Prognostic Significance of Multidrug Resistance Protein in Adult T-cell Leukemia

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ABSTRACT

The response of adult T-cell leukemia (ATL) to chemotherapy is poor, and a major obstacle to successful treatment is intrinsic or acquired drug resistance. To determine the clinical significance of multidrug resistance protein (MRP) 1 in ATL, we studied MRP1 expression and its association with clinical outcome. The expression of MRP1 mRNA in leukemia cells from 48 ATL patients was studied by slot blot analysis. The expression level of MRP1 mRNA in chronic-type ATL was significantly higher than that in lymphoma-type ATL (P = 0.033). There was no correlation between MRP1 expression and age, gender, WBC count, LDH, hypercalcemia, blood urea nitrogen, or performance status. However, the expression of MRP1 mRNA correlated only with peripheral blood abnormal lymphocyte counts (P = 0.008). The transporting activity of MRP1 was assessed using membrane vesicles. Membrane vesicles prepared from ATL cells with high expression of MRP1 mRNA showed a higher ATP-dependent leukotriene C4 uptake than did those with low expression of MRP1 mRNA. This uptake was almost completely inhibited by LTD4 antagonists ONO-1078 and MK571. In acute- and lymphoma-type ATL, high expression of MRP1 mRNA at diagnosis correlated with shorter survival, and Cox regression analysis revealed that MRP1 expression was an independent prognostic factor. These findings suggest that functionally active MRP1 is expressed in some ATL cells and that it is involved in drug resistance and has a possible causal relationship with poor prognosis in ATL. Multidrug resistance-reversing agents, such as ONO-1078 and MK571, that directly interact and inhibit the transporting activity of MRP1 may be useful for treating ATL patients.

INTRODUCTION

ATL3 is thought to be caused by HTLV-I; however, the mechanisms of leukemogenesis are not fully understood. ATL is divided into four clinical subtypes (smoldering, chronic, lymphoma, and acute ATL; Ref. 1). Although smoldering ATL and chronic ATL have a mild clinical course without intensive chemotherapy, the outcome of chemotherapy for acute and lymphoma ATL remains poor even when regimens of conventional chemotherapy for malignant lymphoma (2) or regimens of weekly exchanged, non-cross-resistant drugs (3) are performed. The poor response to chemotherapy may be attributed mainly to the resistance of ATL cells to chemotherapeutic drugs. Leukemia cells are often refractory to chemotherapy at initial presentation and acquire resistance when ATL patients are at their terminal or relapsed stages (4). One of the mechanisms for drug resistance is overexpression of a membrane glycoprotein termed P-gp (4). Overexpression of P-gp in ATL cells was observed in 8 of 20 ATL patients at initial presentation and in all ATL patients at relapse (4). Enhanced MDR1 mRNA expression was observed in 9 of 10 HTLV-I-infected subjects (5). However, Ikeda et al. (6) could not detect MDR1 mRNA expression in 28 ATL patients by semiquantitative reverse transcription-PCR.

Another factor involved in MDR is MRP1. MRP1 cDNA was cloned by Cole et al. (7) from an Adriamycin-resistant lung cancer cell line (H69) and sequenced. MRP1 is a M, 190,000 membrane protein belonging to the ATP-binding cassette (ABC) transporter superfamily. It transports some glutathione, glucuronate, and sulfate conjugates (8) and confers MDR (9). PBMCs from acute- and chronic-type ATL patients reportedly expressed significantly higher levels of MRP1 and lung resistance-associated protein mRNA than did normal PBMCs (6).

In this study, we show that high expression of MRP1 was associated with unfavorable clinical outcome in ATL. MRP1 expressed in ATL cells has transporting activity that is inhibited by some agents. These agents may be useful in reversing MRP1-mediated MDR in ATL cells.

