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ORIGINAL ARTICLE

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Antitumor activity of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine, a novel deoxyadenosine analog, against human colon tumor xenografts by oral administration

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Abstract 2-Chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine (Cl-F-araA) is a novel deoxyadenosine analog, which inhibits DNA synthesis by inhibiting DNA polymerase α and ribonucleotide reductase. Cl-F-araA shows potent antiproliferative activity against several leukemic cell lines including those of human origin and is also effective against murine solid tumors, in particular being curative against colon tumors. *Purpose*: We therefore decided to investigate whether Cl-F-araA is effective against human colon tumors, in particular by oral administration, since it has improved stability compared with other deoxyadenosine analogs. *Methods*: Antiproliferative activity in vitro was determined from cell counts. Subcutaneously inoculated xenograft models and a liver micrometastases model were used for assessment of antitumor activity in vivo. *Results*: Cl-F-araA showed potent antiproliferative activity against four human colon tumor cell lines (HCT116, HT-29, DLD-1, WiDr), with a 50% growth-inhibitory concentration (IC_{50}) of 0.26 μ*M* with a 72-h exposure. This activity was greater than those of fludarabine desphosphate and cladribine, other deoxyadenosine analogs, which showed IC_{50} values of 19 μ*M* and 0.35 μ*M*, respectively. Cl-F-araA showed potent antitumor activity against four human colon tumor xenograft models (HT-29, WiDr, Co-3, COLO-320DM) in a 5-day daily administration schedule, which was shown to be the most effective of three administration regimens tested (single, twice-weekly, 5-day daily). In particular, oral administration showed significantly superior activity, with a regressive or cytostatic growth curve, compared with intravenous administration. In addition, Cl-F-araA was effective at only one-sixteenth of the maximum dose tested in a 10-day daily administration schedule. Therapeutic efficiency seemed to increase in proportion to the frequency of administration. Cl-F-araA also decreased liver micrometastases created by intrasplenic injection of human colon tumor cells, leading to complete suppression at the maximum dose tested. *Conclusions*: These results suggest that Cl-F-araA might be clinically effective against human colon cancers using a daily oral administration schedule.

Key words Antimetabolite · Antitumor activity · Colon · Oral administration · Schedule

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Introduction

Antimetabolites have been widely used to treat a variety of tumors. 2-Chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine (Cl-F-araA) is the latest deoxyadenosine analog to be synthesized. The drug has a chloride at the C2 position of deoxyadenosine and a fluoride in the 2′-arabino moiety. Therefore, the drug is resistant to deamination and the phosphorolytic cleavage, which are involved in the inactivation of several antimetabolites [3, 15]. To exert its cytotoxic effect, Cl-F-araA requires intracellular phosphorylation by deoxycytidine kinase (DCK) [3], which is a rate-limiting enzyme in the salvage pathway of pyrimidine nucleosides and has broad substrate specificity [1, 2], before finally being metabolized to the corresponding triphosphate form, Cl-F-araATP [29]. Cl-F-araATP inhibits both DNA polymerase α (poly α) and ribonucleotide reductase (RNR) [17, 30], like the triphosphate forms of fludarabine and cladribine [6, 16, 26], which have been used clinically alone and in combination for the treatment of hematological malignancies such as chronic lymphocytic leukemia (CLL) [11, 13, 18], low-grade lymphoma [12, 21], hairy cell leukemia (HCL) [19, 24] and acute myelogenous leukemia [4]. The concentration of Cl-F-araATP required to inhibit poly α is similar to that of fludarabine or cladribine, whereas the concentration for RNR inhibition is

the same as that of cladribine, but tenfold less than that of fludarabine [17].

Cl-F-araA shows potent cytotoxicity against several leukemic cell lines including those of human origin [3, 15], and is effective against murine tumor syngeneic models such as P388 leukemia, colon 36 and mammary 16/c [27]. Interestingly, Cl-F-araA is curative against both early and advanced stages of colon 36 tumors. However, its effect on human solid tumors has not yet been determined.

