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Persistent reversal of P-glycoprotein-mediated daunorubicin resistance by tetrandrine in multidrug-resistant human T lymphoblastoid leukemia MOLT-4 cells

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Abstract

Multidrug resistance (MDR) represents a major problem in cancer chemotherapy. P-glycoprotein (P-gp), the drug efflux pump that mediates this resistance, can be inhibited by compounds with a variety of pharmacological functions, thus circumventing the MDR phenotype. The present study was performed to evaluate a unique MDR-reversal feature of a bisbenzylisoquinoline alkaloid tetrandrine (TET) in a P-gp expressing MOLT-4 MDR line (MOLT-4/DNR) established in our laboratory. Cell viability was determined by an MTT assay. P-gp function was characterized by determining the Rh123 accumulation/efflux capacity. P-gp overexpression in resistant MOLT-4/DNR cells was confirmed by flow cytometry analysis after staining with phycoerythrin-conjugated anti-P-gp monoclonal antibody 17F9. Compared to ciclosporin A (CsA), TET exhibited stronger activity to reverse drug resistance to daunorubicin (DNR), vinblastine (VLB) and doxorubicin (DOX) in MOLT-4/DNR cells. TET showed no cytotoxic effects on parental MOLT-4 cells lacking P-gp expression or on the resistant MOLT-4/DNR cells. TET modulated DNR cytotoxicity even after it was washed with the medium for 24 h, while CsA almost completely lost its reversal capability 24 h after washing. TET and CsA similarly increased the accumulation of Rh123 in resistant MOLT-4/DNR cells. However, TET inhibited Rh123 efflux from resistant cells even after washing with the medium, while CsA rapidly lost its ability to inhibit Rh123 efflux after washing. The current study suggests that TET enhances the cytotoxicity of anticancer drugs in the P-gp expressing MDR cell line by modulating P-gp in a different manner to the well-known P-gp inhibitor CsA.

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Introduction

The success of chemotherapy in human cancer treatment is frequently limited by intrinsic or acquired multidrug resistance (MDR) caused by increased expression of the plasma membrane P-glycoprotein (P-gp) (Naito et al 1992; Fukuda et al 1996). This protein is an ATP-dependent transporter that effluxes a number of structurally unrelated anticancer agents out of cells, thereby reducing intracellular drug concentration, permitting the tumour cells to survive despite high concentrations of drugs that otherwise would be toxic. Inhibiting P-gp function with diverse pharmacological agents circumvents the MDR phenotype. Compounds such as verapamil, ciclosporin A (CsA) and SDZ PSC833, and flavonoids have been reported to modify P-gp and sensitize MDR cells to cytotoxic agents (Boesch et al 1991; Dantzig et al 1996; Di Pietro et al 2002).

Tetrandrine (TET, Figure 1) is a bisbenzylisoquinoline alkaloid isolated from the root of *Stephania tetrandra* S. Moore. It has been shown to enhance the cytotoxicity of anticancer drugs to cells of a colorectal P-gp positive HCT15 cell line, while having no effect on the cytotoxicity of the drugs to P-gp negative SK-OV-3 cells (Choi et al 1998). However, the cause of the TET reversal effect against the P-gp function is not well understood. Information about this unique TET action might be useful in developing an effective strategy against MDR.

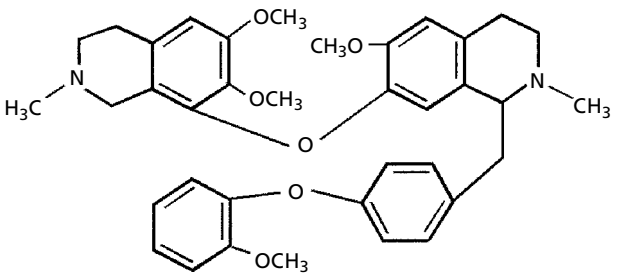

Figure 1 Chemical structure of tetrandrine.

