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Evidence for Transcriptional Control of Human *mdr1* Gene Expression by Verapamil in Multidrug-Resistant Leukemic Cells

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SUMMARY

We investigated the mechanism of verapamil (VRP) effects on *mdr1* gene expression in two leukemic multidrug-resistant (MDR) cell lines, K562/ADR and CEM VLB_{100}. Exposure to VRP for 24 hr resulted in a decrease in *mdr1* mRNA levels that was dose related at concentrations between 15 and 50 μM. The maximal decrease of *mdr1* mRNA levels was found to be 6-fold in the K562/ADR cells and 3-fold in the CEM VLB_{100} cells. The effect of VRP on *mdr1* mRNA levels was, however, biphasic. At 100 μM VRP, which strongly inhibited cell proliferation, a 2-fold increase of *mdr1* mRNA levels was observed in the K562/ADR cells. To determine whether the decrease of mRNA levels resulted from post-transcriptional mechanisms, mRNA stability was studied after blocking of transcription with actinomycin D in VRP-treated cells and in control cells. This study revealed that *mdr1* mRNA was stable in both cell lines and no increase in *mdr1* mRNA degradation was observed in the 30 μM VRP-treated cells versus control cells (half-lives of 23 hr versus 14 hr for the K562/ADR cells and 15.5 hr versus 10.0 hr for the CEM VLB_{100} cells). The suggestion of a transcriptional mechanism was confirmed by nuclear run-on assays. A 4-fold decrease in the *mdr1* gene transcription rate was observed in the 30 μM VRP-treated CEM VLB_{100} cells. The decreased transcription rate could be due to the decrease in *mdr1* proximal promoter activity observed in CEM VLB_{100} cells transiently transfected with the *mdr1* promoter fused to the chloramphenicol acetyltransferase gene. Indeed, after exposure to 30 μM VRP, chloramphenicol acetyltransferase activity was decreased by 2-fold. This study reports for the first time a down-regulation of *mdr1* gene transcription by a pharmacological agent. These results provide further identification of the regulatory mechanisms involved in the overexpression of *mdr1* in MDR cells and may help in the development of new strategies for MDR reversal.

The Pgp is a 170-kDa membrane glycoprotein that is overexpressed in cells with the MDR phenotype. Pgp is capable of reducing intracellular drug concentrations by increasing the efflux, from resistant cells, of a variety of heterocyclic anticancer drugs (i.e., anthracyclines, *Vinca* alkaloids, and act D), thereby reducing their cytotoxic potential (1). Pgp is frequently overexpressed in human tumors (1, 2), and its expression has been associated with poor treatment outcome in hematological malignancies such as acute lymphoblastic and nonlymphoblastic leukemias (3, 4).

A number of investigators have previously reported that a large group of coumpounds, including calcium channel blockers, can serve as chemosensitizers *in vitro* (5). Most of them have been shown by photoaffinity labeling studies to bind directly to Pgp, and they presumably block cytotoxic drug binding and efflux through a competitive inhibition mechanism (6, 7). Another approach to reverse the MDR phenotype may lie in selective down-regulation of Pgp expression in resistant tumor cells by pharmacological agents. Using this approach, we previously established that treatment with VRP (15–30 μM) led to a decrease in *mdr1* mRNA and Pgp levels, as well as a significant improvement of both doxorubicin and vincristine cytotoxicity in two resistant leukemic cell lines, K562/ADR and CEM VLB_{100} (8). To investigate the molecular basis of this pharmacological effect, we studied the effect of VRP on *mdr1* gene expression at both the transcriptional and post-transcriptional levels.

Materials and Methods

Cell lines and cell culture. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM glutamine, 125

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ABBREVIATIONS: Pgp, P-glycoprotein; MDR, multidrug resistance (multidrug-resistant); VRP, verapamil; *MDR1*pp, *mdr1* proximal promoter; VLB, vinblastine; TCA, trichloroacetic acid; bp, base pair(s); act D, actinomycin D; SDS, sodium dodecyl sulfate; SSC, standard saline citrate; CAT, chloramphenicol acetyltransferase; SV40, simian virus 40.

units/ml penicillin, and 125 μg/ml streptomycin. K562 cells and the drug-resistant subline K562/ADR were provided by Dr. Jeanneson (Institut J. Godinot-Reims) (9, 10). CEM cells and the VLB-resistant subline CEM VLB_{100} were provided by Dr. Victor Ling (Ontario Cancer Institue, Toronto, Canada) (11). The cells were grown in the continuous presence of drug and were passaged in drug-free medium 5 days before any experiments.

