Biochemical Pharmacology, Vol. 43, No. 6, pp. 1311–1317, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

ALLEVIATION OF INTESTINAL LESIONS BY COMBINED TREATMENT WITH A 5-FLUORO-2'-DEOXYURIDINE (FUDR) DERIVATIVE AND α-DIFLUOROMETHYLORNITHINE (DMFO) IN TUMOR-BEARING MICE

SHUJI TAKESHITA, HIKARU NAGATOMI and KAZUKO ANDO*
Development Laboratories, Hirakata Center, Marion Merrell Dow K.K., Hirakata, Osaka 573, Japan

(*Received* 3 *September* 1991; *accepted* 25 *November* 1991)

Abstract—α-Difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase, reduced intestinal lesions in tumor-bearing mice caused by treatment with 3*N*-(3-methylbenzoyl)-FUDR (FF-705), a derivative of 5-fluoro-2'-deoxyuridine (FUDR). FF-705 at 32 mg/kg (the effective dose) suppressed tumor growth to about 40% of the control level. At this dose, body weight gain was suppressed slightly when FF-705 was given alone, and this change was milder in the DFMO-supplemented group. Intestinal lesions were suppressed almost completely by concomitant treatment with DFMO. The gross lesion index in the combined treatment group was similar to that in the controls and significantly smaller than in the FF-705-alone group (0.3 and 1.9, respectively). The histological lesion index in the combined treatment group was also significantly smaller than in the FF-705-alone group (7.9 and 23.8, respectively). When FF-705 was given at 64 mg/kg, the intestinal mucosal lesions were more severe, but DFMO supplementation reduced them by approximately 50%. Moreover, maltase and diamine oxidase activities of intestinal epithelium remained higher with combined treatment than with FF-705 alone. With FF-705 at 256 mg/kg (a toxic dose), DFMO had little protective effect against intestinal damage.

Inhibition of cell proliferation has been one of the major ideas in cancer therapy for decades, and many kinds of compounds with various mechanisms have been developed [1]. However, therapy that kills tumor cells or inhibits their growth can also harm normal cells resulting in leukopenia, diarrhea due to damage to the intestinal mucosa, etc. [2].

Combination chemotherapy is often superior to single-drug chemotherapy and α-difluoromethylornithine (DFMO†), a specific inhibitor of ornithine decarboxylase (ODC) and the major rate-controlling enzyme of polyamine biosynthesis [3–5], was introduced as one of the suitable candidates to be used for combination therapy with other antitumor drugs [6]. The strong enhancement in the cytotoxicity of the drugs by DFMO has been reported *in vivo* against L1210 murine leukemia by 1-β-D-arabinofuranosylcytosine (ara-C) [7], against B16 melanoma by interferon [8], and against various animal tumor models by Adriamycin® and vindesine [9], and also in *in vitro* studies [10–12].

These reports described mainly the additive or synergistic cytotoxicity from the view point of the therapeutic effect. We describe another important aspect, side-effects such as intestinal disorders, in this report. Relatively strong intestinal toxicity has been revealed as one of the major side-effects of 5-fluorouracil (5FU) [13] and 5-fluoro-2'-deoxyuridine (FUDR) [14]. A derivative of FUDR, 3*N*-(3-methylbenzoyl)-FUDR (FF-705), was reported to show milder intestinal toxicity than either 5FU or FUDR [15]. Combination of this compound, FF-705, with DFMO was investigated in terms of not only cytotoxic effects but also intestinal toxicity.

MATERIALS AND METHODS

Drugs. DFMO was provided by Marion Merrell Dow Research Institute (Cincinnati, OH, U.S.A.). FF-705 was synthesized in the Chemistry Department of the Development Laboratories, Hirakata, Japan.

Tumors. The tumor cell line, Sarcoma 180, was maintained by transplantation into the abdominal cavity of the mouse. Tumor cells collected from ascites were washed three times with 0.15 M phosphate buffer in physiological saline, and 1×10^7 cells were inoculated subcutaneously into the right inguinal region of test animals.

