Journal of Biochemistry and Molecular Biology, Vol. 31, No. 3, May 1998, pp. 269–273

JOURNAL OF
Biochemistry
and
Molecular Biology

Effect of α-Glycosidase Inhibitor in Multidrug Resistant Cell Lines

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Received 7 February 1998

The objective of this study was to evaluate the reversal of multidrug resistance of human cell lines by specific inhibitors of α-glucosidases and mannosidases that had been reported to be involved in N-linked oligosaccharide processing of glycoproteins. N-methyldeoxynojirimycin, 1-deoxynojirimycin, and castanospermine, which were known to be potent inhibitors of both α-glucosidase I and II, showed no activity against the multidrug resistant phenotype of the cell lines of SNU1DOX, KB-V1, and MCF-7/ADR. In contrast, 1-deoxymannojirimycin, an inhibitor of mannosidase I, resulted in a slight reversal for the vinblastine resistance of the KB-V1 cell line, but did not show any activity toward the other cell lines. Parallel experiments with tunicamycin, an inhibitor of N-linked glycosylation, also resulted in no significant changes in multidrug resistant (MDR) phenotype of the cell lines tested in this work. These observations suggest that the unglycosylation of P-glycoprotein associated with the inhibitor treatments might not be correlated with the reversal of multidrug resistance of the cell lines tested in this study.

Keywords: α-Glucosidase inhibitors, Multidrug resistance, P-glycoprotein.

Introduction

Multidrug resistance (MDR) is a serious problem in the treatment of human cancers. Initially, responsive tumors often develop a drug resistance phenotype after repeated cycles of chemotheraphy. MDR is characterized by cross-resistance to a number of structurally and functionally unrelated drugs, due to overexpression of the *mdr1* gene producing P-gycoprotein. P-glycoprotein is a 170 kDa membrane glycoprotein that acts as an ATP-dependent efflux pump, increasing transport of various anticancer compounds out of cells and decreasing cellular accumulation of drugs (Biedler and Riemhm, 1970; Julinao and Ling, 1976; Riordan *et al.*, 1985). Specifically, *Vinca* alkaloid-resistant leukemic cells have on their surfaces increased amounts of glycoproteins of 170 kDa to 190 kDa and less glycoprotein of 90kDa than do the drug-sensitive cells (Baker and Ling, 1978). Several investigators have reported that certain calcium channel blockers such as verapamil, nifedipine, and the calmoduline antagonist, trifluoperazine, are able to overcome, or at least partially circumvent, MDR (Tsuruo *et al.*, 1982; Rogan *et al.*, 1984; Kessel and Wilberding, 1985; Willingham *et al.*, 1986). Greenberger *et al.* (1987, 1988) have shown that the multiple forms of P-glycoprotein observed in a number of MDR mouse cell lines are likely the products of different P-glycoprotein genes that can undergo differential N-linked glycosylations. Glycosylation of membrane proteins is normally initiated in the rough endoplasmic reticulum with the dolichol pathway. The first step in the processing of the oligosaccharides is the removal of the three glucose residues, an event which occurs shortly after completion of the polypeptide chain while the protein is still in the rough endoplasmic reticulum (Kornfeld and Kornfeld, 1985). Several types of inhibitors of the glycoprotein-processing glucosidases are now available and the proteins glycosylated in the presence of these inhibitors are known to have unprocessed oligosaccharides on their molecules (Saunier *et al.*, 1982; Saul *et al.*, 1985; Gross *et al.*, 1986). Hiss *et al.* (1996) reported that suppression of glycoprotein synthesis with tunicamycin, a potent inhibitior of protein N-glycosylation, enhanced the sensitivity of multidrug resistant KB carcinoma cells and increased their retention of radiolabelled vincristine. Among the α-glucosidase inhibitors, deoxynojirimycin and castanospermine have been shown to inhibit the processing and maturation of glycoprotein of some enveloped viruses (Sunkara *et al.*,

