Effects of Amiodarone, Cyclosporin A, and PSC 833 on the Cytotoxicity of Mitoxantrone, Doxorubicin, and Vincristine in Non-P-glycoprotein Human Small Cell Lung Cancer Cell Lines


ABSTRACT

The multidrug resistance (MDR) modulators amiodarone (AM), cyclosporin A (CsA), and PSC 833 were tested for their potential to modulate cytotoxicity of doxorubicin (DOX), vincristine (VCR), and mitoxantrone (MX) in a sensitive human small cell lung carcinoma cell line GLC4, in its DOX-resistant non-P-glycoprotein subline GLC4-Adr, and in its cisplatin-resistant subline GLC4-CDDP. GLC4-Adr, in which overexpression of the so-called multidrug resistance-associated protein has been demonstrated, is 91-fold resistant for DOX, 22-fold for VCR, and 7.5-fold for MX, compared with its sensitive cell line. AM previously modulated DOX and VCR resistance in the P-glycoprotein-positive human colon cancer cell line COLO 320. Cytotoxicity was studied in the microtiter well tetrazolium assay. In the small cell lung carcinoma cell lines described above, AM did not increase cytotoxicity of DOX, but increased VCR cytotoxicity; moreover, AM was shown to be a potent modulator of MX cytotoxicity. CsA did not potentiate DOX cytotoxicity, but, at a concentration of 4 μM, it modestly increased VCR cytotoxicity in GLC4. However, 0.8 and 4.0 μM CsA protected against MX cytotoxicity in GLC4 and GLC4-CDDP, but no effect was observed in GLC4-Adr. At the much higher ID₅₀ concentration CsA modulated MX cytotoxicity 1.6-fold in GLC4-Adr and slightly in GLC4 and GLC4-CDDP. PSC 833, a nonimmunosuppressive CsA analogue, did not alter the cytotoxicity of DOX or MX in these cell lines, but potentiated VCR cytotoxicity in GLC4-Adr at a concentration of 0.4 μM. The modulation of MX cytotoxicity by AM and the protection by CsA was confirmed in a clonogenic assay. In the colony-forming unit granulocyte-monocyte assay, no additional MX toxicity on normal bone marrow by AM was observed. Flow cytometry of cellular MX fluorescence was performed in order to elucidate the mechanism behind the AM-induced increased MX cytotoxicity. This revealed an increase in cellular MX after 1-h incubation of MX combined with AM and an inhibition of efflux from GLC4 and GLC4-Adr; CsA and PSC 833 had no effect on MX efflux. An increase in MX-induced cleavable complexes by AM in GLC4 was observed using the K⁺/sodium dodecyl sulfate coprecipitation assay, but no effect of CsA was found. In conclusion, AM enhances MX and VCR cytotoxicity in these sensitive, non-P-glycoprotein DOX and cisplatin-resistant small cell lung carcinoma cell lines. It also inhibits efflux of MX and causes more MX-induced cleavable complexes.

INTRODUCTION

The phenomenon of in vitro and in vivo MDR of tumor cells has been investigated extensively in recent years. Intrinsic and acquired resistance for several nonrelated natural cytostatic drugs and the presence of an energy-dependent membrane efflux pump, Pgp, are main features of the classical MDR phenotype (1–3). The best-known members of the MDR drug family are the anthracyclines, Vinca alkaloids, epipodophyllotoxins, colchicine, and actinomycin D. Non-Pgp MDR tumor cells also exist. Several mechanisms have been reported for this form of MDR, such as altered topoisomerase II (4–6), increased detoxification, and more recently overexpression of a new membrane transporter, the multidrug resistance-associated protein (7–11). Various modulators, such as verapamil, quinidine, diprydamole, AM, and CsA of MDR have been tested in vitro and in vivo (12–18).

