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Phase IB and pharmacological study of the novel taxane BMS-184476 in combination with doxorubicin☆

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Abstract

The aim of this study was to define the maximum tolerated dose (MTD) and the pharmacological profile of the paclitaxel analogue BMS-184476 given once every 3 weeks, or on days 1 and 8 every 3 weeks (d1&8), in combination with a fixed dose of 50 mg/m^2 of Doxorubicin (Doxo) administered on day 1 of a 21-day cycle. Adult patients with advanced solid malignancies received escalating doses of BMS-184476 infused over 1 h after bolus Doxo. Pharmacokinetics (PK) of BMS-184476, Doxo and metabolites were investigated. The effect of BMS-184476 on doxorubicinol formation was studied in the cytosol from human myocardium. The MTD of 3-weekly BMS-184476 was 30 mg/m^2. The MTD/recommended Phase II dose was 35 mg/m^2/week (70 mg/m^2 per cycle) in the d1&8 schedule. The dose-limiting toxicity was neutropenia for both schedules. Other toxicities were loss of appetite, asthenia, and mild, cumulative peripheral neuropathy. The objective response rate in 17 previously untreated or minimaletreated patients with breast cancer treated at 35 mg/m^2/week of BMS-184476 was 59% (95% Confidence Interval (CI): 33–82%). Two of the 7 patients not responding to the study regimen later responded to Doxo and paclitaxel. Plasma disposition of BMS-184476 at 30, 35 and 40 mg/m^2 was linear without evidence of a PK interaction with Doxo. In studies with cytosol from human myocardium, the formation of cardiotoxic doxorubicinol was not enhanced by BMS-184476. Dosing of BMS-184476 for 2 consecutive weeks allowed the administration of larger doses of the taxane with a promising antitumour activity in patients with untreated or minimally pretreated breast cancer. The higher than expected myelotoxicity of the 3-weekly schedule is unexplained by the investigated interactions. Lack of enhanced doxorubicinol formation in human myocardium is consistent with the cardiac safety of the regimen.

Keywords: Phase 1; Taxanes; Pharmacokinetic; Anthracycline; BMS-184476; Doxorubicin

1. Introduction

The clinical success of paclitaxel and docetaxel has justified the search for new analogues with a better therapeutic index. 7-methylthiomethyl-paclitaxel (BMS-184476) is an analogue of paclitaxel that experimentally has a greater anticancer activity and lower neurotoxic potential than the parent compound, and is formulated in 80% less Cremophor® EL [1].

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Phase I studies with BMS-184476 were recently completed with weekly and 3-weekly administrations [2,3]. For the latter schedule, neutropenia, diarrhoea and stomatitis were the dose-limiting toxicities (DLT) ; a dose of 60 mg/m^2 given every 3 weeks was recommended for Phase II studies [2]. In the other schedule, BMS-184476 was initially given for 3 consecutive weeks every 4 weeks, but eventually recommended on a day 1 and 8 schedule at a dose of 50 mg/m^2 per week, every 3–4 weeks [3]. BMS-184476 followed linear pharmacokinetics (PK) showing an important antitumour activity in different types of cancer, especially with the weekly schedule [2,3].

In the past, we showed that the combination of doxorubicin (Doxo) and paclitaxel (AT), both given at full therapeutic doses, is very active in women with untreated metastatic breast cancer [4]. However, a higher than expected incidence of anthracycline-like cardiotoxicity required limiting the total Doxo dose to 360–380 mg/m^2 [4,5]. Two mechanisms contribute to the enhanced cardiotoxicity: a PK interaction between the anthracycline and the taxane due to paclitaxel formulation in Cremophor© EL [6], and a biochemical reaction common to paclitaxel and docetaxel causing increased transformation of Doxo to a cardiotoxic species in human myocardium [7].

We considered that the different formulation and the improved antitumour activity in preclinical models of BMS-184476 could lead to a combination with Doxo that has a better therapeutic window than that of AT. The aim of the present study was to define the Maximum Tolerated Dose (MTD) of BMS-184476 with a fixed therapeutic dose of Doxo and also to explore the antitumour activity in patients with minimal prior chemotherapy, no prior exposure to anthracyclines and enrolling preferentially patients with a diagnosis of advanced breast cancer. The study also investigated the PK of BMS-184476 and Doxo, and analysed the effects of the taxane analogue on the metabolism of the Doxo.

