Interactions of Ofloxacin and Erythromycin with the Multidrug Resistance Protein (MRP) in MRP-Overexpressing Human Leukemia Cells

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To investigate interactions between the multidrug resistance protein (MRP) and antimicrobial agents, we examined the effects of 12 agents on vincristine sensitivity and efflux of the calcein acetoxy-methyl ester (calcein-AM) of a MRP substrate in MRP-overexpressing cells. Only ofloxacin and erythromycin enhanced vincristine sensitivity with increased intracellular vincristine accumulation and inhibited the calcein-AM efflux. Our findings suggest that the two agents are possible MRP substrates and may competitively inhibit MRP function as a drug efflux pump.

Novel transporter proteins of the ATP-binding cassette superfamily have been identified, and their substrate specificities and tissue distributions have been investigated (10, 11). Several proteins of the superfamily, such as P-glycoprotein (Pgp) encoded by the MDR1/mdr1 gene, interact directly or indirectly with various drugs and transport drugs in an ATP energy-dependent manner out of cells (6, 12). This leads to the resistance of cancer cells against multiple anticancer drugs, a phenomenon known as multidrug resistance. The multidrug resistance protein (MRP [also called MRP1]), a member of the superfamily, also functions as a drug efflux pump (12). MRP is broadly distributed in normal human tissues, and the highest levels are found in the testes, skeletal muscle, heart, kidney, and lung (3, 12). In vitro studies using membrane vesicles of cells have shown that MRP appears to transport a broad spectrum of anionic substrates, such as glutathione disulfide, 17β-estradiol 17-β-d-glucuronide, and bile salt derivatives (12). Several agents, including calcium channel blockers and immuno-suppressants, reverse Pgp- and MRP-mediated multidrug resistance probably by the competitive inhibition of Pgp and MRP functions (1, 12). Probenecid and the leukotriene receptor antagonist MKS71 are relatively specific reversal agents for MRP rather than for Pgp (12). With regard to antimicrobial agents, erythromycin and difloxacin reverse Pgp- and MRP-mediated multidrug resistance in vitro, respectively (5, 7). Thus, interactions between antimicrobial agents and MRP are of great interest, because MRP is probably involved in biliary and renal excretion of drugs and protection of the physiological blood-cerebrospinal fluid barrier (12, 17).

In this study, to investigate interactions between antimicrobial agents and MRP, we examined the effects of 12 antimicrobial agents on vincristine sensitivity and efflux of the calcein acetoxy-methyl ester (calcein-AM; Molecular Probes, Eugene, Oreg.) of a MRP substrate in MRP-overexpressing human leukemia HL60R cells. Multidrug-resistant HL60R cells were selected from HL60 human promyelocytic leukemia cells by doxorubicin and expressed high levels of MRP mRNA but no MDR1 mRNA (15). HL60R cells are 12 times as resistant as parental HL60 cells to vincristine (15). The cells were cultured at 37°C under a humidified atmosphere of 5% CO2 in complete RPMI 1640 medium. The 12 antimicrobial agents tested were as follows: ofloxacin (Daiichi Pharmaceutical Co., Tokyo, Japan), ciprofloxacin (Bayer Yakuhin, Osaka, Japan), tosufloxacin, erythromycin, pipercillin (all from Toyama Chemical Co., Tokyo, Japan), enoxacin (Dainippon Pharmaceutical Co., Osaka, Japan), clarithromycin (Taiho Pharmaceutical Co., Tokyo, Japan), aspoxicillin (Tanabe Seiyaku Co., Tokyo, Japan), cefotiam (Takeda Chemical Industries, Osaka, Japan), ceftazidime (Glaxo Co., Tokyo, Japan), kanamycin (Meiji Seika Kaisha, Tokyo, Japan), and gentamicin (Schering-Plough, Tokyo, Japan).

