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Potentiation of antitumor agents by calcium channel blockers with special reference to cross-resistance patterns

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Summary. *The calcium channel blockers verapamil, diltiazem, nicardipine, and niludipine potentiated the antitumor activities of mitotic poison antitumor agents, such as vincristine, vinblastine, vindesine, VP16-213, and taxol in P388 leukemia cells resistant to vincristine. The potentiating effect was generally dependent on the extent of cross-resistance seen in the cell line for these drugs. Calcium channel blockers also potentiate the antitumor activities of several DNA-interacting drugs, such as adriamycin, THP-adriamycin, daunomycin, aclacinomycin A, mitomycin C, actinomycin D, mitoxantrone, and nogalamycin derivatives in P388 leukemia resistant to adriamycin. Greater potentiation was observed for those antitumor agents to which the ADM-resistant cell line had become markedly cross-resistant, with the exception of the nogalamycin derivatives. Only a two-fold enhancement was observed for mitomycin C and aclacinomycin, as the cell line was only weakly cross-resistant to these agents. These results suggest the potential for therapeutic gain through the use of calcium channel blockers in combination with classic chemotherapeutic agents.*

Introduction

Previous reports from this laboratory described enhancement by calcium channel blockers of the chemotherapeutic effects of vincristine (VCR) and adriamycin (ADM) in drug-resistant tumor cells of mouse and human origin achieved by way of increasing the accumulation of these drugs in the tumor cells [11, 12, 14–16]. These findings have been confirmed by other groups working with murine tumors [10] and human ovarian tumor cells of mouse and human origin achieved by way of cells resistant to vinca alkaloids and anthracycline antibiotics transport these drugs more actively to the outside of cells [2, 5, 9, 12], and this mechanism is thought to play a major role in the cross-resistance between vinca alkaloids and anthracyclines.

As the calcium channel blockers inhibit the VCR and ADM efflux function of resistant tumor cells [11, 12, 14], they might potentiate the effect of other antitumor agents to which VCR- or ADM-resistant cells have become cross-resistant. We examined this possibility in this study and found that the effectiveness of calcium channel blockers on enhancement of the cytotoxicity of various antitumor agents is associated with the cross-resistance patterns of the cell lines.

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Materials and methods

Tumor cells and culture. P388 leukemia cells were supplied by Simonsen Laboratories, Inc., Gilroy, Calif, under the auspices of the National Cancer Institute, NIH, Bethesda, Md. P388 cells resistant to VCR (P388/VCR) and ADM (P388/ADM) were kindly supplied by the Mammalian Genetics and Animal Production Section, Division of Cancer Treatment, National Cancer Institute, NIH.

The growth medium for these tumor cells was RPMI 1640 supplemented with 10% fetal bovine serum (Grand Island Biological Co., Grand Island, NY), 20 μM 2-mercaptoethanol, and kanamycin (100 μg/ml) [11, 12, 14–16]. The cells were maintained in plastic dishes (Corning Glass Works, Corning, NY) in the growth medium at 37° C in a humidified atmosphere of 5% CO_2.

Drugs. All antitumor agents were formulated for clinical or preclinical use. The drugs were obtained from the following sources: VCR and vinblastine (VLB) from Shionogi and Co., Ltd, Osaka, Japan; ADM, mitomycin C (MMC) from Kyowa Hakko Co. Ltd, Tokyo, Japan; actinomycin D (ACT) from Merck and Co., Rahway, NJ; daunomycin (DAU) and tetrahydropyranyladriamycin (THP-ADM) [13] from Meiji Seika Kaisha, Ltd, Tokyo, Japan; mitoxantrone (MIT) from Ledery Japan Co., Ltd, Tokyo, Japan; aclacinomycin A (ACM) from Sanraku Ocean Co., Ltd, Tokyo, Japan; vindesine (VDS) and etoposide (VP16-213) from Shionogi and Co., Ltd, Osaka, Japan.

Menograrol and taxol were kindly provided by Dr M. Suffness, Division of Cancer Treatment, National Cancer Institute, Bethesda, and arugomycin and AG-2 by Dr N, Otake, University of Tokyo. Menogarol, arugomycin, and AG-2 belong to the same class (nogalamycin derivatives) of antitumor agents [3, 4].

