Anti-Cancer Drugs 1996, 7, pp. 738–744

Effects of modulators of multidrug resistance on the expression of the *MDR1* gene in human KB cells in culture

Yan-Ping Hu, Philippe Pourquier, François Doignon, Marc Crouzet and Jacques Robert
Institut Bergonié and Université de Bordeaux 2, 180 rue de Saint-Genès, 33076 Bordeaux Cedex, France. Tel: (+33) 56 33 33 27; Fax: (+33) 56 33 33 89.

The effect of four modulators of multidrug resistance (MDR) on the expression of the *MDR1* gene was studied in two resistant variants of the KB cell lines, KB V1 and KB A1. This was done using a semi-quantitative assay based on mRNA reverse transcription coupled with polymerase chain reaction of the cDNA obtained. An automatic DNA sequencer was used for the measurement of the fluorescent amplification products and the *MDR1* signal was compared to that of the β-actin gene of the cells. After 24 h incubation with 15 μM of the modulators, *MDR1* gene expression was slightly but significantly decreased by two of them, quinine and cyclosporine A, whereas verapamil and S-9788 had very little effect on this parameter. The effect were more pronounced in the KB A1 line than in the KB V1 line. The effect of quinine was studied over a longer time period (4–48 h) and was shown to be maximum at 24 h. These results favor the existence of a direct effect of some MDR reverters, especially quinine, on the expression of the *MDR1* gene and could partially explain their modulating effect of MDR.

Key words: MDR reversal, MDR1 expression, multidrug resistance.

Introduction

Many cell lines selected for resistance *in vitro* to an anticancer drug also show cross-resistance to other agents which are structurally and functionally unrelated, and this phenomenon has been called multidrug resistance (MDR). Most of these cell lines overexpress the *MDR1* gene and its product, P-glycoprotein (Pgp). Pgp is thought to function as an ATP-dependent drug efflux pump, reducing intracellular drug accumulation.[1,2] Studies on cell lines have shown a good correlation between *MDR1* mRNA levels and the degree of MDR, and *MDR1* expression has been widely observed in many different human tumors and tissues. An increased *MDR1* level was frequently observed at relapse following chemotherapy[3–5] and even very low levels of *MDR1* gene expression can confer a several-fold increase in drug resistance, which may be clinically significant.[6] In human tumors, the overexpression of the *MDR1* gene does not result from gene amplification.[7,8] Thus, intrinsic variations in *MDR1* mRNA levels may be an important determinant of tumor response or of MDR reversal.

The regulation of the expression of this gene has been studied in normal and malignant cells, but is still not clearly understood. Increased expression of *MDR1*/Pgp has been demonstrated following chemotherapy.[9,10] Transient exposure to cytotoxic drugs, including agents that are not transported by Pgp, induced Pgp and *MDR1* mRNA expression in most of the tested cell lines.[11,12] In these experiments, drug-induced *MDR1* expression, associated with a 2- to 3-fold increase in resistance to vinblastine, was maintained in K562 leukemia cells for at least several weeks after removal of drug. This drug-mediated *MDR1* gene induction can be prevented by protein kinase C inhibitors.[12,13] Other agents stimulating *MDR1* expression include heat shock,[14] differentiating agents[15] and protein kinase C activators.[16]

Many investigators have reported that numerous compounds, including calcium channel blockers, can reverse MDR *in vitro*.[17,18] 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 competitive or non-competitive inhibition mechanisms.[19] In some MDR tumor cells, the level of *MDR1*/Pgp expression can be modulated by these pharmacological agents. Muller *et al.*[20] have reported that exposure to verapamil resulted, in two leukemia cell lines, in a decrease in *MDR1* mRNA levels. No effect was observed with nifedipine or diltiazem. This is in marked contrast to the work of Herzog *et al.*,[21] who observed increased levels of *MDR1* mRNA and Pgp

This work was supported by grants from the Ligue nationale française contre le cancer. Y-PH was a recipient of fellowships from the Fondation pour la recherche médicale and from the Comité des Pyrénées-Atlantiques of the Ligue nationale française contre le cancer.

