Biochemical and Biophysical Research Communications **283,** 64–71 (2001)
doi:10.1006/bbrc.2001.4746, available online at http://www.idealibrary.com on IDEAL®

# Double-Edged Sword of Chemosensitizer: Increase of Multidrug Resistance Protein (MRP) in Leukemic Cells by an MRP Inhibitor Probenecid[1]

Hyang-Suk Kim,* Young-Don Min,† and Cheol-Hee Choi*,[2]
**Department of Pharmacology and †Department of General Surgery, Chosun University Medical School, 375 Seosuk-dong, Dong-gu, Kwangju 501-759, South Korea*

Received March 19, 2001

**The multidrug resistance protein (MRP) is a drug efflux membrane pump conferring multidrug resistance to tumor cells. Clinical trials have been undertaken to improve the effectiveness of chemotherapy by adding an MRP inhibitor to the treatment regimen. This study attempted not only to determine novel resistance mechanisms in MRP-overexpressing AML cells (AML-2/DX100) by chronic exposure to doxorubicin in the presence of an MRP inhibitor probenecid but also to find out whether probenecid could increase MRP levels. AML-2/DXPBA cultured in the presence of probenecid (600 $\mu$M) and doxorubicin (100 ng/ml) showed a higher level of the multidrug resistance (MDR) phenotype when compared to AML-2/DX100. AML-2/DXPBA showed increased levels of MRP compared to those of AML-2/DX100. Probenecid increased the MRP levels without an increase in *MRP* mRNA in AML-2/WT in both a time- and dose-dependent manner. Of the MRP inhibitors including probenecid, ofloxacin, erythromycin, and rifampicin used in this study, only probenecid showed a marked chemosensitizing effect in AML-2/DX100 but not in HL-60/Adr, suggesting that the chemosensitizing effects of the MRP inhibitors vary according to the type of resistant cells. The maximum noncytotoxic concentrations of these MRP inhibitors increased the MRP levels to various degrees in both AML-2/WT and HL-60/WT. However, the chemosensitizing effects of the MRP inhibitors were not correlated with their MRP-increasing effects. Altogether, MRP inhibitors such as probenecid have been shown to function as a double-edged sword, indicating that they are not only an effective chemosensitizer of MRP-associated MDR tumor cells but also an MRP activator. Therefore caution should be taken whenever using MRP inhibitors to reverse MRP-mediated multidrug resistance in clinical cancer chemotherapy as well as when used to inhibit MRP expression *in vitro*.** 

The development of multidrug resistance by tumor cells is a major obstacle to successful cancer chemotherapy. Multidrug resistance can be mediated by overexpression of the human *MDR1* and *MRP* genes, the 170 kDa P-glycoprotein and the 190 kDa multidrug resistance protein (MRP) (1, 2). Both P-glycoprotein and MRP function as transmembrane efflux pumps, which decrease intracellular drug accumulation, thereby conferring multidrug resistance on tumor cells. Many chemosensitizers have been developed to overcome the multidrug resistance mediated by the P-glycoprotein and/or MRP. Verapamil and cyclosporin A are well-known chemosensitizers that inhibit P-glycoprotein-mediated multidrug resistance, and consequently increase sensitivity to P-glycoprotein-mediated chemotherapeutic agents (3, 4). MRP inhibitors include difloxacin (5), the leukotriene LTD4 receptor antagonist MK571 (6), probenecid (7), indomethacin (8), the leukotriene LTD4 receptor antagonist ONO-1078 (9), pluronic block copolymer (10), dipyridamol (11), the progesteron antagonist RU486 (12), rifampicin (13), erythromycin and ofloxacin (14).

However, it has been reported that P-glycoprotein inhibitors do not only inhibit the P-glycoprotein-mediated multidrug resistance phenotype but also increase P-glycoprotein gene expression. P-glycoprotein inhibitors, including verapamil, nifedipine, and cyclosporin A increase both the levels of *MDR1* mRNA and its product P-glycoprotein in the human colon carcinoma cell line, LS180, and its drug-resistant sublines, LS 180-Ad50 and LS 180-Vb2 (15). From this, it can be hypothesized that an MRP inhibitor may have an abil-

Abbreviations used: MRP, multidrug resistance-associated protein; MDR, multidrug resistance; AML, acute myelogenous leukemia; DX, doxorubicin; PBA, probenecid; WT, wild type.

[1] This paper was supported by a grant [Life Phenomena & Function Research Group program] (00-J-LF-01-B-09) from the Ministry of Science and Technology, 2000.

[2] To whom correspondence should be addressed. Fax: +82-62-232-4045. E-mail: chchoi@mail.chosun.ac.kr.

0006-291X/01 $35.00

ity to increase MRP. A further motivation for this study was that resistant cells selected in the presence of an MRP inhibitor might express other novel resistance mechanisms except an MRP or P-glycoprotein, which cannot completely explain the multidrug resistant phenotypes resulting from a decreased accumulation of drugs. Actually the MCF-7 human breast cancer cells selected for their resistance to doxorubicin in the presence of verapamil did not express either the P-glycoprotein or MRP (16, 17) but expressed the breast cancer resistance protein BCRP (18). In this study, probenecid was chosen as an MRP inhibitor because in a preliminary experiment, it exhibited a greater chemosensitizing effect against MRP-overexpressing AML-2/DX100. Probenecid was chronically treated to AML-2/DX100 in the presence of 100 ng/ml doxorubicin and this subline was named AML-2/DXPBA. AML-2/DXPBA showed an increased MRP level than AML-2/DX100. Therefore the effect of probenecid on the increase in MRP levels was further investigated.

