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# Antitumor and Radiosensitizing Effects of (*E*)-2′-Deoxy-2′-(fluoromethylene) Cytidine, a Novel Inhibitor of Ribonucleoside Diphosphate Reductase, on Human Colon Carcinoma Xenografts in Nude Mice

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## ABSTRACT

**Antitumor and radiosensitizing effects of (*E*)-2′-deoxy-2′-(fluromethylene) cytidine (FMdC), a novel inhibitor of ribonucleotide reductase, were evaluated on nude mice bearing s.c. xenografts and liver metastases of a human colon carcinoma. FMdC given once daily or twice weekly has a dose-dependent antitumor effect. The maximum tolerated dose in the mice was reached with 10 mg/kg applied daily over 12 days. Twice weekly administration of FMdC reduced its toxicity but lowered the antitumor effect. Treatment of preestablished liver micrometastases obtained via intrasplenic injection of tumor cells, with 5 or 10 mg/kg FMdC, significantly prolonged the survival of the mice as compared to controls ($P < 0.025$ and $P < 0.001$, respectively). Ten mg/kg resulted in longer survival than 5 mg/kg FMdC ($P < 0.05$). Radiotherapy alone of s.c. xenografts (10 fractions over 12 days) yielded the radiation dose required to produce local tumor control in 50% of the treated mice ($TCD_{50}$) of 43.0 Gy. When combined with FMdC, $TCD_{50}$ was reduced to 22.5 and 19.0 Gy at doses of 5 and 10 mg/kg given i.p. 1 h before each irradiation, respectively. The corresponding enhancement ratios were 1.91 and 2.43, respectively. FMdC produced moderate and reversible myelosuppression. When 5 mg/kg FMdC was combined with irradiation, there was no increased skin or hematological toxicity as compared to radiotherapy or FMdC alone. At the 10 mg/kg level, however, lower leukocyte counts were observed. These results show that FMdC appears to be a potent anticancer drug and radiosensitizer.**

## INTRODUCTION

The cure of cancer depends on locoregional control and/or eradication of metastatic disease. Chemoradiation therapy potentially fulfills both aims (1, 2). FMdC[2] (3, 4), a novel compound synthesized to exert irreversible and potent inhibition of RR, has a very effective cytotoxicity against a variety of common human cancers (5–7) and a strong radiosensitizing effects on tumor cells *in vitro* (8).[3] This compound could be an ideal drug for chemoradiation therapy.

The antitumor effect and radiosensitization observed can be explained by the effect on RR and subsequent alteration of the dNTPs. It has been shown by others that RR has an increased activity in rapidly growing tumors (9–11) and that alteration of the dNTP pool is related to the modification of radiation response (12, 13). Other drugs, such as hydroxyurea or 2′,2′-difluoro-2′-deoxycytidine (gemcitabine) acting on the same target, have been shown to be potent radiosensitizers (14–16). Increased *in vitro* cellular sensitivity to both X-ray and UV irradiation after FMdC exposure has been observed (8).[3] *In vivo* data are, however, not yet available. Therefore, we decided to investigate the radiation sensitizing effect of FMdC on human tumor xenografts in nude mice. On the other hand, we determined if at concentrations required to get radiosensitization *in vivo*, an antitumor effect could be obtained on small established liver metastases.

## MATERIALS AND METHODS

**Chemicals and Cell Line.** FMdC (MDL 101,731) was kindly provided by Hoechst Marion Roussel, Inc. (Cincinnati, OH). Cell culture media and supplements were purchased from Life Technologies, Inc. (Basel, Switzerland). FCS was obtained from Fakola.

The WiDr colon carcinoma cell line was obtained from American Type Culture Collection (Rockville, MD). Cells were grown as a monolayer in Eagle's MEM with 10% FCS, 2 mM L-glutamine, and 1% penicillin-streptomycin. To establish tumors in nude mice, cells in exponential growth phase were harvested after a 3-min incubation with trypsin (0.05%)-EDTA (0.02%) solution and resuspended in serum-free MEM. A suspension of about $5 \times 10^6$ cells was inoculated s.c. in the dorsum of the Swiss nude mice. For experiments, animals were implanted with tumors only two or three passages away from the initial source. For establishment of liver metastases, sodium-heparin solution (1 IU/ml) was added to single-cell suspensions. These cells were kept on ice until use within 1.5 h.

**s.c. Tumor Model.** All experiments in nude mice were performed according to Swiss legislation and approved by the official committee of surveillance of animal experiments. Female Swiss homozygous *nu/nu* nude mice, 7–9 weeks of age, were given a s.c. transplantation in the midline of the back at 2 cm from the tail of a volume of about 30 $mm^3$ of freshly excised, minced WiDr colon cancer. Three to 4 weeks after inoculation, the mice bearing tumors of approximately 80–120 $mm^3$ volume with a mean tumor volume of about 100 $mm^3$ were assigned randomly for control or the test treatment groups.

