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Modulation of *MDR1* gene expression in multidrug resistant MCF7 cells by low concentrations of small interfering RNAs

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Abstract

MDR1 overexpression is one form of the multidrug resistance (MDR) phenotype, which can be acquired by patients initially responsive to chemotherapy. Because of the high toxicity of the inhibitors of P-glycoprotein (P-gp), the protein encoded by *MDR1*, attention has been focused on selective modulation of the *MDR1* gene. Small interfering RNAs (siRNAs) were shown to be powerful tools for such a purpose, even when used at low concentrations ($\leq$20 nM) in order to avoid sequence nonspecific effects. Two siRNAs used at 20 nM were shown to lead to efficient down-regulation of *MDR1* at the protein level (only ca. 20% total P-gp expression remaining) in the doxorubicin selected MCF7-R human cell line. Cell surface expression of P-gp was inhibited, leading to reversal of the drug efflux phenotype (about 40% reversal with the most efficient siRNA) and enhancement of chemosensitivity (about 35%). At the mRNA level, the down-regulation of *MDR1* obtained with the most efficient siRNA increased from about 50% (5 nM siRNA) to 60% (10 or 20 nM). The advantage of using a combination of siRNAs instead of a single one has been suggested.

Keywords: Multidrug resistance; Small interfering RNA; P-gp expression; *MDR1* silencing; Low concentrations of siRNA; Combination of siRNAs

1. Introduction

The multidrug resistance (MDR) phenotype acquired by initially drug responsive patients remains a major hindrance to efficient chemotherapy. *MDR1* overexpression is one form of MDR; it encodes for the 170 kDa P-glycoprotein (P-gp), a transmembrane pump that causes efflux of drugs out of cells. A lot of research was focused on MDR reversal. Many compounds modulate this phenotype in vitro by inhibiting the efflux pump activity of P-gp but their clinical application is limited because of their high toxicity. Therefore, attention has been focused on selective down-regulation of *MDR1* expression through antisense [1–4], triple helix [5,6] and, more recently, small interfering RNA (siRNA) strategies [7–12].

SiRNAs are 21–25 nt double-stranded RNA molecules, which induce sequence-specific degradation of mRNAs. Physiologically, RNA interference (RNAi) is triggered by dsRNAs homologous to the silenced genes (for review, see [13,14]). DsRNA is cut by the RNase III-like enzyme Dicer into short 21–23 nt double-stranded siRNAs. These siRNAs are incorporated into the multiprotein RNA induced silencing complex (RISC), where the siRNA duplex is unwound and the antisense strand guides RISC to homologous mRNA target for cleavage. In mammals, dsRNAs longer than 30 nt activate the interferon response, leading to nonspecific mRNA degradation. Tuschl's group [15] showed that chemically synthesised siRNAs with a length of 21–23 nt bypass this nonspecific response when transfected into cultured mammalian cells and trigger RNAi. Since synthetic siRNAs cause only transient knockdown of target genes, more recent reports described DNA vector-based expression of small hairpin RNA [16,17] that allowed stable long-termed RNAi effects to be obtained.

However, a widespread nonspecific effect on mammalian gene expression has been observed using siRNAs at 100 nM, which is the concentration suggested by manufacturers and

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the reduction of the siRNA concentration to 20 nM is now strongly recommended in order to eliminate this nonspecific response [18,19]. Nevertheless, lowering the concentration of siRNAs below 100 nM can reduce their silencing efficiency. It is particularly true when the limiting factor of siRNA efficiency is the high turnover of the target protein [20], like in the case of P-gp. P-gp half-life was found to vary from 16 h [21] to about 72 h [22], depending on cell line and such factors as serum deprivation or high cell density [23].

Efficient siRNAs have been designed against *MDR1* but they were used at rather high concentrations: 40 nM [9–11], 100 nM [8] or 200 nM [7,10].

In a precedent work [12], we have shown that siRNAi efficiency against *MDR1* is favoured in cells growing in an exponential phase in the course of which P-gp half-life is minimized and that MCF7-R cells (a doxorubicin selected human cell line) is a model of choice since its cell cycle duration of about 48 h minimises the siRNA dilution effects due to cellular division. Two siRNAs (si1 and si2) led to an efficient silencing in reducing the total P-gp content when used at a concentration of 20 nM.

The total P-gp content of cells includes cytoplasmic and surface P-gp, the cytoplasmic P-gp pool being consumed for maintaining a steady-state level of surface P-gp. Only surface P-gp are responsible of drug efflux [5].

The present study was focused on down-regulation of *MDR1* by si1 and si2 at concentrations $\leq$20 nM in order to eliminate nonspecific effects. Silencing has been demonstrated (i) at the protein level by Western blotting (total P-gp) and functional assays as daunorubicin accumulation or enhancement of chemosensitivity (active P-gp) and (ii) at the mRNA level by RT-PCR.

