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Dipyridamole Enhancement of Drug Sensitivity in Acute Lymphoblastic Leukemia Cells

Manabu Sotomatsu, Shin-ichirou Yugami, Toshiji Shitara, and Takayoshi Kuroume
Department of Pediatrics, Gunma University School of Medicine, Maebashi, Japan

The effect of dipyridamole (DPM) on cell sensitivity to anticancer drugs was examined in acute lymphoblastic leukemia (ALL) cell lines. We established two ALL cell lines (KMO-90 and KMO-R) from bone marrow samples of a 12-year-old girl with ALL. The drug concentrations needed to reduce optical density to 50% of that of control cells (IC_{50}) showed that KMO-R was about twofold more resistant to doxorubicin (DOX), mitxantrone (MIT), vincristine (VCR), and etoposide (VP-16) than was KMO-90. Considering that both KMO-90 and KMO-R were established from a patient with ALL at the time of presentation and relapse, respectively, these two cell lines might be novel and useful models for research into the acquisition of drug resistance in ALL cells. Although cytotoxicity of DPM in KMO-90 was about 6% at 1 μg/ml, DPM enhanced cell sensitivity to DOX, MIT, VCR, and VP-16 at this concentration. Cytotoxicity of DPM in KMO-R was less than 5% at 1, 5, and 10 μg/ml. In KMO-R, DPM enhanced cell sensitivity to these four drugs in a dose-dependent manner. The plasma concentrations achieved by oral administration of DPM is about 1 μg/ml. At clinically achievable concentrations, DPM enhanced cell sensitivity to DOX, MIT, VCR, and VP-16 in both KMO-90 and KMO-R, thus showing DPM to be a useful agent for potentiating anticancer chemotherapy of hematopoietic malignancy.

Key words: hematopoietic malignancy, multidrug resistance, chemosensitizer

INTRODUCTION

Acute lymphoblastic leukemia (ALL), the most common malignancy of childhood, is considered a chemoresponsive malignancy. Despite impressive improvement in the rate of complete remission, some children with ALL will eventually relapse and so become resistant to various anticancer drugs. The emergence of multidrug resistance is a major obstacle to effective chemotherapy of hematopoietic malignancy.

Published reports have described a number of agents that have enhanced anticancer drug efficacy—drugs such as calcium-channel blockers [1,2], calmodulin inhibitors [3], antiarrhythmics [4], and certain hormones [5,6]—and these reports have documented the optimum concentrations for this enhancement as well above their maximally tolerated plasma levels. Recently, several groups have demonstrated that cyclosporin A potentiates chemotherapy with vinca alkaloids and/or anthracyclines directed against drug-sensitive as well as drug-resistant tumors at a safely achievable concentration [7,8]. Dipyridamole (DPM) has been shown to enhance sensitivity to anticancer drugs in hepatoma [9,10], colon cancer [11,12], ovarian carcinoma cells [13,14], and leukemia/lymphoma [15].

This report reveals that DPM at readily achievable concentrations improved patient sensitivity to doxorubicin (DOX), mitxantrone (MIT), vincristine (VCR), and etoposide (VP-16) in ALL cells.

MATERIALS AND METHODS

Drugs

We purchased DPM from Boehringer-Ingelheim (Japan) Ltd. (Tokyo, Japan); DOX from Kyowa Hakkou Kogyo (Tokyo); MIT from Lederly (Japan), (Tokyo); VCR from Shionogi (Osaka); and VP-16 from Nippon Kayaku (Tokyo).

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Address reprint requests to Dr. Manabu Sotomatsu, Department of Pediatrics, Gunma University School of Medicine, 3-39-15, Showa-machi, Maebashi, Gunma 371, Japan.