2. Patients and methods

2.1. Patients

Patients with a histological or cytological diagnosis of a non-haematological malignancy, potentially sensitive to treatment with an anthracycline/taxane combination were eligible. Inclusion criteria were: age $\geqslant$18 years; Eastern Cooperative Oncology Group (ECOG) performance status $\leqslant$1; normal bone marrow, renal and liver function; left ventricular ejection fraction (LVEF) 55% by echocardiography; a maximum of one prior chemotherapy regimen and no prior anthracyclines. Patients refractory to paclitaxel/docetaxel (defined as progression on therapy or within 6 months from last dose) were excluded. The study was conducted within the framework of the Southern European New Drugs Organization (SENDO) according to Good Clinical Practice rules. All patients had to give their written informed consent.

2.2. Dosage and drug administration

Commercially available Doxo (50 mg/m^2) was administered intravenously (i.v.) over 5-min. BMS-184476 was supplied by Bristol-Myers Squibb (Wallingford, CT), reconstituted and diluted as described in Refs. [2,3], and infused over one hour starting 15 min after the end of Doxo. The combination was given on day 1 of a 3-week cycle and repeated thereafter. The 3-weekly schedule was subsequently amended to administer BMS-184476 on days 1 and 8, while maintaining the Doxo administration on day 1. Routine antiemetic prophylaxis consisted of $5HT_3$ antagonists only. Premedication with H_1- and H_2-blockers given 30 min before BMS-184476 was implemented early during the study.

Baseline evaluations included: physical examination, complete blood cell count (CBC), serum biochemistry, electrocardiogram (ECG), echocardiography with LVEF evaluation, chest X-ray and tumour assessment. Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (version 2.1). Haematological toxicity was assessed based on at least one CBC performed between day 13 and 17 and between day 18 and 22. During treatment, LVEF and tumour assessment were performed every second cycle. DLT was defined as grade 4 neutropenia lasting $>$5 days; febrile neutropenia; platelets $<25\times10^9$ cells/l or bleeding requiring transfusions; uncontrolled nausea and vomiting; grade $>$2 diarrhoea, stomatitis, peripheral neuropathy and hepatic toxicity. Failure to meet criteria for retreatment on day 8 of the first cycle, or for 2 weeks after day 22 was also considered a DLT. The maximum administered dose (MAD) was defined by the first-course (DLT) occuring in more than 2 of 3 to 6 patients. The maximum tolerated dose (MTD) was defined as the dose level at which first-course DLT was seen in less than 2 of 3 to 6 patients. No intrapatient dose escalation was allowed. Patients with a DLT could be retreated at the immediately lower dose. Response status was assessed according to the World Health Organisation (WHO) criteria [8]. Treatment was administered until a cumulative Doxo dose of 360 mg/m^2 was reached. Thereafter, BMS-184476 was given as a single agent.

2.3. Pharmacokinetic studies

Plasma PK of BMS-184476, Doxo and metabolites (BMS-246178, BMS-246180, paclitaxel, doxorubicinol) was assessed in 15 patients. Samples were obtained

before treatment, at the end of the i.v. bolus of Doxo, at 15 (immediately prior to BMS-184476 infusion), 30 and 75 min (immediately after the end of BMS-184476 infusion), and at 13 additional time points until 72 h. When BMS-184476 was given on day 8, samples were collected after the start of the infusion (15 min), and at times matching the same intervals as reported above. Blood was placed in K_3 ethylenediamine tetraacetic acid (K_3EDTA)-containing tubes in chipped ice and protected from light. After centrifugation at 1400×g (10 min at 4 °C) plasma was collected and stored into screw-capped polypropylene tubes at −20 °C.

Taxanes for standard curves were provided by Bristol Myers Squibb; Doxo and doxorubicinol were obtained from Pharmacia & Upjohn (Milano, Italy).

The high-performance liquid chromatography (HPLC) method for measuring BMS-184476 and metabolites has already been described in full in Ref. [3], and was applied in our laboratory after validation according to criteria indicated by the Laboratory of Metabolism and Pharmacokinetics of Bristol Myers Squibb (New Brunswick, NJ), including the preparation in duplicate of a nine-point calibration curve (range: 10 to 5000 nM for BMS-184476 and paclitaxel, 5 to 2500 nM for BMS-246178 and BMS-246180) and three quality control samples (QC) representative of high, medium and low concentrations of BMS-184476 and metabolites for each run. The chromatographic data were collected using the HPChem Software (Agilent Technologies, Palo Alto, CA). Drug peak height to internal standard peak height ratio were calculated and fitted to a linear regression equation for BMS-184476 and metabolites, weighting the values by $1/Y^2$ (Prism Software, GraphPad, San Diego, CA). Doxo and doxorubicinol were measured in plasma with HPLC-fluorescence after on-line extraction as described in Ref. [6].

