(R)- and (S)-Verapamil Differentially Modulate the Multidrug-resistant Protein MRP1*

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The multidrug-resistant protein MRP1 (involved in the cancer cell multidrug resistance phenotype) has been found to be modulated by racemic verapamil (through stimulation of glutathione transport), inducing apoptosis of human MRP1 cDNA-transfected baby hamster kidney 21 (BHK-21) cells and not of control BHK-21 cells. In this study, we show that the two enantiomers of verapamil have different effects on MRP1 activity. Only the S-isomer (not the R-isomer) potently induced the death of MRP1-transfected BHK-21 cells. The decrease in cellular glutathione content induced by the S-isomer, which was not observed with the R-isomer, was stronger than that induced by the racemic mixture, indicating that the R-isomer antagonized the S-isomer effect. Both enantiomers altered leukotriene C4 and calcine transport by MRP1. Thus, the R-isomer behaved as an inhibitor, which was confirmed by its ability to revert the multidrug resistance phenotype toward vincristine. Molecular studies on purified MRP1 using fluorescence spectroscopy showed that both enantiomers bound to MRP1 with high affinity, with the binding being prevented by glutathione. Furthermore, conformational changes induced by the two enantiomers (monitored by sodium iodide accessibility of MRP1 tryptophan residues) were quite different, correlating with their distinct effects. (S)-Verapamil induces the death of potentially resistant tumor cells, whereas (R)-verapamil sensitizes MRP1-overexpressing cells to chemotherapeutics. These results might be of great potential interest in the design of new compounds able to modulate MRP1 in chemotherapy.

Resistance of tumors to multiple structurally unrelated anticancer drugs is one of the major obstacles to successful cancer chemotherapy. Failure to achieve complete and long-lasting responses is a common clinical problem that limits the curative potential of anticancer drugs in clinical oncology. Human multidrug resistance (MDR)2 is frequently associated with the overexpression of three transporters belonging to the ATP-binding cassette (ABC) protein superfamily, P-glycoprotein (ABCB1), the multidrug-resistance protein MRP1 (ABCC1), and the breast cancer resistance protein (ABCG2) (1), which can actively extrude anticancer drugs from the cell at the expense of ATP hydrolysis. MRP1 transports organic anions, many of which are conjugated to GSH, sulfate, or glucuronate; physiological substrates of MRP1 include GSH and leukotriene C4 (LTC4) (2). Cytotoxic drugs in clinical use against cancer such as vincristine, vinblastine, and daunorubicin are cotransported with GSH (2). Although a number of modulators have been found (3), the mechanism of inhibition is poorly understood. In many cases, a competitive inhibition toward substrate was described, as for the leukotriene antagonist MK571, which is considered a reference inhibitor for MRP1 (4). Interesting but complex results have been obtained with two classes of modulators: flavonoids and verapamil. Dietary flavonoids such as apigenin have been shown to interact with MRP1 (5), and quercetin induces reversion of drug resistance (6). Another flavonoid, dehydrodiosylvin, efficiently chemosensitizes cell growth to vincristine (7). Some bioflavonoids have been shown to stimulate glutathione transport by MRP1 (6). Verapamil, which is a P-glycoprotein inhibitor (8), has been reported to be ineffective in restoring drug sensitivity of MRP1-overexpressing cells (9–11). However, although verapamil alone poorly inhibits LTC4 transport by MRP1, the addition of GSH strongly enhances the inhibition (10, 12, 13). Verapamil and its analogs as well as bioflavonoids stimulate glutathione transport by MRP1 (6, 12, 14) but are not transported themselves by MRP1. A new mechanism of modulation of the MDR phenotype induced by MRP1 recently emerged (13) wherein the modulator, i.e. verapamil, triggers apoptosis of resistant cells through stimulation of MRP1-mediated glutathione efflux. This new concept could lead to finding new inducers of GSH depletion as suggested (6, 15).

