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Original Paper

The Dithiane Ro 44-5912 Enhances Vinblastine Sensitivity of Drug Resistant and Parental KB Lines *In Vivo*

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The multidrug resistance modifying activity of a dithiane analogue of tiapamil, Ro 44-5912, was examined *in vivo*. Results of acute toxicity studies in mice indicated that lethal toxicity occurred with doses greater than 1 mmol/kg of body weight. In a preliminary pharmacokinetic investigation, Ro 44-5912 appeared to have a longer half-life in mice than did its (R) enantiomer Ro 44-5911 (3.15 ± 0.02 h versus 2.15 ± 0.14 h) as measured by total radiolabel in plasma. In non-tumour bearing mice, Ro 44-5912 enhanced the toxicity of vinblastine in a manner that was dependent on the dose of both drugs. Vinblastine did not have a significant effect on tumour growth when given to nude mice bearing the parental cell line KB-3-1 at a dose of 1.5 mg/kg once per week for 3 weeks. Combination treatment with Ro 44-5912 markedly enhanced the antitumour activity of vinblastine. Similar results were seen when KB-3-1 tumours were treated with the combination of vinblastine plus cyclosporin A. Another tiapamil analogue, Ro 11-2933, had no enhancing activity with this tumour when used at an equitoxic combination dose. Ro 44-5912 also significantly enhanced vinblastine activity with P-glycoprotein-expressing KB-8-5 tumours. In three independent experiments, Ro 44-5912 enhanced the growth inhibiting activity of vinblastine by a mean of approximately 40%. Neither Ro-11-2933 nor cyclosporin A, at the maximal tolerated doses in combination with vinblastine, led to significant inhibition of KB-8-5 tumour growth compared to treatment with the two vehicles alone. These results show that Ro 44-5912 is an active modulator of drug resistance *in vivo*.

Key words: solid tumour, multidrug resistance, xenograft, resistance modifier
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INTRODUCTION

DESPITE SIGNIFICANT advances in chemotherapy of childhood cancers during recent decades, little progress has been seen with cancers affecting adults. A major reason for this disappointing situation is the refractory nature of cancers, particularly solid tumours that appear with increasing age. Much effort in recent years has been given to the development of strategies that may be able to overcome drug resistance in tumours. Interest has focused on a form of drug resistance called multidrug resistance (MDR), since Tsuruo and associates [1] demonstrated that the calcium channel blocker verapamil could overcome the resistance of multidrug resistant murine leukaemia cells. In particular, cloning of two genes encoding cell surface glycoproteins, responsible for efflux of the affected cytotoxic drugs, P-glycoprotein [2–4] and MDR-associated protein [5], has greatly advanced understanding of the mechanisms by which cells become resistant.

A large number of compounds from various chemical and pharmacological classes have been examined as potential MDR modifiers [6]. The aim has been to identify drugs that would strongly interact with P-glycoprotein on tumour cells and have few other pharmacological effects. We have established *in vitro* assays for MDR modifiers using the resistant human carcinoma cell line KB-8-5 and the sensitive parental line KB-3-1 [7]. Based on assays of over 600 compounds, we have found several dithiane analogues of tiapamil that have very high activity in sensitising

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KB-8-5 cells to vincristine and doxorubicin, at concentrations that had no effect on the proliferation of these cells in the absence of cytotoxic drug [8]. Two of the most active of these compounds are dithiane analogues of tiapamil, the enantiomers Ro 44-5911((R)-N-(3,4-dimethoxyphenethyl)-2-(3,4-dimethoxyphenyl)-beta, N-dimethyl-m-dithiane-2-propanamine (M_r = 506)) and Ro 44-5912 ((S)-N-(3,4-dimenthoxyphenethyl)-2-(3,4-dimethoxyphenyl)-beta, N-dimethyl-m-diathane-2-propanamine (M_r = 506)), which have also been shown sensitise KB-3-1 cells by 3-fold to vincristine and 2-fold to doxorubicin [8].

The vasodilatory activities of Ro 44-5911 and Ro 44-5912 appear to be much less than that of verapamil in tests measuring relaxation of aortic strip contractions *in vitro* [8]. This suggests that these compounds could have significant MDR modifying activity *in vivo*. Most previous studies with resistance modifiers (RM) *in vivo* have been performed using murine leukaemia cells [6]. However, for the model to be more clinically relevant, we used KB-8-5 and KB-3-1 cells grown as subcutaneous solid tumour xenografts in congenitally athymic nude mice. Based on results of preliminary studies on the toxicity and pharmacokinetic properties of these two compounds, Ro 44-5912 was selected for further investigation in the xenograft model. We compared the results to those obtained with two MDR modifiers previously shown to be active in other *in vivo* models, cyclosporin A [9] and another dithiane analogue of tiapamil, Ro 11-2933 (N-(3,4-dimethoxyphenethyl)-N-methyl-2-(2-naphthyl)-M-diathane-2-propylamine (M_r = 482)) [10–12].

