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Short Communication

Reversal of P-glycoprotein-mediated multidrug resistance *in vitro* by doramectin and nemadectin

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Abstract

Objectives Multidrug resistance (MDR) is a serious obstacle encountered in cancer treatment. This study was performed to explore the reversal of MDR by doramectin from the avermectin family and nemadectin belonging to the milbemycin family.

Methods The MTT assay was used to evaluate the abilities of the two compounds to reverse drug resistance in adriamycin-resistant human breast carcinoma cells (MCF-7/adr). Intracellular accumulation of adriamycin was determined by HPLC. The effects of the two compounds on inhibiting P-glycoprotein (P-gp) efflux was demonstrated by accumulation of rhodamine 123 in MCF-7/adr cells. To investigate the mechanism of reversal by the two compounds, the expressions of P-gp and the *MDR1* gene encoding P-gp were tested by flow cytometry and reverse-transcriptase PCR.

Key findings Doramectin and nemadectin at the high dose of 8 μmol/l significantly increased the sensitivity of MCF-7/adr cells to adriamycin by 49.35- and 23.97-fold, respectively. They also increased the intracellular accumulation of adriamycin and rhodamine 123 in MCF-7/adr cells in a dose-dependent manner. Expression of both P-gp and *MDR1* were down-regulated.

Conclusions Doramectin and nemadectin are promising agents for overcoming MDR in cancer therapy. Doramectin was more potent in reversing MDR.

Keywords doramectin; nemadectin; multidrug resistance; P-glycoprotein

Introduction

The resistance of human tumours to multiple anti-cancer drugs (multidrug resistance, MDR) is a major obstacle for successful cancer chemotherapy. A classic mechanism of MDR is overexpression of the 170 kDa P-glycoprotein (P-gp), which is a member of the ATP-binding cassette (ABC) superfamily of membrane transporters.[1,2] In tumour cells, P-gp acts as an efflux pump that extrudes chemotherapeutic agents such as adriamycin, vinblastine, teniposide, paclitaxel and mitomycin C out of cells, decreasing their intracellular concentration.[3] Similarly, P-gp as an efflux pump in the blood–brain barrier can limit entry of some agents (e.g. HIV protease inhibitors) into brain tissue.[4] ABC drug transporters also include the multidrug resistance proteins MRP1 (ABCC1) and MRP7 (ABCC10), and the breast cancer resistance protein (BCRP or ABCG2).[5–7] Other mechanisms play a part in MDR in addition to ABC drug transporters, including enhanced expression of glutathione-S-transferase or glutathione peroxidase, reduced expression of topoisomerase II, cell adhesion to extracellular matrix proteins, and the sharing of common substrates of cytochrome P450 3A with P-gp in cancer cells.[8–11]

In order to regain sensitivity of resistant tumour cells to chemotherapeutics, a number of compounds to reverse MDR have been explored in the past two decades, such as verapamil, PSC 833, trifluoperazine, prednisolone and LY335979.[12] In addition, numerous plant-derived dietary compounds have been reported as modulators that suppress transport of P-gp, for instance ginsenoside Rg_3, cnidiadin and tea catechins.[13–15] Although several chemosensitisers showed potential for reversing MDR *in vitro* and *in vivo*, the results of clinical trials with these compounds were disappointing because of side-effects and/or weak potency. Therefore, development of new and more potent reversal agents with fewer side-effects is a priority for research on MDR.

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Previous studies on the reversal of MDR have investigated macrocyclic lactones, which are highly hydrophobic molecules that have a macrocyclic lactone ring as a common structural feature.[16] They are the most powerful agents used worldwide in livestock to fight against a broad spectrum of ecto- and endoparasites. The binding of macrocyclic lactones to the invertebrate glutamate-gated chloride channel, which is essentially irreversible, keeps the chloride channel open, leading to a very lasting hyperpolarisation or depolarisation of the neurone or muscle cell and therefore blocking further function.[17] Fortunately, macrocyclic lactones are safe for mammals and do not permeate the blood–brain barrier at low concentrations.