Verapamil being a chiral molecule, Loe et al. (14) showed that the enantiomers of various verapamil analogs display differential abilities to stimulate GSH transport. Therefore, in this work, we investigated the individual effects of the two R- and S-enantiomers of verapamil on MRP1-overexpressing baby hamster kidney 21 (BHK-21) cells. Surprisingly, we found that, whereas the S-isomer was responsible for the death of MRP1-overexpressing cells through GSH depletion, in contrast, the

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2 The abbreviations used are: MDR, multidrug resistance; ABC, ATP-binding cassette; LTC4, leukotriene C4; BHK-21, baby hamster kidney 21; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; AM, acetoxymethyl ester; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid; PBS, phosphate-buffered saline.
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R-isomer sensitized the cell growth to vincristine by inhibiting drug transport. In addition, we demonstrate that (R)-verapamil antagonized the (S)-verapamil-induced GSH efflux and that the two isomers directly bound to purified MRP1. The two isomers induced specific conformational changes in MRP1, likely correlating with their different effects on MRP1 activity.

**EXPERIMENTAL PROCEDURES**

**Materials**—Reduced GSH, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 5,5’-dithiobis(2-nitrobenzoic acid), β-NADPH, calcein acetoxyethyl ester (AM), nucleotides, MgCl₂, dithiothreitol, verapamil enantiomers, penicillin/streptomycin, mammalian protease inhibitor mixture, asolectin, and fetal bovine serum were obtained from Sigma (Saint Quentin Fallavier, France). Triton X-100 was from Merck (Darmstadt, Germany); CHAPS was from Eurodex (Soffelwyersheim, France); and methotrexate was from Rhône-Poulenc (Montrouge, France). ATP and glutathione reductase (EC 1.8.1.7) was from Roche Applied Science (Meylan, France). Bradford reagent was from Pierce (Perbio Science France SA).

**Cell Lines**—BHK-21 cells were stably transfected with wild-type MRP1 as described previously (16). No MRP1 expression was detected in control BHK-21 cells by Western blot analysis (16). Cells were grown at 37 °C and 5% CO₂ in culture medium containing 1% (v/v) penicillin/streptomycin, 5% (v/v) fetal bovine serum, and 200 μM methotrexate for transfected cells.

**Cell Proliferation as Determined by MTT Assay**—The MTT colorimetric assay was used to assess the sensitivity of control and MRP1-transfected BHK-21 (MRP1-BHK-21) cells to verapamil enantiomers. Briefly, growth inhibition (IC₅₀) assays were performed by plating cells at a density of 1.0 × 10⁴ cells/well in 96-well plates. Cells were cultured for 17 h before the addition of verapamil enantiomers diluted in complete culture medium in the presence of 0.5% (v/v) Me₂SO. The cells were then incubated for 72 h at 37 °C and 5% CO₂. Surviving cells were quantified by the MTT colorimetric assay as described previously (17). The IC₅₀ values were derived from a typical MTT assay. Experiments were performed in triplicate and on different days. Results were averaged from triplicates of a typical experiment.

**Calcein-AM Assay**—To monitor the inhibitory effect of verapamil enantiomers on the transport of calcein by MRP1, cells were detached using EDTA, harvested by centrifugation at 500 × g, and resuspended in culture medium supplemented with 5 mM EDTA. Calcein-AM (0.2 μM final concentration) was added to 5.0 × 10⁵ cells in 4 ml of culture medium (supplemented with 5 mM EDTA to avoid cell aggregation) containing either 30 μM MK571 or verapamil enantiomers. The treated cells were incubated at 37 °C in the dark for 10 min and then harvested and resuspended in 400 μl of cold phosphate-buffered saline (PBS) containing 0.1% (v/v) bovine serum albumin. Green fluorescence intensity was immediately analyzed using an FL1 channel of a FACSscan flow cytometer (BD Biosciences, Heidelberg, Germany).

