Cancer Chemother Pharmacol (1995) 36: 459–462

ORIGINAL ARTICLE

Kunihiko Koga · Eri Iizuka · Akira Sato · Hisao Ekimoto
Mineaki Okada

Characteristic antitumor activity of cytarabine ocfosfate against human colorectal adenocarcinoma xenografts in nude mice

Received: 21 March 1994 / Accepted: 3 February 1995

Abstract The antitumor activity of cytarabine ocfosfate (SPAC) was tested against human colorectal, gastric and lung adenocarcinoma xenografts in nude mice in comparison with the activities of various antitumor drugs used clinically. SPAC showed higher therapeutic efficacy against human colorectal adenocarcinoma xenografts than against human gastric and lung adenocarcinoma xenografts. SPAC was effective against three of four human colorectal adenocarcinoma xenografts, with efficacy higher than that of 1-β-D-arabinofuranosylcytosine, fluorouracil, cisplatin, doxorubicin, pirarubicin and vindesine sulfate, but lower than that of mitomycin C and cyclophosphamide. These results indicate that SPAC may be useful for induction and/or postoperative chemotherapy against colorectal adenocarcinomas.

Key words Cytarabine ocfosfate · Colorectal adenocarcinoma · Nude mice

Introduction

Cytarabine ocfosfate (Starasid, SPAC) is an orally active depot form of 1-β-D-arabinofuranosylcytosine (Ara-C) [3]. SPAC has been used in patients with adult acute nonlymphocytic leukemia and myelodysplastic syndrome. When administered orally to animals, SPAC is gradually converted to Ara-C in the liver, and then remains in the blood for a sustained period [4]. It is thought that Ara-C is continually incorporated into leukemia cells against which it shows high antitumor activity after conversion to the active metabolite, ara-CTP.

In a previous pharmacokinetic study in mice, the tissue concentration of SPAC in the washed colorectal organ (colon) has been found to remain at about half that in the liver for as long as 12 h after oral administration [5]. Ara-C, the active metabolite of SPAC, is also relatively well distributed in the colorectal organ at a concentration less than that in the liver (data not published).

On the basis of this distribution of SPAC and Ara-C in the colorectal organ, we considered it possible that SPAC would also show antitumor activity against colorectal adenocarcinoma. We therefore studied the antitumor activity of SPAC against human colorectal, gastric and lung adenocarcinoma xenografts in nude mice in comparison with the activities of various antitumor drugs in clinical use.

K. Koga · E. Iizuka · A. Sato · H. Ekimoto · M. Okada (✉)
Anticancer Drugs Department, Pharmaceuticals Group, Nippon Kayaku Co. Ltd., 3-31-12 Shimo, Kita-ku, Tokyo 115, Japan

Materials and methods

Drugs and treatment schedules

Table 1 shows the drugs and treatment schedules used in this study. These drugs are used clinically for the treatment of colorectal, gastric and lung adenocarcinomas either alone or in combination chemotherapy. SPAC (Fig. 1) and cisplatin (CDDP; both Nippon Kayaku Co., Tokyo, Japan), Ara-C (Nippon Shinyaku Co., Kyoto, Japan), fluorouracil (5-FU) and doxorubicin (ADM; both Kyowa Hakko Kogyo Co., Tokyo, Japan) and vindesine sulfate (VDS; Shionogi Co., Osaka, Japan) were dissolved in saline and diluted for use. Pirarubicin (THP; Nippon Kayaku Co.) was dissolved in 5% glucose solution and diluted. Mitomycin C (MMC; Kyowa Hakko Kogyo Co.) and cyclophosphamide (CYC; Shionogi, Co.) were dissolved in distilled water and diluted in saline. Each compound was administered i.v. or orally according to the usual clinical route (Table 1) at an injection volume of 0.1 ml/10 g body weight. The high dose of each compound

Table 1 Drugs and treatment schedules used in this study

Drug	Dose (mg/kg)	Schedule	Route
SPAC	160,'vg; 113	q1dx5	Oral
Ara-C	200, 141	q1dx5	IV
5-FU	20, 14.1	q1dx5	IV
CDDP	5, 3.54	q4dx3	IV
ADM	7.5, 5.3	q4dx3	IV
THP	10, 7.07	q4dx3	IV
VDS	2, 1.41	q4dx3	IV
MMC	6, 4.24	Single	IV
CYC	300, 212	Single	IV