## MATERIALS AND METHODS

*Culture and selection of resistant cells in the presence of both probenecid and doxorubicin.* The OCI-AML-2 line obtained from the Ontario Cancer Institute (Toronto, Canada) was cultured at 37°C in a 5% $CO_2$ atmosphere using $\alpha$-MEM medium (Gibco) with 10% heat inactivated fetal bovine serum (FBS, Sigma). Cells were maintained as a suspension culture, and subcultured at confluence. The previous study revealed that a doxorubicin-resistant AML subline (AML-2/DX100) overexpressed the MDR-associated protein (MRP) but not P-glycoprotein (19). AML-2/DXPBA was selected from AML-2/DX100 by chronic exposure to doxorubicin (100 ng/ml) in the presence of probenecid (600 $\mu$M) over 2 months. The daunorubicin-resistant AML-2 subline AML-2/D100 were selected as described previously (20). Doxorubicin-resistant subline HL-60/Adr was used as the MRP-overexpressing cell (21).

*Cytotoxicity assay.* The *in vitro* cytotoxicity of drugs was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, Sigma] assay described by Pieters *et al.* (22). The 50% inhibitory concentration ($IC_{50}$) for a particular agent was defined as the drug concentration which results in a 50% reduction in cell number to the untreated control. $IC_{50}$ values were determined directly from semilogarithmic dose–response curves. The experiment was undertaken, at least, in triplicate.

*Effects of chemosensitizers on the growth of AML-2/DXPBA.* AML-2/DXPBA of $2 \times 10^5$/ml cells were plated into a 96-well plate containing doxorubicin (100 ng/ml) in the presence of cyclosporin A (3 $\mu$M), verapamil (20 $\mu$M), and probenecid (600 $\mu$M). After 3-day incubation, MTT assay was carried out to determine the effects of chemosensitizers.

*Western blot analysis for MRP and P-glycoprotein.* Plasma membrane proteins from the drug-sensitive and -resistant cells were prepared as previously described (23). Membrane proteins were solubilized and then fractionated by SDS-PAGE. Western blotting was performed by a slight modification of the method first described by Towbin *et al.* (24). Proteins were transferred onto a nitrocellulose membrane by electroblotting at a current of 60 V overnight. The membrane was incubated in blocking solution (5% skim milk) for 1 h at room temperature, washed, and then incubated with primary antibodies (diluted 1:1000), C219 (Signet) for Pgp and Mrpr1 (Signet) for MRP. The membrane was washed, and incubated with horseradish peroxidase-conjugated rabbit-antimouse IgG (diluted 1:2000, Sigma) for 1 h. The membrane was then stained using the detection reagent of the ECL detection kit (Amersham). Protein concentration was determined with a Bio-Rad protein assay kit and standardized with bovine serum albumin.

*RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR) assay.* Total RNA was extracted from cells using the acid guanidium thiocyanate-phenol-chloroform method (25). *MDR1*, *MRP* and *β-actin* mRNA transcripts were detected using the RT-PCR assay. *MDR1* expression was detected with 3′ and 5′ primers corresponding to nucleotides 1179–1201 and 907–930, respectively, of the published cDNA sequence (26), yielding a 295-bp PCR product. *MRP* expression was detected with 3′ and 5′ primers corresponding to nucleotides 4551–4568 and 4180–4197, respectively, of the published cDNA sequence, yielding a 389-bp PCR product. *β-actin* expression as a control of the RNA amount was detected with 3′ and 5′ primers corresponding to nucleotides 2392–2412 and 1912–1932, respectively, of the published cDNA sequence (27), yielding a 501-bp PCR product. RNAs from each sample were reverse transcribed using units of Moloney murine leukemia virus reverse transcriptase (Bethesta Research Laboratories) and oligo (dT) primer for 1 h at 37°C. The resulting cDNA were diluted 1:5 with water and then were amplified with 2.5 units of *Taq* polymerase (Promega, U.S.A.) and 10 pmol of each primers in a GeneAmp PCR9600 (Perkin-Elmer-Cetus) for 21 cycles (but 15 cycles for *β-actin*) of sequential denaturation (at 95°C for 30 s), annealing (at 53°C except at 65°C in *MDR1*), and extension (at 72°C for 30 s). After the last cycle, all PCR products were subjected to a final extension for 5 min at 72°C. For quantitation, 5 $\mu$Ci of [$\alpha$-$^{32}$P]dCTP were added to each reaction mixture. After PCR, PCR products were combined and then electrophoresed on 7.5% nondenaturing polyacrylamide gels. Autoradiographic films of the RT-PCR assay were subjected to densitometric analysis using a densitometer (Pdi, U.S.A.). The autoradiograms were normalized compared with the *β-actin* signal.

*Statistical analysis.* Statistical significance of the data was determined by the Student's *t* test. *P* values less than 0.05 were taken as statistically significant.