Cl-F-araA has the advantages over other deoxyadenosine analogs of being stable in acidic solution and resistant to the phosphorolytic cleavage [3, 15]. A pharmacokinetic study in rats has suggested that the drug might show antitumor activity when administered orally because the bioavailability of Cl-F-araA is approximately 50% [20]. Therefore, Cl-F-araA might be effective when administered orally as well as by the intravenous route. In this study, we studied particularly tumors of the colon and investigated whether Cl-F-araA was effective against human tumors, particularly by oral administration using subcutaneously inoculated xenograft models and a liver micrometastases model.

Materials and methods

Chemicals and tumor cells

Cl-F-araA (Fig. 1) was provided by Southern Research Institute (Birmingham, Ala.). Fludarabine desphosphate (2-fluoroadenine-9-β-D-arabinofuranoside) and cladribine (2-chloro-2′-deoxyadenosine) were purchased from Sigma Chemical Co. (St. Louis, Mo.). Human colon tumor cell lines (HCT116, HT-29, DLD-1, WiDr) were obtained from the American Type Culture Collection (Rockville, Md.). These cell lines were maintained each in its most suitable medium (McCoy 5A for HCT116, RPMI-1640 for HT-29 and DLD-1, and MEM for WiDr) with 10% fetal bovine serum (Life Technologies, Grand Island, N.Y.) at 37 °C in a humidified atmosphere of 5% CO_2 in air for evaluating antiproliferative activity in vitro. HT-29, WiDr and COLO-320DM tumor xenografts were established by inoculating subcutaneously each type of cultured cells into 6–8-week-old male BALB/c-nu/nu mice (nude mice) obtained from Clea Japan (Tokyo, Japan). Co-3, LC-6, QG-56, LX-1, MX-1 and NS-8 tumor xenografts were supplied by the Central Institute of Experimental Animals (Kanagawa, Japan) and maintained subcutaneously in the flank of nude mice.

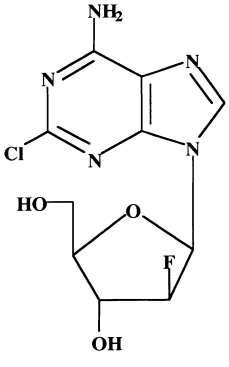

Fig. 1 Structure of Cl-F-araA

Antiproliferative activity

Cells were precultured for 24 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. The cells were exposed to various concentrations of drugs for 72 h. The cells were counted using a microcell counter (Sysmex F-300, Toa Medical Electronics, Hyogo, Japan). The concentration of drugs required for 50% inhibition of cell growth (IC_{50}) was calculated and is taken to represent the antiproliferative activity. The mean IC_{50} is expressed as the mean log IC_{50} of each drug against the four tumor cell lines.

Antitumor activity

Tumor fragments of approximately 8 mm^3 were inoculated subcutaneously into the flank of nude mice ($n = 3$–5). When the tumor reached a volume of between 50 and 300 mm^3, drugs were administered in the indicated schedule. The lengths and widths of the tumors were measured twice weekly and tumor volumes were calculated according to the following formula: length × width × width × 0.5. Drug efficacy against tumor xenografts is expressed in terms of T/C minimum (T/C min) which is the lowest mean value of V/V_0 of the treated group versus that of the control group during the observation period after first drug treatment, where V was the tumor volume on the day of evaluation and V_0 was the tumor volume on the day of first drug treatment (day 0). Body weight change is shown relative to that observed on day 0. The statistical significance of differences was analyzed using the Mann-Whitney *U*-test and the results were considered significant when T/Cmin was less than 50% and the *P*-value was less than 0.05.

Liver micrometastases

Nude mice ($n = 6$) were anesthetized with pentobarbital by intraperitoneal injection and the abdomen was prepared for sterile surgery. A small abdominal incision was made and the spleen was isolated. A single-cell suspension of 5×10^5 HT-29 human colon tumor cells in 0.05 ml Hanks' solution (Nissui Pharmaceutical Co., Tokyo, Japan) containing 1% serum of nude mice and 10 units/ml heparin (Shimizu Pharmaceutical Co., Shizuoka, Japan) was slowly injected into the spleen using a 27-gauge needle. The spleen was returned to the abdominal cavity and the wound was closed with sutures. Drug administration was initiated 6 days after the tumor injection and was given in a 5-day daily oral administration schedule at doses in the range 25–100 mg/kg per day. There were no postoperative deaths. The liver micrometastasis burden at 4 weeks after the tumor injection was scored by counting tumor foci of >2 mm in diameter. Statistical significance was analyzed using the Steel test and the results were considered significant when the *P*-value was less than 0.05.