Maximum plasma concentration (C_{max}) was directly determined from the analytical data; Area Under the Concentration×time curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated by adding the AUC from time zero to the last sampling time (log-trapezoidal rule) to the area of the extrapolated region. Terminal half-life was derived from the formula $T_{1/2} = 0.693/Ke$, where Ke is the slope of the linear equation best fitted to the last 3–4 concentration-time points in a semilog plot. Total Body Clearance (CL_{TB}) was calculated as dose/$AUC_{0-\infty}$.

Within patients comparisons were done with Student's *t*-test for paired data.

2.4. Doxorubicin metabolism in human myocardium

Specimens of normothermic beating myocardium (approximately 0.1 g) from the lateral aspect of right atrium that were routinely disposed of by the surgeons during procedures for aorto-coronary by-pass grafting were obtained and stored at −80 °C until they were used. Cytosol was prepared as described in Ref. [9] from pools of 10–15 anonymous specimens. Drug metabolism was reconstituted in 0.25–0.5 ml incubations containing cytosol (0.3 mg protein/ml), nicotinamide-adenine dinucleotide phosphate reduced (NADPH) (0.25 mM) and ethanol-dissolved 1–10 μM BMS-184476, paclitaxel, docetaxel (Taxotere, Rhone-Poulenc-Rorer S.p.A., Lainate, Milano, Italy) or vinorelbine (Navelbine 50, Pierre-Fabre Pharma, Milano, Italy), as described in Ref. [7]. Aliquots of drug-free ethanol were included as appropriate to permit direct comparisons between all incubations [7]. Because the vinorelbine formulation contained ditartrate, aliquots of vinorelbine-free ditartrate were added as appropriate to adjust the concentration to 25 μM in all incubations. Doxo was added to a final concentration of 25 μM. After 4 h at 37 °C, the reaction mixtures were extracted with a 4-fold excess of (1:1) $CHCl_3/CH_3OH$ and analysed for doxorubicinol by a previously validated two-dimensional thin layer chromatography (TLC) method and fluorescence spectroscopy [9].

3. Results

3.1. Patients, schedules and dose escalation

The main patient characteristics are summarised in Table 1. BMS-184476 was given 3-weekly to 10 and weekly to 22 patients, respectively. All patients had a good performance status, had received minimal prior chemotherapy and no anthracyclines. In particular, 23 patients had advanced breast cancer; 10 of them had received prior adjuvant therapy, one had received therapy for metastatic disease, and 12 were not previously treated. Untreated patients were preferentially enrolled after definition of the MTD, when Doxo was combined

Table 1
Patients' characteristics

	Schedule of BMS-184476		
	3-weekly	d1&8	Total
Age (median in years, range)	61 (57–72)	54 (46–69)	58 (46–72)
Median ECOG PS (range)	0 (0–1)	0 (0–1)	0 (0–1)
Prior chemotherapy			
⩾one regimen	8	6	14
None	2	16	18
Tumour type			
Miscellaneaous	2	2	4
NSCLC	2	3	5
Breast cancer	6	17	23
Total	10	22	32

d1&8, on day 1 and 8 every 3 weeks; NSCLC, non-small cell lung cancer. ECOG PS, Eastern Cooperative Oncology Group Performance Status.

Table 2
First-cycle DLT observed in the cohorts of patients enrolled into the dose-escalation phase of the study

Schedule	3-weekly		d1&8	
BMS-184476 dose (mg/m^2)	40	30	30 + 30	35 + 35
Patients (*n*)	6	4	4	6
Neutropenia				
Grade 4	4	0	1	4
Grade 4 for > 5 days[a]	3	0	0	2
Febrile neutropenia[a]	0	0	0	1
Nadir, median in days (range)	13 (11–19)	15 (5–18)	14 (11–14)	14 (12–15)
Recovery, median day (range)	21 (16–22)	20 (18–22)	18 (17–18)	22 (14–26)

d1&8, BMS-184476 given on day 1 and day 8 every 3 weeks, Doxo given at a fixed dose of 50 mg/m^2 on day 1 only; 3-weekly, both drugs given on day 1 every 3 weeks, Doxo dose: 50 mg/m^2.
[a] DLT.

with the highest safe dose of BMS-184476. Untreated patients without an objective response after two cycles of treatment were removed from the study, and treated according to standard treatment options.