MATERIALS AND METHODS

Animals

Male and female BALB/c and BALB/c *nu/nu* mice were purchased from Nippon SLC (Shizuoka, Japan). Mice were used when 5–8 weeks old.

Drugs

The compounds Ro 44-5911 (MW = 506), Ro 44-5912 (MW = 506), Ro 11-2933 (MW = 482) [13] and cyclosporin A (MW = 1206) were dissolved in 0.15 mM NaCl containing 0.5% (w/v) carboxymethylcellulose, 0.4% (w/v) Tween 80, and 0.5% (w/v) benzyl alcohol and were administered by gavage. Vinblastine (Sigma, St Louis, Missouri, U.S.A.) was dissolved in 0.15 mM NaCl and was injected intraperitoneally (i.p.).

Toxicity studies

Toxicity studies were performed using normal, non-tumour bearing mice with RM alone or in combination with vinblastine. Acute toxicity was examined by giving animals single ascending doses of RM, and mortality was monitored initially for several hours and each day thereafter, when body weights were measured. Combination studies with multiple doses of RM were given in conjunction with a single injection of vinblastine on the same day. Mortality and body weights of the mice were monitored for up to 14 days.

Subacute toxicity was examined by administering Ro 44-5911 or Ro 44-5912 at doses of 0.84 mmol/kg/day, or 0.17 mmol/kg/day to the animals for 14 days. In a second experiment, the animals were given the same top dose and a low dose of 0.34 mmol/kg/day for 14 days. Half of the animals were sacrificed at this time and half were allowed to recover for 14 days without drugs before sacrifice. The mice were observed daily during the dosing period to record mortality and clinical signs. Body weight and food consumption were measured twice per week. At the end of the dosing period or recovery period, animals were anaesthetised and blood samples were collected by cardiac puncture before the animals were sacrificed.

Pharmacokinetic analysis

Normal, non-tumour bearing mice were orally dosed with 0.17 mmol/kg of [^{14}C]Ro 44-5911 or [^{14}C]Ro 44-5912 following a 16 h fasting period. The radiolabelled compounds were synthesised by Dr N. Flück (F. Hoffmann-La Roche, Basel, Switzerland). The specific activity of [^{14}C]Ro 44-5911 was 29.4 mCi/mmol and that of [^{14}C]Ro 44-5912 was 30.6 mCi/mmol. Blood samples were collected serially from the tail vein of the mice. The blood specimens were burned in an automatic sample combustion system and the generated $^{14}CO_2$ was absorbed in Oxisorb^{TM}-CO_2 (NEN, Beverly, Massachusetts, U.S.A.). Radioactivity was determined using a liquid scintillation counter. The results were calculated using a one-compartment model using the NONLIN programme.

Xenograft studies

The human multidrug resistant carcinoma cell line KB-8-5 and its parental line KB-3-1 were kindly provided by Dr M.M. Gottesman (NCI, Bethesda, Maryland, U.S.A.). The cells were cultured as previously described [7] and were harvested by trypsin treatment. Each BALB/c nude mouse was injected in two sites, left rear flank and right upper flank, with 0.1 ml of cell suspension containing 5×10^5 viable cells. Tumour take was 100% in nearly all experiments so that each animal had two separate tumours. After 1–2 weeks, palpable tumours were measured and the mice were randomised into treatment groups.

For each treatment, the RM was given by gavage every 3 h for three administrations. Vinblastine at a dose of 1.5 mg/kg was injected 2 h after the first dose of RM. The treatment was repeated once a week for 3 weeks.

Body weights and tumour sizes were measured twice a week. Tumour volumes were estimated by the formula $V = ab^2/2$, where a is the tumour length and b the tumour width. To compare different treatments over the entire course of the experiment, the area under the growth curve (AUC) for each individual tumour was calculated.

Statistical analysis

All statistical calculations were performed using SigmaStat^{TM} software. Normality of distribution was tested using The Kolmogorov–Smirnov test. If the results were not significantly different from a normal distribution, the differences between treatment groups were analysed using one way ANOVA. Pairwise multiple comparisons were made using the Student–Newman–Keuls method. If the results were not normally distributed, Kruskal–Wallis one way ANOVA on ranks was performed and pairwise multiple comparisons were made using Dunn's test. A value of $P < 0.05$ was considered to be significant. Unless otherwise stated, standard errors of the mean are given.

RESULTS

Toxicity and pharmacokinetics studies

Preliminary single dose acute toxicity studies with Ro 44-5911 and Ro 44-5912 indicated that the two enantiomers had similar toxicities, with some deaths occurring at doses of Ro 44-5911 from 0.9 to 1 mmol/kg and from 1 to 1.3 mmol/kg with Ro 44-5912. Those animals died within a few minutes after administration with signs of gasping, suggesting that residual vasodilatory activity of the compounds was responsible, although

central nervous system effects due to disruption of P-glycoprotein function in the blood–brain barrier cannot be ruled out.