It has been reported that naturally occurring avermectins can modify the sensitivity of tumour cells to the substrates of MRPs. In some experimental systems, the most active avermectin was almost one order of magnitude more effective than the traditional inhibitor of multidrug resistance, ciclosporin.[18] Another macrolide compound, ivermectin, is a dehydrated derivative of avermectin B_{1a}. Millions of humans have been treated with ivermectin for the control of onchocerciasis and lymphatic filiarisis.[19] Because of its high efficacy and low toxicity, ivermectin could be an ideal agent for reversing MDR of tumour cells.[20] Published data show that ivermectin was 4- and 9-fold more potent than ciclosporin and verapamil, respectively, in reversing MDR. In terms of mechanisms of reversing MDR, ivermectin can bind to P-gp and inhibit the efflux pump.[21] Another possible mechanisms is that ivermectin inhibits MRP1 transport function and specifically interacts with MRP1, MRP2 and MPR3.[22] Eprinomectin, doramectin, selamectin and moxidectin have also been shown to inhibit the P-gp efflux pump.[23]

In this work, the macrocyclic lactones doramectin and nemadectin were evaluated for their ability to reverse MDR. Doramectin is a member of the avermectin family and is a broad-spectrum macrocyclic lactone endectocide like ivermectin. It differs from ivermectin by substitution of a cyclohexyl group at the C25 position. Nemadectin is a member of the milbemycin family, bearing a longer unsaturated chain group at C25. Nemadectin differs from the avermectins mainly by the lack of a sugar moiety attached to the C13 of the macrocyclic ring (Figure 1). We investigated the ability of doramectin and nemadectin to reverse MDR in adriamycin-resistant human breast carcinoma cells (MCF-7/adr), and the likely mechanism of this reversal.

Materials and Methods

Materials

Doramectin and nemadectin (purity > 98%) were provided by Zhejiang Hisun Pharmaceutical Co. Ltd (Taizhou, China). MTT and rhodamine 123 (Rh123) were purchased from Sigma-Aldrich Canada (Oakville, Ontario, Canada). Adriamycin was purchased from Pharmacia Italia (Nerviano, Italy). Verapamil was from Harvest (Shanghai, China). R-phycoerythrin-conjugated mouse anti-human monoclonal antibody against P-gp Mdr-1 (UIC2) was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Fetal calf serum (FCS) and RPMI-1640 medium were purchased from Gibco (Gaithersburg, MD, USA).

Cell lines and cell culture

The drug-sensitive human breast carcinoma cell line MCF-7 and its adriamycin-resistant counterpart MCF-7/adr were obtained from Dr Liang (Harbin Medical University, Harbin, China). The MCF-7/adr cell line was established by Dr Liang by exposing the parent MCF-7 cell line to increasing doses of adriamycin.

Cells were cultured in RPMI-1640 medium supplemented with 10% FCS at 37°C, 95% humidity and 5% CO_2. MCF-7/adr cells were maintained in medium containing 0.5 μmol/l adriamycin and were cultured in drug-free medium for 2 weeks before the experiments.

Determination of cytotoxicity and P-glycoprotein inhibitory activity

The in-vitro cytotoxicity of the drugs was determined using the MTT assay.[24] Cells that had been cultured without drug for at least 2 weeks were harvested in the exponential growth phase, and 100 μl aliquots were plated into 96-well plates at 1×10^4 cells per well for MCF-7 cells and 2×10^4 cells per well for MCF-7/adr cells and were pre-incubated for 24 h at