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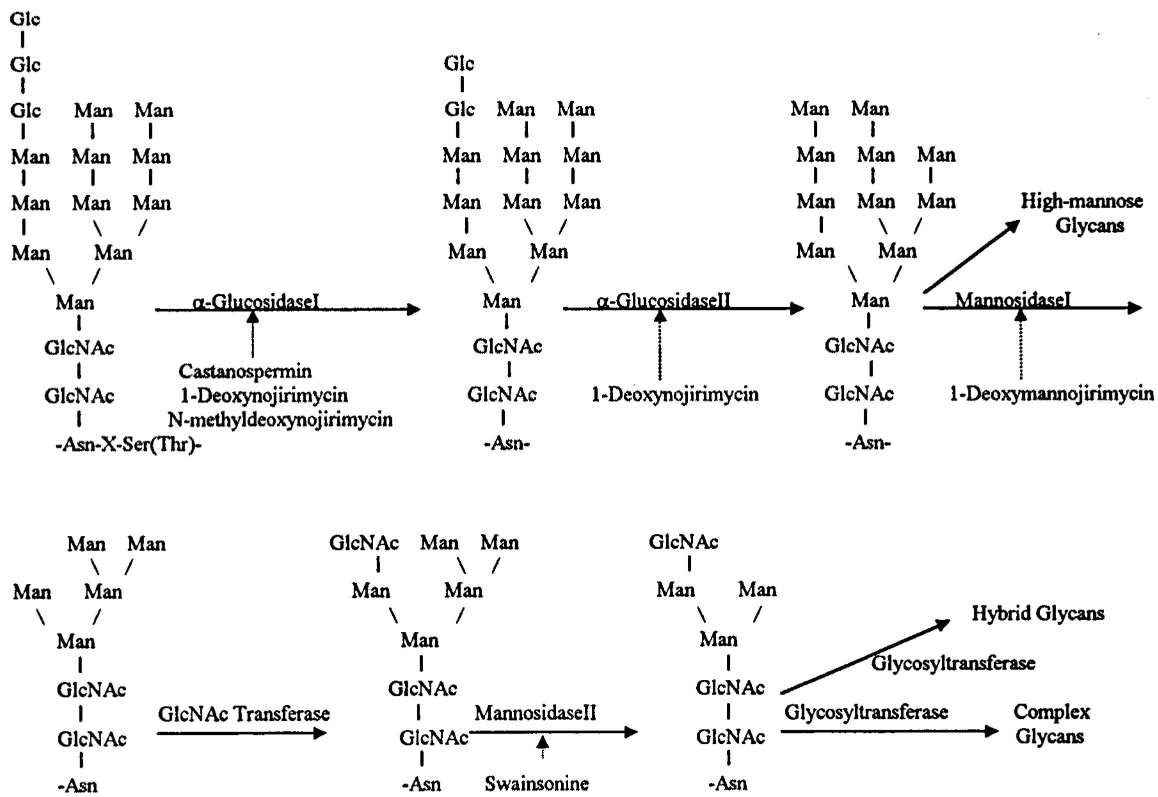

Fig. 1. Schematic representation of glycoprotein processing. Glc = D-glucose; Man = D-mannose; GlcNAc = N-acetyl-glucosamine; Asn = asparagine; Ser = serine; Thr = threonine; ↑, inhibition.

1987). In this study, we have examined whether the modification of P-gylcoprotein by α-glycosidase inhibitors can be usefully applied to overcome MDR (Fig. 1).

Materials and Methods

Chemicals Vinblastine and doxorubicin were obtained from Eli Lilly and Il Dong Pharmaceutical Co., respectively. Verapamil HCl, 1-deoxynojirimycin (DNJ), 1-deoxymannojirimycin (DMJ), N-methyldeoxynojirimycin (N-mDNJ), tunicamycin (TUN), and castanospermin (CAS) were purchased from Sigma Chemical Co. (St. Louis, USA). All other reagents were of analytical grade and obtained from either Merck Chemicals (Darmstadt, Germany) or Sigma Chemical Co.

Cell lines The human epidermoid cell line KB-V1 resistant to vinblastine and its parental KB3-1 cell, the resistant human breast cell line MCF-7/ADR to doxorubicin, and its parental MCF-7 cell were provided by KRIBB (Korea Research Institute of Bioscience and Biotechnology). The human stomach cell line SNU1DOX resistant to doxorubicin and its parental SNU1 were provided by the SNU cancer research center (Seoul University, Seoul, Korea). Each of the MDR cell lines was continuously cultured in the presence of their corresponding concentrations of anticancer agents.