Results

Antiproliferative activity of Cl-F-araA against four human colon tumor cell lines

We investigated the antiproliferative activity of Cl-F-araA and two other deoxyadenosine analogs, fludarabine desphosphate and cladribine, against four human colon tumor cell lines. The human colon cell lines tested and the antiproliferative activity of the drugs in terms of the IC_{50} values are summarized in Table 1. Cl-F-araA showed potent antiproliferative activity against the four human colon tumor cell lines tested, with a mean IC_{50} of 0.26 μ*M* ranging between 0.07 μ*M* and 0.8 μ*M* with a 72-h exposure. The activity was greater

Table 1 Comparative antiproliferative activity of Cl-F-araA, fludarabine desphosphate and cladribine against human colon tumor cell lines. Cells were exposed to various concentrations of the drugs for 72 h. Antiproliferative activity (IC_{50}) of the drugs was determined from cell numbers. Values are the means of three separate experiments

Cell line	Antiproliferative activity IC_{50} (μM)		
	Cl-F-araA	Fludarabine	Cladribine
HCT116	0.12	3.1	0.12
HT-29	0.77	30	3.4
DLD-1	0.07	18	0.09
WiDr	0.67	72	9.5
Log mean	0.26	19	0.35

than that of fludarabine desphosphate and cladribine, which had IC_{50} values of 19 μM and 0.35 μM, respectively.

Optimal administration schedule for antitumor activity

We first sought to determine the optimal administration schedule for Cl-F-araA before the precise evaluation of its antitumor activity. The antitumor activity against subcutaneously inoculated HT-29 human colon tumors in nude mice was evaluated in three intraperitoneal administration schedules: single (administration on day 0), twice-weekly (administration on days 0 and 3), and 5-day daily (administration on days 0–4) (Table 2, Fig. 2). A result was considered significant when T/C was less than 50% and the *P*-value was less than 0.05. The daily administration schedule (Fig. 2C) was evaluated at doses in the range 25–100 mg/kg per day and was dose-dependently effective showing T/Cmin of 48% and 30% at 50 mg/kg per day and 100 mg/kg per day, respectively.

Table 2 Optimal administration schedule for antitumor activity of Cl-F-araA. Cl-F-araA suspended in saline was administered in a single, a twice-weekly or a 5-day daily intraperitoneal schedule to nude mice inoculated with HT-29 human colon tumors. Tumor volumes were followed for 22 days after initial treatment (day 0) and antitumor activity is represented in terms of T/Cmin

Drug	Schedule	Dose (mg/kg/day)	T/Cmin (%)	Mortality
Cl-F-araA	Single	500	–	5/5
		250	85	0/5
		125	82	0/5
	Twice-weekly	500	–	5/5
		250	63	0/5
		125	83	0/5
	Daily	200	5	2/5
		100	30[a]	0/5
		50	48[a]	0/5
		25	52	0/5

[a] The T/C value was less than 50% and the *P*-value from statistical analysis by the Mann-Whitney *U*-test was less than 0.05

On the other hand, the single (Fig. 2A) and the twice-weekly (Fig. 2B) administration schedules showed no significant activity even at 250 mg/kg per day, the maximum dose tested at which no mice died. However, the twice-weekly administration at 250 mg/kg per day showed increased activity compared with the single administration, but the daily schedule showed a more potent effect when compared in terms of the total dose of each schedule. The daily schedule at 50 mg/kg per day (a total dose of 250 mg/kg) showed significant activity whereas the other schedule at the same total dose did not. Therefore, it was concluded that Cl-F-araA showed a schedule-dependent antitumor activity with an increased therapeutic efficacy when administered daily. Similar results were also seen with the oral and intravenous administration schedules (data not shown), suggesting that Cl-F-araA might show time-dependent activity against tumor cells. This is consistent with the results of kinetic analysis of antiproliferative activity, which was analyzed in terms of the relationship between drug concentration and exposure time, reported by Inaba et al. [9]

