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Antitumor activity of prolonged as compared with bolus administration of 2′,2′-difluorodeoxycytidine in vivo against murine colon tumors

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Abstract 2′,2′-Difluorodeoxycytidine (gemcitabine) is a cytidine analogue with established antitumor activity against several experimental tumor types and against human ovarian and non-small-cell lung cancer. Both preclinical studies and most clinical trials involving patients with solid tumors have focused on short-term administration schedules; however, mechanistic studies indicate that a continuous-infusion schedule may be more effective. We determined the maximal tolerated dose (MTD) of gemcitabine in mice using various schedules. At these MTDs we observed considerably better antitumor activity of gemcitabine in two of three murine colon carcinoma lines using a prolonged administration as compared with a standard bolus protocol (i.p. 120 mg/kg q3d × 4). On the latter schedule, Colon 26–10 grown in BALB/c mice was the most sensitive tumor line, showing a growth-delay factor (GDF, number of doubling times gained by the treatment) of 6.7, whereas Colon 38 (grown in C57/B16 mice) was the least sensitive tumor, displaying a GDF of 0.9. Prolonged treatment (q3d × 6) of Colon 26–10 at a lower dose (100 mg/kg) enhanced the antitumor activity (GDF 9.6) while producing similar toxicity. A similar weight loss was found following the continuous infusion (c.i.) of gemcitabine using Alzet osmotic pumps s.c. for 3 or 7 days (2 mg/kg), but the GDF increased to 2.4 in Colon 38 (C57/B16) as compared with that provided by the bolus injections. Continuous infusion of gemcitabine at 15 mg/kg per 24 h q7d × 2 i.v. via the tail vein was more effective than bolus injection against Colon 26–10, with the GDF being > 17.7 and 73% of the tumors regressing completely. However, against Colon 38 tumors this schedule was not effective (GDF 0.4), even with a 25% higher dose. The plasma pharmacokinetics of gemcitabine was determined after one bolus dose (120 mg/kg). The peak concentration of gemcitabine was 225 μ*M* and that of the deaminated catabolite 2′,2′-difluorodeoxyuridine (dFdU) was 79 μ*M*. The elimination of gemcitabine was much faster than that of dFdU, with the $t_{1/2ß}$ values being 15 min and 8 h, respectively. For the c.i. schedules, plasma concentrations were below the detection limit of the assay (< 0.5 μ*M*). Our results suggest that prolonged infusion of gemcitabine can give a better antitumor activity than bolus injections and shows promise of being active in clinical trials.

Key words Gemcitabine · Colon cancer · Continuous infusion · schedule dependence

Introduction

2′,2′-Difluorodeoxycytidine (gemcitabine) is a cytidine analogue that has shown marked activity not only against experimental solid tumors in mice [5, 6, 13] but also against ovarian and non-small-cell lung cancer in patients [1, 14, 17]. For treatment of tumor-bearing animals as well as patients, most schedules have thus far been limited to bolus injections given at different doses and intervals. In clinical trials involving patients with solid tumors the drug was given as a 30-min infusion, weekly times 3, every 4 weeks, at doses varying from 800 to 1,250 mg/m^2 [14, 17]. However, in leukemic blast cells, Grunewald et al. [10, 11] observed a 4-fold higher accumulation of the active triphosphate of gemcitabine, 2′,2′-difluorodeoxycytidine

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triphosphate (dFdCTP), when the infusion time was prolonged. Other antimetabolites such as methotrexate, 6-mercaptopurine, and 1-ß-D-arabinofuranosylcytosine, which are commonly used in leukemia, were more active when given by continuous infusion than when given by bolus injection [2, 12, 18, 23]. Also 5-fluorouracil (5-FU) has a better antitumor effect when given continuously over a longer period [9, 16, 22]. These data are in agreement with the in vitro sensitivity pattern reported for these drugs [7, 30], in which continuous exposure (24–72 h) yielded considerably lower 50% inhibitory concentrations (IC_{50} values) than did short-term exposure (1–4 h). For gemcitabine we also observed that the IC_{50} values decreased considerably at longer exposure times (24–72 h) in ovarian and head and neck squamous-cell carcinoma cell lines as well as colon-cancer cell lines [26]. Therefore, we performed a study to determine whether we could improve the antitumor activity of gemcitabine by using prolonged administration and continuous infusion. For that purpose we used a tumor type that is rather insensitive to gemcitabine given as a bolus, murine colon cancer.