For cell proliferation studies, 1×10^5 cells/ml were grown for 24 hr in the presence of increasing concentrations of VRP. After this time period, cell viability was assessed by the trypan blue dye exclusion test.

[5-^{3}H]Uridine incorporation. Cells (2×10^5) were plated into 24-well plates, with or without VRP (30 μM), 24 hr before the beginning of the experiment. The cells were then pulsed for 2 hr with 2 μCi of [5-^{3}H]uridine (5 mCi/mol; Amersham International). The cells were washed three times with cold phosphate-buffered saline and lysed in 200 μl of 4 M guanidium thiocyanate, 25 mM sodium citrate, pH 7.0, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol. The RNA was then precipitated by addition of 5 ml of 5% TCA in 60 mM sodium pyrophosphate to the 200-μl RNA lysate. Precipates were allowed to form for 30 min on ice. The TCA precipitate was then trapped on a water-saturated glass fiber filter (Whatman GF/F), extensively washed with 1% TCA by filtration, and dried under a heat lamp. The filters were placed into 5 ml of scintillation fluid (Ready-Solv-MP; Beckman Instruments, Fullerton, CA) and counted by scintillation counting.

Northern blotting. Total RNAs were extracted by the method of Chomczynski and Sacchi (12). Two to 8 μg of RNA were then separated on a 1.2% agarose-7% formaldehyde gel. RNA was transferred to nitrocellulose and hybridized as described previously (8), using a *mdr1* cDNA probe that corresponded to the first 5′ third of the *mdr1* mRNA (nucleotides 325–1600) (obtained from Dr. P. Borst, Division of Molecular Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands). After autoradiography (24–72 hr, at −70°, with intensifying screens), the intensity of the signals was quantified by densitometric scanning. As a control for the amount of RNA loaded in each well, the amounts of *36B4* mRNA and rRNA were checked in the same experiments. The *36B4* gene codes for a ribosomal phosphoprotein, and levels of its mRNA are not affected by various treaments such as phorbol esters and estradiol (13). The *36B4* gene expression was revealed using a 600-bp fragment of the gene (a generous gift of Dr. P. Chambon, INSERM U184, Biologie Moleculaire et genie génétique, Strasbourg, France). In addition, before hybridization experiments the transfer membranes were photographed under UV light to assess the amount of rRNA by ethidium bromide fluorescence. A good correlation was found between the two methods, and *36B4* mRNA levels were further used as a control for RNA loading.

RNA stability studies. The optimal act D concentration that inhibits >95% of RNA synthesis in living cells was determined for K562/ADR and CEM VLB_{100} cells as follows. Cells (1×10^5) were plated into 24-well plates containing RPMI 1640 medium plus 10% fetal calf serum. For the VRP-treated cells, the drug was added at a 30 μM concentration 24 hr before the experiment. Serial dilutions of act D (0–60 μg/ml) were added to control and VRP-treated cells for 1 hr. The cells were then pulsed for 2 hr with 2 μCi of [5-^{3}H]uridine and processed as described above.

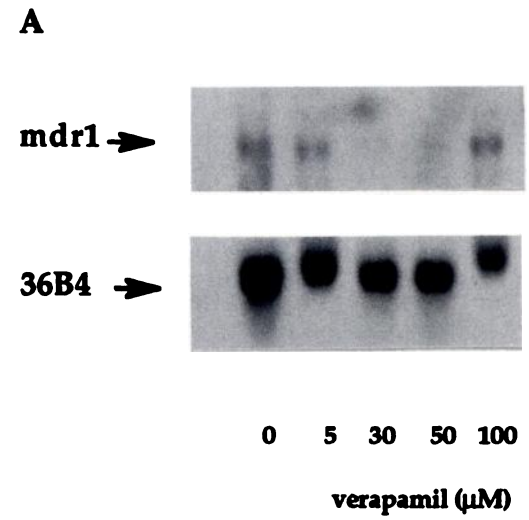
Once the optimal act D concentration for each cell line (with and without VRP) had been determined, mRNA stability was studied. Cells (4×10^6) were plated in a 75-cm^2 tissue culture Falcon flask. Half of the flasks were pretreated with VRP for 24 hr. The control and VRP-treated cells were then further incubated with the optimal act D concentration for 0, 8, 16, or 24 hr, at which time points RNAs were extracted and the level of *mdr1* mRNA was measured by Northern blotting as described above. For the stability studies, controls for the amount of mRNA loaded in each well were performed using a 28 S rRNA probe generated by reverse transcription-polymerase chain reaction (1 min of denaturation at 94°, 1 min of primer annealing at 55°, and 2 min of extension at 72°) in the presence of [α-^{32}P]dCTP, using 0.05 μg of cDNA, as described previously (14). Primers used for the amplification of ribo-specific sequences were 5′-GAAAGATGGTGAACTATGCC-3′ (sense strand, positions 1501–1520) and 5′-TTACCAAAAGTGGCCCACTA-3′ (antisense strand, positions 1827–1846), yielding a 346-bp product.

