Cancer Chemother Pharmacol (2004) 53: 363–369
DOI 10.1007/s00280-003-0745-2

ORIGINAL ARTICLE

**Hans Minderman · Tracy A. Brooks
Kieran L. O'Loughlin · Iwao Ojima
Ralph J. Bernacki · Maria R. Baer**

Broad-spectrum modulation of ATP-binding cassette transport proteins by the taxane derivatives ortataxel (IDN-5109, BAY 59-8862) and tRA96023

Received: 25 April 2003 / Accepted: 24 October 2003 / Published online: 27 January 2004

Abstract *Purpose*: The taxanes paclitaxel and docetaxel are substrates for P-glycoprotein (Pgp), an ATP-binding cassette (ABC) transport protein associated with multidrug resistance (MDR). In contrast, the synthetic taxane ortataxel (BAY 59-8862, IDN-5109) is effective against Pgp-expressing cells by virtue of modulation of Pgp-mediated transport. The synthetic taxane tRA96023 also modulates Pgp and is noncytotoxic due to removal of the tubulin-binding side chain at the C-13 position of the taxane backbone. We studied the effects of ortataxel and tRA96023 on the other MDR-associated ABC transport proteins, multidrug resistance protein (MRP-1) and breast cancer resistance protein (BCRP, MXR, ABCG2). *Methods*: Modulation of mitoxantrone, daunorubicin and doxorubicin retention and cytotoxicity by ortataxel and tRA96023 was studied in established cell lines overexpressing Pgp, MRP-1 and wild type ($BCRP^{R482}$) and mutant ($BCRP^{R482T}$) BCRP, and was compared with modulation by the established Pgp-, MRP-1- and BCRP-specific modulators PSC-833, probenecid and fumitremorgin C, respectively. *Results*: Ortataxel effectively modulated drug retention and cytotoxicity in cell lines overexpressing MRP-1 and $BCRP^{R482}$, in addition to Pgp. tRA96023 modulated drug retention and cytotoxicity in cell lines overexpressing $BCRP^{R482}$ and Pgp, but not those overexpressing MRP-1. Neither ortataxel nor tRA96023 modulated $BCRP^{R482T}$. *Conclusions*: The synthetic taxane derivatives ortataxel and tRA96023 are broad-spectrum ABC protein modulators. Further studies will seek to identify a non-cytotoxic synthetic taxane that modulates Pgp, MRP-1 and BCRP.

Keywords Taxanes · P-glycoprotein · Multidrug resistance protein · Breast cancer resistance protein · Modulation

This work was supported by grants R21 CA098457 and R21 CA89938 (to M.R.B.), R01 CA73872 (to R.J.B.) and T32 CA09072-27 from the National Cancer Institute and R01 GM42798 from the National Institute of General Medical Sciences (to I.O.), by a Leukemia and Lymphoma Society Translational Research Program grant (to M.R.B.), by shared resources of the Roswell Park Cancer Center Support Grant (P30 CA16056) and by the Leonard S. LoVullo Memorial Fund for Leukemia Research and the Dennis J. Szefel, Jr Endowed Fund for Leukemia Research at Roswell Park Cancer Institute.

H. Minderman · K. L. O'Loughlin · M. R. Baer (✉)
Leukemia Section, Department of Medicine,
Roswell Park Cancer Institute,
Elm and Carlton Streets, Buffalo,
New York 14263, USA
E-mail: maria.baer@roswellpark.org
Tel.: +1-716-8455975

T. A. Brooks · R. J. Bernacki · M. R. Baer
Department of Pharmacology and Therapeutics,
Roswell Park Cancer Institute,
Elm and Carlton Streets, Buffalo,
New York 14263, USA

I. Ojima
Department of Chemistry,
State University of New York at Stony Brook,
Stony Brook, New York 11794-3400, USA

Introduction

Tumor cells frequently exhibit multidrug resistance (MDR) mediated by the ATP-binding cassette (ABC) membrane proteins P-glycoprotein (Pgp), multidrug resistance protein (MRP-1) and breast cancer resistance protein (BCRP) [10]. Noncytotoxic molecules, termed MDR modulators, inhibit drug efflux mediated by these proteins, restoring cellular drug sensitivity. PSC-833 [2], probenecid [9] and fumitremorgin C [33] modulate Pgp, MRP-1 and BCRP, respectively; cyclosporin A (CsA) has activity against MRP-1 and BCRP, as well as Pgp [26]. Structural properties responsible for broad-spectrum, versus ABC protein-specific, modulation are unknown.

The taxanes paclitaxel and docetaxel, semisynthetic chemotherapy drugs that inhibit microtubule depolymerization [34, 38], are substrates for Pgp [3, 16,

39]. Limited availability of natural resources led to the development of synthetic taxanes [5], and active efflux of natural product taxanes by Pgp prompted attempts to synthesize analogs that are not susceptible to Pgp-mediated drug resistance [31]. Ortataxel (formerly BAY 59-8862 and IDN 5109) [31] is a cytotoxic taxane [4] that modulates Pgp, blocking its own efflux from Pgp-overexpressing cells [17, 28, 29, 30, 31, 32, 45]. tRA96023 also modulates Pgp [12], but is noncytotoxic due to removal of the side chain at the C-13 position of the taxane backbone, which is required for tubulin binding [32]. Ortataxel is in clinical trials [6], and tRA96023 is in preclinical development.

To determine whether ortataxel and tRA96023 might be broad-spectrum MDR modulators, we studied their effects on drug retention and cytotoxicity in resistant cell lines overexpressing MRP-1 and BCRP, as well as Pgp. The drug retention assay measures short-term effects on drug transport, while the cytotoxicity assay measures long-term effects on cell survival.

