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P-Glycoprotein-Dependent Multidrug Resistance in Cancer Cells

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1. Introduction

Clinical resistance to chemotherapeutic drugs is a major problem in the treatment of cancer. Cells selected for resistance with one drug display significant cross-resistance to the other drugs, including natural products such as the anthracyclines, *Vinca* alkaloids, podophyllotoxins, colchicine, *etc.* This fairly consistent pattern of cross-resistance is termed the *Multidrug Resistance* (MDR).

The MDR cells overexpress P-glycoprotein in the plasma membrane, which works as a drug efflux pump in an energy-dependent manner. Consequently, the MDR cells have been shown to display decreased drug accumulation. There is an *mdr* gene family, consisting *mdr*1 and *mdr*3(2) in man and *mdr*1a, *mdr*1b, and *mdr*2 in mouse. Until now, only *mdr*1-encoded P-glycoprotein has been shown to contribute to MDR. P-glycoprotein encoded human *mdr*1 gene is 1,280 amino acids in length, with 12 transmembrane domains, *N*-linked glycosylation, and two ATP-binding sites, and is thought to form a channel-like structure in the plasma membrane. Although the *mdr*3-encoded protein closely resembles P-glycoprotein in size, structure, and amino acid sequence (about 76% identity), its physiological function remains unclear.

2. Regulation of P-Glycoprotein Function

P-glycoprotein possesses ATPase activity and plays as an active outward transporter of anticancer drugs having broad specificity, shown in Table I. Since Garman *et al.* (1983) reported that P-glycoprotein in the plasma membrane of Chinese hamster lung cells resistant to adriamycin (ADR) is a phosphoprotein and its hyperphosphorylation becomes biologically inactive, many investigations have been performed to see the significance of phosphorylation for the function of P-glycoprotein. The serine residue in P-glycoprotein has been shown to be phosphorylated by not only Ca^{2+}/phospholipid-dependent protein kinase (PKC) but cyclic AMP-dependent protein kinase (PKA). Sato *et al.* (1990) reported that staurosporine, a non-selective protein kinase inhibitor, inhibits the phosphorylation of P-glycoprotein by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and the pump action is inhibited.

Then, we attempt to develop selective protein kinase inhibitors to see the

involvement of protein kinases in the regulation of P-glycoprotein and expression of *mdr* gene. We developed selective PKA and PKC inhibitors, H-87 (an isoquinolinesulfonamide compound) and NA-382 from staurosporine, respectively. However, these agents directly interacted with vinblastine (VBL) on the P-glycoprotein-binding site and reversed the drug resistance and the significance of phosphorylation of P-glycoprotein could not be clarified (Wakusawa *et al.*, 1992; Miyamoto *et al.*, 1993a)

On the other hand, Kim *et al.* (1993) have reported inhibition of *mdr*1 gene expression by H-87. NA-382 also inhibited the expression of mouse *mdr*1a mRNA induced by TPA (unpublished data). These evidence support that the promoter region of mouse *mdr*1a gene has cyclic AMP- and TPA-responsible elements (Hsu *et al.* , 1990).

Table I. Cross-resistance of MDR cell lines and its reverters

Anticancer drug	MDR reverter
Vinca alkaloids	Calcium channel blockers
Vinblastine	Verapamil
Vincristine	Nicardipine, Azidopine etc.
Vindesine	Calmodulin inhibitors
Anthracylines	Trifluoperazine, Fluphenazine etc.
Adriamycin	Cardiovascular drugs
Daunorubicin	Dipyridamol
Aclacinomycin A	Quinidine
Actinomycin D	CNS drugs
Mitoxantrone	Amitriptyline
Camptotheca alkaloids	Chlorpromazine
Camptothecin	Reserpine & *Rauwolfia* alkaloids
SN-38	Cyclosporines
Podophyllotoxins	Cyclosporine A, FK506, SDZ PSC-833
Etoposide	Steroidal agents
Teniposide	Tamoxifen
Taxus alkaloid	Progesterone
Taxol	Protein kinase inhibitors
Colchicine	Staurosporine derivatives (NA-382 etc.)
	Calphostin
	Isoquinolinesulfonamides (H-87 etc.)
	Miscellaneous drugs
	Quinine, Chloroquine, Quinacrine
	Cepharanthin, Cefoperazone

3. Reversal of MDR

A major goal in experimental as well as clinical investigations of drug resistance is to discover unique methods to reverse or circumvent it. Therefore, many investigators have focused on the pharmacological reversal of MDR. The pharmacological agents used to date both *in vitro* and *in vivo* for the reversal of MDR are listed in Table I. In general, these agents competitively inhibit the

binding of anticancer drugs to P-glycoprotein, increase the drug accumulation in MDR cells, resulting complete or partial reverse of the drug resistance, but cause little or no potentiation of cytotoxicity of the drug in sensitive cells. Although these agents share only broad structural similarities, most are lipophilic, heterocyclic, amphipathic substances.