2. Materials and methods

2.1. siRNAs

RNAs (21 nt) were synthesised and PAGE purified by Eurogentec. They contained a 3′-dTdT extension. They were solubilised in TE buffer (10 mM Tris pH 7.8, 1 mM EDTA in diethyl pyrocarbonate (DEPC)-treated water). The duplexes were formed according to the supplier protocol. Single strands (20 μM) were incubated in annealing buffer (50 mM Tris, pH 7.5–8.0, 100 mM NaCl in DEPC-treated water) for 2 min at 95 °C then slowly cooled down to ambient temperature. Duplex formation was confirmed by PAGE electrophoresis (SYBR gold nucleic acid gel stain (Molecular Probes) detection). si1 was a siRNA targeting the human *MDR1* mRNA [24] at the level of the region 88–108 nt relative to the start codon (sense strand 5′-GAAACCAACUGUCAGUGUA). si2 was directed toward the region 162–182 nt (sense strand 5′-CUUUGGCUGCCAUCAUCCA). si3 was a control-scrambled si1 (sense strand 5′-GAUACGAAUUGACACCGUA) [12]. A BLAST search (NCBI database) was carried out to check that the only target of si1 and si2 was on *MDR1* and that si3 had no target in the human genome.

2.2. Cells and transfection

MCF7-S (the parental human mammary adenocarcinoma cell line) and MCF7-R (the doxorubicin–resistant line) were a gift from M.F. Poupon (Paris, France) [25]. Cells were grown in RPMI medium supplemented with 10% decomplemented foetal bovine serum (FBS), 50 U/ml penicillin, 50 μg/ml streptomycin, 2 mM glutamine in a 5% CO_2 atmosphere.

Transfection with Oligofectamine® (Invitrogen) was carried out as directed by the manufacturer with the modifications proposed by Elbashir et al. [26]. Cells (3×10^5/well) were plated in six-well plates. They were transfected at 30–40% confluence. The final concentration in siRNA was 5, 10 or 20 nM. Transfection was performed in OPTI-MEM1® (Invitrogen) without serum or antibiotics in a final volume of 2.5 ml. After 4 h incubation at 37 °C, FBS (10% final concentration), 50 U/ml penicillin and 50 μg/ml streptomycin were added.

2.3. Western blotting

Cells were lysed 72 h after transfection. Nontreated MCF7-R cells were used as reference of resistant cells. Cells were trypsinised, washed in phosphate buffered saline (PBS), counted, and resuspended in RIPA lysis buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% SDS, 1% nonidet-P40, 0.5% deoxycholate) containing 5 mM EDTA and protease inhibitors (PMSF, leupeptin, aprotinin) at the ratio of 100 μL buffer for 3×10^6 cells. After 30 min on ice with some vortexing, the lysates were centrifuged at $13{,}000 \times g$ for 20 min at 4 °C. Protein concentration was determined with a protein assay kit (Bio-Rad). Equal amounts of proteins (20 μg) were mixed with SDS reducing buffer. Protein samples were separated on 7.5% acrylamide SDS-PAGE then transferred onto PVDF membrane (Hybond-P, Amersham Pharmacia Biotech). The membrane was blocked with 5% nonfat dry milk in 0.1% Tween-PBS and treated with 0.65 μg/ml C219 monoclonal anti-P-gp antibody (DAKO) or 2 μg/ml AC-74 monoclonal anti β-actin antibody (Sigma). Detection by enzyme-linked chemiluminescence was performed according to the manufacturer's protocol (ECL plus®, Amersham Pharmacia Biotech). P-gp expression was quantified by NIH Image software.

2.4. Flow cytometry

Forty-eight or 72 h after transfection, cells were washed with OPTI-MemI® (Invitrogen) and incubated for 1 h with 1 μM daunorubicin in 5% CO_2 atmosphere. Nontreated MCF7-R cells have also been incubated or not in 20 μM

verapamil (30 min at 37 °C) before daunorubicin addition. Cells were then harvested at 4 °C with 3 mM EDTA and analysed with a FACSCalibur flow cytometer (Becton Dickinson) (FL2-H detector). 10,000 cells were investigated. Data were processed with the Cell Quest Pro® software. According to the manufacturer, the coefficient of variation of the full peak is <3%.

2.5. Chemosensitivity to daunorubicin

MCF7-S and MCF7-R cells were seeded in 12-well plates (1.2×10^5 cells per well). After attachment, MCF7-S cells were treated with increasing amounts of daunorubicin (9–22 μM). After 24 h incubation at 37 °C in 5% CO_2, cells were trypsinised and counted in 10% trypan blue. After attachment, MCF7-R cells were treated or not with siRNAs. 72 h after transfection, they were washed in PBS buffer then incubated in 22 μM daunorubicin for a further 24 h. They were trypsinised and counted in 10% trypan blue.

2.6. RT-PCR

Forty-eight and 72 h after transfection, total RNA was extracted (RNeasy kit, Qiagen) and cDNA was synthesised using Omniscript reverse transcription kit (Qiagen) and 0.5 μM gene-specific reverse primers of *MDR1* and β-actin which was chosen as an internal control. Hot Star Taq PCR kit (Qiagen) was used for DNA amplification, which was kept in its exponential phase (23 cycles). Primers used for *MDR1* amplification (0.5 μM) were: sense, 5′-d(TCTTGAAGGGCCTGAACCTG) and reverse, 5′-d(AGTCATAGGCATTGGCTTCC). Primers for β-actin amplification (0.3 μM) were: sense, 5′-d(ACCAACTGGGACGACATGGA) and reverse, 5′-d(CTCCTTAATGTCACGCACGA). Amplification products were separated on 1.8% agarose gel stained with ethidium bromide (6 μg/ml). Gene expression level was quantified by NIH Image software.