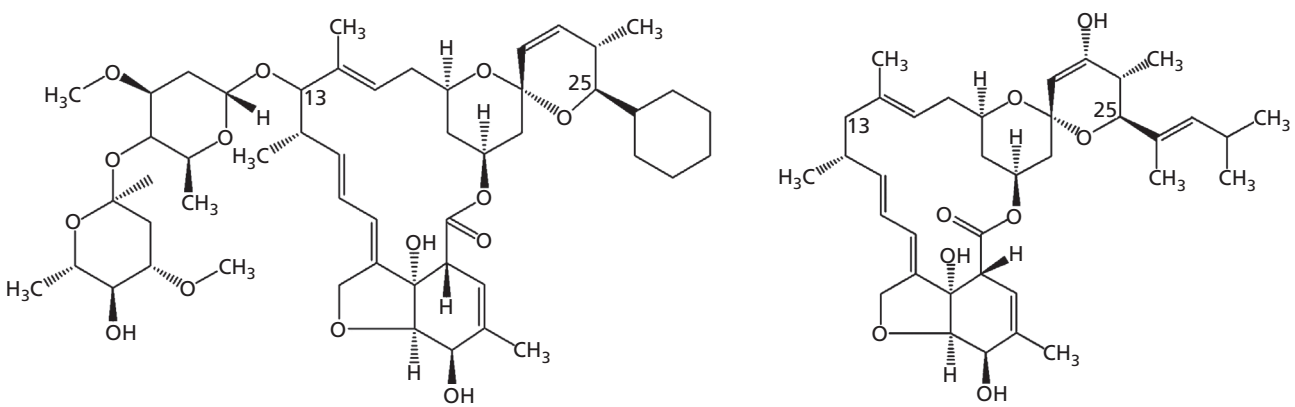

Figure 1 Structures of the macrocyclic lactones

37°C. After pre-incubation, the cells were treated with various concentrations of doramectin and nemadectin for 48 h. Then, 20 μl of freshly prepared MTT was added to each well and incubated for 4 h at 37°C. Cells were centrifuged for 15 min at 3500 rpm; 200 μl of medium was carefully removed and 150 μl DMSO added. Cells were shaken for 10 min until no particulate matter was visible. The absorbance was measured on a microplate reader (Bio-Rad Model/550, Hercules, CA, USA) with a wavelength of 570 nm. Concentrations of modulators that inhibited cell growth by 10%, 15% and 50% (IC10, IC15, IC50, respectively) were calculated using SPSS software (Bizinsight Information Technology Co. Ltd, Beijing, China). The concentration ranges of doramectin and nemadectin used in other experiments were below the IC15.

The reversal effects of modulators were investigated using the same method. MCF-7 and MCF-7/adr cells were seeded into 96-well plates and treated with various concentrations of adriamycin in the absence and presence of doramectin and nemadectin at 1, 4 and 8 μmol/l for 48 h. Triplicate experiments with triplicate samples were performed. The inhibition of cell growth was determined from the IC50 value of adriamycin. The reversal-fold value, a parameter of reversal potency, was calculated by dividing the IC50 of adriamycin in combination with modulators by the IC50 of adriamycin alone. Control medium included an equivalent amount of DMSO (as solvent control), but this applied dose did not affect cell growth or drug sensitivity. Verapamil (5 and 10 μmol/l) was used as a positive control.

Rhodamine 123 accumulation assay

MCF-7 and MCF-7/adr cells were seeded into 24-well plates at 1.0×10^5 cells per well and cultured for 6 h at 37°C in an atmosphere containing 5% CO_2. Then, 400 μl of fresh media containing 1, 4 or 8 μmol/l doramectin or nemadectin or 10 μmol/l verapamil was added, and incubated at 37°C for 1 h. Subsequently, 2.5 μg of Rh123 was added to each well, and the plates were incubated for 1 h at 37°C. The accumulation of Rh123 was stopped by washing the cells five times with ice-cold phosphate-buffered saline (PBS, pH 7.2), and the cells were lysed with 0.1% Triton X-100 at room temperature.[25] The fluorescence of the cell lysates was measured with a F-7000 spectrofluorometer (Hitachi Seisakusho, Tokyo, Japan) at an excitation wavelength of 485 nm and emission wavelength of 538 nm. The experiment was carried out in triplicate with three parallel samples.