More recently, specific inhibitors of P-glycoprotein-mediated MDR have been synthesized, such as S9788 and a nonimmunosuppressive analogue of CsA, PSC 833 (19–21). PSC 833 has been shown to be a 10-fold more potent than CsA in circumvention of resistance for Vinca alkaloids and anthracyclines in Pgp-positive tumors (18, 22–25). Barrand et al. (26) also recently described the efficacy of PSC 833 to restore the daunorubicin accumulation partially in a Pgp-negative MDR lung cancer cell line with the 190-kDa membrane protein and with a drug accumulation deficit. Until now, specific modifiers of non-Pgp-mediated MDR have not been described. MX does not strictly belong to the group of cytostatic MDR-related drugs (27), although cross-resistance with cell lines made resistant for DOX often exists (28, 29) and circumvention of MX cross-resistance by verapamil and CsA has been described in Pgp-positive tumor cell lines (17). Cell lines that have been made resistant for MX often demonstrate cross-resistance to DOX. Moreover, they are generally Pgp negative and although they frequently express the MDR phenotype, circumvention of MX resistance with classical MDR blockers is rare (30–33). MX resistance has been ascribed to reduced topoisomerase II catalytic activity and to reduced MX-induced cleavage of DNA by topoisomerase II in nuclear extracts (34–36). In clinical cross-over studies, no complete cross-resistance between DOX and MX exists (37, 38). Therefore MX seems to be a good alternative in some cases of DOX resistance. In order to evaluate whether the cytotoxicity of MX could be potentiated in sensitive and non-Pgp cells with the MDR phenotype, we tested AM, CsA, and PSC 833 at clinically relevant and ID₅₀ concentrations for their effect on MX cytotoxicity.

Their effect on the cytotoxicity of classical MDR drugs such as DOX and VCR was also monitored. The cytotoxicity assays were performed in a human sensitive, small cell lung carcinoma cell line, GLC4, in a non-Pgp DOX-resistant subline which overexpresses multidrug resistance-associated protein, GLC4-Adr (8, 9), and in a cisplatin-resistant subline, GLC4-CDDP. The mechanisms behind the observed phenomena were evaluated by monitoring the effect of these modulators on cellular MX fluorescence. In addition the effect of AM and CsA on the formation of MX-induced DNA-topoisomerase II cleavable complexes was studied since this is one of the main mechanisms of the cytostatic action of MX (39, 40).
MATERIALS AND METHODS

Chemicals

AM was kindly provided by Sanofi (Maassluis, The Netherlands), PSC 833 and CsA were donated by Sandoz (Basel, Switzerland). DOX was obtained from Farmitalia Carlo Erba, VCR from Eli Lilly (Indianapolis, IN), teniposide (VM26) from Bristol-Myers Squibb (Woesp, The Netherlands), and MX from Lederle (Etten-Leur, The Netherlands). [3H]thymidine was purchased from New England Nuclear (Boston, MA), RPMI 1640 medium from Gibco (Paisley, UK), and Dulbecco's modified Eagle medium (DME), Ham's F12, and Iscove's medium from Flow Labs (Irvine, CA). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and deionized bovine serum albumin were obtained from Sigma (St. Louis, MO). Agar was purchased from Difco (Detroit, MI), agarose from FMC (Rockland, ME), and LymphoPrep from Nycomed Pharma AS (Oslo, Norway). Methylcellulose was obtained from Dow Chemicals Co. (Midland, MI), FCS for all experiments, with the exception of the CFU-GM assay, was purchased from Sanbio (Uden, The Netherlands); FCS used in the CFU-GM assay was obtained from Hyclone (Logan, UT).

Cell Lines

GLC4 is a human small cell lung carcinoma cell line, derived in our laboratory from malignant pleural effusion. GLC4-Adr is the DOX-resistant subline of GLC4. This cell line has the so-called atypical MDR phenotype, with a lowered topoisomerase II activity compared with GLC4 (4, 5). Recently, a n transfection of a TCR7 gene into GLC4 has been demonstrated (7, 9, 41). GLC4-CDDP is a cisplatin-resistant subline of GLC4 that was established in this cell line (7, 9, 41). GLC4-CDDP is a cisplatin-resistant subline of GLC4 with an increased nuclear topoisomerase II activity compared with GLC4 (42, 43). In these three cell lines the same topoisomerase I activity was demonstrated (5). The cell lines were cultured in RPMI 1640 medium-10% FCS in a humidified atmosphere with 10% CO2 at 37°C.

