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Antitumor drug cross-resistance in vivo in a cisplatin-resistant murine P388 leukemia

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Summary. Since 1978, over 50 clinically useful antitumor drugs or new candidate antitumor agents have been evaluated in vivo against cisplatin-resistant P388 leukemia (P388/DDPt) in our laboratories. Analysis of this data base has yielded insights into the cross-resistance, collateral sensitivity, and mechanisms of resistance of P388/DDPt. P388/DDPt was cross-resistant or marginally cross-resistant to eight agents [carmethizole · HCl, rhizoxin, dibromodulcitol, spirohydantoin mustard, hepsulfam, arabinosyl-5-azacytosine (ara-AC), tiazofurin, and deoxyspergualin]. Of these eight agents, the latter six have entered various phases of clinical trials. For these trials, it may be important to exclude or to monitor with extra care patients who have previously been treated with cisplatin. P388/DDPt was collaterally sensitive to six agents [fludarabine phosphate (2-F-ara-AMP), amsacrine (AMSA), mitoxantrone, etoposide (VP-16), batracylin, and flavone acetic acid] and, possibly, to two others (merbarone and echinomycin). These observations of collateral sensitivity suggest that a combination of cisplatin plus any one of these drugs might exhibit therapeutic synergism. Therapeutic synergism has been observed in animal models for combinations of cisplatin plus VP-16, AMSA, or mitoxantrone. The observation of collateral sensitivity for P388/DDPt to four agents (AMSA, mitoxantrone, merbarone, and VP-16) that have been reported to interact with DNA topoisomerase II suggests the possible involvement of the latter in cisplatin resistance. Both the increased sensitivity of P388/DDPt to these agents and a portion of its resistance to cisplatin could be the result of an increase in DNA topoisomerase II activity.

Abbreviations: AMSA, amsacrine; ara-AC, arabinosyl-5-azacytosine; DTP, Developmental Therapeutics Program; DCT, Division of Cancer Treatment; 2-F-ara-AMP, fludarabine phosphate; GSH, glutathione; ILS, increase in life span; LD_{10}, dose producing lethality in 10% of the test group; NCI, National Cancer Institute; P388/DDPt, cisplatin-resistant P388 leukemia; P388/0, cisplatin-sensitive P388 leukemia; VP-16, etoposide

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Introduction

Teicher and co-workers [25] recently published results of their studies that revealed a difference between the resistance of tumor cells to alkylating agents when in vivo and in vitro models were compared. Tumor cells were selected for alkylating-agent resistance by treatment of successive in vivo passages with selected drugs for up to 6 months. The resulting tumor lines exhibited 6- to 21,000-fold resistance in vivo (as compared with the parent line and depending on the dose) to the drug for which resistance was selected. When these drug-resistant cells were cultured, no resistance was observed. Cells maintained in culture for up to 6 weeks exhibited unattenuated drug resistance in vivo when they were reimplanted in mice. These results emphasize the importance of studying drug resistance in animal models as well as in cultured cells and of distinguishing, if possible, which models more closely resemble the clinical situation.

The most extensive data on in vivo models of drug resistance and cross-resistance have come from Schabel and co-workers [22]. Their initial report included results of in vivo cross-resistance studies on 79 antitumor drugs in 8 lines of murine L1210 leukemia (the parent, sensitive line plus 7 drug-resistant lines) and 74 drugs in 13 lines of P388 leukemia (the parent, sensitive line plus 12 drug-resistant lines). The drug-resistant lines were developed, maintained, and studied in vivo [22]. We have continued to use these in vivo models for evaluation of new drugs of potential clinical interest [4, 6–9, 16, 30].

P388/DDPt has been of particular interest to us because of related studies from our group exploring the mechanism(s) of cisplatin resistance in murine leukemia [29]. Additionally, we wanted to test the hypothesis that the in vivo cross-resistance profile of P388/DDPt, coupled with knowledge of the known mechanisms of action of various antitumor drugs, would yield insights into the cross-resistance, collateral sensitivity, and mechanisms of resistance of P388/DDPt. We report herein that the in vivo cross-resistance profile of P388/DDPt has enabled the identification of potentially useful guides for patient selection for