Cell culture KB3-1 and KB-V1 cells were suspended in DMEM medium (Gibco, Grand Island, USA), or RPMI1640 medium (Gibco) in the case of the other cells, supplemented with 10% fetal calf serum and cultured in the absence or presence of an anticancer agent and α-glycosidase inhibitor. The cells were cultured in a humidified incubator maintained at 95% air/5% CO_2 atmosphere for 48 h at 37°C . The cultured cells were counted under a microscope, and the effects of anticancer agents were expressed by their 50% growth inhibiting concentration (IC_{50}).

Cytotoxicity assay Viability of the cancer cell lines to each anticancer agent and α-glycosidase inhibitor was measured in accordance with the SRB (sulforhodamin B) method. Stock solutions of α-glycosidase inhibitor were prepared by dissolving with distilled-deionized water. The prepared solutions were sterilized by passing through a 0.22 μm disposable filter (Millipore, Millex-GV). After 48 h incubation of the cancer cell line in 96-well microtiter plates, a 50 ml of cold 50% trichloroacetate solution was added to each well of the microplates and the plates were incubated at 4°C for 1 h to immobilize surviving cells. The microplates were washed five times with tap water and dried. Then, the cells were stained with SRB (0.4% w/v in 1% acetic acid) solution for 30 min, washed four times with 1% acetic acid solution to remove the remaining SRB solution, and then dissolved in 10 mM Tris buffer (pH 10.5). The absorbency of each well of the microplates was measured at 540 nm. The degree of growth inhibition was expressed as a percentage of the absorbency of the control well to the absorbency of the well treated with each anticancer agent and the 50% inhibitory concentration (IC_{50}) of the anticancer agents to each cell line was obtained by the Probit method (Tallarida,

1987). The relative resistance of each cell line against each anticancer agent was calculated according to the following equation.

$$\text{Relative resistance} = \frac{IC_{50} \text{ of the MDR cell line}}{IC_{50} \text{ of the sensitive cell line}}$$

Evaluation of the activity of the α-glycosidase inhibitor to reverse the multidrug resistance of cancer cells In order to determine the activity of α-glycosidase inhibitor for reversing the MDR, each cell line was treated with each of the anticancer agents alone or in combination with the α-glycosidase inhibitor at 60 μM or less concentrations, and the IC_{50} of each anticancer agent in the absence or presence of α-glycosidase was obtained according to the SRB method described above. Verapamil was used as the control overcomer-agent of MDR. Thereafter, the degree of potentiation (D.P.) of the cytotoxicity of each anticancer agent to the resistant cell was calculated according to the following equation.

$$\text{D.P.} = \frac{IC_{50} \text{ of the anticancer agent alone}}{IC_{50} \text{ of the anticancer agent in the presence of α-glycosidase inhibitor}}$$

The data of D.P. were presented as the average of three experiments ± SE.

Results and Discussion

The relative resistance of the MDR cell line The relative resistance of the three MDR cell lines used in this work was evaluated against anticancer agents, doxorubicin and vinblastine, and the values obtained are shown in Table 1. The highest levels of relative resistance were displayed by the MCF-7/ADR cell line which were 258 and 452 for doxorubicin and vinblasine, respectively. Interestingly, we found that the MCF-7/ADR cell line, originally selected by its resistance to doxorubicin, showed higher relative resistance for vinblastin rather than doxorubicin. In the case of the KB-V1 cell line, the relative resistance against doxorubicin and vinblastine were 36 and 200, respectively. The SNU1DOX cell line established the weakest cellular MDR phenotype, the relative resistance against doxorubicin and vinblastine being 27 and 4, respectively. From these results, we concluded that the three cell lines had sufficient multidrug resistance for their use in this study.

Cytotoxicity of the α-glycosidase inhibitors In order to assess the cytotoxicity of the α-glycosidase inhibitors used in this work, the IC_{50} values of the inhibitors for the three cell lines were determined, and comparisons were made with those for verapamil. As seen in Table 2, all the glycosidase inhibitors gave fairly low cytotoxicity even at the doses higher than 200 μM, regardless of the sensitivity of the target cell lines against the anticancer agents. The

Table 1. Relative resistance of MDR cell lines.