Materials and methods

Cell lines

Established resistant cell lines overexpressing Pgp (8226/Dox6, MCF7/R), MRP-1 (HL60/Adr) or wild type (8226/MR20) or mutant (MCF7/AdVp3000) BCRP were studied [8, 14, 24, 25]. Wild type ($BCRP^{R482}$) and mutant ($BCRP^{R482T}$) BCRP, with arginine and threonine in the amino acid 482 position, respectively, both efflux mitoxantrone, but only $BCRP^{R482T}$ effluxes anthracyclines [15]. Wild type HL60 cells served as negative controls for all three proteins [25] since the parental 8226 and MCF7 cell lines express low levels of $BCRP^{R482}$ [25, 27].

ABC protein expression

Expression of Pgp, MRP-1 and BCRP was confirmed by staining with the MRK-16 (Kamiya Biomedical Company, Tukwila, Wash.), MRPm6 (Kamiya), BXP-21 and BXP-34 (from Dr. R.J. Scheper, Free University, The Netherlands, as previously described [25]) antibodies, respectively, followed by flow cytometric detection (Fig. 1). The demonstrated expression of the MDR-associated ABC proteins in Fig. 1 is consistent with previous reports in the literature [1, 8, 14, 25].

Drugs

Drugs used in retention and cytotoxicity assays included mitoxantrone, daunorubicin and doxorubicin (Sigma, St. Louis, Mo.). All are substrates for Pgp [22, 25], MRP-1 [22, 25] and $BCRP^{R482T}$ [15, 22, 25], but only mitoxantrone is also a substrate for $BCRP^{R482}$ [15].

Modulators

Ortataxel (Indena, Milan, Italy) and tRA96023 (SB-RA-310124, synthesized as described previously [30]), were compared with PSC-833 (Novartis, East Hanover, N.J.), *p*-(dipropylsulfamoyl)benzoic acid (Probenecid, Sigma) and fumitremorgin C (FTC, from Dr. Susan Bates, National Cancer Institute, Bethesda, Md.) at concentrations of 2.5 μM, 1 mM and 10 μM, respectively, which were previously described to be most effective for modulation without cytotoxicity [1, 9, 35]. Ortataxel was minimally fluorescent at high concentrations; tRA96023 was not fluorescent (Fig. 2).

Drug retention studies

Drug retention was studied as previously described [25]. Briefly, drug uptake was achieved by incubating cells with drug (3 μM) for 30 min. Drug efflux was then studied by comparing drug retention following a 90-min incubation in drug-free medium in the presence and absence of modulator. All experiments were performed in triplicate. Cellular drug content was measured by flow cytometry

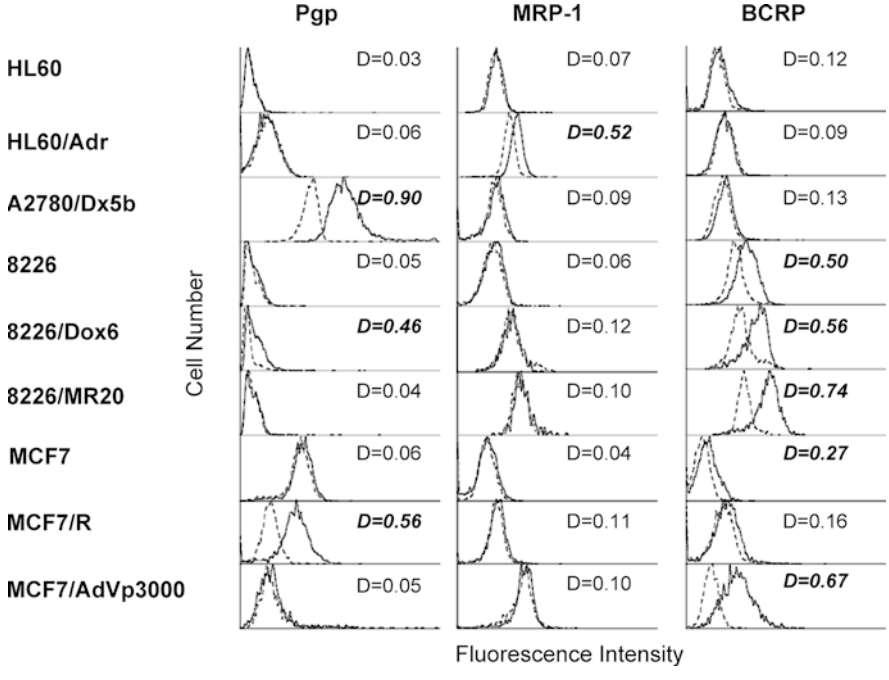


Fig. 1 ABC protein expression in cell lines, measured by flow cytometry. Cells were stained with the MRK-16, MRPm6 and BXP-21 antibodies (*solid lines*), which are specific for Pgp, MRP-1 and BCRP, respectively, and with isotype controls (*broken lines*). Staining with antibody was compared with staining with isotype control by the Kolmogorov-Smirnov statistic, expressed as a D-value ranging from 0 to 1. D-values indicating positive staining are in bold italics. Representative comparisons are shown from triplicate experiments. Staining with BXP-34, a second BCRP-specific antibody, was similar to staining with BXP-21 (data not shown)

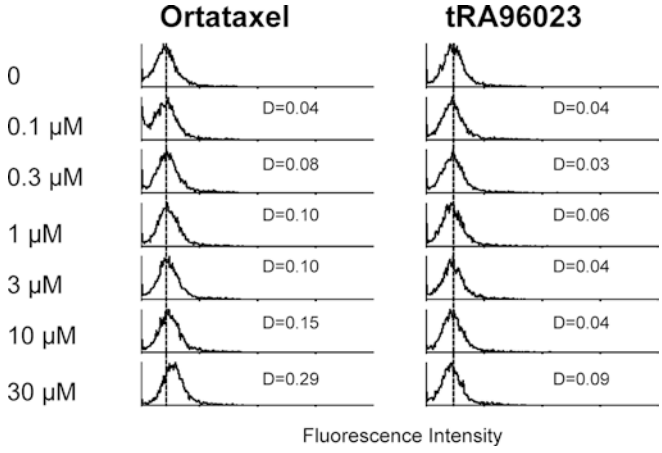


Fig. 2 Measurement of intrinsic fluorescence of ortataxel and tRA96023. HL60 cells were incubated for 30 min with ortataxel (*left*) and with tRA96023 (*right*) at the concentrations shown. Fluorescence of cells incubated with ortataxel or tRA96023 was compared with that of cells incubated without modulator by the Kolmogorov-Smirnov statistic, expressed as D-values ranging from 0 to 1. Experiments were performed in triplicate; a representative experiment is shown. Ortataxel was minimally fluorescent at high concentrations; tRA96023 was not fluorescent

