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# (E)-2′-Deoxy-2′-(fluoromethylene) Cytidine Potentiates Radioresponse of Two Human Solid Tumor Xenografts[1]

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## ABSTRACT

**Antitumor and radiosensitizing effects of (E)-2′-deoxy-2′-(fluoromethylene) cytidine (FMdC), a novel inhibitor of ribonucleotide reductase, were evaluated on nude mice bearing s.c. human C33-A cervix cancer and U-87 MG glioblastoma xenografts. FMdC given once daily has a dose-dependent antitumor effect. The maximum tolerated dose in the mice was reached with 10 daily i.p. administrations of 10 mg/kg over 12 days. In the case of radiotherapy (RT) alone (10 fractions over 12 days), the radiation dose required to produce local tumor control in 50% of the treated C33-A xenografts was 51.0 Gy. When combined with FMdC, the radiation dose required to produce local tumor control was reduced to 41.4 and 38.2 Gy, at respective doses of 5 and 10 mg/kg given i.p. 1 h before each irradiation. The corresponding enhancement ratios (ERs) were 1.2 and 1.3, respectively. In U-87 MG xenografts, when 5–20 mg/kg FMdC combined with 30 or 40 Gy of RT, the combination treatment produced a significantly increased growth delay as compared with RT alone ($P \leq 0.002$). The ERs of 5, 10, and 20 mg/kg FMdC at a dose of 30 Gy were 2.0, 1.4, and 1.8, respectively. At the 40-Gy level, ERs of 10 and 20 mg/kg FMdC were 1.4 and 1.7. When FMdC was combined with 50 Gy of RT, an increased long-term remission rate of 80–88.9% was observed, as compared with 25% for RT alone ($P < 0.05$). FMdC produced moderate myelosuppression in the mice bearing cervix cancer, whereas leukocytosis occurred in the mice bearing glioblastoma at a low dose. Slightly increased skin toxicity (only with U-87 MG tumor) was observed, as compared with RT alone. In conclusion, FMdC is a potent cytotoxic agent and able to modify the radiation response of C33-A and U-87 MG xenografts.**

## INTRODUCTION

FMdC[5] (1, 2), a novel compound synthesized to exert irreversible and potent inhibition of RR, is a very effective cytotoxic agent against a variety of common human cancers (3–7). Moreover, it has been shown to act as a radiosensitizer both *in vitro* (8) and *in vivo* (7).

The antitumor effect and radiosensitization can be explained by the effect on RR and subsequent alteration of the dNTPs. It has been shown by others that RR, an enzyme that catalyzes a rate-limiting step in DNA synthesis, has an increased activity in rapidly growing tumors (9) and that alteration of the dNTP pool is related to the modification of radiation response (10). Takahashi *et al.* (6) showed recently that FMdC induced long-lasting inhibition of RR and resulted in a depletion of the dCTP, dATP, and dGTP pools and an increase in the TTP pool. Other drugs such as hydroxyurea or gemcitabine, acting on the same target, also have been shown to be potent radiosensitizers (11–13). Recently, We have observed that the radiosensitizing effect of FMdC is evident in a human colon cancer WiDr xenograft model (7); however, whether FMdC also has a similar radiosensitizing effect on other types of human cancers *in vivo* is not clear. In this study, we decided to further evaluate the capacity of FMdC to act as a radiation sensitizer in a human cervix cancer C33-A with a mutated p53 (14) and a glioblastoma U-87 MG with a wild type of p53 (15) xenografted in nude mice.

## MATERIALS AND METHODS

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

The human C33-A cervix cancer cell lines were obtained from the American Type Culture Collection (Manassas, VA). The human glioblastoma U-87 MG cell line was a gift from the Department of Neurosurgery at our hospital. 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 the experiments, animals were implanted with tumors at least three passages away from the initial source.

**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 tumor tissues. Three to 4 weeks after inoculation, the mice bearing tumors of approximately 80–160 $mm^3$ volume, with a mean tumor volume of about 120 $mm^3$, were assigned randomly for the control or the test treatment groups.

**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/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 degrees at alternate treatments. The treatment regime consisted of 10 fractions over 12 days (5 fractions/week comparable with a clinical fractionation schedule).

**Antitumor Effects of FMdC.** Nude mice with s.c. tumor xenografts were treated i.p. with 1, 5, 10, and 20 mg/kg FMdC, once daily, 5 days/week for up to 2 weeks.

**Radiosensitizing Effect of FMdC.** 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 during the weekend. All RT or RT-FMdC combined groups were evaluated in two blocks, each block including half of the mice of each group with similar-sized tumors. Each group consisted of 8–13 mice.

**Experimental End Points.** After treatment, three perpendicular diameters of each tumor were measured with calipers twice a week. Complete or partial

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[5] The abbreviations used are: FMdC, (E)-2′-deoxy-2′-(fluoromethylene) cytidine; RR, ribonucleotide reductase; dNTP, deoxyribonucleotide; RT, radiotherapy; AGD, absolute growth delay; NGD, normalized growth delay; $TCD_{50}$, the radiation dose required to produce local tumor control in 50% of the treated mice; ER, enhancement ratio; gemcitabine, 2′,2′-difluorocytidine.