We have developed an MDR cell line MOLT-4/DNR from a T lymphoblastoid leukemia MOLT-4 cell line by exposing the parent cells to increasing concentrations of daunorubicin (DNR) over 3 months (Liu et al 2002). MDR in MOLT-4/DNR has been shown to be closely related to the expression of P-gp and MDR1 mRNA (Liu et al 2002) and therefore this subline is a suitable model for the investigation of the agents that modify P-gp function and drug resistance. Thus, the present study was undertaken to evaluate the TET action that sensitizes this P-gp expressing MDR cell line to anticancer agents, and to provide an insight into the pharmacological modulation of the MDR phenotype.

Materials and Methods

Reagents

RPMI-1640 medium and fetal bovine serum (FBS) were purchased from Gibco BRL Co. (Grand Island, NY). The Cell Proliferation Kit I (MTT) was purchased from Roche Mol. Biochem. (Indianapolis, IN). Trypan blue, rhodamine 123 (Rh123) and anticancer agents daunorubicin (DNR), vinblastine sulfate (VLB) and doxorubicin hydrochloride (DOX) were obtained from Sigma Chemical Co. (St Louis, MO). CsA was a gift from Novartis Pharma. Co. (Basel, Switzerland). TET was purchased from Aldrich Chemical Co. (Milwaukee, WI). Concanavalin A was obtained from Seikagaku Kogyo Co. (Japan). Ficoll-Hypaque was from Nakarai Co. (Japan). [^{3}H]Thymidine (5.55×1011 Bq mmol^{-1}) was from New England Nuclear Corporation (USA). The other reagents were of the best available grade.

Cell lines and culture conditions

Human acute lymphoblastoid leukemia cell line MOLT-4 was purchased from ICN Biomed. Inc. (Tokyo, Japan). The resistant MOLT-4/DNR subline was developed from the parental MOLT-4 cell line by exposing it to increasing concentrations of DNR stepwise from 10 nM to 50 nM over 3 months at our laboratory. These cells were revealed to express 61-fold resistance to DNR compared to the parental cell line, MOLT-4 (Liu et al 2002). The sensitive (parent) and the resistant cells were maintained in RPMI-1640 medium containing 10% FBS, 100 000 IU L^{-1} penicillin and 100 mg L^{-1} streptomycin.

Cell proliferation assays

Cell viability was determined by an MTT assay using the Cell Proliferation Kit I. Cells were harvested during the logarithmic growth phase, washed and resuspended in the medium at a density of 5×10^5 cells mL^{-1}. The viable cells were counted in a hemocytometer using a trypan blue exclusion procedure. Aliquots of 196 μL of this cell suspension were placed in a 96-well flat-bottom plate (Iwaki, Co. Chiba, Japan), and 4 μL of each anticancer drug solution or 2 μL of each anticancer drug solution plus 2 μL of each modulator (i.e. CsA or TET) solution in ethanol were added to give final serial concentrations. Four microlitres of ethanol was added into the control wells. The cells were then incubated for 72 h at 37 °C in a humidified chamber containing 5% CO_2/air. After the incubation period, 10 μL of the MTT reagent was added and the mixture was incubated for a further 4 h. After this 100 μL of the solubilization solution was added to each well, and the plate was allowed to stand for 12–18 h in the incubator. The spectrophotometrical absorbance of the samples was measured using a microplate reader. Absorbance of the formazan product was measured using a wavelength of 570 nm, with a reference wavelength of 630 nm. The dose–response curve was plotted and the concentration that gave 50% cell growth inhibition (IC_{50}) was calculated as described previously (Hirano et al 1994, 1995, 1996).

MDR reversal assays

The experiment was performed as described by Newman et al with slight modifications (Newman et al 2000). In brief, MOLT-4/DNR cells at a density of 5×10^5 cells mL^{-1} in medium were incubated with either 5 μM CsA, or 3 or 0.5 μM TET for 24 h at 37 °C in a humidified chamber containing 5% CO_2/air. Aliquots of ethanol were added to the control. After being washed 0 or 3 times in medium and incubated for 0, 4, 8 or 24 h at 37 °C, cells were washed once in medium and treated with varying concentrations of DNR or vehicle, then incubated for an additional 72 h. Subsequently, cell viability was determined using an MTT assay procedure. The IC_{50} value was calculated from the dose–response curve.