Correspondence to J Robert

in a human colon carcinoma cell line and its drug-resistant sublines after treatment with the Pgp antagonists, verapamil, nifedipine and cyclosporine A. The increase in *MDR1* mRNA and Pgp was maintained for the duration of treatment up to several months, with a rapid decline after removal of the P-gp antagonists. Increased *MDR1* mRNA levels were also seen with nicardipine and diltiazem, but not with quinidine or chlorpromazine.[21]

These discrepancies emphasized the fact that the effect of various drugs on *MDR1*/Pgp expression is highly dependent on the model used, or cell type, and method of measuring *MDR1*. Insufficient sensitivity of an assay, together with the often limited sample material available from tumors, or its degradation, has often hampered the detection of what may be clinically significant amounts of *MDR1* mRNA. In addition, reliable detection of *MDR1* gene expression is further complicated by the existence of a homologous gene called *MDR2*,[22] which is not associated with resistance to chemotherapeutic agents.[23] These findings prompted us to examine the effects of several MDR modulators, i.e. verapamil, S-9788, cyclosporine A and quinine, on the expression of the *MDR1* gene in MDR KB cells, by a semi-quantitative assay based on RT-PCR using an automatic DNA sequencer for the measurement of the fluorescent amplification products. This question is of importance, since a down-regulation of *MDR1* gene expression by MDR modulators could well participate in their mechanism of action and amplify their direct inhibition of Pgp.

Materials and methods

Drugs and products

Doxorubicin was a gift from Pharmacia (St-Quentin-en-Yvelines, France); verapamil was used as a clinical formulation (Isoptine®; Laboratoires Biosedra, Malakoff, France); cyclosporine A was obtained from Sandoz (Rueil-Malmaison, France) and S-9788 from Servier (Courbevoie, France). The oligonucleotide primers specific for *MDR1* and β-actin genes were provided by Eurogentec (Angers, France). PCR kits were purchased from Perkin Elmer (St-Quentin-en-Yvelines, France).

Cell culture and drug treatment

The KB 31 cell line originates from a human cervix carcinoma. Several MDR variants had been selected for the early characterization of MDR.[7] We have obtained the KB V1 and the KB A1 variants from Dr MM Gottesman (National Cancer Institute, Bethesda, MD). The KB V1 and KB A1 lines were maintained in 0.6 μg/ml vinblastine and 1 μg/ml doxorubicin, respectively. These cell lines were grown in Dulbecco's modified Eagle medium, supplemented with 10% fetal calf serum, 100 U/ml penicillin and 100 μg/ml streptomycin, at 37°C in 5% CO_2. Cells were transferred in drug-free medium 4 days before any experiment.

For the evaluation of the effects of modulators on *MDR1* gene expression, we used a fixed concentration of the four modulators studied (15 μM) and an exposure time of 24 h. In addition, the effects of quinine were evaluated over a higher range of exposure times (4–48 h). Incubations were always performed in KB V1 and KB A1 cells during the exponential phase of growth. In order to have a positive control for the validity of our approach, we have in addition incubated sensitive KB 31 cells with 0.2 μg/ml doxorubicin for exposure times of 4–48 h.

Doxorubicin cytotoxicity

A colorimetric assay using the tetrazolium salt MTT[24] was used to assess cytotoxicity after 2 h exposures to doxorubicin in the presence or absence of the four modulators. Briefly, appropriate numbers of KB V1 and KB A1 cells were plated in 96-well plates (Nunc, Denmark) in a volume of 200 μl of culture medium. After incubations of 48 h, the cells were exposed for 2 h to the appropriate concentrations of doxorubicin and verapamil, cyclosporine A, quinine or S9788. The culture medium was then removed, the cell layers were rinsed twice with phosphate-buffered saline, fresh culture medium was added and the cells allowed to grow for a further 3–4 days. These conditions have been established after a careful study of the growth curves of the cells, in order to keep the cells in the exponential phase of growth over the 7 days of culture. At the end of the incubation, 200 μl of MTT-containing medium was added and maintained for 4 h; after elimination of the medium, 200 μl dimethylsulfoxide was added to dissolve the formazan crystals. Absorbance was then immediately measured on a two-wavelength microplate photometer (Bio-Tek Instruments) set at 570 and 630 nm. All experiments were performed three times in triplicate. The concentration of doxorubicin required for 50% growth inhibition (IC_{50}) was estimated as that giving a 50%

decrease of absorbance as compared with controls incubated simultaneously without doxorubicin. The cytotoxicity of the modulator itself was estimated by comparing the cell survival obtained with the various concentrations of the modulator in the absence of doxorubicin. The resistance factor was estimated from the ratio of the IC_{50} of doxorubicin in the resistant line and in the sensitive one. The residual resistance factor in the presence of a modulator was determined as the ratio of the IC_{50} of a cytotoxic drug with the reverter in the resistant line and without reverter in the sensitive line.