Animals. Four-week-old male ICR mice obtained from the Japan Charles River Breeding Laboratories were housed in polyorefin cages with wooden chips for 1 week prior to the experiments. Each group was composed of eight mice, and two groups were made into one and used as the control.

Administration of drugs. FF-705 suspended in 5% acacia solution was administered orally to animals

* Corresponding author: Dr. Kazuko Ando, Department of Scientific Administration, Development Laboratories, Hirakata Center, Marion Merrell Dow K.K., 3-11 Shodai-Tajika, Hirakata, Osaka 573, Japan. Tel. 720-56-9523; FAX 720-56-9538.

† Abbreviations: DFMO, α-difluoromethylornithine; FF-705, 3*N*-(3-methylbenzoyl)-FUDR; FUDR, 5-fluoro-2'-deoxyuridine; 5FU, 5-fluorouracil; ODC, ornithine decarboxylase; WBC, white blood cell; T/C, tumor weight in treated group/tumor weight in control group; H&E, hematoxylin and eosin; and ara-C, 1-β-D-arabinofuranosylcytosine.

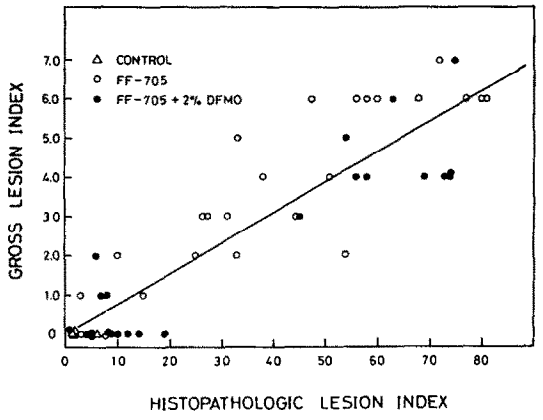

Fig. 1. The relationship between the gross and histopathological lesion indices. The scores for each animal are shown as follows: (△) control data, (○) data from animals treated with FF-705 alone, and (●) data from animals treated with FF-705 plus DFMO. The correlation coefficient was 0.9018, and the regression equation was $Y = 0.0787X + 0.0006$ for all the data.

Table 1. Antitumor effects of FF-705 alone and in combination with DFMO

	Dose (mg/kg)	No. of animals	Tumor weight (mg)	T/C*
Control 5% acacia		16	628 ± 57	1.00
FF-705	32	8	228 ± 21†	0.38
	64	8	256 ± 32†	0.41
	256	8	133 ± 16†	0.21
2% DFMO		8	565 ± 75	0.90
FF-705 +2% DFMO	32	8	291 ± 48†	0.46
	64	8	249 ± 32†	0.40
	256	8	157 ± 12†	0.25

Sarcoma 180 cells (1×10^7) were inoculated subcutaneously into ICR mice, and drugs were administered for 7 consecutive days from 24 hr after the inoculation. Animals were killed on day 8 to assess tumor growth and other items. Values are means ± SEM.

* T/C = tumor weight ratio (treated group/control group).

† $P < 0.001$ compared to the control.

at 0.1 ml/10 g body weight for 7 consecutive days starting at 24 hr after tumor cell inoculation. The doses of FF-705 used were 32, 64, and 256 mg/kg. Control animals received 5% acacia solution alone. Throughout the experiments, animals had free access to solid food and drinking water (tap water or 2% DFMO solution for the DFMO-treated groups). DFMO treatment was started on the same day as FF-705 treatment.

Observation. The body weights of all animals and the intake of 2% DFMO solution by each group were measured daily for 8 days. Blood samples were collected from a cervical blood vessel after the last dose, and the white blood cell (WBC) count was determined. Solid tumor tissues that grew subcutaneously in the inguinal region were removed and weighed. The ratio of the tumor weight of the treated group (T) to that of the control group (C) was used as an index of antitumor activity (T/C).