Intracellular adriamycin accumulation

MCF-7 and MCF-7/adr cells were harvested and seeded into 24-well plates at 1.0×10^5 cells per well and cultured for 6 h at 37°C in an atmosphere containing 5% CO_2. The cells were then treated with 1, 4 or 8 μmol/l doramectin or nemadectin or 10 μmol/l verapamil for 1 h. The cells were then exposed to 15 μmol/l adriamycin. Controls were treated with 15 μmol/l adriamycin alone. After incubation for 3 h, the cells were washed three times with ice-cold PBS. The cells were resuspended in 0.3 mol/l HCl in 60% ethanol overnight,[26] and the mixture was centrifuged for 10 min at 12 000 rpm.

The intracellular adriamycin concentration was determined by HPLC using an Agilent 1100 (San Francisco, CA, USA), XDS-C18 column (4.6 × 150 mm, 5 μm).[27] The mobile phase was 5 mmol/l phosphoric acid/methanol/2-propanol/acetonitrile (8 : 7 : 3 : 2 v/v) delivered at a flow rate of 1 ml/min. Fluorescence detection was at excitation and emission wavelengths of 233 nm and 560 nm, respectively. Adriamycin concentrations were determined from a standard curve. Experiments were performed in triplicate, and triplicate samples analysed.

RNA extraction and RT-PCR

Total RNA from MCF-7 and MCF-7/adr cells treated with 5 μmol/l doramectin or nemadectin for 24 h was isolated with the TRIzol reagent of TaKaRa RNAiso Plus, and RT-PCR was performed. The primers used for *MDR1* and β-actin genes were as follows: *MDR1*, forward 5′-CCATCATTGCA ATAGCAGG-3′ and reverse 5′-AGTCCTCGTCTTCAAAC TTG-3′ for a 157 bp product, and β-actin, forward 5′-CTAC AATGAGCTGCGTGTGGC-3′ and reverse 5′-CAGGTCCA GACGCAGGATGGC-3′ for a 270 bp product. Amplification was performed for 35 cycles of sequential denaturation (94°C, 30 s), annealing (56°C, 30 s) and extension (72°C, 1 min). The amplified fragments were detected by 2% (w/v) agarose gel electrophoresis and stained with ethidium bromide. Bands were analysed using an image analysis system Tanon 2500 (Tanon Science and Technology Co. Ltd, Shanghai, China). The specific gene expression level was determined semi-quantitatively by calculating the ratio of densitometric value from specific genes expressed in relation to the internal standard (*MDR1* gene expression/β-actin expression). Duplicate experiments were performed, with analysis of triplicate samples.

Detection of P-glycoprotein expression

Cell-surface P-gp levels were measured by immunofluorescence flow cytometry.[28] MCF-7 and MCF-7/adr cells were seeded into six-well plates at a density of 2×10^5 cells per well and cultured for 24 h. Cells were then exposed to 4 and 8 μmol/l doramectin and nemadectin for a further 24 h. Cells were harvested, washed twice with ice-cold PBS, counted and then labelled with R-phycoerythrin-conjugated mouse anti-human monoclonal antibody against P-gp, according to the manufacturer's instructions. The fluorescent intensity was determined using flow cytometry (BD FACS, Aria, San Diego, CA, USA). Duplicate experiments were performed and triplicate samples analysed.

Statistical analysis

Data are presented as means ± SD. The Kruskal–Wallis test and Dunn's test were used for individual differences between the various treatments. Linear regression and factorial designed one-way analysis of variance (ANOVA) were used to determine relationships among many variables. $P < 0.05$ was considered significant.