Table 1. Cross-resistance of P388/DDPt to alkylating agents

Drug	NSC number	Optimal i.p. dosage ($\leq LD_{10}$, mg/kg per dose)	Schedule	Therapeutic response						Comments
				P388/0 Leukemia			P388/DDPt Leukemia			
				Median % ILS (dying mice only)	Approx. log_{10} change in tumor burden after last treatment[a]	45-day survivors	Median % ILS (dying mice only)	Approx. log_{10} change in tumor burden after last treatment[a]	45-day survivors	
L-phenylalanine mustard	8806	20	Day 1	+109	–7	8/10	+148	–7	3/10	No cross-resistance
		15	Day 1	+190	–7	6/10	+115	–7	3/10	
Cyclophosphamide	26271	265	Day 1	+318	–7	5/10	+108	–7	2/10	No cross-resistance
		265	Day 1	+100	–6	5/ 9	+119	–7	4/10	
Mitomycin C	26980	8	Day 1	+122	–7	0/10	+59	–7	0/10	No cross-resistance
		8	Day 1	+78	–7	0/10	+36	–6	0/10	
Dibromodulcitol	104800	120	Days 1–5	+95	–4	0/10	+23	+1	0/10	Cross-resistance
Cisplatin[b]	119875	5.3	Days 1, 5, 9	+167	–6	0/10	+16	+2	0/10	Resistance
Spirohydantoin mustard	172112	9	Days 1–9	+139	–4	0/10	+42	+1	0/10	Cross-resistance
		9	Days 1–9	+159	–7	0/10	+92	–2	0/10	
cis-Dichloro-(1,2-diamino-cyclohexane) platinum(II)	194814	15[c]	Day 1	+90	–7	0/10	+61	–5	0/10	No cross-resistance
		7.5[c]	Days 1, 5, 9, 13	+122	–1	0/10	+71	0	0/10	
Carboxyphthalato-1,2-diamino-cyclohexane platinum	271674	40[c]	Day 1	+65	–5	0/10	+34	–3	0/10	No cross-resistance
		20	Days 1, 5, 9, 13	+86	+1	0/10	+56	+2	0/10	
Teroxirone	296934	22	Days 1–5	+135	–6	0/10	+92	–6	0/10	No cross-resistance
		33	Days 1–5	+160	–7	4/10	+84	–6	1/10	
Hepsulfam	329680	67	Days 1–5	+72	–4	0/10	+29	0	0/10	Cross-resistance
		67	Days 1–5	+90	–5	0/10	+33	–1	0/10	
Clomesone	338947	75	Days 1–5	–	–8	10/10	+155	–7	2/10	No cross-resistance
		100	Days 1–5	–	–8	10/10	+166	–7	5/10	
Piperazine, 1-(2-chloroethyl)-4-(3-chloropropyl)-, dihydrochloride	344007	13	Days 1–5	+130	–7	0/10	+66	–6	0/10	No cross-resistance
		9	Days 1–5	+93	–3	0/10	+65	–3	0/10	
Cyclodisone	348948	50	Days 1–5	+136	–7	5/10	+70	–6	0/10	No cross-resistance
		50	Days 1–5	+168	–7	8/10	+118	–8	6/10	
Mitozolomide	353451	22	Days 1–5	+311	–7	8/10	–	–8	10/10	No cross-resistance
		10	Days 1–5	+170	–7	2/10	+75	–7	0/10	
Pyrazine diazo-hydroxide	361456	38	Days 1–9	+201	–7	1/10	+119	–7	0/10	No cross-resistance
		38	Days 1–9	+168	–6	0/10	+132	–7	0/10	
Tetraplatin	363812	8	Days 1, 5, 9	+140	–4	0/10	+103	–4	0/10	No cross-resistance
		8	Days 1, 5, 9	+110	–2	0/10	+96	–3	0/10	
1,2-Ethanediamine, *N,N*-bis(2-chloroethyl)-, dihydrochloride	364989	1.5	Days 1–5	+136	–7	2/10	+88	–8	2/10	No cross-resistance
BCNU	409962	30	Day 1	+268	–7	8/10	+74	–8	6/10	No cross-resistance
		30	Day 1	+136	–7	9/10	+60	–7	1/10	
Carmethizole · HCl	602668	50	Days 1–5	+103	–4	0/10	+36	–1	0/10	Cross-resistance
		51	Days 1–5	+86	–4	0/10	+26	0	0/10	

[a] Log_{10} change in viable tumor cell population at the end of therapy as compared with that at the start of therapy, based on the median day of death among the animals that died. For treatment with drugs that yielded only survivors, the log_{10} change is based on the day of evaluation (day 45)