Antitumor activity of Cl-F-araA administered by the oral and intravenous routes

We next investigated the antitumor activity of Cl-F-araA administered orally and intravenously against the HT-29 tumor xenograft model in a 5-day daily schedule (Table 3, Fig. 3). The oral administration schedule was evaluated at doses in the range 25–200 mg/kg per day. Two of five mice had died by day 7 at 200 mg/kg per day (Table 3). Cl-F-araA was significantly effective in the dose range 50–100 mg/kg per day, particularly showing marked activity with a T/Cmin of 8% at 100 mg/kg per day. As shown in Fig. 3A, 100 mg/kg per day of Cl-F-araA showed a regressive growth curve by day 16. Significant body weight loss was not observed with any of the doses tested compared with the body weight of each group on day 0. On the other hand, the intravenous administration schedule was evaluated at doses in the range 25–100 mg/kg per day because five of five mice died at 200 mg/kg per day in an independent experiment (data not shown). Cl-F-araA was also significantly effective administered intravenously at doses in the range 50–100 mg/kg per day, but did not cause regression of tumor volume (Fig. 3B). Slight body weight loss was observed on day 4 of the last treatment at 100 mg/kg per day, but it immediately recovered (Fig. 3B). Therefore, oral administration showed superior activity to intravenous administration, particularly at 100 mg/kg per day.

A dose of 200 mg/kg per day was not tolerated by mice on both the oral and intravenous administration schedules. Therefore, the maximum tolerated dose (MTD) of Cl-F-araA administered orally might be almost the same as when administered intravenously. This suggests that Cl-F-araA could have excellent oral bioavailability in mice in our system.

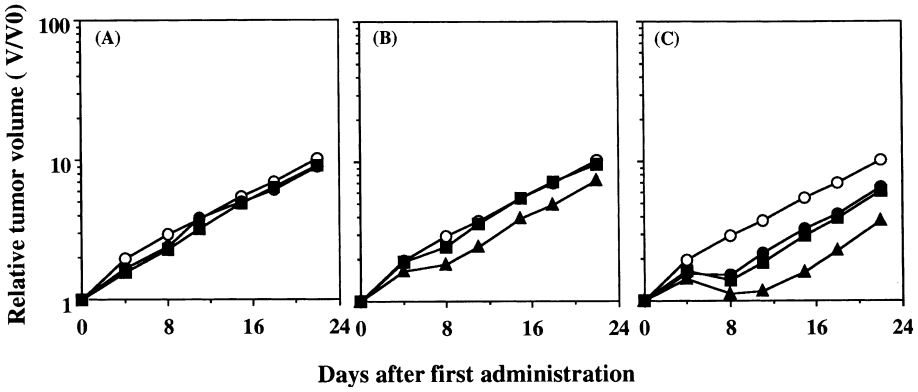

Fig. 2A–C Optimal administration schedule for antitumor activity of Cl-F-araA. Cl-F-araA suspended in saline was administered by a single (**A** ○ control, ● 125 mg/kg per day, ■ 250 mg/kg per day), twice weekly (**B** ○ control, ■ 125 mg/kg per day, ▲ 250 mg/kg per day) or 5-day daily (**C** ○ control, ● 25 mg/kg per day, ■ 50 mg/kg per day, ▲ 100 mg/kg per day) intraperitoneal schedule to nude mice inoculated with HT-29 human colon tumors. Mean tumor volume of each group on day 0 was 144 ± 21 mm^3. Tumor volumes were measured twice weekly and studied for 22 days after initial treatment (day 0)

Antitumor activity of Cl-F-araA against human colon tumors

To investigate whether Cl-F-araA shows antitumor activity against other human colon tumor xenograft models, additional experiments with colon tumor xenografts WiDr, Co-3 and COLO-320DM were performed using a 5-day daily oral administration schedule. As shown in Table 4, Cl-F-araA showed significantly superior antitumor activity with a T/Cmin of less than 10% at a dose of 100 mg/kg per day, showing regressive or cytostatic activity for all the colon tumor models tested (Fig. 4). Interestingly, the COLO-320DM tumor xenograft did not propagate during the observation period whereas HT-29, WiDr and Co-3 tumor xenografts began to propagate 16–20 days after initial treatment.