RT-PCR

Total cellular RNAs from exponentially growing drug-treated or untreated cells were extracted by 6 M guanidine isothiocyanate and centrifuged at 150 000 *g* for 17 h on a 5.7 M cesium chloride layer.[25] RNAs were washed twice in 70% ethanol and resuspended in TES buffer (Tris 10 mM, pH 7.4, EDTA 5 mM, SDS 1%). They were quantified by spectrometric absorbance measurements at 260 and 280 nm, precipitated in ethanol/3 M sodium acetate (2/1, v/v) and stored at −20°C until use. Complementary DNAs (cDNAs) were synthesized with 1 μg of total cellular RNA and 2.5 μM random hexamers in 20 μl of a solution containing 1 × PCR buffer, 5 mM $MgCl_2$, 1 mM of each deoxynucleoside triphosphate, 1 U RNase inhibitor and 2.5 U reverse transcriptase. Reverse transcription was carried out at 42°C for 1 h. The samples were then heated at 99°C for 10 min, followed by a 4°C quick chill.

A 157 bp *MDR1*-specific fragment was amplified using two oligonucleotides with the following sequences, according to Noonan *et al.*:[5] sense, 5′-CCC ATC ATT GCA ATA GCA GG-3′; antisense, 5′-GTT CAA ACT TCT GCT CCT GA-3′, with a fluorescein label at the 5′ end of the sense primers. PCR was performed by adding 5 μl of the RT products to 45 μl of amplification reaction buffer (1 × PCR buffer, 250 ng each primer, 2.5 U Ampli Taq DNA polymerase, 1.35 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate). Samples were first incubated at 94°C for 5 min and submitted to a various number of cycles: 15, 20, 25 or 30, using a Perkin-Elmer 480 thermocycler. Each cycle included the following steps: 94°C, 1 min; 58°C, 15 s; 72°C, 15 s. Then, a final extension of 5 min at 72°C was performed and samples were cooled down to 4°C before loading. As a control, a 80 bp fragment of the β-actin cDNA was amplified in a separate tube under the same conditions as above using the following primers chosen after the gene sequence:[26] sense, 5′-GAG AAG ATG ACC CAG ATC ATG T-3′; antisense: 5′-CAG AGG CGT ACA GGG ATA GCA C-3′, with a fluorescein label at the 5′ end of the sense primers.

Separation and quantification of the amplification products

RT-PCR products were qualitatively characterized by loading 10 μl of the reaction volume on a 8% polyacrylamide gel stained with ethidium bromide. For quantitative purposes the fluorescent PCR products were separated on a sequencing gel and analyzed using an automated DNA sequencer (ALF; Pharmacia Biotech, Uppsala, Sweden).[27] Areas under the peaks were evaluated using the integration software Smart Manager (Pharmacia Biotech).

The *MDR1*/β-actin ratio after 25 cycles of PCR in cells treated with modulators was compared to that evaluated in untreated cells. This percentage represented the effect of modulators on the expression of *MDR1* gene.

$$\% \text{ effect} = \frac{MDR1/\beta\text{-actin (with modulator)}}{MDR1/\beta\text{-actin (without modulator)}} \times 100$$