Leukemic Cell Lines

We established two cell lines using bone marrow samples from a 12-year-old girl with ALL carrying 1;19 chromosome translocation. One line (KMO-90), reported previously [16], was established from a bone marrow sample at the time of presentation; the second line (KMO-R) at the time of the first relapse. The same methods were used to establish both cell lines [16]. The KMO-R cells were positive for markers characteristic of early cells of B lineage, such as CD10, CD19, and HLA-DR, and negative for markers of T lymphoids, granulocytes, and monocytes (data not shown). For 60 and 35 weeks, respectively, we maintained KMO-90 and KMO-R cells in RPMI-1640 (Nissui, Tokyo) supplemented with 10% fetal calf serum (FCS; Cell Culture Laboratory, Ohio) 2 mM glutamine (GIBCO, Grand Island, NY, 100 μg/ml streptomycin (Meiji Seika Kaisha, Tokyo), and 100 U/ml penicillin G (Toyo Jozo, Shizuoka).

MTT Assay

A colorimetric assay using the tetrazolium salt, MTT (Sigma Chemical Co., St. Louis, MO) [17], was used to assess cytotoxicity. We seeded the KMO-90 and KMO-R cells into 96-well round-bottom plastic plates (#25850, Corning, NY) at 3×10^4 cells/well in 100 μl of medium including various concentrations of anticancer drug with or without DPM and cultured at 37°C in a humidified atmosphere of 5% CO_2 in air. After 72 hr of incubation, 10 μl of 5 mg/ml MTT was added to each well. After another 4 hr of incubation, we added 100 μl of isopropyl alcohol with 0.04 N HCl to each well to dissolve the formazan crystals produced by the viable cells. To measure each well's optical density (OD) we used the Immuno-Reader NJ-2000 (Inter-Med, Tokyo), a scanning microplate photometer, with a 570-nm test wavelength and a 620-nm reference wavelength. Each experiment was performed in four replicate wells. The presence of DPM had no effect on the MTT assay.

Data were expressed as percentage of survival compared with control (with or without DPM). The IC_{50} for each drug, alone or in combination with DPM, was defined as the drug concentration that reduced OD to 50% of control cells (with no anticancer drugs). We determined the sensitization factor (SF) by dividing the IC_{50} for each anticancer drug alone by the IC_{50} in the presence of DPM.

Statistical Analysis

We used Student's t-test to detect differences in drug sensitivity between KMO-90 and KMO-R and between anticancer drugs with DPM and controls (anticancer drugs alone).

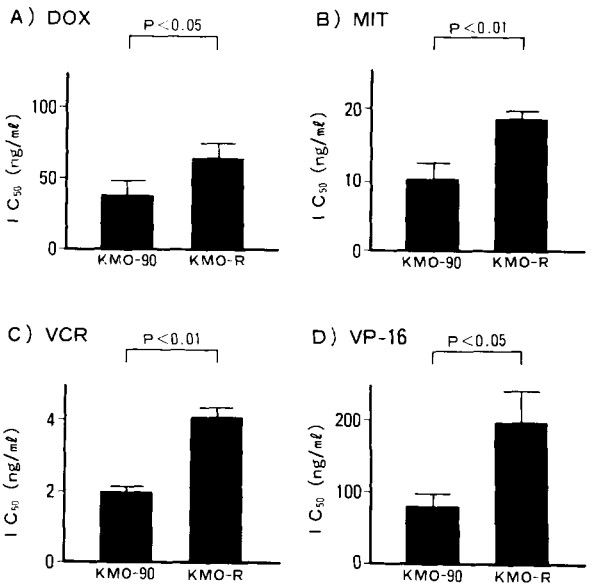

Fig. 1. IC_{50} for DOX (A), MIT (B), VCR (C), and VP-16 (D) in KMO-90 and KMO-R. Cytotoxicity was determined using the MTT assay. Each column represents the mean of three separate experiments using quadruplicate cultures. Bars = SD. The difference in IC_{50} for each drug between KMO-90 and KMO-R was statistically significant ($P < 0.01$ or $P < 0.05$).

RESULTS

We examined cell sensitivity to DOX, MIT, VCR, and VP-16 in KMO-90 and KMO-R. IC_{50} showed KMO-R to be less sensitive to these four drugs than was KMO-90 (Fig. 1). This difference between KMO-90 and KMO-R was statistically significant. The ratios of IC_{50} in KMO-R to IC_{50} in KMO-90 for DOX, MIT, VCR, and VP-16 were 1.7, 1.9, 2.0, and 2.3, respectively, showing that KMO-R was about 2-fold more resistant to these four anticancer drugs than was KMO-90.