The abbreviations used are: ATL, adult T-cell leukemia; MRP, multidrug resistance protein; HTLV-I, human T-cell lymphotrophic virus type I; P-gp, P-glycoprotein; PBMC, peripheral blood mononuclear cell; MDR, multidrug resistance; PS, performance status; cMOAT, canalicular multispecific organic anion transporter; LT, leukotriene; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; PVDF, polyvinylidene difluoride; VCR, vincristine; AML, acute myelogenous leukemia; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; DIG, digoxigenin.
Table 1  Patient characteristics

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a Data were not available from all patients.
b Caused by hypercalcemia.

MATERIALS AND METHODS

Patients. Between July 1989 and July 1998, we studied 48 ATL patients (27 males and 21 females) with a mean age of 62.5 years (age range, 41–86 years). According to previously reported diagnostic criteria (1), 32 of these patients had acute-type ATL, 9 had chronic-type ATL, and 7 had lymphoma-type ATL. Ten patients had hypercalcemia (corrected Ca level with serum albumin, >5.5 meq/liter). PS was based on the five-grade scale of the WHO. During this study, we treated acute- and lymphoma-type ATL patients using combined chemotherapy regimens such as the response-oriented multidrug protocol (3), CHOP (cyclophosphamide, doxorubicin, VCR, and prednisone) protocol, or LSG15 protocol (10). There were no significant survival differences among these protocols in this study (data not shown). Response was evaluated according to previously reported criteria (10). All chronic-type ATL patients did not require treatment with intensive chemotherapy. After informed consent, 43 samples were obtained at initial presentation, and 5 samples were obtained at relapse.

Leukemia Cells. PBMNCs separated by Ficoll-Conray density gradient centrifugation were collected from 26 patients with acute-type ATL and 9 patients with chronic-type ATL, and lymph node samples were taken from 6 patients with acute-type ATL and 7 patients with lymphoma-type ATL. The characteristics of the patients are summarized in Table 1. All samples contained >80% abnormal lymphocytes, and they were stored at −110°C. We also obtained PBMNCs from healthy non-HTLV-I carriers as healthy controls.

Cell Lines and Cell Culture. Human epidermoid KB carcinoma cells were grown as monolayers in MEM (Nissui Seiyaku, Tokyo, Japan) containing 10% newborn calf serum (Sera-Lab, Crawley Down, United Kingdom), 1 mg/ml bacteropentone (Difco, Detroit, MI), 0.292 mg/ml glutamine, and 100 IU/ml penicillin. KB cells were subcloned twice, and a single recloned line, KB-3-1 (11), was used as a drug-sensitive parental cell line. CV60 cells (12) overexpress MRP1 and were maintained in MEM with 1 μg/ml cepharanthine and 60 ng/ml VCR. KB-C2 cells that overexpress P-gp were originally isolated from KB-3-1 cells with increasing concentrations of colchicine and maintained in medium containing 2 μg/ml colchicine. KB/MRP1 (13) was obtained from Dr. K. Ueda (Kyoto University, Kyoto, Japan). LLC-PK1/cMOAT cells, pig kidney epithelial LLC-PK1 cells transfected with human cMOAT cDNA, were obtained from Dr. M. Kuwano (Kyushu University, Fukuoka, Japan). KUT-1 (14) is a HTLV-I-infected cell line established from the PBMNCs of an ATL patient.