[b] Values for % ILS, log_{10} change in tumor burden, and number of survivors are the means of 32 experiments

[c] Highest dosage tested

Table 2. Cross-resistance of P388/DDPt to antimetabolites

Drug	NSC number	Optimal i.p. dosage ($\leq LD_{10}$, mg/kg per dose)	Schedule	Therapeutic response						Comments
				P388/0 Leukemia			P388/DDPt Leukemia			
				Median % ILS (dying mice only)	Approx. log_{10} change in tumor burden after last treatment[a]	45-day survivors	Median % ILS (dying mice only)	Approx. log_{10} change in tumor burden after last treatment[a]	45-day survivors	
Methotrexate	740	3	Days 1–9	+112	–3	0/10				No cross-resistance
		2.5	Days 1–9				+103	–7	0/10	
		3.5	Days 1–9	+121	–4	0/10	+88	–4	0/10	
5-Fluorouracil	19893	35	Days 1–5	+103	–4	0/10	+88	–6	0/10	No cross-resistance
		30	Days 1–5	+77	–4	0/10	+78	–3	0/10	
PalmO-ara-C	135692	150	Day 1	+189	–7	1/10	+142	–7	0/10	No cross-resistance
		150	Day 1	+210	–7	0/10	+123	–7	1/10	
Trimetrexate	249008	90	Days 1–9	+83	–1	0/10	+49	+1	0/10	No cross-resistance
		60	Days 1–9	+70	0	0/10	+32	+1	0/10	
Triciribine phosphate	280594	80	Days 1–5	+20	+1	0/10	+11	+1	0/10	No cross-resistance
		80	Days 1–5	+40	0	0/ 5	+50	–2	0/ 5	
Ara-AC	281272	100[b]	Days 1–9	+171	–7	0/10	+84	–3	0/10	Cross-resistance
		150[b]	Days 1–9	+184	–7	0/10	+79	–4	0/10	
Tiazofurin	286193	500[b]	Days 1–9	+118	–5	0/10	+51	+1	0/10	Cross-resistance
		500[b]	Days 1–9	+100	–2	0/10	+43	+1	0/10	
2-F-ara-AMP	312887	134	Days 1–9	+105	–2	0/10	+85	–3	0/10	Collateral sensitivity
		200	Days 1–9	+113	–4	0/10	+161	–7	0/10	
		134	Days 1–9	+103	–2	0/10	+165	–7	1/ 9	

[a] Log_{10} change in viable tumor cell population at the end of therapy as compared with that at the start of therapy, based on the median day of death among the animals that died

[b] Highest dosage tested

clinical trials of new antitumor drugs, of possible noncross-resistant drug combinations with cisplatin, and of a possible biochemical mechanism of resistance to cisplatin. A portion of the data has previously been reported in an abbreviated form [22].

Materials and methods

Drugs. Antitumor drugs were provided by either the Drug Synthesis and Chemistry Branch, DTP, DCT, NCI (Bethesda, Md.), or the Organic Chemistry Department, Southern Research Institute.

Mice and P388 leukemia. BALB/c × DBA/2 F_1 ($CD2F_1$) mice were obtained from various commercial suppliers and housed in open-top, stainless steel cages. Mice were allowed commercial mouse food and water ad libitum. P388/0 was obtained from the DTP Tumor Repository, DCT, NCI (Frederick, Md.), whereas P388/DDPt, which was selected for cisplatin resistance in vivo, was obtained from Dr. J. Burchenal (Memorial Sloan-Kettering Cancer Center, New York, N. Y.).

Evaluation of antitumor activity. $CD2F_1$ mice were implanted i.p. with 10^6 cells of either P388/0 or P388/DDPt. The day of tumor implantation was designated as day 0. Drugs were given i.p. according to the schedules listed in the tables. Each drug was evaluated at several doses (ranging from toxic to nontoxic), with each dose being given to 10 mice; tumor-bearing control mice (20/experiment) were left untreated. Mice were observed for increases in life span. In each experiment, tumor-bearing groups were treated with a range of doses of cisplatin to confirm the resistance of P388/DDPt. Moreover, in each experiment P388/DDPt was compared directly with P388/0, and the parallel groups of mice were treated identically with a single-drug preparation.