**Establishment of Liver Metastases.** The method for establishment of liver metastases has been described previously (17, 18). Briefly, mice were given 6 Gy of total body irradiation 3 days before grafting to inhibit natural killer activity. The pretreated mice were anesthetized with 0.25 ml tribromoethanol (25.5 mg/ml) injected i.p. (Aldrich, Gilligham, United Kingdom). A small left subcostal incision was made, the spleen was isolated, and a single-cell suspension of $2 \times 10^6$ WiDr tumor cells in 0.05 ml heparinated MEM was slowly injected into the spleen, using a 0.45-mm needle. Three min after injection, the spleen vessels were ligatured, a splenectomy was performed, and the abdomen was closed with sutures.

**Irradiation of Tumors.** X-rays were generated by a Philips RT 250 operating at 200 kV and 20 mA. The beam was filtered with 0.5-mm Cu (half-value layer = 1 mm Cu). Up to six mice per irradiation were restrained in 3-mm lead jigs designed with a cutout 20 × 14 mm to expose their lower dorsum. The jigs were placed in a perspex box with an additional lead shield with 60 × 17-mm openings; in each field, two mice were exposed tail-on-tail. This setup gives minimal scatter to the animals placed at 52.5 cm from the source. The X-ray beam hits the tumors tangentially to the dorsum. The dose rate in this setup was 0.64 Gy/min with a dose heterogeneity of ±5.5% for an 8-mm tumor. To obtain dose homogeneity, the mice were rotated through 180° at alternate treatments. The treatment regime consisted of 10 fractions over 12 days (5 fractions per week comparable to a clinical fractionation schedule; Ref. 19).

**Antitumor Effects of FMdC.** Nude mice with s.c. WiDr tumor xenografts were treated i.p. with 1, 5, 10, 20, and 40 mg/kg FMdC, once daily, 5 days/week, or treated i.v. with 25, 50, and 100 mg/kg FMdC, twice weekly,

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[2] The abbreviations used are: FMdC, (*E*)-2′-deoxy-2′-(fluoromethylene) cytidine; RR, ribonucleotide reductase; RT, radiotherapy; dNTP, deoxyribonucleotide; RD, (absolute tumor) regrowth delay; MV, minimal volume; $TCD_{50}$, the radiation dose required to produce local tumor control in 50% of the treated mice.

[3] P. A. Coucke, Y-X. Li, N. Paschoud, and R-O. Mirimanoff. The ribonucleoside diphosphate reductase inhibitor (*E*)-2′-deoxy-2′-(fluoromethylene) cytidine acts as a cytotoxic radiosensitizer on various human cancer cell lines *in vitro*, manuscript in preparation.

both for up to 2 weeks. The mice with liver metastases 3 or 7 days after intrasplenic injection of tumor cells were treated i.p. with 5 or 10 mg/kg FMdC or saline for control, once daily, 5 days/week, for up to 2 weeks.

**Radiosensitizing Effect of FMdC.** Two experiments were done; the first experiment was done with two constant drug doses (5 or 10 mg/kg daily) and varying radiation doses (experiment A), and in the second experiment, with two constant radiation doses (2 or 3 Gy daily) and varying drug doses (experiment B). FMdC dissolved in saline and sterilized by filtration through Millipore 0.22 μm was administrated i.p. 1 h before each irradiation for 2 weeks with a 2-day rest on the weekend. All RT or RT combined FMdC groups were evaluated in two blocks, each block including half of the mice of each group with similarly sized tumors. Each group consisted of 8–14 mice.

**Experimental End Points.** After treatment, three perpendicular diameters of each tumor were measured with calipers twice a week. Complete or partial regressions were assessed once per week. The tumor volume was calculated using the formula: $V$ = length × width × thickness/2. The time required for tumor volume to increase by three times the initial treatment size was calculated for each mouse, and the absolute tumor RD was obtained by subtraction of the mean RD in untreated mice (8.8 ± 1.8 days, $n$ = 10). The MV was defined as the smallest tumor volume after treatment in the percentage of tumor volume at day 0. The effect of graded doses of radiation given alone or in combined regimens was evaluated as the radiation dose required to produce local tumor control in 50% of the treated mice ($TCD_{50}$). The absence of palpable tumor mass at 120 days after the end of the treatment was taken as an indication of local control. Local tumor controls were verified by pathological analysis; no residual tumor cells were observed. All regrowing tumors were recorded as relapse in the analysis whether or not the tumor diameter was smaller than that of the first day of irradiation. The percentage of controlled tumors at 120 days was plotted for each group, and the data were fitted by logit analysis. For the liver metastases, the survival times after intrasplenic tumor cell grafting were recorded for the control and treated mice. Survival of more than 150 days without macroscopically observed liver metastases after intrasplenic tumor cell grafting was defined as long-term survival.

**Toxicity Evaluation.** Local skin toxicity of RT was evaluated by inspection three times per week for the first 5 weeks and then twice per week. The skin toxicity in the radiation field was scored as follows: I, faint redness; II, partial necrosis; and III, complete necrosis. Toxicity following injections of FMdC alone or combined to RT was evaluated by body weight measurements and peripheral WBC counting. Body weight was measured three times weekly from the first injection of FMdC until 4 weeks after the end of the treatment. Peripheral WBCs were monitored 1 day after the end of FMdC treatment and 3 days after finishing radiation treatment alone or combined to FMdC (correlated with the nadir of WBC at those moments). WBCs were counted in 15 μl of blood (obtained from the tail vein) diluted 1:10 in Turck solution and manually counted.