Results

Effect of modulators on doxorubicin cytotoxicity in KB cells

The sensitivity of KB sublines to doxorubicin in the absence or presence of MDR modulators was studied after 2 h exposure to the drugs. It was found that, in the KB sensitive lines (wild-type cells), there was no significant effects of verapamil, cyclosporine A, quinine or S-9788, up to 30 μM, on the cytotoxicity of doxorubicin; in all resistant cell lines, there was a dose-dependent increase in doxorubicin cytotoxicity in the presence of the modulators (data not shown). We have calculated the ratio of the IC_{50} obtained with modulators in the resistant lines to the IC_{50} in the sensitive line; this 'residual resistance factor' is shown in Table 1 for one representative concentration of the modulators (10 μM). S-9788 and cyclosporine A strongly reversed the resistance of KB V1 and A1 cell lines, with residual resistances of about 3–4 (KB V1) to 7–9 (KB A1). In contrast, reversal by verapamil and quinine was 2-fold less pronounced, with residual resistances ranging between 6–9 (KB V1) and 15–22 (KB A1).

Table 1. MDR-reversing effect of modulators

Cell lines	Doxorubicin IC_{50} (*resistance factor*)	Doxorubicin IC_{50} in the presence of modulators at 10 μM (*residual resistance factor*)			
		Verapamil	Quinine	S-9788	Cyclosporine A
KB 31	0.1 (*1*)	NA	NA	NA	NA
KB A1	11.7 (*117*)	1.53 (*15.3*)	2.2 (*22*)	0.72 (*7.2*)	0.89 (*8.9*)
KB V1	10.1 (*101*)	0.63 (*6.3*)	0.91 (*9.1*)	0.31 (*3.1*)	0.40 (*4.0*)

Results calculated from three to five independent determinations.

MDR1 gene expression in KB cell lines

We first determined qualitatively the overexpression of the *MDR1* gene in the KB resistant variants. This was done using 30 cycles of PCR in the conditions described in Materials and methods. Figure 1 shows a representative electrophoresis of RT-PCR products originating from KB 31, KB V1 and KB A1 mRNAs, and colored with ethidium bromide. Strong signals were present in the resistant cells and no signal was detectable for the sensitive line. In order to have a positive control, we treated KB 31 cells with 0.2 μg/ml doxorubicin for 4, 12, 24 and 48 h, and *MDR1* gene expression was analyzed. Doxorubicin was able to induce *MDR1* mRNA expression in the sensitive cells, and this induction was especially important after 48 h of contact.

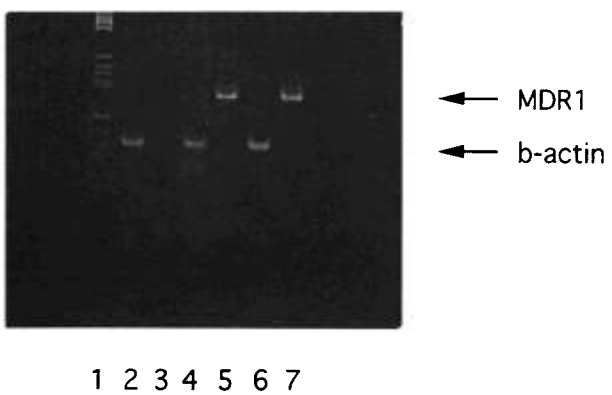

Figure 1. Electrophoresis of the RT-PCR products obtained from the RNAs originating from KB cell lines. Lane 1, DNA markers; lane 2, KB 31, β-actin; lane 3, KB 31, *MDR1*; lane 4, KB A1, β-actin; lane 5, KB A1, *MDR1*; lane 6, KB V1, β-actin; lane 7, KB V1, *MDR1*.

Kinetics of RT-PCR amplification of *MDR1* and β-actin sequences in resistant KB lines

To establish the optimal conditions for the quantitative evaluation of eventual changes in *MDR1* gene expression, we performed a kinetic analysis of *MDR1*-specific and β-actin-specific amplification in RNA preparations from the KB A1 and KB V1 cells, using the quantification methods presented in Materials and methods. After reverse transcription, both *MDR1* and β-actin cDNAs were amplified using 15, 20, 25 and 30 cycles of PCR. Results are shown in Figure 2. In all cases, there was a good log-linearity