Materials and methods

Materials

Gemcitabine and 2′,2′-difluorodeoxyuridine (dFdU) were kindly provided by Lilly Research Center Ltd. (Indianapolis, Ind., USA). An ampoule contained gemcitabine HCl equivalent to 500 mg gemcitabine, 500 mg mannitol, and 80 mg sodium acetate. The gemcitabine powder was dissolved in 0.9% NaCl to reach a final concentration of 12 mg/ml. Hypnorm anesthetic was obtained from Janssen Pharmaceutica (Tilburg, The Netherlands). Deoxycytidine (CdR) was purchased from Sigma Chemical Co. (St. Louis, Mo., USA). All other chemicals were of analytical grade and were commercially available.

Tumors

The sources and characteristics of the murine Colon 26 and Colon 38 (adeno) carcinomas have been described elsewhere [20]. The Colon 26–10 tumor was established by injecting $3–5 \times 10^6$ C26–10 cells into both flanks of BALB/c mice. The C26–10 cells were derived from a Colon 26 tumor and provided by Dr. W.D. Klohs (Ann Arbor, Mich., USA) [15, 21]. The resulting tumor line, Colon 26–10, was significantly different from the original Colon 26 tumor line in, e.g., its enhanced sensitivity to 5-FU [31, 32]. Moreover, Colon 26–10 tumors did not cause cachexia, in contrast to Colon [26, 31]. The Colon 26 and Colon 26–10 tumors were grown in female BALB/c mice and the Colon 38 tumor, in female C57/B16 mice (Harlan/Olac, Zeist, The Netherlands). The mice were kept in an area maintained on a standardized light/dark cycle and had access to food (RMH-B 10 mm code 2100, Hope Farms, Woerden, The Netherlands) and water ad libitum. Tumors were transplanted s.c. in both flanks in the thoracic region in small fragments of 1–5 mm^3. When tumors reached a volume of 50–150 mm^3, treatment was started. Tumor size was determined by caliper measurement (length × width × height × 0.5) twice a week, which was shown to be the most reliable method [20, 29]. The volume of the tumors was expressed relative to that determined on the 1st day of treatment (day 0). Before treatment, mice were randomized into several groups, one group serving as a control group and the others, as treatment groups. Each group consisted of at least 6 mice, corresponding to 12 tumors. Antitumor activity was evaluated by calculation of the T/C values, i.e., by dividing the relative tumor volume of treated mice by that of the control mice; the growth-delay factor (GDF) was defined as the mean number of tumor-doubling times gained by treatment and was calculated as the doubling time of treated tumors minus the doubling time of control tumors divided by the doubling time of controls. The increase in life span (ILS) was calculated because mice bearing Colon 26 suffer from cachexia when the tumor mass exceeds 500 mm^3, leading to death of the mice. The reduction in cachexia observed during gemcitabine treatment resulted in a prolonged life span and was a parameter for the efficacy of the treatment. ILS was defined as the median life span of the treated group divided by the median life span of the control group times 100%, using the day of transplantation as day 0. The day of death was defined as the day at which mice were taken off the experiment when the cachexia-induced weight loss was $> 15\%$ and was associated with a total tumor volume of 800 mm^3. Mice bearing Colon 38 tumors were killed because of their tumor burden ($> 2,000$ mm^3). Differences in significance between the antitumor effects of a particular treatment were determined by means of Student's *t*-test.

Doses and schedules

Mice were treated by i.p. bolus injection, s.c. continuous infusion and i.v. continuous infusion. The maximal tolerated dose (MTD) was assessed in non-tumor-bearing BALB/c and C57/B16 mice and was defined as the dose that caused a maximal weight loss of 15%. One part of the continuous-infusion studies was performed by giving the drug s.c. with Alzet Micro-Osmotic Pumps (Alza Corporation, Palo Alto, Calif. USA). The osmotic pumps were implanted s.c. and delivered the drug for 3 or 7 days (models 1003D and 1007D, respectively). Pumps were removed after 3 or 7 days, at the end of the infusion. After their removal we checked whether the pumps were empty by transferring them into a vial containing 0.9% NaCl and measured an eventual gemcitabine release by high-performance liquid chromatography (HPLC) using a μBondapack C_{18} column with Pic B, (Waters) in 15% methanol (final concentration of heptane sulfonic acid 5 m*M*) at pH 3.1. Peaks were detected and quantitated according to their absorption at 254 and 280 nm. The determined postinfusion release was within the margins reported by Alzet ($< 5\%$).