Fig. 3 Concentration-dependent effects of ortataxel (*left*) and tRA96023 (*right*) on efflux of mitoxantrone in the 8226/Dox6, HL60/Adr and 8226/MR20 cell lines, overexpressing Pgp, MRP-1 and wildtype BCRP ($BCRP^{R482}$), respectively. Cells were incubated with 3 μ*M* mitoxantrone for 30 min (*uptake*), washed and resuspended in drug-free medium, then incubated for 90 min without modulator (*efflux*) and with ortataxel or tRA96023 at the concentrations shown. Efflux in the presence of ortataxel or tRA96023 at each concentration was compared with efflux in the absence of modulator by the Kolmogorov-Smirnov statistic, expressed as D-values ranging from 0 to 1. A representative example from triplicate experiments is shown. Ortataxel modulated all cell lines, while tRA96023 had no effect in HL60/Adr cells. Both modulators increased mitoxantrone retention in a concentration-dependent manner, effectively blocking efflux at 10 μ*M*

and analyzed using the WinList program (Verity Software House, Topsham, Me.). In experiments with ortataxel, cellular fluorescence was compensated to account for that associated with the modulator.

Flow cytometry data analysis

Labeling with specific antibody and with isotype control was compared by the Kolmogorov-Smirnov (KS) statistic, expressed as a D-value ranging from 0 to 1 [46]. Antibody positivity was defined by D-values ≥0.1 and ≥0.2 for staining of unfixed (MRK-16 [1]) and fixed (other antibodies [25]) cells, respectively. Drug retention in the presence and absence of modulator was also compared by the KS statistic; D-values ≥0.2 were considered to be indicative of modulation [25].

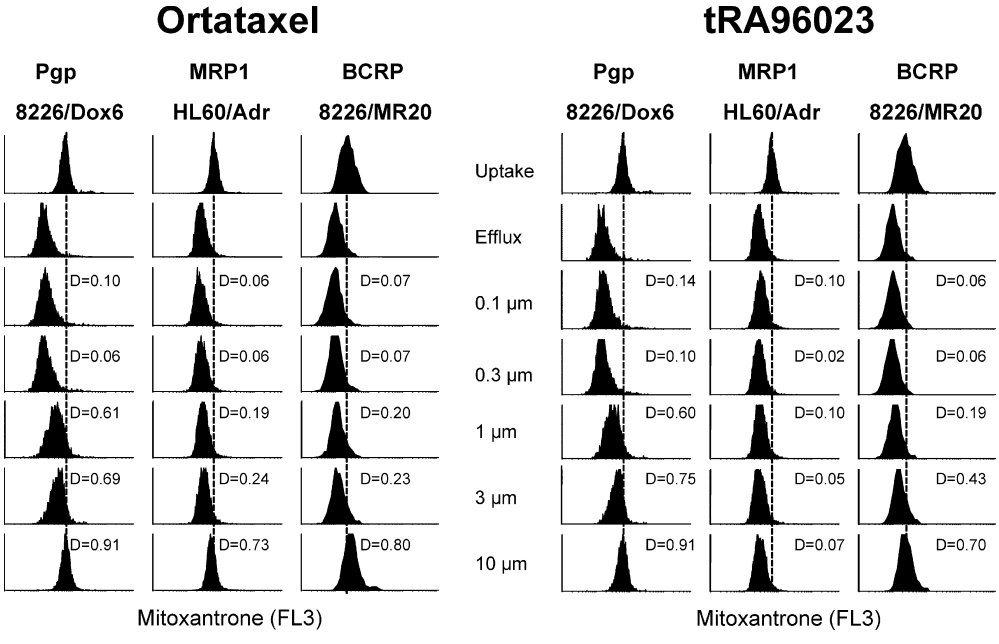
Cytotoxicity

Cells were cultured for 96 h in 96-well plates at densities of 10,000 (suspension) or 1,500 (adherent) cells per well in RPMI 1640 medium with 10% FBS, 2 m*M* L-glutamine, 20 U/ml penicillin, 20 μg/ml streptomycin and drugs at a range of doses in the absence and presence of modulators, in triplicate. Cell growth was assessed by the WST-1 assay (Roche Diagnostics, Mannheim, Germany) for suspension cells [36, 42] and the sulforhodamine-B (SRB) assay for adherent cells [40]. IC_{50} values, or drug concentrations inhibiting cell growth by 50% compared to untreated cells, were determined using curve-fitting software, as previously described [11]. Relative resistance (RR) was calculated as the ratio (IC_{50} resistant cells/IC_{50} wild type cells). The resistance modifying factor (RMF) was calculated as the ratio (IC_{50} drug/IC_{50} drug + modulator).