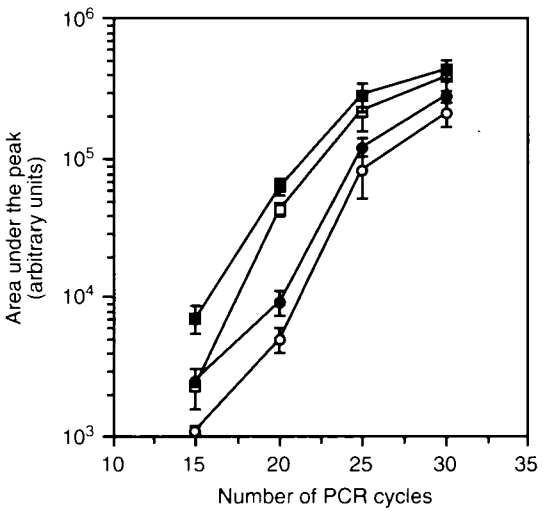

Figure 2. Effect of the number of PCR cycles on the amount of amplification products after retrotranscription of KB A1 and KB V1 mRNAs, using *MDR1* and β-actin specific primers. ■, KB V1, β-actin; □, KB A1, β-actin; ●, KB V1, *MDR1*; ○, KB A1, *MDR1*. Results are means ± SD of two independent RNA extractions, with which three RT-PCR were performed.

between the number of PCR cycles up to 25 and the amount of material amplified, estimated as the area under the peak of the fluorescent DNA spot obtained after electrophoresis. Beyond 25 cycles, the yield of the specific products approaches a plateau. We have, therefore, performed the quantitation of the changes in *MDR1* gene expression induced by modulators using 25 cycles of PCR.

Effects of modulators on the expression of the *MDR1* gene

We have evaluated the ratio of *MDR1* to β-actin amplification products after 25 cycles of PCR in KB V1 and KB A1 cells treated with modulators (15 μM) for 24 h. The results are presented on Table 2. The four modulators studied showed a small inhibition of *MDR1* expression in KB V1 cells. Quinine and cyclosporine A showed a 20% reduction in *MDR1* expression in these conditions. In KB A1 cells, the inhibitory effect of modulators on *MDR1* gene expression was higher; verapamil and S-9788 reduced *MDR1* expression by 25%, and cyclosporine A and quinine by about 40%.

We have then evaluated *MDR1* gene expression in KB A1 cells incubated with quinine at 15 μM for different periods of time (4, 12, 24 and 48 h). The results (Figure 3) show that *MDR1* expression begins to decrease after 12 h of exposure to 15 μM quinine. The maximal inhibition was reached after 24 h exposure and was maintained until 48 h.

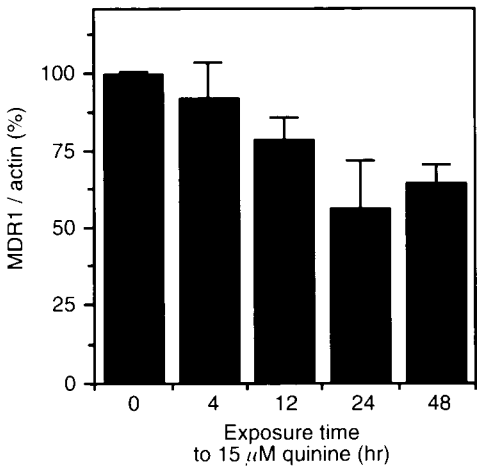

Figure 3. Effect of exposure time to quinine on the expression of the *MDR1* gene in KB A1 cells, evaluated as the *MDR1*/β-actin ratio. Results are means ± SD of two independent RNA extractions, with which three RT-PCR were performed.

Discussion

Numerous techniques are available for the estimation of *MDR1* gene expression. Northern blots or dot blots with various *MDR1* probes have been extensively used in early studies, especially in human tumor samples.[4] However, this technique does not appear sensitive enough for the detection of very low levels of expression, which may be, however, of clinical importance. RT-PCR represents a valuable alternative to mRNA blotting, as shown by Noonan *et al.*[5] However, the precise quantification of gene expression might be difficult by RT-PCR because of the exponential increase of PCR products, which may rapidly lead to the loss of proportionality between the number of starting mRNA molecules and the number of PCR products detected on the gels. Several means have been used for solving this problem. We have chosen to use as a standard the mRNA of the β-actin gene, which was retrotranscribed and amplified simultaneously with that of the *MDR1* gene, the amplification products being run on the same electrophoresis gel. This technique, however, does not avoid several problems, related for instance to differences in reverse transcriptase activity on the different mRNAs or to differences in Taq polymerase activity on different oligonucleotide pairs. This technique cannot be considered, therefore, to be able to evaluate absolute amounts of specific mRNAs and only competitive techniques can do so.[28] However, for comparative evaluations of gene expression in a given cell line subjected to various treatments, this semi-quantitative technique appeared quite satisfactory. It assumes, however, that the treatment has no effect on the expression of the gene selected as an internal standard and that there is a good log-linearity between the number of amplification cycles used and the amount of PCR products present at the end of the amplification.