**Histopathological Studies.** The s.c. tumors that were untreated or treated with 5 or 10 daily administrations of 5 mg/kg FMdC (once daily, 5 days/week) were removed from nude mice 8 h after the last treatment and fixed in 4% buffered formalin. Livers with metastases from mice killed 3 and 7 days after intrasplenic tumor grafting and splenectomy were also fixed in 4% buffered formalin. The specimens were embedded in paraffin, and 4-μm thick sections were stained sequentially with H&E for microscopic examination.

**Statistical Analysis.** Tumor RD, MV, and hematological toxicity in the various conditions of therapy have been evaluated using Student's $t$ test. $TCD_{50}$ was calculated according to the logit analysis. For the liver metastases, Kaplan-Meier survival curves were estimated, and the log-rank test was used to compare the survival.

## RESULTS

**Effects of FMdC on s.c. Xenografts with Once Daily i.p. or Twice Weekly i.v. Administrations.** The tumor response to once daily i.p. administrations of FMdC was dose dependent from 1 to 10 mg/kg ($P \leq 0.002$; Fig. 1 and Table 1). No significant weight loss (less than 1.5%) was observed at these lower doses. However, at higher doses of FMdC, *i.e.*, 20 and 40 mg/kg, there was no further increase in RD and MV as compared to 10 mg/kg ($P > 0.05$ for both RD and MV). On the contrary, there was a significant weight loss

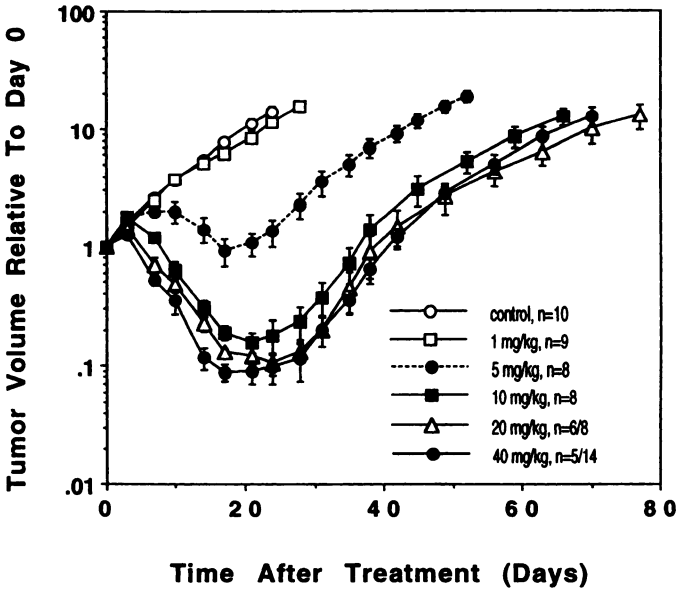


Fig. 1. The effects of FMdC given as once daily administrations (10 times over 12 days) on the growth of WiDr xenografts in nude mice. The mean tumor volume relative to the initial tumor volume at day 0 is plotted *versus* time for each group; *bars*, SE. In the 20-mg/kg group (eight mice), the two mice that died of toxicity are not included, and in the 40-mg/kg group (14 mice), the nine mice that died of toxicity are not included.

(14.7 ± 8.2% for 20 mg/kg and 22.1 ± 9.0% for 40 mg/kg) and an increasing incidence of toxic death (Table 1). Weight losses occurred 10–14 days and 5–14 days after treatment for 20 and 40 mg/kg, respectively. Two of 8 mice (25%) treated with 20 mg/kg and 9 of 14 mice (64%) treated with 40 mg/kg died 7–14 days after treatment. The leukocytes of the nude mice were moderately reduced after FMdC treatment (Table 2). The difference in leukocyte counts for doses ranging from 1 to 10 mg/kg was not significant. On the other hand, doses of 20 or 40 mg/kg resulted in a significant reduction of leukocyte counts as compared to 10 mg/kg FMdC ($P < 0.05$). No petechiae were observed in any of the groups. The hematological toxicity might not be the main reason of death, because five mice treated with 40 mg/kg FMdC were given bone marrow transplantation with blood transfusion 5 and 8 days after treatment, but three of five mice still died 7–14 days after treatment. Mice surviving higher doses of FMdC (20 or 40 mg/kg) recovered their body weight within 7–10 days and their leukocyte counts completely within 10–14 days after the end of the treatment (data not shown). No late toxicity was observed with FMdC alone.

When the mice with s.c. tumors were treated with twice weekly i.v. administrations of FMdC at a dose ranging from 25 to 100 mg/kg, up to 2 weeks, the tumor responses were also dose dependent ($P < 0.05$; Fig. 2 and Table 1). No petechiae, toxic death, or significant weight loss were observed. The hematological toxicity was mild (Table 2).