Flow cytometry analysis for P-gp function and expression

Aliquots of 1 mL of cell suspension containing 5×10^5 cells were incubated with 2 μM Rh123 dye in the presence or absence of CsA or TET for 1 h at 37 °C in a humidified chamber containing 5% CO_2/air. The incubations were terminated by rapid chilling with ice-cold medium and two wash steps. The cells were then resuspended in ice-cold phosphate-buffered saline and kept on ice in the

dark until analysis. The intracellular Rh123 mean fluorescence intensity was analyzed using a FACSCalibur analyzer (Becton Dickinson, CA). Green fluorescence for the gated population was collected with a bandpass filter (FL1) to obtain histogram plot data. These data were further calculated using CellQuest software (Becton Dickinson) to obtain the mean intracellular Rh123 fluorescence intensity for each sample.

Double labelling was performed with Rh123 and phycoerythrin-conjugated mouse anti-P-gp monoclonal antibody 17F9 (PharMingen Co., San Diego). Cells were initially stained with Rh123 as described above. Following 40 min of dye efflux in Rh123-free medium with or without modulator, the cells were washed twice in ice-cold PBS and treated with 20 μL of phycoerythrin-conjugated mouse anti-P-gp monoclonal antibody 17F9 or 20 μL of phycoerythrin-conjugated mouse IgG2b isotype control monoclonal antibody (PharMingen) for 30 min at 4 °C. After incubation, cells were washed twice in ice-cold PBS and kept on ice in the dark until analysis. The spectral overlap between Rh123 and phycoerythrin was electronically compensated for using cells stained only with Rh123 or phycoerythrin-conjugated antibody 17F9 to establish the degree of electronic subtraction. Samples were analyzed with a FACSCalibur analyzer (Becton Dickinson, CA), with Rh123 collected in FL1 and phycoerythrin (PE) in FL2 to obtain contour intensity maps. The wavelengths of FL1 and FL2 were 515–545 and 564–606 nm, respectively. A total of 20 000 non-gated cells was analyzed.

Isolation and culture of peripheral blood mononuclear cells

After informed consent was obtained, 20 mL of venous blood was taken from a healthy subject and heparinized. The heparinized blood was loaded onto 6 mL of Ficoll-Hypaque, centrifuged at 1300 × *g* for 15 min, and peripheral blood mononuclear cells (PBMCs) were separated as described previously (Hirano et al 2000). The cells were washed and resuspended in RPMI 1640 medium containing 10% fetal calf serum, 100 000 IU L^{-1} penicillin and 100 mg L^{-1} streptomycin to a final density of 1×10^6 cells mL^{-1}. Two hundred microlitres of this cell suspension was placed into each of the 96 flat-bottomed wells of a microtiter plate. Concanavalin A was added to each well as the mitogen to a final concentration of 5.0 μg mL^{-1}. Subsequently, 4 μL of an ethanol solution containing each agent was added to give corresponding final agent concentrations. Four microlitres of ethanol were added to a control well. The plate was incubated for 96 h in 5% CO_2/air at 37 °C. The cells were pulsed with 18.5 KBq $well^{-1}$ of [^{3}H]thymidine for the last 16 h of incubation and then collected on glass-fibre filter paper (Futaba Medical Co., Japan) using a multiharvester device (Labo Science Co., Japan) and dried. The radioactivity retained on the filter was further processed for liquid scintillation counting. The liquid scintillator was 4% omnifluor (Packard Instrument Co., USA) dissolved in toluene. The mean of the counts for duplicates of each sample was determined. The percentages of PBMC-blastogenesis in the presence of the agent were calculated.

Statistical analysis

For comparison of means, the data were first analyzed by one-way ANOVA. Statistics were carried out using the Bonferroni/Dun multiple comparison and the Kruskal–Wallis test. Calculated P values of less than 0.05 were considered to be significant.

Results

Modulation of drug resistance by TET

The IC_{50} values for DNR, VLB and DOX against the in-vitro growth of parent MOLT-4 cells were 36.3, 6.5 and 31.5 nM, respectively, whereas the IC_{50} values for these drugs against the growth of resistant MOLT-4/DNR cells were 2210, 522 and 949 nM, and were 60.9, 80.4 and 23.9 times higher than those against the growth of parent cells. The effects of the modulator on the cytotoxicity of anticancer drugs were calculated as the 'fold shift', which is the ratio of the IC_{50} measured from the absence up to the presence of the modulator. The effects of TET on the cytotoxicity of these anticancer drugs against MDR cells were examined and compared to those of a well-known P-gp modulator, CsA (Sikic 1997).