Table 2. Comparison of the effects of MDR modulators on the ratio of *MDR1*/β-actin expression in KB cells

Modulator (15 μM)	KB V1 (%)	KB A1 (%)
Control	100	100
Verapamil	90.3 ± 14.3	75.4 ± 14.8
S-9788	92.5 ± 15.9	73.1 ± 8.5
Cyclosporine A	82.2 ± 12.1	64.0 ± 13.3
Quinine	79.8 ± 9.5	56.1 ± 16.5

Results are means ± SD of two independent RNA extractions with which three RT-PCR were performed.

Although we could not definitively assess that the expression of β-actin was unchanged during cell treatment with modulators, we think that this method was satisfactory enough for our purpose. Several advantages of this technique should be emphasized: the fluorescent primers are stable at −20°C for more than 1 year; fluorescence detection is easier and safer than radioactivity counting of gel fragments; and the use of an automatic sequencer allows the separation and the quantitation of individual PCR products at same time.[27]

In preliminary experiments, we have shown that, as expected, resistant KB cell lines did overexpress the *MDR1* gene and that no expression could be detected in the KB 31 line. We also showed that *MDR1* gene expression could be induced in KB 31 sensitive cells by relatively brief exposures to doxorubicin, as already shown by Chaudhary and Roninson.[12] This enabled us to use the same methodology for evaluating eventual changes in *MDR1* gene expression upon the action of modulators. Studies on the effect of modulators on *MDR1* gene expression led to apparently conflicting results. Herzog *et al.*[21] have shown a transient increase in *MDR1* mRNAs occurring upon treatment of colon carcinoma cells by verapamil and other MDR modulators, but not all. This increase was neither mediated by an increase in the transcription rate nor by a stabilization of the mRNA species. It was attributed to post-transcriptional regulations occurring within the nucleus.[21] In contrast, Muller *et al.*[20] observed in two MDR leukemic cell lines that verapamil induced a decrease in P-gp expression at the mRNA level, which was associated with a sensitization of the cells to the anticancer drugs. The other modulators tested (nifedipine and diltiazem) had no effect on *MDR1* gene expression. This decrease was shown to be due to a decreased transcription rate at the level of the proximal promoter activity.[29] In our experiments, we have shown that MDR modulators could decrease *MDR1* gene expression in MDR KB cell lines. This effect was, however, more important in the KB A1 than in the KB V1 cell line and was in both lines much lower than that observed by Muller *et al.*[20,29] in leukemic cell lines. In our hands, verapamil had only a very limited effect on *MDR1* mRNA levels and this is the same with a new modulator in development, S-9788.[30] In contrast, cyclosporine A and quinine had a more pronounced effect, reaching a near 2-fold decrease in our experimental conditions. It appears from our work and that of Muller *et al.*[20,29] and Herzog *et al.*[21] that the effect of modulators on *MDR1* gene expression is both cell line-dependent and modulator-dependent, although not strictly modulator-specific.