Nuclear run-on assays. For nuclear run-on experiments, exponentially growing CEM VLB_{100} cells (1.5×10^5 cells/ml) were exposed to 30 μM VRP. After 24 hr, cells were centrifugated at 1200 rpm for 15 min at 4°, and pellets were washed twice with cold phosphate-buffered saline. Cells were then resuspended in 2 ml of buffer A (10 mM Tris·HCl, pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% Nonidet P-40) and chilled on ice for 5 min. Suspensions were then added to tubes containing 5 ml of buffer B (50 mM Tris, pH 8.3, 5 mM $MgCl_2$, 0.1 mM EDTA, 30% sucrose), and purified nuclei were obtained at the bottom of the tubes after centrifugation for 5 min at 2000 rpm. Purified nuclei were resuspended in nuclear storage buffer (50 mM Tris·HCl, pH 7.3, 5 mM $MgCl_2$, 0.1 mM EDTA, 40% glycerol) and stored in liquid nitrogen until use. For transcription run-on assays, the nuclei were quickly thawed and incubated for 30 min at 30° in 10 mM Tris·HCl, pH 8.0, 5 mM $MgCl_2$, 300 mM KCl, 0.5 mM ATP, 0.5 mM GTP, 0.5 mM CTP, with 10 μl of [^{32}P]UTP (specific activity, 3000 Ci/ml; Amersham International). The mixture was further incubated for 5 min at 30° with 200 IU of RNase-free DNase and for 1 hr at 42° in the presence of 0.15% SDS and 0.1 mg/ml proteinase K. The RNA was then purified on a G-50 Sephadex column. Hybridization of labeled RNA (about 15×10^6 dpm) was carried out on nitrocellulose filters (to which 5 μg of alkali-denatured DNA plasmid-containing probes had been immobilized) in Church buffer (0.5 mM sodium phosphate, pH 7.1, 7% SDS, 0.1 mM EDTA) for 72 hr at 65°. The following DNA probes were used for nuclear run-on experiments: *mdr1* fragments from nucleotide 325 to nucleotide 1600 (probe 1) or from nucleotide 1600 to nucleotide 4777 (probe 2), a *36B4* fragment, and vector alone (PuC 9) as a negative control. The blots were first washed in 0.1× SSC (Standard Saline Citrate: 15 mM NaCl, 1.5 mM Na_3 citrate)/1% SDS at room temperature for 30 min and then quickly rinsed with 2× SSC before being incubated for 15 min in 2× SSC in the presence of 2 μg/ml RNase. A final washing was performed at room temperature in 0.1× SSC/0.1% SDS for 30 min, and blots were exposed for autoradiography at −70° for 2–10 days, using intensifying screens, and were analyzed by densitometry.

DNA transfection and CAT assays. Transient transfection of *MDR1*pp into leukemic cells was performed using *MDR1*pp containing CAT as a tracer gene. The following plasmid constructs were used: *MDR1*pp-CAT (633 bp upstream of the major site of initiation of transcription inserted at the *Sal*I cloning site in the 5′ end of the CAT gene in pSb1), pSVECAT (containing the CAT gene under SV40 promoter control), and pCH110 (containing the functionnal *lacZ* gene under SV40 promoter control) (the latter two provided by the American Type Culture Collection). Transient DNA transfection and CAT activity assays were performed as described previously (15). β-Galactosidase activity was measured in each sample, as a control for transfection efficiency, and was used to normalize CAT activity. The effect of VRP treatment on the *mdr1* promoter activity was studied after 24-hr exposure.