3. Results

3.1. Down-regulation of MDR1 gene expression with specific siRNAs

3.1.1. Protein level

3.1.1.1. Total protein. The efficiency of two siRNAs (si1 and si2) in reducing P-gp has been evaluated after a 72 h treatment (Fig. 1). The total siRNAs concentration was kept as low as 20 nM in order to eliminate the possibility of nonspecific effects. The siRNAs were separately transfected or co-transfected for combination experiments into MCF7-R cells. A very efficient decrease in P-gp expression was obtained with only ca. 20% remaining P-gp expression after treatment with si1, si2 or the combination of both of them; si3 was without effect.

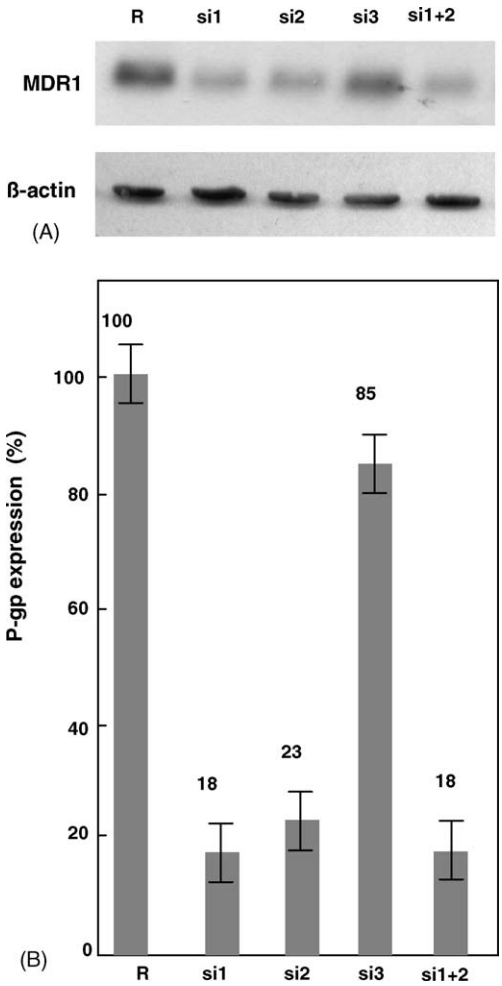

Fig. 1. Down-regulation of total P-gp expression by siRNAs. (A) Western blotting analysis at 72 h of MCF7-R cells without treatment (*R*) or treated with si1, si2, si3, si1 + si2; [total si] = 20 nM. β-actin was used as an internal control. (B) Average of the remaining total P-gp expression at 72 h including standard deviation. All experiments were performed at least in duplicate and three Western blots were performed for each cell lysis preparation.

3.1.1.2. Daunorubicin accumulation. The effect of si1 (20 nM), si2 (20 nM) and the combination of both (10 nM each) on daunorubicin accumulation was determined by flow cytometry. Fig. 2 shows FACS histograms of gated living cells with distribution of cell fluorescence in inset: grey cells had a non-significant and black cells a significant fluorescence. Marker M1 was placed around the peak of cell autofluorescence (MCF7-R cells without daunorubicin) (a). Marker M2 was placed to the right of M1 to designate positive events. Positive events were at a maximum when cells were treated with 20 μM verapamil (P-gp inhibitor) before daunorubicin addition (b). Such a concentration of verapamil induced a daunorubicin accumulation similar to that obtained in sensitive MCF7-S cells (not shown). Even in the absence of siRNA treatment, there was some daunorubicin accumulation in MCF7-R cells (c).