The intestine was removed and the extent of damage was examined according to the following criteria based on the water content in the intestine. Damage to the small intestine and cecum was graded as 0 for normal, 1 for slightly watery, and 2 for moderately watery. Damage to the colon and rectum was assessed using four grades (grade 0 to grade 2 were the same as above and grade 3 was severely watery feces). Then the intestine was fixed in 10% neutral formalin. The duodenum, jejunum, ileum, colon, and rectum were examined histopathologically with hematoxylin and eosin (H&E) staining and histopathological lesions were graded from 0 to 3. All animals in the treated groups and four animals selected randomly from the control group were examined. All observations in this experiment were carried out in a blinded manner.

The severity of the lesions as assessed by gross and histopathological observation correlated well ($r = 0.9018$, $Y = 0.0787X + 0.0006$) ($P < 0.001$) (Fig. 1). Therefore, the gross intestinal lesion index was confirmed to provide a good estimate of the intestinal damage caused by antitumor therapy.

Enzyme activities in intestinal tissues. Animals treated with 64 mg/kg of FF-705 were killed by exanguination under light anesthesia. The ileum was removed, opened, and immediately washed three times in cold 0.9% NaCl solution. Tissues were homogenized in 9 vol. of cold 0.9% NaCl solution, and enzyme activities were measured in the supernatant obtained by centrifugation at 15,000 rpm for 30 min. Disaccharidase activity was determined by the method of Dahlqvist [16] with minor modification. Diamine oxidase activity was measured by the method of Pietta *et al.* [17] with slight modification. Specific activities were calculated on the basis of the amount of the protein determined by the method of Lowry *et al.* [18].

Statistical analysis. Gross and histological observations and enzyme activities were compared between the DFMO-free groups (FF-705 without DFMO) and DFMO-treated groups (FF-705 combined with DFMO). Statistical significance was determined by the Mann–Whitney U-test [19] for gross and histopathological data and by Student's *t*-test for enzyme activities.

RESULTS

Antitumor activity. Tumor cells inoculated subcutaneously proliferated rapidly to form solid tumors weighing approximately 600 mg in 7 days in the control group. FF-705 inhibited solid tumor growth giving T/C ratios of 0.38, 0.41, and 0.21 at doses of 32, 64, and 256 mg/kg, respectively. The T/C ratio showed that DFMO alone also had a weak antineoplastic effect (T/C = 0.90). Combined treatment with FF-705 and DFMO had an antitumor effect similar to that of FF-705 alone (Table 1).

Table 2. Changes in body weight and WBC count after treatment with FF-705 alone or the combination of FF-705 and DFMO

	Dose (mg/kg)	No. of animals	Body weight* (%)	WBC count ($\times$ 100 mm^3)
Control 5% acacia		16	6.4 ± 0.7	46 ± 5
FF-705	32	8	−2.2 ± 1.9†	21 ± 2‡
	64	8	−7.9 ± 2.8†	13 ± 3†
	256	8	−22.2 ± 2.3†	5 ± 1†
2% DFMO		8	6.8 ± 1.1	52 ± 4
FF-705 +2% DFMO	32	8	1.9 ± 1.2‡	26 ± 4§
	64	8	−5.7 ± 2.9†	14 ± 1†
	256	8	−30.2 ± 1.6†‖	7 ± 2†

Values are means ± SEM.
* Body weight = (final body wt − initial body wt)/(initial body wt) × 100.
† $P < 0.001$ compared to the control.
‡ $P < 0.01$ compared to the control.
§ $P < 0.05$ compared to the control.
‖ $P < 0.05$ compared to the same dose of FF-705 alone.

Table 3. Intake of water and 2% DFMO solution

	Dose (mg/kg)	Water or 2% DFMO intake (mL/body/day)	DFMO intake (mg/body/day)
Control 5% acacia		5.6 ± 0.2	
FF-705	32	3.9 ± 0.2*	
	64	3.8 ± 0.3*	
	256	2.5 ± 0.6*	
2% DFMO		5.0 ± 0.5	100 ± 10
FF-705 +2% DFMO	32	4.2 ± 0.3†	84 ± 6†
	64	3.3 ± 0.6†	66 ± 12†
	256	1.2 ± 0.3*	24 ± 6*

Water intake was monitored for each group every day and water consumption was calculated per capita every day. Values are means ± SEM.
* $P < 0.001$ compared to the control.
† $P < 0.01$ compared to the control.

