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Short communication

Cross-resistance of drug-resistant murine P388 leukemias to taxol in vivo*

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Summary. The antimicrotubule agent taxol (NSC 125973) has shown clinical antitumor activity against several classically refractory tumors. We developed a drug-resistance profile for taxol using ten drug-resistant P388 leukemias to identify potentially useful guides for patient selection for further clinical trials of taxol and possible non-cross-resistant drug combinations with taxol. Multidrug-resistant P388 leukemias exhibited either clear (leukemia resistant to amsacrine) or marginal cross-resistance (leukemias resistant to doxorubicin, actinomycin D, and mitoxantrone) to taxol. Leukemias resistant to vincristine (non-multidrug-resistant leukemia), camptothecin, melphalan, cisplatin, 1-β-D-arabinofuranosylcytosine, and methotrexate were not cross-resistant to taxol. The data suggest that (1) it may be important to exclude or to monitor with extra care patients who have previously been treated with amsacrine, doxorubicin, actinomycin D, or mitoxantrone and (2) a combination of one of the non-cross-resistant drugs and taxol might exhibit therapeutic synergism.

Introduction

The antimicrotubule agent taxol (NSC 125973) has shown clinical antitumor activity against several classically refractory tumors, including cisplatin-resistant ovarian carcinoma (phase II trials) and malignant melanoma and non-small-cell lung carcinoma (phase I trials) [7]. Clinical trials of taxol and of the related drug taxotere are under way in this country and abroad. Drug resistance that may be either inherent or acquired seems likely to be encountered in these trials. Development of a drug-resistance profile for taxol may identify potentially useful guides for patient selection for further clinical trials of taxol and possible non-cross-resistant drug combinations with taxol. This information should aid in the design of strategies for the optimal use of the drug. In vivo models may be preferable for the development of drug-resistance profiles because of evidence that drug resistance may not be comparable between in vitro and in vivo models of the same cell line [9]. This report describes an in vivo cross-resistance profile for taxol that we developed using drug-resistant murine leukemias.

Materials and methods

The in vivo sensitivity of P388/0 and ten drug-resistant P388 leukemias to taxol was determined as previously described for other antitumor agents [10]. $CD2F_1$ mice were implanted i.p. with 10^6 cells of either P388/0 or P388/drug-resistant leukemia on day 0. Taxol was evaluated at several i.p. dose levels (ranging from toxic to nontoxic). There were 10 mice in each dose group; tumor-bearing control mice (20/experiment) were left untreated. Mice were observed for increase in life span (ILS). In each experiment, additional groups were treated with a range of doses of an appropriate drug to confirm the resistance of a P388/drug-resistant leukemia. Moreover, in each experiment a P388/drug-resistant leukemia was compared directly with the parent or wild-type P388/0 leukemia, and the parallel groups of mice were treated identically with a single-drug preparation.

Antitumor activity was assessed on the basis of the percentage of median ILS and the net $\log_{10}$ cell kill. Calculations of the net $\log_{10}$ cell kill were made from the tumor-doubling time that was determined from an internal tumor titration consisting of implants from serial 10-fold

Abbreviations: ILS, increase in life span; P388/ACT-D, actinomycin D-resistant P388 leukemia; P388/ADR, doxorubicin-resistant P388 leukemia; P388/AMSA, amsacrine-resistant P388 leukemia; P388/ARA-C, 1-β-D-arabinofuranosylcytosine-resistant P388 leukemia; P388/CPT, camptothecin-resistant P388 leukemia; P388/DDPt, cisplatin-resistant P388 leukemia; P388/DIOHA, mitoxantrone acetate-resistant P388 leukemia; P388/L-PAM, melphalan-resistant P388 leukemia; P388/MTX, methotrexate-resistant P388 leukemia; P388/VCR, vincristine-resistant P388 leukemia; P388/0, parental P388 leukemia

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Table 1. Activity of taxol against drug-resistant P388 leukemias in vivo

Experiment	Resistant leukemia[a]	Optimal i.p. dose[b] ($\leq LD_{10}$, mg/kg)	Therapeutic response				Cross-resistance?
			Sensitive leukemia		Resistant leukemia[d]		
			Median % ILS	Approx. $\log_{10}$ change in tumor burden[c]	Median % ILS	Approx. $\log_{10}$ change in tumor burden[c]	
1	P388/ADR	15.0	+36	0.0	+4	+1.5	Marginal
2		15.0	+50	−1.2	0	+1.4	
1	P388/AMSA	22.5	+54	−1.6	−4	+1.4	Yes
2		15.0	+45	−0.6	0	+1.6	
1	P388/VCR	22.5	+40	Toxic	+60	−1.5	No
		15.0	+50	−0.7	+40	0.0	
2		15.0	+50	−1.1	+40	0.0	
3		10.0	+40	0.0	+40	0.0	
1	P388/ACT-D	22.5	+55	−1.0	+20	+1.2	Marginal
2		22.5	+33	+0.3	+22	+1.3	
3		22.5	+50	−0.7	+20	+1.3	
1	P388/DIOHA	15.0	+36	0.0	+25	+1.1	Marginal
2		15.0	+55	−0.9	+38	+0.5	
3		15.0	+55	−1.1	+20	+1.3	
1	P388/CPT	22.5	+60	−1.3	+63	−2.2	No
2		15.0	+55	−1.1	+57	−1.6	
1	P388/L-PAM	15.0	+37	−0.4	+17	Toxic	No
		10.0	+16	+1.3	+31	−0.3	
2		10.0	+45	−0.4	+46	−1.1	
1	P388/DDPt	15.0	+55	−1.0	+72	−3.4	No
2		15.0	+50	−0.8	+58	−2.4	
1	P388/ARA-C	15.0	+36	0.0	+18	+1.3	No
2		10.0	+40	−0.4	+35	+0.3	
1	P388/MTX	10.0	+31	+0.4	+70	−2.3	No
2		22.5	+50	−1.1	+54	Toxic	
		15.0	+36	0.0	+50	−1.1	