Quantitation of antitumor activity. 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 dilutions [20]. 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 treatment, the survival difference between treated and control groups was adjusted to account for regrowth of tumor cell populations that may occur between individual treatments [13]. The net log_{10} cell kill was calculated as follows:

$$\text{Net } \log_{10} \text{ cell kill} = \frac{(T\text{–}C) - (\text{duration of treatment in days})}{3.32 \times T_d}$$

where (T–C) is the difference in the median day of death between the treated (T) and the control (C) groups and T_d is the mean tumor-doubling time (in days) calculated from a log-linear least-squares fit of the implant sizes and the median days of death of the titration groups. Log_{10} values for net cell kill were rounded to whole numbers.

Cross-resistance and collateral sensitivity. Cross-resistance was defined as a decrease in the sensitivity (by >2 log_{10} units of cell kill) of P388/DDPt leukemia to a drug 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. Collat-

Table 3. Cross-resistance of P388/DDPt to DNA-binding agents

Drug	NSC number	Optimal i.p. dosage ($\leq LD_{10}$, mg/kg per dose)	Schedule	Therapeutic response						Comments
				P388/0 Leukemia			P388/DDPt Leukemia			
				Median % ILS (dying mice only)	Approx. log_{10} change in tumor burden after last treatment[a]	45-day survivors	Median % ILS (dying mice only)	Approx. log_{10} change in tumor burden after last treatment[a]	45-day survivors	
Actinomycin D	3053	0.75	Day 1	+85	–6	0/10	+38	–4	0/10	Marginal cross-resistance?
Camptothecin	94600	10	Days 1, 5	+141	–7	0/10	+127	–7	5/10	No cross-resistance
		15	Days 1, 5	+147	–7	4/10	+116	–7	4/10	
Doxorubicin	123127	15	Day 1	+109	–7	0/10	+81	–7	3/10	No cross-resistance
		15	Day 1	+166	–7	3/10	+50	–7	9/10	
VP-16	141540	40	Days 1, 5, 9	+141	–6	6/10	+208	–7	8/10	No cross-resistance
		40	Days 1, 5, 9	+152	–5	6/10	+95	–4	6/10	
AMSA	249992	30	Days 1, 5, 9	+96	–2	0/10	+133	–7	0/10	Collateral sensitivity
		45	Days 1, 5, 9	+122	–4	0/10	+165	–7	6/10	
Menogaril	269148	15[b]	Days 1–9	+181	–7	1/10	+292	–7	1/10	No cross-resistance
Mitoxantrone	301739	1.8	Days 1, 5, 9	+130	–3	4/10	–	–8	10/10	Collateral sensitivity
Acodazole	305884	100	Days 1–5	+87	–3	0/10	+74	–4	0/10	No cross-resistance
		67	Days 1–5	+60	–1	0/10	+35	–2	0/10	
Amonafide	308847	16	Days 1–9	+81	–1	0/10	+63	–1	0/10	No cross-resistance
		16	Days 1–9	+100	–1	0/10	+73	–2	0/10	
Pibenzimol	322921	67	Days 1–9	+54	+1	0/10	+40	+1	0/10	No cross-resistance
		100	Days 1–9	+65	+1	0/10	+57	0	0/10	
Merbarone	336628	150[b]	Days 1–9	+145	–5	3/ 9	+156	–7	6/ 9	–[c]
Piroxantrone	349174	85[b]	Day 1	+185	–7	6/10	–	–8	10/10	No cross-resistance
		85[b]	Day 1	+185	–7	4/10	+235	–7	8/10	
Echinomycin	526417	0.10	Days 1–5	+80	–4	0/10	+66	–6	1/10	–[c]

[a] Log_{10} change in viable tumor cell population at the end of therapy as compared with that at the start of therapy, based on the median day of death among the animals that died. For treatment with drugs that yielded only survivors, the log_{10} change is based on the day of evaluation (day 45)

[b] Highest dosage tested

[c] The results of this single study suggest possible collateral sensitivity; however, an unequivocal conclusion requires additional studies

eral sensitivity was defined as an increase in the sensitivity (by >2 log_{10} units of cell kill) of P388/DDPt leukemia to a drug over that concurrently observed for P388/0 leukemia.