Results

Dose-effect curve for the effects of VRP treatment on *mdr1*/Pgp expression. We previously established that treatment of K562/ADR and CEM VLB_{100} cell lines with 15 μM VRP for 72 hr led to a 2–3-fold decrease in Pgp expression, with a marginal effect on cell proliferation (8). A decrease in *mdr1* mRNA expression levels was observed after 16 hr of treatment with VRP, and the effect was found to be maximal at 24 hr (8). Here, we studied the dose-effect curve for VRP treatment (5–100 μM) effects on *mdr1* mRNA levels after 24-hr incubation. As shown in Fig. 1A, exposure to VRP resulted in a decrease in *mdr1* mRNA levels that was dose related at



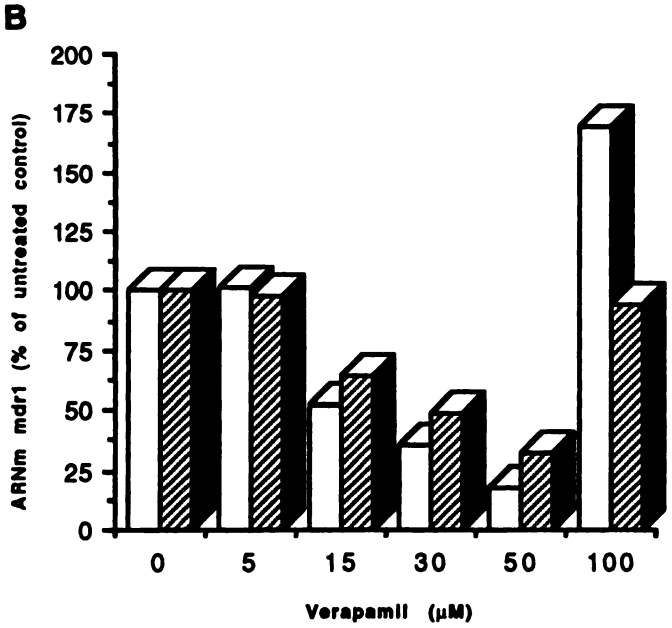


Fig. 1. Dose-effect curve for effects of 24-hr VRP treatment on *mdr1* mRNA levels. Exponentially growing cells were cultured in the presence of increasing VRP concentrations, and RNA was extracted after 24-hr incubations with the drug. A, *Upper*, representative Northern blot obtained with the K562/ADR cells, using a *mdr1* probe (nucleotides 325–1600). *Lower*, same blot, hybridized with a *36B4* probe. B, Representation of the results obtained after densitometric analysis of autoradiographs (mean of at least two experiments). Results are expressed as percentage of the *mdr1* mRNA levels in untreated controls. □, K562/ADR cells; ■, CEM VLB_{100} cells. ARNm = mRNA.

concentrations between 15 and 50 μM. From two separate experiments performed with the K562/ADR cell line, densitometric analysis revealed that a maximal decrease occurred at 50 μM (6-fold diminution), whereas a 3-fold diminution was observed at 30 μM. Similar results were obtained with the CEM VLB_{100} cell line; however, the effect was less pronounced when a 50 μM concentration was used (3-fold decrease) (Fig. 1B). Noteworthy is the fact that, in both cell lines, the effect was biphasic. Indeed, at a 100 μM concentration, the *mdr1* mRNA level was unaffected in the CEM VLB_{100} cells, whereas a 2-fold increase was noted in the K562/ADR cells.

In parallel, the dose-effect curve for VRP effects on cellular proliferation was studied. As shown in Fig. 2, whatever the leukemic cell line considered, no effect on cell proliferation was observed for concentrations below 35 μM, whereas at higher concentrations K562/ADR cells were more sensitive to VRP than were CEM VLB_{100} cells. Indeed, VRP at 100 μM strongly inhibited K562/ADR cell proliferation but only slightly inhibited that of CEM VLB_{100} cells (35% versus 70% of the untreated control, respectively). Based on these results, we used 30 μM VRP for further studies, given that at this concentration, in both cell lines, VRP decreased *mdr1* mRNA expression levels without showing any effect on cell proliferation.

Effect of VRP treatment on *mdr1* mRNA stability in CEM VLB_{100} and K562/ADR cells. To investigate the *mdr1* mRNA stability in the two cell lines, treated or not with 30 μM VRP, the half-life of the *mdr1* mRNA was estimated in Northern blot analyses, by measuring the decay of the specific *mdr1* signal in total cellular RNA from cells treated with the RNA polymerase inhibitor act D over a period of 24 hr. We first established the optimal act D concentration that blocks >95% of RNA synthesis. This step was especially important because act D can be efficiently transported by Pgp (1), which is expressed at high levels in the K562/ADR and CEM VLB_{100} cells. In fact, both cell lines were cross-resistant to act D (330-fold resistance and 670-fold resistance for the K562/ADR and CEM VLB_{100} cells, respectively, when incubated for 24 hr with the drug) (data not shown). A 95% inhibition of K562/ADR cell RNA synthesis (Fig. 3) was obtained at 20 μg/ml act D, compared with 0.5 μg/ml act D in the parental cell line K562 (data not shown). When the K562/ADR cells were incubated for 24 hr with 30 μM VRP, 5 μg/ml act D inhibited >95% of RNA synthesis. This result is explained by the fact that VRP increased act D accumulation via direct binding to Pgp, thereby inhibiting drug efflux (6). For the CEM VLB_{100} cell line, the