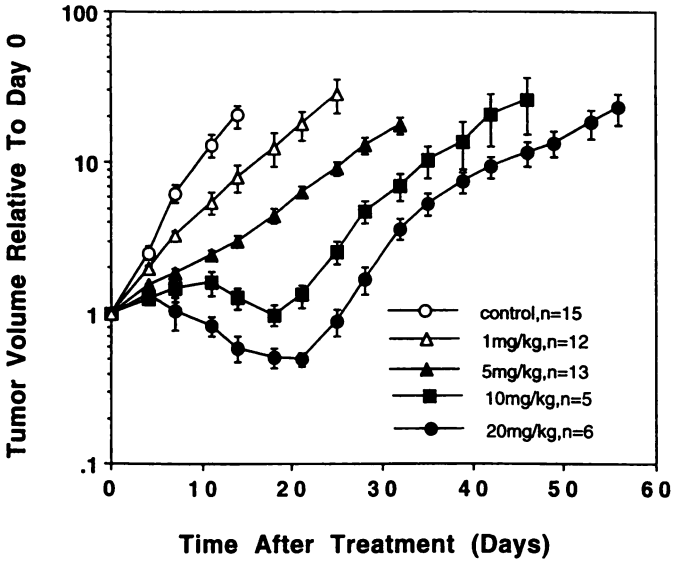


Fig. 1. The effects of FMdC given as once daily i.p. administrations (10 times over 12 days) on the growth of C33 carcinoma xenografts in nude mice. The mean tumor volume relative to the initial tumor volume at day 0 is plotted *versus* time for each group (± SE). The tumor volumes at day 0 were 82.7 ± 38.1, 81.1 ± 32.9, 102.7 ± 19.7, 118.5 ± 37.5, and 128.8 ± 39.9 $mm^3$ for groups of control, 1, 5, 10, and 20 mg/kg FMdC, respectively.

regressions were assessed once/week. The tumor volume was calculated using the formula: V = length × width × thickness/2. The relative tumor volume (V/Vo) was calculated by dividing the measured tumor volume (V) by the initial tumor volume (vo) at day 0.

Tumor responses were quantified by tumor regrowth delay, local control, and long-term remission. Tumor regrowth delay was expressed as AGD and NGD. AGD was defined as the time for tumor volume in the treated groups to increase by five times the initial treatment size minus the time in the untreated control group to reach the same size. NGD was defined as the time for tumor volume to increase by five times the initial treatment size in the mice treated by the combination of FMdC and radiation minus the time for tumors to reach the same size in the mice treated with FMdC alone. Local control for the C33-A tumor is defined as absence of palpable tumor mass at 120 days after the end of the treatment. 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. The effect of graded doses of radiation given alone or in combined regimens was evaluated as the $TCD_{50}$. The ER for C33-A tumor was calculated as the ratio of $TCD_{50}$ produced by radiation alone to $TCD_{50}$ produced by combined FMdC and radiation. Because U-87 MG glioblastoma lacks an apoptotic cell death pathway (16) and is a very radioresistant tumor that has a $TCD_{50}$ of 75.2 Gy under clamp hypoxia using single-dose irradiation (17), the local control rate was not available. Therefore, we used long-term remission for the U-87 MG tumor, which was defined as absence of palpable or residual tumor mass <2 times of initial volume 180 days after the end of treatment. To calculate the ER, we also included these long-term remission tumors and arbitrarily took 180 days as the time for these tumors to grow to five times the initial treatment size. The ER for the U-87 MG glioblastoma was calculated as the ratio of the NGD produced by combined FMdC and radiation to the AGD produced by radiation alone.

**Toxicity Evaluation.** Local skin toxicity of RT was evaluated by inspection three times/week for the first 5 weeks, and then twice/week. The skin toxicity in the radiation field was scored as follows: I, local erythema; II, moist desquamation; and III, complete ulceration. Toxicity after injections of FMdC alone or combined with 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, and the weight loss was expressed as a percentage of the initial weight (<initial weight − lowest weight>/initial weight × 100%). Peripheral WBCs were monitored 1 day after the end of FMdC treatment and 3 days after finishing radiation treatment alone or combined with 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. Lower limb paralysis was recorded after treatment.

**Histopathological Studies.** The s.c. tumors that were untreated or treated with 10 daily administrations of 10 mg/kg FMdC (once daily, 5 days/week) were removed from nude mice 24 h after the last treatment and fixed in 4% buffered formalin. The residual tumor masses (which were defined as long-term remission) were also fixed in 4% buffered formalin, 6 months after the end of treatment. The specimens were embedded in paraffin, and 4-μm thick sections were stained sequentially with H&E for microscopic examination.

**Potential Doubling Time and Volume Doubling Time.** The potential doubling times of C33-A and U-87 MG tumors were measured by iododeoxyuridine labeling and flow cytometry. The mice bearing C33-A or U-87 MG tumors were given i.p. injections of iododeoxyuridine 6 h before the tumors were removed. The dose administrated was 150 mg/kg. The s.c. tumors were fixed in 70% ethanol and stored at 4°C in the dark until analysis. Preparation of samples for flow cytometry was according to the method of Begg (18). Briefly, the tumor samples were cut into small pieces in Petri dishes and digested with 0.4 mg/ml pepsin for 1 h at 37°C, which produced a suspension of nuclei. DNA of the nuclei was denatured with 2N HCl for 20 min at 37°C. Antibody staining consisted of the mouse anti-iododeoxyuridine antibody (1:100 dilution; Partect AG, Basel, Switzerland) followed by an FITC-conjugated goat antimouse IgG antibody (1:50 dilution; Partect AG). Total DNA was stained with 50 μg/ml propidium iodide. Flow cytometric analysis was carried out using FACScan flow cytometry (Becton Dickinson). The methods for calculating potential doubling time has been described previously (19). The volume doubling times were individually estimated from the growth curves of the tumors xenografted in the mice.

