Cancer Chemother and Pharmacol (1996) 38: 178–180

ORIGINAL ARTICLE

William R. Waud · Karen S. Gilbert
Gerald B. Grindey · John F. Worzalla

Lack of in vivo crossresistance with gemcitabine against drug-resistant murine P388 leukemias

Received: 14 October 1994/Accepted: 9 October 1995

Abstract Gemcitabine, a novel pyrimidine nucleoside antimetabolite, has shown clinical antitumor activity against several tumors (breast, small-cell and non-small-cell lung, bladder, pancreatic, and ovarian). We have developed a drug-resistance profile for gemcitabine using eight drug-resistant P388 leukemias in order to identify potentially useful guides for patient selection for further clinical trials of gemcitabine and possible noncrossresistant drug combinations with gemcitabine. Multidrug-resistant P388 leukemias (leukemias resistant to doxorubicin or etoposide) exhibited no crossresistance to gemcitabine. Leukemias resistant to vincristine (not multidrug resistant), cyclophosphamide, melphalan, cisplatin, and methotrexate were also not crossresistant to gemcitabine. Only the leukemia resistant to 1-β-D-arabinofuranosylcytosine was crossresistant to gemcitabine. The results suggest that (1) it may be important to exclude or to monitor with extra care patients who have previously been treated with 1-β-D-arabinofuranosylcytosine and (2) the lack of crossresistance seen with gemcitabine may contribute to therapeutic synergism when gemcitabine is combined with other agents.

Key words Gemcitabine · In vivo crossresistance

Abbreviations *ILS* increase in life span, *P388/ADR* doxorubicin-resistant P388 leukemia, *P388/ARA-C* 1-β-D-arabinofuranosylcytosine-resistant P388 leukemia, *P388/CPA* cyclophosphamide-resistant P388 leukemia, *P388/DDPt* cisplatin-resistant P388 leukemia, *P388/L-PAM* melphalan-resistant P388 leukemia, *P388/MTX* methotrexate-resistant P388 leukemia, *P388/VCR* vincristine-resistant P388 leukemia, *P388/VP-16* etoposide-resistant P388 leukemia, *P388/0* parental P388 leukemia

W.R. Waud (✉) · K.S. Gilbert
Experimental Therapeutics Department, Southern Research Institute, Birmingham, Alabama 35255–5305, USA

G.B. Grindey · J.F. Worzalla
Lilly Research Laboratories, Indianapolis, Indiana 46285–0542, USA

Introduction

Gemcitabine (2′, 2′-difluorodeoxycytidine) is a novel pyrimidine nucleoside antimetabolite, which differs from ara-C by only two fluorine atoms (Fig. 1). The compound was initially synthesized as an antiviral agent [8]; however, the in vivo therapeutic index was not sufficient for further development. Gemcitabine was concurrently tested for antitumor activity and was found to have greater activity against both murine solid tumors and human tumor xenografts than ara-C [9]. Furthermore, the compound exhibited a broader therapeutic index than ara-C [9] and superior cytotoxicity when administered intermittently instead of daily, like ara-C [7]. During phase II clinical trials, gemcitabine showed activity against advanced breast cancer [2], small-cell [4] and non-small-cell [1, 11] lung cancer, advanced bladder cancer [14], refractory ovarian cancer [12, 13], and pancreatic cancer [3, 17]. Additional clinical trials 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 gemcitabine may identify potentially useful guides for patient selection for further clinical trials of gemcitabine and possible noncrossresistant drug combinations with gemcitabine. This information should aid in the design of strategies for the optimal use of the drug. In vivo models may be preferable for developing 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 [16]. We report here the lack of in vivo crossresistance seen for gemcitabine against drug-resistant murine leukemias.