**Total Cellular Glutathione Determination**—The total cellular glutathione content was measured following the enzymatic method as described (18) and adapted (19). Cells were seeded in 96-well plates at a density of 1.0 × 10⁵ cells/well and cultured for 17 h before the addition of verapamil enantiomers, with the Me₂SO concentration not exceeding 0.5% (v/v). After various incubation times, cells were washed twice with 200 μl of cold PBS and then lysed with 50 μl of PBS supplemented with 0.1% (v/v) Triton X-100. The lysate was homogenized, and two aliquots of 5 and 10 μl were transferred to new plates for GSH titration and protein quantification, respectively. GSH titration was performed by adding 120 μl of PBS containing 221.3 μM NADPH, 462.6 μM 5,5’-dithiobis(2-nitrobenzoic acid), and 11 units/ml GSH reductase. The plate was then placed in a microplate reader, and the absorbance was measured at 412 nm every 30 s for 2 min. The slope was calculated for each sample, and the GSH concentration was determined by comparison with known glutathione standards. Protein titration was performed by the Bradford assay according to the manufacturer’s instructions. The total glutathione concentrations measured were expressed as nmol/mg of protein, and the results were averaged from triplicates. The experiment was repeated on different days.

**LTC₄ Transport Assay**—ATP-dependent LTC₄ transport was assayed by a rapid filtration technique (20, 21) as described (22) in the absence or presence of 3 mM GSH, racemic (RS)-verapamil, or each enantiomer at 0, 1, 4, 16, 64, and 256 μM. All experiments were performed in triplicate in the presence of either AMP or ATP at 4 mM. The amount of LTC₄ bound to the membrane vesicles in the presence of 4 mM AMP was considered as the background and subtracted from the corresponding reaction in the presence of 4 mM ATP.

**MRP1 Purification**—MRP1 purification was performed according to Chang et al. (16). BHK-21 cells expressing MRP1 with a C-terminal His₁₀ tag were treated with 4 mM sodium butyrate for 17 h to enhance MRP1 expression. Cells were then washed twice with cold PBS, detached with 5 mM EDTA, and harvested by centrifugation at 500 × g. The pellet was resuspended in hypotonic buffer containing 10 mM Tris-HCl (pH 7.4), 10 mM KCl, and 5 mM EDTA (1.5 ml/15-cm diameter dish) and supplemented with a protease inhibitor mixture. Crude membranes were prepared by homogenizing cells with a Potter-Elvehjem homogenizer (Fisher Scientific Bioblock, Illkirch, France). Nuclei were removed by centrifugation at 1000 × g for 15 min. The supernatant was then centrifuged at 45,000 × g for 45 min to collect the membranes. The pellet was resuspended in buffer A (20 mM Tris-HCl (pH 7.9), 0.5 M NaCl, 10 mM CHAPS, 20% (v/v) glycerol, and 5 mM imidazole) to obtain a final protein concentration of 2 mg/ml. The membranes were solubilized for 30 min at 4 °C under gentle shaking, and the sample was centrifuged at 10,000 × g for 15 min. The supernatant was then mixed with nickel-nitritriacetic acid-agarose (pre-equilibrated with buffer A) and gently shaken for 1 h at 4 °C. The packed nickel-nitritriacetic acid-agarose column was washed with 10 column volumes of buffer A containing 5 mM CHAPS and 25 mM imidazole and then with 10 column volumes of buffer A containing 5 mM CHAPS, 10% (v/v) glycerol, and 40 mM imidazole. The protein was eluted with 3 column volumes of buffer A containing 300 mM imidazole and 0.05% (v/v) β-mercaptoethanol. The eluate was extensively dialyzed in buffer B (20 mM Tris-HCl (pH 7.4), 0.5 M NaCl, 5 mM CHAPS, 10% (v/v) glycerol, and 0.05% (v/v) β-mercaptoetha-
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mol), 0.2% (w/v) asolectin was then added. The protein concentration was determined by the Bradford assay. Purified MRP1 was used no later than 24 h after dialysis and was subjected to SDS-PAGE (6% (v/v) polyacrylamide gel), immunoblotted on a nitrocellulose membrane, and probed with antibody MRPm6 (Alexis Biochemicals Corp., San Diego, CA).