We also investigated the antitumor activity of Cl-F-araA against other xenografts including lung, breast and stomach tumors (Table 4). Cl-F-araA showed the greatest superior activity against the LX-1 tumor xenograft, as it did against colon tumors, of three lung tumor xenografts tested. It also showed moderate activity against MX-1 breast tumor xenograft. However, it showed no activity against NS-8 stomach or the other two lung tumor xenografts.

Table 3 Antitumor activity of Cl-F-araA administered orally or intravenously against HT-29 human colon tumors. Cl-F-araA dissolved in 26% PEG 400 aqueous solution was administered in a 5-day daily oral or intravenous schedule to nude mice incubated with HT-29 human colon tumors. Tumor volumes were followed for 23 days after initial treatment (day 0) and antitumor activity is represented in terms of T/Cmin. Body weight change was determined on day 4

Drug	Route	Dose (mg/kg/day)	T/Cmin (%)	Body weight change (g)	Mortality
Cl-F-araA	Oral	200	3	−2.6	2/5
		100	8[a]	−0.3	0/5
		50	32[a]	0.1	0/5
		25	55	−0.1	0/5
	Intravenous	100	34[a]	−1.5	0/5
		50	41[a]	−0.5	0/5
		25	55	−0.1	0/5

[a] The T/C value was less than 50% and the *P*-value from statistical analysis by the Mann-Whitney *U*-test was less than 0.05

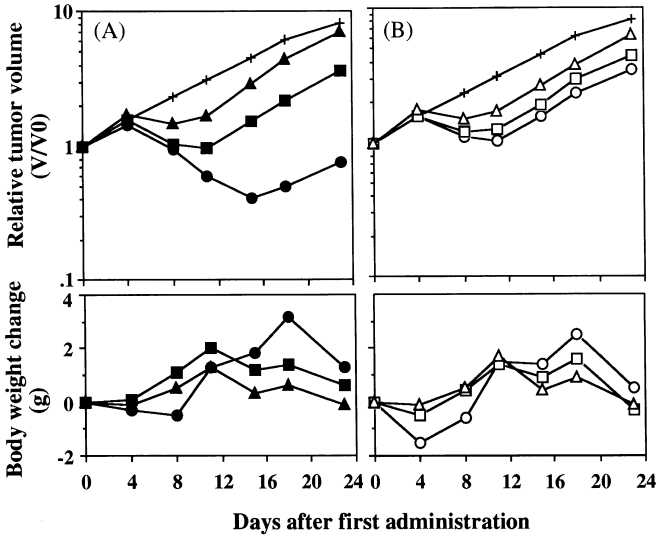

Fig. 3A,B Antitumor activity of and body weight change induced by Cl-F-araA administered by the oral (**A**) and intravenous (**B**) routes. Cl-F-araA dissolved in 26% PEG 400 aqueous solution was administered in a 5-day daily oral (**A** + control, ▲ 25 mg/kg per day, ■ 50 mg/kg per day, ● 100 mg/kg per day) or intravenous (**B** + control, △ 25 mg/kg per day, □ 50 mg/kg per day, ○ 100 mg/kg per day) administration schedules at doses in the range 25–100 mg/kg per day to nude mice inoculated with HT-29 human colon tumors. The mean tumor volume of each group on day 0 was 159 ± 34 mm^3. Tumor volumes were measured twice weekly after initial treatment (day 0) and are represented as the mean relative tumor volume (V/V_0). Body weight change (g) is expressed relative to that at the first treatment with the drug

Table 4 Antitumor activity of Cl-F-araA against human colon and other tumors. Cl-F-araA dissolved in 26% PEG 400 aqueous solution was administered in a 5-day daily oral schedule at 100 mg/kg per day. Antitumor activity is represented in terms of T/Cmin

Drug	Organ	Tumor	T/Cmin (%)
Cl-F-araA	Colon	HT-29	8[a]
		WiDr	4[a]
		Co-3	8[a]
		COLO-320DM	0.1[a]
	Lung	LC-6	85
		QG-56	60
		LX-1	2[a]
	Breast	MX-1	18[a]
	Stomach	NS-8	68