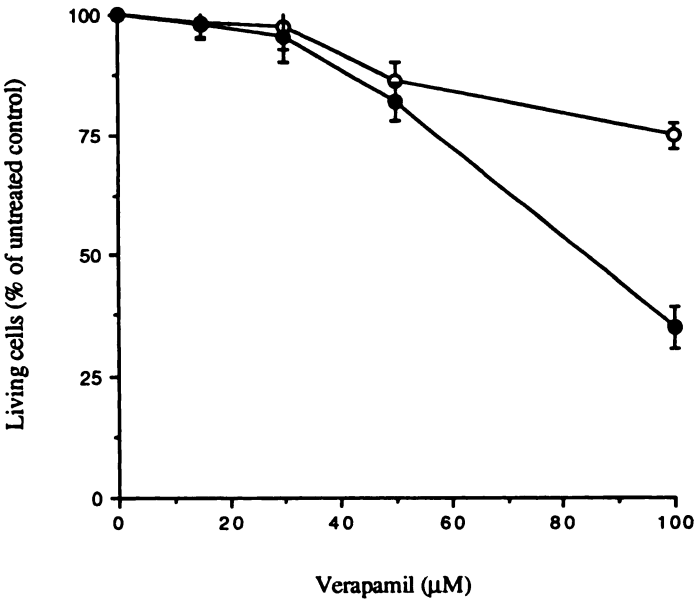


Fig. 2. Effect of VRP treatment on K562/ADR and CEM VLB_{100} cell proliferation. Exponentially growing cells were incubated in the presence of increasing concentrations of VRP for 24 hr, and then living cells were counted. Results are the mean of three experiments. ●, K562/ADR cells; ○, CEM VLB_{100} cells.

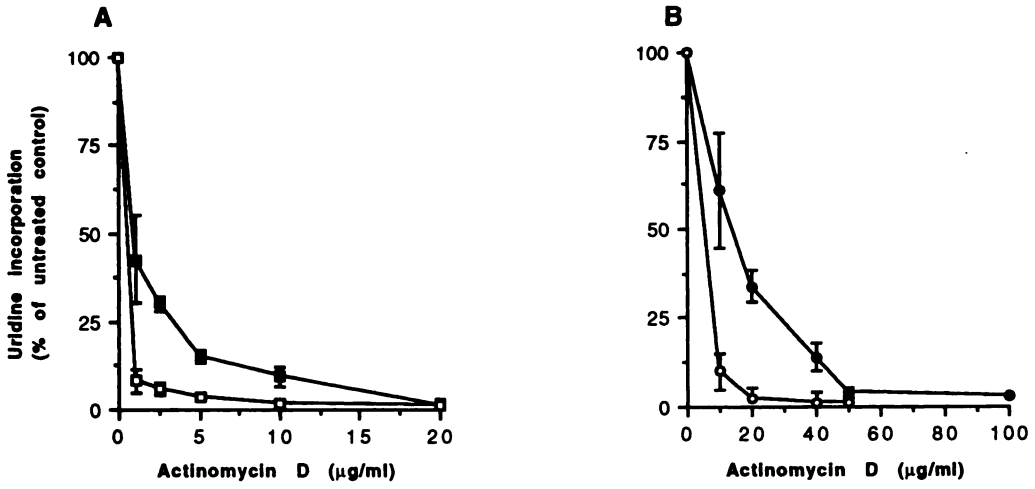


Fig. 3. Inhibition of [^{3}H]uridine incorporation in the presence of act D. Cells were grown for 24 hr, with or without 30 μM VRP. Serial dilutions of act D were added to control and VRP-treated cells for 1 hr, and the cells were then pulsed for 2 hr with 2 μCi of [5-^{3}H]uridine and processed as described in Materials and Methods. A, ■, Control K562/ADR cells; □, treated cells; B, ●, control CEM VLB_{100} cells; ○, treated cells.

act D concentrations were determined to be 50 μg/ml in the control cells and 20 μg/ml in the VRP-treated cells.

Preliminary experiments showed that the rates of decay of *mdr1* mRNA and *36B4* mRNA were very similar (half-lives between 8 and 16 hr); the latter probe then appeared to be inadequate to evaluate the amount of mRNA loaded in each well, to ensure quantitative measurement of the *mdr1* mRNA half-life. Thus, in our study of *mdr1* mRNA degradation we used a 28 S ribosomal probe generated as an internal control by reverse transcription-polymerase chain reaction, because a previous study reported that its half-life was >72 hr (16).

