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Lack of correlation between mitotic arrest or apoptosis and antitumor effect of docetaxel

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Abstract *Purpose*: To determine, as we did for paclitaxel, whether mitotic arrest and apoptosis induced in murine tumors in vivo by docetaxel correlate with the drug's antitumor effect and whether the antitumor efficacy of docetaxel depends on p53 mutational status of tumors. *Methods*: C3Hf/Kam mice were implanted with one of the following 15 syngeneic tumors: seven adenocarcinomas (MCa-4, MCa-29, MCa-35, MCa-K, OCa-I, ACa-SG, and HCa-I), two squamous cell carcinomas (SCC-IV and SCC-VII), five sarcomas (FSa, FSa-II, Sa-NH, NFSa, and Sa-4020) and one lymphoma (Ly-TH). When the tumors had grown to 8 mm in diameter, the mice were treated with 31.3 mg/kg docetaxel i.v. Tumor growth delay was the endpoint of docetaxel's antitumor effect. In separate groups of mice, mitotic arrest and apoptosis were determined micromorphometrically 1 to 72 h after docetaxel treatment. Tumors were assayed for their p53 status by sequence analysis of RNA prepared from freshly excised tumors. *Results*: Docetaxel caused statistically significant growth delay in six of seven adenocarcinomas, three of five sarcomas, and the lymphoma, but not in either of the squamous cell carcinomas. The drug induced mitotic arrest in all tumor types, but to various degrees ranging from 6.4 +/− 0.4% to 25.1 +/− 0.1%. In contrast, docetaxel induced appreciable apoptosis in only 5 of 15 tumors, with 10.3 +/− 1.6% being the highest apoptotic value. Neither mitotic arrest nor apoptosis were significantly correlated with tumor growth delay. However, tumors that responded to docetaxel by significant tumor growth delay histologically displayed massive cell destruction by cell lysis, and four of these tumors also showed marked infiltration with mononuclear lymphoid cells. Of the 15 tumors only 3 had mutant p53. *Conclusions:* Docetaxel exhibited a strong antitumor effect in two-thirds of murine tumors, and on a milligram per kilogram basis was more effective than paclitaxel against the same tumors. The drug was a potent inducer of mitotic arrest but a weak inducer of apoptosis, neither of which correlated with its antitumor effect. Tumor cell lysis appeared to be a major mode of tumor cell destruction and can be regarded as the main mechanism underlying antitumor efficacy of docetaxel. In contrast, paclitaxel's antitumor efficacy is related to its ability to induce apoptosis. At the molecular level, there was no dependency of antitumor efficacy of docetaxel on p53 mutational status of tumors.

Key words Docetaxel · Mouse tumors · Mitotic arrest · Apoptosis · Cell lysis · p53

Introduction

Taxanes (paclitaxel and docetaxel) are antimicrotubule agents effective in the treatment of common cancers in humans [44, 51, 53]. They are mitotic spindle poisons and promote accelerated assembly of excessively stable microtubules, thus affecting microtubule-dependent cellular functions like the control of mitosis and intracellular transport [2, 13, 46]. As a result of this unique mechanism of action, proliferating cells are blocked in mitosis. In addition, taxanes induce cell death by apoptosis in cell cultures [1, 18, 24, 48], tumors in vivo [31, 37, 40], and normal tissues [30, 31]. Recently, we have shown that paclitaxel induces mitotic arrest to various degrees in 16 different mouse tumors but significant apoptosis in only 8 [40]. However, it is paclitaxel-induced apoptosis and not mitotic arrest that correlates with the antitumor effect of paclitaxel as measured by tumor growth delay. Paclitaxel significantly delayed tumor growth in eight of the 16 tumors.

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Recently, we reported that docetaxel enhances the radioresponse of a murine mammary carcinoma, designated MCa-4 [31], more so than does paclitaxel [36, 38, 41]. The MCa-4 tumor is sensitive to docetaxel alone and displays histological features after treatment with docetaxel that are not observed to the same degree in paclitaxel-treated tumors [31]. Docetaxel-treated tumors demonstrate a lytic, nonapoptotic mode of cell death that dominates compared to apoptotic cell death. In addition, massive tumor infiltration by mononuclear lymphoid cells is seen after treatment with docetaxel [31], but not in any of 16 tumors treated with paclitaxel [40]. This observation suggests that the mechanisms of docetaxel's antitumor activity might differ from that of paclitaxel's.

The present study quantified mitotic arrest and apoptosis induced by docetaxel in 15 different murine tumors in order to determine whether they relate to the drug's antitumor efficacy expressed by tumor growth delay. In addition, the treated tumors were analyzed for infiltration by mononuclear cells to see whether this reaction, observed previously in MCa-tumor [31], is a general phenomenon after treatment with docetaxel. Further, we compared the results obtained with docetaxel with the effects of paclitaxel already characterized for this same group of tumors [40].