Drug Sensitivity Assays

The Microculture Tetrazolium Assay. The MTA was performed as described previously (44, 45). A total volume of 0.1 ml was used per microculture well. Before the assays were performed the linear relationship of cell number to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide formazan production was checked and cell growth studies were performed. Cells (5,000/well for GLC4, 12,500/well for GLC4-Adr, and 15,000/well for GLC4-CDDP) in logarithmic growth phase were incubated in culture medium with the chemotherapeutic drugs alone or with the combination of the chemotherapeutic drugs and modulators. The concentrations of the modulators were chosen according to previous reports: 10 μM AM has previously proven its in vitro modulating efficacy and is a clinically achievable concentration (16). CsA, 0.8 μM (1 μg/ml), a commonly used MDR modulation dose in vitro, is considered 10-fold less potent than PSC 833, resulting in a concentration of 0.08 μM (0.1 μg/ml) of PSC 833 (25). In order to evaluate whether the eventual modulating effect is modulator dose related, the effect of a 5-fold higher concentration of each modulator was also studied. Moreover, ID10 concentrations of CsA and PSC 833 were tested in combination with MX. Experiments were performed after a 1-h drug incubation time. After the incubation period, cells were washed three times by removal of medium after centrifugation (10 min, 180 X g) followed by the addition of fresh medium. On day 4, cell survival was measured (45).

Clonogenic Assay. Underlayers of 0.5% agar in DME/F12 nutrient mixture-20% FCS, in a 35-mm Petri dish were used in all experiments. GLC4 cells were incubated for 1 h with MX minus or plus 10 μM AM or 0.8 μM CsA in RPMI 1640 medium plus 10% FCS. After the incubation period, the cells were washed three times with phosphate-buffered saline, and a suspension was prepared in 0.3% agarose in DME/F12-20% FCS without the drug. This suspension was plated as the upper layer. For GLC4 3000 viable cells (trypan blue dye exclusion) were cloned per dish (4). On day 8 colonies containing more than 40 cells were counted.

CFU-GM Assay. The MX-induced bone marrow toxicity was tested in both the presence and absence of AM by using a clonogenic bone marrow assay as described before (40). Six samples of normal bone marrow samples from four different patients were tested in duplicate after orally informed consent. Mononuclear cells were isolated from the bone marrow by using density gradient centrifugation with Lymphoprep. The in vitro colony assay for the normal myeloid progenitor cells was performed with 1.1% methylcellulose, 20% FCS, 1% deionized bovine serum albumin, and Iscove's medium. Mononuclear cells (3.10^6/ml) were incubated with medium (RPMI 1640 plus 10% FSC) as control, MX (0.075 μM, 0.01 μM, 0.015 μM, or 0.2 μM), or MX plus 10 μM AM. After 1-h incubation, the colonies were washed three times with RPMI 1640 medium plus 10% FCS. Subsequently 1 x 10^3 mononuclear cells were plated in methylcellulose, and, after 14 days, the myeloid colony formation (CFU-GM) was counted.

Intracellular Mitoxantrone Accumulation

Fluorescence excitation spectra indicated that the emission maximum of MX was 690 nm. Forward and side scatter was measured using a 488-nm argon laser. MX was excited with a helium-neon laser (Spectra Physics, 633 nm, power 40 mW) in a separated dual beam flow cytometer (Couler Epics Elite) with a gated amplifier for delaying the upper beam signals. Cellular MX was measured with the standard OMEGA 675 filter, having a band pass range of 40 nm. Cells (2.5 x 10^5 with 2.5 x 10^5 MX) were incubated for 1 h with MX minus or plus 10 μM AM, 0.8 μM CsA, or 0.08 μM PSC 833 at 0°C and 37°C. GLC4 and GLC4-Adr cells were incubated with 4.3 μM and 32.5 μM MX, respectively, resulting in a good measurable fluorescence signal. Intracellular MX accumulation was calculated by subtracting the MX fluorescence values measured at 0°C (autofluorescence plus extracellular bound MX) from the corresponding values measured at 37°C. The cellular MX resulting from a 1-h incubation with MX alone was taken as the 100% value. After the incubation period, the cells were centrifuged at 0°C for 10 min, the supernatant was removed, and the pellet was resuspended in 0.5 ml ice-cold phosphate-buffered saline. The MX fluorescence was then measured immediately. To study MX efflux, cells incubated for 1 h were resuspended in fresh medium and incubated for another hour at 37°C. After this hour the same procedure was followed as described above.