**Statistical Analysis.** Tumor AGD, NGD, and hematological toxicity in the various conditions of therapy have been compared using the Student's *t* test (two-tailed). For comparison of long-term remission rates, the $\chi^2$ test was used. $TCD_{50}$ was calculated according to the logit analysis.

## RESULTS

**Antitumor Effects of FMdC on C33-A and U-87 MG Tumor Xenografts.** The response of C33-A xenografts to FMdC was dose-dependent (Fig. 1). Even at the low dose (*i.e.*, 1 mg/kg), we observed a significant antitumor effect as compared with untreated control ($P < 0.0001$). Significant higher tumor shrinkage was observed at 20 mg/kg FMdC. No significant body weight loss (≤4.0%) was observed at the dose <5 mg/kg, whereas a 7.0% and 13.0% weight loss were observed at the dose of 10 and 20 mg/kg FMdC, respectively (*P*

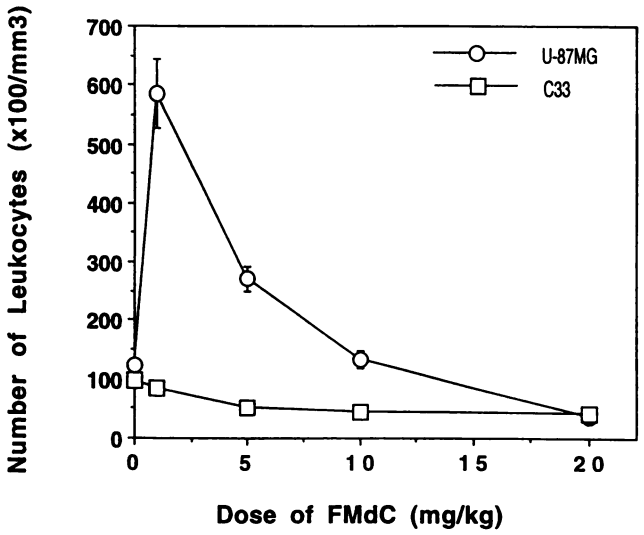


Fig. 2. Peripheral blood leukocyte analysis of mice bearing C33-A and U-87 MG tumors 1 day after the end of FMdC treatment (± SE). The leukocyte counts of normal mice without tumors were 10,900 ± 680/$mm^3$ ($n$ = 12; mean ± SE).

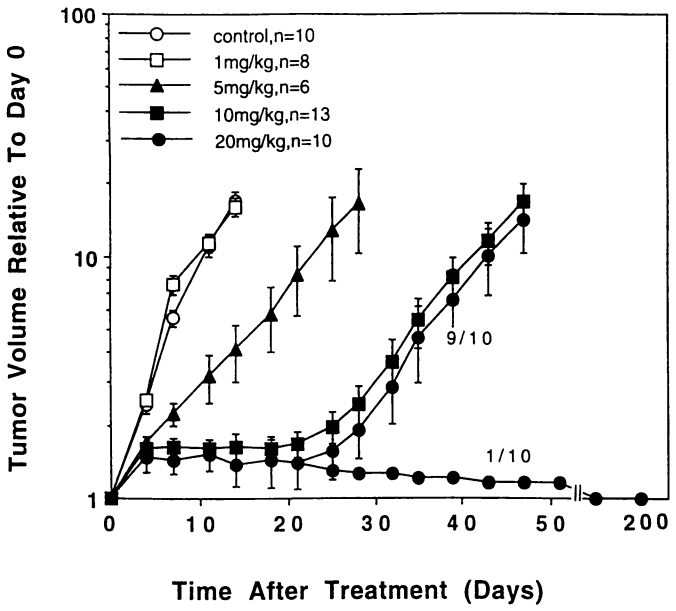


Fig. 3. The effects of FMdC given as once daily i.p. administrations (10 times over 12 days) on growth of U87 MG xenografts in nude mice (± SE). In the 20-mg/kg group, 1 of 11 mice died of acute toxicity (data not included).

<0.05). Even at a dose as high as 20 mg/kg, we did not observe toxic death. As far as hematological toxicity was concerned, we did observe a significant reduction of leukocyte counts from doses >5 mg/kg as compared with untreated controls ($P$ <0.001), but no further decrease at higher doses (Fig. 2). Petechiae did not appear.

For U-87 MG xenografts, the antitumor effect became significant at doses >5 mg/kg (Fig. 3). However, no further increase in AGD was observed at a higher dose (*i.e.*, 20 mg/kg), as compared with 10 mg/kg ($P$ = 0.18). There was also a moderate weight loss (9.5%) at a dose of 20 mg/kg ($P$ <0.05). In contrast to the mice bearing C33-A xenografts, we had to face toxic death in 1 of 11 mice (9.1%) treated at the higher dose level of 20 mg/kg. Tumor shrinkage did not appear, even up to a dose of 20 mg/kg, but significant prolonged AGDs were observed at doses of 5–20 mg/kg as compared with the untreated control ($P$ ≤0.0001). In contrast to the C33-A model, a rise of leukocyte counts was observed at lower doses of 1 and 5 mg/kg in the U-87 MG model ($P$ <0.0001). However, at the higher dose level, the leukocyte counts were significantly decreased compared with untreated control mice ($P$ <0.0001; Fig. 2).