	Relative resistance	
Cell line	Doxorubicin	Vinblastin
SNU1DOX	27	4
KB-V1	36	200
MCF-7/ADR	258	452

Table 2. Cytotoxicity of α-glycosidase inhibitors.

	IC_{50} (μM)					
Cell line	CAS	DNJ	DMJ	N-mDNJ	TUN	Verapamil
SNU1	200>	200>	200>	200>	0.8	19.2
SNU1DOX	200>	200>	200>	200>	4.5	18.4
KB3-1	200>	200>	200>	200>	1.1	75.9
KB-V1	200>	200>	200>	200>	1.0	65.3
MCF-7	200>	200>	200>	200>	1.5	60.9
MCF-7/ADR	200>	200>	200>	200>	1.1	155

IC_{50} value of tunicamycin was estimated to be less than 4.5 μM for all of the cell lines tested, while the IC_{50} values for verapamil revealed somewhat higher levels ranging from 18.4 (for SNU1DOX) to 155 (for MCF-7/ADR). Thereafter, the concentrations of the α-glycosidase inhibitors added to the culture media were chosen to be at lower than 60 μM, and that of tunicamycin was lower than 0.4 μM. These concentrations were found to have little or no effect on cell viability, although all the α-glycosidase inhibitors exhibited high inhibitory activities for glycosidases at the concentration mentioned above.

Potentiation of anticancer-agent cytotoxicity by the α-glycosidase inhibitors In order to determine whether the carbohydrate component of P-glycoprotein influences the cells' drug-sensitivity, we examined the effects of the α-glycosidase inhibitors on the viability of the drug-sensitive and drug-resistant cell lines by continuously exposing the test cells for 48 h to different concentrations of the inhibitors. Table 3 shows the effects of the inhibitors on the treatment of doxorubicin of the cultures of SNU1 and SNU1DOX cells. Even at the highest concentration tested the α-glycosidase inhibitors did not significantly alter SNU1DOX cells' ability to reverse doxorubicin-resistance. In contrast, verapamil effectively reduced the doxorubicin-resistance phenotype, giving a D.P. value of 8.7 at the 5 μM concentration. Next, we examined the effects of the inhibitors on the vinblasine treatment of KB3-1 and KB-V1 cell lines. As shown in Table 4, a slight reversal phenomenon with 1-deoxymannojirimycin was observed in a concentration-dependent manner only for the vinblastine resistance of the KB-V1 cell line. At 120 μM, the concentration of 1-deoxymannojirimycin, with a D.P. value

Table 3. Effects of the α-glycosidase inhibitor on reversal of MDR of the human stomach cell lines.

Cell line	α-glycosidase inhibitor (μM)	D.P.					
		CAS	DNJ	DMJ	N-mDNJ	TUN	Verapamil
SNU1	15	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.2	0.9 ± 0.2[a]	1.2 ± 0.3[d]
	30	0.9 ± 0.2	1.0 ± 0.3	1.0 ± 0.3	1.1 ± 0.1	0.9 ± 0.3[b]	–
	60	1.0 ± 0.4	1.0 ± 0.2	1.1 ± 0.3	1.1 ± 0.2	1.0 ± 0.3[c]	–
SNU1DOX	15	1.0 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.4	0.8 ± 0.1 [a]	8.7 ± 0.4 [d]
	30	1.0 ± 0.2	1.1 ± 0.1	1.0 ± 0.2	1.1 ± 0.4	0.9 ± 0.2 [b]	–
	60	1.0 ± 0.3	1.2 ± 0.2	1.0 ± 0.2	1.2 ± 0.1	0.9 ± 0.3 [c]	–

D.P. was obtained in comparison with the IC_{50} of doxorubicin in the presence of the α-glycosidase inhibitor.
[a] 0.1 μM;
[b] 0.2 μM;
[c] 0.4 μM;
[d] 5 μM.

Table 4. Effects of the α-glycosidase inhibitor on reversal of MDR of the human epidermoid cell lines.