Material and methods

Mice

C3Hf/Kam mice from our own specific pathogen-free mouse colony were used. They were 3–4 months old and weighed 27 to 34 g (mean 32 g). The mice were housed five or six per cage and fed sterilized pelleted food (Prolabs Animal Diet, Agway Syracuse, N.Y.) and sterilized water *ad libitum* and exposed to a 12-h light/dark cycle. The experimental protocol was approved by the Institutional Animal Care and Use Committee. Mice were maintained in a fully accredited animal facility (American Association for Accreditation of Laboratory Animal Care) and in accordance with current regulations and standards of the US Department of Health and Human Services.

Tumors

Solitary tumors were generated in the muscle of the right leg of the mouse by inoculation of 5×10^5 viable tumor cells in suspension. Tumor cell suspensions were prepared by mechanical disruption and enzymatic digestion of nonnecrotic tumor tissue. The method has been fully detailed previously [33].

We studied the following tumors: seven adenocarcinomas (four mammary carcinomas designated MCa-4, MCa-29, MCa-35, and MCa-K, used in their fourth isotransplant generations, or eighth in the case of MCa-K; an ovarian carcinoma designated OCa-I, used in its sixth isotransplant generation; a salivary gland adenocarcinoma designated ACa-SG, used in its fifth isotransplant generation; and a hepatocellular carcinoma designated HCa-I, also used in its fifth isotransplant generation); two squamous cell carcinomas designated SCC-IV and SCC-VII, used in their eighth (SCC-IV) and sixth (SCC-VII) isotransplant generation; five soft tissue sarcomas designated FSa, FSa-II, Sa-NH, NFSa, and Sa-4020, used in their seventh (FSa), fifth (FSa-II), fourth (Sa-NH), twelfth (NFSa), and sixth (Sa-4020) isotransplant generation; and one lymphoma designated Ly-TH, used in its sixth isotransplant generation. All tumors were syngeneic to this strain of mouse. The MCa-4, MCa-29, MCa-35, ACa-SG, HCa-I, SCC-VII, FSa-II, Sa-NH, and Ly-TH tumors originally arose spontaneously, and the MCa-K, OCa-I, NFSa, and Sa-4020 tumors developed outside the treated volume in irradiated mice. The SCC-IV and FSa tumors were induced by 3-methylcholanthrene. Responses of these tumors to radiation and a number of chemotherapeutic agents other than docetaxel have been previously reported [32, 35, 36, 38–41].

Docetaxel

Docetaxel (Taxotere®) was obtained from Rhone-Poulenc Rhorer (Vitry Sur Seine Cedex, France) as a pure crystalline powder and stored at 4 °C. Stock solutions of 50 mg/ml were prepared in absolute ethanol (Aaper, Shebyville, Ky.) and stored at −20 °C for the duration of the experiments. Treatment solutions were prepared by mixing 1 volume of the ethanolic stock solution, 1 volume of polysorbate 80 (Sigma, St. Louis, Mo.), and 18 volumes of 5% glucose in water. The intravenous (i.v.) injection volume per mouse was 0.4 ml, or 31.3 mg/kg for a 32-g mouse. Treatment solutions were kept on ice and injected within 5 min of formulation.

Tumor growth delay

The antitumor effect of docetaxel was determined by its ability to delay tumor growth. Palpable tumors were measured daily in three orthogonal diameters with vernier calipers. Mice whose tumors had grown to 8 mm in diameter were then treated with docetaxel (31.3 mg/kg i.v.) or not (controls) and allocated to either the tumor growth delay arm or the histologic analysis arm of the study.

For each histologic tumor type, the effect of docetaxel was assessed by the measurement of tumor growth in untreated control and docetaxel-treated groups at daily intervals until tumors had grown to at least 12 mm in diameter. The general experimental design for tumor growth delay was to allocate at least six mice to each of the control and treatment groups for each histologic tumor type. In the case of SCC-IV only five mice per group were used. A total of 208 mice were used, 106 in the control groups and 102 in the treatment arms.

The effect of treatment on tumor growth is expressed as absolute growth delay (AGD), defined as the time in days for the tumors in the treated groups to grow from 8 to 12 mm in diameter minus the time in days for untreated control tumors to grow from 8 to 12 mm in diameter.

