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Original article

Antitumor activity of taxotere (RP 56976, NSC 628503), a new taxol analog, in experimental ovarian cancer

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Summary

Background: The new cytostatic agent taxol has clearly demonstrated its effectiveness in ovarian cancer patients. The synthesis of drugs related to taxol could overcome its limited natural supply and may have additional benefits, such as greater efficacy or better solubility. Taxotere (RP 56976, NSC 628503) is such a compound. We investigated the drug for its antitumor activity in human ovarian cancer xenografts.

Materials and methods: Five human ovarian cancer lines were selected with respect to differences in histological subtypes, growth rates and chemosensitivity to conventional cytostatic agents. Tumors were implanted as fragments s.c. into both flanks of female nude mice (Hsd: athymic nude-nu). Treatment was started in groups of 5–8 mice at the time mean tumor volume measured 50–150 mm^3. Taxotere was injected i.v. weekly × 2. Drug efficacy was expressed as the maximum percentage of growth inhibition of treated tumors as compared to control tumors.

Results: At the maximum tolerated dose of 15–20 mg/kg for weekly i.v. × 2 injections, taxotere induced a mean weight loss of 10%–15% of the initial weight within 2 weeks after the first injection. The maximum percentage of growth inhibition obtained was ⩾50% in 4/5 lines and ⩾90% in 3/5 lines. In 2 lines, taxotere appeared more effective than cisplatin, cyclophosphamide or doxorubicin, drugs studied previously at maximum tolerated doses in the same tumor lines.

Conclusion: Our findings in human ovarian cancer xenografts hold promise for the efficacy of taxotere in this type of disease in the clinic.

Key words: antitumor activity, human tumor xenografts, ovarian cancer, taxotere

Introduction

Only recently, taxol has emerged as an important cytostatic agent in the treatment of cancer. Interest in taxol started in the late 1960s, when a crude extract of the bark from the Pacific yew *Taxus brevifolia L.* was shown to have a broad cytotoxicity profile in the large screening program conducted in murine tumors at the National Cancer Institute [1]. The active constituent of the bark extract, taxol, was found to bind preferentially to microtubules, induce tubulin polymerization and form extremely stable and non-functional microtubules [2]. Preclinical development of taxol has proceeded slowly, because of its scarcity and the difficulty of large-scale isolation, extraction, and preparation. In addition, poor aqueous solubility has hampered the development of a suitable formulation.

Phase I clinical trials of taxol started in 1983 [3]. Hypersensitivity reactions were of major concern, but continuous infusions and anti-allergic premedications limited the incidence and severity of these side effects [4]. Neutropenia was the principal dose-limiting effect, followed by mucositis, peripheral neurotoxicity, and transient asymptomatic bradycardia. Phase II clinical trials are not yet completed, but taxol was clearly demonstrated to be effective in advanced and cisplatin-resistant ovarian cancer patients [3, 5].

Natural supplies of taxol are limited, but synthesis of drugs related to taxol (taxoids) could overcome this problem while bestowing additional benefits, such as greater efficacy or better solubility. Such a compound is taxotere, which was obtained by semisynthesis from a non-cytotoxic precursor extracted from the needles of the tree, *Taxus baccata L.* [6, 7]. Taxotere was evaluated for antitumor activity in a variety of transplantable murine tumors and found to be effective against 9/11 of them [8, 9]. Its mechanism of action appeared to be similar to that of taxol [10]. Phase I clinical trials recently completed showed neutropenia as the dose-limiting side effect [11–15]. The reasonable toxicity profile and the preliminary signs of antitumor activity warranted introduction of the drug into phase II trials in a variety of malignancies.

In an extension of the preclinical antitumor activity analysis of taxotere in murine tumors we have focused on the potential efficacy of the drug against human ovarian cancer. For this purpose 5 human ovarian cancer lines were used, which were grown as s.c. xenografts in nude mice. The growth inhibition obtained with taxotere was compared with previous data on cisplatin, cyclophosphamide and doxorubicin to obtain insight into the particular cytotoxic effects of this taxol analog.

Materials and methods

Animals

Female nude mice (Hsd: athymic nude-nu) were purchased from Harlan Cpb, Zeist, The Netherlands, at the age of 6 weeks. The animals were maintained in cages with paper filter covers under controlled athmospheric conditions. Cages, covers, bedding, food and water were changed and sterilized weekly. Animals were handled in a sterile manner in a laminar down-flow hood.