If we compare the antitumor effects and toxicities of FMdC given as once daily and twice weekly administrations at the same total dose level, the antitumor effects of once daily administrations seemed more efficient than that of twice weekly administrations, whereas the toxicities were contrary (Figs. 1 and 2 and Tables 1 and 2). For example, at the same total dose of 100 mg/kg level, the antitumor effects expressed as RD and MV of once daily administration of 10 mg/kg FMdC, 10 times, were more efficient than that of twice weekly administration of 25 mg/kg, 4 times ($P < 0.001$); however, the leukocytes were slightly lowered in mice treated with once daily administrations ($P < 0.05$).

**Effects of FMdC on Liver Metastatic Tumors.** The intrasplenic injection of tumor cells followed by immediate splenectomy was well

Table 1 *Antitumor effect and host toxicity of FMdC given as once daily i.p. or twice weekly i.v. administrations*

| Groups | Total dose (mg) | No. of mice | RD[a] (days) | MV[b] (%) | No. of mice that died (%) |
|---|---|---|---|---|---|
| 1 mg/kg[c] 10 times | 10 | 9 | 1.8 ± 4.8 | 100.0 ± 0.0 | 0 |
| 5 mg/kg[c] 10 times | 50 | 8 | 22.8 ± 6.3 | 67.1 ± 26.7 | 0 |
| 10 mg/kg[c] 10 times | 100 | 8 | 38.7 ± 6.8 | 13.7 ± 9.1 | 0 |
| 20 mg/kg[c] 10 times | 200 | 6/8[d] | 42.6 ± 8.1[d] | 9.6 ± 4.2[d] | 2 (25) |
| 40 mg/kg[c] 10 times | 400 | 5/14[e] | 43.5 ± 4.4[e] | 8.4 ± 3.2[e] | 9 (64) |
| 25 mg/kg[f] 4 times | 100 | 7 | 16.3 ± 6.1 | 87.9 ± 21.2 | 0 |
| 50 mg/kg[f] 4 times | 200 | 7 | 23.2 ± 4.6 | 76.8 ± 25.1 | 0 |
| 100 mg/kg[f] 4 times | 400 | 8 | 34.4 ± 12.7 | 25.3 ± 26.2 | 0 |

[a] Mean absolute tumor RD (±SD).
[b] Mean tumor MV (± SD).
[c] Given as once daily i.p. administrations, 10 times over 12 days.
[d] Mean RD or MV from six mice that survived of 8.
[e] Mean RD or MV from five mice that survived of 14.
[f] Given as twice weekly i.v. administrations (on Monday and Thursday), 4 times over 11 days.

Table 2 *Peripheral blood leukocyte analysis of mice 1 day after end of FMdC treatment with once daily i.p. or twice weekly i.v. administrations*

| Groups | Total dose (mg) | No. of mice | Leukocytes no./mm$^3$ (mean ± SD) |
|---|---|---|---|
| Untreated | 0 | 13 | 8380 ± 1560 |
| 1 mg/kg[a] 10 times | 10 | 9 | 4580 ± 720 |
| 5 mg/kg[a] 10 times | 50 | 8 | 4460 ± 1160 |
| 10 mg/kg[a] 10 times | 100 | 8 | 4510 ± 1110 |
| 20 mg/kg[a] 10 times | 200 | 6/8[b] | 2620 ± 1730 |
| 40 mg/kg[a] 10 times | 400 | 5/14[c] | 1510 ± 990 |
| 25 mg/kg[d] 4 times | 100 | 7 | 6533 ± 213 |
| 50 mg/kg[d] 4 times | 200 | 7 | 5275 ± 241 |
| 100 mg/kg[d] 4 times | 400 | 8 | 4819 ± 1572 |

[a] Given as once daily i.p. administrations, 10 times over 12 days.
[b] Results from six mice that survived of eight.
[c] Results from five mice that survived of 14.
[d] Given as twice weekly i.v. administrations, 4 times over 11 days.

tolerated. There were no postoperative deaths. Liver tumor engraftment rates were 90% (9 of 10) as assessed macroscopically 4 weeks after grafting. The numbers of macroscopically detectable metastases, approximately 1–7 mm in diameter, varied from 1.0 to 67.0 (median was 17.0). The histological assessment of liver micrometastases 3 and 7 days after grafting has been shown in Fig. 7, *D–F*.

Fig. 3 shows the survival curves for untreated and treated mice. The median survival of control mice was 68.5 days. When the treatment was initiated 3 days after grafting, 10 daily administrations of 5 mg/kg FMdC resulted in a median survival of 94 days and a long-term survival of 25% (two of eight), whereas 10 mg/kg FMdC resulted in a long-term survival of 75% (six of eight). Treatment with 5 or 10 mg/kg FMdC significantly prolonged the actuarial survival of the treated mice as compared to the control mice ($P < 0.025$ for 5 mg/kg and $P < 0.001$ for 10 mg/kg, respectively). Ten mg/kg treatment resulted in longer survival than 5 mg/kg FMdC ($P < 0.05$). However, 1 of 9 mice and 2 of 10 mice died 11–14 days after treatment in groups of mice treated with 5 and 10 mg/kg FMdC, respectively. These mice with early toxic death were not censored for survival analysis. The reason of death is probably an interval too short between whole body irradiation (6 Gy) and the start of FMdC treatment (only 6 days apart). The bone marrow had no time to recover before FMdC treatment was started.