Histological determination of mitotic and apoptotic indices

In general at 1, 3, 6, 9, 12, 24, 48, 72, and 96 h after docetaxel administration, three mice were killed by cervical dislocation. In the case of MCa-4 the mice were killed 1, 3, 6, 9, 12, 16, 24, 48, and 72 h after treatment with the drug, and in the case of SCC-IV only two mice per time-point were killed. No tumor in Ly-TH lymphoma-bearing mice was clinically or histologically visible later than 24 h after docetaxel treatment; thus 24 h was the latest time-point included for this tumor. All tumors were removed and placed in neutral buffered formalin. For each histologic type, tumors were also removed from untreated mice (two mice for SCC-IV and three mice for all other histologic types). The total number of mice used for this part of the experiment was 440. The tissue was embedded in paraffin blocks, and 4-μm sections were cut from these blocks and stained with hematoxylin-eosin.

The morphologic features used to identify mitosis and apoptosis in murine tumors have been described previously [37]. Five fields of nonnecrotic areas were selected randomly across each tumor section, and in each field, apoptotic bodies and cells in mitotic arrest were expressed as a percentage based on the scoring of 1500 nuclei (1000 nuclei for SCC-IV) at each interval after treatment. Micromorphometric scoring of histologic tumor sections was

conducted without knowledge of the results of the tumor growth delay assay.

Sequence analysis of p53 status

Total RNA was prepared from freshly excised tumors by the method of Chomczynski and Sacchi [8] using commercially available reagents (RNAzol B, Tel-Test, Friendswood, Tx.) and reverse transcribed with random hexamer primers. The p53 cDNA was selectively amplified by 30 cycles of PCR using oligonucleotide primers that flank the coding region (TTGGGACCATCCTGGCTGTAG and AAGGGACCGGGAGGATTGTGT). The amplication products were purified by agarose gel electrophoresis and then cycle sequenced using [γ-^{32}P]-labeled dideoxynucleotide terminators (Amersham, Arlington Heights, Ill.) and the original PCR primers as sequencing primers. The products of the sequencing reaction were resolved by electrophoresis on a GenomyxLR DNA sequencing system. Each reaction was assayed on two gels, the first designed to resolve from the primer to 350 bases and the second designed to resolve out to 800 bases. Following electrophoresis, the dried sequencing gels were exposed to X-ray film for detection.

Statistical methods

For each histologic tumor type a plot was constructed that showed the percentage of mitotic cells and apoptotic bodies as a function of time after docetaxel treatment. From these plots, the baseline levels of mitosis and apoptosis in untreated tumors and the temporal change in these levels after treatment were determined. The latter were expressed as peak level, defined as the maximum level occuring within the first 48 h after treatment, and the net level, defined as the peak level minus the baseline level.

The statistical significance of the differences in the time for untreated and treated tumors to grow from 8 to 12 mm in diameter was determined by Student's *t*-test [56]. Tests were two-tailed and assumed unequal variance; the *P*-values were two-sided and considered significant if they were less than or equal to 0.05. Scatter plots were used to determine the correlation between docetaxel-induced peak levels of mitosis and apoptosis and the antitumor efficacy of docetaxel and between antitumor effects of paclitaxel and docetaxel [56]. Data were fitted by simple linear regressions. Data for paclitaxel were those published in reference 40.

Results

The antitumor efficacy of docetaxel varied among tumor types (Table 1). Statistically significant growth delay was found in 10 (67%) of the 15 tumors. Adenocarcinomas were the most likely to respond: 6 of 7 (86%) showed significant growth delay. Three of 5 sarcomas (60%) and 0 of 2 squamous cell carcinomas showed significant growth delay. The longest growth delay was for the lymphoma Ly-TH: 19.7 days ($P < 0.0001$).

All tumors were scored histologically for mitotic arrest and apoptosis at successive time-points (1–96 h; MCa-4 1–72 h) after docetaxel treatment (Table 2). Docetaxel induced mitotic arrest in all tumors, but the magnitude of arrest varied greatly, from 6.4% +/− 0.4% (Sa-NH sarcoma) to 25.1% +/− 0.1% (Ly-TH lymphoma). In general, higher values of mitotic arrest were observed in the adenocarcinomas and squamous cell carcinomas than in the sarcomas. Induction of apoptosis ranged from almost no increase (SCC-IV, SCC-VII, FSa, Fsa-II, Sa-NH, NFSa, Sa-4020) to 10.3% +/− 1.6% in the MCa-4 adenocarcinoma (Table 2). The peak levels in tumors responding with apoptosis were lower in every case than those previously observed in paclitaxel-treated tumors (Table 2) [40].