Continuous i.v. infusion was performed via the tail vein. The mice were anesthesized (i.m.) with Hypnorm (dose 0.02 ml/mouse weighing 20 g) and laid on a water-heated bed. The tail vein was punctured with a 21-gauge needle. A catheter (Intramedic polyethylene tubing, medical formulation PHF; inside diameter 0.58 mm, outside diameter 0.97 mm; Clay Adams) was inserted into the tail vein and flushed with 0.9% NaCl. Both tail and catheter were splinted. Catheter and splint were protected by a plastic tube. Each mouse was put separately in one cage, which was put on a heated water bed to prevent hypothermia. The catheter was connected to a syringe, which was placed in an infusion pump. Each mouse received 1.2 ml solution containing gemcitabine in 0.9% NaCl per 24 h. After the 24-h infusion the catheter was removed. In a control experiment to check whether the catheters were inserted in the vein the right way, diluted India ink was injected. After a few minutes the color of the eyes and feet of the mouse became greyish. When the mouse was killed and opened we observed that organs such as the liver and spleen had become completely black, indicating that the infusion was indeed i.v.

Treatment was initiated at day 10 (Colon 26 and Colon 26–10) and day 19 (Colon 38) after transplantation. All protocols were approved by the ethics committee for animal experiments of the Free University of Amsterdam.

Plasma concentrations of gemcitabine and dFdU

Plasma levels of gemcitabine and dFdU were determined in mice injected i.p. with 120 mg/kg gemcitabine. For each time point, blood was sampled from three mice by cardiac puncture. Blood samples were collected in heparinized tubes containing tetrahydrouridine (THU) to prevent deamination of gemcitabine to dFdU. The blood samples were immediately centrifuged and the supernatant was frozen and stored at $-20\,^{\circ}C$ until analysis. During the continuous infusions we also sampled blood at several time points (days 0, 1, 2, and 3) and determined the possible gemcitabine and dFdU concentrations.

Gemcitabine and dFdU were extracted from the plasma by the addition of 0.5 ml plasma to the internal standard deoxycytidine. Subsequently, 2 ml isopropylalcohol was added and allowed to stand for 5 min, after which 5 ml ethylacetate was added and the suspension was mixed and centrifuged for 5 min at 4,000 rpm. The supernatant was transferred to another tube and blown to dryness at 40°C under N_2. Before analysis the pellet was dissolved in 250 µl HPLC mobile phase. Compounds were injected onto an HPLC system consisting of an Econosphere-5-NH_2 column (length 25 cm, internal diameter 4.6 mm, pore size 5 µm), a Waters M-45 pump, and a Waters fixed-wavelength detector (M-440) set at 254 and 280 nm. The mobile phase consisted of a cyclohexane/dichloroethane/methanol/water/acetic acid mixture (60/15/25/0.1/0.05, by vol.) and the flow rate was 2 ml/min. Peak heights were measured and the concentrations of the compounds were quantitated by calculation of the ratio between deoxycytidine and dFdC/dFdU. A calibration curve ranging from 10 to 1,000 ng/ml was obtained by spiking normal plasma. The limit of quantitation was about 0.5 µ*M* for dFdC and dFdU.

Pharmacokinetic parameters were calculated with PC-NONLIN software (SCI software) using a two-compartment model. The area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal rule. Total clearance was calculated by dividing the dose by the AUC.

Results

Bolus-injection schedules

Table 1 summarizes the experiments performed to determine the MTDs of the various schedules. Schedule 2 (120 mg/kg gemcitabine q3d × 4) was considered to be the standard i.p. schedule. This schedule had previously been shown to be the most active bolus i.p. schedule for treatment of mice bearing various types of xenografts [4, 5, 13]. With this schedule, weight loss in BALB/c mice varied between the experiments but was not more than 15%. The weight loss was usually acute and observed at 1 day after treatment. On the 2nd day the mice recovered completely from the treatment. In tumor-bearing mice, toxic deaths were occasionally observed with schedule 2 (Table 1). The weight loss for C57/B16 mice was lower, which allowed a substantial increase in dose intensity (Schedules 4, 5, 6, and 8).

In Table 2 the antitumor activities for all experiments are summarized on schedule 2, Colon 26–10 (Fig. 1) was the most sensitive tumor and Colon 38, the least sensitive (GDF 6.5 and 0.9, respectively). Although in C57/B16 mice the dose could be increased to 300 mg/kg (schedule 6; Fig. 2), this hardly improved the antitumor activity (GDF 1.7). Since in Colon 26–10, regrowth of the responding tumors was observed after the last i.p. treatment, the number of injections was increased, although the dose had to be decreased to 100 mg/kg gemcitabine (schedule 7). The antitumor activity of this schedule, with similar toxicity, was significantly better than that of the standard treatment ($P < 0.01$; GDF 9.6 and 6.5, respectively). The ILS was also longer for mice that had been treated six times than for those that had been treated four times.