Calcium channel blockers [15] were obtained from: verapamil, the Eizai Co., Ltd, Tokyo, Japan; diltiazem, Tanabe Seiyaku Co., Ltd, Osaka, Japan; nicardipine, Yamanocuchi Pharmaceutical Co., Ltd, Tokyo, Japan; niludipine, Bayer AG, Wuppertal-Elberfeld, W. Germany.

Drug treatment. Cells (2×10^4) were cultured at 37° C for 5 h in Falcon No. 2054 culture tubes containing 2 ml growth medium per tube. Then they were treated with graded concentrations of antitumor agents (0.01–10 m*M*) in the absence or presence of a calcium channel blocker. Concentrations of calcium channel blockers were 35 μ*M* for diltiazem and 10 μ*M* for the

other blockers; at these concentrations the drugs were not cytotoxic to tumor cells. The cells were cultured in the presence of the chemotherapeutic drugs with and without calium channel blockers and counted with a Coulter counter 3 days after treatment. Three tubes were used for each drug concentration. The cytotoxic activity of the drug was measured by determining the IC_{50}, which was obtained by plotting the logarithm of the drug concentration versus the growth rate (percentage of control) of the treated cells. The initial cell number was subtracted in the calculation.

Results

Cross-resistance patterns of P388/VCR and P388/ADM to various antitumor agents

VCR, VLB, and VDS showed similar cytotoxicity against P388 leukemia cells (Table 1). VP16-213 and taxol were only weakly cytotoxic against P388 leukemic cells compared with the above three agents. P388/VCR possessed 20-fold resistance to VCR when compared with the IC_{50} value of VCR of P388 cells. The cells were cross-resistant to VLB and especially to VDS. The cells were also resistant to taxol; however, they showed rather marginal corss-resistance to the podophyllotoxin derivative VP16-213.

The cross-resistance patterns in P388/ADM toward DNA-interacting antitumor agents can be classified into three groups (Table 2), i.e., (1) a highly resistant group including THP-ADM, DAU, and MIT; (2) a moderately resistant group including ACT, AG2, and menogarol; and (3) a marginally or non-cross-resistant group including ACM, MMC, and arugomycin. It is of interest that P388/ADM was only marginally cross-resistant to ACM, although ADM and ACM are similar structurally. Arugomycin, AG-2, and menogarol also have similar structures (nogalamycin derivatives), differing in the number of sugar moieties; their cytotoxicity in P388/ADM is dependent on the number of sugar moieties insofar as the drug with fewer sugar moieties in the molecule (menogarol) showed less activity in the adriamycin-resistant P388 cells.

Potentiation of the effect of mitotic poisons by calcium channel blockers

Four calcium channel blockers potentiated the cytotoxic effects of VRC, VLB, and VDS in P388/VCR cells (Table 3). The highest potentiation was obtained for VDS and the lowest, for VLB. This pattern correlates with the extent of cross-resistance of P388/VCR to these agents (Table 1).

Verapamil at 10 μM potentiates the taxol cytotoxicity 21-fold; however, verapamil potentiates the cytotoxicity of VP16-213 only 4.7-fold (Table 4). Of the mitotic poisons tested in P388/VCR, the cells showed the weakest cross-resistance to VP16-213, and this drug's cytotoxicity was potentiated the least by verapamil.

Table 1. Inhibitory effects of mitotic spindle poisons on in vitro growth of mouse leukemia P388 and P388/VCR

Drug	IC_{50} (nM) of the drug against	
	P388	P388/VCR
Vincristine	1.6 ± 0.2[a]	32 ± 2.3 (20)[b]
Vinblastine	0.54 ± 0.1	6.3 ± 0.3 (12)
Vindesine	1.4 ± 0.1	49 ± 3.4 (35)
VP-16-213	29 ± 1.2	140 ± 8.3 (4.8)
Taxol	21 ± 0.1	290 ± 23 (14)

[a] Mean ± SD of three determinations
[b] Figures in parentheses show the resistance index (x-fold) for each drug