As recently described [31], two additional histological features were noted in tumors responding to docetaxel by significant tumor growth delay. A massive amount of

Table 1 The antitumor effect of docetaxel against different types of murine tumors. The times in days to grow from 8 to 12 mm (arithmetic mean diameter) are presented as group means +/− standard error. Mice bearing 8-mm tumors in the right leg were treated with docetaxel (31.3 mg/kg intravenously). Tumor growth was determined by daily measurement of three orthogonal diameters with vernier calipers. (*WT* wildtype p53)

Tumor type		p53 status	Time in days for tumor to grow from 8 to 12 mm in diameter		Absolute growth delay[a]	*P*-value (*t*-test)
			Untreated	Treated		
Adenocarcinoma	MCa-4	WT	7.0 +/−0.6	11.5 +/−0.6	4.5	< 0.0001
	MCa-29	WT	5.6 +/−0.7	11.9 +/−0.8	6.3	< 0.0001
	MCa-35	Mutant	8.4 +/−0.5	14.9 +/−1.0	6.5	< 0.0001
	MCa-K	Mutant	4.5 +/−0.4	20.0 +/−1.1	15.6	< 0.0001
	OCa-I	WT	11.4 +/−1.1	29.1 +/−1.9	17.7	< 0.0001
	ACa-SG	WT	4.1 +/−0.3	6.5 +/−0.3	2.4	< 0.0001
	HCa-I	WT	5.1 +/−0.6	4.9 +/−0.5	−0.2	0.86
Squamous cell carcinoma	SCC-IV	WT	7.2 +/−1.5	7.2 +/−0.8	0.2	1.0
	SCC-VII	WT	3.1 +/−0.1	3.9 +/−0.4	0.8	0.15
Sarcoma	FSa	Mutant	3.1 +/−0.3	4.0 +/−0.2	0.9	0.01
	FSa-II	WT	2.4 +/−0.2	3.1 +/−0.3	0.7	0.06
	Sa-NH	WT	4.4 +/−0.2	6.9 +/−0.3	2.5	< 0.0001
	NFSa	WT	4.0 +/−0.2	4.0 +/−0.3	0.0	0.57
	SA-4020	WT	3.8 +/−0.1	5.9 +/−0.3	2.1	0.0004
Lymphoma	Ly-TH	WT	3.6 +/−0.3	23.3 +/−0.8	19.7	< 0.0001

[a] Defined as the time in days for tumors treated with docetaxel (31.3 mg/kg intravenously) to grow from 8 to 12 mm minus the time in days for untreated tumors to grow from 8 to 12 mm

Table 2 Induction of mitotic arrest and apoptosis by docetaxel in different types of murine tumors. Mice bearing 8-mm tumors in the right leg were treated with docetaxel (31.3 mg/kg intravenously). The percentage of cells in mitosis or apoptosis was scored from histologic sections prepared from tumors excised 1 to 96 h after treatment. The baseline level of mitosis and apoptosis was determined in untreated tumors; shown are the group means +/− standard error. The peak levels of mitosis and apoptosis were determined from the time course of treated tumors; shown are the group means +/− standard error; the time (hours) at which the peak was detected is indicated in parentheses (*WT* wild-type p53)

Tumor type		p53 status	Mitosis		Apoptosis	
			Baseline	Peak	Baseline	Peak
Adenocarcinoma	MCa-4	WT	2.4 +/−1.0	22.1 +/−1.2 (9)	3.0 +/−2.4	10.3 +/−1.6 (24)
	MCa-29	WT	0.7 +/−0.3	18.5 +/−0.2 (9)	0.2 +/−0.1	2.7 +/−0.2 (12)
	MCa-35	Mutant	0.9 +/−0.1	11.4 +/−1.5 (12)	0.5 +/−0.1	3.0 +/−0.5 (24)
	MCa-K	Mutant	0.7 +/−0.3	15.4 +/−1.7 (6)	0.1 +/−0.1	1.9 +/−0.4 (48)
	OCa-I	WT	0.9 +/−0.1	13.0 +/−0.8 (9)	0.5 +/−0.1	2.8 +/−0.1 (12)
	ACa-SG	WT	0.8 +/−0.1	18.3 +/−0.4 (9)	0.0	1.4 +/−0.4 (12)
	HCa-I	WT	1.1 +/−0.2	9.3 +/−0.5 (9)	0.1 +/−0.1	0.2 +/−0.1 (12)
Squamous cell carcinoma	SCC−IV	WT	1.4 +/−0.2	12.9 +/−0.7 (6)	0.7 +/−0.3	0.9 +/−0.1 (24)
	SCC−VII	WT	0.4 +/−0.1	18.7 +/−4.6 (6)	0.0	0.1 +/−0.1 (24)
Sarcoma	FSa	Mutant	1.6 +/−0.1	8.5 +/−0.1 (6)	0.1 +/−0.1	0.2 +/−0.1 (12)
	FSa-II	WT	1.5 +/−0.1	6.7 +/−0.9 (3)	0.3 +/−0.1	0.4 +/−0.1 (12)
	Sa-NH	WT	0.7 +/−0.1	6.4 +/−0.4 (6)	0.1 +/−0.1	0.2 +/−0.1 (6)
	NFSa	WT	0.7 +/−0.3	8.2 +/−0.1 (6)	0.2 +/−0.1	0.5 +/−0.1 (12)
	SA-4020	WT	0.9 +/−0.2	11.3 +/−1.1 (6)	0.2 +/−0.1	1.0 +/−0.5 (24)
Lymphoma[a]	Ly-TH	WT	2.3 +/−0.4	25.1 +/−0.1 (6)	0.9 +/−0.3	3.8 +/−2.9 (24)