Chemicals. 4-Oxo-8-[p-(4-phenylbutyloxy)benzoylamino]-2-(tetrazol-5-yl)-4H-1-benzopyran hemihydrate (ONO-1078) was obtained from Ono Pharmaceutical Co. Ltd, and 3-[[3-[[7-chloro-2-quinolinyl]ethyl]phenyl]-[3(dimethylamino-3-oxopropyl-thio)-thio]-methyl][thio]propanoic acid (MK571; Ref. 15) was kindly provided by Dr. A. W. Ford-Hutchinson (Merck-Frosst Center for Therapeutic Research, Pointe Claire-Dorval, Canada). QCRL-1 and QCRL-3 (16), monoclonal antibodies against murine MRP1, were obtained from Dr. Susan P. C. Cole. [3H]LTC4 (150 Ci/mmol) was from DuPont New England Nuclear (Boston, MA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Probe Preparation. To prepare RNA probes for Northern and slot blotting, the 3′-end PstI fragment (660 bp) of human MRP1 cDNA and a 982-bp of the human G3PDH cDNA fragment corresponding to G3PDH nucleotides 6 through 987 obtained by reverse transcription-PCR and a 832-bp fragment (nucleotides 1885 through 2716) of the human MDR1 cDNA were inserted into the pT7T318U multifunctional Phagemid (nucleotides 1885 through 2716) of the human MRP1 cDNA and a 982-bp fragment corresponding to G3PDH nucleotides 6 through 987 obtained by reverse transcription-PCR and a 832-bp fragment (nucleotides 1885 through 2716) of the human G3PDH cDNA were inserted into the pT7T318U multifunctional Phagemid and Bluescript II SK+ (Pharmacia, Uppsala, Sweden) vectors. After the vectors were linearized, single-strand RNA probes were prepared using a DIG RNA labeling kit (Boehringer Mannheim, Mannheim, Germany).

Slot and Northern Blot Analyses. Total cellular RNA was extracted in a single step using Trizol reagent (Life Technologies, Inc., Rockville, MD). The integrity of isolated RNA was monitored by ethidium bromide staining after gel electrophoresis. For slot blotting, 1 and 5 μg of RNA were applied to a nylon membrane (Hybond N+) under a vacuum, and the membrane was dried at room temperature and then fixed with UV irradiation. The membrane was prehybridized for 1 h at 68°C in 50% formamide, 5× SSC [1× SSC = 0.15 m NaCl/15 mM sodium citrate (pH 7.0)], 1% SDS, and 0.05 mg/ml yeast tRNA. The membrane was then hybridized for 16 h at 68°C in 50% formamide, 1% SDS, 5× SSC, 0.05 mg/ml tRNA, and 100 ng/ml DIG-labeled RNA probe.

After hybridization, the membrane was washed twice with 2× SSC and 0.1% SDS for 5 min at room temperature and then washed twice with 0.1× SSC and 0.1% SDS for 1 h at 68°C.
The blot was detected using alkaline phosphatase-labeled anti-DIG antibody and chemiluminescent substrate according to the manufacturer’s instructions (Boehringer Mannheim, Mannheim, Germany). The density of the MRP1 mRNA signal on the autoradiograms was determined using the GS-525 Molecular Imager System (Bio-Rad, Hercules, CA), and the density of the signals for 5 μg of total RNA from KB-3-1 cells was arbitrarily assigned a value of 1 unit. The data are shown relative to MRP1 mRNA in KB-3-1 cells.

For Northern blotting, polyadenylated RNA was extracted from total RNA with a BioMag mRNA Purification Kit (Perseptive Biosystem, Framingham, MA). Polyadenylated RNA (1 μg) was resolved by electrophoresis on a formaldehyde-agarose denaturing gel. After blotting onto a nylon membrane (Hybond N+), hybridization was carried out with the same protocol used for slot blot analysis.

Membrane Vesicle Preparation. Membrane vesicles were prepared from KUT-1, LLC-PK1/cMOAT, and ATL cells as described previously (17). Cells were washed once and resuspended into PBS containing 1% aprotinin (Sigma Chemical Co.). The cells were washed in PBS, centrifuged (4,000 rpm, 10 min), and then suspended in buffer A [1 mM Tris-HCl (pH 7.5), 0.025 M sucrose, and 0.2 mM CaCl2] and equilibrated at 4°C under a nitrogen pressure of 30 kg/cm2 for 15 min. The mixture was then diluted twice with buffer B [10 mM Tris-HCl (pH 7.5) and 0.25 mM sucrose] and centrifuged at 1,000 × g for 10 min to remove nuclei and unlysed cells. EDTA was added to the lysed cell suspension to a final concentration of 1 mM. The supernatant was layered onto a 35% sucrose cushion containing 10 mM Tris-HCl (pH 7.5), 0.25 M sucrose, and 0.2 mM CaCl2, and the radioactivity was determined using a ECL Western blotting detection system (Amersham). The PVDF membrane was rinsed once for 15 min and four times for 5 min with buffer C and then evenly coated using the ECL Western blotting detection system (Amersham) for 1 min. The membrane was immediately exposed to Fuji Rx-U medical X-ray film at room temperature for various periods in a film cassette.