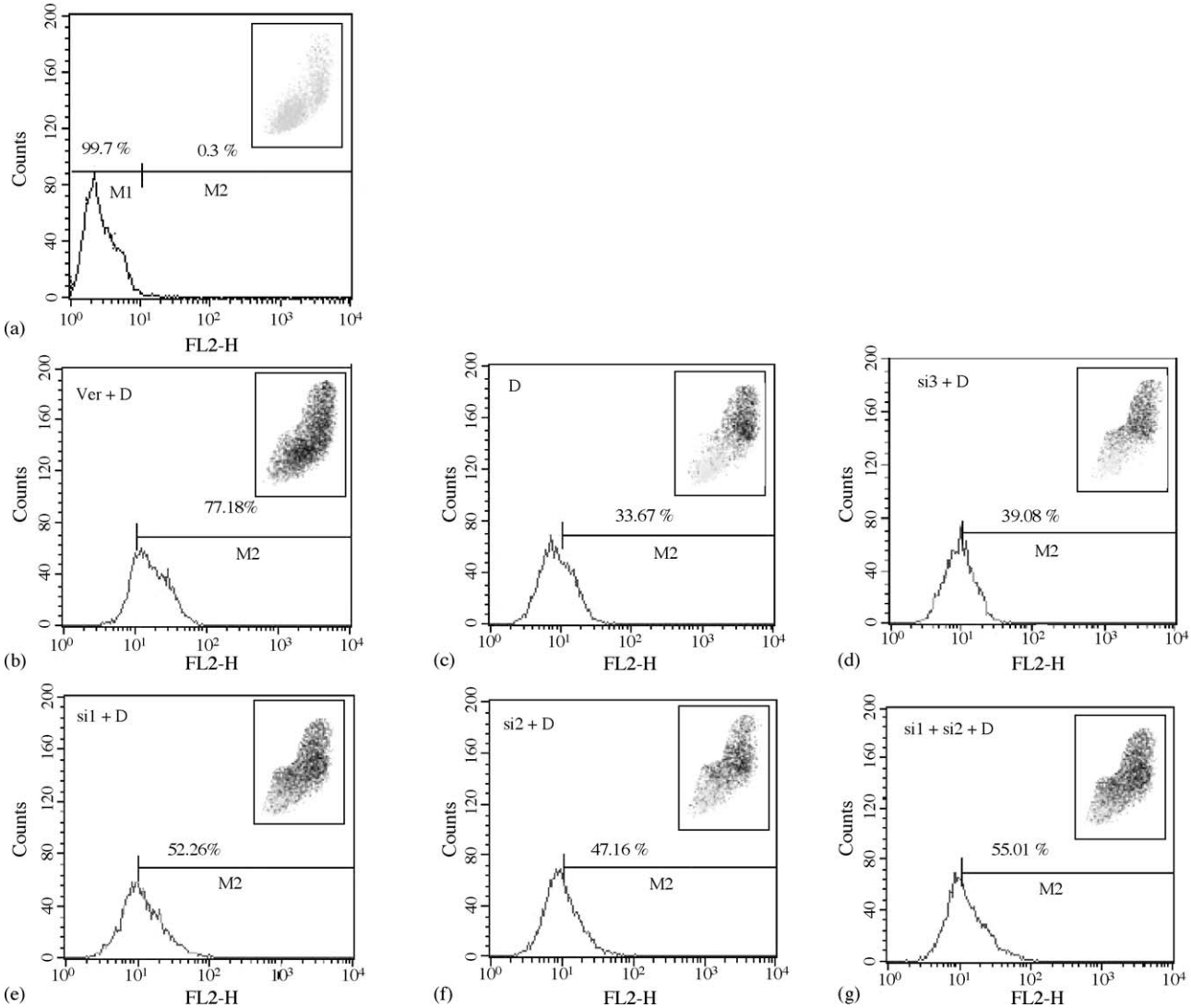

Fig. 2. Daunorubicin accumulation: FACS histograms of MCF7-R cells without any treatment (a) or treated for 1 h with 1 μM daunorubicin, after 30 min incubation in 20 μM verapamil (b), without verapamil (c) and after a 72 h treatment with si3 (d), si1 (e), si2 (f), si1 + si2 (g); [total si] = 20 nM. In inset, distribution of cell fluorescence. Ver: verapamil, D: daunorubicin.

This revealed a MCF7-R cells subpopulation with reduced resistance. It was only after a 72 h treatment with siRNAs that an increase in daunorubicin accumulation was observed. This increase was evaluated considering that treatment with verapamil had led to 100% P-gp inhibition (b) and that the minimal daunorubicin incorporation was that obtained in the absence of siRNA treatment (c). Daunorubicin accumulation in siRNA-treated cells was, thus, in agreement with a P-gp reversal (%) of about $100 \times [(M2 - 33.67)/(77.18 - 33.67)]$. A small effect was observed with the control si3: $12 \pm 3\%$ P-gp reversal (d). P-gp reversal was $43 \pm 3\%$ after treatment with si1 (e). $31 \pm 3\%$ after treatment with si2 (f) and $49 \pm 3\%$ after treatment with si1 + si2 (g).

3.1.1.3. Enhancement of chemosensitivity to daunorubicin. Enhancement of chemosensitivity to daunorubicin was expressed as the decrease of the survival rate after incubation with daunorubicin of siRNA-treated cells compared with siRNA-untreated control cells. As the cells were previously treated with si1, si2 or the combination of both for 72 h, it was first necessary to estimate which daunorubicin dose was adequate to discriminate between cells during a rather fast incubation period (24 h) to avoid dilution of siRNA effect due to cellular division. Colorimetric MTT (3-(4,5-dimethyl-2-thizolyl)-2,5-diphenyl-2H-tetrazolium bromide) assay was not appropriate because the absorbance of daunorubicin at concentrations above 8 μM interfered with that of formazan that was produced when MTT was incubated in live cells (570 nm) [27]. Cell counting in trypan blue (see Section 2) was found more accurate for such a study. Sixty percent MCF7-S cells were killed when incubated for 24 h in 17 μM daunorubicin, 75% in 22 μM (not shown). This last concentration was chosen in the purpose of a fast discrimination between siRNA-treated and nontreated MCF7-R cells. Fig. 3 shows