Results

Effects of ortataxel and tRA96023 on drug efflux mediated by Pgp, MRP-1 and BCRP

Mitoxantrone retention was studied in the absence and presence of increasing concentrations of ortataxel and tRA96023 in cells overexpressing Pgp (8226/Dox6),



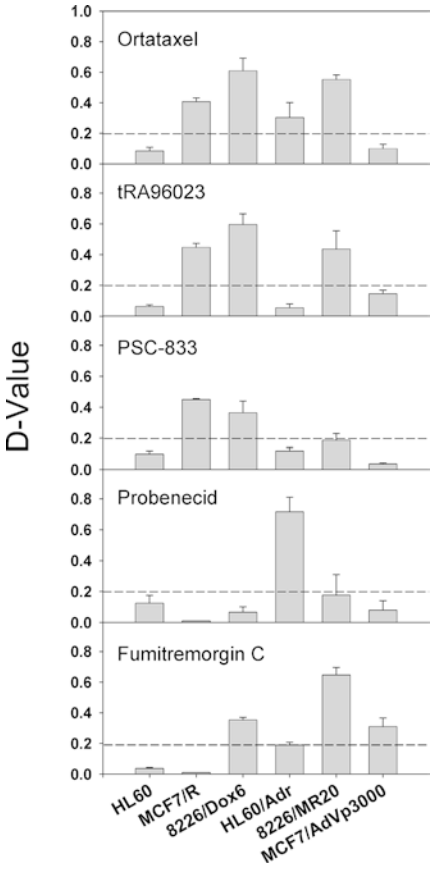


Fig. 4 Modulation of mitoxantrone efflux by ortataxel (10 μ*M*), tRA96023 (10 μ*M*), PSC-833 (2.5 μ*M*), probenecid (1 m*M*) and fumitremorgin C (10 μ*M*) in cell lines expressing different MDR-associated transport proteins: HL60 (none), 8226/Dox6 and MCF7/R (Pgp), HL60/Adr (MRP-1), 8226/MR20 ($BCRP^{R482}$) and MCF7/AdVp3000 ($BCRP^{R482T}$). Cells were incubated with 3 μ*M* mitoxantrone for 30 min (*uptake*), washed and resuspended in drug-free medium, then incubated for 90 min with and without modulator (*efflux*). Retention in the presence of each modulator was compared with retention in the absence of modulator by the Kolmogorov-Smirnov statistic, expressed as a D-value ranging from 0 to 1. D-values ≥0.20 (*dashed lines*) were considered to indicate modulation. The graphs show the mean values from triplicate experiments, with standard errors. Ortataxel and tRA96023 had a broader spectrum of activity than PSC-833, probenecid and fumitremorgin C, but were slightly less potent, as evidenced by lower D-values

MRP-1 (HL60/Adr) and BCRP (8226/MR20) (Fig. 3). Ortataxel modulated retention in all three cell lines, but tRA96023 had no effect in HL60/Adr cells. Both modulators increased mitoxantrone retention in a concentration-dependent manner, effectively blocking efflux at 10 μ*M*. The effects of ortataxel and tRA96023 on mitoxantrone retention were compared with those of specific modulators of Pgp, MRP-1 and BCRP (Fig. 4). The taxane derivatives had a broader spectrum of activity but were slightly less potent MRP-1 and BCRP modulators than probenecid and FTC, as evidenced by lower D-values.

Modulation of efflux of daunorubicin and doxorubicin, which are additional established substrates of Pgp, MRP-1 and $BCRP^{R482T}$, was also studied (Fig. 5).

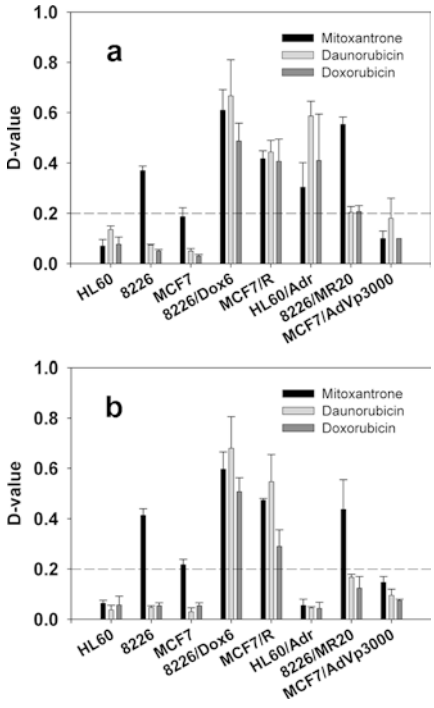


Fig. 5a, b Modulation of efflux of mitoxantrone, daunorubicin and doxorubicin by ortataxel (**a**) and by tRA96023 (**b**) in cell lines expressing different MDR-associated transport proteins. Parental cell lines: HL60 (none), 8226 ($BCRP^{R482}$) and MCF7 ($BCRP^{R482}$). MDR cell lines: 8226/Dox6 and MCF7/R (Pgp), HL60/Adr (MRP-1), 8226/MR20 ($BCRP^{R482}$) and MCF7/AdVp3000 ($BCRP^{R482T}$). Cells were incubated with 3 μ*M* mitoxantrone, 3 μ*M* daunorubicin or 3 μ*M* doxorubicin for 30 min (*uptake*), washed and resuspended in drug-free medium, then incubated for 90 min with and without 10 μ*M* ortataxel or 10 μ*M* tRA96023 (*efflux*). Retention of each drug in the presence of each modulator was compared with retention in the absence of modulator by the Kolmogorov-Smirnov statistic, expressed as a D-value ranging from 0 to 1. D-values ≥0.20 (*dashed lines*) were considered to indicate modulation. The graphs show the mean values from triplicate experiments, with standard errors. Ortataxel (**a**) effectively increased mitoxantrone, daunorubicin and doxorubicin retention in cell lines overexpressing Pgp (8226/Dox6, MCF7/R) and MRP-1 (HL60/Adr). It also increased retention of mitoxantrone, but not anthracyclines, in 8226/MR20 cells, which overexpress $BCRP^{R482}$, and to a lesser extent in the parental 8226 and MCF7 cells, which also express $BCRP^{R482}$. This modulation profile is in agreement with the fact that anthracyclines are not substrates for $BCRP^{R482}$. Moreover, ortataxel had no effect in MCF7/AdVp3000 cells, which overexpress $BCRP^{R482T}$. The effects of tRA96023 were similar to those of ortataxel in all cells except HL60/Adr (MRP-1) (**b**)

Ortataxel (Fig. 5a) effectively increased retention of daunorubicin and doxorubicin, in addition to mitoxantrone, in cell lines expressing Pgp (8226/Dox6, MCF7/R) and MRP-1 (HL60/Adr). As expected, it did increase retention of mitoxantrone but not of anthracyclines in 8226/MR20 cells and the parental 8226 and MCF7 cells, which express $BCRP^{R482}$, for which the anthracyclines are not substrates. Moreover, ortataxel had no effect in MCF7/AdVp3000 cells, which overexpress $BCRP^{R482T}$. tRA96023 had effects similar to those of ortataxel, except that it had no effect in HL60/Adr cells (MRP-1) (Fig. 5b).