To reduce the early toxic death, we started the same treatment as mentioned above (5 or 10 mg/kg FMdC, once daily, 10 times over 12 days) 1 week after intrasplenic tumor cell grafting and splenectomy (Fig. 3). The treatment with 5 mg/kg FMdC resulted in a median survival of 94.5 days and a long-term survival in 10% (1 of 10) of treated mice. However, this is not significantly different from saline-treated mice ($P > 0.05$). In contrast, the treatment with 10 mg/kg FMdC significantly prolonged the actuarial survival of treated mice with a median survival of 121.5 days and a long-term survival of 40% (4 of 10), when compared to control ($P < 0.001$) and 5 mg/kg-treated groups ($P < 0.05$).

**Radiosensitizing Effect of FMdC.** The responses of s.c. tumors to RT were dose dependent, and the $TCD_{50}$ for RT alone was 43.0 ± 1.2 Gy (Fig. 4). In experiment A, when 5 mg/kg FMdC was given i.p. 1 h before each irradiation, the dose-effect curve was significantly shifted to the left, and the $TCD_{50}$ dropped to 22.5 ± 1.3 Gy (Fig. 4). When 10 mg/kg FMdC were used, the radiosensitizing effect was even more significant, and the $TCD_{50}$ reached 17.7 ± 1.3 Gy (Fig. 3). The enhancement ratios at $TCD_{50}$ level were 1.91 and 2.43 for 5 and 10 mg/kg FMdC, respectively.

In experiment B, a fixed total dose of 20 or 30 Gy in, respectively, 10 fractions of 2 or 3 Gy, 5 fractions per week over 12 days, has been applied. The dose of FMdC varied from 1 to 20 mg/kg, i.p. administrated 1 h before each irradiation. One mg/kg FMdC did not have any radiosensitizing effect at both daily 2- and 3-Gy levels. In contrast, 5, 10, or 20 mg/kg FMdC increased progressively local tumor control whatever the fractionation used (Fig. 5). According to the logit model, the dose of FMdC required to get a local tumor control in 50% of treated mice at total doses of 20 or 30 Gy was 7.6 and 1.9 mg/kg, respectively.

At the 5-mg/kg level, the irradiated mice showed neither significant weight loss (data not shown) nor enhanced hematological toxicity (Fig. 6) as compared to FMdC treatment alone. Increased toxicity, however, was observed at a higher radiation dose level (50 Gy; $P < 0.004$). At 10 mg/kg, the mice treated with RT and

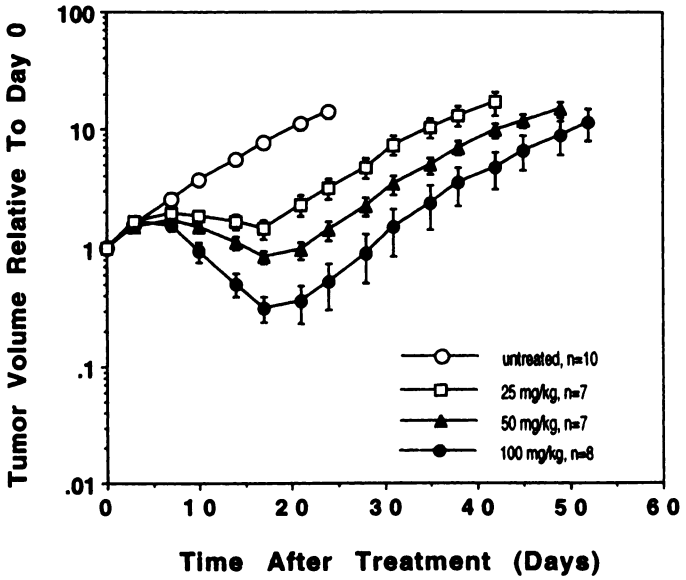


Fig. 2. The effects of FMdC given as twice weekly administrations (4 times over 11 days) on growth of WiDr xenografts in nude mice. The mean tumor volume relative to the initial tumor volume at day 0 is plotted *versus* time for each group; *bars*, SE.

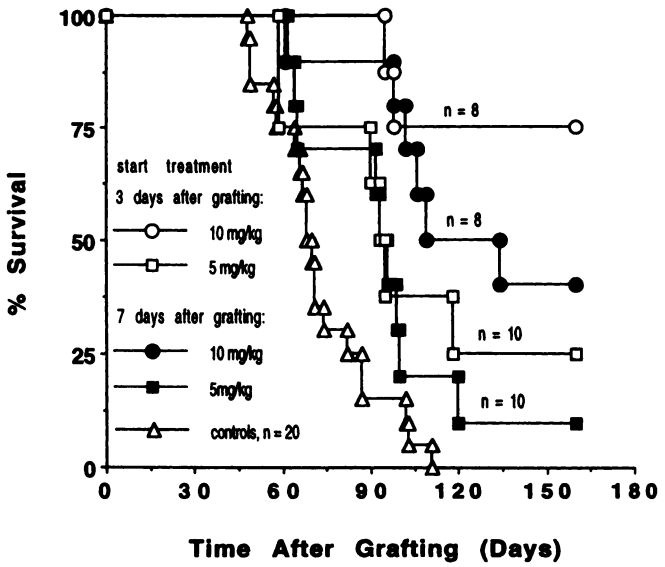


Fig. 3. Actuarial survival of mice bearing liver metastases and treated with 10 daily administrations of saline or 5 or 10 mg/kg FMdC over 12 days. The treatment was started 3 days after grafting (○ and □) *versus* 7 days after grafting (● and ■).