Table 1 Determination of the MTD of gemcitabine for various schedules of administration[a]

Number	Schedule	Route	Toxicity			
			MWL[b] (%)	(day)	Deaths (*n*)	(day)
BALB/c:						
1	100 mg/kg (q3d × 4)	i.p.	12	(8)	0/5[e]	
2	120 mg/kg (q3d × 4)	i.p.	10	(8)	6/36[c,e]	(9)
3	150 mg/kg (q3d × 4)	i.p.	15	(10)	0/3[d]	
4	180 mg/kg (q3d × 4)	i.p.	27	(13)	1/3[d]	(11)
5	240 mg/kg (q3d × 4)	i.p.	25	(10)	2/3[d]	(10)
7	100 mg/kg (q3d × 6)	i.p.	11	(8)	0/6[e]	
9	2 mg/kg/24 h (3d pump)	s.c.	11	(6)	2/10[e]	(3)
10	3.5 mg/kg/24 h (3d pump)	s.c.	24	(4)	5/5[d]	(4)
12	2 mg/kg/24 h (7d pump)	s.c.	20	(7)	5/5[d]	(7)
13	5 mg/kg/24 h (q7d × 2)	i.v.	4	(2)	0/3[d]	
14	10 mg/kg/24 h (q7d × 2)	i.v.	9	(2)	0/3[d]	
15	15 mg/kg/24 h (q7d × 2)	i.v.	16	(5)	0/12[e]	
C57/B16:						
2	120 mg/kg (q3d × 4)	i.p.	4	(7)	0/12[c]	
4	180 mg/kg (q3d × 4)	i.p.	8	(7)	0/3[d]	
5	240 mg/kg (q3d × 4)	i.p.	4	(7)	0/3[d]	
6	300 mg/kg (q3d × 4)	i.p.	4	(7)	1/6[e]	(9)
8	120 mg/kg (q3d × 6)	i.p.	4	(6)	0/6[e]	
9	2 mg/kg/24 h (3d pump)	s.c.	0	(1)	0/4[e]	
11	1 mg/kg/24 h (7d pump)	s.c.	4	(6)	0/4[e]	
12	2 mg/kg/24 h (7d pump)	s.c.	8	(11)	0/4[e]	
15	15 mg/kg/24 h (q7d × 2)	i.v.	9	(9)	0/3[d]	
16	20 mg/kg/24 h (q7d × 2)	i.v.	6	(8)	0/6[e]	
17	25 mg/kg/24 h (q7d × 2)	i.v.	18	(9)	0/3[d]	

[a] Initial dosing studies were performed in BALB/c mice because of the generally higher toxicity; the MTD was subsequently used as a starting point for C57/B16 mice
[b] Maximal weight loss (%) as compared with the 1st day of treatment (day 0), including the day after first treatment at which this was observed
[c] Toxic deaths were found only in tumor-bearing mice
[d] Only non-tumor-bearing mice
[e] tumor-bearing and non-tumor-bearing mice

Table 2 Summary of the antitumor activity of gemcitabine given at various doses and schedules.