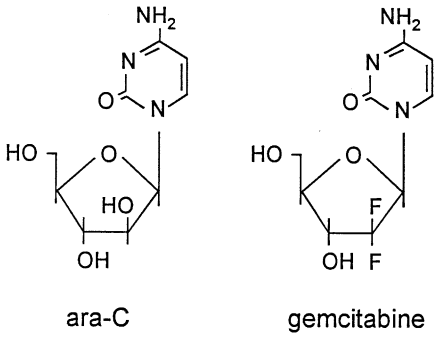

Fig. 1 Structures of ara-C and gemcitabine

Materials and methods

Gemcitabine was supplied as a hydrochloride salt (LY264368) by Lilly Research Laboratories. The compound was dissolved in sterile saline (13.5 mg/ml) and appropriate dilutions were made with saline. Dosing solutions of gemcitabine were prepared fresh every 4 days and were stored at 4–8°C when not in use.

The in vivo sensitivity of P388/0 and eight drug-resistant P388 leukemias to gemcitabine was determined as previously described for other antitumor agents [18]. $CD2F_1$ mice were implanted i.p. with 10^6 cells of either P388/0 or P388/drug-resistant leukemia on day 0. Gemcitabine was evaluated at three i.p. dose levels (ranging from toxic to nontoxic). There were 10 mice in each dose group; tumor-bearing control mice (20 per experiment) were untreated. Mice were observed for life span. In each experiment, additional groups were treated with two doses of an appropriate drug to confirm the resistance of a P388/drug-resistant leukemia. In each experiment two P388/drug-resistant leukemias were compared directly with the parent or wild-type P388/0 leukemia, and the three parallel groups of mice were treated identically with a single-drug preparation. Procedures were approved by the Southern Research Institute's Institutional Animal Care and Use Committee which conforms to the current Public Health Service *Policy on Humane Care and Use of Laboratory Animals* and the *Guide for the Care and Use of Laboratory Animals*.

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 tenfold dilutions of P388 cells [15]. 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 have occurred between individual treatments [10]. The net $\log_{10}$ cell kill was calculated as follows:

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

where (T − C) is the difference in the median day of death between the treated (T) and the control (C) groups, 3.32 is the number of doublings required for a population to increase 1 $\log_{10}$ unit, and T_d is the mean tumor doubling time (days) calculated from a log-

Table 1 Activity of gemcitabine against drug-resistant P388 leukemias in vivo (*ADR* doxorubicin, *VP-16* etoposide, *VCR* vincristine, *CPA* cyclophosphamide, *L-PAM* melphalan, *DDPt* cisplatin, *ARA-C* 1-β-D-arabinofuranosylcytosine, *MTX* methotrexate).

			Therapeutic response						
			P388/0 leukemia			Resistant leukemia[c]			
Expt. No.	Resistant leukemia	Optimal i.p. dosage[a] ($\leq LD_{10}$, mg/kg/dose)	Median % ILS	Net $\log_{10}$ cell kill[b]	45-day survivors/ total	Median % ILS	Net $\log_{10}$ cell kill[b]	45-day survivors/ total	Cross-resistance?
7	P388/ADR	180	+ 180	+ 5.1	1/10	+ 218	+ 6.8	1/10	No
8		180	+ 130	+ 2.9	0/10	+ 135	+ 2.9	0/10	
3	P388/VP-16	120	+ 150	+ 4.4	0/10	+ 130	+ 7.0	0/10	No
6		120	+ 145	+ 5.0	2/10	+ 129	+ 5.0	0/10	
2	P388/VCR	120	+ 165	+ 4.7	0/10	+ 142	+ 3.5	0/10	No
5		180	+ 136	+ 4.8	0/10	+ 161	+ 4.2	0/10	
3	P388/CPA	180	+ 160	+ 5.2	0/10	+ 220	+ 6.6	1/10	No
6		120[d]	+ 145	+ 5.0	2/10	+ 166	+ 5.0	1/10	
1	P388/L-PAM	180	+ 152	+ 4.3	0/10	+ 107	+ 5.5	0/10	No
4		180	+ 152	+ 5.0	0/10	+ 93	+ 4.3	0/10	
7	P388/DDPt	120	+ 160	+ 4.0	0/10	+ 107	+ 4.5	0/10	No
8		180	+ 130	+ 2.9	0/10	+ 100	+ 2.6	0/10	
2	P388/ARA-C	180	+ 170	+ 5.0	0/10	+ 4	− 2.2	0/10	Yes
5		180	+ 136	+ 4.8	0/10	− 5	− 2.4	0/10	
1	P388/MTX	180	+ 152	+ 4.3	0/10	+ 166	+ 5.5	0/10	No
4		180	+ 152	+ 5.0	0/10	+ 126	+ 3.8	0/10	