Body weight and WBC count. Body weight decreased slightly on day 2 after the transplantation of tumor cells and this was followed by a normal increase until the end of the experiment in both the control and DFMO-only groups (Table 2). FF-705 treatment at both 32 and 64 mg/kg slightly suppressed the increase in body weight. This change was milder when DFMO was given with FF-705. The highest dose of FF-705 (256 mg/kg) greatly reduced body weight in the groups with or without DFMO supplementation.

The WBC count was decreased in a dose-dependent manner by FF-705 and DFMO did not have any effect on this change.

Intake of water or 2% DFMO solution. Addition of DFMO to the drinking water led to reduced intake compared with the control, probably because of its bitter taste (Table 3). Treatment with FF-705 at 32 and 64 mg/kg brought about a decrease in the intake of both water and 2% DFMO solution to the same extent. The dose of 256 mg/kg had the greatest effect on fluid intake, and animals with DFMO supplementation drank very little, only half as much as those without DFMO treatment. This decrease in fluid intake led to a low DFMO intake, and the average DFMO doses were 84, 66, and 24 mg/body/day for the FF-705 doses of 32, 64, and 256 mg/kg groups, respectively. Therefore, in evaluating the effect of DFMO, this difference in the water intake and the dose of DFMO had to be taken into consideration.

Intestinal lesion index. Animals treated with FF-705 suffered dose-dependent damage in the intestinal tract. This damage was moderate at 32 mg/kg giving an index of 1.9, fairly severe at 64 mg/kg (3.8), and highly severe at 256 mg/kg (5.6) (Table 4). Addition

Table 4. Gross intestinal lesion index after treatment by FF-705 alone and the combination of FF-705 and DFMO

	Dose (mg/kg)	DFMO intake (mg/body/day)	Gross lesion index Index	Relative value (%)
Control 5% acacia			0.4 ± 0.2	
FF-705	32		1.9 ± 0.4†	
	64		3.8 ± 0.7*	
	256		5.6 ± 0.5*	
2% DFMO		100 ± 10	0.8 ± 0.3	
FF-705 +2% DFMO	32	84 ± 6	0.3 ± 0.2‡	16
	64	66 ± 12	1.1 ± 0.6§	29
	256	24 ± 6	4.8 ± 0.4*	86

Immediately after killing the animals on day 8, the intestinal tract was removed and lesions were examined grossly. The index for small intestine and cecum is grade 0 for normal, grade 1 for slightly watery, and grade 2 for moderately watery. Similarly, damage to the colon and rectum was graded from grade 0 to grade 3 (grade 3 was severely watery). The sum of indices for each animal is designated as the intestinal toxicity, and mean values and SEM are shown for each group (eight animals for treated groups and sixteen animals for the control group). Relative values are ratios between indices with and without DFMO treatment at the same FF-705 dose.

* $P < 0.001$ compared to the control.
† $P < 0.01$ compared to the control.
‡ $P < 0.01$ compared to the same dose of FF-705 alone.
§ $P < 0.05$ compared to the same dose of FF-705 alone.

of DFMO to FF-705 therapy significantly alleviated the intestinal damage caused by FF-705. There was no damage noted at 32 mg/kg of FF-705 and only slight damage at 64 mg/kg (an index of 1.1). At 256 mg/kg, DFMO did not provide much useful protection, and the index was only slightly smaller than that of the group without DFMO treatment.

Histopathological findings. The changes in the intestinal tract caused by FF-705 resembled those caused by 5FU [20].