**ATPase Activity Assay**—The ATPase activity of purified MRP1 was measured using a colorimetric ascorbic acid/ammonium molybdate assay (23). Briefly, 1.5 μg of purified MRP1 was incubated at 37 °C for 4 h in 100 μl of assay buffer containing 20 mM Tris-HCl (pH 7.4), 1 mM dithiothreitol, 10 mM MgCl₂, and 5 mM ATP in the presence or absence of 5 mM GSH or 1 μM verapamil enantiomers. All experiments were performed twice in triplicate and corrected for the background.

**Quenching of the Intrinsic Trp Fluorescence of MRP1 by Verapamil Enantiomers**—Fluorescence spectra were recorded with a Photon Technology International QuantaMaster I spectrofluorometer thermostatted at 25 °C using a 2-nm excitation and emission band-pass filter. All experiments were corrected for buffer contribution to fluorescence, and inner filter effects were measured on N-acetyltryptophanamide. Measurements were performed using 0.2 μM purified MRP1 in 500 μl of buffer B with an excitation wavelength of 295 nm and an emission scan range of 310−360 nm. The quenching studies were performed twice. Curve fitting and determination of dissociation constants were performed with the GraFit program (Erithacus Software).

**Measurements of Trp Accessibility to Sodium Iodide**—Modifications of tryptophan accessibility to sodium iodide from 0 to 400 mM were tested under the same conditions as those used for the quenching of intrinsic fluorescence. The ionic strength effect was controlled with NaCl, and no modification of the MRP1 fluorescence was detected. Quenching data were analyzed using the Stern-Volmer equation: $F_0/F = 1 + K_{SV}[Q]$, where $F_0$ and $F$ are the fluorescence intensities in the absence and presence of the quencher, respectively; $K_{SV}$ is the Stern-Volmer constant; and [Q] is the quencher concentration. The accessibility fraction ($f_a$) was obtained from the Lehrer equation: $f_a/ΔF = 1/(f_aK_{q}[Q]) + 1/f_a$, where $ΔF$ is the difference between $F_a$ and $F$ and $K_q$ is the accessibility constant. All the measurements were performed three times.

**RESULTS**

Differential Toxicity of Verapamil Enantiomers in MRP1-BHK-21 Cells—Racemic verapamil was shown previously to specifically kill MRP1-BHK-21 cells by triggering apoptosis through glutathione extrusion by MRP1 (13). In this study, we investigated the individual effects of each enantiomer by comparison with the racemic mixture. The cellular effects were tested on both control and MRP1-BHK-21 cells (Fig. 1). In agreement with previous results (13), racemic verapamil was cytotoxic for MRP1-BHK-21 cells at concentrations <10−20 μM, which were ineffective in control BHK-21 cells. The R- and S-enantiomers were highly discriminated because (R)-verapamil did not exhibit any cytotoxicity for the two types of cell, whereas (S)-verapamil displayed a killing activity, even more potent than that observed with the racemic mixture at the same concentration.

**Selectivity of Effects of Verapamil Enantiomers on the Intracellular GSH Content**—We checked the kinetics of intracellular GSH extrusion upon the addition of either racemic verapamil or its isolated enantiomers to the cell cultures. Fig. 2A shows an expected strong and rapid decrease in the total intracellular GSH content in the presence of racemic verapamil, as reported previously (13). Interestingly, the R-enantiomer had no effect on the intracellular GSH content, whereas the S-enantiomer induced a strong decrease in the intracellular GSH content, in agreement with the cytotoxicity shown in Fig. 1. In addition, 5 μM (S)-verapamil exerted a stronger effect on the intracellular GSH content compared with 10 μM racemic verapamil, which should also contain 5 μM (S)-verapamil, suggesting that the R-enantiomer might antagonize the S-enantiomer-induced modulation of MRP1 activity. To test this hypothesis, we investigated the effect of the R-enantiomer on the kinetics of the intracellular GSH decrease induced by the S-enantiomer. Double-reciprocal plot analysis indeed showed an inhibition in the micromolar range with mixed-type characteristics (Fig. 2B).