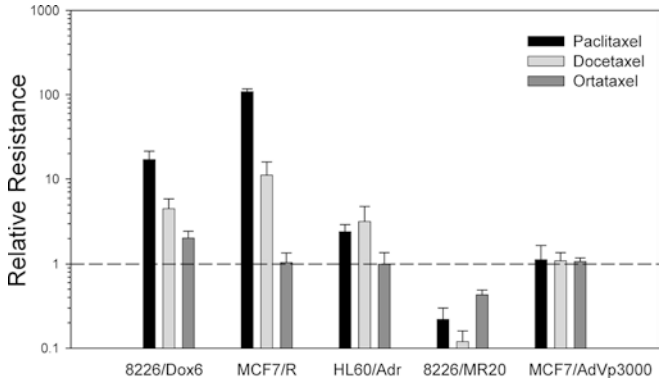


Fig. 6 Relative resistance of cell lines overexpressing Pgp (8226/Dox6, MCF7/R), MRP-1 (HL60/Adr) and wild type (8226/MR20) and mutant (MCF7/AdVp3000) BCRP to paclitaxel, docetaxel and ortataxel, compared to parental cells. Relative resistance was calculated as the ratio (IC_{50} resistant cells/IC_{50} wild type cells). The graphs show the mean values from triplicate experiments, with standard errors. 8226/Dox6 and MCF7/R cells, which overexpress Pgp, were highly resistant to paclitaxel and docetaxel, but only slightly resistant to ortataxel. HL60/Adr cells, which overexpress MRP-1, had slight resistance to paclitaxel and docetaxel, but none to ortataxel. 8226/MR20 and MCF7/AdVp3000 cells, which overexpress BCRP, were not resistant to any of the taxanes tested

Effects of ortataxel and tRA96023 on resistance mediated by Pgp, MRP-1 and BCRP

Because ortataxel is cytotoxic, activity was further characterized by comparing its cytotoxicity to those of paclitaxel and docetaxel in cell lines overexpressing Pgp, MRP-1 and BCRP (Fig. 6). 8226/Dox6 and MCF7/R cells, which overexpress Pgp, were highly resistant to paclitaxel and docetaxel, but only slightly resistant to ortataxel. HL60/Adr cells, which overexpress MRP-1, had slight resistance to paclitaxel and docetaxel, but none to ortataxel. 8226/MR20 and MCF7/AdVp3000 cells, which overexpress BCRP, were not resistant to any of the taxanes tested. Thus ortataxel is not a substrate for BCRP or MRP-1 and has a lower resistance profile in Pgp-overexpressing cell lines, as compared to paclitaxel and docetaxel, most likely due to its demonstrated modulation of Pgp and MRP-1.

Because tRA96023 is noncytotoxic, its activity as a modulator was assessed by studying its potentiating effect on mitoxantrone and anthracycline cytotoxicity in cell lines overexpressing Pgp, MRP-1 and BCRP. The IC_{50} values of mitoxantrone, doxorubicin and daunorubicin were compared in the presence and absence of 10 μM tRA96023 in the resistant cell lines, in relation to parental cell lines (Table 1). Concordant with the drug retention data, the presence of tRA96023 decreased relative mitoxantrone resistance of cells expressing Pgp (8226/Dox6, MCF7/R) and $BCRP^{R482}$ (8226/MR20) but not those expressing MRP-1 (HL60/Adr) or $BCRP^{R482T}$ (MCF7/AdVp3000), and decreased relative resistance to doxorubicin and daunorubicin only in cells expressing Pgp. Resistance modifying factors are shown graphically in Fig. 7.

Discussion

We demonstrated that synthetic taxanes modulate resistance mediated by the MDR-associated ABC transport proteins MRP-1 and $BCRP^{R482}$, in addition to their known modulation of Pgp. Ortataxel, which is cytotoxic, increased mitoxantrone and anthracycline retention and was more cytotoxic than paclitaxel and docetaxel in cell lines overexpressing Pgp, MRP-1 and $BCRP^{R482}$. tRA96023, which is noncytotoxic, enhanced mitoxantrone and anthracycline retention and cytotoxicity in cell lines overexpressing Pgp and $BCRP^{R482}$, but not those overexpressing MRP-1. Thus synthetic taxane modulators may sensitize cancers with MDR mediated by multiple ABC proteins. The finding that neither taxane modulated drug transport mediated by $BCRP^{R482T}$, in contrast to their effective modulation of $BCRP^{R482}$, indicates that specific mutations in this transport protein may affect not only substrate specificity [15], but also modulator specificity.