Mice surviving a higher dose of FMdC (*i.e.*, 20 mg/kg) recovered their body weight within 7–10 days and their leukocyte counts completely within 10–14 days after the end of treatment (data not shown).

**Radiosensitizing Effect of FMdC.** The responses of C33-A tumors to radiotherapy were dose-dependent, and the $TCD_{50}$ for RT alone was 51.0 ± 1.1 Gy (± 95% confidence limits; Fig. 4). When 5 mg/kg FMdC was given i.p. 1 h before each irradiation, the dose effect curve was shifted to the left and the $TCD_{50}$ dropped to 41.4 ± 1.1 Gy (Fig. 4). When 10 mg/kg FMdC was used, the $TCD_{50}$ was reduced to 38.2 ± 1.1 Gy (Fig. 4). The ERs at the $TCD_{50}$ level were 1.2 and 1.3 for 5 mg/kg and 10 mg/kg FMdC, respectively. The irradiated mice showed neither significant weight loss (data not shown) nor enhanced hematological toxicity (Fig. 5*A*), as compared with FMdC treatment alone. There was no increased skin toxicity with combined FMdC and RT as compared with RT alone (data not shown).

The response of U-87 MG tumors to RT were also dose-dependent (Fig. 6). When 5, 10, or 20 mg/kg FMdC were combined with 30 Gy of RT (Fig. 7), the tumor AGDs were significantly increased as compared with RT alone ($P$ <0.002, $P$ ≤0.0007, and $P$ ≤0.0001 for 5, 10, and 20 mg/kg FMdC, respectively). If the AGDs produced by combined FMdC and RT were corrected for AGDs produced by FMdC alone, the corresponding ERs at the doses of 5, 10, and 20 mg/kg FMdC were 2.0, 1.4, and 1.8, respectively (Table 1). The results were confirmed in a second experiment, in which a RT of 40 Gy was combined with 10 or 20 mg/kg FMdC (Fig. 8). Again, the AGDs produced by combined RT and FMdC were significantly prolonged as compared with RT alone ($P$ <0.002). The corresponding ERs at the doses of 10 and 20 mg/kg FMdC were 1.4 and 1.7, respectively (Table 1). However, as mentioned earlier, the toxic death was observed at the dose of 20 mg/kg. The long-term remission rate was also increased from 20% in cases of RT alone to 50% in cases of RT combined with 20 mg/kg FMdC (Fig. 8), but this difference was not significant ($P$ >0.05). When FMdC was combined to 50 Gy of RT, a significantly increased long-term remission rate was observed as compared with RT alone ($P$ <0.05; Table 2). However, the long-term remission rate was similar at the dose of 5 mg/kg as compared with 20 mg/kg.

Side effects of combined RT and FMdC in the mice bearing U-87 MG tumors were not the same as in the mice bearing C33-A tumors. At the dose of 5 mg/kg, the irradiated mice showed decreased leukocyte counts as compared with FMdC treatment alone ($P$ <0.05); however, the leukocyte counts were still higher than or similar to that of untreated control, even at a dose of 50 Gy (Fig. 5*B*). At the doses of 10 mg or 20 mg/kg, the irradiated mice showed no enhanced hematological toxicity as compared with FMdC alone (Fig. 5*B*). Increased toxicity, however, was observed at a higher radiation dose level (50 Gy) when 10 mg/kg FMdC was combined with RT ($P$ <0.001). No significant weight loss was observed in all groups as compared with FMdC alone (data not shown). When 20 mg/kg FMdC was combined with RT, 9–18% of the treated mice died of toxicity 10–14 days after treatment (see Table 2 and legends to Figs. 7 and 8). The hematological toxicity might not be the main reason of death, because bone marrow transplantation with blood tranfusion did not reduce the death rate (7). When FMdC was combined with RT, skin toxicity was slightly increased as compared with RT alone, especially

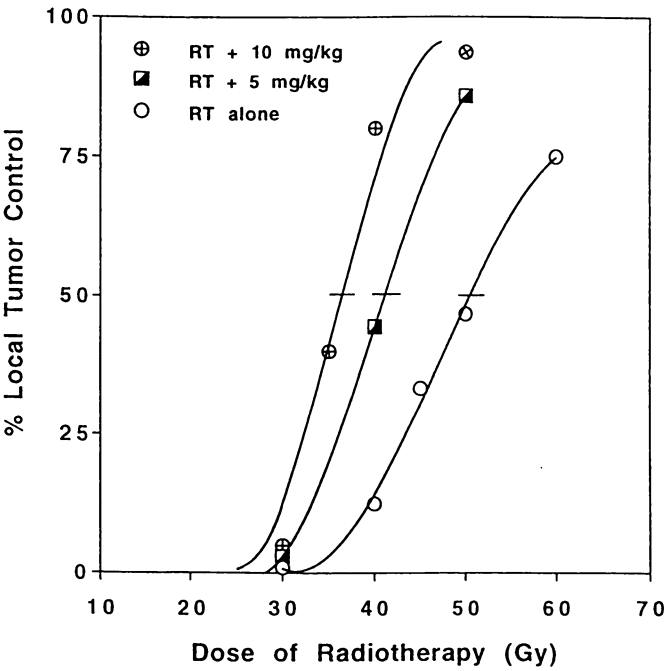


Fig. 4. Local tumor control of C33 xenografts to RT combined to daily 5 or 10 mg/kg FMdC. RT was given in 10 fractions over 12 days, and FMdC was given i.p. 1 h before each irradiation (± 95% confidence limits).