[a] The T/C value was less than 50% and the *P*-value from statistical analysis by the Mann-Whitney *U*-test was less than 0.05

Therapeutic efficacy in terms of frequency of administration

It has been recently reported that drugs that can be activated by deoxycytidine kinase and are not inactivated by deaminases would show interspecies differences in MTD between humans and mice [22]. The MTD of these drugs, such as fludarabine phosphate and cladribine, in humans is approximately 10- to 50-fold lower than in mice [8, 14, 28]. Therefore,we wondered whether Cl-F-araA would show antitumor activity with a T/C of less than 50% at much lower doses than the MTD in mice in order to predict clinical efficacy. It is noteworthy that, as shown in Table 2, the MTD of Cl-F-araA in three administration schedules appeared to be relatively constant regardless of administration frequency. We expected that a dose of 100 mg/kg per day might be tolerable in mice particularly with more frequent administration. The antitumor activity of Cl-F-araA in a 10-day daily oral administration schedule was investigated using the WiDr human colon tumor xenograft model. Cl-F-araA was evaluated at doses in the range 3.1–100 mg/kg per day in a 10-day daily administration schedule (Fig. 5B). One of three mice died receiving 100 mg/kg per day, a dose that was tolerated by mice treated on a 5-day daily schedule (Table 5). However, the therapeutic efficacy was increased because Cl-F-araA showed potent antitumor activity even at 3.1 mg/kg per day, one-sixteenth of 50 mg/kg per day, whereas it was effective at 12.5 mg/kg per day, one-eighth of 100 mg/kg per day, in a 5-day daily administration schedule. As shown in Fig. 5B, Cl-F-araA showed a regressive growth curve even at 12.5 mg/kg per day in a 10-day daily administration schedule. These results suggest that the therapeutic efficacy of Cl-F-araA might be increased by more frequent administration.

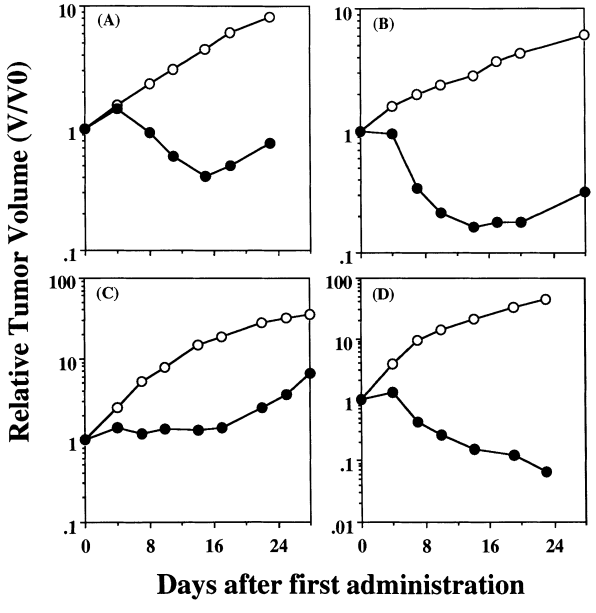

Fig. 4A–D Antitumor activity of Cl-F-araA against human colon tumors. Cl-F-araA dissolved in 26% PEG 400 aqueous solution was administered in a 5-day daily oral administration schedule at 100 mg/kg per day (○ control, ● treated group) into nude mice inoculated with a human colon tumor (**A** HT-29, **B** WiDr, **C** Co-3, **D** COLO-320DM). Tumor volumes were measured twice weekly after initial treatment (day 0) and are represented as the mean relative tumor volume (V/V_0). The mean volumes of the HT-29, WiDr, Co-3 and COLO-320DM tumors on day 0 were 159 ± 34, 227 ± 54, 101 ± 33, and 129 ± 28 mm^3, respectively