Cell line	α-glycosidase inhibitor (μM)	D.P.					
		CAS	DNJ	DMJ	N-mDNJ	TUN	Verapamil
KB 3-1	15	0.9 ± 0.1	0.7 ± 0.2	1.1 ± 0.3	0.7 ± 0.3	0.8 ± 0.3 [a]	2.1 ± 0.2 [d]
	30	0.8 ± 0.2	0.8 ± 0.1	1.6 ± 0.4	0.6 ± 0.4	0.7 ± 0.3 [b]	–
	60	0.7 ± 0.4	0.9 ± 0.3	1.7 ± 0.4	0.9 ± 0.2	0.8 ± 0.3 [c]	–
KB V-1	15	1.1 ± 0.1	0.8 ± 0.3	1.1 ± 0.2	1.2 ± 0.1	1.1 ± 0.1 [a]	180 ± 10 [d]
	30	1.0 ± 0.1	0.8 ± 0.3	2.7 ± 0.2	1.0 ± 0.1	1.3 ± 0.2 [b]	–
	60	1.2 ± 0.3	0.9 ± 0.2	5.3 ± 0.1	1.3 ± 0.3	1.7 ± 0.2 [c]	–

D.P. was obtained in comparison with the IC_{50} of vinblastine in the presence of the α-glycosidase inhibitor.
[a] 0.1 μM;
[b] 0.2 μM;
[c] 0.4 μM;
[d] 5 μM.

Table 5. Effects of the α-glycosidase inhibitor on reversal of MDR of the human breast cell lines.

Cell line	α-glycosidase inhibitor (μM)	D.P.					
		CAS	DNJ	DMJ	N-mDNJ	TUN	Verapamil
MCF-7	15	0.7 ± 0.2	0.6 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.8 ± 0.2[a]	1.6 ± 0.3[d]
	30	0.7 ± 0.1	0.6 ± 0.3	0.9 ± 0.3	0.8 ± 0.2	0.8 ± 0.1[b]	–
	60	0.8 ± 0.3	0.7 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	1.5 ± 0.3[c]	–
MCF-7/ADR	15	1.0 ± 0.2	0.8 ± 0.3	1.0 ± 0.1	1.0 ± 0.3	1.0 ± 0.1[a]	24 ± 5[d]
	30	0.9 ± 0.3	0.8 ± 0.3	1.1 ± 0.3	1.1 ± 0.3	0.9 ± 0.1[b]	–
	60	1.3 ± 0.2	0.8 ± 0.2	1.7 ± 0.1	1.4 ± 0.1	1.1 ± 0.2[c]	–

D.P. was obtained in comparison with the IC_{50} of doxorubicin in the presence of the α-glycosidase inhibitor.
[a] 0.1 μM;
[b] 0.2 μM;
[c] 0.4 μM;
[d] 5 μM.

of 14.7 (data not shown), and the other inhibitors did not display any detectable activities to reverse the drug resistance of the KB-V1 cell line. In contrast, verapamil showed a D.P. value of 180 at the concentration of 5 μM. Although the results of this experiment do not provide a comprehensive answer to the MDR reversal mechanism of the KB-V1 cells, they may be useful in analyzing the effects of glycosylation on the functionality of P-glycoprotein. Finally, in the examination of the MCF-7 and MCF/7ADR cell lines, we also did not find any appreciable effects of the α-glycosidase inhibitors on reducing the doxorubicin resistance (Table 5). In contrast to our observation described above, Kramer *et al.* (1995) demonstrated that the inhibition of glycosylation processing of P-glycoprotein by treatment with tunicamycin resulted in an increased drug accumulation in the human cell line. Beck and Cirtain (1982) suggested that exposure of the drug-resistant cell line CEM/VLB100 to tunicamycin inhibits only N-glycosylation of P-glycoprotein without diminishing the activity of drug efflux in these cells. Therefore, our results obtained in the present study are consistent with those of Beck and Cirtain (1982), even though we have no clear evidence that the α-glycosidase inhibitors had actually altered the glycosylation processing of P-glycoprotein. Also, the observations described in this study could be interpreted by the hypothesis that not all glycoproteins become unstable or nonfunctional in the unglycosylated form (Elbein, 1987), and still sustain biological activity of P-glycoprotein in MDR under the deficiency in its carbohydrate (Chou and Kessel, 1981).

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