In order to develop an appropriate dosing schedule for testing these compounds *in vivo*, it was important to have some idea about their pharmacokinetics in mice. Therefore, [^{14}C]-labelled Ro 44-5911 and Ro 44-5912 were prepared and administered orally to mice. Blood was sampled at various times thereafter and total radioactivity was measured. The reults are presented in Figure 1. The results for Ro 44-5912 fit best to a one-compartment model, whereas the results for Ro 44-5911 could fit either one- or two-compartment models. For purposes of comparison, we analysed both sets of data using one-compartment kinetics. The maximal concentration was reached at the 1 h sampling point with Ro 44-5911 and at 2 h with Ro 44-5912. The half-life of Ro 44-5912 was significantly longer (3.15 ± 0.02 h) than that of Ro 44-5911 (2.15 ± 0.14 h). The AUC was significantly greater as well (82.2 ± 1.5 μg equivalents h/ml versus 65.1 ± 2.8 μg equivalents h/ml).

The compound Ro 44-5912 was selected for further studies because of its superior pharmacokinetic profile and its slightly lower toxicity. Vinblastine was chosen as the cytotoxic drug because it had greater antitumour effects than vincristine, doxorubicin, or actinomycin D against KB-3-1 xenografts in preliminary experiments when administered once a week. Several different schedules were tested for toxicity in combination with vinblastine. The results of one experiment are shown in Figure 2. In this experiment, animals were given a loading dose of Ro 44-5912 at time 0. This was followed by administration of vinblastine 2 h later, when the concentration of Ro 44-5912 should be maximal. One hour after vinblastine, a maintenance dose equal to half of the loading dose was given and this was followed 3 h later by the same dose of RM. As evidenced by the body weight loss shown in Figure 2a, Ro 44-5912 alone had no toxicity up to a cumulative dose of 0.8 mmol/kg. When given alone, 2.5 mg/kg vinblastine was the maximum tolerated dose in tumour bearing mice, with deaths of animals occurring with 3.0 mg/kg. The toxicity of vinblastine was increased with increasing doses of RM (Figures 2b and c), so that the maximum tolerated dose of vinblastine was decreased by 40% to 1.5 mg/kg

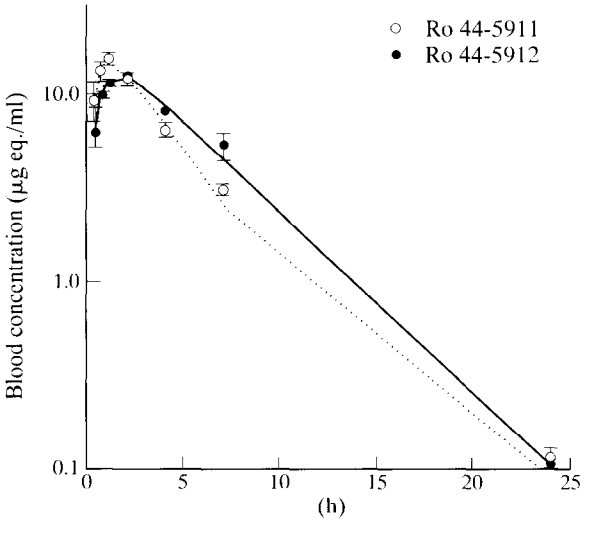

Figure 1. Pharmacokinetics of Ro 44-5911 and Ro 44-5912 in mice. The points represent mean levels of total blood [^{14}C] radioactivity measured at various times after administration of 0.17 mmol/kg of each RM. Vertical bars represent ± 1 S.D.

in the combinations. Similar dose related increases in vinblastine toxicity were found using three equal doses of Ro 44-5912 administered at the same times (data not shown). Higher cumulative doses of RM with this schedule, up to 1.2 mmol/kg (0.6 + 0.3 + 0.3 or 0.4 × 3), also had no evident toxicity when given alone. Repetition of these treatment schedules at weekly intervals for three administrations was not toxic if the dose of vinblastine was less than 2.0 mg/kg. The major sign of toxicity in the combination treated animals was weight loss, and there were no visible differences between these animals and those given equitoxic doses of vinblastine alone.

The single agent toxicities of Ro 44-5911 and Ro 44-5912 were also examined in 2 week repeated administration studies in mice. No toxic effects were seen with the lower doses below 0.84 mmol/kg/day. At the high dose of 0.84 mmol/kg/day, however, some clinical signs of gasping were observed with both compounds. In histopathological examinations, slight fatty changes in the liver were observed only in the high dose group. These appeared to be fatty change of centrilobular hepatocytes and were reversed during a 2 week recovery period.