As shown in Table 5, all regions of the intestine were damaged by FF-705. No region escaped from the cytotoxic action of FF-705 and the small intestine, especially the ileum, suffered severe damage. The total of the indices for all the intestinal regions demonstrates the overall severity of damage. DFMO protected every region against damage by FF-705 at doses of 32 and 64 mg/kg, with the lesion index being decreased from 23.8 to 7.9 by 84 mg/body/day of DFMO and from 32.8 to 19.1 by 66 mg/body/day of DFMO, respectively. The effect of DFMO depended on the dose given in a similar manner to gross observation and no effect was observed with DFMO treatment at 24 mg/body/day.

Enzymatic activity of intestinal tissue. Diamine oxidase activity in the ileum decreased significantly with FF-705 treatment, and was markedly restored by DFMO supplementation (Fig. 2). This was in good agreement with the intestinal lesion indices obtained by gross and histological observation, Maltase activity showed the same pattern of change, except that DFMO, alone also increased the activity.

DISCUSSION

There are many antimetabolites that have a strong antineoplastic activity, but they can cause adverse effects such as diarrhea due to intestinal mucosal damage. FF-705 is an FUDR derivative that has a lower intestinal toxicity than FUDR itself.

DFMO is a specific inhibitor of ornithine decarboxylase, which has been studied in many laboratories and found to inhibit cell proliferation reversibly by arresting it at certain stages of the cell cycle through causing polyamine deficiency. Cells can resume their original cell cycle activity when polyamines are supplied [21]. This inhibition leads to an antitumor effect against various animal neoplasms, but at the same time causes diarrhea in dogs and monkeys because it delays the maturation of intestinal epithelial cells and impairs the development of microvilli [22]. In regenerating tissues, such as those of newborn rats or tissues recovering from damage by chemotherapy, DFMO delays cell growth and recovery as well as decreasing maltase and diamine oxidase activity [23].

The combination of DFMO with other anticancer drugs has a synergistic antineoplastic action [7–12, 24]. In this study, FF-705 was used in combination therapy with DFMO against an animal tumor in mice (Sarcoma 180). Although the antitumor effect was almost the same as with FF-705 alone, intestinal toxicity was reduced significantly according to both gross and histological observations and biochemical observations in the changes of intestinal enzyme activities, which can be important indices of intestinal impairment. The mother compound of FF-705, FUDR, and also 5FU were compared with FF-705 in the DFMO combination studies. The intestinal toxicity caused by these compounds was also reduced by the combination

Table 5. Histopathological lesion index of the intestinal tract after treatment with FF-705 alone and FF-705 plus 2% DFMO

	Dose (mg/kg)	DFMO* (mg/day)	No. of animals	Histopathological lesion index					
				Deuodenum	Jejunum	Ileum	Colon	Rectum	Total†
Control 5% acacia			4	0.3 ± 0.3	0.3 ± 0.3	1.0 ± 0.4	0.5 ± 0.3	0.3 ± 0.3	2.3 ± 1.3
FF-705	32		8	6.0 ± 1.7‖	5.5 ± 2.0	9.1 ± 2.1‡	2.3 ± 0.6	0.9 ± 0.4	23.8 ± 6.3‖
	64		8	4.9 ± 0.9§	8.3 ± 1.4§	10.9 ± 1.6§	5.4 ± 1.3‖	3.4 ± 1.2	32.8 ± 5.6§
	256		8	14.1 ± 0.6‡	15.0 ± 0.9‡	16.9 ± 0.8‡	11.9 ± 1.0‡	10.8 ± 1.0‡	68.8 ± 3.7‡
FF-705 +2% DFMO	32	84 ± 6	8	2.8 ± 1.1	1.1 ± 0.6	2.0 ± 0.5¶	1.6 ± 0.4	0.1 ± 0.1	7.9 ± 2.1**
	64	66 ± 12	8	3.9 ± 1.9	4.5 ± 1.9	6.0 ± 1.9	2.9 ± 1.2	1.9 ± 0.9	19.1 ± 7.2
	256	24 ± 6	8	15.4 ± 0.7‡	15.5 ± 0.8‡	15.9 ± 0.9‡	9.8 ± 1.1‡	10.8 ± 0.9‡	67.3 ± 3.0‡

Each portion of the intestinal tract was fixed in 10% formalin and examined histopathologically with H&E staining. Histopathological lesions were graded as grade 0 for normal, grade 1 for slight changes, grade 2 for moderate changes, and grade 3 for severe changes. Values are means ± SEM.