## RESULTS

### *Selection of Resistant Cells in the Presence of Both Probenecid and Doxorubicin*

It was suggested that in the presence of an MRP inhibitor, the MRP mechanism and possibly the Pgp mechanism cannot develop. As a result, the selection of other resistance mechanisms is possible. P-glycoprotein was rarely expressed at doxorubicin concentrations <100 ng/ml (19). The doxorubicin-resistant AML subline (AML-2/DX100) overexpressed the MDR-associated protein (MRP) but not P-glycoprotein (19). In AML-2/DX100, the overexpression of MRP resulted from an increased *MRP* mRNA level transcribed from the amplified *MRP* gene (data not shown). Therefore, AML-2/DX100 resistant to doxorubicin in the presence of probenecid was selected by chronic exposure of the doxorubicin-resistant AML subline (AML-2/DX100) to gradually increasing concentrations of probenecid in the presence of doxorubicin. The probenecid concentrations were used from 300 $\mu$M to 600 $\mu$M as severe cytotoxicity occurs at concentrations >600 $\mu$M even in the absence of doxorubicin. The doxorubicin-resistant cells selected in the presence of 600

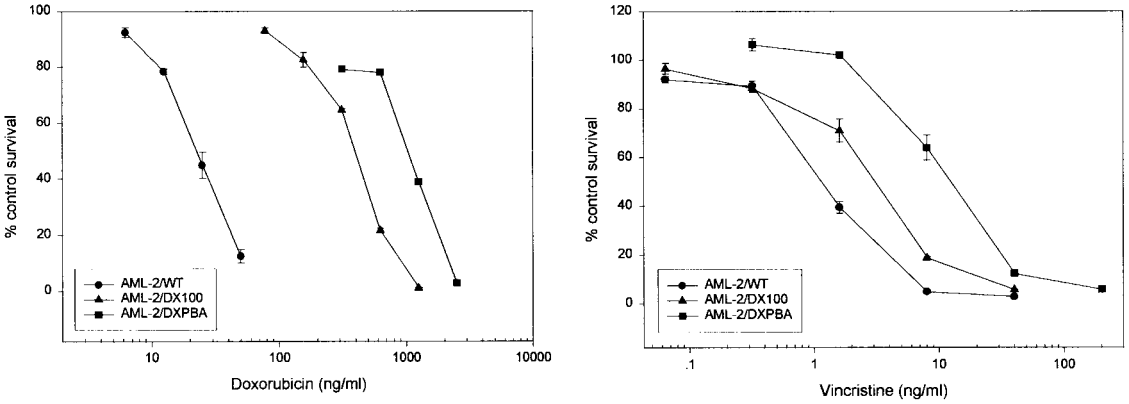


**FIG. 1.** The resistance profile of doxorubicin-resistant AML-2 sublines to doxorubicin or vincristine. The doxorubicin or vincristine sensitivities of AML-2/DX100 and AML-2/DXPBA were determined by an MTT assay as described under Materials and Methods. The data points are means of results from three separate experiments. Bars, SE.

$\mu$M probenecid were designated AML-2/DXPBA, which appeared to be grossly similar to AML-2/DX100 in cell size.

### *The Dose–Response Effects of Probenecid on the Doxorubicin Sensitivity of AML-2/DX100 and HL-60/Adr*

The dose–response effects of probenecid in the presence or absence of doxorubicin were examined in both drug-sensitive parental leukemic cells and MRP-overexpressing resistant cells. The probenecid $IC_{50}$s in AML-2/DX100 and HL-60/Adr, in the absence of doxorubicin, were 860 and 2887 $\mu$M, respectively. In contrast, the probenecid $IC_{50}$s in AML-2/DX100 and HL-60/Adr in the presence of 100 ng/ml doxorubicin were 202 and 2680 $\mu$M, respectively. Thus probenecid reversed AML-2/DX100 resistance to 100 ng/ml doxorubicin in a concentration-dependent manner (data not shown). However, this was not observed in HL-60/Adr, suggesting that probenecid has differential effects depending on the type of resistant cells. In addition, the probenecid concentration that showed a >80% AML-2/DX100 survival rate in the absence of doxorubicin, was approximately 600 $\mu$M. However, with HL-60/Adr, the concentration was 2000 $\mu$M.

### *Resistance Profile of AML-2/DXPBA in the Presence of Doxorubicin or Vincristine*

In advance, the AML-2/DX100 sensitivity to doxorubicin and vincristine was determined by using the MTT assay. Doxorubicin and vincristine were chosen as an AML-2/DX100 selecting drug and as a good substrate for MRP, respectively. The $IC_{50}$s of doxorubicin were 22.4 ng/ml in AML-2/WT, 392.9 ng/ml in AML-2/DX100 and 1021.4 ng/ml in AML-2/DXPBA (Fig. 1). With respect to the relative resistance, AML-2/DX100 and AML-2/DXPBA resulted in an 18-fold and 46-fold increase in resistance to doxorubicin, respectively, when compared to AML-2/WT. The $IC_{50}$s of vincristine were 1.13 ng/ml in AML-2/WT, 3.27 ng/ml in AML-2/DX100 and 12.5 ng/ml in AML-2/DXPBA (Fig. 1). With respect to the relative resistance, AML-2/DX and AML-2/DXPBA showed a 3-fold and 11-fold resistance to vincristine, respectively, when compared to AML-2/WT. The AML-2/DXPBA resistance to either doxorubicin or vincristine in the presence of 600 $\mu$M probenecid may suggest the development of non-MRP resistant mechanisms.

### *Effects of Chemosensitizers on the Growth of AML-2/DXPBA in the Presence of Doxorubicin*

Agents such as verapamil or cyclosporin A inhibit the efflux of anticancer drugs by binding to P-glycoprotein. These result in a greater intracellular accumulation of the anticancer drugs, thereby overcoming drug resistance (3, 4, 28, 29). The effects of either P-glycoprotein inhibitors (cyclosporin A or verapamil) or an MRP inhibitor (probenecid), on the growth of AML-2/DXPBA in the presence of doxorubicin were determined using the MTT assay. A cyclosporin A concentration of 3 $\mu$M, a verapamil concentration of 20 $\mu$M, and a probenecid concentration of 600 $\mu$M were used to completely reverse the P-glycoprotein or MRP in this study. As shown in Fig. 2, either cyclosporin A or verapamil completely reverses the resistance of P-glycoprotein overexpressing AML-2/D100 to daunorubcin whereas probenecid does not. In MRP-overexpressing AML-2/DX100, verapamil and probenecid reverse the resistance to doxorubicin in a moderate and marked degree, respectively, but cyclosporin A does not. This result is consistent with the result that MRP is less sensitive than P-glycoprotein to the inhibition effects of cyclosporin or verapamil (30). At the same time, the concentrations of the three chemosensitizers used in this study did not reverse the resistance of AML-2/DXPBA to doxorubicin (Fig. 2). However, the resistance of AML-2/DXPBA to doxorubicin was completely reversed with