As shown in Table 1, 5 μM CsA enhanced the cytotoxicity of DNR, VLB and DOX from 22- to 66-fold against the drug-resistant MOLT-4/DNR cells. In contrast, the same dose of TET enhanced the cytotoxicity of these agents by 24- to 83-fold. Even 3 μM TET showed a higher activity of restoring the sensitivity of the resistant cells to the anticancer drugs than that of 5 μM CsA. The modulating activity of TET at 0.5 μM was approximately 50% as that of the same agent at 5 μM whereas the fold shift of CsA at 0.5 μM was 1.7, and thus CsA at this concentration exhibited little effect to restore the sensitivity of the resistant cells to DNR. The presence of 5 μM CsA or TET had little to no effect on the cytotoxicity of these anticancer drugs to cells of the parental MOLT-4 line. The IC_{50} values for TET alone against growth of MOLT-4/DNR and its parental MOLT-4 cells as assessed with an MTT assay were 17.2 and 16.7 μM, respectively. Similarly, the IC_{50} values for CsA against the growth of these cells were 13.5 and 14.5 μM, respectively. Thus, the cytotoxicity of TET as well as of CsA appeared to be low, and was equal to that of MOLT-4/DNR cells and parent MOLT-4 cells.

Duration of TET action

The length of time TET was active as a modulator was evaluated and compared with the action of CsA in MOLT-4/DNR cells. The results demonstrate that the modulating activity of 5 μM CsA reduces significantly and rapidly after it has been washed out from the medium (Table 2). The fold shift of DNR IC_{50} changed from 53-fold, determined in the presence of the drug, to 3-fold,

Table 1 Modulation of cytotoxicity of MOLT-4 and MOLT-4/DNR cells.

	Modulator	Concn (μM)	DNR		VLB		DOX	
			IC_{50} (nM)	Fold shift	IC_{50} (nM)	Fold shift	IC_{50} (nM)	Fold shift
MOLT-4								
	None		36.3 (4.6)	–	6.5 (2.1)	–	31.5 (14.3)	–
	CsA	5	34.7 (16.9)	1.1 (0.1)	5.7 (2.7)	1.1 (0.4)	31.6 (23.7)	1.0 (0.7)
	TET	5	37.8 (9.4)	1.0 (0.5)	5.0 (1.3)	1.3 (0.2)	29.2 (9.3)	1.1 (0.7)
		3	34.2 (20.8)	1.1 (0.3)	5.3 (3.3)	1.2 (0.5)	30.7 (14.8)	1.0 (0.4)
MOLT-4/DNR								
	None		2210.1 (233.0)	–	522.4 (244.9)	–	746.4 (173.9)	–
	CsA	5	56.2 (23.7)	39.3 (25.2)	7.9 (4.9)	66.1 (48.5)	33.6 (7.3)	22.2 (5.0)
	TET	5	33.9 (26.6)	65.2 (42.8)	6.3 (1.9)	82.9 (29.7)	31.1 (18.1)	24.0 (16.7)
		3	38.8 (26.7)	57.0 (46.2)	6.7 (2.5)	78.8 (33.3)	33.0 (22.7)	22.6 (15.7)
		1	43.3 (19.0)	51.0 (28.5)	10.5 (5.8)	49.8 (25.6)	58.5 (24.8)	12.8 (6.9)
		0.5	71.8 (4.4)	30.8 (1.8)	14.2 (7.5)	36.8 (18.0)	65.2 (31.5)	11.4 (6.5)

Fold shift is the ratio of the IC_{50} measured in the absence (none) to the presence of the modulator. Values are the means determined in three independent experiments. Numbers in parentheses indicate standard deviations.

determined immediately after washing of CsA, and the modulating activity of CsA had almost disappeared by 4 h after its removal from the medium. The reversal activity of 3 μM TET, on the other hand, persisted throughout the 24-h time period after its removal from the medium. In contrast, a significant reduction in modulating activity was seen after washing TET when the cells were treated with 0.5 μM TET. However, a somewhat stronger activity was observed with 0.5 μM TET as compared to 5 μM CsA after washing these agents from the medium (Table 2).