Results

The cross-resistance profile of P388/DDPt to 18 different alkylating agents is shown in Table 1. The P388/DDPt line was not cross-resistant to most of the alkylating agents studied. The agents for which cross-resistance was observed were dibromodulcitol, spirohydantoin mustard, hepsulfam, and carmethizole · HCl. The three cisplatin analogs (NSC numbers 194814, 271674, and 363812) studied, which contain the diaminocyclohexane moiety, were effective against the P388/DDPt line.

The effect of eight different antimetabolites on P388/DDPt is shown in Table 2. The cisplatin-resistant line was not cross-resistant to methotrexate, 5-fluorouracil, palmO-ara-C, trimetrexate, or triciribine phosphate and was collaterally sensitive to 2-F-ara-AMP. Interestingly, the line was cross-resistant to ara-AC and tiazofurin.

The sensitivity of P388/DDPt to 13 agents that interact with DNA and/or its associated proteins is shown in Table 3. The P388/DDPt line was not cross-resistant to any of the agents studied (with the possible exception of actinomycin D) and was collaterally sensitive [at an optimal ($\leq LD_{10}$) i.p. dosage] to at least two of the agents (AMSA and mitoxantrone) and, possibly, to two others (merbarone and echinomycin). The line also exhibited collateral sensitivity to VP-16 at suboptimal i.p. dosages (see Table 4).

Table 4. Sensitivity of P388/DDPt to VP-16

i. p. dosage[a] (mg/kg per dose)	Therapeutic response					
	P388/0 Leukemia			P388/DDPt Leukemia		
	Median % ILS (dying mice only)	Approx. $\log_{10}$ change in tumor burden after last treatment[b]	45-day survivors	Median % ILS (dying mice only)	Approx. $\log_{10}$ change in tumor burden after last treatment[b]	45-day survivors
40	+152	–5	6/10	+95	–4	6/10
27	+134	–4	2/10	+153	–7	6/10
18	+120	–3	1/10	+131	–7	6/10
40	+221	–7	6/10	+58	Toxic	0/10
27	+147	–6	2/10	+139	–7	6/10
18	+145	–6	0/10	+109	–7	8/10
12	+111	–3	0/10	+116	–7	2/10

[a] VP-16 was given on days 1, 5, and 9

[b] $\log_{10}$ change in viable tumor cell population at the end of therapy as compared with that at the start of therapy, based on the median day of death among the animals that died

Table 5. Cross-resistance of P388/DDPt to tubulin binders and a protein synthesis inhibitor

Drug	NSC number	Optimal i.p. dosage ($\leq LD_{10}$, mg/kg per dose)	Schedule	Therapeutic response						Comments
				P388/0 Leukemia			P388/DDPt Leukemia			
				Median % ILS (dying mice only)	Approx. $\log_{10}$ change in tumor burden after last treatment[a]	45-day survivors	Median % ILS (dying mice only)	Approx. $\log_{10}$ change in tumor burden after last treatment[a]	45-day survivors	
Vincristine	67 574	1.5	Days 1, 5, 9	+181	–7	1/10	+126	–5	2/10	No cross-resistance
		1.5	Days 1, 5, 9	+120	–3	0/10	+100	–3	0/10	
Homoharringtonine	141 633	2.25	Days 1–9	+123	–3	0/10	+102	–5	0/10	No cross-resistance
		2	Days 1–9	+109	–3	0/10	+76	–3	0/10	
Vindesine	245 467	1.5	Days 1, 5, 9	+90	–1	0/10	+61	0	0/10	No cross-resistance
		2	Days 1, 5, 9	+172	–7	1/10	+173	–7	4/10	
Rhizoxin	332 598	0.30[b]	Days 1–5	+65	–2	0/10	+24	+1	0/10	Marginal cross-resistance
		0.45[b]	Days 1–5	+71	–2	0/10	+28	–1	0/10	
1-Deaza-7,8-dihydropteridine[c]	370 147	3.3	Day 1	+31	–3	0/10	+12	–2	0/10	No cross-resistance
		5	Day 1	+36	–2	0/10	+28	–3	0/10	

[a] $\log_{10}$ change in viable tumor cell population at the end of therapy as compared with that at the start of therapy, based on the median day of death among the animals that died

[b] Highest dosage tested

[c] Ethyl 5-amino-1,2-dihydro-2-methyl-3-phenylpyrido[3,4-*b*]pyrazin-7-ylcarbamate, 2-hydroxyethanesulfonate, hydrate

The cross-resistance profile of P388/DDPt to four tubulin binders and one protein-synthesis inhibitor is shown in Table 5. The cisplatin-resistant line exhibited marginal cross-resistance to rhizoxin but was not cross-resistant to vincristine, homoharringtonine, vindesine, or NSC 370 147. The sensitivity of P388/DDPt to four agents whose mechanisms of action are not known is shown in Table 6. The P388/DDPt line was not cross-resistant to penclomedine and was collaterally sensitive to batracylin and flavone acetic acid (two of three experiments); however, it was cross-resistant to deoxyspergualin.