[a]Gemcitabine was administered on days 1, 4, 7, and 10 using an injection volume of 0.2 ml/10 g animal body weight
[b]Net $\log_{10}$ reduction in the tumor cell population between the beginning and the end of therapy, based on the median day of death of mice that died
[c]In these studies, the average degree of resistance of a drug-resistant subline in comparison to the parental line was as follows: ADR, 5 $\log_{10}$ units; VP-16, 8 $\log_{10}$ units; VCR, 6 $\log_{10}$ units; CPA, 6 $\log_{10}$ units; L-PAM, 7 $\log_{10}$ units; DDPt, 8 $\log_{10}$ units; ARA-C, 8 $\log_{10}$ units; and MTX, 1 $\log_{10}$ unit
[d]LD_{30} for resistant leukemia portion of the study

linear least-squares fit of the implant sizes and the median days of death of the titration groups.

Crossresistance was defined as a decrease in the sensitivity (> 2 $\log_{10}$ units of cell kill) of P388/drug-resistant leukemia to gemcitabine as compared with that concurrently observed for P388/0 leukemia.

Results and discussion

The in vivo crossresistance profile for gemcitabine is shown in Table 1. Multidrug-resistant P388 leukemias (P388/ADR and P388/VP-16) exhibited no crossresistance to the drug. P388/VCR (not multidrug resistant), P388/CPA, P388/L-PAM, P388/DDPt, and P388/MTX were also not crossresistant to gemcitabine. Only P388/ARA-C exhibited crossresistance to the drug.

As new agents enter phase II and III clinical trials, the selection of patients, most of whom have been treated previously with one or more drugs, may be critical to the success of the trials [6]. Information on the patterns of crossresistance among various antitumor agents may be helpful in the selection of patients for treatment with gemcitabine. For these trials, it may be important to exclude or to monitor with extra care patients who have previously been treated with ara-C.

The observation of a lack of crossresistance of P388/ADR, P388/VP-16, P388/VCR, P388/CPA, P388/L-PAM, P388/DDPt, and P388/MTX leukemias to gemcitabine suggests that a combination of doxorubicin, etoposide, vincristine, cyclophosphamide, melphalan, cisplatin, or methotrexate with gemcitabine might exhibit therapeutic synergism. Phase I and II clinical trials have shown gemcitabine to be active against cisplatin-resistant ovarian carcinoma [12, 13] and previously treated breast (regimens not given) and bladder (treated with methotrexate, vincristine, doxorubicin, and cisplatin) cancer [2, 14]. Preliminary results for the combination of gemcitabine plus cisplatin also suggest possible therapeutic synergism in non-small-cell lung cancer [5]. 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.