* Values are calculated per capita from 2% DFMO intake.

† The total is the sum of indices for five regions of the intestinal tract.

‡ $P < 0.001$ compared to the control.

§ $P < 0.01$ compared to the control.

‖ $P < 0.05$ compared to the control.

¶ $P < 0.01$ compared to the same dose of FF-705 alone.

** $P < 0.05$ compared to the same dose of FF-705 alone.

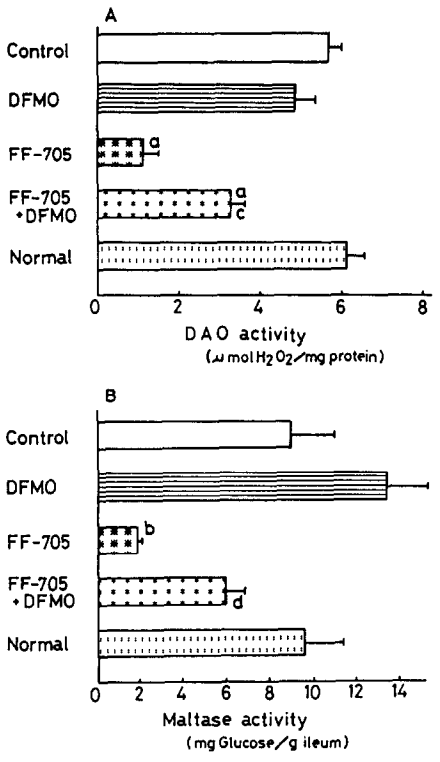

Fig. 2. Effects on ileal enzyme activity of FF-705 alone and the combination of FF-705 and DFMO in tumor-bearing mice (five animals per group). The data obtained from non-tumor-bearing untreated mice are described as normal. The dose of FF-705 was 64 mg/kg and the concentration of DFMO was 2%. Diamine oxidase activity (A) was determined after 1 hr of incubation at 37° and maltase activity (B) was determined after incubation for 10 min. The results are expressed as means ± SEM. Significant difference from the control: (a) ($P < 0.001$), and (b) ($P < 0.01$); significant difference from the same dose of FF-705 alone: (c) ($P < 0.005$), and (d) ($P < 0.01$).

with DFMO similarly to that for FF-705, although the toxicity by FUDR and 5FU was greater than that by FF-705.

When animals were treated with FF-705 alone for 7 consecutive days, intestinal epithelial cells must have been exposed in their S phase to 5FU or FUDR derived from FF-705 [25–27], and many cells should have suffered serious cytotoxic damage through interference in DNA synthesis. Use of DFMO may have altered this situation by possibly blocking the forward progression of the cell cycle from the G_1 to S phase [28, 29]. The majority of cells, therefore, should have remained in the G_1 or G_2 phase and these cells in addition to those in the G_0 phase should have escaped the cytotoxic effect of FF-705.

Although the intestinal toxicity was reduced significantly by this DFMO combination therapy, intestinal conditions reflected only partly to animal body weight, that is, rather moderate improvement by DFMO. In addition to intestinal conditions, there are other important factors, such as water and food intake, the bitter taste of DFMO, WBC count, and probably some unknown factors.

Previous *in vitro* studies have shown some interesting schedule-dependent aspects in the combination treatment with DFMO: cytotoxicity against the 9L rat brain tumor cell line by ara-C [30] was greatly suppressed by DFMO pretreatment, and similar suppression by DFMO pretreatment 1 hr before *cis*-diamminedichloroplatinum(II) and stronger suppression 48 hr before [31]. Additive cytotoxicity, however, by 5FU and DFMO was reported when they were added at the same time to the culture of a human colon adenocarcinoma cell line [10]. We studied briefly how administration schedules affect two focussed important factors, efficacy and side-effect, but pretreatment with DFMO did not give better results than those obtained by simultaneous administration. *In vivo* studies deal with the very complex whole-body system: many different kinds of cells at various cell stages in their individual environments, which should affect the antitumor activity and overall protection of normal cells such as intestinal cells.