**Inhibition by (S)- and (R)-Verapamil of MRP1-mediated Calcein-AM Transport**—The above mixed-type inhibition pattern indicated that both enantiomers were able to interact with MRP1. We investigated their effects on the transport activity of MRP1 by flow cytometry using calcein-AM as a substrate. The control BHK-21 cells accumulated similar amounts of calcein regardless of whether verapamil was present or not (Fig. 3A). In contrast, both R- and S-enantiomers induced an increase in calcein accumulation in MRP1-overexpressing cells, similar to the MRP1-specific inhibitor MK571 (Fig. 3B), implying that either the R- or S-enantiomer can efficiently inhibit the function of MRP1.

**Inhibition of LTC₄ Transport by (S)- and (R)-Verapamil in the Presence of GSH**—The racemic mixture inhibited LTC₄ transport in a concentration-dependent manner only in the presence of GSH (Fig. 3C). (GSH alone inhibited the transport of ~10%, in agreement with Loe et al. (12).) In agreement with the results.
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FIGURE 2. Effect of racemic verapamil and its enantiomers on the total cellular glutathione content. A, control BHK-21 cells (open symbols) and MRP1-BHK-21 cells (closed symbols) were cultured in triplicate with 10 μM racemic verapamil (○ and ●), 5 μM (R)-verapamil (△ and △), or 5 μM (S)-verapamil (□ and □). Total glutathione levels were determined (the amount of GSH in untreated cells was taken as 100%), and data were analyzed by hyperbola fitting. B, the total glutathione level in MRP1-BHK-21 cells was measured in the presence of increasing concentrations of (S)-verapamil without (▼) or with either 2 μM (R)-verapamil (▼) or 4 μM (R)-verapamil (▲). Glutathione efflux was calculated as the difference between the initial cellular GSH content and the GSH content after 30 min at 37 °C in the presence of the modulator, and results were fitted using a double-reciprocal plot (Lineweaver-Burk representation). Data points represent the means of triplicate determinations in a typical experiment. Error bars indicate S.D.

obtained with the racemic mixture, each individual enantiomer could not efficiently inhibit LTC4 transport in the absence of GSH (Fig. 3C). Upon the addition of 3 mM GSH, (R)-verapamil only slightly inhibited LTC4 transport, whereas (S)-verapamil more strongly inhibited it compared with the racemic mixture. These results are consistent with those derived from Fig. 3 (A and B) on calcine accumulation.

Reversal of MRP1-induced Multidrug Resistance by (R)-Verapamil—Because (R)-verapamil inhibited MRP1 transport activity (Fig. 3) but showed no cytotoxicity for BHK-21 or MRP1-BHK-21 cells (Fig. 1), we speculated that (R)-verapamil might sensitize MRP1-BHK-21 cells to anticancer drugs. To test this hypothesis, the IC50 values of vincristine for BHK-21 or MRP1-BHK-21 cells were determined in the absence or presence of 5 or 15 μM R-enantiomer. Indeed, (R)-verapamil had no significant effect on the IC50 values of vincristine for control BHK-21 cells, whereas these values for MRP1-BHK-21 cells decreased from 9.42 to 2.87 nM in the presence of 5 μM R-enantiomer and 1.02 nM in the presence of 15 μM R-enantiomer (Table 1). 15 μM R-enantiomer led to complete reversion of the resistance to vincristine.