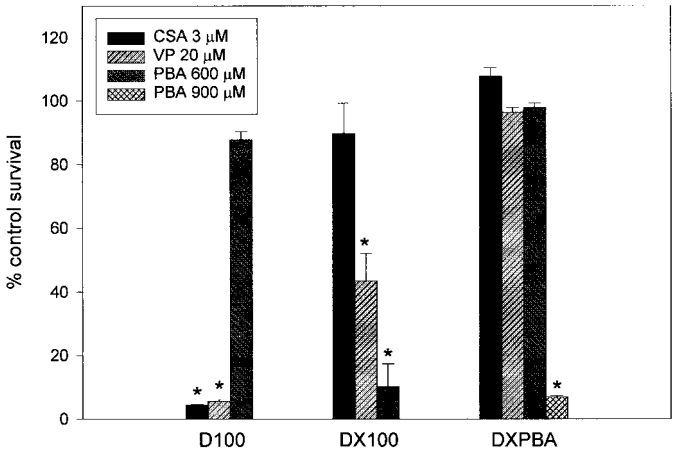


**FIG. 2.** The effects of chemosensitizers on the growth of drug-resistant cells in the presence of each selecting drug. The effects of chemosensitizers on AML-2/D100 and AML-2/DX100 were tested in the presence of each selecting drug, 100 nM daunorubicin and 100 ng/ml doxorubicin, respectively. The MTT assay was performed 3 days after treatment of the chemosensitizers with each selecting drug. The data represent an average of triplicate determinations. Bars, SE; *, significant difference (at $P < 0.05$) from control values by Student's *t* test. D100, AML-2/D100; DX100, AML-2/DX100; DXPBA, AML-2/DXPBA; CSA, cyclosporin A; VP, verapamil; PBA, probenecid.

>900 μM probenecid. This result suggests that the increased resistance of AML-2/DXPBA to doxorubicin only results from increased MRP levels.

### *Evaluation of Expression of Resistance Proteins in AML-2/DXPBA*

To confirm if either the P-glycoprotein or MRP was involved in AML-2/DXPBA resistance to doxorubicin, P-glycoprotein and MRP expression was evaluated by both Western blot and RT-PCR analyses. As shown in Fig. 3A, AML-2/WT expresses a low level of MRP but not P-glycoprotein whereas AML-2/DX100 overexpresses MRP but not P-glycoprotein. But AML-2/DXPBA did not express P-glycoprotein, either (data not shown). On the other hand, AML-2/DXPBA exhibited a higher MRP level than AML-2/DX100 (Fig. 3A). To determine whether the increased MRP level was due to a higher *MRP* mRNA level, reverse transcription-PCR was performed. The RT-PCR data indicates no *MDR1*/P-glycoprotein mRNA expression (data not shown). The *MRP* mRNA level increased in AML-2/DX100 when compared to AML-2/WT. However, no differences in *MRP* mRNA levels were observed between AML-2/DX100 and AML-2/DXPBA (Fig. 3B).

### *Time- and Dose-Dependent Effects of Probenecid on MRP Expression in AML-2/WT*

It was noticed that the chronic exposure to probenecid could increase the MRP levels in AML-2/DX100 even in the presence of doxorubicin. We examined whether the short-term treatment with probenecid in the absence of doxorubicin could increase MRP in drug-sensitive parental cells AML-2/WT as a function of time and concentration. As shown in Fig. 4, treatment of AML-2/WT with 600 μM probenecid from 6 h to 48 h shows a steady increase in MRP with increasing time. AML-2/WT treated with 600 μM probenecid for 48 h exhibited a 4-fold increase in MRP level when compared to the control. However, the *MRP* mRNAs levels decreased slightly with increasing time (Fig. 4B).

The effect of dosage was investigated to evaluate the effects of probenecid concentrations <600 μM for 48 h (Fig. 5). This duration of treatment was chosen since the preliminary experiment indicated a peak increase in MRP levels after the 48-h treatment. The effects of probenecid on MRP expression in AML-2/WT were determined as a function of concentration. Four probenecid concentrations, 100, 200, 400 and 600 μM, were used. As shown in Fig. 5, the MRP levels in AML-2/WT increase with increasing probenecid concentration. During this time, no change in *MRP* mRNA levels was detected when >400 μM probenecid was used although the *MRP* mRNA levels increase slightly at both 100 μM to 200 μM probenecid.