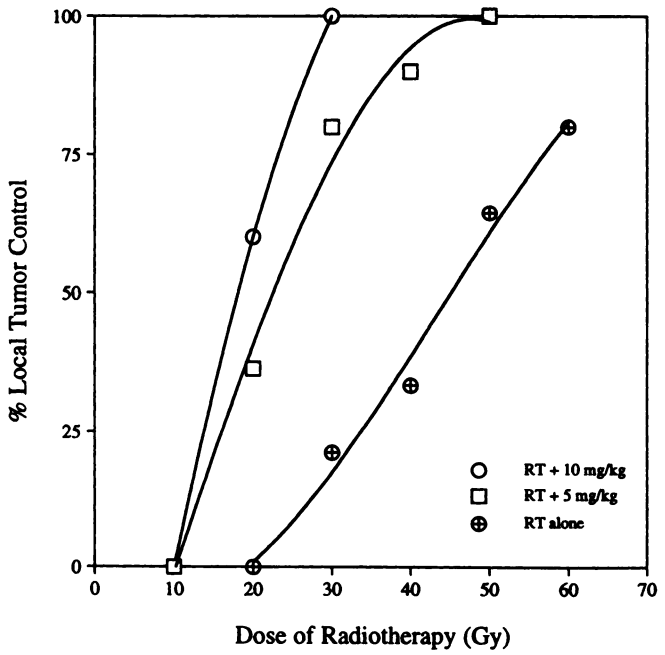


Fig. 4. Experiment A, local tumor control to RT combined to daily 5 or 10 mg/kg FMdC. RT was given 10 fractions over 12 days, and FMdC was given i.p. 1 h before each irradiation.

FMdC had lower leukocyte counts as compared to FMdC alone ($P < 0.05$; Fig. 6), but no significant weight loss was observed (data not shown). There was no increased skin toxicity with combined FMdC and RT as compared to RT alone (data not shown).

**Histopathological Studies.** Histology of untreated s.c tumor showed that necrotic areas were observed only at 10 or more cell layers distant from supportive tissue (Fig. 7*A*). Some swollen cells and necrotic areas adjacent to supportive tissue were observed after five daily i.p. treatments of 5 mg/kg FMdC (Fig. 7*B*). Treatment with 10 daily i.p. administration of 5 mg/kg FMdC over 12 days resulted in significant necrosis of tumor tissues (Fig. 7*C*).

Microscopical liver metastases containing 5–15 tumor cells were observed 3 days after intrasplenic tumor grafting (Fig. 7*D*). Seven days after intrasplenic tumor grafting, the metastatic nodules in livers increased in size (50–300 cells; Fig. 3, *E* and *F*). Some of these metastases were located in small portal veins (Fig. 7*F*) and resulted in embolic liver infarction (Fig. 7*D*).

## DISCUSSION

The results of these *in vivo* studies provide experimental evidence that FMdC may be an effective anticancer agent in the treatment of human colon carcinoma. This compound induces regression of s.c. tumors and dramatic prolongation of survival in mice bearing liver metastases in a dose-dependent manner. Progressive tumor regrowth after the end of FMdC treatment alone, indicates that the once daily or twice weekly administrations of FMdC are not curative for s.c. tumors. However, for

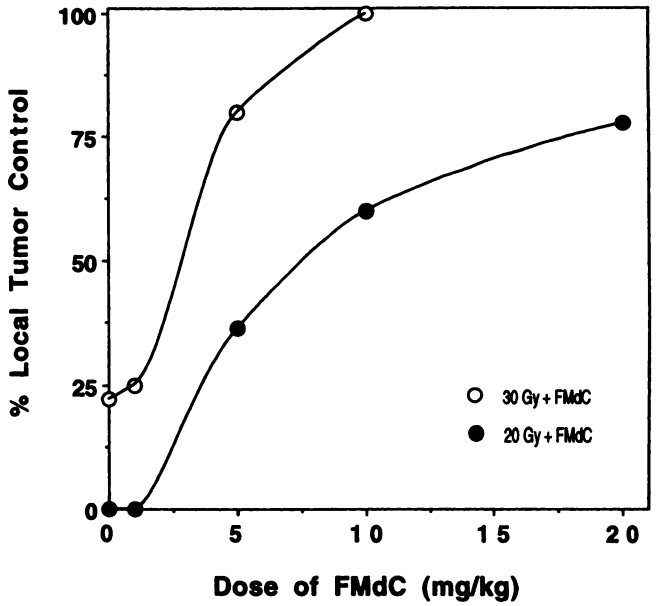


Fig. 5. Experiment B, local tumor control to various doses of FMdC tested at the total dose of 20 or 30 Gy in 10 fractions over 12 days. FMdC was given i.p. 1 h before each irradiation.

