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NOTE

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Suppression of Multidrug Resistance by Migrastatin

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Abstract Migrastatin (MGS) is a *Streptomyces* metabolite that inhibits cancer cell migration. In this study, we found that MGS also enhanced the cytotoxicity of vinblastine, vincristine, and taxol in P-glycoprotein-overexpressing VJ-300 cells and P388/VCR cells. Furthermore, MGS increased the intracellular concentration of labeled vinblastine, vincristine, and taxol in both VJ-300 cells and P388/VCR cells. P-glycoprotein was photolabeled with [^{3}H]azidopine, but this photolabeling was significantly inhibited in the presence of MGS. These results indicated that MGS directly interacts with and inhibits P-glycoprotein, thereby sensitizing drug-resistant cells to anticancer drugs.

Keywords migrastatin, multi drug resistance, P-glycoprotein, anticancer drugs

Introduction

The development of drug resistance in the treatment of cancer continues to challenge oncologists. The hallmark of multidrug resistance is cross-resistance to multiple structurally unrelated compounds, such as the anthracyclines, taxanes, vinca alkaloids, and epipodophilotoxins [1, 2]. The *MDR-1* gene mediates one of the most extensively studied mechanisms of drug resistance [3]. The protein product of the *MDR-1* gene, P-glycoprotein (Pgp), is a 170-kDa ATP-dependent efflux pump located in the plasma membrane [4, 5]. Expression of this pump decreases intracellular drug concentration rendering tumor cells drug resistant. Because P-glycoprotein would be an important molecular target for cancer therapy, we searched for a compound that modulates its function in a library of natural products. In this paper, we report that migrastatin (MGS), which was originally isolated from *Streptomyces* sp. MK929-43F1 as an inhibitor of cancer cell migration [6~9], enhances the cytotoxicity of anticancer drugs in P-glycoprotein-overexpressing cells.

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Materials and Methods

Materials

Migrastatin (MGS) was isolated from *Streptomyces* sp. as described previously [6]. Vinblastine, vincristine, and taxol were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). [G-^{3}H]vinblastine (289 GBq/mmol), [G-^{3}H]vincristine (181 GBq/mmol), and [^{3}H]azidopine (2.22 TBq/mmol) were purchased from Amersham Japan Ltd (Tokyo, Japan). [^{3}H]taxol (740 GBq/mmol) was purchased from American Radiolabeled Chemical, Inc (St. Louis, MO).

Cell Culture

Human epidermoid carcinoma KB/S cells, vincristine-resistant VJ-300 cells [10], mouse leukemia P388/S cells and vincristine-resistant P388/VCR cells [11] were kindly provided by Dr. M. Hayashi (Kitasato Institute). VJ-300 cells and P388/VCR cells were cultured in medium containing 10 ng/ml vincristine to maintain their drug resistance.

MTT Cell Growth Assay

The cells were seeded at 5×10^3 cells in 200 μl of growth medium in 96-well plates. Drugs were added, and the cells were further incubated for 3 days. Ten microliters of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) in phosphate-buffered saline (PBS) was added to each well, and the plate was incubated at 37°C for 4 hours. Then, 100 μl of DMSO was added to cells. The

amount of Formazan formed was measured at 570 nm using a MRP-A4 micro plate reader (Tosho).

Measurement of Intracellular Concentration of Anti-cancer Drugs in Cancer Cells

The cells were seeded at 5×10^4 cells in 24-well plates and incubated at 37°C for 24 hours. Then, the cells were pretreated with or without MGS for 1 hour. Next, 7.4 KBq/ml (17 nM) [^{3}H]vinblastine, 7.4 KBq/ml (17 nM) [^{3}H]vincristine, or 7.4 KBq/ml (17 nM) [^{3}H]taxol was added. After 2 hours, cells were washed with cold PBS and dissolved in 0.5 N NaOH, and the cell-associated radioactivity was measured with a liquid scintillation counter.

Preparation and Photoaffinity Labeling of Plasma Membrane

VJ-300 cells were homogenized in buffer A (10 mM Tris-HCl buffer, pH 7.5, 25 mM sucrose, 0.2 mM $CaCl_2$ and 1 mM phenylmetylsulfonyl fluoride (PMSF)). The cell homogenate was diluted 4 fold with buffer B (10 mM Tris-HCl, pH 7.5, and 25 mM sucrose) and then centrifuged at $1000\times g$ for 10 minutes. The supernatant was layered onto a 35% sucrose solution (10 mM Tris-HCl, pH 7.5, 35% sucrose, and 1 mM EDTA) and centrifuged at $10{,}000\times g$ for 30 minutes. The membrane fraction at the interface was collected, resuspended in buffer B, and then centrifuged at $100{,}000\times g$ for 60 minutes. The pellets (plasma membrane) were resuspended in buffer C (10 mM Tris-HCl, pH 7.5, 250 mM sucrose, and 1 mM PMSF) and incubated with 8.3 nM [^{3}H]azidopine and drugs for 1 hour at 4°C. The mixture was irradiated for 15 minutes at 4°C with an UV lamp (312 nm), and photolabeled membranes were analyzed by SDS-PAGE. The gel was fixed in a mixture of 30% acetic acid and 10% methanol, treated with Autoradiography Enhancer (PerkinElmer, Boston, MA) for 1 hour, dried, and then exposed for 2 weeks at −80°C using Kodac XAR-5film.