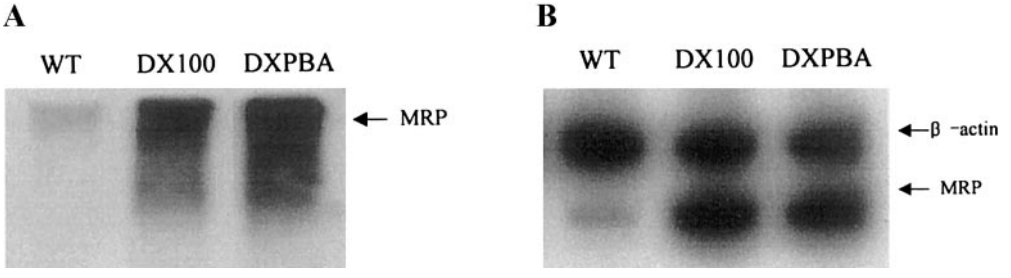


**FIG. 3.** The Western blot and RT-PCR analyses for MRP in AML-2/WT and its doxorubicin-resistant sublines. (A) Western blot analysis; the protein samples were extracted from cells and then 50 μg aliquots of protein from each sample were subjected to 7% SDS–PAGE. The MRP was detected by immunoblotting with rat monoclonal antibody Mrpr1. (B) RT-PCR; an aliquot of RNA was transcribed, and the cDNA was subjected to PCR using a primer specific for the *MRP* and *β-actin*. The PCR for *β-actin* performed on cDNA is included as a positive control and indicates that approximately equal amounts of cDNA were loaded for each condition examined.

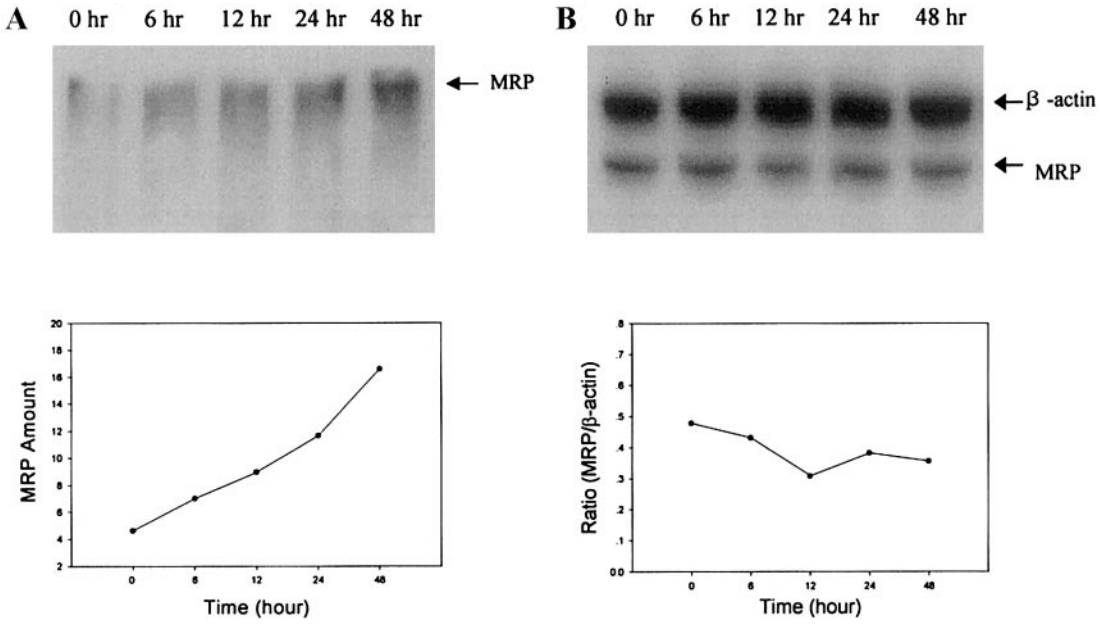


**FIG. 4.** Time effects of probenecid on MRP expression in AML-2/WT. AML-2/WT was treated for 6, 12, 24, and 48 h with 600 $\mu$M probenecid. MRP expression was determined by Western blot (A) and RT-PCR (B) analyses.

*Effects of Various MRP Inhibitors on the MRP Expression in AML-2/WT*

To determine whether other MRP inhibitors may increase the MRP levels, the effects of various MRP inhibitors were tested in AML-2/WT. In advance, the chemosensitizing effects of other MRP inhibitors including probenecid, ofloxacin, erythromycin and rifampicin were evaluated in both AML-2/DX100 and HL-60/Adr. The maximum noncytotoxic concentration (more than 90% of control survival) of each drug was

