurosporine derivatives did not relate to the VBL accumulation in MDR cells, but the reversal of MDR was dependent on the binding affinity for P-glycoprotein.

References

1) Sato, W.; K. Yusa, M. Naito & T. Tsuruo: Staurosporine, a potent inhibitor of C-kinase, enhances drug accumulation in multidrug-resistant cells. Biochem. Biophys. Res. Commun. 173: 1252~1257, 1990

2) Miyamoto, K.; S. Wakusawa, K. Inoko, K. Takagi & M. Koyama: Reversal of vinblastine resistance by a staurosporine derivative, NA-382, in P388/ADR cells. Cancer Lett. 64: 177~183, 1992

3) Chambers, T. C.; E. M. McAvoy, J. W. Jacobs & G. Eilon: Protein kinase C phosphorylates P-glycoprotein in multidrug resistant human KB carcinoma cells. J. Biol. Chem. 265: 7679~7686, 1990

4) Koshino, H.; H. Osada & K. Isono: A new inhibitor of protein kinase C, RK-1409 (7-oxostaurosporine). II. Fermentation, isolation, physico-chemical properties and structure. J. Antibiotics 45: 195~198, 1992

5) Murakata, C; A. Sato, M. Kasai, M. Morimoto & S. Akinaga (Kyowa Hakko Kogyo): Staurosporine derivatives. WO 89,07105, Aug. 10, 1989

6) Safa, A. R.; C. J. Glover, J. L. Sewell, M. B. Meyers, J. L. Biedler & R. L. Felsted: Identification of the multidrug resistance-related membrane glycoprotein as an acceptor for calcium channel blockers. J. Biol. Chem. 262: 7884~7888, 1987

7) Wakusawa, S.; K. Miyamoto, T. Yanaoka & R. Koshiura: Sensitivity to vinblastine and mechanisms of intracellular accumulation of vinblastine by reserpine in rat ascites hepatoma cells. Yakugaku Zasshi 104: 1288~1294, 1984