K+/SDS Coprecipitation Assay of Protein-DNA Complexes

The role of AM (10 μM) and CsA (0.8 μM) in the formation of MX-induced topoisomerase II-DNA complexes was measured by performing the K+/SDS coprecipitation assay as described (41, 47). GLC4 cells in culture were labeled for 24 h with [3H]thymidine (2 Ci/mmol) to a final concentration of 0.4 μCi([H]thymidine/ml. One hour before drug exposure the cells were washed twice and resuspended in fresh culture medium to a final concentration of 10^5 cell/ml. Subsequently the cells were incubated with MX, in the absence or presence of the modulating agent, at 37°C for 1 h. As a control of each experiment, cells were incubated with 25 μM VM26, which has previously been shown to yield cleavable complexes (41). After the incubation period the cells were pelleted at 900 x g for 5 min. Medium was removed and cells were lysed by addition of 1 ml of prewarmed (65°C) lysis solution, containing 1.25% SDS, 5 mm EDTA (pH 8.0), and 0.4 mg/ml salmon sperm DNA. After 10 min 250 μl 325 mM KCl were added and the suspension was vortexed vigorously for 15 s to fragment DNA. One ml of the lysate was transferred to a 1.5-ml Eppendorf tube, cooled on ice for 10 min, and centrifuged at 4°C in a microfuge for 10 min. The pellet was resuspended by heating at 65°C for 10 min with periodic mixing in 1 ml of wash solution containing 10 mm Tris-HCl (pH 8.0), 100 mm KCl, 1 mm EDTA, and 0.1 mg/ml of salmon sperm DNA. The tubes were then placed on ice for 10 min and centrifuged again at 4°C. The pellet was resuspended in 500 μl H2O at 65°C and added to 4 ml Picofluor-30 for radioactivity measurements. The remainder of the cell lysate was used to determine the total amount of radioactivity in 1 ml cell lysate. The amount of coprecipitated [H]-DNA was expressed as the percentage of the total amount of radioactivity present in 1 ml lysate.

Statistics

Statistical significance was determined with the paired Student's t test; P < 0.05 was considered significant.
RESULTS

Fig. 1 shows the results of the ID_{50} values of DOX, MX, and VCR with each cell line as measured with the MTA. Of the three cell lines tested, GLC4-Adr is not only the most resistant for DOX, but also for VCR and MX, as is shown in the logarithmically expressed results of the ID_{50} of each chemotherapeutic drug. The cross-resistance factors relative to GLC4, determined from the ID_{50} at 1-h incubation in the MTA, are, for DOX, MX, and VCR, 91, 7.5, and 22, respectively, with GLC4-Adr and 0.5, 0.7, and 3.0, respectively, with GLC4-CDDP. In Fig. 2, the DMFs on MX cytotoxicity by 10/50 μM AM and 0.8/4 μM CsA in the three cell lines are shown. AM demonstrated its strong potentiating effect on MX cytotoxicity in the MX-sensitive cell lines as well as in the MX-resistant GLC4-Adr, whereas CsA had no effect on GLC4-Adr and even significantly protected for MX cytotoxicity in the GLC4 and GLC4-CDDP. Table 1 shows the DMFs of three different concentrations of the three modulators on MX cytotoxicity in the three cell lines. By using the ID_{10} concentration of CsA, which is much higher than the commonly used modulation concentration, the protecting effect of CsA disappeared in GLC4 and GLC4-CDDP, and changed in a small, but significant potentiating effect of CsA in GLC4 and in a nonsignificant increase of MX cytotoxicity in GLC4-Adr. PSC 833 had no effect on the cytotoxicity of MX or on the cytotoxicity of DOX in these cell lines (data not shown). Only the highest concentration tested, 0.4 μM, had a modest, but significant, modulation effect on the cytotoxicity of VCR in GLC4-Adr, giving a DMF at ID_{50} of 1.2. Fig. 3 shows the DMFs of 10 and 50 μM AM on VCR cytotoxicity. AM had a modest, but significant VCR cytotoxicity-potentiating effect in all three cell lines. In contrast to the effects of AM on MX cytotoxicity, the effect of AM on VCR cytotoxicity by AM was dose dependent. CsA only induced in GLC4 a DMF of 1.4 at ID_{50} for VCR at the highest tested dose of 4 μM, but had no effect on VCR cytotoxicity in GLC4-Adr and GLC4-CDDP. CsA as well as AM did not modulate DOX cytotoxicity in any of the cell lines tested. Because of the interesting AM-induced increase and CsA-induced decrease in MX cytotoxicity, another cytotoxicity assay was performed in order to rule out effects on mitochondrial activity. In the clonogenic assay (n = 2 in triplicate) the percentage of colonies of clonogenic cells was taken as control (100 ± 7.0%). GLC4 cells cultured in the presence of AM demonstrated a slight increase in the number of clonogenic cells (109 ± 7.7%, P < 0.025), whereas in the presence of AM did not modulate DOX cytotoxicity in any of the cell lines tested. In order to elucidate the mechanisms behind these modulation effects on MX cytotoxicity, cellular accumulation of MX was studied by measurement of MX fluorescence. In Fig. 4 and 5 the percentage of MX accumulation is shown in GLC4 and GLC4-Adr after 1-h incubation with MX alone or in the presence of one of the modulators. AM increased cellular MX in both GLC4 and GLC4-Adr, whereas CsA and PSC 833 did not influence cellular MX. Efflux of MX was inhibited by AM in GLC4 and GLC4-Adr (n = 3, in duplicate); in GLC4 41 ± 11% efflux of MX was observed in the first hour after 1-h incubation versus 33 ± 4% after incubation with MX + AM (not significant). In GLC4-Adr the 1-h efflux of MX was 54 ± 12% after incubation with MX alone versus 30 ± 14% efflux after coincubation of MX and AM (P < 0.0025). No effect of CsA and PSC 833 on MX efflux was observed in these cell lines.