Results

Intrinsic cytotoxicity

IC50 values are typically used to express the intrinsic toxicity of drugs. The IC50 values of doramectin and nemadectin in

MCF-7/adr cells were 14 μmol/l and 19.7 μmol/l, respectively. IC15 values were 8.4 μmol/l and 11 μmol/l. The concentration ranges of these drugs used in further experiments were below the individual IC15s, that is, 1–8 μmol/l. At this concentration range *in vitro*, doramectin and nemadectin did not appear to affect cell survival.

Multidrug resistance reversal efficacy of doramectin and nemadectin

MCF-7/adr cells were approximately 61-fold resistant to adriamycin compared with MCF-7 cells. Modulators enhanced the cytotoxicity of adriamycin in MCF-7/adr cells but had little effect on cytotoxicity in drug-sensitive MCF-7 cells. Table 1 shows the IC50 of adriamycin in MCF-7/adr cells in the presence of increasing concentrations of doramectin and nemadectin. The cytotoxicity of adriamycin in MCF-7/adr cells was increased 49.35-fold with 8 μmol/l doramectin. This concentration of doramectin was approximately 2-fold better than nemadectin and verapamil at potentiating the toxicity of adriamycin. Linear regression analysis revealed a significant negative correlation between the IC50 and the dose of doramectin ($r = -0.878$; $P < 0.01$) or nemadectin ($r = -0.99$; $P < 0.01$). Factorial designed ANOVA showed that doramectin differed significantly from nemadectin in terms of the IC50 of adriamycin ($P < 0.01$). Overall, the IC50 of adriamycin was decreased significantly by doramectin compared with verapamil and nemadectin and doramectin was more powerful than equivalent concentrations of nemadectin in terms of fold-reversal of MDR.

Effects on intracellular adriamycin accumulation

The intracellular accumulation of adriamycin was approximately 3.5 times more in MCF-7 cells than in MCF-7/adr cells. The intracellular accumulation of adriamycin in MCF-7/adr cells was markedly increased following treatment with doramectin or nemadectin (1, 4 and 8 μmol/l) whereas the accumulation of adriamycin in MCF-7 cells was not remarkably affected by these compounds. Compared with untreated MCF-7/adr cells, the enhancement of adriamycin accumulation in MCF-7/adr cells treated with 1, 4 and 8 μmol/l doramectin was approximately 1.31-, 1.78- and 2.33-fold, respectively, and 1.29-, 1.73- and 2.1-fold with the same concentrations of nemadectin (Figure 2). Linear regression analysis showed a significant positive correlation between adriamycin accumulation and the concentration of doramectin ($r = 0.986$; $P < 0.01$) or nemadectin ($r = 0.944$; $P < 0.01$). Furthermore, the data showed that doramectin had a significantly stronger effect than nemadectin on intracellular adriamycin accumulation ($P < 0.01$).

Effects of modulators on intracellular rhodamine 123 accumulation

MCF-7/adr and MCF-7 cells were also used to study the effects of doramectin and nemadectin on P-gp function by determining the intracellular Rh123-associated mean fluorescence intensity (MFI). As shown in Table 2, doramectin and nemadectin enhanced intracellular Rh123 accumulation in a concentration-dependent manner (doramectin: $r = 0.937$; $P < 0.01$; nemadectin: $r = 0.939$; $P < 0.01$). The data also indicate that doramectin had a significantly stronger effect on intracellular Rh123 accumulation than nemadectin ($P < 0.01$). In particular, MCF-7/adr cells treated with 8 μmol/l doramectin or nemadectin showed a notably higher intracellular MFI than untreated MCF-7/adr cells. No such increase in MFI was observed in MCF-7 cells treated with doramectin or nemadectin.