The only DLT observed was neutropenia (Table 2), which occurred in 3 of 6 patients treated at the starting dose level (40 mg/m^2 BMS-184476 and 50 mg/m^2 Doxo) of the 3-weekly schedule. This was defined as the MAD. BMS-184476 was later given at a reduced dose of 30 mg/m^2 to 4 additional patients without observing any further DLT. The combination of 50 mg/m^2 of Doxo and of 30 mg/m^2 of BMS-184476, both given every 3 weeks, was then defined as the MTD according to protocol. In all of the above patients neutropenia had resolved by day 21 (Table 2).

In order to increase the total dosage of BMS-184476 given in a cycle, the study was then amended to deliver the taxane analogue in two separate administrations on days 1 and 8 every 3 weeks. At the first dose level of the new schedule (30 mg/m^2 of BMS-184476) no DLT was observed. Six patients were then treated at 35 mg/m^2 of BMS-184476 and 3 DLTs were observed (1 patient with febrile neutropenia and 2 patients with grade 4 neutropenia lasting > 5 days). This dose level, by definition the MAD, was also defined as the MTD for the d1&8 schedule, and 12 additional patients with previously untreated breast cancer were then enrolled at this dose level.

3.2. Toxicity

Overall, a total of 160 cycles was administered on the two schedules (Table 3). The median number of cycles per patient in the d1&8 schedule was 4 (range: 1–10). The median cumulative dose of Doxo was 112 mg/m^2 (87–300 mg/m^2) in the 3-weekly and 300 mg/m^2 (50–350 mg/m^2) in the d1&d8 schedule, respectively. Five patients with breast cancer received a maximum cumulative dose of 350 mg/m^2 of Doxo.

Haematological toxicity was assessed over the 160 cycles (31 cycles of the 3-weekly and 129 cycles of the d1&8 schedule) (Table 3), and was mostly characterised by neutropenia. Grade 3 thrombocytopenia only occurred in one patient treated with 40 mg/m^2 BMS-184476. Long-lasting neutropenia and febrile neutropenia were also the most frequent and severe toxicities of the d1&8 schedule (Table 3). In the 18 patients with minimal or no prior chemotherapy receiving 35 mg/m^2 of BMS-184476 twice per cycle, neutropenia grade 4 occurred in 33% of the cycles, and lasted more than 5 days in 13% of the cycles. In addition, for non-haematological toxi-

Table 3
Evaluable cycles with toxicities

Schedule	3-weekly		d1&8	
BMS-184476 dose (mg/m^2)	40	30	30 + 30	35 + 35
Patients	6	4	4	18
Evaluable cycles	23	8	16	113
% of cycles with decreased dose	22	0	0	37
Neutropenia (% of cycles)				
Grade 4	39	0	37.5	33
Grade 4 for > 5 days	13	0	25	13
Febrile neutropenia	0	0	6	3.5
Stomatitis (% of cycles)				
Grade 2	0	0	0	3
Grade 3	0	0	0	1
Peripheral neuropathy (% of cycles)				
Grade 1	39	0	19	22
Grade 2	0	0	0	1
Myalgia/arthralgia (% of cycles)				
Grade 1	9	25	0	11
Grade 2	9	0	0	5
Fatigue/asthenia (% of cycles)				
Grade 2	9	12.5	19	7
Grade 3	4	0	6	0
Anorexia (% of cycles)	4	0	44	6
Vomiting (grade 3) (% of cycles)	0	0	0	1
Diarrhoea (grade ⩾2) (% of cycles)	9	12.5	6	7

d1&8, BMS-184476 given on day 1 and day 8 every 3 weeks, Doxo given at 50 mg/m^2 on day 1 only; 3-weekly, both drugs given on day 1 every 3 weeks, Doxo dose: 50 mg/m^2.