Using these experimental conditions, the *mdr1* mRNA half-life was determined to be 14 hr in the K562/ADR cells, compared with 23 hr in the 24-hr VRP-treated cells (Fig. 4, A and C). Similarly, in CEM VLB_{100} cells, the half-life corresponded to 10.0 hr in the control cells and 15.5 hr in the VRP-treated cells (Fig. 4, B and D). Thus, we were unable to show any decrease of mRNA stability after VRP treatment that could explain the decrease of *mdr1* mRNA steady state levels.

Nuclear transcription run-on assays of the *mdr1* gene in control and VRP-treated CEM VLB_{100} cells. The absence of variation in *mdr1* mRNA stability in control and VRP-treated cells prompted us to study the effect of VRP treatment on the *mdr1* transcription rate in CEM VLB_{100} cells, using the nuclear run-on technique. Nuclear run-on assays were performed using a human *mdr1* cDNA fragment corresponding to the 5′ region (encompassing nucleotide 325 to nucleotide 1600; probe 1). Because we determined by Northern blotting that *36B4* mRNA levels were unaffected by VRP treatment, this cDNA probe was used as a control for transcriptional activity in treated and untreated cells. As shown in Fig. 5, a 4-fold decrease of the *mdr1* signal was seen in the VRP-treated cells, compared with untreated controls. Furthermore, we determined that the *36B4* signal in treated cells was similar to that in control cells. α-Amanitin treatment (2 μg/ml) abrogated the signal detected with both *mdr1* and *36B4* probes (data not shown). Together, these results suggest that VRP effects on *mdr1* mRNA levels in the CEM VLB_{100} line are mediated by a transcriptional mechanism.

Effect of VRP on *MDR1*pp activity. Based on the run-on experiment results, we further studied the effect of VRP treatment on *MDR1*pp activity. The effects of 24-hr treatment with VRP at doses that led to a decrease in *mdr1* mRNA

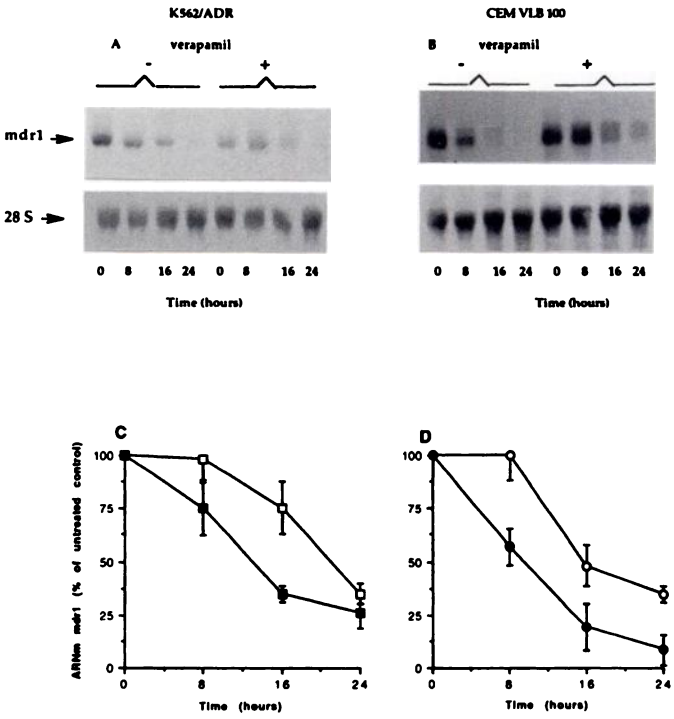


Fig. 4. Stability of *mdr1* mRNA in control and VRP-treated K562/ADR cells and CEM VLB_{100} cells. A and B, Autoradiographs. Cells were grown for 24 hr in the presence or in the absence of VRP and were further incubated with act D at a concentration that inhibited >95% of the [5-^{3}H]uridine incorporation. At the time points indicated, RNAs were extracted and the level of *mdr1* gene expression (*upper*) or 28 S gene expression (*lower*) was estimated by Northern blot analysis, as described in Materials and Methods. A, K562/ADR cells; B, CEM VLB_{100} cells. C and D, Densitometric analysis of autoradiographs (the results are expressed as percentage of the signal obtained before incubation with act D). C, ■, Control K562/ADR cells; □, treated cells; D, ●, control CEM VLB_{100} cells; ○, treated cells. ARNm = mRNA.