The sensitivity to vincristine (Sigma Chemicals, St. Louis, Mo.) of a good substrate for MRP was determined by using the tetrazolium dye assay (13), as reported previously (15). HL60R cells (1,500 cells/well) were seeded onto 96-well plates with various concentrations of vincristine in the presence or absence of an antimicrobial agent. In preliminary experiments, we measured the cytotoxicity of each agent in HL60R cells and determined the concentration of the agent to ensure that the cytotoxicity was less than 5%. The IC50 was defined as the concentration of vincristine that reduced the absorbance in each test by 50%. The reversal effect was expressed as the ratio of the IC50 in the presence and absence (control) of each agent. Each study was repeated three times. For comparison, peak levels of vincristine in plasma are approximately 0.4 μM in humans following the infusion of a conventional dose of 1.4 mg/m² (19). The maximum nontoxic concentration (in milligrams per liter) of each agent tested here was as follows: ofloxacin, 21.7; ciprofloxacin, 1.2; tosufloxacin, 11.9; enoxacin, 20.8; pipercillin, 307; aspoxicillin, 1,248; cefotiam, 12.0; ceftazidime, 82.8; kanamycin, 40.8; gentamicin, 630; clarithromycin, 11.2; and erythromycin, 14.7. These concentrations were used in the present study. The IC50s of vincristine were 41.2 ± 4.20
FIG. 1. Reversal effects of ofloxacin and erythromycin on the vincristine sensitivity of HL60R cells. The vincristine sensitivity of HL60R cells was examined in the presence and absence (control) of each agent. The data represent the means ± standard deviations of three independent experiments.

In the present study, only one of four quinolones (ofloxacin) modulated MRP. Considered with the substrate specificity of MRP (12), this difference among quinolones may be due to differences in the hydrophobic and/or anionic structural characteristics. With respect to ofloxacin, Okano et al. (16) suggested the interaction between ofloxacin and cationic transporters.
system in rat renal brush-border membranes in vitro, but Foote and Halstenson (4) reported the involvement of both cationic and anionic systems in rat renal clearance in vivo. These findings suggest that ofloxacin is possibly eliminated by both cationic and anionic transport systems; however, biochemical analysis using membrane vesicles overexpressing each transporter protein should be performed to confirm this hypothesis.

We also demonstrated that erythromycin is a possible substrate for MRP. To date, the metabolism of most macrolides has been explained by cytochrome P450 enzymes (14, 18, 24), and only a few studies have described the involvement of drug transporter proteins (20–23). The latter studies showed that erythromycin was a likely substrate for Pgp and reversed Pgp-mediated multidrug resistance (7, 20, 21). Thus, erythromycin seems to be a substrate for both MRP and Pgp. On the other hand, Wakasugi et al. (23) demonstrated that clarithromycin inhibited the efflux of digoxin as a substrate for Pgp and increased its intracellular accumulation in vitro. Considering this observation together with the present results, clarithromycin may be at least a substrate for Pgp but not MRP. Thus, it is

**FIG. 2.** Effects of ofloxacin and erythromycin on intracellular accumulation and efflux of calcein-AM in HL60R cells. Fluorescence of intracellular calcein was analyzed by flow cytometry. The accumulation phase was examined in the presence (shaded) and absence (dotted line) of agents. The efflux phase was also examined in the presence (bold line) and absence (dashed line) of agents. PROB, probenecid (2 mM); OFLX, ofloxacin (21.7 mg/liter); EM, erythromycin (14.7 mg/liter); CPFX, ciprofloxacin (1.2 mg/liter).
likely that different transporter proteins are involved in the elimination of each macrolide.

In conclusion, our results suggested that ofloxacin and erythromycin are possible substrates for MRP, indicating possible interactions between ofloxacin and erythromycin and between them and other substrate drugs for MRP, such as vincristine. The concentrations of ofloxacin and erythromycin used here were similar to those achieved clinically. Accordingly, the above interactions should be taken into consideration when these agents are used clinically and in pharmacological studies.

REFERENCES