Down-regulation of *MDR1* gene and P-glycoprotein expression

The *MDR1* gene encodes the drug efflux pump P-gp, the over-expression of which is associated with the development of MDR. The *MDR1* genes of MCF-7 cells and MCF-7/adr cells were assessed with semi-quantitative RT-PCR, and the relative densitometric value calculated relative to β-actin expression. Figure 3a shows that, as expected, there was no apparent *MDR1* expression in MCF-7 cells, whereas *MDR1* expression was clearly observed in MCF-7/adr cells with acquired MDR. Compared with untreated MCF-7/adr cells,

Table 1 Modulation of the sensitivity of MCF-7/adr and MCF-7 cells to adriamycin by doramectin and nemadectin

Modulator and concn (μmol/l)	IC50 of adriamycin (μmol/l)		Fold-reversal of MDR	
	MCF-7/adr cells	MCF-7 cells	MCF-7/adr cells	MCF-7 cells
Control	34.725 ± 1.162	0.567 ± 0.038		
Verapamil				
5	4.296 ± 0.256**	0.554 ± 0.026	8.09	1.02
10	1.304 ± 0.175**	0.571 ± 0.047	26.65	0.99
Doramectin				
1	4.956 ± 0.155**	0.583 ± 0.029	7.03	0.97
4	1.208 ± 0.073**	0.575 ± 0.033	28.76	0.99
8	0.704 ± 0.132**	0.533 ± 0.047	49.35	1.06
Nemadectin				
1	10.167 ± 0.272**	0.55 ± 0.022	3.42	1.03
4	7.429 ± 0.355**	0.638 ± 0.025	4.68	0.89
8	1.45 ± 0.088**	0.571 ± 0.052	23.97	0.99

IC50, concentration of adriamycin required to inhibit cell growth by 50%. Values are means ± SD ($n = 3$). Fold-reversal of multidrug resistance (MDR), ratio of the IC50 for adriamycin alone vs IC50 for adriamycin in the presence of inhibitor. **$P < 0.01$ vs control group (Dunn's test).

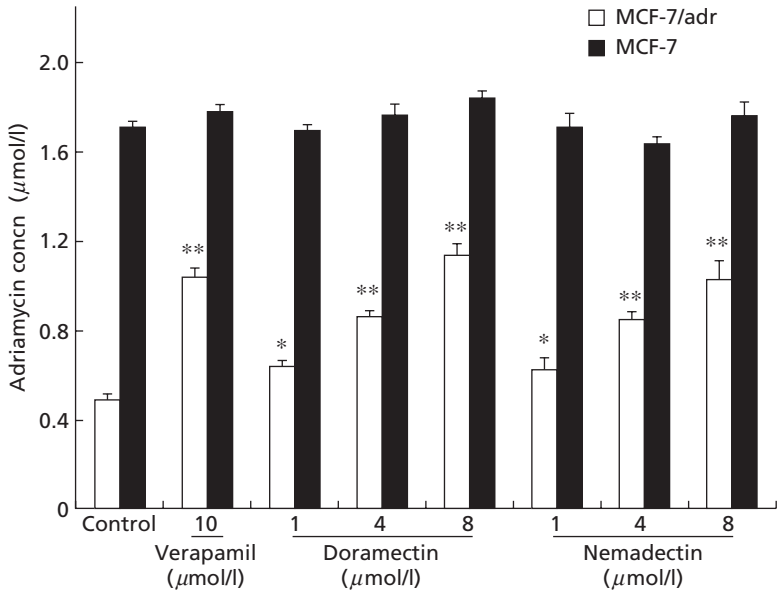

Figure 2 Effects of doramectin and nemadectin on adriamycin accumulation in MCF-7 cells and MCF-7/adr cells. Bars represent means ± SD of triplicate determinations. $^{*}P < 0.05$; $^{**}P < 0.01$ vs MCF-7/adr control group (Dunn's test)

Table 2 Effects of doramectin and nemadectin on the intracellular accumulation of rhodamine 123 in MCF-7/adr and MCF-7 cells

Concn (μmol/l)	Rh123-associated MFI	
	MCF-7/adr cells	MCF-7 cells
Control	517 ± 2	1239 ± 32
Verapamil (10)	697 ± 33**	1269 ± 33
Doramectin		
1	565 ± 23*	1255 ± 9
4	616 ± 36**	1264 ± 38
8	852 ± 44**	1261 ± 29
Nemadectin		
1	531 ± 8*	1236 ± 34
4	570 ± 15**	1240 ± 29
8	807 ± 40**	1267 ± 25

Values are mean fluorescence intensity (MFI) ± SD of least three independent experiments. Rh123, rhodamine123. $^{*}P < 0.05$; $^{**}P < 0.01$ vs control group (Dunn's test).