[a] Lymphoma was not visible either clinically or histologically 24 h after the treatment; thus, mitosis and apoptosis counts were determined only within 24 h of treatment

mitotically arrested cells were disrupted, with nuclear material spilling into the interstitial space. This nonapoptotic mode of cell death has also been noted in tumors treated with paclitaxel [37, 40], but not to such an extent. Although this docetaxel-induced cellular lysis could not be quantified, the process as shown on histologic sections may have contributed more to tumor cell death than apoptosis. The second notable histologic feature was massive tumor infiltration by mononuclear lymphoid cells, first noted at 48 h and becoming particularly evident 72 h after treatment with docetaxel. This feature was found in four tumors: MCa-4, MCa-29, MCa-K, and OCa-I. While in MCa-4, MCa-29 and OCa-I tumors the majority of infiltrating cells displayed histologic characteristics of macrophages, in MCA-K they were predominantly lymphocytes.

The mitotic arrest and apoptosis plots for a strongly docetaxel-responsive tumor (MCa-4) and a nonresponsive tumor (SCC-VII) are shown in Fig. 1. In the case of MCa-4 (Fig. 1A), high mitotic arrest was followed by histologically visible cell death due to either apoptosis or necrosis. Despite a high mitotic arrest for SCC-VII (Fig. 1B), no apoptosis could be observed histologically. The peak of mitotic arrest occurred 6–12 h after docetaxel administration, while the peak of apoptosis occurred 12–48 h later (Table 2).

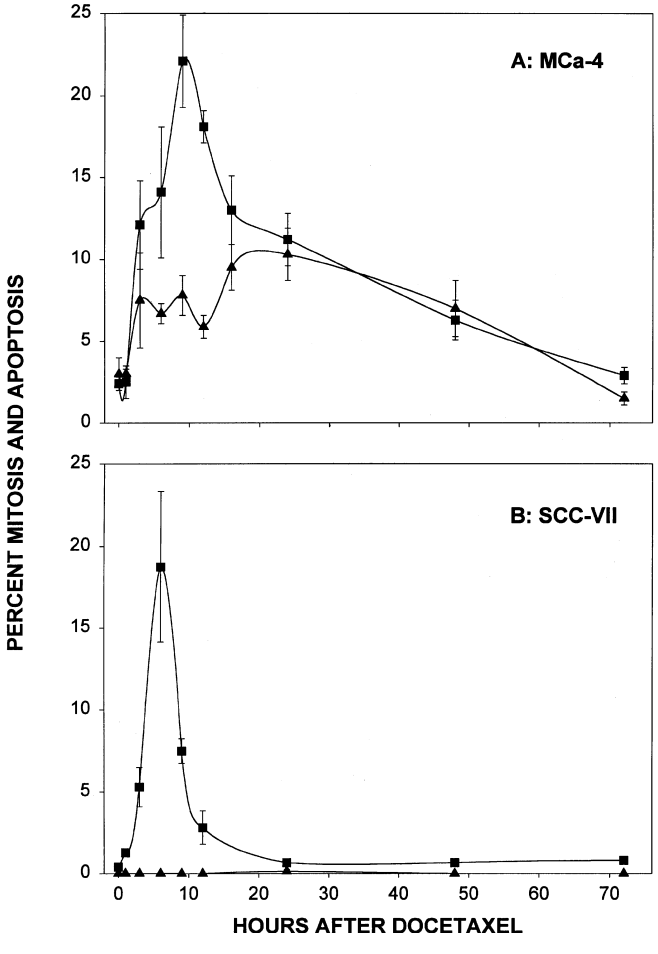

Fig. 1A,B Percentage of mitosis (*squares*) and apoptosis (*triangles*) induced in tumors following treatment with docetaxel (31.3 mg/kg i.v.) at different time-points up to 72 h (MCa-4,**A**) and 96 h (SCC-VII,**B**). Vertical bars are SE of the mean values

To determine whether there was a relationship between docetaxel-induced mitotic arrest or apoptosis and the antitumor efficacy of docetaxel, the AGD was plotted against the peak of mitotic arrest or apoptosis (Fig. 2) and the correlation coefficients calculated. The plots showed no significant correlation between either the induced mitotic arrest or induced apoptosis and the antimor effect of docetaxel. However, there was a trend toward higher mitotic arrest with greater tumor growth delay. No such trend existed for apoptosis.. These findings differ from those reported for paclitaxel [40], which show that increased levels of induced apoptosis highly correlate with increased ability of paclitaxel to delay tumor growth.