Results

We examined the effect of MGS on the sensitivity of KB/S cells and their P-glycoprotein-overexpressing variant (VJ-300) to vinblastine (VBL). As shown in Fig. 1A and Table 1, VBL induced cell death with an IC_{50} of 2.0 ng/ml in KB/S cells and of 216 ng/ml in VJ-300 cells. MGS did not show significant cytotoxicity up to 50 μg/ml, however, it enhanced the sensitivity to VBL in VJ-300 cells in a dose-dependent manner. Furthermore, MGS at 30 μg/ml enhanced the sensitivity to other anti-cancer drugs, such as vincristine (VCR) and taxol (TXL), in VJ-300 cells (Table 1). P-glycoprotein was also overexpressed in VCR-resistant mouse leukemia P388/VCR cells, and the sensitivity of P388/VCR to VBL was 21-fold lower than that of parental P388/S cells. However, the resistance to VBL in P388/VCR cells was also reduced by the addition of MGS in a dose-dependent manner (Fig. 1B). These results indicated that MGS might be of potential use for the circumvention of MDR.

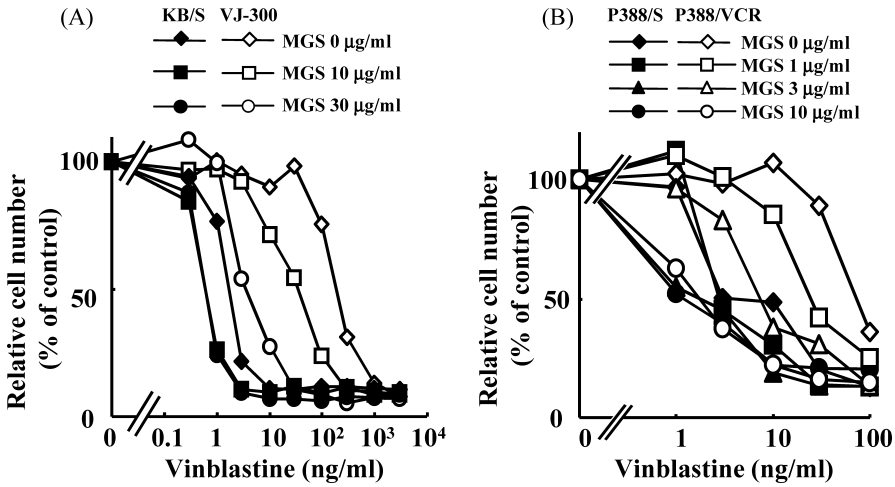

Fig. 1 Effect of MGS on sensitivity of drug-sensitive and drug-resistant tumor cells to vinblastine.

(A) KB/S cells or VJ-300 cells were incubated with VBL in the presence of various concentrations of MGS for 3 days. (B) P388/S or P388/VCR cells were incubated with VBL in the presence of various concentrations of MGS for 3 days. Cells were enumerated by MTT assay. The 100% values are the cell numbers with MGS alone in the absence of VBL. Values are means of two determinations.

Table 1 Enhancement of the cytotoxicities of vinblastine, vincristine, and taxol by MGS

Drugs	MGS (μg/ml)	IC_{50} (ng/ml)	
		KB/S	VJ-300
Vinblastine	0	2.0 (1.0)[a)]	216 (108)
	10	0.72 (0.4)	40 (20)
	30	0.71 (0.4)	4.1 (2.1)
Vincristine	0	2.2 (1.0)	665 (302)
	30	0.69 (0.3)	17 (11)
Taxol	0	6.0 (1.0)	7680 (1280)
	30	4.6 (0.8)	151 (25)

a) Numbers in parentheses, relative resistance.

Table 2 Enhancement of the intracellular concentrations of vinblastine, vincristine, and taxol by MGS

Drugs	MGS (μg/ml)	Intracellular concentration (% of control)[a)]	
		KB/S	VJ-300
Vinblastine	0	100	17
	30	219	81
Vincristine	0	100	14
	30	188	71
Taxol	0	100	6
	30	124	26

a) The 100% values are for the intracellular concentration of drugs in KB/S cells in the absence of MGS.