The relevance of MDR proteins has been most fully studied in acute myeloid leukemia (AML), because of the ease of obtaining tumor cells for study and the ability to correlate MDR protein expression and function with heterogeneous treatment response. Pgp [7, 13,

Table 1 Modulation of cytotoxicity of mitoxantrone, daunorubicin and doxorubicin by tRA96023 (10 μM) in cell lines overexpressing Pgp, MRP-1, $BCRP^{R482}$ or $BCRP^{R482T}$ and the corresponding parental cell lines. Resistance modifying factors (RMF) were calculated as the ratios of the IC_{50} values in the absence and presence of tRA. Each IC_{50} and RMF was calculated as the mean ± SD of triplicate experiments. IC_{50} values are in micromoles

Cell line	Resistance mechanism	Mitoxantrone		Daunorubicin		Doxorubicin	
		IC_{50}	RMF	IC_{50}	RMF	IC_{50}	RMF
8226 (parental)	$BCRP^{R482}$	0.347 ± 0.001	1.0 ± 0.2	0.879 ± 0.126	0.8 ± 0.2	0.582 ± 0.098	1.3 ± 0.3
8226/Dox6	Pgp, $BCRP^{R482}$	1.23 ± 0.09	12.5 ± 1.2	1.17 ± 0.2	3.3 ± 0.7	1.37 ± 0.29	6.5 ± 0.9
8226/MR20	$BCRP^{R482}$	2.4 ± 0.8	61.0 ± 26.5	0.913 ± 0.081	1.3 ± 0.3	0.776 ± 0.174	1.9 ± 0.23
HL60 (parental)	None	0.018 ± 0.007	1.8 ± 0.2	0.007 ± 0.002	2.7 ± 0.8	0.014 ± 0.006	1.5 ± 0.3
HL60/Adr	MRP-1	4.7 ± 0.66	1.6 ± 0.3	0.647 ± 0.094	1.1 ± 0.4	5.2 ± 0.62	1.35 ± 0.05
MCF7 (parental)	$BCRP^{R482}$	0.040 ± 0.01	2.7 ± 0.9	0.012 ± 0.001	1.02 ± 0.2	0.052 ± 0.019	3.15 ± 1.2
MCF7/R	Pgp	0.504 ± 0.17	6.6 ± 2.2	0.415 ± 0.068	19.7 ± 7.8	2.002 ± 0.31	14.1 ± 3.9
MCF7/AdVp3000	$BCRP^{R482T}$	134.7 ± 7.4	2.8 ± 0.5	2.79 ± 0.27	1.62 ± 0.6	24.5 ± 5.15	3.7 ± 1.4

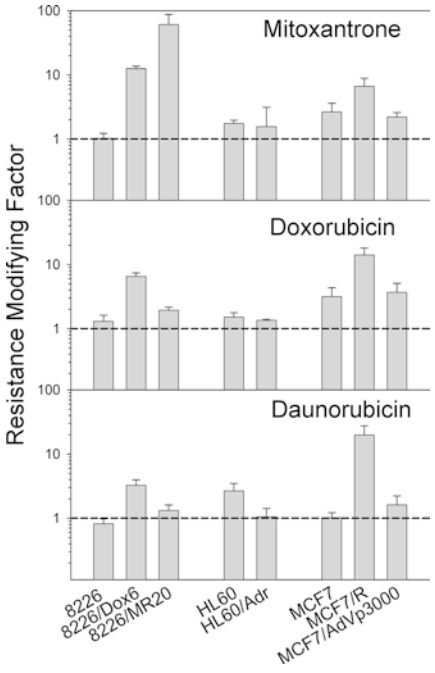


Fig. 7 tRA96023 modulation of mitoxantrone, doxorubicin and daunorubicin cytotoxicity in cell lines overexpressing Pgp (8226/Dox6, MCF7/R), MRP-1 (HL60/Adr) and wild type (8226/MR20) and mutant (MCF7/AdVp3000) BCRP. The resistance modifying factor was calculated as the ratio (IC_{50} drug/IC_{50} drug + modulator). The graphs show the mean values from triplicate experiments, with standard errors. Consistent with the drug retention data, tRA96023 modulated mitoxantrone cytotoxicity only in 8226/Dox6 and MCF7/R cells, which express Pgp, and in 8226/MR20 cells, which express $BCRP^{R482}$, and modulated anthracycline cytotoxicity only in Pgp-expressing cells

18, 19, 20, 21, 23] and MRP-1 [18, 19, 21] have clinical relevance in AML, and the relevance of BCRP has also been suggested [37, 41, 43, 44]. BCRP has been wild type ($BCRP^{R482}$) in all cases of AML studied to date [41]; whether mutant BCRP is present in other tumor types, or only in cell lines, is yet unknown. Broad-spectrum modulators have an obvious theoretical advantage in this and other malignancies with expression of multiple ABC transport proteins.

Most MDR modulation clinical trials to date have yielded disappointing results. Trials have only targeted Pgp, despite the fact that additional ABC proteins contribute to clinical MDR. The synthetic taxanes may be promising agents for clinical application because of their broad spectrum of modulation. Nevertheless, the safety and efficacy of clinical application of broad-spectrum modulators remains to be demonstrated.

The data presented here serve to demonstrate that taxane derivatives have the potential to modulate all three MDR pumps. The efficacy of ortataxel as a cytotoxic chemotherapeutic agent is currently being evaluated [6]. The present data suggest that this drug should be effective against tumors expressing MRP-1 and BCRP, as well as those expressing Pgp [45]. Nevertheless, due to the cytotoxic properties of ortataxel, it is not strictly an MDR modulator. tRA96023 is noncytotoxic, but its lack of modulation of MRP-1 makes it less than optimal for development as a broad-spectrum clinical modulator. However, tRA96023 is one of a large library of noncytotoxic taxane derivatives lacking the C13 tubulin-binding side chain [30, 32]. This library is currently being screened to identify noncytotoxic modulators of all three MDR-associated ABC proteins.