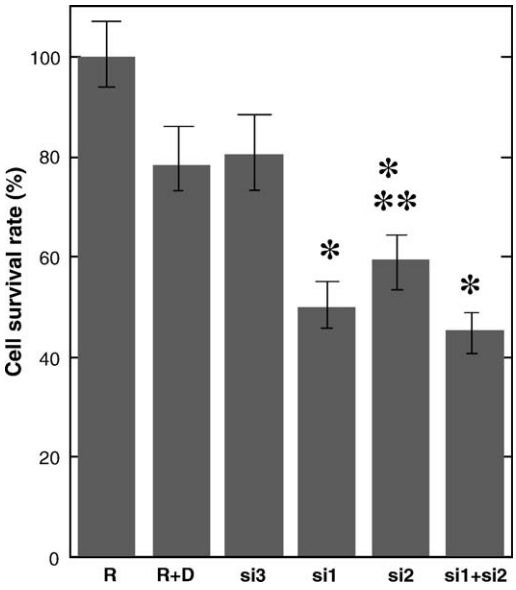

Fig. 3. Enhancement of chemosensitivity: cytotoxicity of daunorubicin in MCF7-R cells without treatment (*R*), treated with 22 μM daunorubicin (*R* + *D*) or treated with 22 μM daunorubicin after a 72 h treatment with si1, si2, si3, si1 + si2; [total si] = 20 nM. Each treatment was in triplicate and the three wells were counted. Each set of experiments (without treatment or treated with si1, si2, si3, si1 + si2) was repeated four times. The mean results are represented including standard deviations; $^{*}p < 0.01$ compared to (R + D) cells; $^{**}p < 0.05$ compared to si1 or si1 + si2 cells.

the cell survival rate of MCF7-R cells without incubation with daunorubicin (*R*) or with incubation in 22 μM daunorubicin but without siRNA treatment (*R* + *D*), or with incubation in 22 μM daunorubicin after siRNA treatment (si3, 20 nM; si1, 20 nM; si2, 20 nM; si1 + si2, 10 nM each). Even without siRNA treatment, MCF7-R cells were sensitive to such a high daunorubicin concentration (78.4 ± 7.2% cell survival). An MCF7-R cells subpopulation with reduced resistance was once again suggested (see Section 3.1.1.2). The control si3 was without further effect (80.7 ± 8.1% cell survival). Significant enhancement of chemosensitivity ($p < 0.01$) was observed after treatment with si1 (49.8 ± 5.5% cell survival), si2 (59.8 ± 4.8% cell survival) and si1 + si2 (45.7 ± 3.4% cell survival). si1 was slightly more efficient than si2 ($p < 0.05$). The percentage enhancement of chemosensitivity was calculated as the decrease of cell survival rate (%) = 100 × [(78.4% − % cell survival after siRNA treatment)/78.4%]. si1 led to an increase of chemosensitivity of about 35%, si2 of about 25%, si1 + si2 of about 40%, si3 was without effect.

3.1.2. mRNA level

Down-regulation at *MDR1* mRNA level has been evaluated after 48 or 72 h treatment by RT-PCR (Fig. 4). The sensitivity of this method allowed the efficiency of si1, si2 or their combination to be investigated at concentrations comprised between 5 and 20 nM. At 48 h, a significant ca. 50% inhibition was obtained with as low as 5 nM si1 or with the combination si1 + si2 (10 nM total siRNA concentration, i.e. 5 nM each si). The maximum of *MDR1* mRNA reduction of expression was about 60% and was obtained at 10 nM si1 without further improvement at 20 nM si1 or with the combination si1 + si2 (20 nM total siRNA concentration, i.e. 10 nM each si). si2 was shown to be less efficient than si1: only ca. 35% inhibition was obtained at 10 or 20 nM. The control si3 was without effect. The β-actin encoding mRNA was not affected. At 72 h, the initial levels of *MDR1* mRNA were restored except in the case of si1 treatment for which it was only ca. 70% (respectively ca. 60%) of its initial value after si1 treatment with 10 nM (respectively 20 nM).

4. Discussion

Two siRNAs have been found to specifically down-regulate *MDR1* at the protein and at the mRNA levels. Because nonspecific effects have been described with as low as 100 nM siRNA [18], ≤20 nM siRNA was used in this study despite the high turnover of the target protein. In our previous study [12], it had already been shown that, in favourable conditions i.e. when cells have a long cell cycle duration like MCF7-R cells and are in their exponential phase, 20 nM of si1 or si2 led to efficient reduction of the total P-gp cell content after a 72 h treatment. In the present study, we have shown that this decrease was drastic and led to about 20% remaining P-gp expression after transfection of a single siRNA or after co-transfection of both siRNAs (total concentration = 20 nM) (Fig. 1). As high as 500 nM antisense oligonucleotide was necessary to obtain 30% remaining P-gp expression in the same cells [12].