DPM itself is cytotoxic in many kinds of cells [10,15,18]. Its cytotoxicity in KMO-90 at concentrations of 1, 5, and 10 μg/ml was 6.2 ± 4.4, 12.4 ± 8.1, and 18.0 ± 4.9%, respectively. The cytotoxicity of DPM in KMO-R at the same concentrations was less than 5%. It was interesting that KMO-R became more resistant to DPM than did KMO-90.

At only 1μg/ml, DPM improved cell sensitivity to DOX in KMO-90 and KMO-R. Then, in DPM concentrations increasing from 1 to 10 μg/ml, sensitivity to DOX increased in a dose-dependent manner (Fig. 2). In both KMO-90 and KMO-R this effect of DPM was also identified in MIT, VCR, and VP-16 (Fig. 2). IC_{50} showed that DPM's enhancement of sensitivity to these four drugs was statistically significant at concentrations of 1, 5, and 10 μg/ml in both KMO-90 and KMO-R (Table I).

Whereas SFs for DOX in KMO-R were 2.8, 3.4, and 3.2 at 1, 5, and 10 μg/ml of DPM, respectively, SFs for

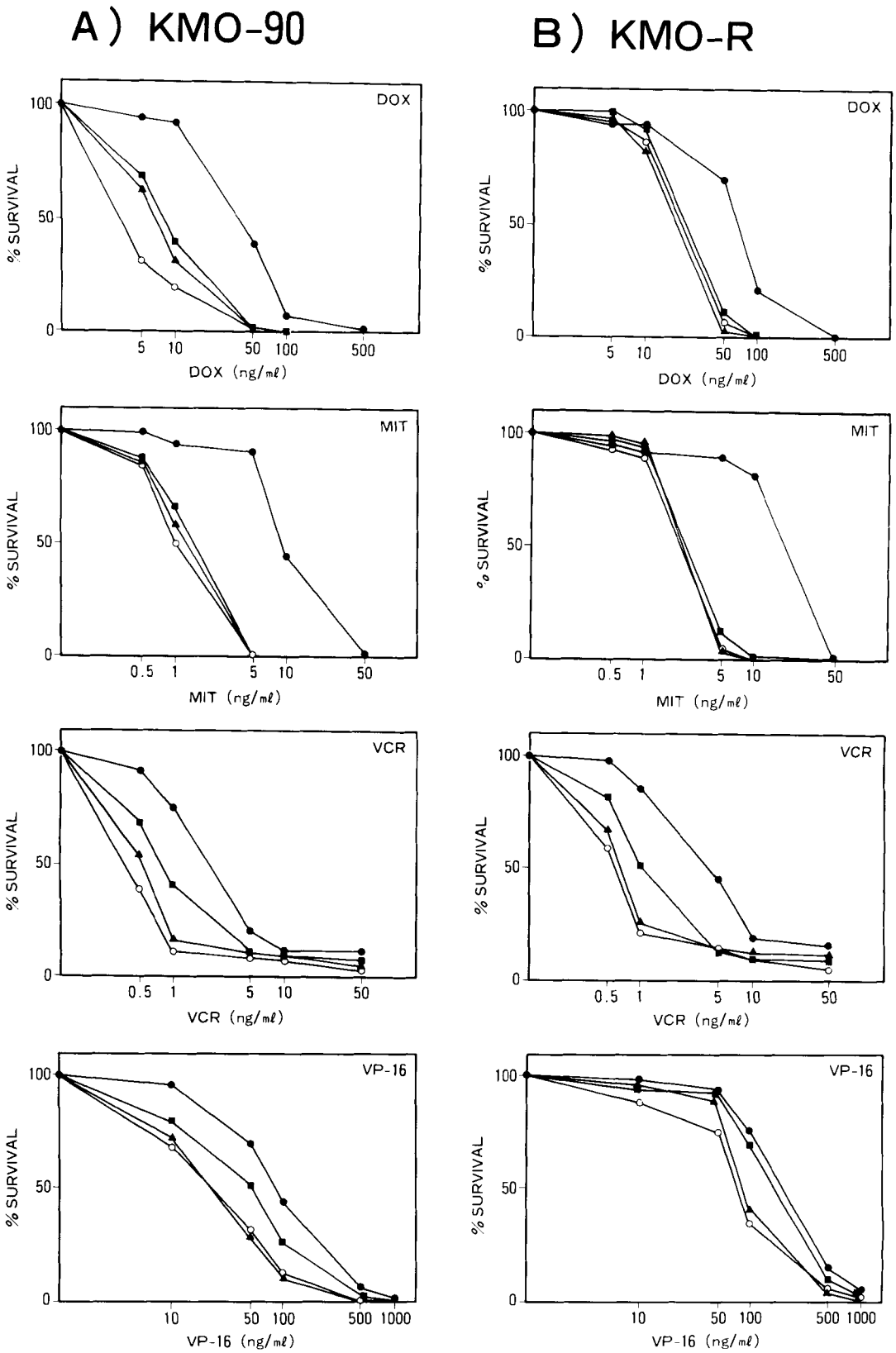

Fig. 2. Dose-response curves for killing of KMO-90 (A) and KMO-R (B) cells by DOX, MIT, VCR, and VP-16 in the absence (●) and presence of DPM 1 μg/ml (■), 5 μg/ml (▲), and 10 μg/ml (○). Cytotoxicity was determined using the MTT assay. Data are expressed as survival percentage compared with control cultures containing no anticancer drug (with/without DPM). Each point represents the mean of three separate experiments using quadruplicate cultures.

TABLE I. Effects of DPM on Sensitivity to DOX, MIT, VCR, and VP-16 in KMO-90 and KMO-R