Table 2. Inhibitory effects of DNA-interacting agents on in vitro growth of mouse leukemia P388 and P388/ADM

Drug	IC_{50} (nM) of the drug against	
	P388	P388/ADM
Adriamycin	31 ± 1.2[a]	850 ± 97 (27)[b]
THP-Adriamycin	3.4 ± 0.4	105 ± 5.9 (31)
Daunomycin	8.7 ± 0.4	165 ± 19 (19)
Aclacinomycin A	14 ± 2.0	18 ± 0.5 (1.3)
Mitomycin C	38 ± 3.3	58 ± 0.6 (1.5)
Actinomycin D	0.73 ± 0.1	6.9 ± 0.4 (9.5)
Mitoxantrone	0.61 ± 0.1	10 ± 0.6 (16)
Arugomycin	523 ± 23	1,970 ± 107 (3.7)
AG-2	166 ± 24	1,260 ± 78 (7.6)
Menogarol	28 ± 3.6	244 ± 29 (8.7)

[a] Mean ± SD of three determinations
[b] Figures in parentheses show the resistance index (x-fold) for each drug

Table 3. Effect of calcium channel blockers on cytotoxicity of vincristine, vinblastine, and vindesine in P388/VCR cells

Ca channel blocker	Concentration (μM)	IC_{50} (nM) for the drug in P388/VCR cells		
		Vincristine	Vinblastine	Vindesine
None		32 ± 2.3[a]	6.3 ± 0.3	49 ± 3.4
Verapamil	10	0.79 ± 0.1[b] (40)[c]	0.92 ± 0.1 (6.8)	0.42 ± 0.1 (117)
Diltiazem	35	1.1 ± 0.1 (29)	0.75 ± 0.1 (8.4)	0.68 ± 0.1 (72)
Nicardipine	10	0.46 ± 0.1 (70)	0.89 ± 0.1 (7.1)	0.84 ± 0.1 (58)
Niludipine	10	1.3 ± 0.1 (25)	0.88 ± 0.1 (7.2)	1.9 ± 0.1 (26)

[a] Mean ± SD of three determinations
[b] All values obtained in the presence of calcium channel blockers are significantly smaller ($P < 0.05$, Student's t-test) than those obtained for each drug in the absence of calcium channel blockers
[c] Increase in cytotoxicity (x-fold)

Potentiation of the effect of DNA-interacting agents by calcium channel blockers

Calcium channel blockers enhanced the cytotoxicity of DNA-interacting drugs in P388/ADM cells, although the degree of enhancement was less than that observed for VCR in P388/VCR cells (Table 5). Enhancement of the cytotoxicity 9- to 27-fold was observed for ADM. Approximately 8- to 4-fold enhancement occurred with DAU, ACT, and THP-ADM. However, enhancement of the cytotoxicity of ACM and MMC was marginal (around 2-fold), and these were the drugs to which P388/ADM had become only weakly cross-resistant.

Verapamil at 10 μ*M* potentiates the effect of MIT 7.7-fold, and this enhancement is similar to that observed for DAU (Table 6). Among the nogalamycin derivatives, the most potent effect was observed for AG-2 and the weakest effect was for menogarol. This pattern, however, did not correlate well with the cross-resistance of P388/ADM cells to these drugs (Table 2), as the cells were markedly cross-resistant to menogarol.

Table 4. Effect of verapamil on cytotoxicity of VPl6-213 and taxol in P388/VCR cells

Verapamil (μ*M*)	IC_{50} (n*M*) of the drug in P388/VCR cells	
	VP16-213	Taxol
0	140 ± 8.2[a]	290 ± 23
3.5	38 ± 0.2[b]	22 ± 0.8
10	30 ± 0.2 (4.7)[c]	14 ± 0.6 (21)

[a] Mean ± SD of three determinations
[b] All values obtained in the presence of verapamil are significantly smaller ($P < 0.05$, Student's *t*-test) than those obtained for each drug in the absence of verapamil
[c] Increase in cytotoxicity (x-fold)