This paper will show the effects of newly synthesized compounds on MDR P388 cells and the parent sensitive cells. H-87 significantly potentiated the cytotoxic effects of ADR, daunorubicin, vincristine, and VBL on MDR P388 murine leukemic cells acquired by prolonged exposure to ADR (P388/ADR), but hardly influenced the effects of mitomycin C, 5-fluorouracil, and cisplatin on P388/ADR cells and P388 phenotypes resistant to the corresponding anticancer drugs (Miyamoto *et al.*, 1990). When cells were incubated with VBL in the presence of H-87 for 30 min at 37℃, H-87 increased the accumulation of VBL much more in P388/ADR cells than in the sensitive cells. The extrusion of the anticancer drug from the cells was significantly reduced in the presence of H-87. This compound almost completely inhibited the binding of a photoaffinity ligand of VBL to P-glycoprotein. Another isoquinolinesulfonamide compound having PKA inhibitory activity, H-8, did not show these activities (Wakusawa *et al.*, 1992). Consequently, this reversal by H-87 in a brief treatment can elucidate from the direct action on P-glycoprotein, but not from the PKA inhibition. Then, we designed bis-benzenesulfonamide compounds without PKA inhibitory activity (Sawanishi *et al.*, 1994). One of these compounds, SM-64, potentiated the cytotoxic effects of *Vinca* alkaloids, anthracyclines, and an active camptothecin derivative SN-38 *in vitro*. However, both H-87 and SM-64 did not show any anticancer combined effect with VBL *in vivo*.

In the process of development of selective PKC inhibitor, we synthesized many staurosporine derivatives and got NA-382 (Miyamoto *et al.*, 1993a; Wakusawa *et al.*, 1993). When cells were incubated with VBL and a compound for 30 min at 37℃, all compounds promoted the VBL accumulation in MDR P388 cells, but not in the sensitive cells. There were not any correlations between the increase of VBL accumulation and inhibitory activities on PKA and PKC. Among these compounds, NA-382 significantly potentiated the *in vitro* and *in vivo* anticancer effects of VBL on P388/ADR, without toxicity, while verapamil affected the effect of VBL *in vitro*, but did not *in vivo* even at the maximum tolerated dose (Miyamoto *et al.*, 1993b; Miyamoto *et al.*, 1995).

4. Physiological Function of P-Glycoprotein

P-glycoprotein has been also indicated to be an intrinsic plasma membrane protein present in several normal tissues, where its function is not completely certain. It is of interest that the P-glycoprotein in brain capillary endothelial cell transports drugs including anticancer drugs from the basal side to the apical side (Tsuji *et al.*, 1993). This function of P-glycoprotein in brain capillary should

involve in a part of the complex function of the blood-brain barrier. Therefore, MDR reverters such as NA-382 may be useful for the treatment of the brain cancer as well as MDR cancers. Combination chemotherapy with MDR reverters should be also considered the adverse reactions on organs expressing P-glycoprotein. While P-glycoprotein overexpressed in the MDR cancer cell and the blood-brain barrier is encoded by *mdr*1 gene, *mdr*3-encoded P-glycoprotein has been demonstrated in other normal organs including liver, kidney, and adrenal (Fojo *et al.*, 1987). These P-glycoproteins have a similar structure, but it is possible that the function mechanisms and substrate affinities are different. These are now in progress aggressively.

5. Conclusion

P-glycoprotein is thought to constitute a defense system of not only cancer cells but normal tissues against foreign matters or metabolites. To improve cancer chemotherapy, reversal of MDR of cancer is important, and influence on the normal tissues should be also considered. Additionally, modulation of normal function of P-glycoprotein may be possible to achieve drug therapy for other diseases. One may hope that a full understanding of the biochemical differences between cancer cells and normal tissues may lead to the development of rationally designed drugs that exploit these differences more effectively than the empirical drug therapy now available.

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