The effect of AM or CsA on cleavable complex formation by MX was studied in GLC4 using the K^{+}/SDS coprecipitation assay (n = 3, in duplicate). The percentage of cleavable complex formation with 0.5 μM MX alone was 17.2 ± 2.6. Addition of AM resulted in 27.0 ± 1.0% MX-induced cleavable complexes, an increase in cleavable complex formation of 59% (P < 0.01), whereas adding CsA to MX resulted in 15.6 ± 6.0% cleavable complexes, which is a slight decrease of 5% (not significant) relative to MX-induced cleavable complex formation alone. In the control experiments 25 μM VM26
induced $50.7 \pm 3.0$ cleavable complexes, which corresponds with previous results (41). Higher concentrations of MX (1 and 5 $\mu M$) resulted in 23 and 33% cleavable complexes, respectively ($n = 2$ in duplicate). At these concentrations no effect of CsA on the cleavable complex formation of MX was observed, whereas the increase induced by AM was 11% at 1 $\mu M$ MX and 22% at 5 $\mu M$ MX ($n = 2$, in duplicate).

In the CFU-GM assay, AM alone was not bone marrow toxic as was shown previously (16). Six samples of normal bone marrow from four different patients were tested in duplicate. The untreated controls, taken as 100%, varied between 136 and 408 CFU-GM/1 $\times 10^3$ mononuclear cells on day 14. Two-tenths $\mu M$ MX alone and 0.2 $\mu M$ MX + 10 $\mu M$ AM induced 25% survival; 0.15 $\mu M$ MX induced 85% survival and 83% after MX + AM; 0.1 $\mu M$ MX resulted in 84% survival and 82% after MX + AM; and 0.075 $\mu M$ MX yielded 85% survival and 80% with 0.075 $\mu M$ MX + AM. Thus no additional MX-induced bone marrow toxicity by AM was observed.

**DISCUSSION**

From previous reports it is known that AM is a potent modulator of classical, Pgp-mediated MDR in vitro (15, 16). AM was able to reverse DOX cytotoxicity in Pgp-positive tumor cells, whereas no effect on DOX cytotoxicity in the non-Pgp MDR tumor cell line GLC4-Adr was observed (16). AM also enhanced VCR cytotoxicity in Pgp-positive tumor cells (16). In the present study, it has been demonstrated that AM not only reverses cytotoxicity in Pgp-positive cells, but cytotoxicity of VCR was also potentiated by AM in a sensitive cell line GLC4, in a cisplatin-resistant cell line GLC4-CDDP, and in the non-Pgp cell line GLC4-Adr which expresses 22-fold cross-resistance for VCR compared with GLC4. High-dose CsA and PSC also significantly potentiated VCR cytotoxicity in GLC4 and GLC4-Adr, respectively, although the DMFs were very low. Shalinsky et al. (48) previously reported about the synergistic effect of dipyridamole on vinblastine cytotoxicity in HT1080/DR4 cells, a DOX-resistant human fibrosarcoma cell line with a MDR phenotype, but lacking MDR1 expression. However, they found much more modulation efficacy of dipyridamole on vinblastine cytotoxicity in the KB-GRCF cell line which expresses the MDR1 gene (14). No clear-cut explanation for the significant potentiation of VCR cytotoxicity in our cell lines, presumably by AM, can be given. Previously in the Pgp-positive cell line COLO 320, the effect of AM on the cytotoxicity of VCR was much more pronounced with DMFs of 2.8 and 12.4 after incubation with 10 $\mu M$ and 50 $\mu M$ AM, respectively, which confirms the observations of Shalinsky et al. (16, 48).