Nevertheless, this reduced amount in total P-gp content did not prejudge reversal of the MDR phenotype since only cell surface P-gp are responsible for the drug efflux [5]. Functional assays as daunorubicin accumulation or enhancement of chemosensitivity to daunorubicin have shown the efficiency of siRNAs in inhibiting cell surface expression of P-gp (Figs. 2 and 3). Whereas, correlation between decrease of P-gp cell surface expression and reduction of total P-gp content had been observed after siRNA treatment [7–10] and in the present study and after antisense treatment [1–3], this has not been described in a triple-helix strategy [5].

In both methods, functional P-gp inhibition was obtained at 72 h and si1 was more efficient than si2 (about 40% P-gp reversal with si1 versus 30% with si2 (Fig. 2); about 35% enhancement of chemosensitivity to daunorubicin with si1 versus 25% with si2 (Fig. 3)). P-gp inhibition occured after down-regulation at the mRNA level that was obtained at 48 h (Fig. 4). This 24 h gap resulted from P-gp long half-life and was described previously [8,12]. si1 was more efficient than si2 in decreasing the mRNA amount (respectively ca. 60% and ca. 35% inhibition) (Fig. 4). Both siRNAs satisfied conditions of efficient siRNAs. They were directed against a mRNA region downstream of the start codon (respectively 88–108 nt

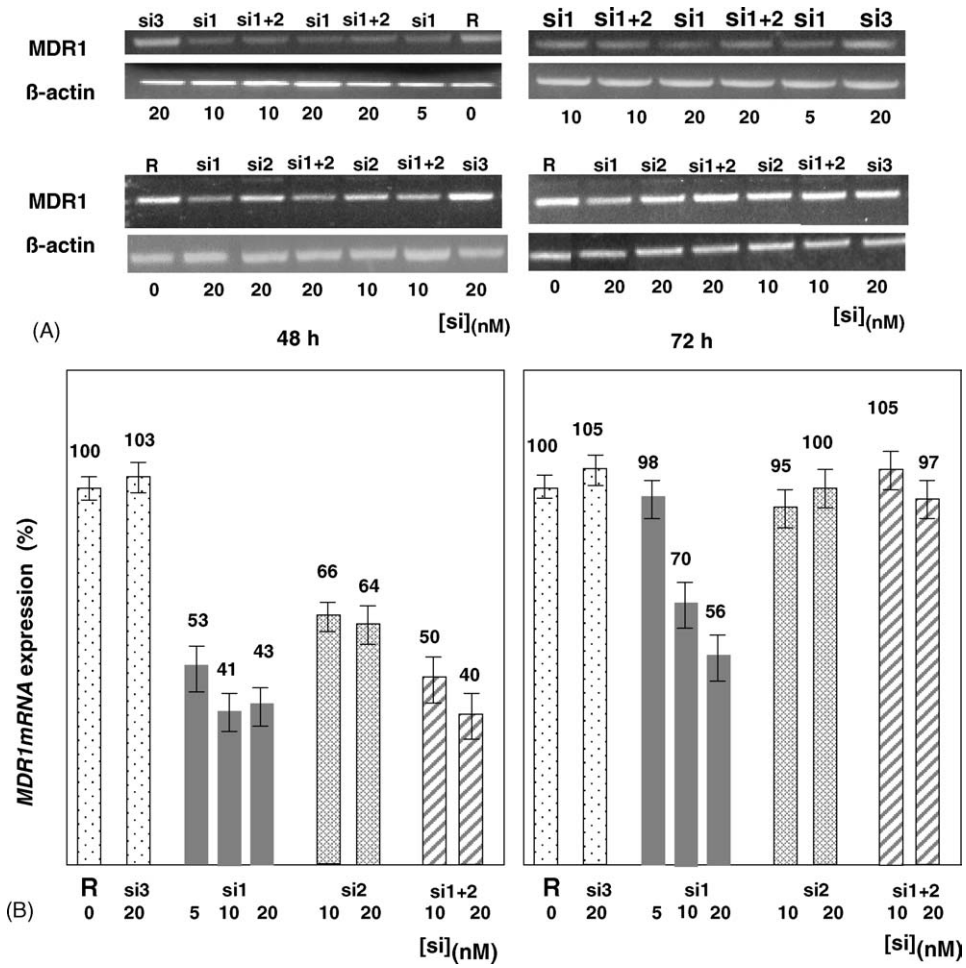

Fig. 4. Down-regulation of *MDR1* mRNA expression by siRNAs. (A) RT-PCR analysis at 48 and 72 h of MCF7-R cells without treatment (*R*) or treated with si1, si2, si3 or si1 + si2; [total si] as indicated on the figure. β-actin was used as an internal control. (B) Average of the remaining mRNA expression at 48 and 72 h including standard deviation. Four transfection experiments have been performed and PCR was in duplicate for each of them.

and 162–182 nt) and had about 50% G/C content (respectively 42 and 53%) [26]. Both siRNAs had low internal stability at the antisense strand 5′-terminus: U at the 5′-end of the antisense strand, C or G at the 5′-end of the sense strand and AU-richness in the 5′-terminal third of the antisense strand [28]. However, some characteristics associated with siRNA functionality and related to sense strand base preference were in favour of si1: an A at position 3 and an U at position 10 [29].