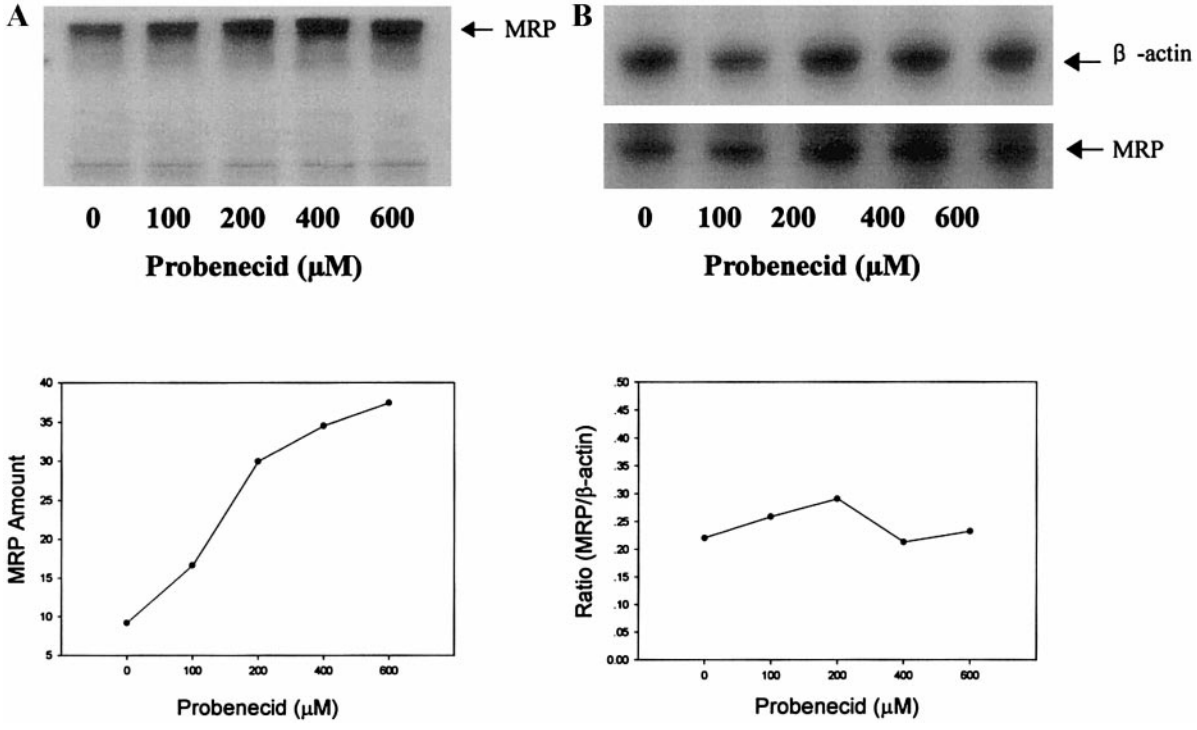


**FIG. 5.** The concentration-dependent effects of probenecid on MRP expression in AML-2/WT. A dose–response relationship was determined by evaluating the effects of 100–600 $\mu$M probenecid for 48 h. MRP expression was determined by Western blot (A) and RT-PCR (B) analyses.

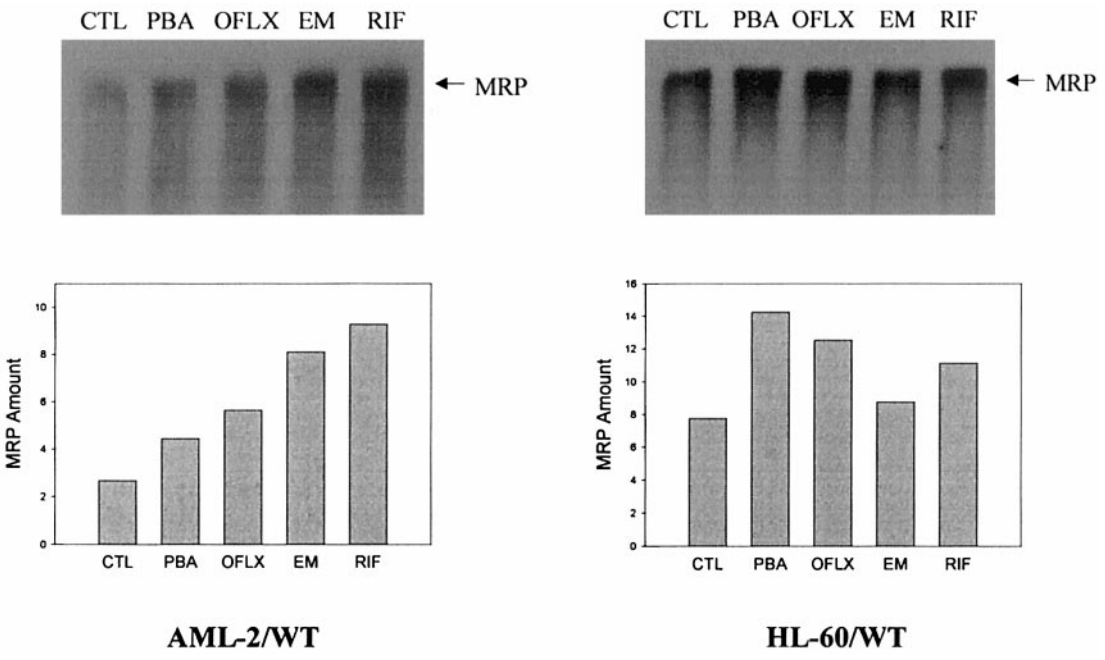


**FIG. 6.** Effects of various MRP inhibitors on the MRP expression in AML-2/WT and HL-60/WT. The maximum noncytotoxic concentration (more than 90% of control survival) of each MRP inhibitor such as ofloxacin, erythromycin, and rifampicin was administered to AML-2/WT and HL-60/WT for 48 h. Subsequently, Western blot and RT-PCR analyses were performed as described in the legend to Fig. 3. CTL, control; PBA, probenecid; OFLX, ofloxacin; EM, erythromycin; RIF, rifampicin.

used in order to avoid the possibility of preexisting cells with higher MRP levels being selected. The maximum noncytotoxic concentrations of the MRP inhibitors in AML-2/WT were as follows: probenecid 600 $\mu$M, ofloxacin 70 $\mu$M, erythromycin 45 $\mu$M and rifampicin 40 $\mu$M. Probenecid and ofloxacin showed significant and slightly chemosensitizing effects in AML-2/DX100, respectively. However, this effect was not observed in HL-60/Adr. In contrast, both erythromycin and rifampicin had no effect in either of the two cell lines. AML-2/WT was treated with the maximum noncytotoxic concentration of MRP inhibitors for 48 h. As shown in Fig. 6, the four MRP inhibitors that were used in this study resulted in 1.67- to 3.49-fold MRP increase in AML-2/WT.