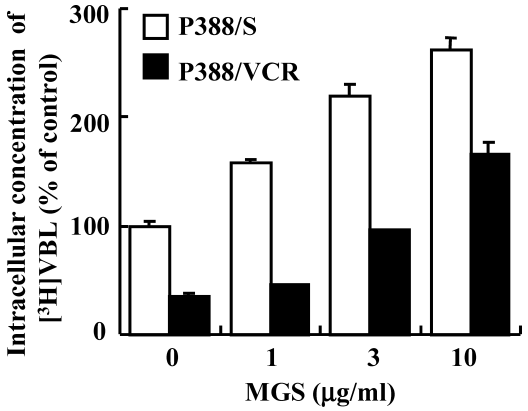

Fig. 2 Enhancement of intracellular concentration of vinblastine by MGS in P388/VCR cells.

P388/S or P388/VCR cells were pretreated with MGS for 1 hour and treated with [^{3}H]VBL for 2 hours. Cells were washed and resolved in 0.5 N NaOH, and measured for radioactivity. Values are means of four independent determinations; bars, SD. The 100% values are for the intracellular concentration of [^{3}H]VBL in P388/S cells in the absence of MGS.

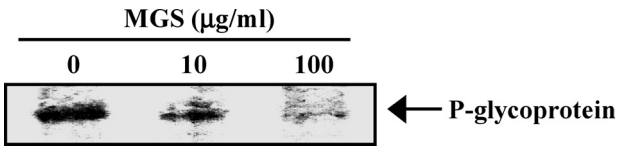

Fig. 3 Effect of MGS on photolabeling of P-glycoprotein with [^{3}H]azidopine.

The membrane vesicles (150 mg of protein) of VJ-300 cells were photolabeled with 8.3 nM of [^{3}H]azidopine for 15 minutes at 4°C in the presence of the indicated concentrations of MGS, and analyzed by SDS-PAGE and Fluorography.

Since the intracellular concentration of anticancer drugs is decreased in P-glycoprotein-overexpressing tumor cells, the intracellular concentration of anticancer drugs in KB/S and VJ-300 cells was measured in the presence or absence of MGS. As shown in Table 2, the intracellular concentration of [^{3}H]VBL, [^{3}H]VCR, and [^{3}H]TXL in VJ-300 cells was 17%, 14% and 6% of that in KB/S cells, respectively. A significant increase in intracellular concentration of [^{3}H]VBL, [^{3}H]VCR, and [^{3}H]TXL was observed following the addition of 30 μg/ml of MGS in VJ-300 cells. MGS also increased the intracellular concentration of [^{3}H]VBL in P388/VCR cells (Fig. 2). These results indicated that the potentiation of the cytotoxicity of anticancer drugs by MGS in P-glycoprotein-overexpressing cells was due to an enhancement of the intracellular concentration of the drugs by MGS.

It was reported that P-glycoprotein is photolabeled with azidopine, and this labeling is inhibited in the presence of compounds that are substrates of P-glycoprotein [12, 13]. Thus, we examined whether MGS inhibited the photolabeling of P-glycoprotein with [^{3}H]azidopine. As shown in Fig. 3, MGS inhibited the binding of [^{3}H]azidopine to P-glycoprotein in VJ-300 cells in a dose-dependent manner. Hence, the reversing effect of MGS on MDR tumor cells is due, at least in part, to its direct interaction with P-glycoprotein.

Discussion

In the present study, we demonstrated that MGS overcame the resistance to anticancer drugs in P-glycoprotein-

overexpressing tumor cells. Several studies have suggested that direct interaction with cytotoxic drug-binding sites on P-glycoprotein was involved in the mechanism of reversing MDR with chemosensitizers. MGS inhibited the binding of [^{3}H]azidopine to P-glycoprotein, therefore, MGS would directly inhibit the function of P-glycoprotein. There are several sites on P-glycoprotein for drug-binding and transport that interact in a cooperative manner [14]. R site preferentially recognizes rhodamine-123, doxorubicin, and daunorubicin. H site preferentially recognizes Hoechst 33342 and colchicine. VBL, actinomycin D, and etoposide interact equally with both sites [14]. Inhibition of the photolabeling by VBL suggests that azidopine interacts with P-glycoprotein at the same site as VBL [15]. Our finding that MGS inhibited the photolabeling with azidopine indicates MGS directly interacts at the azidopine- and VBL-binding site of P-glycoprotein.

Because MGS is an effective inhibitor of P-glycoprotein, the results of more advanced *in vivo* tests will be pivotal in contemplating further development of MGS as a clinically useful pharmaceutical agent.

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