Efficacy studies with Ro 44-5912

Given alone at a dose of 1.5 mg/kg once per week for 3 weeks, vinblastine had a small, non-significant inhibitory effect on the growth of KB-3-1 xenograft tumours (Figure 3, Tables 1 and 2). In separate experiments examining the antitumour activities of various doses of vinblastine, the maximum tolerated dose of vinblastine was slightly, but not significantly more active than the dose of 1.5 mg/kg with this tumour. Ro 44-5912 clearly enhanced the antitumour activity of vinblastine against this cell line in a dose-dependent manner (Figures 3 and 4, Table 1). Maximal enhancing activity was obtained with cumulative doses of approximately 0.9 mmol per treatment. Higher doses had little additional effect on tumour reduction (Table 1, experiment 2). With optimal doses of RM, inhibition of tumour growth was significantly greater than treatment with vinblastine alone in this tumour. The pooled results for the groups treated with cumulative doses of 0.75, 0.8 or 0.9 mmol/kg/week (Table 1) were analysed, and the mean inhibition of tumour growth of the combination treated groups, relative to controls, was 86 ± 7%. Compared to the vinblastine alone treated groups, the reduction was 79 ± 10%. The differences in the extent of this enhancement seen between the two experiments using similar doses of Ro 44-5912 (Table 1, experiments 1 and 3), may be related to the fact that the tumours were larger (140 mm^3 versus 30 mm^3) at the start of treatment in the experiment where the effect was less marked (experiment 1).

Ro 44-5912 also enhanced the activity of vinblastine against KB-8-5 tumours, although the effect was not as marked as that observed with KB-3-1 xenografts (Figures 3 and 4). Combination treatment resulted in a mean inhibition of tumour growth from three experiments (Table 1) equal to 57 ± 11% compared to the control group given only vehicles. When the results of combination treatment were compared to those of the group treated with vinblastine alone, the combination still resulted in a statistically significant reduction in growth of 38 ± 16%.

Efficacy studies with other resistance modifiers

For the purposes of comparison, two other RMs demonstrated to be active *in vivo* were tested with KB-3-1 and KB-8-5 xenografts. One of these was Ro 11-2933, a dithiane with structural similarity to Ro 44-5912 [13] and the other, representing a completely different structural class, was cyclosporin

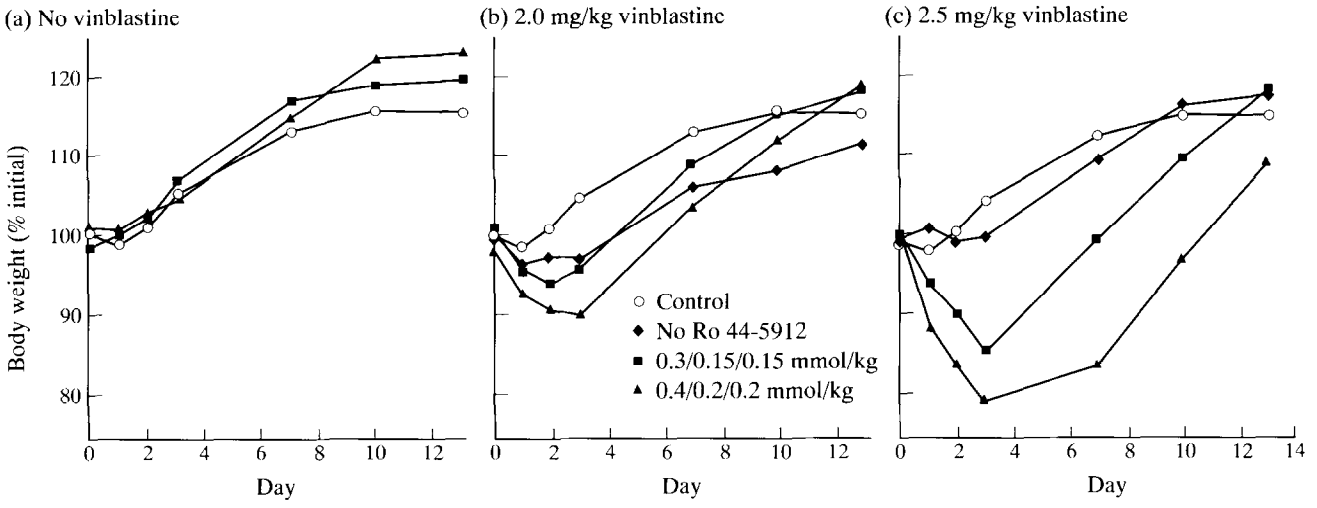

Figure 2. Body weights of mice given Ro 44-5912 alone (a) and in combination with 2.0 mg/kg (b) or 2.5 mg/kg (c) of vinblastine. Normal BALB/c mice were given drugs on day 0 and body weights were monitored for the following 2 weeks. Open circles represent results from control animals treated with the two vehicles alone and are reproduced in each panel for comparison.