Discussion

The selection and proliferation of specific drug-resistant tumor cells during treatment are a major problem with current cancer chemotherapy [7, 8]. The utilization of calcium channel blockers might help in overcoming this problem. Clinical trials and preclinical examination based on the clonogenic assay are currently in progress [1, 6]. Modern cancer chemotherapy is seldom confined to the use of a single agent; rather, different types of drugs with different modes of action are used in combination. Thus, it is important to know which drugs' activities can be potentiated by calcium channel blockers. In this study, we found that calcium channel blockers were effective with drugs for which the P388-resistant variants P388/VCR and P388/ADM had become cross-resistant, and the extent of enhancement was roughly dependent on the extent of cross-resistance to the drugs with the exception of the nogalamycin derivatives. This gives a theoretical rationale for using calcium channel blockers with combination chemotherapy which includes mitotic poisons, anthracyclines, and their derivatives.

It is of interest that potentiation of the drug effect was associated with the extent of cross-resistance of P388/ADM or P388/VCR to the drug. Both vinca alkaloids and anthracycline antibiotics are actively transported out of the resistant tumor cells [2, 5, 9, 12], and calcium channel blockers inhibit VCR and ADM efflux from such tumor cells [11 12, 14]. Calcium

Table 5. Effect of calcium channel blockers on cytotoxcity of DNA-interacting agents in P388/ADM cells

Ca channel blocker	Concentration (μ*M*)	IC_{50} (n*M*) of the drug in P388/ADM cells					
		Adriamycin	THP-Adriamycin	Daunomycin	Aclacinomycin A	Mitomycin C	Actinomycin D
None		850 ± 97[a]	105 ± 5.9	165 ± 19	18 ± 0.5	58 ± 0.6	6.9 ± 0.4
Verapamil	10	90 ± 4.2[b] (9.4)[c]	26 ± 0.5 (4.0)	19 ± 1.4 (8.7)	7.2 ± 0.3 (2.5)	24 ± 0.8 (2.4)	1.3 ± 0.2 (5.3)
Diltiazem	35	93 ± 5.2 (9.1)	21 ± 0.3 (5.0)	34 ± 1.5 (4.9)	8.2 ± 0.4 (2.2)	37 ± 1.2 (1.6)	1.4 ± 0.1 (4.9)
Nicardipine	10	31 ± 1.7 (27)	26 ± 1.0 (4.0)	19 ± 0.7 (8.7)	7.3 ± 0.4 (2.5)	27 ± 0.3 (2.1)	0.85 ± 0.2 (7.4)
Niludipine	10	87 ± 3.5 (9.8)	29 ± 1.7 (3.6)	48 ± 3.1 (3.4)	7.9 ± 0.2 (2.3)	25 ± 1.2 (2.3)	0.90 ± 0.1 (7.0)

[a] Mean ± SD of three determinations
[b] All values obtained in the presence of calcium channel blockers are significantly smaller ($P < 0.05$, Student's *t*-test) than those obtained for each drug in the absence of calcium channel blocker
[c] Increase in cytotoxicity (x-fold)

Table 6. Effect of verapamil on cytotoxicity of mitoxantrone and nogalamycin derivatives in P388/ADM cells

Verapamil (μ*M*)	IC_{50} (n*M*) of the drug in P388/ADM cells			
	Mitoxantrone	Arugomycin	AG-2	Menogarol
0	10 ± 0.6[a]	1,970 ± 107	1,260 ± 78	244 ± 29
3.5	1.7 ± 0.1[b]	462 ± 15	213 ± 7	141 ± 10
10	1.3 ± 0.7 (7.7)[c]	351 ± 7 (5.1)	174 ± 5 (7.2)	134 ± 1.6 (1.8)

[a] Mean ± SD of three determinations
[b] All values obtained in the presence of verapamil are significantly smaller ($P < 0.05$, Student's *t*-test) than those obtained for each drug in the absence of verapamil
[c] Increase in cytotoxicity (x-fold)

antagonists were generally more potent in combination with antitumor agents for which cells had become moderately to highly cross-resistant. This suggests that the mechanism of cross-resistance among structurally unrelated antitumor agents could be explained at least partly by efflux mechanisms akin to those operative in ADM- and VCR-resistant tumor cells.

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