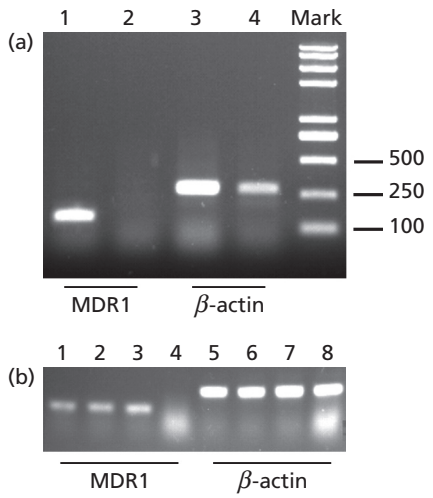

Figure 3 RT-PCR analysis of *MDR1* mRNA expression. The sizes of the specific RT-PCR products were 157 bp for *MDR1* and 270 bp for β-actin. (a) Lanes 1 and 3, untreated MCF-7/adr cells; lanes 2 and 4, untreated MCF-7 cells. (b) Lanes 4 and 8, untreated MCF-7 cells; lanes 3 and 7, untreated MCF-7/adr cells; lanes 1 and 5, MCF-7/adr cells treated with 5 μmol/l doramectin; lanes 2 and 6, MCF-7/adr cells treated with 5 μmol/l nemadectin

MDR1 expression in MCF-7/adr cells treated with 5 μmol/l doramectin or nemadectin for 24 h was decreased by 40.21 ± 4.57% and 29.17 ± 2.14%, respectively (Figure 3b, $P < 0.01$).

To further confirm whether doramectin and nemadectin could down-regulate P-gp expression, the expression level of P-gp in MCF-7 cells and MCF-7/adr cells was analysed by flow cytometry. MCF-7 cells showed virtually no fluorescence intensity labelled by anti-P-gp monoclonal antibody whereas MCF-7/adr cells exhibited a strong fluorescent area corresponding to P-gp. In MCF-7/adr cells cultured for 24 h with 4 or 8 μmol/l doramectin, the expression of P-gp was decreased by 44.86 ± 4.76% and 65.11 ± 3.26%, respectively, compared with untreated MCF-7/adr cells ($P < 0.01$). Similarly, expression of P-gp in MCF-7/adr cells cultured for 24 h with 4 or 8 μmol/l nemadectin was decreased by 33.26 ± 2.17% and 57.39 ± 4.12%, respectively ($P < 0.01$). These results indicate that doramectin and nemadectin decrease the expression of P-gp in MCF-7/adr cells.

Discussion

MDR of tumour cells is often associated with overexpression of P-gp and leads to chemotherapeutic failure. The macrolide compounds ivermectin and avermectins are inhibitors of P-gp and are even more efficient than verapamil

and ciclosporin.[18,21] A previous study found that the relative efficacy of inhibition of tumour cell MDR by avermectins depends on the type of cell and the substrate of transport proteins.[18]

In the current study we have tested the effects of doramectin (from the avermectin family) and nemadectin (from the milbemycin family) on reversal of MDR in MCF-7/adr cells using the MTT assay, accumulation of adriamycin and Rh123, and analysing expression of the *MDR1* gene and P-gp expression, and also analysed the relationships between reversal of MDR and chemical structure of the two compounds. The results showed that these two macrocyclic lactones could effectively reverse the MDR of MCF-7/adr tumour cells. Our findings using the MTT assay demonstrated that doramectin and nemadectin could both potently reverse MDR and that doramectin was more effective than nemadectin and verapamil *in vitro*. The concentrations of doramectin and nemadectin used (1, 4 and 8 μmol/l) was similar to other macrcyclic lactones,[20,22,23] although these concentrations may be too high for use *in vivo*. We found that 1 μmol/l doramectin and nemadectin could reverse the MDR (7.03- and 3.42-fold reversal, respectively). Therefore, we may decide concentrations for use *in vivo* on this basis.