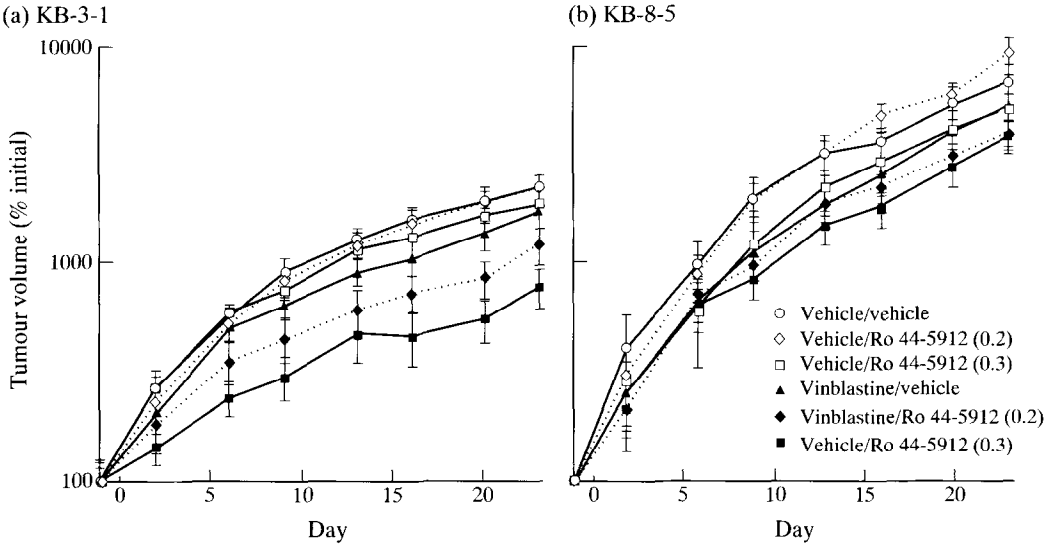

Figure 3. The effect of different doses of Ro 44-5912 on antitumour efficacy of vinblastine in xenografts of KB-3-1 (a) and KB-8-5 (b). The initial volume of the KB-3-1 tumours was 141 ± 11 mm³ and that of the KB-8-5 tumours was 30 ± 4 mm³.

A. The dose of each compound used in this experiment was considered to be the maximal tolerated dose when given in combination with 1.5 mg/kg of vinblastine, that is, it was half of the dose that resulted in the death of animals given combination treatment with vinblastine plus RM, but not of animals given the RM alone.

As can be seen from the growth curves depicted in Figure 5 and the summary in Table 2, Ro 11-2933 did not enhance the activity of vinblastine with either cell line. Cyclosporin A, significantly enhanced the antitumour effect of vinblastine against the KB-3-1 line. The level of enhancement was comparable to that seen with Ro 44-5912, resulting in 87% inhibition of growth relative to the control group and 77% inhibition compared to the vinblastine alone treatment group. However, cyclosporin A did not increase the sensitivity of the KB-8-5 line to vinblastine with this treatment protocol (Figure 6 and Table 2).

DISCUSSION

We developed an *in vivo* model with human carcinoma cells to examine the activity of MDR modifiers in solid tumours. The KB-8-5 and KB-3-1 lines that we use for our *in vitro* assays [7, 8] were grown as xenografts in nude mice. There have been only a few reports of xenograft models for testing low molecular weight MDR modifiers [12, 14, 15]. We attempted to design a treatment protocol that might be clinically relevant, using bolus treatment with cytotoxic drug once per week for 3 weeks. Vinblastine was selected as the cytotoxic drug because it binds to P-glycoprotein [16], and because it had the best growth inhibiting activity against KB-3-1 cells in our preliminary experiments, although these tumours were quite resistant to all the drugs we tested *in vivo*.

A relatively short duration of treatment with the MDR modifiers was used in order to minimise possible toxicity to

Table 1. Enhancement of vinblastine antitumour activity at day 21 of treatment by Ro 44-5912 in xenografts of KB-3-1 and KB-8-5 (three separate experiments)

Ro 44-5912 (mmol/kg)	Vinblastine (1.5 mg/kg)	KB-3-1		KB-8-5	
		Mean ± S.E.M.	Median	Mean ± S.E.M.	Median
Experiment 1					
None	−	100 ± 13	99	100 ± 21	125
0.2 × 3	−	97 ± 15	91	115 ± 16	114
0.3 × 3	−	87 ± 6	95	73 ± 29	39
None	+	70 ± 12	73	68 ± 11	74
0.2 × 3	+	50 ± 10	38*†	56 ± 6	56
0.3 × 3	+	25 ± 8	20*†‡	36 ± 6	32*‡
Experiment 2					
None	−	100 ± 10	98	100 ± 16	94
0.4/0.2/0.2	−	105 ± 19	96	130 ± 38	84
0.6/0.3/0.3	−	140 ± 60	149	92 ± 15	88
None	+	67 ± 28	34	74 ± 12	78
0.4/0.2/0.2	+	28 ± 11	22	60 ± 11	50
0.6/0.3/0.3	+	17 ± 12	−0.6*	64 ± 11	72
Experiment 3					
None	−	100 ± 27	79	100 ± 32	56
0.25 × 3	−	95 ± 15	67	80 ± 16	68
None	+	68 ± 16	58	66 ± 10	67
0.25 × 3	+	1.5 ± 0.4	0.8*†‡	28 ± 7	25*†

*Significantly different from vehicle only controls; †Significantly different from RM only group; ‡Significantly different from vinblastine only group.