Effects of MRP inhibitors on MRP expression in another leukemic cell line HL-60 Multidrug-resistant HL-60/Adr cells were selected from drug-sensitive parental HL-60/WT human promyelocytic leukemia cells by exposure to 100 ng/ml doxorubicin. They expressed MRP mRNA but no MDR1 mRNA (21). To determine whether MRP inhibitors had similar effects in another leukemic cell line HL-60, MRP expression was examined in HL-60 after treatment with MRP inhibitors such as rifampicin, erythromycin, ofloxacin, and probenecid. The maximum noncytotoxic concentrations of the MRP inhibitors in HL-60/WT were as follows: 1000 $\mu$M probenecid, 70 $\mu$M ofloxacin, 400 $\mu$M erythromycin and 50 $\mu$M rifampicin in HL-60/WT. After exposure to the maximum noncytotoxic concentrations of MRP inhibitors for 48 h, the four MRP inhibitors tested in this study resulted in a 1.13- to 1.83-fold MRP increase in AML-2/WT without an increase in MRP levels (Fig. 6).

## DISCUSSION

AML-2/DXPBA was selected for resistance of MRP-overexpressing AML-2/DX100 to 100 ng/ml doxorubicin in the presence of 600 $\mu$M probenecid. AML-2/DXPBA showed increased resistance to doxorubicin when compared with AML-2/DX100 and survived in the presence of chemosensitizers such as cyclosporin A (3 $\mu$M), verapamil (20 $\mu$M) and probenecid (600 $\mu$M). These results suggest no involvement of either P-glycoprotein or possibly MRP. However, AML-2/DXPBA resistance to doxorubicin was completely reversed by more than 900 $\mu$M probenecid. This result suggests a possibility that increased MRP levels can be overcome by increased concentrations of probenecid in AML-2/DXPBA. Western blot analysis revealed that AML-2/DXPBA increased the MRP level but not P-glycoprotein. This result mimics the increases in *MDR-1*/P-glycoprotein expression after treatment with the P-glycoprotein antagonists, verapamil, nifedipine, and cyclosporin A. P-glycoprotein antagonists increased P-glycoprotein expression with increases in *MDR1* mRNA levels (15). Short-term treatment of probenecid also increased MRP levels in drug sensitive parental cells, AML-2/WT, in both a concentration- and time-dependent manner. AML-2/DX100 overexpressed

MRP resulting from the increased *MRP* mRNA level following gene amplification. On the other hand, both AML-2/DXPBA and probenecid-treated AML-2/WT showed increased MRP protein levels without an increase in the *MRP* mRNA level. Although the mechanism by which the MRP levels were induced by MRP inhibitors is unknown, this result suggests that the increased MRP level may be due to post-transcription or post-translation mechanisms such as an increased translation efficiency or increased MRP stability. P-glycoprotein can influence hepatic expression of CYP3A or other cytochromes P-450 (P-450s) because Pgp can transport endogenous regulators of these cytochromes (31). It could be therefore hypothesized that probenecid, an inhibitor of organic anion transport (7), may inhibit the transport of endogenous substances capable of increasing the MRP levels. The exact molecular mechanism remains to be determined.

Of the MRP inhibitors including probenecid, ofloxacin, erythromycin, and rifampicin used in this study, only probenecid showed a marked chemosensitizing effect in AML-2/DX100 but not in HL-60/Adr. This result suggests that the chemosensitizing effects of the MRP inhibitors vary according to the type of resistant cells. The maximum noncytotoxic concentrations of these MRP inhibitors were used to examine their effects on MRP expression in AML-2/WT and HL-60/WT. The maximum noncytotoxic concentrations of these MRP inhibitors increased the MRP levels in various degrees. However, the chemosensitizing effects of the MRP inhibitors were not correlated with the effects of increasing MRP levels.

To overcome multidrug resistance in clinical situations, many chemosensitizers that inhibit P-glycoprotein and/or MRP have been developed. Thus far, clinical trials have been undertaken to improve the effectiveness of chemotherapy by adding these chemosensitizers to the chemotherapy regimen. In addition to their clinical use, chemosensitizers have been used in a strategy to determine novel resistant mechanisms as the multidrug resistance phenotypes resulting from the decreased accumulation of drugs cannot be completely explained with P-glycoprotein and MRP. This strategy was motivated by a recent report showing that AML cells contain a novel form of an energy-dependent drug efflux mechanism, which has not yet been identified (32). Therefore, selection for resistance to doxorubicin in the presence of an MRP inhibitor may be attempted with the anticipation of expressing any novel resistance gene(s) except an MRP and P-glycoprotein. AML-2/DXPBA unexpectedly showed an increased levels of MRP. Thus such a strategy should be employed after determining whether or not a chemosensitizer could increase the resistance protein levels. In the present study, AML-2/DXPBA provided a good example.

Chemosensitizers for MRP and P-glycoprotein have their own pharmacological effects. Therefore when these chemosensitizers are administered to cancer patients, their MRP-increasing effects should be considered with regard to their resistance to chemotherapy. In particular, probenecid can be used with chemotherapy to treat hyperuricemia induced by tumor cell lysis after chemotherapy (33). It should be noted that probenecid can increase the MRP levels, thereby conferring resistance to cancer cells in this situation.

Overall, MRP inhibitors such as probenecid have been shown to function as a double-edged sword, indicating that they are not only an effective chemosensitizer of MRP-associated multidrug resistance tumor cells but also an MRP activator. Therefore, caution should be taken when using MRP inhibitors for reversing MRP-mediated multidrug resistance in clinical cancer chemotherapy as well as for inhibiting MRP expression.

## ACKNOWLEDGMENTS

The authors gratefully acknowledge and thank Dr. Centor (Kansas State University, U.S.A.) for providing the HL-60 and HL-60/Adr. The authors thank T. B. Lee and Y. K. Seo of Department of Pharmacology (Chosun University, Kwangju) for their excellent technical assistance.