expression were measured after transfection of the *MDR1*pp-CAT plasmid into CEM VLB_{100} cells. In CEM VLB_{100} cells co-transfected with 15 μg of *MDR1*pp-CAT in the presence of pCH110, a plasmid expressing β-galactosidase, CAT activity decreased after VRP treatment at concentrations between 15 and 50 μM. As shown in Fig. 6, CAT activity was reduced in a dose-dependant manner, with the greater effect being obtained with 50 μM VRP (2.4-fold decrease). Similar experiments were

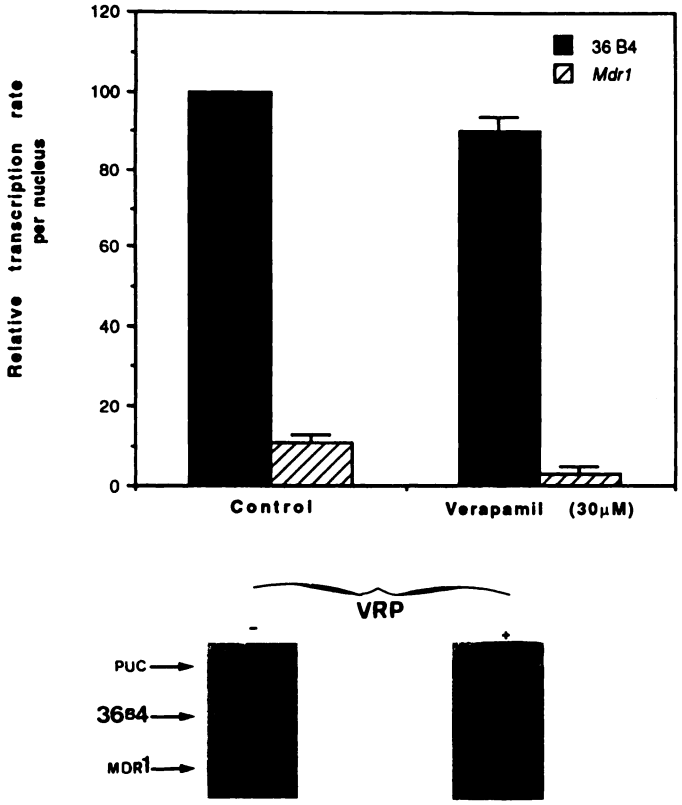


Fig. 5. Effect of VRP on the *mdr1* gene transcription rate in CEM VLB_{100} cells. Nuclei were isolated from control and VRP-treated cells. The nuclear run-on products were hybridized to immobized DNA plasmids containing *mdr1* fragments (nucleotides 325–1600), a *36B4* fragment, or PuC 9 as a negative control. Transcription of the *36B4* gene in control nuclei was set at 100 in control. *Upper*, data representing two different experiments on two separate isolations each of control and VRP-treated nuclei; *lower*, photograph of the autoradiograph from one representative experiment.

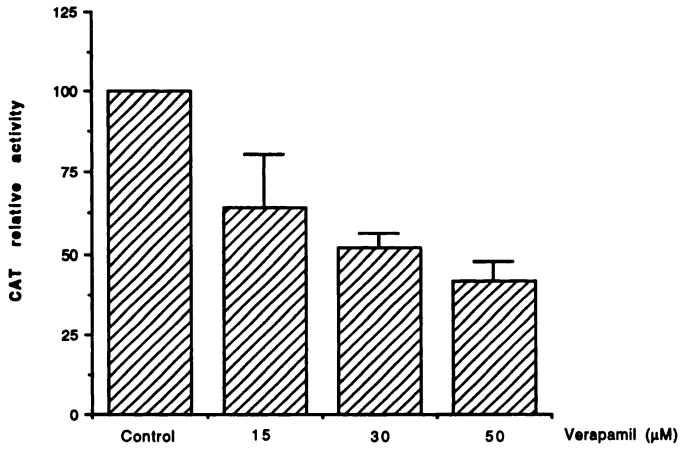


Fig. 6. CAT activity of the *MDR1*pp-CAT plasmid construct transfected into CEM VLB_{100} cells. Cells were incubated in the presence of the indicated VRP concentrations after transient transfection of the *MDR1*pp-CAT plasmid construct. CAT activity, expressed as the ratio of acetylated (mono- and diacetylated)/total [^{14}C]chloramphenicol, was 1.8 ± 0.038 × 10^{-2} cpm in the untreated control (mean ± standard deviation of three separate experiments), which was arbitraly set at 100.

performed using a plasmid construct of the SV40 promoter linked to the CAT gene. After VRP treatment at similar concentrations, no decrease in CAT activity was observed (data not shown).