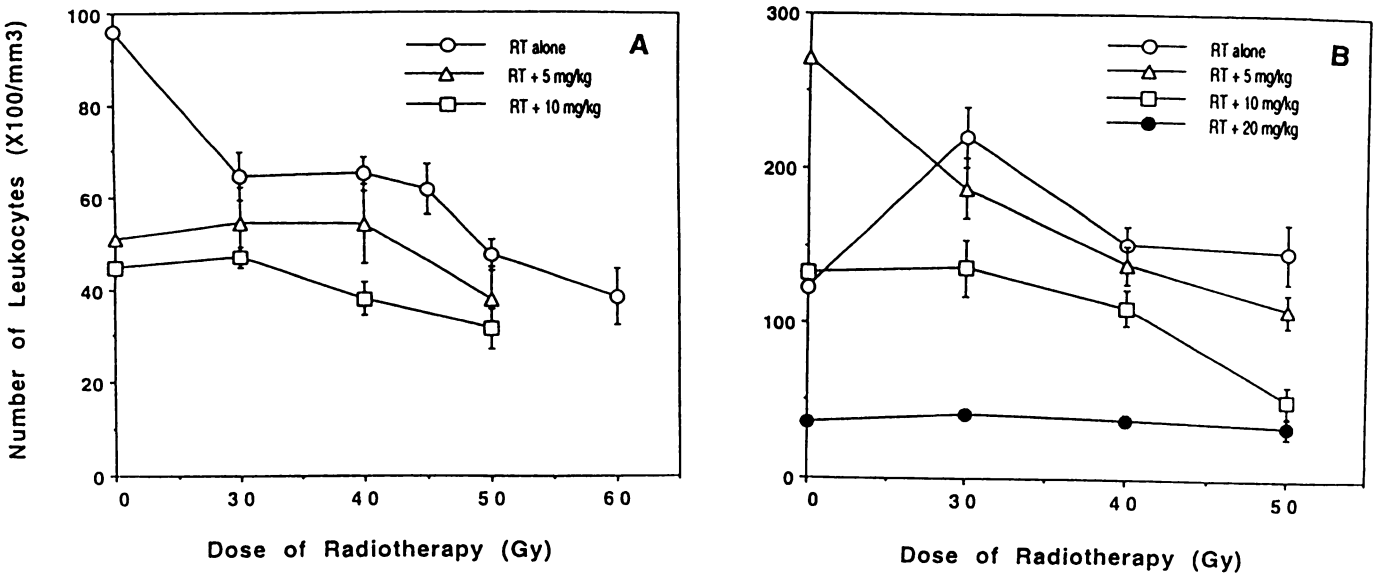


Fig. 5. Peripheral blood leukocyte analysis of mice bearing C33-A (*A*) and U-87 MG (*B*) xenografts 3 days after the end of treatment with combined FMdC and RT (± SE).

at the 40-Gy level (see Fig. 8 legend). Local acute skin reactions appeared about 12 days after treatment initiation, and the healing of radiation-damaged skin generally occurred 2–3 weeks after the end of treatment. One of 10 mice died of lower limb paralysis 3 months after the end of treatment in the group of 20 mg/kg FMdC + RT of 50 Gy.

**Histopathological Studies.** No significant necrotic areas were observed in the untreated s.c U-87 MG tumors (Fig. 9*A*), unless in the center of some tumor nodules (data not shown). Swollen and/or necrotic tumor cells, as well as infiltrated inflammatory cells, were observed after 10 daily i.p. treatments of 10 mg/kg FMdC (Fig. 9*B*). Mitotic figures were evident in untreated control tumors, but not in the tumors treated with FMdC (data not shown). Significant interstitial fibrosis and concurrent inflammatory cell infiltration were observed in the residual U-87 MG tumor mass 6 months after the end of treatment with combined RT (40 Gy) and FMdC (20 mg/kg; Fig. 9*C*, *top*). Enlarged or swollen tumor cells with enlarged irregular nuclei containing chromatin clumps were observed in some residual tumor masses (Fig. 9*C*, *top*). In those samples containing no residual tumor cells, only fibrosis without inflammatory cells was observed (Fig. 9*C*, *bottom*). This may indicate that inflammatory cells are involved in the process of removing dead tumor cells, because apoptotic cell death is not apparent in U-87 MG glioblastoma (16).

Swollen and/or necrotic tumor cells were observed in C33-A tumors treated with 10 daily i.p. treatments of 10 mg/kg FMdC, however, no infiltrated inflammatory cells were visible (Fig. 9, *E* and *F*). Apoptotic cells were visible after FMdC treatment (Fig. 9*F*).