Table 4
Antitumour activity of BMS-184476 and Doxo in patients with measurable breast cancer

Schedule	Patients	Best response			Subsequent AT*	Response to AT
		CR	PR	NR		
3-weekly	6			6	5	2 PR
d1&8q3wks	17	1	9	7	2	1 PR, 1 CR

AT, *Doxorubicin (70 mg/m^2) and paclitaxel (200 mg/mg^2) in combination; CR, PR, NR, complete, partial or no response; q, every; wks, weeks.

city the pattern was similar between the two schedules (Table 3). Nausea and vomiting were well controlled by the antiemetic prophylaxis. The incidence of Grade 2 diarrheoa was low (Table 3). However, it tended to be more common in patients receiving the 35 mg/m^2 dose with the d1&8 schedule (7 of 16 patients, including one case of grade 3) than with the same schedule at the lower dose (1 of 4 patients on on cycle with grade 2). Other toxicities were myalgia/arthralgia, stomatitis and cumulative fatigue/asthenia. Peripheral neuropathy also occurred and was cumulative, but did not appear to worsen with continuous treatment, rarely reached grade 2 severity, and was reversible.

Myocardial contractility was investigated at baseline and at the end of treatment in all patients, but one. No decrease of LVEF to less than 50% was reported. An asymptomatic decline of resting LVEF between 10%–20% from baseline was reported in 7 patients treated with BMS-184476 35 mg/m^2 in the d1&8 schedule. In these 7 patients, a median LVEF decrease of 11% (range: 10 to 17%) was reported, followed by spontaneous recovery after treatment discontinuation.

3.3. Antitumour activity

The 3-weekly combination was given to 10 patients, 6 of whom had breast cancer. Treatment with the d1&8 schedule was administered to 22 patients, 17 of whom had breast cancer. A previously untreated patient with malignant mesothelioma had disease stabilisation for four 3-weekly cycles. None of the breast cancer patients treated with the 3-weekly schedule responded (Table 4). Interestingly, two of them later obtained a partial response after subsequent treatment with AT. In the 17 patients treated with the d1&8 schedule, 9 (1 pt at BMS/DOXO dose level 30/50, the remainder at 35/50; 2 pts previously treated with chemotherapy) achieved a partial response and one (BMS/DOXO dose level 35/50) a complete response for an overall response rate of 59% (95% Confidence Intervals: 33–82%). Two patients with stable disease as their best response after two cycles of treatment with BMS-184476 and Doxo received AT, and achieved a partial and a complete response, respectively. Responses were observed at the primary tumour, lymph node metastases, and visceral metastases.

3.4. Pharmacokinetic results

The plasma PK of BMS-184476 was assessed in 15 patients. The drug disposition could be fitted to a three-compartment model. The main PK parameters of BMS-184476 are listed in Table 5. Analysis of the relationship between the different parameters and administered dose was linear (data not shown). The metabolites BMS-246178, BMS-246180 and paclitaxel were measurable in very low concentrations for a brief interval after the end of the infusion. An unknown compound, which consistently appeared in the chromatograms of samples collected after starting the BMS-184476 infusion, followed a time-course typical for a metabolite. Its elution time was consistent with higher polarity than BMS-184476, and the ultraviolet-visual (UV-VIS) spectrum recorded during the elution was similar to BMS-184476 (data not shown). Plasma concentrations were calculated attributing to the unknown compound the same absorbance at 230 nm of paclitaxel, and fitting the value of integrated area under the chromatographic peak to the standard curve for paclitaxel. Plasma exposure to the putative metabolite corresponded to approximately 15% of the total AUC of BMS-184476.

Within-patient comparisons of the PK parameters of BMS 184476 when given with or without Doxo did not show any difference, ruling out an effect of the anthracycline on the disposition of BMS-184476 and the metabolites. Fig. 1 illustrates the results for the AUC.

Table 5
Main pharmacokinetic parameters of BMS-184476 at the three tested doses during the concomitant administration of 50 mg/m^2 of Doxo

	BMS-184476					Metabolites of BMS-184476		
Dose of BMS-184476 (mg/m^2)	AUC 0–last (nM·h/m^2)	AUC 0–∞ (nM·h/m^2)	C_{max} (nM)	$t_{1/2}\gamma$ (h)	CL_{TB} (L/h/m^2)	BMS-246178 C_{max} (nM)	BMS-246180 C_{max} (nM)	Paclitaxel Cmax (nM)
30	1853±1263	2419±1681	431±111	23±13	18±10	11±7	7±4	9±6
35	1962±176	2524±479	558±168	29±14	16±3	15±10	9±6	9±4
40	2479±789	4446±2785	1092±296	49±57	12±5	24±12	16±8	10±4

AUC, area under the curve of concentration per time; $t_{1/2}\gamma$, terminal half-life; Cl_{TB}, total body clearance; C_{max}, highest plasma concentration. Data are means±standard deviations.