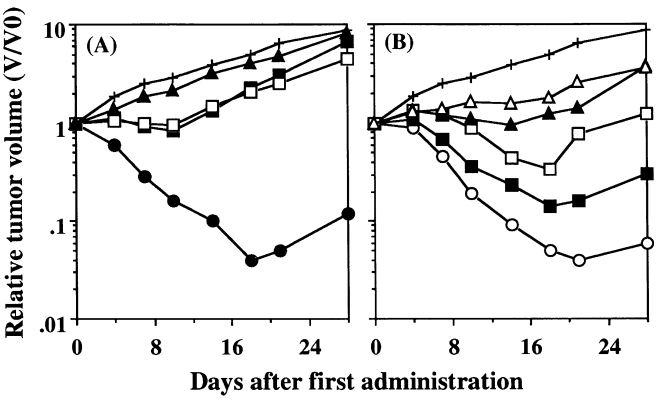

Fig. 5A,B Therapeutic efficacy of Cl-F-araA in terms of frequency of administration. Cl-F-araA dissolved in 26% PEG 400 aqueous solution was administered in a 5-day daily (**A** + control, ▲ 6.3 mg/kg per day, □ 12.5 mg/kg per day, ■ 25 mg/kg per day, ● 100 mg/kg per day) or a 10-day daily (**B** + control, △ 3.1 mg/kg per day, ▲ 6.3 mg/kg per day, □ 12.5 mg/kg per day, ■ 25 mg/kg per day, ○ 50 mg/kg per day) oral schedule to nude mice inoculated with WiDr human colon tumors. Tumor volumes were measured twice weekly and are represented as the mean relative tumor volume (V/V_0). The mean tumor volume of each group on day 0 was 170 ± 44 mm^3

Effect of Cl-F-araA on liver micrometastases of colon tumor cells

We investigated the effect of Cl-F-araA on liver micrometastases of colon tumors (Fig. 6). The liver is frequently the first and only site of dissemination of colon cancer in clinical practice and it has been reported that liver metastases develop in 90% of patients who die of

Table 5 Therapeutic efficiency of Cl-F-araA in terms of frequency of administration. Cl-F-araA dissolved in 26% PEG 400 aqueous solution was administered in a 5-day daily or a 10-day daily oral schedule to nude mice inoculated with WiDr human colon tumors. Tumor volumes were followed for 28 days after initial treatment (day 0) and antitumor activity is represented in terms of T/Cmin. The heighest body weight change was determined on day 4–10

Drug	Schedule	Dose (mg/kg/day)	T/Cmin (%)	Body weight change (g)	Mortality
Cl-F-araA	5-day daily	100	1	−1.7	0/3
		25	29	−0.5	0/3
		12.5	33	−0.5	0/3
		6.3	74	−1.7	0/3
	10-day daily	100	0.4	−2.9	1/3
		50	0.6	−0.9	0/3
		25	2	−0.1	0/3
		12.5	7	0	0/3
		6.3	22	−0.4	0/3
		3.1	36	−1.5	0/3

colon cancer [25]. HT-29 human colon tumor cells, more than 60% of which reach the liver by 5 min after intraspleenal injection [5], were injected into the spleen to produce the model of liver micrometastases,. Six days after the intraspleenal injection, Cl-F-araA was administered in a 5-day daily oral schedule at doses in the range 25–100 mg/kg per day, and tumor foci in the liver were scored after a further 3 weeks. The number of tumor foci formed in the untreated control group was 38 ± 15 under our conditions and Cl-F-araA decreased the formation of tumor foci at doses in the range 50–100 mg/kg per day in a dose-dependent manner. The drug completely suppressed the formation of tumor foci in the liver at a dose of 100 mg/kg per day.

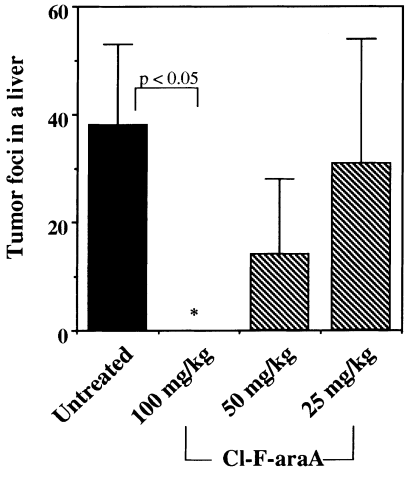

Fig. 6 Effect of Cl-F-araA on liver micrometastases of colon tumor cells. A single-cell suspension of 5×10^5 HT-29 human colon tumor cells in 0.05 ml Hanks' solution containing 1% serum of nude mice and 10 units/ml heparin was slowly injected into the spleen. Administration of the drug was initiated 6 days after tumor injection and was given at doses in the range 25–100 mg/kg per day in a 5-day daily oral administration schedule. The liver micrometastis burden at 4 weeks after tumor injection was evaluated by counting tumor foci of >2mm in diameter. Statistical significance was determined by the Steel test (*$P < 0.05$)