The response of tumors to chemotherapeutic agents is considered to be controlled by oncogenes, and in this regard chemoresistance is often attributed to the presence of mutant p53 gene [25, 28]. However, studies on the influence of p53 on cell cytotoxicity and tumor efficacy by paclitaxel have provided conflicting information [10, 45, 49, 52, 54]. To determine whether the antitumor effect of docetaxel depended on p53, all 15 tumors were analyzed for p53 mutational status (Tables 1 and 2). Only three tumors had mutant p53, MCa-35, MCa-K and FSa, and all three responded to docetaxel. In FSa and MCa-35 the change resulted in substitution of methionine for valine at position 169. In MCa-K tumor, the change resulted in deletion and base changes from threonine at position 275 to proline at position 285. Of 12 wildtype p53 tumors, 7 responded to docetaxel, implying that p53 is not likely to be the major genetic determinant of docetaxel's antitumor activity.

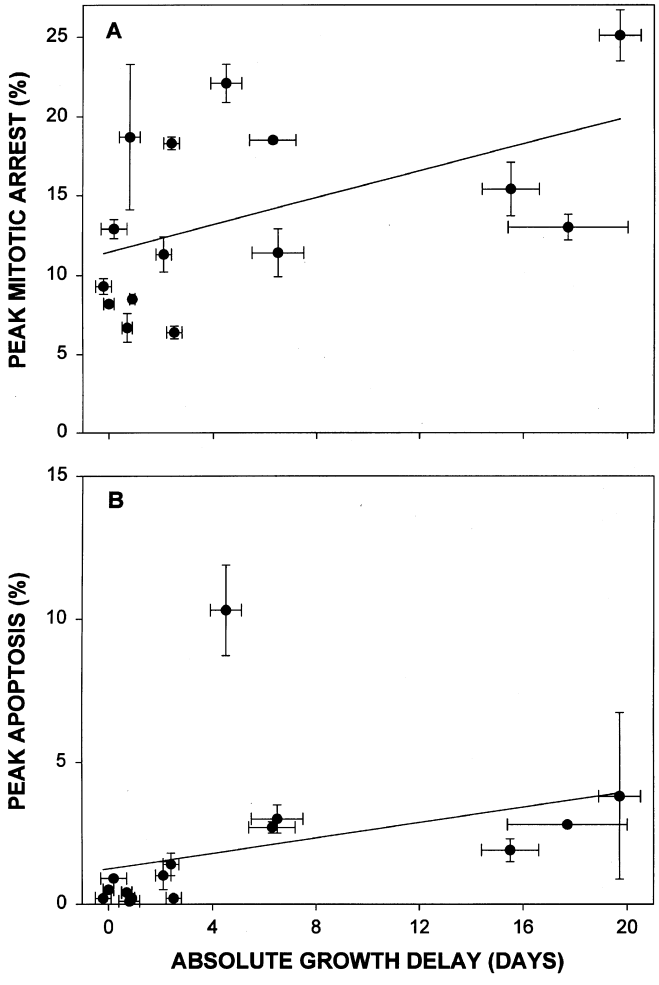

Fig. 2A,B Correlation plots of the peak level of mitotic arrest (**A**) and peak level of apoptosis (**B**) versus the absolute growth delay (in days) induced by docetaxel (31.3 mg/kg given i.v.). Each point represents the mean value for a given tumor type, and the bars represent the corresponding standard errors of the mean. The *solid lines* are the simple linear regression fits to the data. No significant correlation was observed, either for mitotic arrest ($R^2 = 0.250$; $P = 0.57$) or for apoptosis ($R^2 = 0.125$; $P = 0.194$)