In summary, P388/DDPt was cross-resistant or marginally cross-resistant to eight agents (dibromodulcitol, spirohydantoin mustard, hepsulfam, carmethizole · HCl, ara-AC, tiazofurin, rhizoxin, and deoxyspergualin) that represent at least three functionally different classes of antitumor agents. The line was collaterally sensitive to six agents (2-F-ara-AMP, AMSA, mitoxantrone, VP-16, batracylin, and flavone acetic acid) and, possibly, to two others (merbarone and echinomycin). Of these eight agents, four have been reported to interact with DNA topoisomerase II [3, 10, 15, 17].

Table 6. Cross-resistance of P388/DDPt to miscellaneous agents

Drug	NSC number	Optimal i.p. dosage ($\leq LD_{10}$, mg/kg per dose)	Schedule	Therapeutic response						Comments
				P388/0 Leukemia			P388/DDPt Leukemia			
				Median % ILS (dying mice only)	Approx. log_{10} change in tumor burden after last treatment[a]	45-day survivors	Median % ILS (dying mice only)	Approx. log_{10} change in tumor burden after last treatment[a]	45-day survivors	
Batracylin	320846	50	Days 1–9	+5	+2	0/10	+73	–3	1/10	Collateral sensitivity
		50	Days 1–9	+25	+2	0/10	+59	0	0/10	
Penclomedine	338720	90	Days 1–5	+51	–1	0/10	+28	+1	0/10	No cross-resistance
		90	Days 1–5	+59	–1	0/10	+34	0	0/10	
Flavone acetic acid	347512	150	Days 1–9	+102	–1	0/10	+98	–3	0/10	Collateral sensitivity
		150	Days 1–9	+105	–2	0/10	+121	–7	0/10	
		100	Days 1–9	+70	+1	0/10	+48	+1	0/10	
Deoxyspergualin	356894	15[b]	Days 1–9	+115	–2	0/10	+52	+1	0/10	Cross-resistance
		22.5	Days 1–9	+112	–2	0/10	+33	+1	0/10	

[a] Log_{10} change in viable tumor cell population at the end of therapy as compared with that at the start of therapy, based on the median day of death among the animals that died

[b] Highest dosage tested

Discussion

Since 1978, Southern Research Institute has evaluated over 50 clinically useful antitumor drugs or new candidate antitumor agents in vivo against P388/DDPt. Analysis of this data base has revealed (a) potentially useful guides for patient selection for clinical trials of new antitumor drugs, (b) possible noncross-resistant drug combinations with cisplatin, and (c) a possible mechanism of resistance to cisplatin.

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 [5]. Timely information on the patterns of cross-resistance or collateral sensitivity among the various antitumor agents may be helpful to the clinician in the selection of patients for treatment with the new candidate drugs. Of the eight agents to which P388/DDPt exhibited cross-resistance, six (dibromodulcitol, spirohydantoin mustard, hepsulfam, ara-AC, tiazofurin, and deoxyspergualin) have entered various phases of clinical trials (M. Christian, NCI, personal communication). For these trials, it may be important to exclude or to monitor with extra care patients who have previously been treated with cisplatin.

Of the agents to which P388/DDPt exhibited collateral sensitivity, three (AMSA, mitoxantrone, and VP-16) are clinically useful drugs. These observations of collateral sensitivity suggest that a combination of cisplatin plus one of these drugs might exhibit therapeutic synergism. Synergy at the therapeutic level has been reported for the combination of cisplatin plus VP-16 [21]; however, synergy at the cellular level has not been found for this combination [26]. Possibly, the observed therapeutic synergism is due in part to the killing of cisplatin-resistant cells by VP-16. Therapeutic synergism has also been observed in animal models for the combination of cisplatin plus mitoxantrone [21] and for the combination of cisplatin plus AMSA (Southern Research Institute, unpublished data). Merbarone, echinomycin, 2-F-ara-AMP, and flavone acetic acid have entered clinical trials (M. Christian, personal communication). If these agents are approved for clinical use, their use in combination with cisplatin 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.