## REFERENCES

1. Riordan, J. R., Deuchars, K., Kartner, N., Alon, N., Trent, J., and Ling, V. (1985) *Nature* **316,** 817–819.
2. Cole, S. P., Bhardwaj, G., Gerlach, J. H., Mackie, J. E., Grant, C. E., Almquist, K. C., Stewart, A. J., Kurz, E. U., Duncan, A. M., and Deeley, R. G. (1992) *Science* **258,** 1650–1654.
3. Tsuruo, T., Iida, H., Naganuma, K., Tsukagoshi, S., and Sakurai, Y. (1983) *Cancer Res.* **43,** 808–813.
4. Slater, L. M., Sweet, P., Stupecky, M., and Gupta, S. (1986) *J. Clin. Invest.* **77,** 1405–1408.
5. Gollapudi, S., Thadepalli, F., Kim, C. H., and Gupta, S. (1995) *Oncol. Res.* **7,** 213–225.
6. Gekeler, V., Ise, W., Sanders, K. H., Ulrich, W. R., and Beck, J. (1995) *Biochem. Biophys. Res. Commun.* **208,** 345–352.
7. Gollapudi, S., Kim, C. H., Tran, B. N., Sangha, S., and Gupta, S. (1997) *Cancer Chemother. Pharmacol.* **40,** 150–158.
8. Draper, M. P., Martell, R. L., and Levy, S. B. (1997) *Br. J. Cancer* **75,** 810–815.
9. Nakano, R., Oka, M., Nakamura, T., Fukuda, M., Kawabata, S., Terashi, K., Tsukamoto, K., Noguchi, Y., Soda, H., and Kohno, S. (1998) *Biochem. Biophys. Res. Commun.* **251,** 307–312.
10. Miller, D. W., Batrakova, E. V., and Kabanov, A. V. (1999) *Pharm. Res.* **16,** 396–401.
11. Curtin, N. J., and Turner, D. P. (1999) *Eur. J. Cancer* **35,** 1020–1026.
12. Payen, L., Delugin, L., Courtois, A., Trinquart, Y., Guillouzo, A., and Fardel, O. (1999) *Biochem. Biophys. Res. Commun.* **258,** 513–518.
13. Courtois, A., Payen, L., Vernhet, L., de Vries, E. G., Guillouzo, A., and Fardel, O. (1999) *Cancer Lett.* **139,** 97–104.
14. Terashi, K., Oka, M., Soda, H., Fukuda, M., Kawabata, S., Nakatomi, K., Shiozawa, K., Nakamura, T., Tsukamoto, K., Noguchi, Y., Suenaga, M., Tei, C., and Kohno, S. (2000) *Antimicrob. Agents Chemother.* **44,** 1697–1700.

15. Herzog, C. E., Tsokos, M., Bates, S. E., and Fojo, A. T. (1993) *J. Biol. Chem.* **268,** 2946–2952.
16. Chen, Y. N., Mickley, L. A., Schwartz, A. M., Acton, E. M., Hwang, J. L., and Fojo, A. T. (1990) *J. Biol. Chem.* **265,** 10073–10080.
17. Lee, J. S., Scala, S., Matsumoto, Y., Dickstein, B., Robey, R., Zhan, Z., Altenberg, G., and Bates, S. E. (1997) *J. Cell Biochem.* **65,** 513–526.
18. Doyle, L. A., Yang, W., Abruzzo, L. V., Krogmann, T., Gao, Y., Rishi, A. K., and Ross, D. D. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 15665–15670.
19. Choi, C. H., Kim, H. S., Rha, H. S., Jeong, J. H., Park, Y. H., Min, Y. D., Kee, K. H., and Lim, D. Y. (1999) *Mol. Cells* **9,** 314–319.
20. Choi, C. H., and Ling, V. (1997) *Mol. Cells* **7,** 170–177.
21. McGrath, T., and Center, M. S. (1998) *Cancer Res.* **48,** 3959–3963.
22. Pieters, R., Huismans, D. R., Leyva, A., and Veerman, A. J. P. (1988) *Cancer Lett.* **41,** 323–332.
23. Riordan, J. R., and Ling, V. (1979) *J. Biol. Chem.* **254,** 12701–12705.
24. Towbin, H., Staehelin, T., and Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4350–4354.
25. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
26. Chen, C. J., Chin, J. E., Ueda, K., Clark, D. P., Pastan, I., Gottesman, M. M., and Roninson, I. B. (1986) *Cell* **47,** 381–389.
27. Nakajima-Iijima, S., Hammada, H., Reddy, P., and Kakunaga, T. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6133–6137.
28. Cornwell, M. M., Pastan, I., and Gottesman, M. M. (1987) *J. Biol. Chem.* **262,** 2166–2170.
29. Tamai, I., and Safa, A. R. (1990) *J. Biol. Chem.* **265,** 16509–16513.
30. Cole, S. P., Sparks, K. E., Fraser, K., Loe, D. W., Grant, C. E., Wilson, G. M., and Deeley, R. G. (1994) *Cancer Res.* **54,** 5902–5910.
31. Schuetz, E. G., Umbenhauer, D. R., Yasuda, K., Brimer, C., Nguyen, L., Relling, M. V., Schuetz, J. D., and Schinkel, A. H. (2000) *Mol. Pharmacol.* **57,** 188–197.
32. Hedley, D. W., Xie, S. X., Minden, M. D., Choi, C. H., Chen, H., and Ling, V. (1997) *Leukemia* **11,** 48–53.
33. Hutchison, J. C., Wilkinson, W. H., and McMahon, F. G. (1971) *J. Med.* **2,** 45–59.