Transport of [3H]LTC4 into Membrane Vesicles. LTC4 uptake by the vesicles was measured by filtration as described by Ishikawa (19). The standard incubation medium contained membrane vesicles (25 μg of protein), 0.25 M sucrose, 10 mM Tris-HCl (pH 7.4), 10 mM MgCl2, 1 mM ATP or AMP, 10 mM creatine phosphate, and 100 mg/ml creatine kinase in a final volume of 50 μl. The reaction was carried out at 37°C and stopped with 1 ml of ice-cold stop solution [0.25 M sucrose, 100 mM NaCl, and 10 mM Tris-HCl (pH 7.4)]. The diluted samples were passed through Millipore filters (GVWP, 0.22-mm pore size) under light suction. The filters were washed, and radioactivity was measured by liquid scintillation counting.

Drug Accumulation. Intracellular VCR accumulation in KUT-1 and ATL cells was measured as described previously (20). Cells were incubated with 1 mM [3H]VCR in RPMI 1640 with or without ONO-1078 (100 μM) and MK571 (20 μM) for 60 min at 37°C. After washing twice with ice-cold PBS, cells were solubilized in 1% Triton X-100 and 0.2% SDS in 10 mM phosphate buffer (pH 7.4), and the radioactivity was determined.

Statistical Analysis. Differences between groups were analyzed by one-way ANOVA or Student’s t test. P < 0.05 was considered significant. We used the Kaplan-Meier method to estimate survival rates and the log-rank test to compare the two groups for difference in survival rates. Survival duration was measured from the time of chemotherapy to the time of death. The Cox proportional hazards model was used in the univariate and multivariate survival analyses. Maximum likelihood parameter estimates and likelihood ratio statistics were obtained. We
calculated Wald-type confidence intervals. All Ps presented were two-sided.

RESULTS

MRP1 mRNA Expression in ATL Cells. The levels of MRP1 mRNA in 48 ATL samples, in KB cell lines, and in KUT-1 were examined by slot blotting. The expression levels were examined by slot blotting. The results of typical slot blots are shown in Fig. 1. The density of the signal for 5 μg of total RNA from KB-3-1 cells was arbitrarily assigned a value of 1 unit. ATL cells from cases 1, 13, 24, 27, 38, 42, 44, and 46 expressed 0.24-, 1.38-, 3.70-, 8.75-, 2.71-, 0.01-, 0.24-, and 1.08-fold higher levels of MRP1 mRNA, respectively, than did KB-3-1 cells. A healthy control was 0.87 (Fig. 1).

The specificity of the probe was confirmed by Northern blots. A 6.5-kb MRP1 mRNA was detected in the examined samples (samples 14, 25, and 46) and in CV60 and KB-3-1 cells (Fig. 2).

The expression levels of MRP1 mRNA in all ATL samples were quantified by slot blot analysis. The expression levels ranged from 0.10–8.75 unit(s) (median, 1.53 units). The expression levels were not significantly different from those in healthy controls (Fig. 3A). The median expression level in acute, chronic, and lymphoma ATL was 1.53 ± 2.12, 2.70 ± 2.14, and 1.06 ± 0.62, respectively. The expression levels in chronic-type ATL were significantly higher than those in lymphoma-type ATL (P = 0.033; Fig. 3B).

Next we studied whether MRP1 expression was correlated with clinical and laboratory parameters. The expression of MRP1 mRNA was unrelated to age, gender, WBC counts, LDH, hypercalcemia, BUN, and PS (data not shown). However, MRP1 expression was significantly correlated with peripheral blood abnormal lymphocyte counts (P = 0.008; Fig. 3C).