Discussion

In this paper we have shown that VRP treatment decreased *mdr1* gene expression through modulation of *mdr1* gene promoter activity. In a previous work (8), we showed that 15 μM VRP decreased both Pgp and *mdr1* mRNA levels after 72 hr of incubation. However, a plateau in the mRNA level was obtained after 24 hr of incubation. The present study shows that the effect of VRP on *mdr1* mRNA is dose related at concentrations between 15 and 50 μM. However, it should be noted that for higher concentrations (i.e., 100 μM) the effect appeared to be opposite, with an increased *mdr1* mRNA level, compared with the untreated control, at least in the K562/ADR cell line. Because 24-hr VRP treatment at 100 μM strongly inhibited cell proliferation (25% of the untreated control) (Fig. 2), the increase in mRNA *mdr1* levels likely results from nonspecific cellular toxicity, as has been reported for heat shock, arsenite exposure (17), and exposure to cytotoxic agents (18). In contrast, when VRP was used at a noncytotoxic concentration (30 μM) a decrease in *mdr1* mRNA levels was observed. The latter concentration was then used to further investigate the VRP mechanism of action at a molecular level.

To address the question of a possible effect of VRP on *mdr1* mRNA stability, we have measured mRNA half-life in the VRP-treated cells, compared with control cells. Thus, once the experimental conditions for studying *mdr1* mRNA stability were established, we first determined *mdr1* mRNA half-life in two selected cell lines, K562/ADR and CEM VLB_{100}. In both cell lines, we found that the *mdr1* mRNA half-life appeared to be longer than 10 hr. Our results were unexpected, because analysis of the cDNA sequence of the 3′ untranslated region of the *mdr1* gene revealed several characteristics common to unstable messages, such as $(U)_nA$ sequences and an AUUUA motif (19–22), strongly suggesting that *mdr1* mRNA may be very unstable. However, in accordance with our results, Ince and Scotto (23) reported in a preliminary study that *mdr1* mRNA was a very stable message, with a half-life of >12 hr.

By comparing *mdr1* mRNA stability in VRP-treated and untreated cells, we could determine that the observed decrease in *mdr1* mRNA expression levels does not result from enhanced mRNA degradation. However, it is interesting to note that, in both cell lines, VRP treament slightly increased mRNA stability (from 14 hr to 23 hr in the 24-hr VRP-treated K562/ADR cells and from 10.0 hr to 15.5 hr in the VRP-treated CEM VLB_{100} cells). Thus, one may propose that the 2–3-fold decrease in steady state levels of mRNA observed by Northern blot analysis is actually the net result of a 4-fold decrease in transcription and slightly (<2-fold) increased message stability.

As a matter of fact, to our knowledge, our study is the first to report a negative regulation of *mdr1* gene transcription through down-regulation of *MDR1*pp activity in MDR cells by a pharmacological agent. VRP in similar dose ranges has been previously reported to down-regulate the expression of several genes, including the metalloproteinase gene (24), an actin gene (25), and the prolactin gene, with the latter effect occurring through down-regulation of prolactin gene promoter activity

(26). However, the exact molecular mechanism by which VRP exerts its effect remains unknown.

Studies of VRP effects on *mdr1* gene expression led to apparently conflicting results. Indeed, previous authors (27) reported an increase in *mdr1* mRNA levels in colon carcinoma cells after exposure to VRP. In contrast, in accordance with our results, Biedler *et al.* (28) recently reported that VRP treatment (at toxic concentrations) decreased Pgp expression in a series of hamster lung cancer cell lines possibly through down-regulation of the expression of the amplified Pgp genes. VRP effects on *mdr1* gene expression may be dependent on the cell type; another working hypothesis is that VRP may down-regulate *mdr1* gene expression only in some selected mutants overexpressing Pgp with an abnormally regulated gene, because our data provide direct evidence of VRP effects on *mdr1* gene transcription. Thus, the identification of *mdr1* promoter sequences responsive to VRP may provide additional information on the regulation of the gene in MDR cells.

In conclusion, this study presents evidence that, in leukemic cells with the MDR phenotype, VRP (an inhibitor of Pgp activity) may also down-regulate *mdr1* gene transcription through down-regulation of *MDR1*pp activity. Thus, VRP may exert its effect as a reversing agent according to a two-step regulation process, i.e., inhibition of Pgp activity and inhibition of Pgp synthesis. It would be of interest to use CAT activity assays to screen for new MDR-reversing agents with similar activity.

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