Reversal of MDR has for many years been the subject of intensive research, aimed at the identification of compounds able to restore tumor sensitivity in the clinical setting.[17,18] Only limited success has been obtained until now, mainly in myelomas and non-Hodgkin's lymphomas,[31,32] which shows, nevertheless, the validity of the concept. Despite this research activity, we still have only limited information on the mechanism of action of MDR modulators. Their first target is Pgp itself; it has been shown that most of them are able to bind to Pgp and to displace the photoaffinity labeling of Pgp by various analogs.[19] This might allow the identification of several pharmacophores on Pgp thus enabling the design of new modulators. However, some modulators like quinine display a very weak interaction with Pgp, without even restoring anticancer drug accumulation, despite their important MDR reversing activity, which is relevant in the clinical setting.[33] The existence of other targets for MDR modulation is, therefore, most probable. We have tried to identify such targets at the level of Pgp activation by phosphorylation (Hu and Robert, submitted) because some modulators appear as protein kinase C antagonists. We show in this study that an effect on *MDR1* gene expression cannot be excluded, especially for quinine. This effect could contribute to the MDR reversal exhibited by quinine and may explain why this agent acts in synergism with compounds directly targeting Pgp, such as verapamil.[34,35] These results confirm the interest of the association of several modulators for the reversal of MDR in clinics, since they appear to display different and complementary mechanisms of action.

References

1. Endicott JA, Ling V. The biochemistry of P-glycoprotein-mediated multidrug resistance. *Annu Rev Biochem* 1989; **58**: 137–71.
2. Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* 1993; **62**: 385–427.
3. Fojo AT, Ueda K, Slamon DL, Poplack DG, Gottesman MM, Pastan I. Expression of a multidrug-resistance gene in human tumors and tissues. *Proc Natl Acad Sci USA* 1987; **84**: 265–9.
4. Goldstein LJ, Galski H, Fojo A, *et al.* Expression of a multidrug resistance gene in human cancers. *J Natl Cancer Inst* 1989; **81**: 116–24.
5. Noonan KE, Beck C, Holzmayer TA, *et al.* Quantitative analysis of MDR1 (multidrug resistance) gene expression in human tumors by polymerase chain reaction.