[3H]LTC4 Uptake in Membrane Vesicles. To investigate whether the expressed MRP1 was functional in ATL cells,
we examined ATP-dependent [3H]LTC4 uptake in membrane vesicles from KUT-1 and PBMCs from two ATL patients (patients 4 and 25; Fig. 4A). The number of ATL cells from other patients was not sufficient to prepare membrane vesicles. MRP1 mRNA expression in patient 4, patient 25, and KUT-1 cells was 0.54 unit, 5.29 units, and 0.53 unit, respectively. ATP-dependent [3H]LTC4 uptake by membrane vesicles from the ATL sample of patient 25 was significantly higher than that from the ATL sample of patient 4 and that from KUT-1 cells (P = 0.023 and 0.017, respectively). cMOAT also transports LTC4. Although MRP1 but not cMOAT was detected by immunoblot analysis in the membrane vesicles from the patient 25 ATL sample (Fig. 4B), we examined LTC4 transport in the presence of a specific antibody against MRP1, QCRL-3, to confirm that LTC4 was transported by MRP1 and not by cMOAT in the ATL membrane vesicles. QCRL-3 (0.148 μg QCRL-3/10 mg membrane vesicles) reduced LTC4 uptake by 90.0% in the patient 25 ATL sample, but ATP-dependent LTC4 uptake by membrane vesicles from LLC-PK1/cMOAT cells that overexpress cMOAT was not inhibited by QCRL-3 (Fig. 4A). ONO-1078 and MK571, two MDR-reversing agents that inhibit the transporting activity of both MRP1 and cMOAT, reduced the ATP-dependent LTC4 uptake into membrane vesicles from both the patient 25 ATL sample (P = 0.016 and 0.016, respectively) and LLC-PK1/cMOAT cells.

**Accumulation of [3H]VCR.** We examined the accumulation of VCR in cells from two ATL patients and in KUT-1 cells with or without the MDR-reversing agents. In the MRP1-positive ATL samples (samples 23 and 24; MRP1 mRNA expression was 4.49 and 3.70), VCR accumulation was increased by 100 μM ONO-1078 (P < 0.05), but it was not enhanced by ONO-1078 in KUT-1 cells (Fig. 5).

**Prognostic Relevance.** We studied the correlation of MRP expression and survival period. The survival curves of patients with acute-type ATL and patients with lymphoma-type ATL were not significantly different (median, 5.5 versus 5.5 months; P = 0.444; Fig. 6A). Patients with chronic-type ATL reportedly had a longer survival period than those with acute-type and lymphoma-type ATL (1). Therefore, we divided the 27 acute-type and 7 lymphoma-type ATL patients into two groups according to their MRP1 mRNA expression at diagnosis and compared their survival periods (Fig. 6B). We designated the specimens as MRP1-high when MRP1 mRNA expression was higher than twice the median expression level of healthy controls and as MRP1-low when it was lower. The survival period of MRP1-high patients was compared with that of MRP1-low patients, and the prognosis of MRP1-high patients was worse than that of the MRP1-low patients (median, 4.4 versus 6.1 months; P = 0.019; Fig. 6B). We also studied the expression of MDR1 mRNA in 26 ATL patients (19 acute-type and 7 lymphoma-type ATL patients) and 3 healthy controls by slot blot analysis and designated the specimens as MDR1-high and MDR1-low by following the same criteria as described for MRP1 mRNA expression. The survival periods of these two groups were not significantly different (data not shown). High abnormal lymphocyte counts were not associated with short survival (data not shown).