P-gp function and expression

P-gp function was characterized by determining the Rh123 accumulation/efflux capacity. In Rh123 accumulation studies, resistant MOLT-4/DNR cells were shown to decrease accumulation of Rh123 (Figure 2). The average percentage of Rh123 accumulation was 23% compared with that of the parental MOLT-4 cells (100%). Three microlitres and 5 μM of TET significantly increased Rh123 accumulation about 3.7- to 4.0-fold compared to the control ($P = 0.0025$), as analyzed by the Bonferroni/Dun multiple comparison ($P = 0.056$ by the Kruskal–Wallis test), and CsA showed similar activity ($P = 0.0074$ by the Bonferroni/Dun multiple comparison and $P = 0.061$ by the Kruskal–Wallis test; Figure 2B). In parental MOLT-4 cells, TET and CsA had no effect on Rh123 accumulation under the experimental conditions (Figure 2A). To ensure that increased resistance to anticancer drugs and decreased Rh123 accumulation in MOLT-4/DNR cells was due to P-gp overexpression, P-gp expression was determined with flow cytometry analysis using a monoclonal antibody specific to P-gp, PE-conjugated 17F9 (Aihara et al 1991). Parental MOLT-4 cells did not express P-gp, while P-gp expression was observed in resistant MOLT-4/DNR cells. In the presence of TET and CsA, Rh123 efflux was inhibited in resistant MOLT-4/DNR cells, while the retention of Rh123 distinctly decreased after washing CsA. However, a prolonged inhibition of Rh123 efflux was observed following cell treatment with TET and after washing TET. In contrast, no dye efflux was observed in sensitive MOLT-4 cells.

Effects of TET on mitogen-induced blastogenesis of human PBMCs

To examine TET effects on normal human immune-response in vitro, PBMCs obtained from a healthy subject were cultured in the presence of concanavalin A as a mitogen and 0.5–5 μM TET, and the blastogenesis of PBMCs was estimated (Figure 3A). TET exhibited little or no effect on the blastogenesis of PBMCs at these concentrations. We further examined the modifying effects of TET on the immunotoxicity of DNR. As shown in Figure 3B, TET at 1, 3 and 5 μM did not influence the suppressive effect of DNR against the blastogenesis of PBMCs.

Table 2 Persistence of MDR reversal in MOLT-4/DNR cells after incubation and washout of CsA and TET.

Treatment schedule	DNR IC_{50} (nM)	Fold shift
Control	2300.3	
	(2339.0)	
CsA 5 μM		
No wash, 0 h	43.1	53.4
	(17.0)	(32.5)
Wash, 0 h	840.4	2.7
	(70.0)	(0.2)
Wash, 4 h	1800.0	1.3
	(794.0)	(0.9)
TET 0.5 μM		
No wash, 0 h	73.5	31.3
	(21.7)	(11.6)
Wash, 0 h	445.2	5.2
	(66.5)	(0.8)
Wash, 4 h	922.7	2.5
	(405.5)	(1.3)
TET 3 μM		
No wash, 0 h	36.1	63.7
	(15.0)	(32.9)
Wash, 0 h	39.5	58.2
	(13.7)	(37.3)
Wash, 4 h	41.3	55.7
	(17.1)	(37.3)
Wash, 8 h	49.2	46.8
	(3.0)	(3.0)
Wash, 24 h	58.8	39.1
	(16.8)	(13.8)

The fold shift is the ratio of the IC_{50} measured in the absence (control) to the presence of the modulator. Each of the values represent the mean in triplicate experiments. Numbers in parentheses indicate standard deviations.