Tumor lines

The tumor lines grown as s.c. xenografts were of human ovarian cancer origin and were selected from a larger panel based on differences in histological subtype, growth rate and chemosensitivity to conventional cytostatic agents [16, 17]. The tumor lines were the following: Ov.Pe, a moderately differentiated mucinous adenocarcinoma with a volume doubling time (T_D) of 8 days; Ov.Sh, a poorly differentiated serous adenocarcinoma with a T_D of 15 days; FMa, a poorly differentiated mucinous adenocarcinoma with a T_D of 5.5 days; FKo, a moderately differentiated serous adenocarcinoma with a T_D of 12 days; MRI-H-207, an undifferentiated carcinoma of ovarian origin with a T_D of 3.5 days. Tumors were transplanted s.c. as fragments with a diameter of 2–3 mm through a small skin incision and closed with a metal clamp in both flanks of the animals. Upon growth, tumors were measured weekly or twice a week (for MRI-H-207) in 3 dimensions with a vernier caliper by the same observer. The volume was calculated by the equation length × width × thickness × 0.5, and expressed in mm^3.

Treatment

Taxotere (RP 56976) was provided by Rhône-Poulenc Rorer and was dissolved in ethanol as a stock solution of 50 mg/ml. On each injection day the stock solution (1 part) was mixed with polysorbate 80 (1 part) after which glucose 5% (18 parts) was added.

At the start of treatment (day 0), groups of 5–8 tumor-bearing mice were formed to provide a mean tumor volume between 50–150 mm^3 in each group. Mean initial weight of the animals was 24.9 g (SD ± 0.9 g). Taxotere was injected i.v. weekly × 2 at the maximum tolerated dose (MTD). This dose resulted in a mean weight loss of 10%–15% of the initial weight within 2 weeks after the first injection. Deaths occurring within 2 weeks after the last injection were considered as toxic deaths; these animals were excluded from evaluation of drug efficacy.

For evaluation of drug efficacy, the tumor volumes were converted to values related to the initial volume [17]. This relative tumor volume was expressed by the formula V_T/V_O, where V_T is the volume on any given day and V_O the volume at the start of treatment. The ratio of the mean relative volume of treated tumors over that of control tumors multiplied by 100% (T/C%) was assessed on each day of measurement. From the lowest T/C% obtained within 5 weeks after the last injection, growth inhibition (100%-T/C%) was calculated to express drug efficacy. A drug was considered to be active if the growth inhibition obtained in a given human tumor line was ⩾50%, very active ⩾75%, and inactive if inhibition of growth was <50% [17, 18]. Complete remission was defined as the disappearance of tumors upon treatment for a period of at least one month.

Antitumor effects were evaluated with Student's t-test.

Results

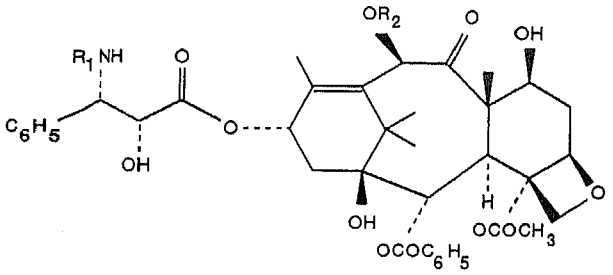
The MTD of taxotere for i.v. injections was determined weekly × 2, first in non-tumor-bearing mice. Doses of 30 mg/kg, 25 mg/kg, 20 mg/kg and 15 mg/kg resulted in a median weight loss of 20%, 13%, 9% and 7%, respectively. Initially, the 20 mg/kg dose was chosen. At this dose, excessive weight loss and 5/7 toxic deaths were observed in Ov.Pe-bearing mice, and because of this the taxotere dose was reduced to 15 mg/kg i.v. weekly × 2. Only Ov.Sh and MRI-H-207-bearing mice could tolerate the 20 mg/kg dose (Table 1). In the last experiments the nadir of weight loss was reached on day 14 after the start of treatment. In general, mice had recovered at day 21 and showed no signs of late toxicity.

The antitumor activity of taxotere expressed as the maximum percentage of growth inhibition reached on a particular day of measurement after the start of treatment (day 0) is given in Table 1. The growth curves of treated and control tumors are represented in Fig. 2. In 4/5 tumor lines growth inhibition ⩾50% was obtained.

Table 1. Growth inhibition and toxicity of taxotere studied i.v. weekly × 2 in 5 human ovarian cancer lines grown s.c. in nude mice.