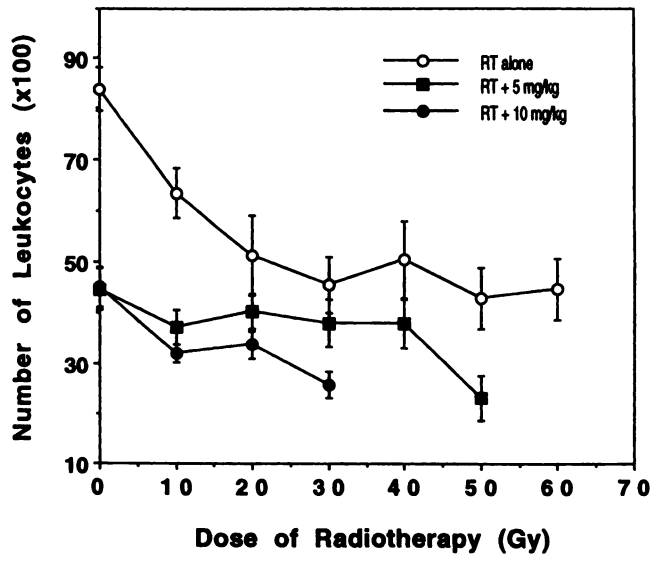


Fig. 6. Peripheral blood leukocyte analysis of mice 3 days after the end of RT or RT combined with 5 or 10 mg/kg FMdC (means; *bars*, SD). Each group consisted of 8–14 mice. For FMdC treatment alone, leukocytes were counted 1 day after the end of treatment. RT was given 10 fractions over 12 days, and FMdC was given i.p. 1 h before each irradiation.