Studies concerning the biochemical basis of tumor resistance to cisplatin suggest that such resistance is multifactorial. Although a variety of mechanisms have been proposed, the published data are primarily used to support three mechanisms: (1) decreased accumulation of drug, (2) increased drug detoxification through increased levels of intracellular protein and nonprotein sulfhydryl molecules such as metallothionein and GSH and through increased activity of the GSH transferase system, and (3) increased repair of cisplatin-induced damage to DNA. The observation of collateral sensitivity for P388/DDPt to various agents provides insight into the latter mechanism of cisplatin resistance.

Increased DNA repair as a mechanism of acquired resistance to cisplatin has been reported for L1210 and P388 leukemia, rat ovarian carcinoma, human ovarian carcinoma, and human colon carcinoma [1]. Increased levels of DNA polymerase α and β, enzymes that are implicated in excision repair, have been found in cisplatin-resistant HCT-8 human colon carcinoma [19] and A2780 human ovarian carcinoma [18] as compared with the corresponding parental cell lines. DNA polymerase β activity has been reported to be elevated in P388/DDPt cells, which were selected in vivo prior to chronic in vitro exposure to

cisplatin [12]. Furthermore, an inhibitor of DNA polymerase α, aphidicolin, has been shown to sensitize cisplatin-resistant but not cisplatin-sensitive A2780 cells to cisplatin [14]. Some cisplatin-resistant cells have been reported to contain elevated levels of enzymes that are involved in folate metabolism (i.e., thymidine kinase, thymidylate synthase, and dihydrofolate reductase) as compared with the parental cell lines [1]. Elevated levels of these enzymes may be required for increased DNA repair.

The observation of collateral sensitivity for P388/DDPt to four agents that have been reported to interact with DNA topoisomerase II suggests the possible involvement of the latter in cisplatin resistance. A possible explanation for the increased sensitivity of P388/DDPt to these agents is an increase in DNA topoisomerase II activity in the cell line. If DNA topoisomerase II is involved in the repair of cisplatin-induced DNA damage, the increased topoisomerase activity could account for a portion of the resistance of P388/DDPt to cisplatin. Increased DNA topoisomerase II activity has been reported for a cisplatin-resistant human small-cell lung carcinoma cell line [11], an intrinsically cisplatin-resistant human breast carcinoma cell line [23], and a nitrogen-mustard-resistant human Burkitt's lymphoma cell line [24] as compared with the corresponding drug-sensitive lines. We assayed P388/0 and P388/DDPt cells for DNA topoisomerase II activity. P388/DDPt cells exhibited more activity than did the parental cells (unpublished data). Studies are currently being conducted to explore further the involvement of DNA topoisomerase II in the resistance of P388/DDPt to cisplatin.

The observation of collateral sensitivity for P388/DDPt to 2-F-ara-AMP, echinomycin, flavone acetic acid, and batracylin may provide insights into the mechanisms of action of these agents. After its conversion to 2-F-ara-ATP, 2-F-ara-AMP appears to exert its antitumor effect by inhibition of DNA polymerase α and ribonucleotide reductase [27]. The collateral sensitivity of P388/DDPt to this agent could result from inhibition of DNA polymerase α, possibly similar to that observed for aphidicolin. Echinomycin acts by binding to DNA via the mechanism of bifunctional intercalation [28]; however, the relationship between its mechanism of action and the collateral sensitivity of P388/DDPt to the agent is unclear. The mechanism(s) by which flavone acetic acid exerts its antitumor effect is unknown. Either flavone acetic acid or a metabolite does act as an immunomodulator and does reduce tumor blood flow [2]; however, a direct relationship of either effect to the antitumor activity and, therefore, to the collateral sensitivity of P388/DDPt to the agent is unclear. The mechanism(s) of action of batracylin is unknown. Possibly, one or more of these drugs may exert a portion, if not all, of their antitumor effect through interaction with DNA topoisomerase II.

In summary, the in vivo cross-resistance profile of P388/DDPt to >50 clinically useful antitumor drugs or new candidate antitumor agents has enabled the identification of potentially useful guides for patient selection for clinical trials of new antitumor drugs, of possible noncross-resistant drug combinations with cisplatin, and of a possible biochemical mechanism of resistance to cisplatin.

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