Tumor line	Dose/inj. mg/kg	Antitumor activity			Weight loss at nadir mean ± SEM	Toxic deaths
		Mean RV[a] (range)	Day[b]	GI[c]		
Ov.Pe	0	4.11 (2.00–8.70)	20			
	15	0.28 (0.00–0.88)	20	93%[d]	9% ± 3%	0/6
Ov.Sh	0	3.59 (2.64–4.77)	28			
	15	3.23 (1.88–5.05)	28	10%	0%	0/7
Ov.Sh	0	5.36 (2.03–13.1)	42			
	20	2.09 (0.76–3.24)	42	61%	15% ± 4%	0/8
FMa	0	11.77 (3.00–23.4)	29			
	15	1.14 (0.32–3.05)	29	90%[d]	16% ± 4%	0/6
FKo	0	5.98 (2.10–10.6)	35			
	15	3.66 (2.22–5.79)	35	39%[d]	16% ± 3%	0/8
MRI-H-207	0	23.38 (10.6–49.3)	20			
	15	1.05 (0.14–5.79)	20	95%[d]	11% ± 2%	0/6
MRI-H-207	0	25.84 (12.7–44.6)	17			
	20	0.75 (0.00–3.21)	17	97%[d]	15% ± 4%	1/7

[a] RV, relative tumor volume; [b] Day after start of treatment on which maximum growth inhibition was calculated; [c] GI, growth inhibition measured as 100%-T/C%; [d] Significantly different from control tumors, $p < 0.05$.



Taxotere: R_1 = $-COOC(CH_3)_3$; R_2 = H
Taxol: R_1 = $-COC_6H_5$; R_2 = $-COCH_3$

Fig. 1. Taxotere (RP 56976, NSC 628503); *N*-debenzoyl-*N*-*tert*-butoxycarbonyl-10-deacetyl taxol.

Table 2. Comparative data of growth inhibition obtained by taxotere and conventional cytostatic agents[a] in 5 human ovarian cancer lines.

Tumor line	Taxotere	Cisplatin	Cyclophosphamide	Doxorubicin
Ov.Pe	93[b]	37[b]	62[b]	54[b]
Ov.Sh	61	94[b]	94[b]	91[b]
FMa	90[b]	75[b]	64[b]	53[b]
FKo	39[b]	42[b]	4	8
MRI-H-207	97[b]	CR[c]	CR[c]	CR[c]

[a] Cisplatin 5 mg/kg i.v. weekly × 2; cyclophosphamide 150 mg/kg i.p. 2-weekly × 2; doxorubicin 8 mg/kg i.v. weekly × 2.
[b] Significantly different from control tumors, $p < 0.05$.
[c] CR, complete remission.