Molecular Effects of the Two Verapamil Enantiomers—To investigate the direct interaction of the two enantiomers with the transporter, we purified the MRP1 protein by a procedure adapted from Chang et al. (16) in the presence of lipids. Fig. 4A shows that only the purified MRP1 protein was detected on SDS-polyacrylamide gel stained with Coomassie Blue. The purified MRP1 protein was further confirmed on a Western blot probed with the MRP1-specific antibody MRPm6 (Fig. 4B). Quenching of MRP1 tryptophan fluorescence was proportional to the concentration of the S-enantiomer (Fig. 4C) or the R-enantiomer (Fig. 4D), indicating that both enantiomers indeed bound to MRP1. These results also suggested that the inhibition of MRP1 transport activity by the enantiomers (shown in Fig. 3), resulted from a direct interaction with the transporter. The Ks values for the R- and S-isomers were 35 ± 7 and 113 ± 24 nM, respectively, indicating that the two isomers tightly bound to purified MRP1. The addition of GSH prevented the S- and R-isomers from binding, more efficiently for the S-isomer (Fig. 4C) than for the R-isomer (Fig. 4D). The direct binding of GSH could not be measured because of the absence of any apparent effect on tryptophan fluorescence. Furthermore, the modification of MRP1 tryptophan accessibility to sodium iodide in the presence of the S- and R-enantiomers was different (Fig. 4E). Indeed, in the absence of ligand, the emitting tryptophan residues appeared to be equally accessible to sodium iodide as shown by a linear Stern-Volmer plot, with KSV = 1.46 ± 0.23 m−1. In the presence of either (S-) or (R)-verapamil, the Stern-Volmer plots were curved, indicating that the emitting tryptophan residues were not equally accessible to sodium iodide (fa = 0.42 and 0.48, respectively, as determined from the Lehrer equation). In both cases, the binding of the isomers led to distinct conformational changes because, in the Lehrer equation, the slope obtained in the presence of (S)-verapamil was 0.25, whereas it was 0.16 in the presence of (R)-verapamil. This likely correlates with the differential effects produced on MRP1 activity as described above.

An additional functional characterization was achieved by measuring the ATPase activity of MRP1. The basal vanadate-sensitive ATPase activity was slightly stimulated by either (S-) or (R)-verapamil (Fig. 5). GSH stimulated MRP1 ATPase activity, in agreement with the GSH stimulation reported by Hooijberg et al. (24) and Manciu et al. (25). The S-isomer completely reversed the GSH stimulation, whereas the R-isomer had no effect.

DISCUSSION

In this work, we have presented important new features of verapamil enantiomers, one of which revealed two different effects on MRP1. These new findings provide insight into the molecular mechanism of this ABC transporter and could be of great interest in reversion of MRP1-dependent multidrug resistance.

Although verapamil is a well known inhibitor of the P-glycoprotein-induced MDR phenotype (8), the results are controversial for MRP1. Indeed, although Cole et al. (9) did not observe any reversion of chemoresistance, Davey et al. (11) and Cullen et al. (10) described a small chemosensitization to daunomycin. However, verapamil has been clearly demonstrated to stimulate GSH transport (10, 12, 26) and to strongly inhibit LTC4 transport by MRP1 in the presence of GSH (12, 13). This duality reflects the complex mechanism of verapamil interaction as suggested previously (27). The inhibition of calcine transport observed here with MRP1-BHK-21 cells agrees with previous
mixed-type inhibition between the two isomers. The isolated R-enantiomer inhibited calcein-AM transport as efficiently as MK571 (Fig. 3) and exhibited an efficient sensitization of MRP1-transfected cell growth to vincristine at the concentrations used in this work. The S-enantiomer also inhibited LTC₄ transport and did so much more strongly compared with the R-isomer (Fig. 3C).

To our knowledge, this is the first time that two enantiomers of an MDR phenotype modulator have been described to display different effects. Indeed, for P-glycoprotein, there is no apparent difference in the inhibitory effects of either verapamil enantiomer on daunomycin transport (28) or of tricyclic compounds on drug efflux from MDR cells (29), whereas (R)-propranolol, but not (S)-propranolol, inhibits daunomycin transport (28). In contrast, for MRP1, we demonstrated here the dual activity of chiral verapamil, i.e. (i) the strong killing effect of the S-isomer and (ii) the chemosensitizing effect of the R-isomer. They are both potentially interesting for MDR phenotype modulation, the first to specifically kill potentially resistant tumor cells (13) and the second to increase the sensitivity of tumor cells to chemotherapy. Because the enantiomers bind to the MRP1 protein with high affinity and the bound verapamil is not transported by MRP1 (12), we expect that low concentrations of each enantiomer would have a long-lasting effect without the necessity of reaching high concentrations in chemotherapeutic treatments. However, the two isomers need to be used in separated forms, not in a mixture, because we have shown here that they antagonize each other.