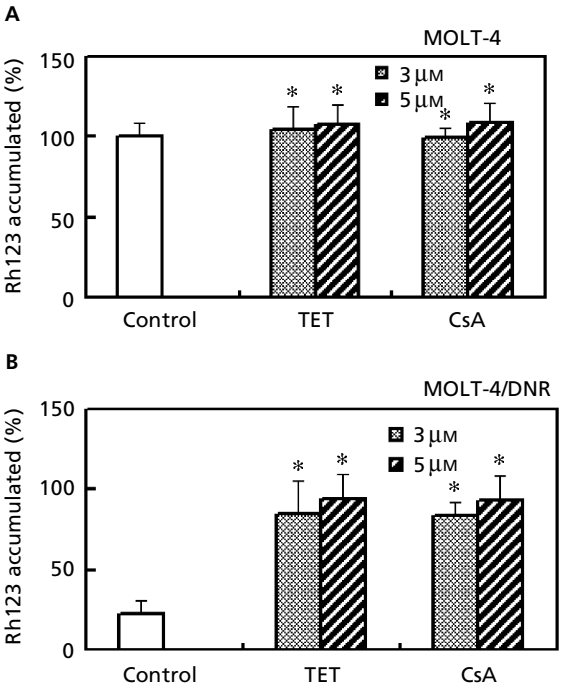

Figure 2 Histograms of Rh123 accumulation in (A) the parental MOLT-4 cells and (B) the resistant MOLT-4/DNR cells in the presence or absence of modulator. Cells were stained with Rh123 for 1 h with or without TET or CsA, washed and Rh123 mean fluorescence intensities were detected by flow cytometry analysis. The mean fluorescence intensities were normalized to the parental MOLT-4 cell level (100%), and the results expressed as the means obtained from three independent experiments; bars, SD. *TET vs control: $P = 0.0025$ by the Bonferroni/Dun multiple comparison, $P = 0.056$ by the Kruskal–Wallis test. *CyA vs control: $P = 0.0074$ by the Bonferroni/Dun multiple comparison and $P = 0.061$ by the Kruskal–Wallis test.

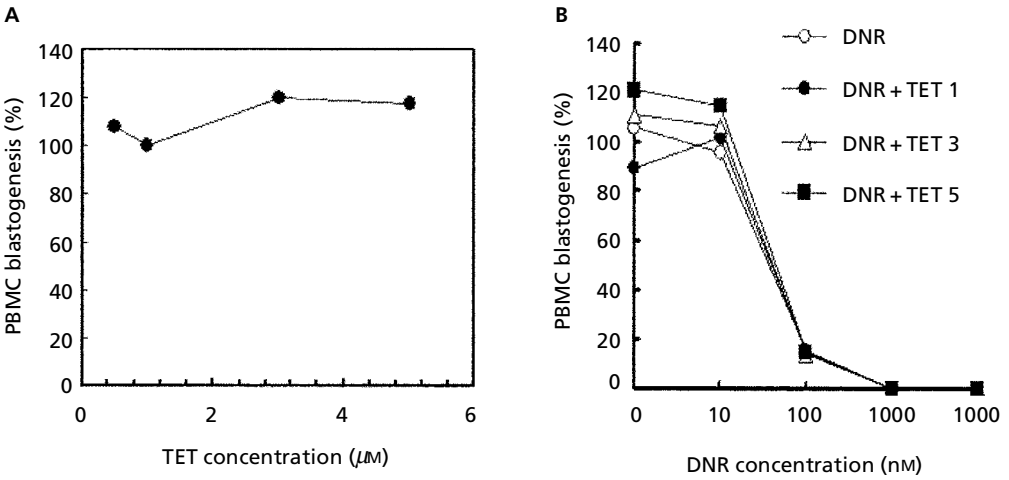

Figure 3 Effects of (A) TET alone or (B) in combination with DNR on the blastogenesis of PBMCs from a healthy subject. In (B) the blastogenesis-suppressive effects of DNR alone at 1–10 000 nM and those of DNR at these concentrations in the presence of 1, 3 and 5 μM TET were compared. Data are the means of two independent experiments.

Discussion

As described above, TET showed a greater enhancement of the cytotoxicity of DNR, VBL and DOX against resistant MOLT-4/DNR cells compared to the same dose of CsA. In contrast, TET at 5 μM exhibited no toxic effect on the ability of normal human PBMCs to proliferate in response to the stimulation by a T-cell mitogen, concanavalin A. In addition, TET shows no apparent effect on the cytotoxicity of DNR against PBMCs.