[a] ADR, doxorubicin; AMSA, amsacrine; VCR, vincristine; ACT-D, actinomycin D; DIOHA, mitoxantrone acetate; CPT, camptothecin; L-PAM, melphalan; DDPt, cisplatin; ARA-C, 1-β-D-arabinofuranosylcytosine; MTX, methotrexate

[b] Taxol was given to mice on days 1–5

[c] $\log_{10}$ change in the tumor cell population between the beginning and the end of therapy, based on the median day of death of mice that died

[d] In these studies, the average degree of resistance of a drug-resistant subline in comparison with its parental line was as follows: ADR, 5 $\log_{10}$ units; AMSA, 5 $\log_{10}$ units; VCR, 5 $\log_{10}$ units; ACT-D, 3 $\log_{10}$ units; DIOHA, 6 $\log_{10}$ units; CPT, 7 $\log_{10}$ units; L-PAM, 5 $\log_{10}$ units; DDPt, 8 $\log_{10}$ units; ARA-C, 7 $\log_{10}$ units; and MTX, 3 $\log_{10}$ units

dilutions of P388 cells [8]. Long-term (45-day) survivors were excluded from calculations of ILS and tumor cell kill. For the assessment of tumor cell kill at the end of the treatment, the survival difference between treated and control groups was adjusted to account for the regrowth of tumor cell populations that may occur between individual treatments [6]. Cross-resistance was defined as a decrease in the sensitivity (>2 $\log_{10}$ units of cell kill) of P388/drug-resistant leukemia to taxol as compared with that concurrently observed for P388/0 leukemia. Similarly, marginal cross-resistance was defined as a decrease in sensitivity of approximately 2 $\log_{10}$ units. Collateral sensitivity was defined as an increase in the sensitivity (by >2 $\log_{10}$ units of cell kill) of P388/drug-resistant leukemia to taxol over that concurrently observed for P388/0 leukemia.

Results and discussion

The in vivo cross-resistance profile for taxol is shown in Table 1. Multidrug-resistant P388 leukemias exhibited either clear (P388/AMSA) or marginal cross-resistance (P388/ADR, P388/ACT-D, and P388/DIOHA) to the drug. P388/VCR (non-multidrug-resistant leukemia), P388/CPT, P388/L-PAM, P388/DDPt, P388/ARA-C, and P388/MTX were not cross-resistant to taxol. The data suggest that P388/DDPt is possibly collaterally sensitive to the drug.

Johnson and co-workers [5] have shown that another in vivo P388/ADR leukemia is also cross-resistant to taxol and that P388/AMSA and P388/CPT (the same leukemias that were used in the present studies) are not cross-resistant to taxol [2, 4]. The cross-resistance profile of P388/AMSA has changed since the original characterization of the leukemia; P388/AMSA is now cross-resistant to actinomycin D, vincristine, vinblastine, and taxol [10].

As new agents enter phase II clinical trials, the selection of patients, most of whom have previously been treated

with one or more drugs, may be critical to the success of the trials [3]. Information on the patterns of cross-resistance or collateral sensitivity among various antitumor agents may be helpful in the selection of patients for treatment with taxol. For these trials, it may be important to exclude or to monitor with extra care patients who have previously been treated with amsacrine, doxorubicin, actinomycin D, or mitoxantrone.

The observation of possible collateral sensitivity of P388/DDPt to taxol suggests that a combination of cisplatin and taxol might exhibit therapeutic synergism and that mechanistic studies may be warranted. Phase II trials have shown taxol to be active against cisplatin-resistant ovarian carcinoma [7]. P388/DDPt has been reported to be collaterally sensitive to other antitumor agents (amsacrine, batracylin, echinomycin, etoposide, flavone acetic acid, fludarabine phosphate, merbarone, and mitoxantrone) [11]. Interestingly, P388/VCR was not cross-resistant to taxol. Some vincristine-resistant Chinese hamster ovary cell lines (thought to be membrane mutations) are cross-resistant to taxol, whereas others (thought to be tubulin mutations) are not [1]. Because taxol inhibits microtubule disassembly and vincristine inhibits microtubule assembly, the non-cross-resistant drug combination of taxol and vincristine should be explored for possible therapeutic synergism. As always, none of the above approaches may be applied clinically without caution and concern for the recognized gap between preclinical prediction and clinical validation.

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