Schedule number	Schedule	Exp[l]	Route	Antitumor effect					
				Dt[a] (days)	GDF[b]	Max T/C[c]	(day)	CR	ILS[d]
Colon 26:									
Control				2.8					
2	120 mg/kg (q3d × 4)	1	i.p.	13.2[f]	5.1	0.63[i]	(3)	0/8	264
9	2 mg/kg/24 h (3d pump)	2	s.c.	9.8[f]	2.2	0.35[j]	(6)	0/5	135
15	15 mg/kg/24 h (q7d × 2)	3	i.v.	8.7[f]	2.8	0.29[j]	(2)	0/5	173
2	120 mg/kg (q3d × 4)	4	i.p.	16.7[f]	3.6	0.25[j]	(8)	0/7	167
15	15 mg/kg/24 h (q7d × 2)	4	i.v.	14.6[f]	3.0	0.59[i]	(12)	1/6	167
Colon 26-10:									
Control				2.8					
2	120 mg/kg (q3d × 4)	1	i.p.	20.1[f]	6.7	0.02[j]	(10)	1/9	144
2	120 mg/kg (q3d × 4)	2	i.p.	21[f]	6.5	0.03[j]	(11)	1/7	159
1[e]	100 mg/kg (q3d × 4)	2	i.p.	22.6[f]	7.1	0.03[j]	(11)	2/10	156
7	100 mg/kg (q3d × 6)	2	i.p.	29.7[f,g]	9.6	0.02[j]	(18)	1/10	207
9	2 mg/kg/24 h (3d pump)	3	s.c.	8.4[f]	1.5	0.27[k]	(6)	0/5	100
2	120 mg/kg (q3d × 4)	4	i.p.	> 36[f]	6.1	0.041[j]	(10)	3/13	163
15	15 mg/kg/24 h (q7d × 2)	4	i.v.	20.7[f]	13.6	0.044[j]	(14)	4/11	242
2	120 mg/kg (q3d × 4)	5	i.p.	19.6[f]	6.5	0.025[j]	(10)	0/9	216
15	15 mg/kg/24 h (q7d × 2)	5	i.v.	48.6[f,h]	> 17.7	0.007[j]	(14)	8/11	> 1, 000[m]
Colon 38:									
Control				7.5					
2	120 mg/kg (q3d × 4)	1	i.p.	16.6[f]	0.9	0.29[k]	(17)	0/8	
2	120 mg/kg (q3d × 4)	2	i.p.	14.7[f]	1.1	0.29[k]	(9)	0/8	
8	120 mg/kg (q3d × 6)	2	i.p.	18[f]	1.5	0.18[i]	(16)	0/8	
6	300 mg/kg (q3d × 4)	2	i.p.	19.3[f]	1.7	0.10[k]	(9)	0/8	
9	2 mg/kg/24 h (3d pump)	3	s.c.	10.8[f]	2.4	0.49[i]	(12)	0/5	
12	2 mg/kg/24 h (7d pump)	3	s.c.	10.3[f]	2.3	0.27[k]	(8)	0/6	
16	20 mg/kg/24 h (q7d × 2)	4	i.v.	9.3	0.4	0.52[k]	(14)	0/7	

[a] Mean doubling time of tumors
[b] GDF = (doubling time of treated tumors - doubling time of control tumors)/doubling time of controls. Values of > 1 indicate that the tumor is sensitive
[c] T/C = relative tumor volume of treated animals/relative tumor volume of controls
[d] ILS = Median life span of the treated group/median life span of the control group × 100%, using the day of transplantation as day 0
[e] 100 mg/kg q3d × 4 was included to provide a direct comparison with 100 mg/kg q3d × 6
Doubling-time statistics:
[f] Significantly different from control tumours ($P < 0.001$)
[g] 100 mg/kg q3d × 6 significantly different from 100 mg/kg q3d × 4 ($P < 0.01$)
[h] 15 mg/kg/24 h q7d × 2 significantly different from 120 mg/kg q3d × 4 ($P < 0.001$)
Tumor-volume statistics:
[i] Significantly different from control tumors ($P < 0.05$)
[j] Significantly different from control tumors ($P < 0.001$)
[k] Significantly different from control tumors ($P < 0.01$)
[l] Arbitrary numbering of separate experiments
[m] since most mice were cured, exact ILS could not be determined
CR complete remission

Continuous-infusion doses and schedules

The initial doses for the continuous infusions were based on the ratio between the IC_{50} values determined for 1 and 48 h. Additionally, it had been observed earlier that more frequent administration (once daily) necessitated a considerable decrease in the dose. In the experiments in which BALB/c mice were treated with the s.c. osmotic pumps, the MTD for a 3-day infusion was 2 mg/kg gemcitabine per 24 h (schedule 9); for the 7-day infusion, 2 mg/kg per 24 h (schedule 12) was well above the MTD (20% weight loss; Table 1). The toxicity observed in BALB/c mice bearing Colon 26–10 and treated for 3 days was comparable with that of the i.p. 120 mg/kg q3d × 4 (schedule 2) treatment. The antitumor activity (Table 2), however, was lower. For Colon 26-bearing animals (Fig. 3) the toxicity of the 3-day infusion (schedule 9) was also comparable with that of