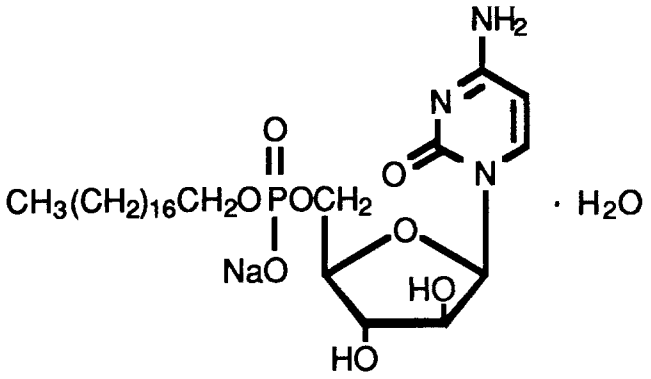

Fig. 1 Structure of cytarabine ocfosfate (Starasid)

was set at 80% of its MTD, as determined in extensive panel studies in our laboratory. The low dose was reduced by a factor of $\sqrt{2}$.

Tumor models

The following human in vitro cell lines were used for xenografts: four human colorectal adenocarcinomas (DLD-1, COLO320HSR, HCT-8 and SW620) purchased from Dainippon Seiyaku Co. (Osaka, Japan), and two human gastric adenocarcinomas (4-1st and SC-7) and one human lung adenocarcinoma (LC-11) purchased from the Central Institute for Experimental Animals (Kanagawa, Japan). These tumors were maintained subcutaneously by serial passage in carrier mice. In each antitumor study, a 1 × 1 × 2-mm tumor fragment was implanted subcutaneously into female nude mice (BALB/c-nuA, 6 to 8 weeks old; Japan SLC, Shizuoka, Japan or Clea Japan, Tokyo, Japan). The mice were housed in plastic cages covered with a filter cap under class 10 K SPF conditions with food (CL-2; Clea Japan) and water available ad libitum. When the tumor volumes reached about 100 mm^3, mice were divided into treatment groups (3 to 5 mice/group) such that the tumor sizes were similar in each group. The controls for each xenograft group comprised 6–12 mice.

Evaluation of antitumor activity against xenografts

Each xenograft was measured with calipers at least twice weekly, depending on the tumor growth rate, until the xenograft reached about 50–70 times the initial volume. Solid tumor volume (mm^3) was estimated from two tumor measurements (mm): tumor volume = (length) × (width)^2/2. The growth curve of each xenograft represents the mean increase in tumor volume relative to that at the start of treatment. The antitumor activity of the drugs tested was calculated as the mean relative tumor volume in the treatment group/ the mean relative tumor volume in the control group × 100. The resulting values are referred to as T/C(%) values.

Statistical analysis

The mean relative tumor volumes in the treatment and control groups were compared using Student's *t*-test at several points after treatment. A drug was considered effective against the xenograft when T/C(%) was less than 50% with $P < 0.05$ versus the control.

Results

Minimum T/C(%) values in each tumor line are shown in Table 2. SPAC showed a higher growth inhibitory activity against human colorectal adenocarcinoma xenografts than

Table 2 Antitumor activity of SPAC and antitumor drugs against human xenografts (*ND* not determined)