References

1. Baer MR, George SL, Dodge RK, O'Loughlin KL, Minderman H, Caligiuri MA, Anastasi J, Powell BL, Kolitz JE, Schiffer CA, Bloomfield CD, Larson RA (2002) Phase III study of the multidrug resistance modulator PSC-833 in previously untreated patients 60 years of age and older with acute myeloid leukemia: Cancer and Leukemia Group B study 9720. Blood 100:1224–1232
2. Boesch D, Gaveriaux C, Jachez B, Pourtier-Manzanedo A, Bollinger P, Loor F (1991) In vivo circumvention of P-glycoprotein-mediated multidrug resistance of tumor cells with SDZ PSC 833. Cancer Res 51:4226–4233
3. Casazza AM, Fairchild CR (1996) Paclitaxel (taxol) mechanisms of resistance. In: Hait WN (ed) Drug resistance. Kluwer Academic Publishers, Boston, pp 149–171
4. Cassinelli G, Lanzi C, Supino R, Pratesi G, Zuco V, Laccabue D, Cuccuru G, Bombardelli E, Zunino F (2002) Cellular bases of the antitumor activity of the novel taxane IDN 5109 (BAY59-8862) on hormone-refractory prostate cancer. Clin Cancer Res 8:2647–2654
5. Cragg GM, Snader KM (1991) Taxol: the supply issue. Cancer Cells 3:233–235
6. Creaven PJ, Eckhardt SG, Hamm J, Schwartz G, Sutton JW, Kaidbey J, Lathia C, Bernacki R, Ramnath NA (2002) Phase I and pharmacokinetic study of a novel taxane BAY-59-8862. Proc Am Soc Clin Oncol 21:100a
7. Del Poeta G, Stasi R, Aronica G, Venditti A, Cox MCA, Bruno A, Buccisano F, Masi M, Tribalto M, Amadori S, Papa G (1996) Clinical relevance of p-glycoprotein expression in de novo acute myeloid leukemia. Blood 87:1997–2004
8. Fairchild CR, Ivy SP, Kao-Shan CS, Whang-Peng J, Rosen N, Israel MA, Melera PW, Cowan KH, Goldsmith ME (1987) Isolation of amplified and overexpressed DNA sequences from adriamycin-resistant human breast cancer cells. Cancer Res 47:5141–5148
9. Gollapudi S, Kim CH, Tran BN, Sangha S, Gupta S (1997) Probenecid reverses multidrug resistance in multidrug resistance-associated protein-overexpressing HL60/AR and H69/AR cells but not in P-glycoprotein-overexpressing HL60/Tax and P388/ADR cells. Cancer Chemother Pharmacol 40:150–158
10. Gottesman MM, Fojo T, Bates SE (2002) Multidrug resistance in cancer: role of ATP-dependent transporters. Nat Rev Cancer 2:48–58
11. Greco WR, Park HS, Rustum YM (1990) An application of a new approach for the quantitation of drug synergism to the combination of cis-diamminedichloroplatinum and 1-beta-D-arabinofuranosylcytosine. Cancer Res 50:5318–5327
12. Gruol DJ, Bernd J, Phippard AE, Ojima I, Bernacki RJ (2001) The use of a novel taxane-based P-glycoprotein inhibitor to identify mutations that alter the interaction of the protein with paclitaxel. Mol Pharmacol 60:104–113
13. Guerci A, Merlin JL, Missoum N, Feldmann L, Marchal S, Witz F, Rose C, Guerci O (1995) Predictive value for treatment outcome in acute myeloid leukemia of cellular daunorubicin accumulation and p-glycoprotein expression simultaneously determined by flow cytometry. Blood 85:2147–2153