The MOLT-4/DNR cell line was developed from the parental MOLT-4 cell line by exposing cells to increasing concentrations of DNR stepwise over 3 months in our laboratory (Liu et al 2002). This cell line has been shown to express high amounts of P-gp and MDR1 mRNA, and this is closely related to its resistance to the cytotoxic effects of DNR (Liu et al 2002). TET at 5 μM fully reversed the multidrug resistance in the MOLT-4/DNR cell line, and it still kept 50% of its reversal activity at 0.5 μM. Furthermore, the reversal activity of TET in DNR cytotoxicity assays against MOLT-4/DNR cells persisted for more than 24 h, which suggested that TET remains in the cells in an active form for prolonged periods of time after its removal from the medium. On the other hand, CsA lost its ability to sensitize DNR resistant cells after being removed from the medium 24 h later. It has been reported that CsA reverses the MDR phenotype by binding directly to P-gp (Foxwell et al 1989), and the MDR-reversing effect is lost immediately upon cell wash (Saeki et al 1993; Boesch & Loor 1994; Archinal-Mattheis et al 1995; Newman et al 2000). Our present results confirm these observations. The results also demonstrate that the concentrations of TET required to reverse drug resistance in vitro are much lower than its cytotoxic concentrations in the resistant MOLT-4/DNR line.

Rh123 has been observed to accumulate in the mitochondria of cells and is used as a standard functional indicator of MDR (Chaudhary & Roninson 1991). MDR modulators, such as verapamil and CsA, inhibit Rh123 efflux and increase Rh123 accumulation in P-gp expressing cells (Ludescher et al 1992). TET, as for CsA, significantly enhanced the accumulation of Rh123 in MOLT-4/DNR cells in comparison with the control, and had no effect on Rh123 accumulation in the parental MOLT-4 cells. On the other hand, the efflux of Rh123 was inhibited in the presence of TET and CsA in MOLT-4/DNR cells after being incubated in Rh123-free medium, while TET continued to inhibit the efflux of Rh123 from cells even after it had been washed from the efflux medium. This finding is consistent with our present observation that the reversal activity of TET against DNR resistance is of long duration in DNR cytotoxicity assays using MOLT-4/DNR cells whereas CsA rapidly loses its function of inhibiting Rh123 efflux after it is washed from the efflux medium.

A number of non-cytotoxic compounds have been found to sensitize MDR cells to anticancer reagents. First-generation modulators were developed from their therapeutic indications, such as verapamil and CsA (Dantzig et al 1996). Second-generation modulators, such as SDZ PSC833 (Boesch et al 1991) and VX710 (Germann et al 1997), have more potential than the first generation and lack their own pharmacological activity other than P-gp modulating ability. Third-generation modulators, such as LY335979 (Dantzig et al 1996) and OC144-093 (Newman et al 2000), are novel inhibitors of P-gp and have no significant pharmacokinetic interaction with anticancer drugs, unlike some of the second-generation modulators. The comparison of TET and CsA as P-gp modulators in the present study gave some insight into TET's action mechanism as one of the unique MDR reversing agents. The current study suggests that TET is not actively removed from the cell as a substrate of P-gp or is only a very weak substrate, and that it possesses a higher affinity for P-gp compared to CsA, which may result in prolonging the suppression of P-gp function after washing the cells. These characteristics of TET suggest that the agent will be an efficient reversing agent against P-gp related MDR in leukemia cells compared to other P-gp modulators, such as CsA, that are transported by P-gp.

Conclusions

The present study was performed to evaluate a unique MDR-reversal feature of a bisbenzylisoquinoline alkaloid tetrandrine (TET) in a P-gp expressing MOLT-4 MDR line (MOLT-4/DNR) established in our laboratory. TET showed greater enhancement of the cytotoxicity of DNR, VBL and DOX against resistant MOLT-4/DNR cells compared to the same dose of CsA. In contrast, TET exhibits no toxic effect on the ability of normal human PBMCs to proliferate in response to stimulation by a T-cell mitogen concanavalin A. In addition, TET shows no apparent effect on the cytotoxicity of DNR against PBMCs. We conclude that TET enhances the cytotoxicity of anticancer drugs by persistently inhibiting P-gp function in a different manner to the well-known P-gp inhibitor CsA in cells of the MOLT-4/DNR cell line. The results also raised the possibility that TET will be an efficient reversing agent against P-gp-related MDR compared to other P-gp modulators, such as CsA, that are transported by P-gp.

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