The initial level of *MDR1* mRNA was restored at 72 h (Fig. 4), except after treatment with si1 (only ca. 60% *MDR1* mRNA expression at 72 h after treatment with 20 nM si1). Return to an initial level of mRNA expression at 72 h was nevertheless expected for a single dose of siRNA [8,12]. The further action of si1 in the present work suggested that its stability was particularly great and more likely that of its antisense strand. Although with lower efficiency than double-stranded siRNAs, siRNA antisense strands can themselves be incorporated into RISC complex and lead to mRNA cleavage [30]. After unwinding of si1 in the RISC complex, its particularly stable antisense strand could thus be processed several times. Because such a stability of si1 had not been observed in NIH-3T3 cell line [12], a different enzymatic composition in MCF7 cells is suggested, probably enriched in endonucleases, which have a greater specificity for si2 than for si1.

When si1 and si2 (10 nM each) were used in combination (i) 49 ± 3% P-gp reversal was obtained, which was comparable to the effect of 20 nM si1 (43 ± 3% P-gp reversal) (Fig 2) and (ii) ca. 40% enhancement of chemosensitivity was obtained versus ca. 35% with 20 nM si1 (Fig. 3). These results would suggest an enhanced effect of si1 + si2 compared with the expected average effect of the addition of both siRNAs since si2 (10 nM in the combination) is less efficient than si1. Nevertheless, the absence of improvement of *MDR1* mRNA inhibition between 10 and 20 nM si1 or si2 (Fig. 4) would be coherent with the observation that cells have a limited capacity to assemble RISC on exogenous siRNAs [31]. In this study like in the present work, the authors kept siRNAs concentrations ≤25 nM in order to avoid sequence-independent changes in gene expression. About 60% *MDR1* mRNA inhibition was obtained with si1 + si2 (10 nM each) or si1 alone (10 nM); 50% with si1 + si2 (5 nM each) or si1 alone (5 nM) (Fig. 4). The apparent loss of effect of si2 in the

combination si1 + si2 suggests competition between both siRNAs, the most efficient of them binding faster on RISC. The better efficiency of si1 implied that its antisense strand was preferentially involved not only in RISC formation, which includes siRNA unwinding, but also preferentially led to mRNA recognition [32]. Thus, it seems reasonable to assume that, when the combination of siRNAs includes 10 nM si1, most of the observed mRNA inhibition, P-gp reversal and increase of chemosensitivity effects are the result of si1 efficiency.

In conclusion, siRNA strategy was shown to be an efficient alternative to P-gp inhibitors to reverse the MDR phenotype and restore sensitivity to chemotherapy, even at low concentration ($\leq$20 nM). It is noteworthy that avoiding nonspecific effects through the control of siRNA concentration seems uncertain when using vector-based continuous expression of hairpin siRNAs; synthetic siRNAs seem more appropriate for such a purpose. Because of the limited capacity of RISC to assemble on siRNAs and the apparent competition between siRNAs, we suggest to use a combination of siRNAs instead of a single one, which could reveal not to have the best efficiency.

References

[1] Alahari SK, Dean NM, Fisher MH, Delong R, Manoharan M, Tivel KL, et al. Inhibition of expression of the multidrug resistance-associated P-glycoprotein by phosphorothioate and 5′ cholesterol-conjugated phosphorothioate antisense oligonucleotides. Mol Pharmacol 1996;50:808–19.

[2] Bertram J, Palfner K, Killian M, Brysch W, Schlingensiepen KH, Hiddemann W, et al. Reversal of multidrug resistance in vitro by phosphorothioate oligonucleotides and ribozymes. Anti Cancer Drugs 1995;6:124–34.

[3] Liu C, Qureshi IA, Ding X, Shan Y, Huang Y, Xie Y, et al. Modulation of multidrug resistance gene (mdr-1) with antisense oligodeoxynucleotides. Clin Sci 1996;91:93–8.

[4] Brigui I, Djavanbakht-Samani T, Jolles B, Pigaglio S, Laigle A. Minimally modified phosphodiester antisense oligodeoxyribonucleotide directed against the multidrug resistance gene *MDR1*. Biochem Pharmacol 2003;65:747–54.

[5] Labroille G, Belloc F, Bilhou-Nabera C, Bonnefille S, Bascans E, Boisseau MR, et al. Cytometric study of intracellular P-gp expression and reversal of drug resistance. Cytometry 1998;32:86–94.

[6] Scaggiante B, Morassutti C, Tolazzi G, Michelutti A, Baccarani M, Quadrifoglio F. Effect of unmodified triple helix-forming oligodeoxyribonucleotide targeted to human multidrug-resistance gene *MDR1* in MDR cancer cells. FEBS Lett 1994;352:380–4.

[7] Wu H, Hait WN, Yang JM. Small interfering RNA-induced suppression of *MDR1* (P-glycoprotein) restores sensitivity to multidrug-resistant cancer cells. Cancer Res 2003;63:1515–9.