Table 2. MDR modifying activity of Ro 11-2933 and cyclosporin A in xenografts of KB-3-1 and KB-8-5 at day 21 of treatment

RM (mmol/kg)	Vinblastine (1.5 mg/kg)	KB-3-1		KB-8-5	
		Mean ± S.E.M.	Median	Mean ± S.E.M.	Median
Ro 11-2933					
None	−	100 ± 26	77	100 ± 25	69
0.1 × 3	−	118 ± 36	74	101 ± 26	60
None	+	71 ± 16	53	72 ± 26	40
0.1 × 3	+	67 ± 16	55	71 ± 14	55
Cyclosporin A					
None	−	100 ± 17	90	100 ± 21	80
0.005 × 3	−	120 ± 37	77	128 ± 40	86
None	+	56 ± 13	45	69 ± 10	71
0.005 × 3	+	13 ± 3	10*,†	62 ± 20	40

RM, resistance modifiers.
*Significantly different from vehicle only controls; †Significantly different from RM only group; ‡Significantly different from vinblastine only group.

normal organs. This was based on the fact that P-glycoprotein is expressed on normal cells, particularly bile canaliculi, renal tubules [17, 18] and haemopoietic stem cells [19]. In our 2 week toxicity studies, a slight effect of these compounds on liver morphology was evident on histopathological examination, which might be related to disruption of normal liver function. Several clinical studies using continuous infusion of cyclosporin A [20–24] and one study where quinine was given by continuous infusion [25] have noted reversible hyperbilirubinaemia, suggesting that MDR modifiers do affect normal liver function in patients.

The final treatment schedules and selection of Ro 44-5912 as the best compound for *in vivo* efficacy studies were based upon the results of preliminary pharmacokinetic studies in mice measuring total radioactivity in plasma. Because nothing is known about the metabolism or possible activity of metabolites, we consider this only to be an approximation for the true pharmacokinetics. Using our final treatment schedule, Ro 44-5912 significantly increased the toxicity of vinblastine at doses that had no evident toxicity when Ro 44-5912 was given alone. The major toxicity with high doses of Ro 44-5912 in the absence of cytotoxic drug appeared to be cardiovascular, reflecting the

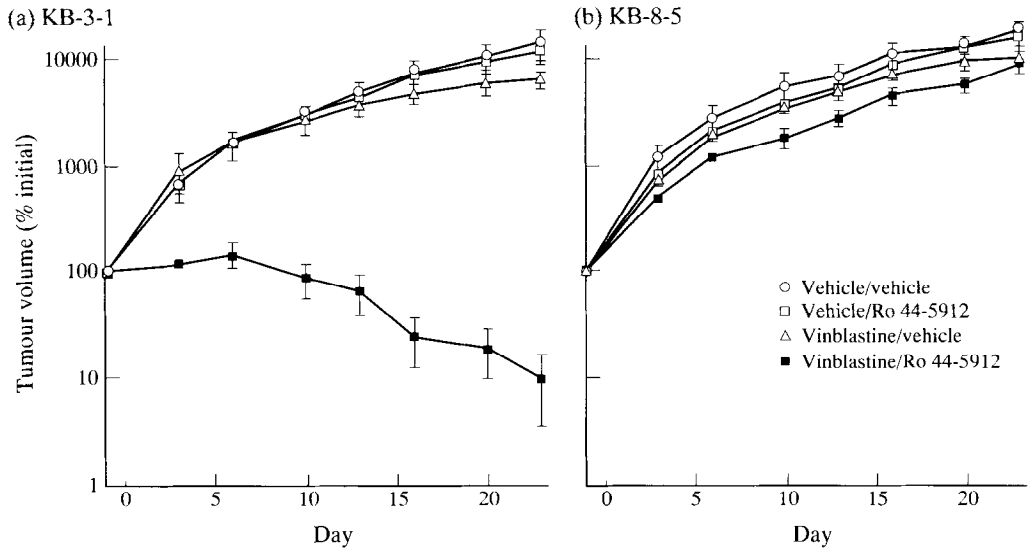

Figure 4. Resistance modifying activity of Ro 44-5912 (0.25 mmol/kg ×3) in combination with vinblastine against xenografts of KB-3-1 (a) and KB-8-5 (b). The initial volume of the KB-3-1 tumours was 31 ± 3mm³ and that of the KB-8-5 tumours was 42 ± 5 mm³.