**Potential Doubling Time and Volume Doubling Time.** Under our experimental conditions, the potential doubling times of untreated C33-A and U-87 MG tumors were 2.1 ± 0.6 ($n$ = 4) and 1.6 ± 0.4 ($n$ = 4) days, and the volume doubling times were 3.4 ± 0.9 ($n$ = 15) and 3.3 ± 0.7 ($n$ = 10) days, respectively.

## DISCUSSION

The results of these *in vivo* studies provide experimental evidence that FMdC may be an effective anticancer agent in the treatment of human cervix cancer and glioblastoma. This compound induces regression of s.c. tumors in a dose-dependent manner. Progressive tumor regrowth after the end of FMdC treatment alone, indicates that the daily administrations of FMdC are not curative for s.c. tumors. 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 >10 mg/kg for 10 times failed to produce more antitumor effects in the U-87 tumor, but resulted in a moderate weight loss and increased toxic death. As compared with untreated controls, the leukocyte counts in the mice bearing C33-A tumor were significantly reduced after treatment with a dose >5 mg/kg, whereas the leukocyte counts were significantly increased after treatment with 1–5 mg/kg FMdC in the mice bearing U-87 glioblastoma. However, at a higher dose (*i.e.*, 20 mg/kg), the reduced leukocyte counts were also observed in the U-87 MG model. The reduced leukocyte counts in the mice bearing C33-A tumors could be explained by direct cytotoxicity of FMdC to bone marrow. However, the exact mechanism by which leukocytosis occurs in the mice bearing U-87 MG tumor treated with a low dose of FMdC is not clear (20, 21). Our results correlate with prior xenograft studies of FMdC in

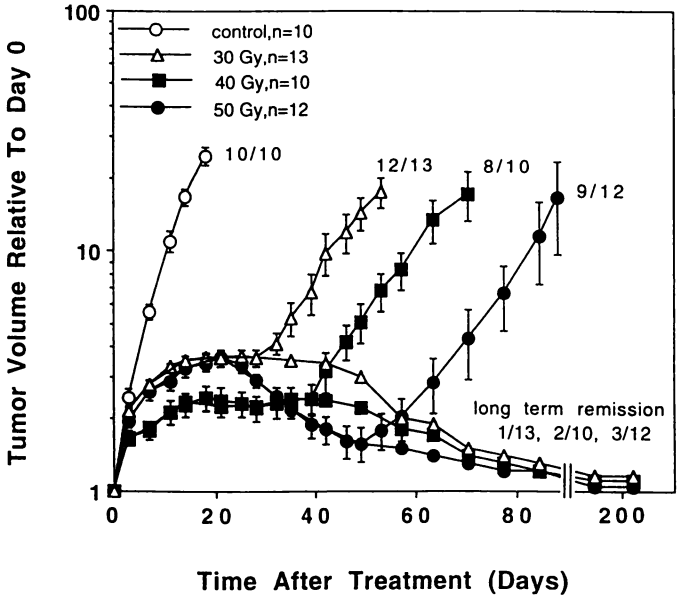


Fig. 6. The effects of RT at different total doses on the growth of U-87 MG xenografts. RT was given in 10 fractions over 12 days (± SE).

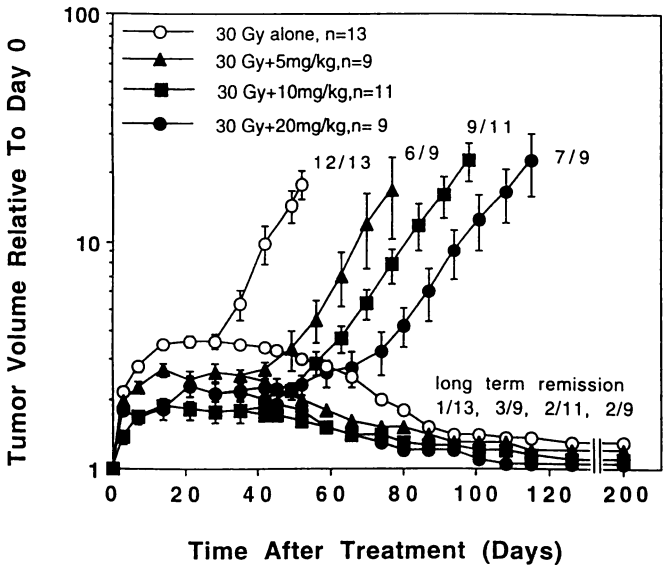


Fig. 7. The effects of 30 Gy of RT alone or combined with FMdC on the growth of U-87 MG xenografts (± SE). In the group of 30 Gy + 20 mg/kg FMdC, 2 of 11 mice died of acute toxicity 1–2 weeks after treatment (data not included).