Drug	Dose (mg/kg)	Minimum T/C (%)						
		Colon				Stomach		Lung
		DLD-1	COLO320 HSR	HCT-8	SW620	4-1st	SC-7	LC-11
SPAC	160	11.9[a]	5.7[a]	50.9	35.2[a]	79.2	74.6	61.4
	113	12.7[a]	6.2[a]	56.1	42.6[a]	69.2	66.1	63.1
Ara-C	200	58.2	40.8[a]	63.8	77.8	54.3	83.9	81.6
	141	68.5	56.6	82.5	83.3	54.1	78.2	87.0
5-FU	20	81.0	88.9	79.5	81.3	51.2	70.5	59.5
	14.1	70.4	96.4	85.9	100.0	55.7	77.9	79.7
CDDP	5	49.2[a]	58.2	46.2	18.3[a]	0.9[a]	73.7	ND
	3.54	49.2[a]	62.5	57.3	81.5	18.7[a]	62.6	ND
ADM	7.5	21.5[a]	61.7	50.8	54.8	29.4[a]	12.2[a]	22.4[a]
	5.3	25.6[a]	31.8[a]	60.0	79.7	50.9	19.9[a]	57.8
THP	10	31.4[a]	59.8	55.7	70.4	22.7[a]	16.8[a]	69.6
	7.07	68.7	79.8	54.1	86.4	44.8[a]	27.4[a]	93.2
VDS	2	52.3	75.5	14.0[a]	79.6	52.0	28.0[a]	3.3[a]
	1.41	60.8	84.6	42.1[a]	50.0	58.5	ND	22.5[a]
MMC	6	19.2[a]	34.2[a]	52.6	16.5[a]	24.7[a]	3.8[a]	27.6[a]
	4.24	36.4[a]	35.2[a]	70.2	18.5[a]	36.8[a]	26.5[a]	46.4
CYC	300	47.3[a]	57.9	46.5[a]	40.7[a]	ND	ND	ND
	212	45.8[a]	50.2	40.4[a]	52.8	ND	ND	ND

[a] Minimum T/C < 50% and $P < 0.05$ versus control (Student's *t*-test)

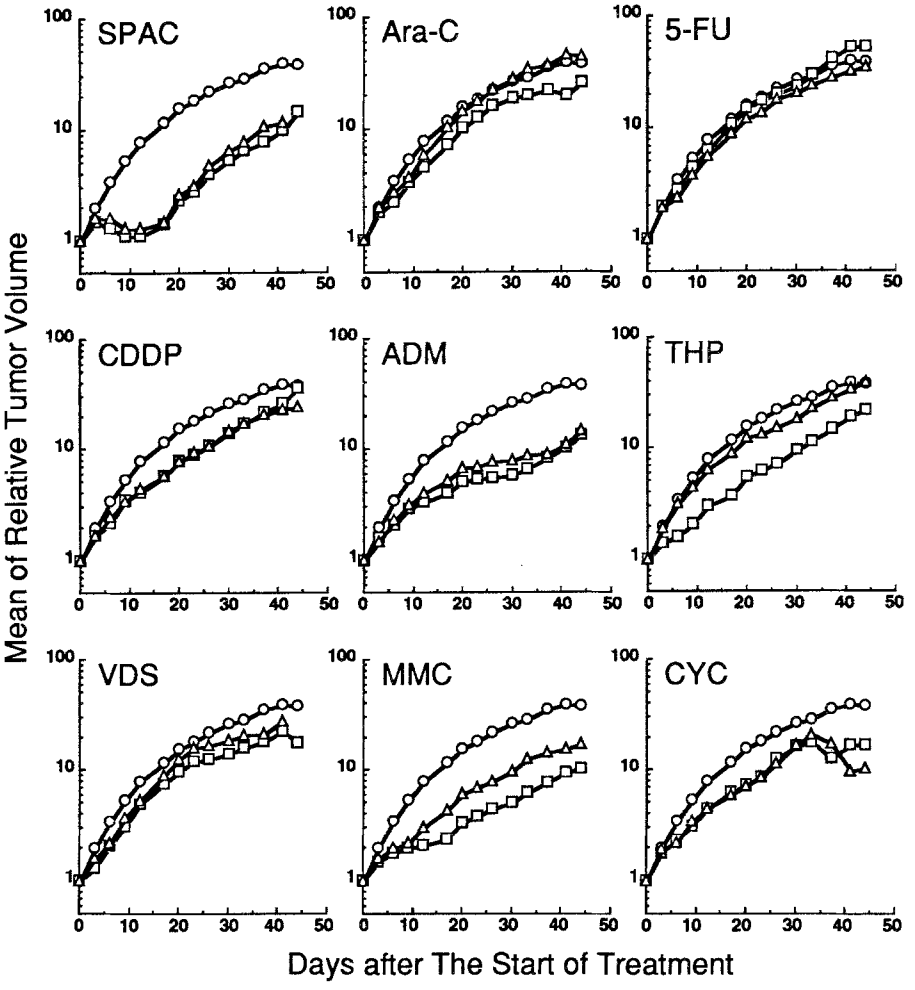

Fig. 2 Antitumor activity of each drug against DLD-1 human colorectal adenocarcinoma xenograft. The tumor volumes of the mice treated with SPAC, Ara-C, 5-FU, CDDP, ADM, THP, VDS, MMC and CYC are shown in comparison with the tumor volume of the control growth (○). Each drug was administered i.v. or orally at a high dose (□) and at a low dose (△) according to the schedule shown in Table 1. The graphs show the mean of tumor volumes relative to those at the start of treatment (day 0)