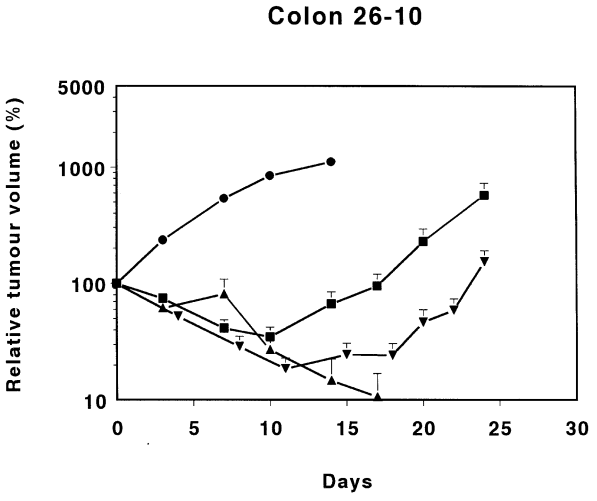

Fig. 1 Antitumor activity against Colon 26-10 of dFdC given at several schedules and doses. Tumor volumes are depicted as mean values ± SEM (*Circles* Control growth [SEM are within the symbols], *squares* i.p. 120 mg/kg dFdC q3d × 4, *inverted triangles* i.p. 100 mg/kg q3d × 6, *triangles* continuous infusion 15 mg/kg per 24 h q7d × 2). The *curves* generated for the treatments derive from separate experiments, in which the control tumors grew at a comparable rate. At day 14, 15 mg/kg per 24 h q7d × 2 was significantly different from 120 mg/kg q3d × 4 ($P < 0.05$)

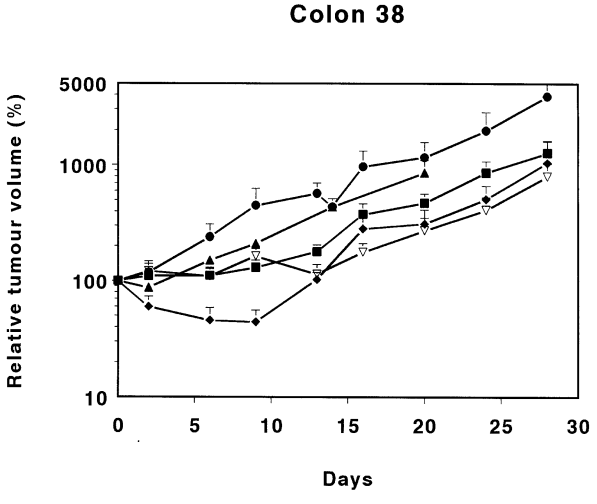

Fig. 2 Antitumor activity against Colon 38 of dFdC given at several schedules and doses. Tumor volumes are depicted as mean values ± SEM (*Circles* Control growth, *squares* i.p. 120 mg/kg dFdC q3d × 4, *white inverted triangles* i.p. 120 mg/kg q3d × 6, *diamonds* i.p. 300 mg/kg q3d × 4, *black triangles* continuous infusion 20 mg/kg per 24 h q7d × 2). The *curves* generated for the treatments derive from separate experiments, in which the control tumors grew at a comparable rate. At day 9, 300 mg/kg q3d × 4 was significantly different from 120 mg/kg q3d × 4 ($P < 0.01$) ; at day 8, 2 mg/kg per 24 h (7-day pump) was not significantly different from 2 mg/kg per 24 h (3-day pump)

the 120 mg/kg q3d × 4 schedule (schedule 2), and, as in Colon 26–10, schedule 9 was less effective than the i.p. treatment (schedule 2). The MTD in C57/B16 mice was higher than that in BALB/c mice (Table 1). A significantly better antitumor effect was obtained for

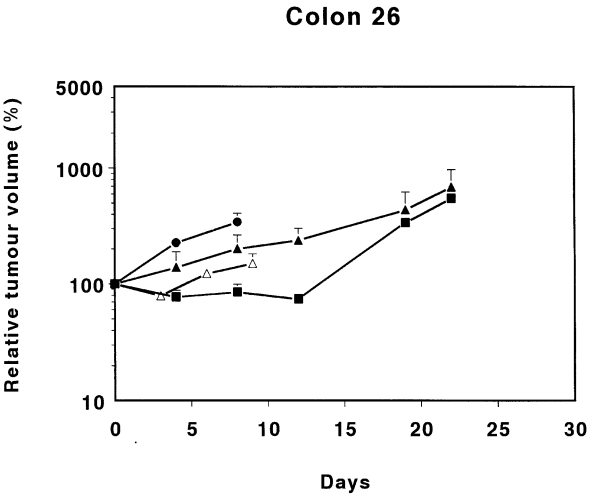

Fig. 3 Antitumor activity against Colon 26 of dFdC given at several schedules and doses. Tumor volumes are depicted as mean values ± SEM. (*Circles* Control growth, *squares* i.p. 120 mg/kg dFdC q3d × 4, *white triangles* continuous infusion s.c. 2 mg/kg per 24 h for 3 days, *black triangles* continuous infusion i.v. 15 mg/kg per 24 h q7d × 2). The *curves* generated for the treatments derive from separate experiments, in which the control tumors grew at a comparable rate. At day 8, 120 mg/kg q3d × 4 was not significantly different from 15 mg/kg per 24 h q7d × 2

Colon 38 with both s.c. infusion schedules (3 days [schedule 9] and 7 days [schedule 12]) than with the i.p. treatment ($P < 0.05$; GDF 2.4, 2.3, and 0.9, respectively). All tumors started to regrow after discontinuation of the treatment.