Cell line	DPM (μg/ml)	DOX		MIT		VCR		VP-16	
		IC_{50} (ng/ml)[a]	SF[b]	IC_{50} (ng/ml)	SF	IC_{50} (ng/ml)	SF	IC_{50} (ng/ml)	SF
KMO-90	0	39.6 ± 9.10[c]	1.0	10.0 ± 2.13	1.0	2.08 ± 0.10	1.0	86.0 ± 10.2	1.0
	1	7.83 ± 0.97*	5.1	1.44 ± 0.37**	6.9	0.86 ± 0.18**	2.4	47.3 ± 10.6*	1.8
	5	6.50 ± 1.36*	6.1	1.26 ± 0.41**	7.9	0.52 ± 0.09**	4.0	22.8 ± 3.01**	3.8
	10	3.35 ± 0.74*	11.8	1.13 ± 0.27**	8.8	0.39 ± 0.08**	5.3	22.0 ± 6.97**	3.9
KMO-R	0	66.3 ± 8.50	1.0	18.4 ± 1.36	1.0	4.10 ± 0.21	1.0	196.6 ± 47.8	1.0
	1	23.3 ± 2.05*	2.8	2.38 ± 0.13**	7.9	1.24 ± 0.57**	3.3	168.3 ± 39.6	1.2
	5	19.5 ± 0.71*	3.4	2.23 ± 0.04**	8.3	0.67 ± 0.08**	6.1	99.3 ± 28.7	2.0
	10	21.0 ± 0.81*	3.2	2.10 ± 0.24**	8.8	0.59 ± 0.04**	6.9	79.6 ± 12.1*	2.5

[a]IC_{50} was defined as the drug concentration that reduced OD to 50% of that of control cells with no anticancer drug. Cytotoxicity was determined using the MTT assay.

[b]SF, the sensitization factor, was determined by dividing the IC_{50} for each anticancer drug alone by the IC_{50} in the presence of DPM.

[c]Mean ± SD.

*Statistically significant ($P < 0.05$) by Student's t-test compared with that of the control culture containing no DPM.

**Statistically significant ($P < 0.01$).

DOX in KMO-90 ranged from 5.1 to 11.8 in increasing concentrations of DPM (Table I). This showed that KMO-R became more resistant to DPM's improving sensitivity to DOX than did KMO-90. The mechanism for this enhancement resistance is unknown. The same effect in KMO-R was not identified in MIT, VCR, and VP-16.