[8] Nieth C, Priebsch A, Stege A, Lage H. Modulation of the classical multidrug resistance (MDR) phenotype by RNA interference (RNAi). FEBS Lett 2003;545:144–50.

[9] Duan Z, Brakora KA, Seiden MV. Inhibition of ABCB1 (*MDR1*) and ABCB4 (MDR3) expression by small interfering RNA and reversal of paclitaxel resistance in human ovarian cancer cells. Mol Cancer Ther 2004;3:833–8.

[10] Xu D, Kang H, Fisher M, Juliano RL. Strategies for inhibition of *MDR1* gene expression. Mol Pharmacol 2004;66:268–75.

[11] Celius T, Garberg P, Lundgren B. Stable suppression of *MDR1* gene expression and function by RNAi in Caco-2 cells. Biochem Biophys Res Commun 2004;324:365–71.

[12] Stierle V, Laigle A, Jolles B. The reduction of P-glycoprotein expression by small interfering RNAs is improved in exponentially growing cells. Oligonucleotides 2004;14:191–8.

[13] Hammond SM, Caudy AA, Hannon GJ. Post-transcriptional gene silencing by double-stranded RNA. Nat Rev Genet 2001;2:110–9.

[14] Arenz C, Schepers U. RNA interference: from an ancient mechanism to a state of the art therapeutic application? Naturwissenschaften 2003; 90:345–59.

[15] Elbashir SM, Harborth J, Lendeckel W, Yalcin A, Weber K, Tuschl T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. Nature 2001;411:494–8.

[16] Brummelkamp TR, Bernards R, Agami R. Stable suppression of tumorigenicity by virus-mediated RNA interference. Cancer Cell 2002;2:243–7.

[17] Paddison PJ, Caudy AA, Hannon GJ. Stable suppression of gene expression by RNAi in mammalian cells. Proc Natl Acad Sci USA 2002;99:1443–8.

[18] Semizarov D, Frost L, Sarthy A, Kroeger P, Halbert DN, Fesik SW. Specificity of short interfering RNA determined through gene expression signatures. Proc Natl Acad Sci USA 2003;100:6347–52.

[19] Persengiev SP, Zhu X, Green MR. Nonspecific, concentration-dependent stimulation and repression of mammalian gene expression by small interfering RNAs (siRNAs). RNA 2004;10:12–8.

[20] Harborth J, Elbashir SM, Bechert K, Tuschl T, Weber K. Identification of essential genes in cultured mammalian cells using small interfering RNAs. J Cell Sci 2001;114:4557–65.

[21] Cohen D, Yang CP, Horwitz SB. The products of the *MDR1a* and *MDR1b* genes from multidrug resistant murine cells have similar degradation rates. Life Sci 1990;46:489–95.

[22] Richert ND, Aldwin L, Nitecki D, Gottesman MM, Pastan I. Stability and covalent modification of P-glycoprotein in multidrug-resistant KB cells. Biochemistry 1988;27:7607–13.

[23] Muller C, Laurent G, Ling V. P-glycoprotein stability is affected by serum deprivation and high cell density in multidrug-resistant cells. J Cell Physiol 1995;163:538–44.

[24] Chen CJ, Clark D, Ueda K, Pastan I, Gottesman MM, Roninson IB. Genomic organization of the human multidrug resistance (*MDR1*) gene and origin of P-glycoproteins. J Biol Chem 1990;265:506–14.

[25] Raynaud S, Nemati F, Miccoli L, Michel P, Poupon MF, Fourneau C, et al. Antitumoral effects of squamocin on parental and multidrug resistant MCF7 (human breast adenocarcinoma) cell lines. Life Sci 1999;65:525–33.

[26] Elbashir SM, Harborth J, Weber K, Tuschl T. Analysis of gene function in somatic mammalian cells using small interfering RNAs. Methods 2002;26:199–213.

[27] Li J, Xu LZ, He KL, Guo WJ, Zheng YH, Xia P, et al. Reversal effects of nomegestrol acetate on multidrug resistance in adriamycin-resistant MCF7 breast cancer cell line. Breast Cancer Res 2001;3:253–63.

[28] Naito Y, Yamada T, Ui-Tei K, Morishita S, Saigo K. siDirect: highly effective, target-specific siRNA design software for mammalian RNA interference. Nucl Acids Res 2004;32:W124–9.

[29] Reynolds A, Leake D, Boese Q, Scaringe S, Marshall WS, Khvorova A. Rational siRNA design for RNA interference. Nat Biotechnol 2004; 22:326–30.

[30] Holen T, Amarzguioui M, Wiiger MT, Babaie E, Prydz H. Positional effects of short interfering RNAs targeting the human coagulation trigger tissue factor. Nucl Acids Res 2002;30:1757–66.

[31] Hutvagner G, Simard MJ, Mello CC, Zamore PD. Sequence-specific inhibition of small RNA function. PLoS Biol 2004;2:465–75.

[32] Ui-Tei K, Naito Y, Takahashi F, Haraguchi T, Ohki-Hamazaki H, Juni A, et al. Guidelines for the selection of highly effective siRNA sequences for mammalian and chick RNA interference. Nucl Acids Res 2004;32:936–48.