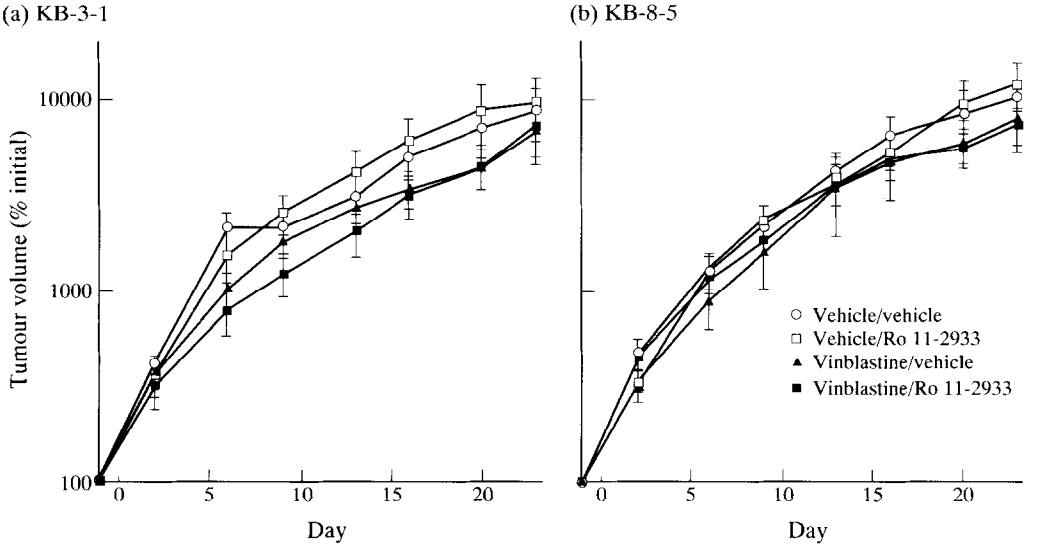

Figure 5. Resistance modifying activity of Ro 11-2933 (0.1 mmol/kg ×3) in combination with vinblastine against xenografts of KB-3-1 (a) and KB-8-5 (b). The initial volume of the KB-3-1 tumours was 48 ± 6 mm³ and that of the KB-8-5 tumours was 44 ± 5 mm³.

fact that it has approximately 5% of the activity of verapamil with aortic muscle *in vitro* [8]. We have also observed the same phenomenon of increased toxicity of cytotoxic drugs when combined with other classes of MDR modifiers, as have other groups [26]. These results clearly indicate that interfering with the function of P-glycoprotein on normal tissues increases the toxicity of MDR-related cytotoxic drugs. Similar conclusions have been drawn from phase I clinical trials with MDR modulators [20, 23, 24].

In three experiments with the P-glycoprotein-expressing KB-8-5 cells, Ro 44-5912 enhanced the activity of vinblastine by approximately 40%. This was more than was seen with other modifiers that we have tested. However, the overall degree of growth inhibition was relatively small, only approximately 60%. One explanation for the low level of enhancement by Ro 44-5912 is that, although the *MDR1* gene is not amplified in KB-8-5 cells, it is highly overexpressed [27] and the degree of overexpression at the mRNA level is greater than that measured in all but 10–15% of human tumour samples [28–34]. Therefore, even though the compound may inhibit P-glycoprotein function *in vivo*, it still may be insufficient to overcome the high degree of resistance of this line relative to the increased toxicity to normal tissues. Another point is that the parental KB-3-1 line is quite resistant *in vivo* itself, suggesting that the KB cell line is intrinsically resistant due to factors other than P-glycoprotein expression. Overall, these results indicate that KB-8-5 cells may have a much higher resistance than do resistant tumours in patients, and thus may not be a truly representative model for the clinical situation.

Very marked growth inhibition was evident in mice bearing KB-3-1 xenografts treated with the combination of vinblastine and Ro 44-5912 as well as with the combination with cyclosporin