In this study, the potentiating cytotoxic effect of MX by AM was interesting in the sensitive GLC4 and the cisplatin-resistant subline GLC4-CDDP. Although the dose-modifying factor of AM on MX cytotoxicity in GLC4-Adr is smaller than in GLC4 and GLC4-CDDP, the absolute reduction in $ID_{50}$ is much more pronounced in this MX cross-resistant cell line. The main cytotoxicity mechanisms of MX are thought to be related to DNA binding and the trapping of DNA topoisomerase II complexes on cellular DNA (40). The cross-resistance of MX with DOX in GLC4-Adr may be explained by the lowered topoisomerase II activity of this cell line. Another explanation might be a decreased accumulation of MX in GLC4-Adr, as is also the case for DOX in this cell line (5). The increase in the amount of MX-induced topoisomerase II-DNA complexes in the presence of AM in GLC4 might be attributable to a higher intracellular MX concentration, but may also be the result of a direct action on the formation of topoisomerase II-DNA complexes. The results of the MX accumulation studies suggest that the potentiation in cytotoxicity...
effects of MDR modulators on MDR drugs in non-Pgp cell lines

of MX is caused by an inhibition of MX efflux. An alternative explanation of the AM-induced increase in MX and also VCR cytotoxicity might be that AM displaces the protein-bound chemotherapeutic drug and thus increases the intracellular pool of free drug.

CsA and PSC 833 did not potentiate cytotoxicity of DOX in these Pgp-negative small cell lung carcinoma cell lines. The protective effect of clinically achievable CsA concentrations on MX cytotoxicity in the MTA for GLC4 and GLC4-CDDP, as described in the clonogenic assay for GLC4, is noteworthy. This effect could neither be ascribed to a decrease in cellular uptake of MX by CsA or to an increase in efflux, nor to a decrease in the number of cleavable complexes. Moreover, when ID₁₀ concentrations of CsA were used, this protective effect of CsA disappeared. Therefore, the mechanism behind this phenomenon is not yet completely elucidated. In the only cell line in which cross-resistance for MX was found, GLC4-Adr, only the ID₁₀ concentration of CsA, which again is much higher than a clinically achievable plasma concentration, potentiated MX cytotoxicity. Freedman et al. (49) using flow cytometry recently demonstrated an increase in MX cellular fluorescence by adding either verapamil or CsA to the Pgp-positive ovarian cancer cell lines SKVCR and SKVLB (49). They did not see more MX fluorescence after coincubation with these modulators in the Pgp-negative sensitive cell line SKOV. The fact that the cellular fluorescence did not decrease, even though we found a protective effect of CsA on MX cytotoxicity in GLC4, only demonstrates that there is no clear correlation between whole cell fluorescence and cytotoxicity. A change in intracellular compartmentalization of MX might be of more importance. The role of a change in subcellular distribution of MX in relation to the sensitivity of MDR cells to MX has also been suggested by Smith et al. (50).

The combination of MX and AM might be interesting for the clinic, although side effects of long-lasting amiodarone administration should be taken into account, and the indication for such a combination regimen should be determined first. It is reassuring to know that in vitro MX-induced bone marrow toxicity did not increase when AM was added. On the other hand, the fact that at more or less standard concentrations, CsA had a protective effect on MX cytotoxicity is a warning not to extrapolate the promising in vitro circumvention effects of one modulator to the whole group of MDR modulators, or to consider one effective MDR modulator for a certain MDR chemo-therapeutic drug also effective for the whole group of MDR(-related) drugs. Combinations of classical MDR drugs, such as VP16 with MX, are administered especially in leukemias. Therefore, studies with CsA to circumvent drug resistance in this context should be considered with extra caution. In conclusion, AM is not only an effective modulator of classical MDR in vitro, but has also proven its efficacy in potentiation of MX and VCR cytotoxicity in non-Pgp cell lines. The observed inhibition of MX efflux in GLC4 and GLC4-Adr might be related to the presence of a non-Pgp pump.

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References


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