Further studies are necessary to determine whether these findings have any relevance to clinical practice. The results and ideas presented in this paper may suggest another advantage of combination therapy in reducing the adverse effects of chemotherapy by consideration of mechanisms of action of antitumor agents.

Acknowledgements—The authors would like to thank Drs. T. H. Tsai, P. P. McCann, M. V, Aylott, and B. Yasui for their helpful advice and comments throughout the work and during the preparation of the manuscript. We are also grateful to Mr. I. Hashimoto and Miss H. Asai for their excellent technical assistance.

REFERENCES

1. Carter SK, Bakowcki MT and Hellman K, *Chemotherapy of cancer*. John Wiley, New York, 1977.
2. Cadmam E, Toxicity of chemotherapeutic agents: In: *Chemotherapy, Cancer* (Ed. Becker FF), Vol. 5, pp. 59–111. Plenum Press, New York, 1977.
3. Metcalf BW, Bey P, Danzin C, Jung MJ, Casara P and Vevert JP, Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analogues. *J Am Chem Soc* **100**: 2551–2553, 1978.
4. Prakash NJ, Schechter PJ, Mamont PS, Grove J, Koch-Weser J and Sjoerdsma A, Inhibition of EMT6 tumor growth by interference with polyamine biosynthesis; Effects of α-difluoromethylornithine, an irreversible inhibitor of ornithine decarboxylase. *Life Sci* **26**: 181–194, 1980.
5. Pegg AE and McCann PP, Polyamine metabolism and function. A brief review. *Am J Physiol* **243**: C212–C221, 1982.
6. Porter CW and Janne J, Modulation of antineoplastic drug action by inhibitors of polyamine biosynthesis. In: *Inhibition of Polyamine Metabolism* (Eds. McCann PP, Pegg AE and Sjoerdsma A). Academic Press, Orlando, FL, 1987.
7. Prakash NJ and Sunkara PS, *Combination chemotherapy involving α-difluoromethylornithine and 1-β-D-arabinofuranosylcytosine* in murine L1210 leukemia. *Cancer Res* **43**: 3192–3196, 1983.
8. Sunkara PS, Prakash NJ, Mayer GD and Sjoerdsma