Table 1 *Effect of FMdC on radioresponse of U-87MG xenografts*

| Treatment groups | Time for tumor to grow to 5 times initial treatment size (days)[a] | Tumor growth delay (days) | ER |
|---|---|---|---|
| Untreated control | 6.3 ± 1.3 | | |
| 5 mg/kg[b] alone | 20.1 ± 1.4 | 13.8[c] | |
| 10 mg/kg alone | 35.7 ± 1.2 | 29.4[c] | |
| 20 mg/kg alone | 39.8 ± 1.3 | 33.5[c] | |
| 30 Gy[d] alone | 41.0 ± 1.6 | 34.7[c] | |
| 40 Gy alone | 64.8 ± 1.7 | 58.4[c] | |
| 30 Gy + 5 mg/kg | 91.2 ± 1.8 | 71.1[e] | 2.0 |
| 30 Gy + 10 mg/kg | 84.2 ± 1.5 | 48.5[e] | 1.4 |
| 30 Gy + 20 mg/kg | 103.1 ± 1.4 | 63.3[e] | 1.8 |
| 40 Gy + 10 mg/kg | 118.6 ± 1.5 | 82.9[e] | 1.4 |
| 40 Gy + 20 mg/kg | 140.6 ± 1.4 | 100.8[e] | 1.7 |

[a] Geometric mean ± SD.
[b] FMdC was given as once daily i.p. injections, 10 times over 12 days.
[c] AGD.
[d] RT was given in 10 fractions over 12 days.
[e] NGD.

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 and liver metastases, as well as a reduction of the number of lung metastases (3–5, 7).

More importantly, FMdC significantly increased radiation response of human cervix cancer and glioblastoma xenografts. The ERs for C33-A tumor at $TCD_{50}$ levels were 1.2 and 1.3 for 5 and 10 mg/kg FMdC applied daily, respectively. However, the ERs for C33-A tumor are less important as compared with WiDr tumors in which ERs of 1.9 and 2.4 were observed (7). One of the reasons for the differences may be that C33-A is a more aggressive tumor than the WiDr tumor. The potential doubling time and volume doubling time were 2.1 and 3.4 days for the C33-A tumor and 3.0 and 6.4 days for the WiDr tumor, respectively. Significantly increased AGDs and long-term remission rates were observed in U-87 MG tumors when 5–20 mg/kg FMdC were combined with RT. Combining FMdC (5–20 mg/kg) with RT (30 or 40 Gy) produces a better therapeutic effect, with an ER ranging from 1.4–2.0. When 5–20 mg/kg FMdC were combined with 50 Gy of RT, significantly increased long-term remission rates of the treated tumors (80–88.9%) were observed, as compared with RT alone (25%). However, the high dose of FMdC (*i.e.*, 20 mg/kg) is not able to further increase a long-term remission rate as compared with 5 mg/kg.

The addition of FMdC to irradiation did not increase skin toxicity, weight loss, or hematological toxicity in the mice bearing C33-A tumors. A moderate increase of hematological toxicity was observed in the mice bearing U-87 MG tumors, when a higher dose of RT (50 Gy) was combined with 10 mg/kg FMdC. However, the hematological toxicity was well-tolerated and was reversible. We do not know why the slightly increased skin toxicity was observed in the mice bearing U-87 MG tumors and not in the mice bearing C33-A and WiDr tumors (7).

Because of direct measurements of RR, dNTP pools and DNA repair were not performed in our studies, the precise mechanism 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 (6, 22–25) or chain termination after being converted to the triphosphate, as shown for gemcit-

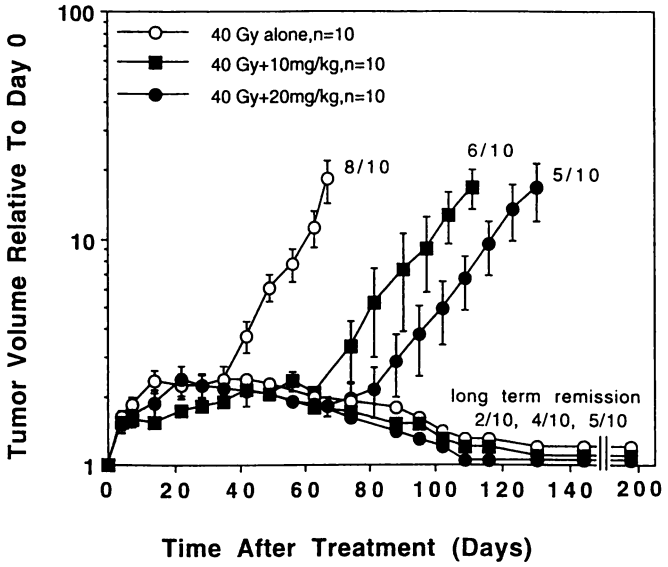


Fig. 8. The effects of 40 Gy of RT alone or combined with FMdC on the growth of U-87 MG xenografts (± SE). In the group of 40 Gy + 20 mg/kg FMdC, 1 of 11 mice died of acute toxicity 2 weeks after treatment (data not included). In the group of RT alone, 1 of 10 mice had skin toxicity grade I. In the group of RT + 10 mg/kg FMdC, 1 of 10 mice had skin toxicity grade II, and 4 mice had grade I. In the group of RT + 20 mg/kg FMdC, 1 of 10 survived mice had skin toxicity grade III, 3 mice had grade II, and 6 mice had grade I.