against human gastric and lung adenocarcinoma xenografts. Its growth inhibitory activity against human colorectal adenocarcinoma xenografts was higher than that of all other antitumor drugs except MMC and CYC. That is, SPAC, Ara-C, 5-FU, CDDP, ADM, THP, VDS, MMC and CYC were effective against 3, 1, 0, 2, 2, 1, 1, 3 and 3 of the 4 tumor lines, respectively. SPAC was particularly effective against DLD-1 and COLO320HSR as compared with the other drugs (Figs. 2 and 3). In contrast' SPAC was less active than the other drugs against human gastric and lung adenocarcinoma xenografts, with SPAC, Ara-C, 5-FU, CDDP, ADM, THP, VDS and MMC effective against 0, 0, 0, 1, 3, 2, 2 and 3 of the 2 or 3 tumor lines, respectively.

Discussion

As a result of the findings that SPAC and Ara-C via SPAC are well distributed in the colorectal organ, we studied the antitumor activity of SPAC against human colorectal adenocarcinoma xenografts in comparison with that against human gastric and lung adenocarcinoma xenografts. SPAC showed higher antitumor activity against colorectal adenocarcinomas than against gastric and lung adenocarcinomas. Ara-C, which is not indicated for clinical use alone [1, 2, 6], showed little antitumor activity against xenografts of these tumor types in nude mice. In contrast, SPAC showed significant antitumor activity against colorectal adenocarcinoma xenografts. These results strongly suggest that the activity of SPAC is affected not only by the distribution of the drug but also by its metabolism. In other words, the biotransformation of SPAC to Ara-C plays a more important role in colorectal adenocarcinomas than it does in gastric and lung adenocarcinomas. We consider that this may be its main mechanism of action against colorectal adenocarcinoma. Indeed, we also confirmed the uptake of SPAC in colorectal adenocarcinomas and the presence of ara-U, which is thought to be converted to Ara-C from SPAC by the action of cytidine deaminase (data not shown). A better understanding of the mechanism of action of SPAC against colorectal adenocarcinoma may soon be gained from a detailed study of its metabolism now being done in various tumor cells.

Since SPAC is an oral drug and less toxic than Ara-C, it is possible that the blood concentration of Ara-C may be maintained and the tumor cell concentrations of SPAC and Ara-C increased by repeated administration over longer periods. Stronger antitumor activity of SPAC against colo-