Discussion

Taxanes are being increasingly used in cancer therapy, as they have shown potent activity against many common cancers in humans [44, 51]. Even so, determining who is likely to respond to these drugs has proved difficult, as there exists a broad range of responses to these agents among tumors of different as well as among tumors of the same histological type. The reasons for this variability are unclear and are likely to be multiple, including molecular and cellular determinants. In the case of paclitaxel, we have recently shown that mitotic arrest and apoptosis are the drug's major cellular effects, but that only apoptosis correlates with the antitumor efficacy of paclitaxel [40]. In addition, spontaneous apoptosis in untreated tumors correlates with paclitaxel-induced apoptosis and with the degree of tumor growth delay produced by this drug. Based on this observation we concluded that both pretreatment and induced apoptosis could help predict the outcome of treatment with paclitaxel. This conclusion has been recently supported by findings that higher spontaneous levels of apoptosis in cervix carcinoma are associated with more successful therapy of those patients receiving paclitaxel [42]. The present investigation assessed whether mitotic arrest or apoptosis were major cellular determinants of response to docetaxel, another potent taxane, and whether they are related to the antitumor effect of docetaxel in preclinical tumor models.

In the 15 different mouse tumors used in the present study, docetaxel exhibited broad variability in its antitumor effect, much as paclitaxel did against the same tumors [40]. Docetaxel significantly delayed tumor growth in 10 of the 15 tumors (Table 1). The adenocarcinomas of mammary origin were the most sensitive tumor category, as all four tumors showed significant growth delay. Significant growth delay occurred in three of five sarcomas (60%). AGDs were longer in mammary carcinomas (4.5–15.5 days) than in the responding sarcomas (0.9–2.5 days). Docetaxel was most effective against the OCa-I ovarian carcinoma and Ly-TH lymhoma, delaying their growth 17.7 and 19.7 days, respectively. In contrast, docetaxel had no measurable

effect on tumor growth of the two squamous cell carcinomas (SCC-IV and SCC-VII).

Docetaxel was effective against the tumors that responded to paclitaxel; in addition, it significantly delayed growth of two paclitaxel-resistant tumors, adenocarcinoma MCa-35 and sarcoma FSa [40]. Further, although the dose of docetaxel (31.3 mg/kg) was lower than that of paclitaxel (40 mg/kg), docetaxel usually produced longer tumor growth delays. Our findings are in concordance with the result of a number of studies by others demonstrating that docetaxel is more cytotoxic in vitro [22, 27, 43] and more effective against tumors in vivo [27] than paclitaxel. The better efficacy of docetaxel has been attributed to its higher affinity for microtubules, higher intracellular concentration, and slower cellular efflux [27].

Published clinical results for docetaxel have shown the highest objective response rates in breast and ovarian carcinoma patients. Response rates ranging from 25% to 41% have been described for advanced ovarian carcinomas [9, 17, 51]. In breast carcinoma, docetaxel has shown significant activity as first-line therapy in metastatic disease, with response rates of 52–68% [7, 23, 50]. The drug has also demonstrated impressive activity in non-small-cell lung cancer (NSCLC), with dose-dependent cumulative response rates between 19% and 25% [15–17]. Additionally, response rates ranging from 32% to 42% in advanced head and neck cancer have been reported with docetaxel [6, 12]. As is the case for paclitaxel [40], this clinical success appears to conflict with our results in tumor models, where docetaxel failed to induce growth delay in murine squamous cell carcinomas. However, we examined only two types of syngeneic murine squamous cell carcinomas, and as has been shown for paclitaxel in vivo [14], considerable heterogeneity of response exists for human squamous cell carcinomas.

At the cellular level, docetaxel induced mitotic arrest in all tumors tested, but the extent of arrest varied greatly (Table 2). Carcinomas responded with higher arrest than sarcomas. However, the magnitude of mitotic arrest failed to show a significant correlation with the antitumor effect of docetaxel, although there was a trend in this direction. These findings, both with respect to the extent of induced mitotic arrest and its lack of correlation with antitumor efficacy, are similar to those reported for paclitaxel [40].

Docetaxel differed from paclitaxel in its ability to induce apoptosis. Docetaxel was less effective in this respect, inducing only a modest increase in the magnitude of apoptosis: the apoptotic index was less than 4% in all tumors with the exception of MCa-4, where it was 10.3%. The levels of neither induced nor spontaneous apoptosis correlated with docetaxel's antitumor effect. In contrast, paclitaxel is efficient in inducing apoptosis, and the degree of apoptosis significantly correlates with its anititumor efficacy [40].

Histological analysis of docetaxel-responding tumors revealed massive destruction of cells by another type of cell death, cell lysis. This mode of cell death was characterized by lysis of the cell membrane and spillage of altered nuclear material into the extracellular space, cellular disintegration, and intense eosinophilia. It also occurred to a lesser extent after treatment with paclitaxel [37, 40]. Lytic cell death was most extensive between 12 and 24 h after treatment with docetaxel.