Next, we investigated whether MRP1 positivity is an independent prognostic variable. A Cox proportional hazards model was constructed using established prognostic factors (21), MDR1 expression and MRP1 expression. Univariate analysis showed that BUN (P = 0.028), PS (P = 0.006), and MRP1 (P = 0.024) were significant prognostic factors (Table 2). Next, we performed a multivariate analysis that included all parameters that were significantly associated with outcome in the univariate analysis. Multivariate survival analysis showed that MRP1 (P = 0.005) alone was a significant prognostic factor (Table 2).
DISCUSSION

In our study, we demonstrated the clinical significance of MRP1 expression and that high MRP1 expression correlated with short survival in patients with acute-type and lymphoma-type ATL. Abnormal lymphocyte counts were correlated with the level of MRP1 expression but were not related to overall survival, and the survival difference between MRP1-high and MRP1-low patients cannot be explained by high abnormal lymphocyte counts in patients with high MRP1. The survival period of patients with lymphoma-type ATL was not significantly longer than that of patients with acute-TAT in this study (Fig. 6A). In chronic-type ATL, the samples showed the highest mean expression level of MRP1 among the three subtypes. Because chronic-type ATL patients did not require treatment with intensive chemotherapy in this study, it is unclear whether overexpression of MRP1 is involved in drug resistance in these patients.

In univariate and multivariate analyses, overexpression of MRP1 was a poor prognostic factor in this study. These findings strongly suggest a possible causal relationship between MRP1 expression and poor survival of patients with acute-type and lymphoma-type ATL. Additional clinical trials with reversing agents are required to clarify the role of MRP1.

The expression of MRP1 mRNA in leukemia cells from AML, acute lymphocytic leukemia, and chronic lymphocytic leukemia patients was also reported (22–26). In de novo AML, expression of MRP1 was reportedly variable, and MRP1 was commonly overexpressed in leukemic cells from relapsed, secondary, or refractory AML and relapsed acute lymphocytic leukemia patients (22, 23). In chronic lymphocytic leukemia, 71% of patients had high expression levels of the MRP1 gene (24). AML associated with the inversion chromosome 16 [inv(16)(p13;q22)] has a favorable prognosis and is chemosensitive. The MRP1 gene is located at chromosome 16p13.13, and the inversion results in loss of the MRP1 gene in some inv(16) AML patients (25). Legrand et al. (26) reported that functional testing of MRP1 activity using calcein acetoxymethyl ester and cyclosporin A revealed a significant prognostic value of MRP1 in AML patients, suggesting that MRP1 is involved in drug resistance in AML.

MRP1 in the plasma membrane (27) is thought to transport anticancer agents outside the cells. To assess the function of MRP1, we used membrane vesicles from ATL cells to directly investigate the transporting activity of MRP1 in ATL cells. LTC4 is a good substrate for MRP1 and is actively transported into MRP1-expressing membrane vesicles (28). CMOAT also transports LTC4 (29). LTC4 was transported more actively into MRP1-high ATL membrane vesicles than into MRP1-low ATL membrane vesicles. A monoclonal antibody against MRP1, QCRL-3, inhibited ATP-dependent LTC4 uptake by membrane vesicles prepared from MRP1-positive ATL cells but did not inhibit LTC4 uptake by membrane vesicles prepared from LLC-PK1/cMOAT cells. In the ATL sample, MRP1, but not cMOAT or P-gp, was detected by immunoblotting. These findings indicate that the MRP1 expressed in ATL cells can actively transport LTC4.

ONO-1078 is an antagonist of the LTD4 receptor and is used clinically for treatment of asthma patients. By directly interacting with MRP1, ONO-1078 completely inhibited ATP-dependent LTC4 uptake into membrane vesicles prepared from CV60 cells that overexpress MRP1 (28). MK571 is another LTD4 receptor antagonist with a structure that differs from that of ONO-1078, and it also completely reversed VCR resistance in MRP1-mediated MDR cells (30). Both ONO-1078 and MK571 almost completely inhibited ATP-dependent LTC4 up-
take by membrane vesicles prepared from MRP1-positive ATL cells. Moreover, VCR accumulation in MRP1-positive ATL cells from two cases was enhanced by ONO-1078. ONO-1078 or MK571 in combination with anticancer agents may be useful for treating patients with MRP1-positive ATL cells.

In conclusion, MRP1 may be one of the factors that causes drug resistance in ATL patients and affects the response to chemotherapy in ATL. Treatment with MRP1 modulators may be useful in MRP1-mediated drug resistance in patients with ATL.

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REFERENCES


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