We showed previously that iodination of verapamil leads to a strong increase in the killing potency of racemic verapamil without any increase in the toxicity for control cells (13). Furthermore, iodination has been achieved on the B-phenyl ring of the molecule, known to be important for cardiotoxicity (30). Therefore, the synthesis of the iodinated R- and S-enantiomers of verapamil is in progress. We hope to decrease the effective concentration of each isomer, especially the S-isomer, to escape their cardiotoxic effects. Such verapamil analogs, which reverse the MDR phenotype induced by P-glycoprotein, were found by Pereira et al. (31). Safety tests of iodinated derivatives will be performed on cardiomyocytes to determine whether iodination of the B-phenyl ring decreases toxicity.

Although GSH has been shown to prevent the binding of the R- and S-isomers, we cannot conversely make conclusions

TABLE 1
Reversion of the MDR phenotype by (R)-verapamil in MRP1-BHK-21 cells

| IC₅₀ for vincristine | 0 µM (R)-verapamil | 5 µM (R)-verapamil | 15 µM (R)-verapamil |
|---------------------|---------------------|---------------------|---------------------|
| BHK-21              | 1.51 ± 0.4          | 1.48 ± 0.27         | 1.06 ± 0.38         |
| MRP1-BHK-21         | 9.42 ± 0.23         | 2.87 ± 0.42         | 1.02 ± 0.18         |

Results showing an increase in daunomycin accumulation induced by verapamil in MRP1-overexpressing cells (10). Our new findings could explain these divergent observations because, with our cell type, racemic verapamil stimulation of GSH transport by MRP1 leads to apoptosis of MRP1-overexpressing cells (13). This cell death was shown here to be specifically induced by the S-isomer. In the racemic mixture, the latter masked the specific inhibition of (R)-verapamil. Indeed, the kinetics of the intracellular GSH decrease (Fig. 2B) showed a
about any effect of verapamil enantiomers on the affinity for GSH because GSH binding cannot be directly monitored by tryptophan fluorescence measurements. However, Karwatsky et al. (32) showed that a GSH photoaffinity analog is enhanced in the presence of verapamil. (R)-Verapamil, (S)-verapamil, or GSH alone moderately stimulated the ATPase activity of purified MRP1 (Fig. 5), implying that binding of each individual compound to MRP1 induced a conformational change in the protein. (R)-Verapamil + GSH enhanced the ATPase activity to the same level as GSH alone (Fig. 5). In contrast, (S)-verapamil + GSH abolished the enhancement effect of GSH (Fig. 5). In the presence of (S)-verapamil, no more stimulation of ATPase activity was observed, whereas (S)-verapamil stimulated GSH transport. We can conclude that the effect of (S)-verapamil does not correlate with ATPase activity. All these results suggest that the S-isomer, but not the R-isomer, could interfere with the GSH site, either directly or through induced conformational changes. This interference could have an impact on the GSH stimulation of ATPase activity. Comprehension of this phenomenon at the molecular level would bring insight to the transport mechanism of MRP1.

We showed previously that cellular GSH efflux leads to apoptosis of MRP1-overexpressing BHK-21 cells (13). A similar phenomenon was described in H69AR and HeLa/ABCC1 cells (33), and Hammond et al. (27) demonstrated that glutathione export during apoptosis requires multidrug-resistant proteins. However, other studies reported a GSH extrusion, but cell death was not observed (10, 12, 26). The cell-type dependence for each verapamil enantiomer is therefore under investigation.

Our results could be of great potential interest in future therapy. The verapamil enantiomers exert different effects on MRP1 activity. (S)-Verapamil acts as a “killer” by activation of MRP1-mediated GSH efflux, leading to the death of potentially resistant tumor cells. In contrast, (R)-verapamil acts as an inhibitor to block MRP1-mediated transport, resulting in chemosensitization of MRP1-overexpressing cells to anticancer drugs. In practice, we have to choose between the two enantiomers to obtain the highest modulatory effect of verapamil on cells overexpressing MRP1. The possibility of such a major difference in MRP1-interacting agents raises the important possibility of constructing different GSH transport and drug resistance modifiers. Furthermore, these new findings may prove to be critically important in interpreting data obtained using either verapamil or its derivatives.

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