Table 6
Main pharmacokinetic parameters of Doxo and its metabolite after the administration of 50 mg/m^2 of Doxo in combination with three different BMS-184476 doses

	Doxo					Doxorubicinol		
BMS-184476 dose (mg/m^2)	AUC 0–last (nM·h/m^2)	AUC 0–∞ (nM·h/m^2)	C_{max} (nM)	$t_{1/2\gamma}$ (h)	CL_{TB} (L/h/m^2)	AUC 0–last (nM·h/m^2)	AUC 0–∞ (nM·\h/m^2)	C_{max} (nM)
30	1417±321	2522±808	2556±291	58±5	46±18	562±68	951±133	15±2
35	1490±308	1771±446	3907±1521	51±18	51±9	547±113	833±216	16±4
40	1798±509	2170±584	4714±2070	46±3	42±10	557±284	954±356	18±6

AUC, area under the curve of concentration per time; $t_{1/2\gamma}$, terminal half-life; Cl_{TB}, total body clearance; C_{max}, highest plasma concentration. Data are mean±standard deviation.

Similar results were observed for total body clearance, terminal half-life and exposure to the metabolites (data not shown).

The PK profile of Doxo and doxorubicinol (Table 6) was similar at BMS-184476 doses from 30 to 40 mg/m^2, indicating that, with the available data, the taxane had no measurable effect on these two concentrations of the two anthracyclines.

3.5. Effects on doxorubicin metabolism in human myocardium

Paclitaxel and docetaxel are capable of enhancing the intracardiac formation of doxorubicinol, which plays a major role in the progression of doxorubicin-induced chronic cardiomyopathy [7]. Such a reaction may concur in explaining the enhanced cardiac toxicity of AT [4]. We compared the effects of BMS-184476 with those of paclitaxel, docetaxel and the tubulin active, non-cardiotoxic vinorelbine on the conversion of Doxo to doxorubicinol. As shown in Fig. 2a, doxorubicinol formation was increased by paclitaxel and docetaxel according to a bell-shaped pattern. Vinorelbine and BMS-184476 had no effect (Fig. 2b). Different reductase activity of different myocardial cytosol preparations affect the paclitaxel- or docetaxel-induced doxorubicinol formation which for paclitaxel persists over a broad range of reductase activity, while for docetaxel is limited to samples with intermediate levels of reductase activity [7]. As shown in Fig. 2b, experiments performed in the present study confirmed the difference between paclitaxel and docetaxel, and showed that vinorelbine and BMS-

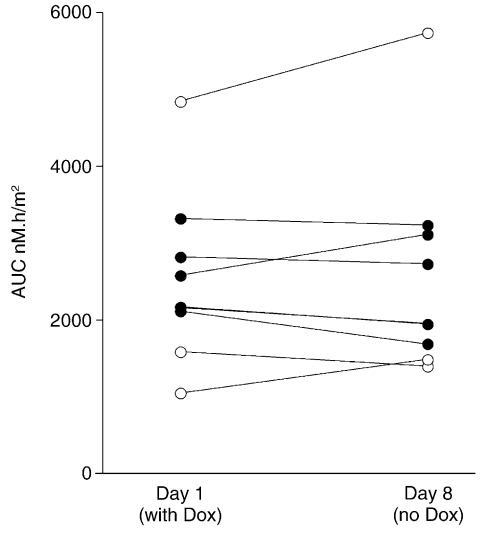

Fig. 1. Comparison of plasma/exposure to BMS-184476 (open circles for 30 mg/m^2 dose; closed circles for 35 mg/m^2) when given with or without Doxo. Student's *t*-test for paired data was applied ($P>0.05$). Doxo, doxorubicin; AUC, area under the concentration× time curve.