At 1 μg/ml of DPM, SFs for DOX, MIT, VCR, and VP-16 in KMO-R were 2.8, 7.9, 3.3, and 1.2, respectively, showing that DPM enhanced sensitivity to each drug differently at the same concentration.

DISCUSSION

Our finding that IC_{50} for DOX, MIT, VCR, and VP-16 in KMO-R were about 2-fold higher (1.7–2.3) than those in KMO-90 showed KMO-R to be more resistant to these four drugs than was KMO-90. It was interesting that KMO-R was not only resistant to the chemotherapeutic agent used before relapse (VCR), but also to drugs which were not used (DOX, MIT, VP-16). This finding may highlight a serious problem for treatment of hematopoietic malignancy. Considering that both KMO-90 and KMO-R were established from a patient with ALL at the times of presentation and relapse, respectively, these two cell lines may be novel and useful models for research into the mechanism of acquired drug resistance in ALL cells.

Although its cytotoxicity in KMO-90 was about 6% at 1 μg/ml, DPM enhanced sensitivity to DOX, MIT, VCR, and VP-16 at this concentration. DPM's cytotoxicity in KMO-R was less than 5% at 1, 5, and 10 μg/ml. In KMO-R, DPM enhanced sensitivity to these four drugs in a dose-dependent manner. These data show that DPM enhanced sensitivity to anticancer drugs at noncytotoxic concentrations. Our study further showed that KMO-R became more resistant to DPM's improving sensitivity to DOX than did KMO-90, and that DPM enhanced differently the sensitivities to DOX, MIT, VCR, and VP-16 at the same concentrations in both KMO-90 and KMO-R. These facts must be considered when using DPM clinically as a chemosensitizer. Although the mechanism through which KMO-R became more resistant to DPM's improving sensitivity to DOX was interesting, it remains to be shown whether this mechanism is identical to that through which hematopoietic malignant cells acquire resistance to anticancer drugs.

Although a number of compounds, such as calcium-channel blockers [1,2], calmodulin inhibitors [3], antiarrhythmics [4], and certain hormones [5,6], enhance anticancer drug efficacy in vitro, the optimum concentrations for this enhancement are usually well above the maximally tolerated plasma levels for many of these chemosensitizers. Cyclosporin A has been shown to enhance sensitivity to anticancer drugs at a safely achievable concentration [7,8]. However, we have obtained the data indicating that cyclosporin A does not enhance sensitivity to DOX, MIT, VCR, and VP-16 in both KMO-90 and KMO-R at this concentration (data not shown). The plasma concentration achieved by oral administration of DPM is about 1 μg/ml [19]. We found that at only 1 μg/ml DPM enhanced sensitivity to DOX, MIT, VCR, and VP-16 in both KMO-90 and KMO-R, thus pointing to DPM as a useful agent for potentiating anticancer chemotherapy against resistant hematopoietic malignancies. We also demonstrated that DPM acted as a chemosensitizer in KMO-90 established from a sample at the time of presentation and so may be useful for initial treatment.

DPM inhibits not only nucleoside-membrane transport [12,20] but also cyclic AMP (cAMP) phosphodiesterase [21]. Howell et al. [14] proposed that the effect of DPM as a chemosensitizer did not result from these two mechanisms and suggested that there might be a newly identified mechanism of action for this agent. It also has been

shown that the synergistic effects of DPM with anticancer drugs were the result of increased intracellular concentrations of anticancer drugs [22,23]. Whereas little is known of a relationship between the expression of P-glycoprotein and DPM-improved sensitivity to anticancer drugs, Shalinsky et al. [24] reported that there was a P-glycoprotein-independent mechanism of synergy for DPM in some cases although a P-glycoprotein-dependent mechanism was apparent in other cases. Neither KMO-90 nor KMO-R expressed P-glycoprotein in our study (data not shown) and the mechanism by which DPM enhances cell sensitivity to the four drugs studied in both KMO-90 and KMO-R remains to be defined.

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