The MTD for a 24-h i.v. infusion of gemcitabine in BALB/c mice appeared to be 15 mg/kg per 24 h (schedule 15) and that in C57/B16 mice, 20 mg/kg per 24 h (schedule 16) when treatment was repeated after 1 week (Table 1). The highest weight loss was found after 3 days, but the mice recovered before the next infusion. Again, for Colon 26 tumors the 24-h infusion gave results comparable with those of the 3-day continuous infusion. Against Colon 38 this 24-h infusion was less effective than the i.p. and 3-day continuous-infusion treatment. Against Colon 26–10, however, the 15 mg/kg per 24 h q7d × 2 i.v. gemcitabine schedule (schedule 15) was superior to any other treatment ($P < 0.001$; GDF > 17.7) as observed in several separately performed experiments. No direct regrowth was observed after cessation of the i.v. treatment, in contrast to the i.p. schedules; the ILS showed a marked increase.

Plasma concentrations of gemcitabine and dFdU

Plasma concentrations of gemcitabine were determined at the various administration schedules. For the bolus injections the plasma peak concentration of gemcitabine was 225 μ*M* as determined at 10 min after injection, the peak for dFdU (79 μ*M*) was

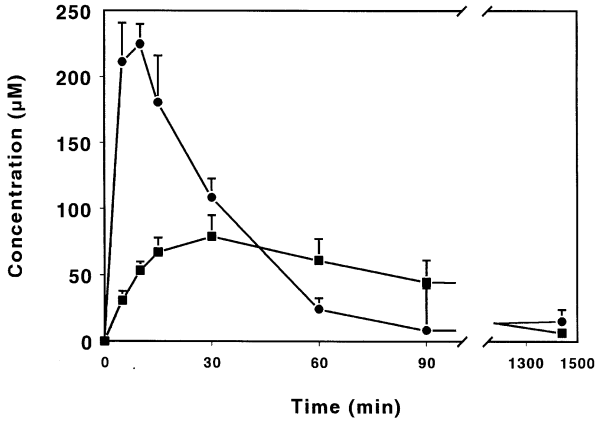

Fig. 4 Plasma concentrations of gemcitabine (*circles*) and dFdU (*squares*) as determined after one bolus injection of 120 mg/kg dFdC. Data represent mean values ± SEM

observed at 30 min (Fig. 4). The elimination half-lives of gemcitabine and dFdU were 15.7 min and 8.2 h, respectively. The clearance calculated for gemcitabine was 1.68 ml/min, but that found for dFdU was 3 times lower. The AUC determined after one bolus injection of gemcitabine was 7,587 μmol min 1^{-1}. A doubling of the dose resulted in a 2-fold increase in the AUC (14,917 μmol min 1^{-1}) without affecting half-lives or elimination. At a weekly interval, 240 mg/kg is the MTD. Plasma concentrations measured for gemcitabine and dFdU after continuous infusions were below the detection limit.

Discussion

This study is the first to demonstrate that continuous infusions of gemcitabine can significantly improve its antitumor effect against solid tumors. From previous studies the antitumor effect of gemitabine was known to be strongly schedule-dependent, but no continuous-infusion schedules were tested [5, 6, 8, 13, 27]. The i.p. schedule of 120 mg/kg q3d × 4 (schedule 2) was very effective, but we now show that either an increase in the dose (schedule 6) or prolongation of the treatment (schedules 7 and 8) can result in a better effect.