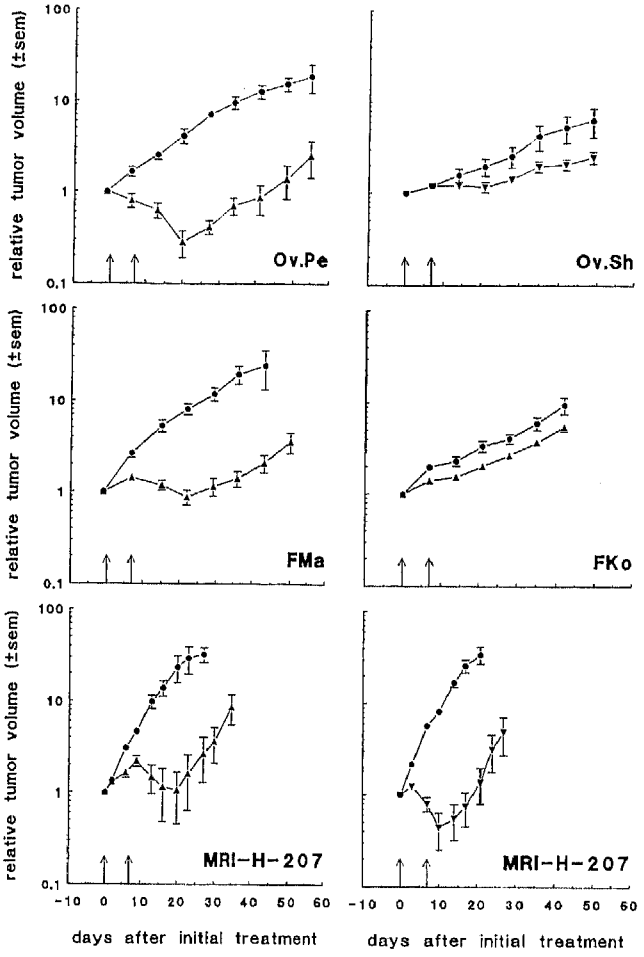


Fig. 2. Treatment results of taxotere 15 mg/kg i.v. weekly × 2 (▲) or 20 mg/kg i.v. weekly × 2 (▼) in 5 human ovarian cancer xenografts, as compared to control tumors (●). The relative tumor volume is the tumor volume on any given day V_T/ the volume at the start of treatment V_0. The graphs were drawn from the mean (± SEM) of the relative tumor volumes.

In terms of drug activity to induce growth inhibition ⩾75%, taxotere appeared very effective in Ov.Pe, FMa and MRI-H-207 tumors, where growth inhibition was 93%, 90% and 97%, respectively. No complete remissions of tumors were observed. In FKo tumors, known to be resistant to conventional cytostatic agents, taxotere was not able to induce any significant growth inhibition.

Table 2 is included for comparison of drug efficacy; it shows the percentage of growth inhibition obtained with the MTD of cisplatin, cyclophosphamide or doxorubicin in the same tumor lines used in previous experiments. Cisplatin induced growth inhibition ⩾50% in 3/5 lines. This percentage was reached in 4/5 lines for both cyclophosphamide and doxorubicin. The 3 drugs were most effective in Ov.Sh and MRI-H-207 tumors, while MRI-H-207 tumors disappeared completely. None of the 3 drugs were effective in FKo tumors.

Discussion

In general, comparison of response rates generated by clinical trials in cancer patients and obtained in xenografts grown s.c. in nude mice reveals a good correlation for the antitumor activity profile of a particular drug [19, 20]. Also, the human tumor xenograft model shows good predictibility for the differential efficacy of analogs of conventional cytostatic agents [21]. In the past, we and others have confirmed the value of a panel of human ovarian cancer lines for preclinical phase II drug screening of both standard and investigational drugs [17, 18, 22, 23].

In the present study, we demonstrated that taxotere can induce growth inhibition ⩾50% in 4/5 human ovarian cancer lines. Taxotere appeared more effective than any of the 3 conventional cytostatic agents, cisplatin, cyclophosphamide and doxorubicin, in both Ov.Pe and FMa tumors. It was found highly active (97% tumor growth inhibition) in MRI-H-207 tumors, but slightly less so than the 3 conventional drugs. In Ov.Sh tumors, taxotere induced a lower percentage of growth inhibition and its activity was insignificant in FKo tumors. Our findings hold promise for the clinical efficacy of taxotere, as taxol was clearly demonstrated to be useful in the treatment of advanced ovarian cancer patients [5].

Only a few studies comparing the antitumor effects of taxotere with those of the parent compound, taxol,

have been carried out. Ringel and Horwitz [10] demonstrated in vitro, that taxotere is a slightly more effective promotor of tubulin polymerization than taxol, it is a 2.5-fold more potent inhibitor of cell replication of the mouse macrophage-like cell line, J774.2, and the mouse lymphocytic leukemia cell line, P388, and at least a 5-fold more potent inhibitor of the taxol-resistant variant cell line, J7.TAX-50. Riou et al. [24] found a 1.3- to 12-fold higher cytotoxicity for taxotere as compared to taxol in several murine and human malignant cell lines in vitro, which could be explained by the fact that its affinity for microtubules is higher than that of taxol. Bissery et al. [9] reported that taxotere had superior activity in vivo in s.c. B16 mouse melanoma upon administration of the drugs at equitoxic doses. All of these observations indicate a greater potency for taxotere than for taxol. Whether this advantage of taxotere will ultimately lead to an improved therapeutic index in the clinic has yet to be seen.

The high efficacy of taxotere found in our human tumor lines derived from ovarian cancer should be confirmed in this disease in the clinic. In phase I trials, partial remissions have occasionally been observed in ovarian cancer patients [11] and reduction of CA125 serum concentrations in others [12, 14]. Notably, in 2 of our lines, both of mucinous origin, taxotere appeared to be superior to the conventional cytostatic agents tested. Thus, provided that side effects are tolerable, taxotere included in combination chemotherapy regimens might ultimately lead to an increased response rate in ovarian cancer.

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