Rh123 is a specific substrate of P-gp and has been used extensively as an indicator of P-gp activity in drug-resistant cell lines with P-gp overexpression.[14,24] We used Rh123 to evaluate the ability of doramectin and nemadectin to modulate the drug transport function of P-gp. As shown in Table 2, treatment of MCF-7/adr cells with doramectin and nemadectin at 1, 4 and 8 μmol/l resulted in a remarkable increase in the fluorescence intensity of Rh123, indicating that doramectin and nemadectin increased the accumulation of adriamycin in these cells by suppressing the drug transport activity of P-gp, and therefore increasing the toxicity of adriamycin. These results indicate that doramectin and nemadectin can inhibit P-gp function and may act as reversing agents for MDR.

We found that doramectin was more potent than nemadectin in reversing the MDR of MCF-7/adr cells. Lespine *et al.*[23] have reported that the chemical structures of macrocyclic lactones influence their affinity for P-gp. The presence of a sugar moiety affects the hydrophobicity of the molecule, and this hydrophobic moiety seems to be important for the interaction of macrocyclic lactones with P-gp. The hydrophobic characteristics of the two compounds were investigated in preliminary experiments by comparing their retention times on reverse-phase HPLC (data not shown). As expected, doramectin had a longer retention time than nemadectin, as it possesses a disaccharide moiety at the C13 of the macrocycle. It is therefore more hydrophobic and has stronger affinity for P-gp, which may be the main reason why doramectin more effectively reversed the MDR of MCF-7/adr cells than nemadectin, consistent with earlier findings.[23] The high affinity for P-gp is one of the mechanisms proposed for reversal of MDR by doramectin and nemadectin (i.e. inhibition of P-gp efflux).

In addition, expression of *MDR1* mRNA and P-gp were analysed by RT-PCR and immunofluorescence flow cytometry, as some studies have proposed that suppressing the expression of P-gp at the transcriptional and/or translational level is a key mechanism for certain modulators or agents that reverse MDR. For example, Li *et al.* found that nomegestrol significantly inhibited the *MDR1* gene and P-gp expressions.[29] Yu *et al.* demonstrated that tryptanthrin inhibited the *MDR1* gene partly through acting on the *MDR1* promoter.[30] Zheng *et al.* suggested that cantharidin could effectively reverse MDR via down-regulation of *MDR1* gene expression.[31] We have shown here that doramectin and nemadectin decrease *MDR1* gene and P-gp expression in MCF-7/adr cells, and doramectin was more potent than nemadectin. The results suggest that doramectin and nemadectin may reduce the expression of P-gp at both the transcriptional and translational levels, reversing the MDR phenotype of MCF-7/adr cells. With regard to the possible mechanism of the reduction on mRNA and protein level, we propose that doramectin and nemadectin may dramatically inhibit the activity of the *MDR1* promoter.[30] We are doing further experiments to elucidate this hypothesis.

Conclusions

We have provided abundant evidence that, *in vitro*, both doramectin and nemadectin effectively reverse MDR of MCF-7/adr cells, via inhibition of the P-gp pump function and down-regulation of the *MDR1* gene and P-gp expression. They may be candidates as effective MDR-reversing agents in cancer chemotherapy. Based on our data, doramectin, which contains a sugar moiety, was more effective than nemadectin as an MDR modulator. Further work is needed to understand the effects of doramectin and nemadectin on reversing MDR of tumour cells *in vitro*.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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