In patients the schedule most often applied is a 30-min infusion of 800–1,250 mg/m^2 q7d × 3, followed by a 1-week rest period. On this weekly schedule, pronounced activity has been observed against several tumor types, such as ovarian and non-small-cell lung cancer [1, 14, 17]. However, other frequently applied antimetabolites such as 1-β-D-arabinofuranosylcytosine and methotrexate are usually given over longer periods, since it has been shown that this provides optimal efficacy [22, 24]. For 5-fluorouracil, administration as a continuous infusion increased the response rate, although the survival of patients was not improved [16, 22]. Also for gemcitabine treatment we could show in our model system that a prolongation of the treatment (schedules 7 and 8) or continuous infusions (schedules 12 and 15) could result in a better effect. A major decrease in gemcitabine dosing was observed when the drug was given for prolonged periods. For i.p. scheduling a more frequent administration led to similar observations (a lower gemcitabine dose) in earlier preclinical [5, 6, 8] and clinical studies [17]. The antitumor activity, however, did not increase. This may be explained by a lower accumulation of dFdCTP, the active metabolite of gemcitabine, in the tumors. However, Grunewald et al. [10] showed that with prolongation of the infusion time from 30 to 60 min, at total dose of 800 mg/m^2 the cellular AUC of dFdCTP in blast cells increased by up to 4 orders of magnitude. In an additional clinical study the infusion times were prolonged further; unfortunately, the response rate was not increased [11]. Both studies, however, were performed in leukemia patients. Since solid tumors seem to respond better to gemcitabine treatment than do leukemias, application of these prolonged infusion schedules to patients with solid tumors, is warranted, also considering the results we obtained in murine colon tumors. In a recent phase I trial applying continuous infusions the MTD for a 24-h infusion was 180 mg/m^2 [3], which is 5–10 times lower than the MTD for the now frequently used weekly bolus-injection schedule [14, 17]. A similar ratio was noted in our studies. It would be worthwhile to test continuous-infusion schedules in more tumor types because when cells are exposed to gemcitabine over a longer period there is a more effective dFdCTP formation and the total retention of dFdCTP is probably longer lasting [26] ; thus, one can expect a better therapeutic effect of the drug. It might even be considered to evaluate 24-h infusions in insensitive tumor types such as colon cancer. Another application for continuous infusions would be the combination of gemcitabine with other agents, similar to that of other antimetabolites with, e.g., cisplatin [4, 19, 28].

There seems to be a threshold for gemcitabine to be active as shown in in vitro studies. In vitro, IC_{50} values for gemcitabine drop dramatically with extension of the exposure period, an effect that is most pronounced in colon-tumor cell lines [26]. In C26-10 cells derived from the Colon 26 tumour, accumulation of dFdCTP, the active metabolite of gemcitabine, was time- and concentration-dependent. Thus, to obtain an optimal therapeutic index for gemcitabine, it is extremely important that the optimal dose be chosen in combination with the best infusion time and dosing interval. From our results it appears that high doses given by bolus injection at longer intervals are best tolerated by normal tissue, whereas for a good antitumor effect a more prolonged infusion seems most promising. To enable the choice of the right schedule, more research is needed on the toxicity patterns of the i.p. schedule as compared with prolonged infusions.

The pharmacokinetic parameters of gemcitabine and dFdU in the mouse (i.p. schedule) and in humans (a 30-min infusion given every week for 3 weeks, followed by 1 week of rest) are of the same order of magnitude as those found in our mice [11, 22]. In humans the elimination from plasma mainly involved dFdU, the deamination product of gemcitabine. The elimination of gemcitabine was rapid ($t_{1/2}$ 8 min) as compared with that of dFdU ($t_{1/2}$ 14 h). In both species the peak concentration of dFdU was observed much later than that of gemcitabine and dFdU was retained much longer than gemcitabine. The AUC value obtained at the weekly MTD (240 mg/kg) in mice is comparable with that achieved in humans treated at 3-weekly intervals (16,968 $\mu mol\,min\,l^{-1}$ at 5,700 mg/m^2) [22].

Mechanisms that may be involved in the difference in sensitivity to gemcitabine could be the activities of the activating and inactivating enzymes deoxycytidine kinase (dCK) and deoxycytidine deaminase (dCDA). In all tumors tested, dCK activity was high enough (data not shown) to activate gemcitabine and there was no correlation between the dCK and dCDA activity and the sensitivity [25]. Another possibility involved in the difference in sensitivity may consist of the difference in the mouse strain used; higher doses could be given to C57/B16 mice. The differences between the tumors may have contributed to the various sensitivity profiles. Colon 38 is a necrotic tumor with a relatively long doubling time, which may explain why relatively long infusions seem more active. Under these conditions, less dFdCTP may be formed, but since the exposure is long, this may be sufficient to obtain an enhancement of the antitumor effect similar to that achieved by long-term exposure in vitro [26]. Colon 26–10, however, is much less necrotic, does not cause cachexia, and is the most sensitive tumor. This tumor is fast-growing, which may explain why bolus injections and relatively short infusions are more effective; higher peak dFdCTP levels can be formed, causing more damage to the fast-growing cells. Colon 26 causes cachexia but is not necrotic. Treatment with gemcitabine apparently reduces the incidence of cachexia, because these mice remain in good condition. This may be related to the increase in adenosine triphosphate (ATP) pools observed following treatment with gemcitabine [26]. Relatively high peak levels of dFdCTP may be required for this.

In conclusion, prolonged infusions of gemcitabine can considerably improve the antitumor activity of the drug against solid tumors and may provide a valuable tool for the treatment of (insensitive) solid tumors.

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