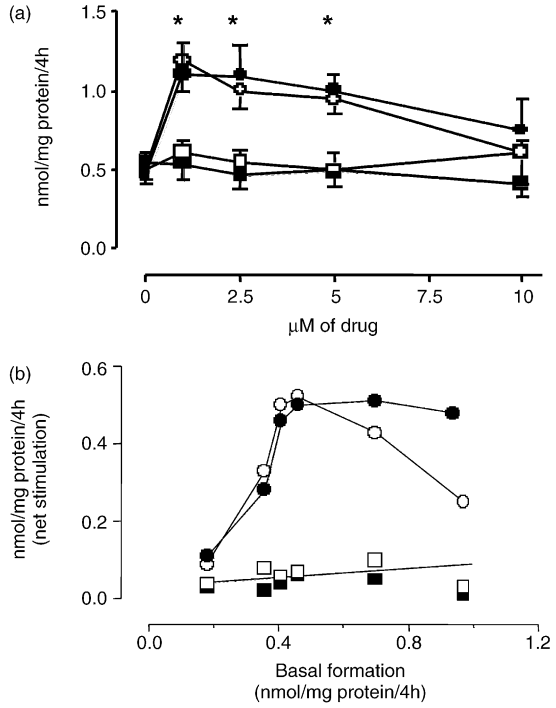

Fig. 2. Effects of tubulin-active agents on the conversion of Doxo to doxorubicinol in human cardiac cytosol. Panel a: Doxorubicinol was measured in incubations prepared as described in the Methods, in the absence or presence of paclitaxel (●), docetaxel (○), BMS-184476 (●), or Vinorelbine (□). Values are means ±standard errors of the mean (SEM) of six experiments. *$P<0.025$ versus corresponding controls (paired Student's *t* test). Panel b: net stimulation of doxorubicinol formation by 1 μM agents as a function of the basal levels of metabolite formation in different cytosol samples.

184476 did not increase doxorubicinol formation, regardless of the basal levels of metabolite formation in different cytosol samples.

4. Discussion

This study investigated whether BMS-184476, that is formulated in 80% less Cremophor® and has greater preclinical antitumour activity than paclitaxel, could partner with Doxo in a combination devoid of cardiac toxicity. This has been observed when combining paclitaxel with doxorubicin, a regimen causing higher than expected cardiac toxicity and requiring the total dose of Doxo to be limited to 360 mg/m^2 when given in combination with the prototype taxane [5]. The results of this study show that the administration of BMS-184476 with a therapeutic dose of Doxo is feasible, devoid of PK and/or the biochemical interactions implicated in the mechanisms of cardiotoxicity of AT, and endowed with a promising antitumour activity in women with previously untreated breast cancer.

The conduct of the study was not uneventful because DLT prompted the reduction of the taxane's dose to 30 mg/m^2 (MTD for the 3-weekly schedule). This generated several considerations. The MTD of BMS-184476 in the combination was half of that recommended as a single agent from prior studies, a dose that also showed no antitumour activity [2]. Furthermore, Doxo and BMS-184476 given as single agents at the doses selected for the starting dose level in this study do not cause limiting bone marrow toxicity [2,3]. Therefore, only a pharmacological interaction between BMS-184476 and Doxo could account for the toxicity. Based on these considerations and on the observation that neutropenia, no matter how severe, had always resolved by day 21, the protocol was later amended in an attempt to reduce the extent of the possible drug-drug interaction by splitting BMS-184476 into 2 weekly doses given on day 1 and 8 every 3 weeks. BMS-184476 could therefore be escalated from 30 to 35 mg/m^2, with 3 DLTs out 6 patients treated at the higher dose level (febrile neutropenia and Grade 4 neutropenia lasting >5 days). In view of the results of the 3-weekly schedule, no further escalation was pursued. In the extended cohort of patients who received the combination, neutropenia was again the most frequent toxicity observed. Other toxicities seen at the MTD level were similar for both schedules, consisting of loss of appetite, myalgia/arthralgia, and infrequent occurrence of diarrhoea. The novel taxane never caused hypersensitivity reactions (HSRs) in our study. However, premedication was implemented after the third patient was enrolled only because HSRs had occurred in other trials with BMS-184476 (privileged information from Bristol Myers Squibb).

The enrollment of patients with minimal or no prior therapy provided the opportunity of administering repeated cycles if there was evidence of therapeutic benefit. This also allowed the investigation of cumulative effects on myocardial contractility and the peripheral nervous system. The asymptomatic decrease of 11% of LVEF relative to baseline after a median total Doxo dose of 247 mg/m^2 was reversible upon discontinuation of therapy, and never went below 50% of the institutional limits. Cumulative, and mostly mild, peripheral neuropathy did occur; it was reversible after treatment discontinuation. In summary, the decision to administer BMS-184476 for 2 consecutive weeks resulted in a more than doubling of the dose of the taxane per cycle, with toxicities similar to those observed with the 3-weekly schedule.