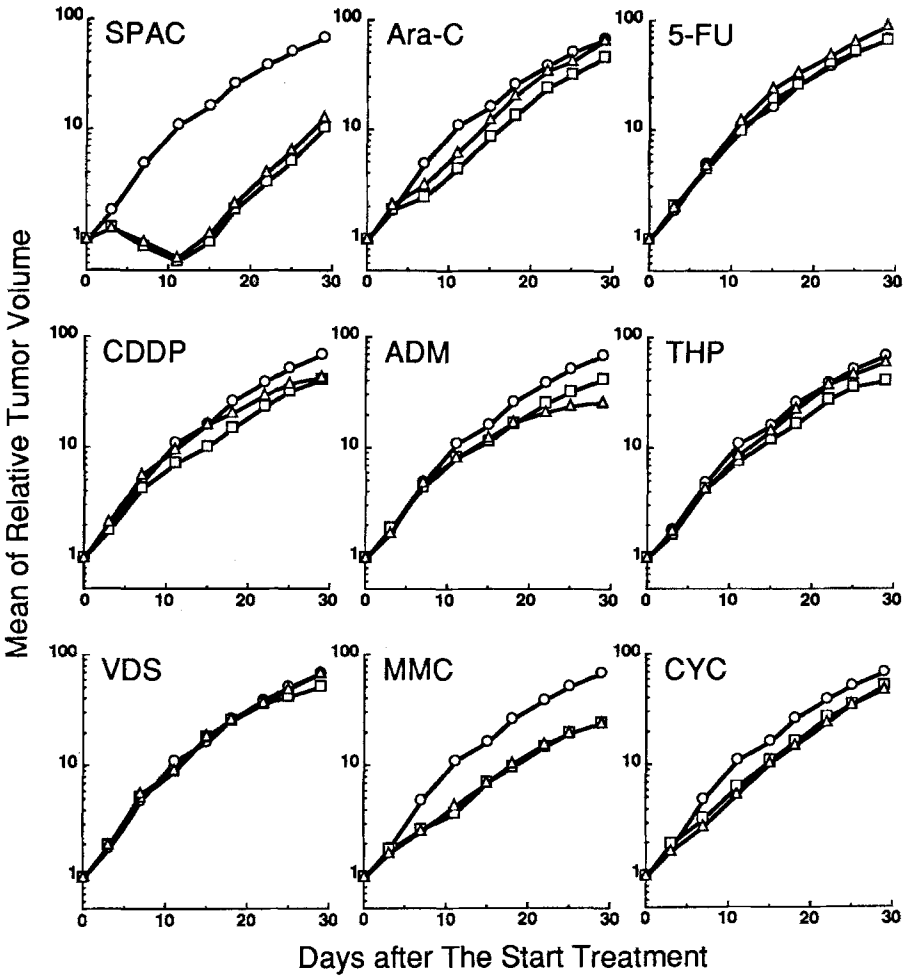

Fig. 3 Antitumor activity of each drug against COLO320HSR human colorectal adenocarcinoma xenograft. The tumor volumes of the mice treated with SPAC, Ara-C, 5-FU, CDDP, ADM, THP, VDS, MMC and CYC are shown in comparison with the tumor volume of the control growth (○). Each drug was administered i.v. or orally at a high dose (□) and at a low dose (△) according to the schedule shown in Table 1. The graphs show the mean tumor volumes relative to those at the start of treatment (day 0)

rectal adenocarcinoma may therefore be expected under such conditions.

Although SPAC has been used clinically only for the treatment of adult acute myelocytic leukemia and myelodysplastic syndrome, these results indicate that SPAC may be useful for induction and/or postoperative chemotherapy against colorectal adenocarcinoma.

References

1. Cullinan SA, O'Connell MJ, Moertel CG, Schutt AJ, Hahn RG, Reitemeier RJ, Frytak S, Rubin J (1977) Phase II study of cytosine arabinoside in advanced large bowel cancer. Cancer Treat Rep 61:1725–1726
2. Davis HL, Rochlin DB, Weiss AJ, Wilson WL, Andrews NC, Madden R (1974) Cytosine arabinoside (NSC 63878) toxicity and antitumor activity in human solid tumors. Oncology 29:190–200
3. Kodama K, Morozumi M, Saitoh K, Kuninaka A, Yoshino H, Saneyoshi M (1989) Antitumor activity and pharmacology of 1-β-D-arabinofuranosylcytosine-5′-stearylphosphate: an orally active derivative of 1-β-D-arabinofuranosylcytosine. Jpn J Cancer Res 80:679–685
4. Sato T, Morozumi M, Kodama K, Kuninaka A, Yoshino H (1984) Sensitive radioimmunoassay for cytarabine and uracil arabinoside in plasma. Cancer Treat Rep 68:1357–1366
5. Takayama H, Nakagawa Y, Ochiai H, Morozumi M, Esumi Y, Takaichi M, Jin Y, Seki H, Gunji S, Takiie S, Matsuura C, Kouno K (1993) Metabolic fate of YNK01(1). Absorption, distribution, metabolism and excretion after single administration of [14C]YNK01 to rats (in Japanese). Xenobio Metabol Dispos 8:1229–1239
6. Wasserman TH, Comis RL, Goldsmith M, Handelsman H, Penta JS, Slavik M, Soper WT, Carter SK (1975) Tabular analysis of the clinical chemotherapy of solid tumors. Cancer Chemother Rep 6:399–419