References

1. Abratt R, Bezwoda W, Falkson G, Goedhals L, Hacking D (1992) Efficacy and safety of gemcitabine in non-small cell lung cancer. Phase II study results. Proc Am Soc Clin Oncol 11:311
2. Carmichael J, Philip P, Rea D, Kerr H, Walling J, Harris AL, Possinger K (1992) Gemcitabine: an active drug in advanced breast cancer. Results of a phase II study. Proc Am Soc Clin Oncol 11:77
3. Casper ES, Green MR, Kelsen DP, Heelan RT, Brown TD, Flombaum CD, Trochanowski B, Tarassoff PG (1994) Phase II trial of gemcitabine (2,2′-difluorodeoxycytidine) in patients with adenocarcinoma of the pancreas. Invest New Drugs 12:29
4. Cormier Y, Eisenhauer E, Muldal A, Gregg R, Ayoub J, Goss G, Stewart D, Tarassoff P, Wong D (1994) Gemcitabine is an active new agent in previously untreated extensive small cell lung cancer (SCLC). A study of the National Cancer Institute of Canada Clinical Trials Group. Ann Oncol 5:283
5. Dunlop DJ, Cameron C, Dabouis G, Lacroix H, Ponzio A, Walling J, Steward WP (1994) Phase I dose-escalation study of cisplatin in combination with gemcitabine in non-small cell lung cancer. Proc Am Soc Clin Oncol 13:342
6. Estey E, Hoth D, Simon R, Marsoni S, Leyland-Jones B, Wittes R (1986) Therapeutic response in phase I trials of antineoplastic agents. Cancer Treat Rep 70:1105
7. Grunewald R, Kantarjian H, Keating MJ, Abbruzzese J, Tarassoff P, Plunkett W (1990) Pharmacologically directed design of the dose rate and schedule of 2′,2′-difluorodeoxycytidine (gemcitabine) administration in leukemia. Cancer Res 50:6823
8. Hertel LW, Kroin JS, Misner JW, Tustin JM (1988) Synthesis of 2-deoxy-2,2-difluoro-D-ribose and 2-deoxy-2,2-difluoro-D-ribofuranosyl nucleosides. J Org Chem 53:2406
9. Hertel LW, Boder GB, Kroin JS, Rinzel SM, Poore GA, Todd GC, Grindey GB (1990) Evaluation of the antitumor activity of gemcitabine (2′,2′-difluoro-2′-deoxycytidine). Cancer Res 50:4417
10. Lloyd HH (1977) Application of tumor models toward the design of treatment schedules for cancer chemotherapy. In: Drewinko B, Humphrey RM (eds) Growth kinetics and biochemical regulation of normal and malignant cells. Williams & Wilkins, Baltimore, p 455
11. Lund B, Anderson H, Walling J, Thatcher N, Hansen HH (1991) Phase II study of gemcitabine in non small cell lung cancer (NSCLC). Lung Cancer [suppl] 7:121
12. Lund B, Hansen OP, Neijt JP, Stroier I, Hansen M (1992) Phase II study of gemcitabine in previously treated ovarian cancer patients. Proc Am Soc Clin Oncol 11:222
13. Morgan-Ihrig C, Lembersky B, Christopherson W, Tarassoff P (1991) A phase II evaluation of difluorodeoxycytidine (dFdC) in advanced stage refractory ovarian cancer. Proc Am Soc Clin Oncol 10:196
14. Pollera CF, Ceribelli A, Crecco M, Calabresi F (1994) Weekly gemcitabine in advanced bladder cancer: a preliminary report from a phase I study. Ann Oncol 5:182
15. Schabel FM Jr, Griswold DP Jr, Laster WR Jr, Corbett TH, Lloyd HH (1977) Quantitative evaluation of anticancer agent activity in experimental animals. Pharmacol Ther [A] 1:411
16. Teicher BA, Herman TS, Holden SA, Wang Y, Pfeffer MR, Crawford JW, Frei E III (1990) Tumor resistance to alkylating agents conferred by mechanisms operative only in vivo. Science 247:1457
17. Tempero M, Capadano M, Tarassoff P (1994) Dose escalation of gemcitabine in previously untreated patients with pancreatic adenocarcinoma. Proc Am Soc Clin Oncol 13:218
18. Waud WR, Harrison SD Jr, Gilbert KS, Laster WR Jr, Griswold DP Jr (1991) Antitumor drug cross-resistance in vivo in a cisplatin-resistant murine P388 leukemia. Cancer Chemother Pharmacol 27:456