Proc Natl Acad Sci USA 1990; **87**: 7160–4.
6. Holzmayer TA, Hilsenbeck S, Von Hoff DD, Roninson IB. Clinical correlates of *MDR1* (P-glycoprotein) gene expression in ovarian and small-cell lung carcinomas. *J Natl Cancer Inst* 1992; **84**: 1486–91.
7. Shen DW, Fojo A, Chin JE, Roninson IB, Richert N, Pastan I. Human multidrug-resistant cell lines: increased *MDR1* expression can precede gene amplification. *Science* 1986; **232**: 643–5.
8. Bourhis J, Goldstein LJ, Riou G, Pastan I, Gottesman MM, Bénard J. Expression of a human multidrug resistance gene in ovarian carcinomas. *Cancer Res* 1989; **49**: 5062–5.
9. Musto P, Melillo L, Lombardi G, Matera R, Di Giorgio G, Carotenuto M. High risk of early resistant relapse for leukaemic patients with presence of multidrug resistance associated P-glycoprotein positive cells in complete remission. *Br J Haematol* 1991; **77**: 50–3.
10. Ma DDF, Scurr RD, Davey RA, *et al.* Detection of a multidrug resistant phenotype in acute non-lymphoblastic leukaemia. *Lancet* 1987; **i**: 135–7.
11. Kato S, Nishimura J, Yufu Y, Ideguchi H, Umemura T, Nawata H. Modulation of expression of multidrug resistance gene (*MDR1*) by adriamycin. *FEBS Lett* 1992; **308**: 175–8.
12. Chaudhary PM, Roninson IB. Induction of multidrug resistance in human cells by transient exposure to different chemotherapeutic drugs. *J Natl Cancer Inst* 1993; **85**: 632–9.
13. Kim SH, Park JI, Chung BS, Kang CD, Hidaka H. Inhibition of *MDR1* gene expression by H-87, a selective inhibitor of cAMP-dependent protein kinase. *Cancer Lett* 1993; **74**: 37–41.
14. Chin KV, Tanaka S, Darlington G, *et al.* Heat shock and arsenite increase expression of the multidrug resistance (*MDR1*) gene in human renal carcinoma cells. *J Biol Chem* 1990; **265**: 221–6.
15. Bates SE, Mickler LA, Chen YN, *et al.* Expression of a drug resistance gene in human neuroblastoma cell lines: modulation by retinoic acid-induced differentiation. *Mol Cell Biol* 1989; **9**: 4337–44.
16. Chaudhary PM, Roninson IB. Activation of *MDR1* (P-glycoprotein) gene expression in human cells by protein kinase C agonists. *Oncol Res* 1992; **4**: 281–90.
17. Ford JM, Hait WN. Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* 1990; **42**: 155–99.
18. Georges E, Sharom FJ, Ling V. Multidrug resistance and chemosensitization: therapeutic implications for cancer chemotherapy. *Adv Pharmacol* 1990; **21**: 185–220.
19. Safa AR. Photoaffinity labeling of P-glycoprotein in multidrug-resistant cells. *Cancer Invest* 1992; **10**: 295–305.
20. Muller C, Bailly JD, Larédo J, Jaffrézou JP, Bordier C, Laurent G. Verapamil decreases P-glycoprotein expression in multidrug-resistant human leukemic cell lines. *Int J Cancer* 1994; **56**: 749–54.
21. Herzog CE, Tsokos M, Bates SE, Fojo AT. Increased *MDR1*/P-glycoprotein expression after treatment of human colon carcinoma cells with P-glycoprotein antagonists. *J Biol Chem* 1993; **268**: 2964–52.
22. Chin JE, Soffir R, Noonan KE, Choi K, Roninson IB. Structure and expression of the human MDR (P-glycoprotein) gene family. *Mol Cell Biol* 1989; **9**: 3808–20.
23. Smit JJ, Schinkel AH, Oude-Elferink RP, *et al.* Homozygous disruption of the murine *mdr2* P-glycoprotein gene leads to a complete absence of phospholipid from bile and to liver disease. *Cell* 1993; **75**: 451–62.
24. Carmichael J, Degraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res* 1987; **47**: 936–42.
25. Chirgwin J, Przybyla A, MacDonald R, Rutter W. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 1979; **18**: 5294–300.
26. Nakajima-Iijima S, Hamada H, Reddy P, Kakunaga T. Molecular structure of the human cytoplasmic β-actin gene: interspecies homology of sequences in the introns. *Proc Natl Acad Sci USA* 1985; **82**: 6133–7.
27. Porcher C, Malinge MC, Picat C, Grandchamp B. A simplified method for determination of specific DNA or RNA copy number using quantitative PCR and an automatic DNA sequencer. *Biotechniques* 1992; **13**: 106–14.
28. Debuire B, Sol O, Lemoine A, May E. Nonisotopic Competitive RT-PCR assay to measure *MDR1* gene expression. *Clin Chem* 1995; **41**: 819–25.
29. Muller C, Goubin F, Ferrandis E, *et al.* Evidence for transcriptional control of human *MDR1* gene expression by verapamil in multidrug-resistance leukemic cells. *Mol Pharmacol* 1995; **47**: 51–6.
30. Huet S, Chapey C, Robert J. Reversal of multidrug resistance by a new lipophilic cationic molecule, S-9788. Comparison with 11 other MDR-modulating agents in a model of doxorubicin-resistant rat glioblastoma cells. *Eur J Cancer* 1993; **55**: 115–21.
31. Dalton WS, Grogan TM, Meltzer PS, *et al.* Drug resistance in multiple myeoma and non-Hodgkin's lymphoma: detection of P-glycoprotein and potential circumvention by addition of verapamil to chemotherapy. *J Clin Oncol* 1989; **7**: 415–24.
32. Wilson WH, Bates SE, Fojo A, *et al.* Controlled trial of dexverapamil, a modulator of multidrug resistance, in lymphomas refractory to EPOCH chemotherapy. *J Clin Oncol* 1995; **13**: 1995–2004.
33. Chauffert B, Pelletier H, Corda C. Potential usefulness of quinine to circumvent the anthracycline resistance in clinical practice. *Br J Cancer* 1990; **62**: 395–7.
34. Lehnert M, Dalton WS, Roe D, Emerson S, Salmon SE. Synergistic inhibition by verapamil and quinine of P-glycoprotein-mediated multidrug resistance in a human myeloma-cell line model. *Blood* 1991; **77**: 348–54.
35. Bennis S, Ichas F, Robert J. Differential effects of verapamil and quinine on the reversal of doxorubicin resistance in a human leukemia cell line. *Int J Cancer* 1995; **62**: 283–90.

(Received 4 July 1996; accepted 6 August 1996)