Such higher dose and intensity may have contributed to the reported antitumour activity. In the past, AT was evaluated in a dose-finding study restricted to patients with untreated metastatic breast cancer [4]. In the present trial, 23 such patients were enrolled. None of the six women who received the intermittent combination responded, while 9 of the 17 patients who received the d1&8 schedule had at least a partial response. Interestingly, four of the 13 patients who did not respond to the combination subsequently responded to AT. Such antitumour activity may have been due to insufficient dosing with BMS-184476 in our study because of the early appearance of limiting toxicity, but could also be explained by the lack of complete cross-resistance between the two taxanes [1].

The pharmacological investigations conducted in our study acquire special interest in view of the clinical observations that were consistent with a toxic interaction. The PK of the taxane and its metabolites were similar to those already described in the literature [2,3]. In particular, concentrations of BMS-184776 proportionally increased with dose in the narrow range that was tested. One peculiar and previously undescribed observation was about the taxane's metabolism. The concentration of the already known metabolites BMS-246178, BMS-246180 and paclitaxel accounted for a minimal fraction of the administered dose of BMS-184776. It is therefore unlikely that they played a major role in determining the pharmacological effects. However, we observed that a compound with UV-VIS spectra similar to that of BMS-184476 appeared in the plasma of all patients, had a time course consistent with that of a metabolite, and an approximate AUC corresponding to 15% of that of BMS-184476. The possible role of this unknown compound as the major plasma metabolite of BMS-184476 in humans suggests the opportunity to better define its biological and toxicological properties.

In the past, we and other investigators showed that concentrations of Doxo and doxorubicinol are

increased by concomitant or prior administration of paclitaxel [5,10]. The effect is attributable to Cremophor® EL interfering with biliary elimination [5,11]. PK studies of Doxo with docetaxel showed that the anthracycline increased plasma concentrations of the taxane [12]. In either case, the PK interaction was partially responsible for the tolerability profile of the combination. The administration of BMS-184476, with and without Doxo in the same cycle, allowed for testing whether the anthracycline interfered with the PK of the taxane; within-patient comparisons did not show any effect of Doxo and ruled out that the early appearance of DLT could be due to a PK interference. Although the design of the study did not allow for formally investigating the effect of BMS-184476, there was no indication that increasing the dose of the taxane had an effect on the exposure to Doxo or doxorubicinol. The range of tested BMS-184476 doses is too narrow (30 to 40 mg/m^2) to conclusively rule out interference; however, the data are sufficient to exclude any major and potentially toxic effect of the taxane on the concentration of the anthracyclines in our study.

Finally, paclitaxel and docetaxel, possibly because of allosteric interaction with cardiac carbonyl- or aldo/keto-reductases, enhance the intracardiac conversion of Doxo to doxorubicinol, the metabolite that is implicated in the transition of anthracycline-induced cardiac damage from an acute and reversible phase to a chronic and irreversible one [13]. In our study, BMS-184476 had no effect on the intracardiac metabolism of Doxo to doxorubicinol. Given the potential role of such a biochemical pathway in the cardiac toxicity of the AT combination [7], the data suggest that BMS-184476 should be applicable in combination with full total doses of Doxo without increased cardiac risks.

In summary, this study has defined the recommended doses of BMS-184476 in combination with Doxo for two different schedules. Both caused neutropenia as the only limiting toxicity. Non-haematological toxicity was clinically manageable and mild to moderate. BMS-184476 for 2 consecutive weeks could be given at larger doses per course, was well tolerated for repeated cycles, and showed promising antitumour activity in women with minimally pretreated or untreated advanced breast cancer. Interestingly, patients who were non-responsive to BMS-184476 and Doxo did respond to subsequent AT. Good tolerability and the therapeutic potential of the new regimen are associated with evidence that the BMS-184476 is not capable of PK and biochemical interactions that have been implicated in the increased cardiac toxicity of Doxo in combination with paclitaxel. In conclusion, the combination of Doxo given on day 1 and BMS-184476 on days 1 and 8, every 3 weeks, appears to be worth testing in future studies.

5. Conflict of interest statement

The authors declare that they have no financial interest which may inappropriately influence the undertaking of this study.

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