A, Tumor suppression with a combination of α-difluoromethylornithine and interferon. *Science* **219**: 851–853, 1983.
9. Bartholeyns J and Koch-Weser J, Effects of α-difluoromethylornithine alone and combined with Adriamycin or vindesine on L1210 leukemia in mice, EMT6 solid tumors in mice, and solid tumors induced by injection of hepatoma tissue culture cells in rats. *Cancer Res* **41**: 5158–5161, 1981.
10. Kingsnorth AN, Russell WE, McCann PP, Diekema KA and Malt RA, Effects of α-difluoromethylornithine and 5-fluorouracil on the proliferation of a human colon adenocarcinoma cell line. *Cancer Res* **43**: 4035–4038, 1983.
11. Hung DT, Deen DF, Seidenfeld J and Marton LJ, Sensitization of 9L rat brain gliosarcoma cells to 1,3-bis(2-chloroethyl)-1-nitrosourea by α-difluoromethylornithine, an ornithine decarboxylase inhibitor. *Cancer Res* **41**: 2783–2785, 1981.
12. Chang BK, Black O Jr and Gutman R, Inhibition of growth of human or hamster pancreatic cancer cell lines by α-difluoromethylornithine alone and combined with *cis*-diamminedichloroplatinum(II). *Cancer Res* **44**: 5100–5104, 1984.
13. Seifert P, Baker LH, Reed ML and Vaitkevigius VK, Comparison of continuously infused 5-fluorouracil with bolus injection in treatment of patients with colorectal adenocarcinoma. *Cancer* **36**: 123–128 1975.
14. Miller E, Sullivan R, Young C and Burchenal JH, Clinical effects of the continuous infusion of antimetabolites—Prevention of toxicity of 5-fluoro-2′-deoxyuridine by thymidine. *Proc Am Assoc Cancer Res* **3**: 251, 1961.
15. Fujii S and Shirasaka T, Development of fluoropyrimidine derivatives. *Jpn J Cancer Chemother* **11**: 2316–2328, 1984.
16. Dahlqvist A, Method for assay of intestinal disaccharidases. *Anal Biochem* **7**: 18–25, 1964.
17. Pietta P, Calatroni A and Colombo R, Determination of diamine oxidase activity by high-performance liquid chromatography. *J Chromatogr* **243**: 123–129, 1982.
18. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.
19. Siegel S, The Mann-Whitney U-test. In: *Nonparametric Statistics for the Behavioral Sciences*, pp. 116–127. McGraw-Hill, New York, 1956.
20. Muggia AL, Wagman E, Milles SS and Spiro HM, Response of the gastrointestinal tract of the mouse to 5-fluorouracil. *Am J Pathol* **42**: 407–414, 1963.
21. Mamont PS, Siat M, Joder-Ohlenbusch AM, Bernhardt A and Casara P, Effects of (2*R*, 5*R*)-6-heptyne-2,5-diamine, a potent inhibitor of L-ornithine decarboxylase, on rat hepatoma cells cultured *in vitro*. *Eur J Biochem* **142**: 457–463, 1984.
22. Yarrington JT, Sprinkle DJ, Loudy DE, Diekema KA, McCann PP and Gibson JP, Intestinal changes caused by DL-α-difluoromethylornithine (DFMO), an inhibitor of ornithine decarboxylase. *Exp Mol Pathol* **39**: 300–316, 1983.
23. Luk GD, Marton LJ and Baylin SB, Ornithine decarboxylase is important in intestinal mucosal maturation and recovery from injury in rats. *Science* **210**: 195–198, 1980.
24. Marton LJ, Levin VA, Hervatin SJ, Koch-Weser J, McCann PP and Sjoerdsma A, Potentiation of the antitumor therapeutic effects of 1,3-bis(2-chloroethyl)-1-nitrosourea by α-difluoromethylornithine, an ornithine decarboxylase inhibitor. *Cancer Res* **41**: 4426–4431, 1981.
25. Bruce WR, Meeker BE Valeriote FA, Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony-forming cells to chemotherapeutic agents administered *in vivo*. *J Natl Cancer Inst* **37**: 233–245, 1966.
26. Lightdale C and Lipkin M, Cell division and tumor growth. In: *Biology of Tumors, Cellular Biology and Growth Cancer* (Ed. Becker FF), Vol. 3, pp. 201–215. Plenum Press, New York, 1975.
27. Cheng H and Leblond CP, Origin, differentiation and renewal of the four main epithelial cell types in the mouse small intestine. I: Columnar cell. *Am J Anat* **141**: 461–480, 1974.
28. Rupniak HT and Paul D, Selective killing of transformed cells by exploitation of their defective cell cycle control by polyamines. *Cancer Res* **40**: 293–297, 1980.
29. Sunkara PS, Fowler SK and Nishioka K, Selective killing of transformed cells in combination with inhibitors of polyamine biosynthesis and S-phase-specific drugs. *Cell Biol Int Rep* **10**: 991–997, 1981.
30. Oredsson SM, Gray JW, Deen DF and Marton LJ, Decreased cytotoxicity of 1-β-D-arabinofuranosylcytosine in 9L rat brain tumor cells pretreated with α-difluoromethylornithine *in vitro*. *Cancer Res* **43**: 2541–2544, 1983.
31. Oredsson SM, Deen DF and Marton LJ, Decreased cytotoxicity of cis-diamminedichloroplatinum(II) by α-difluoromethyl ornithine depletion of polyamines in 9L rat brain tumor cells *in vitro*. *Cancer Res* **42**: 1296–1299, 1982.