We have previously reported [31] massive infiltration of MCa-4 carcinoma by mononuclear lymphoid cells when the tumor is treated with docetaxel. This reaction has not been reported to occur after treatment with paclitaxel. The present study showed that lymphoid infiltration was not a general histological feature of docetaxel-responding tumors. Of the ten responding tumors, it occurred in four (40%): MCa-4, MCa-29, MCa-K and OCa-I. Infiltration by mononuclear lymphoid cells was noted at 48 h and was particularly extensive by 72 h after treatment with docetaxel. The cellular composition of infiltrating cells differed between tumors: the majority of infiltrating cells in MCa-4, MCa-29, and OCa-I tumors had histological characteristics of macrophages; in the MCa-K tumor, the characteristics of most were those of lymphocytes. In some tumors the infiltration was so extensive that individual tumor cells were scarcely visible.

The cause of mononuclear infiltration of tumors after treatment with docetaxel is not clear. The infiltrative reaction, however, resembled that resulting from an antitumor immunological reaction [47]. Of the four tumors showing infiltration, only MCa-K is immunogenic [34], and the observed infiltrate was composed mainly of lymphocytes. Therefore, it is likely that massive destruction of tumor cells in the MCa-K tumor elicited or augmented an antitumor immune rejection. The infiltrative reaction may also have been the result of inflammatory reaction to massive cell lysis, a reaction not associated with the apoptotic mode of cell destruction.

The current literature contains conflicting reports regarding whether taxanes can influence immunomodulation. In one study, paclitaxel, but not docetaxel, has been shown to mimick the effect of lipopolysaccharides on murine macrophages, leading to the production of such cytokines as tumor necrosis factor (TNF) [29]. Other authors have confirmed that paclitaxel induces cytokine gene expression, such as TNF- [4, 5, 11] and interleukin-1 [4], as well as the release of TNF by macrophages [11], whereas docetaxel is unable to induce TNF-α gene expression [5]. Thus, the mechanisms responsible for tumor infiltration with lymphoid cells after docetaxel treatment have not yet been identified. In any case, the infiltrate is not a general feature of docetaxel-responding tumors, and its relationship to docetaxel's antitumor efficacy remains undefined.

Recently several genes or gene products have been shown to be involved in regulating cell and tumor response to cytotoxic agents, including chemotherapy [25, 26, 28, 55]. The tumor suppressor p53 gene, considered a guardian of the genome, appears to play a central role in the response of many cell types. Studies on the role of

p53 in cytotoxicity and antitumor efficacy by taxanes are limited, confined almost exclusively to paclitaxel, and provide discordant information [10, 45, 49, 52, 54]. Depending primarily on the tumor cell line used for the study, paclitaxel's cytotoxicity requires p53 [49, 54] or p53 has no role in the efficacy of paclitaxel [10, 52, 54]. Data from the present study showed that the antitumor efficacy of docetaxel did not depend on the p53 status of murine tumors. Of 15 tumors, 12 had wildtype p53 and 7 of these responded to docetaxel. Three tumors had mutant p53 and they all responded to docetaxel.

Several in vitro studies have explored the role of Bcl-2, an apoptosis-inhibitory oncogene, in the cytotoxicity of taxanes [3, 19–21]. Taxanes induce Bcl-2 phosphorylation, which results in decreased binding of Bcl-2 to the proapoptotic Bax protein, which in turn decreases the Bcl-2/Bax ratio, allowing the proapoptotic action of Bax [19–21]. Docetaxel is more effective than paclitaxel in the induction of Bcl-2 phosphorylation [21]. Clearly, the role of oncogenes in cytotoxicity by taxanes is still poorly defined and further studies are warranted. This is particularly true for antitumor efficacy of taxanes in vivo where multiple factors may be involved in determining tumor response to cytotoxic agents.

Overall, our results show that docetaxel has a wide range of antitumor efficacy, as measured by tumor growth delay in 15 different murine tumors, which was histologically associated with three kinds of induced cellular change. These were mitotic arrest, cell death by apoptosis or cell lysis, and infiltration of tumors with mononuclear lymphoid cells. Mitotic arrest was induced in all tumors, but to various degrees, and this cellular change did not correlate significantly with tumor growth delay. The drug's ability to induce apoptosis was weak, and was not responsible for its antitumor efficacy. In contrast, tumors that responded to docetaxel by significant tumor growth delay histologically displayed massive cell destruction by cell lysis, implying that this mode of cell death is the major mechanism underlying the antitumor efficacy of docetaxel. In some tumors that responded to docetaxel, there was massive tumor infiltration by lymphoid cells. At the molecular level, there was no dependency of antitumor efficacy of docetaxel on p53 mutational status of tumors. In general, on a milligram per kilogram basis docetaxel appeared to be more effective as an antitumor agent than paclitaxel.

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