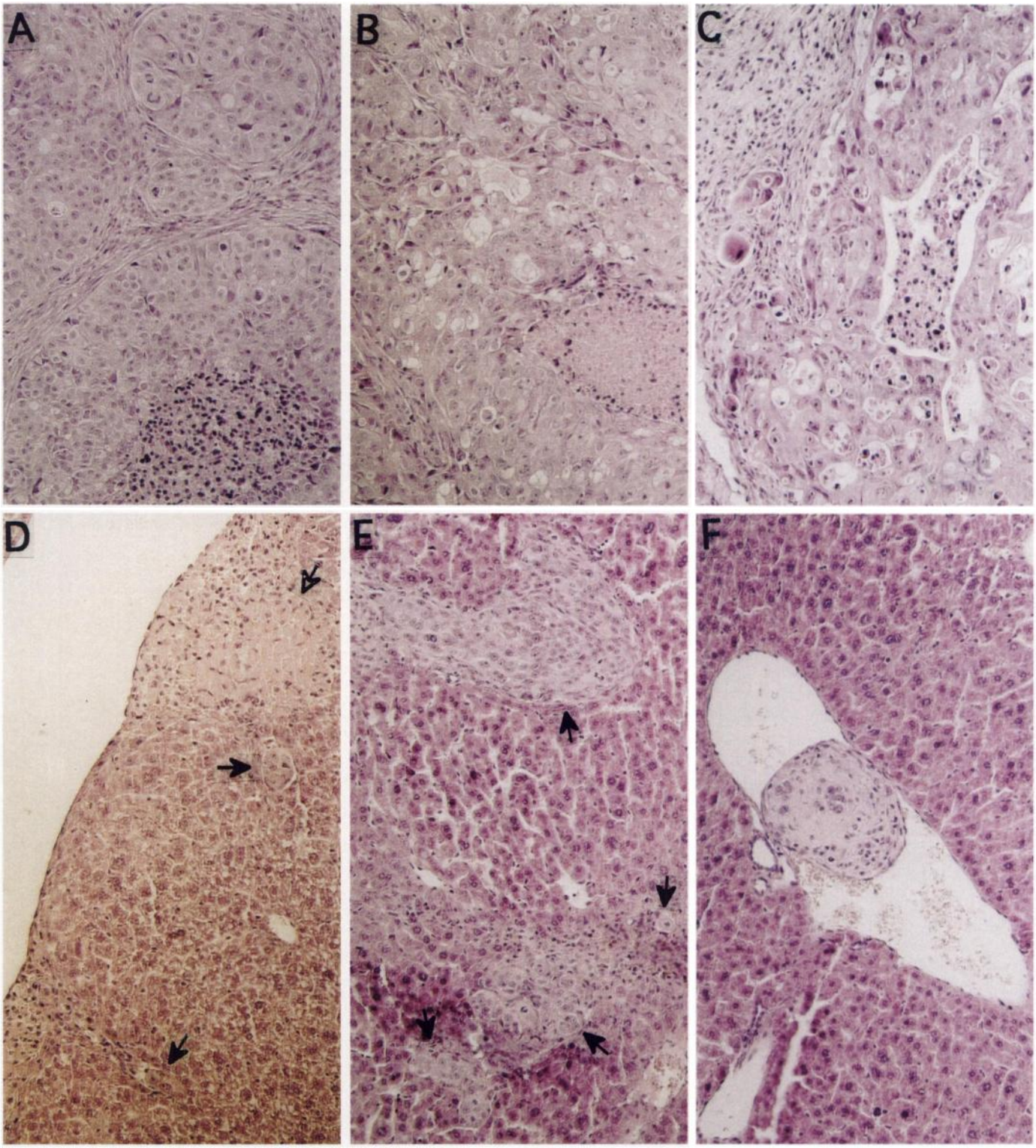


Fig. 7. Histology of human carcinoma WiDr s.c. xenografts (*A–C*) and liver metastases (*D–F*). The s.c. tumors treated with 5 (*B*) or 10 (*C*) daily administrations of 5 mg/kg FMdC (once daily, 5 days/week) were removed for analysis 8 h after the last treatment. Livers with metastases were removed for analysis 3 (*D*) or 7 (*E* and *F*) days after intrasplenic tumor grafting and splenectomy. In untreated tumor (*A*), an intact epithelial tumor structure close to supportive tissue is visible, although some degree of tumor necrosis is present only at a distance from supportive tissue. In *B*, some swollen cells and necrotic areas adjacent to supportive tissue were detectable. In *C*, a significant increase of necrotic tissues even adjacent to supportive tissue is demonstrated. In *D*, microscopical metastatic nodules comprised of 2–15 tumor cells are detectable 3 days after grafting (*filled arrow*), and nearby liver infarction is visible (*empty arrow*). In *E*, one larger metastatic nodule comprised of more tumor cells and many dispersed small nodules (*filled arrow*) are visible 7 days after grafting. In *F*, one larger metastatic nodule is located in the portal vein 7 days after grafting.

the liver metastases, if the treatment is initiated very early, *i.e.*, 3 days after grafting, an increased survival can be observed. As far as toxicity is concerned, we demonstrated that 10 daily administrations of 10 mg/kg FMdC over 12 days was the maximum tolerated dose in the mice. Daily doses higher than 10 mg/kg for 10 times failed to produce more antitumor effects but resulted in a significant weight loss, lower leukocyte counts, and increased toxic deaths. At the same total dose levels, a twice weekly regimen yielded less toxicity but at a price of a slight reduction of

antitumor effect. Our results correlate with prior xenograft studies of FMdC in human malignant breast, colon, prostate, and brain tumors. In these tumors, a dramatic tumor regression of s.c. implanted tumors was obtained, and a significant prolongation of survival was observed in intracerebral implants as well as a reduction of the number of lung metastases (5–7).

More importantly, FMdC significantly increased radiation response of human colon cancer xenografts. The enhancement ratios at $TCD_{50}$ levels were 1.91 and 2.43, respectively, for 5 and 10 mg/kg FMdC applied daily. The radiosensitizing effect of FMdC was dependent on both the doses of the drug and RT. The addition of FMdC to irradiation did not increase skin toxicity nor weight loss. No significantly increased hematological toxicity was observed when daily 5 mg/kg FMdC was combined with fractionated RT, whereas a moderate increase of hematological toxicity was observed when a higher dose (10 mg/kg) of FMdC was combined with RT. However, the hematological toxicity was well tolerated and was reversible.

The combination of FMdC and RT might offer the advantage of spatial co-operation. The drug is both active as a cytotoxic agent and radiosensitizer in the experimental setting we used here. This spatial co-operation is extremely important for various human tumors, especially colorectal cancer. Hepatic metastases are present in 25% of patients at the time of initial colorectal resection, and more than 50% of patients will eventually develop them during the course of their disease. Ninety % of patients who die from colorectal cancer have liver metastases (20). Use of chemotherapeutic agents that have both radiosensitizing and cytotoxic effects will, therefore, make such treatment more effective.

Because direct measurements of RR, dNTP pools, and DNA repair were not performed in our studies, the precise mechanism(s) of FMdC can only be inferred from prior studies. FMdC is a potent member of a class of mechanism-based inhibitors of RR, the enzyme responsible for *de novo* production of dNTPs by reduction of ribonucleotide at the level of diphosphates. The drug acts in a manner similar to other RR inhibitors, such as hydroxyurea and gemcitabine, which has been shown to cause inhibition of DNA synthesis specifically, without significantly inhibiting either protein or RNA synthesis. Inhibition of DNA synthesis with these drugs is most probably due to a decrease in one or more of dNTP pools (9, 21–23) or chain termination after being converted to the triphosphate, as shown for gemcitabine (24). Although not proven in the present study, it is likely that FMdC exerts its radiosensitizing effects through inhibition of irradiation-induced DNA repair processes. The reduced availability of DNA precursors or perturbation of dNTP pools may result in an impairment of radiation-induced DNA repair and may be an important determinant of radiosensitization with FMdC (8). This has been shown in other RR inhibitors such as hydroxyurea (16) and gemcitabine (14, 15). Other drugs active on the dNTP pools have been shown to be potent radiosensitizers, such as fluorodeoxyuridine (inhibition of thymidylate synthase and depletion of dTTP pools; Refs. 13, 25, and 26) and the thymidine analogues, bromodeoxyuridine and iododeoxyuridine (depletion of dCTP and dTTP pools; Ref. 27). Thus, the antitumor and radiosensitizing effects of FMdC may be associated with depletion and intracellular imbalances of dNTP pools. Regardless of the precise mechanism, the above studies show compelling evidence that FMdC may be an effective antitumor and radiosensitizing agent for treatment of colorectal cancer and probably also for other tumors.

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