14. Hazlehurst LA, Foley NE, Gleason-Guzman MC, Hacker MP, Cress AE, Greenberger LW, DeJong MC, Dalton WS (1999) Multiple mechanisms confer drug resistance to mitoxantrone in the human 8226 myeloma cell line. Cancer Res 59:1021–1028
15. Honjo Y, Hrycyna CA, Yan Q-W, Medina-Perez WY, Robey RW, Van de Laar A, Litman T, Dean M, Bates SE (2001) Acquired mutations in the MXR/BCRP/ABCP gene alter substrate specificity in MXR/BCRP/ABCP-overexpressing cells. Cancer Res 61:6635–6639
16. Horwitz SB, Cohen D, Rao S, Ringel I, Shen H-J, Yang C-PH (1993) Taxol: mechanisms of action and resistance. Natl Cancer Inst Monogr 15:55–61
17. Laccabue D, Tortoreto M, Veneroni S, Perego P, Scanziani E, Zucchetti M, Zaffaroni M, D'Incalci M, Bombardelli E, \Zunino F, Pratesi G (2001) A novel taxane active against an orthotopically growing human glioma xenograft. Cancer 92:3085–3092
18. Legrand O, Simonin G, Perrot J-Y, Zittoun R, Marie J-P (1998) Pgp and MRP activities using calcein-AM are prognostic factors in adult acute myeloid leukemia patients. Blood 91:4480–4488
19. Legrand O, Simonin G, Beauchamp-Nicoud A, Zittoun R, Marie J-P (1999) Simultaneous activity of MRP1 and Pgp is correlated with in vitro resistance to daunorubicin and with in vivo resistance in acute myeloid leukemia. Blood 94:1046–1056
20. Leith CP, Kopecky KJ, Godwin J, McConnell T, Slovak ML, Chen I-M, Head DR, Appelbaum FR, Willman CL (1997) Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. Blood 89:3323–3329
21. Leith CP, Kopecky KJ, Chen I-M, Eijdems L, Slovak ML, McConnell TS, Head DR, Weick J, Grever MR, Appelbaum FR, Willman CL (1999) Frequency and clinical significance of the expression of the multidrug resistance proteins MDR1/P-glycoprotein, MRP1, and LRP in acute myeloid leukemia: a Southwest Oncology Group Study. Blood 94:1086–1099
22. Litman T, Brangi M, Hudson E, Fetsch P, Abati A, Ross DD, Miyake K, Resau JH, Bates SE (2000) The multidrug-resistant phenotype associated with overexpression of the new ABC half-transporter, MXR (ABCG2). J Cell Sci 113:2011–2021
23. Marie J-P, Zittoun R, Sikic BI (1991) Multidrug resistance (mdr1) gene expression in adult acute leukemias: correlations with treatment outcome and in vitro drug sensitivity. Blood 78:586–592
24. Marsh W, Sicheri D, Center MS (1986) Isolation and characterization of adriamycin-resistant HL60 cells which are not defective in the initial accumulation of the drug. Cancer Res 46:4053–4057
25. Minderman H, Suvannasankha A, O'Loughlin KL, Scheper RJ, Robey RW, Bates SE, Baer MR (2002) Flow cytometric analysis of breast cancer resistance protein expression and function. Cytometry 48:59–65
26. Minderman H, Suvannasankha A, O'Loughlin KL, Allen J, Schinkel A, Scheper RJ, Scheffer GL, Robey R, Bates SE, Baer MR (2002) P-glycoprotein, multidrug resistance-associated protein and breast cancer resistance protein-mediated transport: substrate and modulator specificity. Proc Am Assoc Cancer Res 43:496
27. Nakanishi T, Karp JE, Tan M, Doyle LA, Peters T, Yang W, Wei D, Ross DD (2003) Quantitative analysis of breast cancer resistance protein and cellular resistance to flavopiridol in acute leukemia patients. Clin Cancer Res 9:3320–3328
28. Nicoletti MI, Colombo T, Rossi C, Monardo C, Stura S, Zucchetti M, Riva A, Morazzoni P, Donati MB, Bombardelli E, D'Incalci M, Giavazzi R (2000) IDN5109, a taxane with oral bioavailability and potent antitumor activity. Cancer Res 60:842–846
29. Ojima I, Slater JC, Michaud E, Kuduk SD, Bounaud PY, Vrignaud P, Bissery MC, Veith JM, Pera P, Bernacki RJ (1996) Syntheses and structure-activity relationships of the second-generation antitumor taxoids: exceptional activity against drug-resistant cancer cells. J Med Chem 39:3889–3896
30. Ojima I, Kuduk SD, Pera P, Weith JM, Bernacki RJ (1997) Syntheses and structure-activity relationships of non-aromatic taxoids: effects of alkyl and alkenyl ester groups on cytotoxicity. J Med Chem 40:279–285
31. Ojima I, Slater JC, Kuduk SD, Takeuchi CS, Gimi RH, Sun CM, Park YH, Pera P, Veith JM, Bernacki RJ (1997) Syntheses and structure-activity relationships of taxoids derived from 14β-hydroxy-10-deacetylbaccatin III. J Med Chem 40:267–278
32. Ojima I, Bounaud PY, Takeuchi C, Pera P, Bernacki RJ (1998) New taxanes as highly efficient reversal agents for multidrug resistance in cancer cells. Bioorg Med Chem Lett 8:189–194
33. Rabindran SK, Ross DD, Doyle LA, Yang W, Greenberger LM (2000) Fumitremorgin C reverses multidrug resistance in cells transfected with the breast cancer resistance protein. Cancer Res 60:47–50
34. Ringel I, Horwitz SB (1991) Studies with RP56976 (taxotere): a semisynthetic analogue of taxol. J Natl Cancer Inst 83:288
35. Robey RW, Honjo Y, Van de Laar A, Miyake K, Regis JT, Litman T, Bates SE (2001) A functional assay for detection of the mitoxantrone resistance protein, MXR (ABCG2). Biochem Biophys Acta 1512:171–182
36. Roche Diagnostics (2003) Cell proliferation agent WST-1 (pack insert). http://www.roche-applied-science.com/pack-insert/1644807a.pdf
37. Sargent JM, Williamson CJ, Maliepaard M, Elgie AW, Scheper RJ, Taylor CG (2001) Breast cancer resistance protein expression and resistance to daunorubicin in blast cells from patients with acute myeloid leukemia. Br J Haematol 115:257–262
38. Schiff PB, Horwitz SB (1980) Taxol stabilizes microtubules in mouse fibroblasts. Proc Natl Acad Sci U S A 77:1561–1565
39. Shirakawa K, Takara K, Tanigawara Y, Aoyama N, Kasuga M, Komada F, Sakaeda F, Okumura K (1999) Interaction of docetaxel (taxotere) with human P-glycoprotein. Jpn J Cancer Res 90:1380–1386
40. Skehan P, Storeng R, Scudiero D, Monk S, McMahon A, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990) New colorimetric cytotoxicity assay for anticancer drug screening. J Natl Cancer Inst 82:1107–1112
41. Suvannasankha A, Minderman H, O'Loughlin KL, Nakanishi T, Greco WR, Ross DD, Baer MR (2002) Breast cancer resistance protein (BCRP/MXR/ABCG2) in acute myeloid leukemia: discordance between expression and function. Blood 100:67a
42. Takenouchi T, Munekata E (1995) Trophic effects of substance P and beta-amyloid peptide on dibutyryl cyclic AMP-differentiated human leukemic (HL-60) cells. Life Sci 56:PL479–484
43. Van den Heuvel-Eibrink MM, Wiemer EAC, Prins A, Meijerink JP, Vossebeld PJM, Van der Holt B, Pieters R, Sonneveld P (2002) Increased expression of the breast cancer resistance protein (BCRP) in relapsed or refractory acute myeloid leukemia (AML). Leukemia 16:833–839
44. Van der Kolk DM, Vellenga E, Scheffer GL, Muller M, Bates SE, Scheper RJ, de Vries EGE (2002) Expression and activity of breast cancer resistance protein (BCRP) in de novo and relapsed acute myeloid leukemia. Blood 99:3763–3770
45. Vredenburg MR, Ojima I, Veith J, Pera P, Kee K, Cabra F, Sharma A, Kanter P, Greco WR, Bernacki RJ (2001) Effects of orally active taxanes on P-glycoprotein modulation and colon and breast carcinoma drug resistance. J Natl Cancer Inst 93:1234–1245
46. Young IT (1977) Proof without prejudice: use of the Kolmogorov-Smirnov test for the analysis of histograms from flow systems and other sources. J Histochem Cytochem 25:935–941