Discussion

Cl-F-araA is a novel deoxyadenosine analog which is activated by DCK [3], without being inactivated by deaminases [15], in the same manner as fludarabine phosphate and cladribine [1, 2]. The drug shows potent cytotoxicity against several leukemic cell lines including those of human origin such as CCRF-CEM and K562 [3, 15, 17], and is also effective against murine tumor syngeneic models such as P388 leukemia, colon 36 and mammary 16/c [27]. Interestingly, Cl-F-araA is curative against both early and advanced stages of colon 36 tumors. Therefore, we studied human colon tumors. Cl-F-araA showed more potent antiproliferative activity than fludarabine desphosphate or cladribine against the human colon tumor cell lines tested and showed marked antitumor activity with regressive or cytostatic activity against all human colon tumor xenografts tested, but was not effective against two lung tumors and a stomach tumor.

In this regard, the level of deaminase in tumors might, in part, play an important role because it has been reported that an antimetabolite activated by DCK is effective against tumors with a high level of deaminase [10]. Cl-F-araA is also included in such antimetabolites. We have preliminary data that human colon tumor xenografts such as HT- 29, WiDr and Co-3 have relatively high deaminase activity compared with some tumor xenografts derived from other organs. It has also been reported that many human colon cancer tissues have high deaminase activity [10]. Therefore, human colon cancers may show high susceptibility to Cl-F-araA. However, there are marked differences in the enzyme activities of tumors [7, 23]. We also have preliminary data that the NS-8 tumor xenograft, which is resistant to Cl-F-araA, has high deaminase activity. Therefore, this aspect requires further exploration.

Drugs that suppress metastases show important effects on survival of patients. The liver is the first and only site of dissemination of colon cancer. At the time of initial colorectal resection, 25% of patients have hepatic metastases, and more than 50% of patients will eventually develop them during their disease. Liver metastases develop in 90% of patients who die of colon cancer [25]. Cl-F-araA showed potent activity against liver micrometastases of colon tumors as well as against subcutaneously inoculated tumors. Therefore, Cl-F-araA may be clinically useful against colon cancers. This effect would be, in part, due to the first pass to the liver of drug administered orally.

Cl-F-araA is active orally because it has the advantages of stability in acid solution and resistance to phosphorolytic cleavage compared with other deoxyadenosine analogs [3, 15]. In fact, Cl-F-araA showed potent antitumor activity following oral administration

in our experiments. The antitumor activity at 100 mg/kg per day in a 5-day daily oral administration schedule was much greater than that at the same dose administered intravenously. Susceptibility differences might be due to unknown mechanisms such as long retention of the drug in mice.

It has been recently reported that drugs catalyzed by DCK show differences in MTD between humans and mice [22]. The MTD of these drugs in humans is approximately over tenfold lower than in mice [8, 14, 28]. Therefore, these drugs would not be expected clinically to show such potent activity in humans as in the mouse model. Cl-F-araA showed potent antitumor activity at a broad of doses range in a 10-day daily oral administration schedule. Its therapeutic efficiency might be increased by more frequent administration. However, we have no evidence on hematological toxicity, particularly cumulative toxicity against bone marrow, resulting from more frequent administration. Therefore, we need to examine this drug using in vitro and in vivo mouse models and if possible, in vitro models using human bone marrow cells.

Preliminary experiments in a mouse model have indicated that Cl-F-araA is more active against CLL than fludarabine [3]. We have also observed that Cl-F-araA shows more potent antiproliferative activity against leukemic cell lines than against carcinoma cell lines. Therefore, we are now investigating human tumors in which Cl-F-araA shows broader therapeutic efficacy.

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