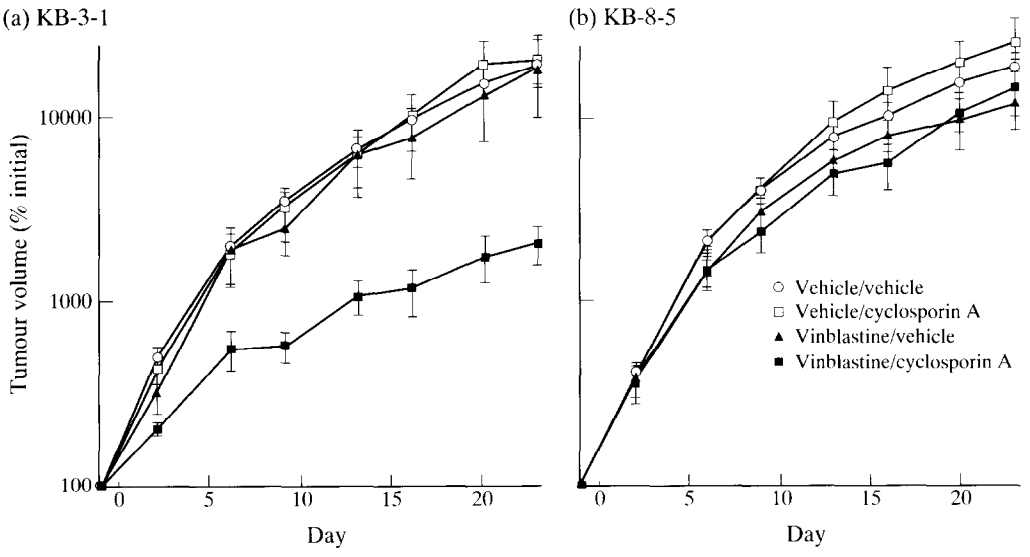

Figure 6. Resistance modifying activity of cyclosporin A (0.005 mmol/kg ×3) in combination with vinblastine against xenografts of KB-3-1 (a) and KB-8-5 (b). The initial volume of the KB-3-1 tumours was 25 ± 3 mm³ and that of the KB-8-5 tumours was 18 ± 1 mm³.

A. Similar enhancement of doxorubicin activity against KB-3-1 has been reported with a dihydropyridine analogue [15]. We have examined KB-3-1 tumours using a sensitive PCR method for detecting mRNA for P-glycoprotein, and have not been able to detect any mRNA. Two potential mechanisms can be envisaged by which these RMs might enhance antitumour activity of these drugs with non-P-glycoprotein expressing cells. The first is that the RMs may affect other resistance pathways. We have shown that Ro 44-5912 enhances vincristine and doxorubicin activity with KB-3-1 cells *in vitro* [8]. A related dithiane analogue, Ro 11-2933, can partially overcome resistance in small cell lung cancer cells that express MDR-associated protein [35]. In addition, Ro 11-2933 inhibits repair of doxorubicin-induced DNA damage [36–38]. The second possible mechanism is suggested by the observation that these RMs enhance the *in vivo* toxicity of anticancer drugs in non-tumour bearing animals, and that the distribution of P-glycoprotein in normal tissues is compatible with it playing a role in clearance of xenobiotics from circulation [17, 18]. Thus, by interacting with P-glycoprotein on normal liver and kidney cells, the RMs would inhibit elimination of the cytotoxic drugs, thereby changing their pharmacokinetics and resulting in increased AUCs. In several clinical trials with cyclosporin A combinations, it has been shown to significantly increase the AUCs for etoposide [39], doxorubicin [23, 24] and epirubicin [40]. Furthermore, changing the pharmacokinetic profile of cytotoxic drugs by changing from bolus to continuous administration can increase their efficacy against clinially resistant cancer cells [41].

We found no enhancing activity of Ro 11-2933 with either the KB-3-1 or KB-8-5 lines at doses that were equitoxic with those of Ro 44-5912 when used in combination with vinblastine. This lack of activity of Ro 11-2933 strongly suggests that the enhanced antitumour effects seen with Ro 44-5912 are specific and not simply due to changes in pharmacokinetics of vinblastine. Others have reported significant resistance modulating effects of Ro 11-2933 *in vivo* against doxorubicin resistant murine leukaemia cells grown as ascites [42], transplantable rat mammary tumours [11] and human ovarian xenografts [12]. It may be that the KB cell lines are more resistant than the lines used in those studies. However, several methodological differences between the protocols may also help explain this discrepancy. The mode of administration may influence the activity of these compounds. We administered the modulators by gavage, whereas other groups have employed intraperitoneal [12, 42] or intravenous [11] administrations. Another aspect is that different cytotoxic drugs were used. The other studies all examined anthracyclines, whereas we used a vinca alkaloid. The administration schedule was also different, a single bolus administration versus once a week adminstration for 3 weeks. As shown by Niwa and associates [15], a single dose of 8 mg/kg of doxorubicin has a considerable inhibitory effect on the growth of KB-3-1 tumours.

Although the KB xenograft system may not completely represent the clinical situation, we have shown that Ro 44-5912, a compound with high selective modification activity in our *in vitro* assay, is also highly active *in vivo* at concentrations that are not overtly toxic. It is much more active against KB-3-1 tumours than the similar compound Ro 11-2933, and it appeared to have a better therapeutic effect against P-glycoprotein-expressing KB-8-5 tumours than did cyclosporin A. Its activity against tumours resistant to chemotherapy due to mechanisms other than P-glycoprotein expression [8], suggests that it could be effective on a wider range of tumours than modulators having only a single mode of action.

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