Table 2 *Antitumor and toxic effects of 50 Gy RT combined with FMdC on U-87MG xenografts*

| Groups | No. of mice | No. of remission (%) | No. of mice that died (%) |
|---|---|---|---|
| RT alone | 12 | 3 (25) | 0 |
| RT + 5 mg/kg[a] | 10 | 8 (80)[b] | 0 |
| RT + 10 mg/kg | 10 | 8 (80)[b] | 0 |
| RT + 20 mg/kg | 10 | 8 (88.9)[c,d] | 1 (10)[e] |

[a] RT was given in 10 fractions over 12 days, and FMdC was given 1 h before each irradiation.
[b] $P < 0.05$ as compared with RT alone.
[c] No. of (long-term) remission from 9 of 10 mice that survived.
[d] $P < 0.001$ as compared with RT alone.
[e] One mouse died of lower limb paralysis 3 months after treatment.

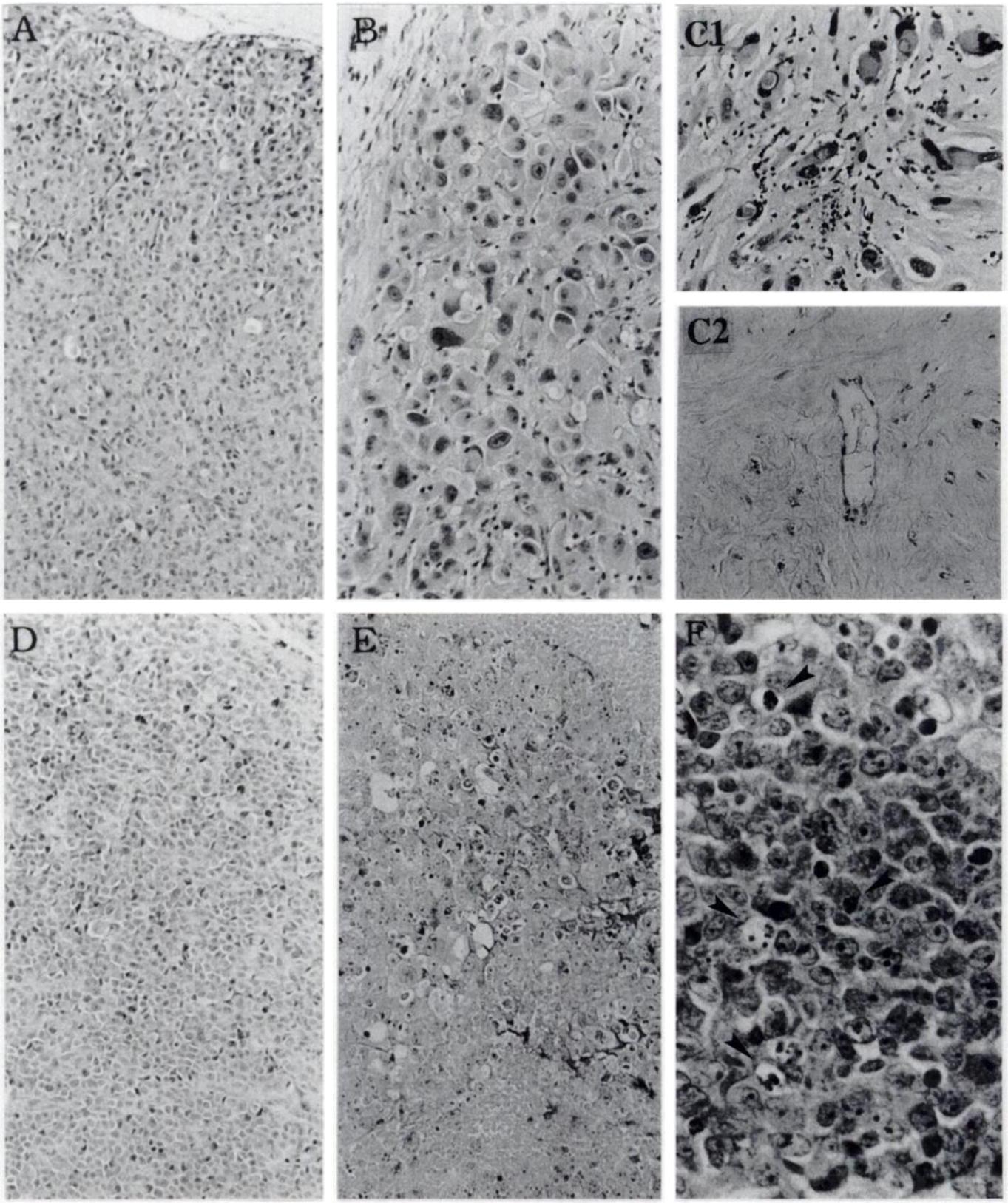


Fig. 9. Histology of human glioblastoma U-87 MG (*A-C*) and human cervix cancer C33-A (*D-F*). The s.c. tumors treated with 10 daily administrations of 10 mg/kg FMdC were removed for analysis 24 h after the last treatment (*B*, *E*, and *F*). The residual tumor masses were removed for analysis 6 months after the last treatment of combined FMdC of 20 mg/kg with RT of 40 Gy (*C1*, *C2*). In untreated tumors (*A* and *D*), tumor cell morphology is well preserved. In FMdC-treated tumors, swollen and/or necrotic tumor cells are observed (*B* and *E*). *C1*, residual abnormal tumor cells with a significant amount of interstitial fibrosis are observed. *C2*, only fibrosis (without residual tumor cells) is observed. *B* and *C1*, infiltrating inflammatory cells are detectable. *F*, apoptotic cells are visible (*arrow head*). Images of *A-E* were captured through a ×10 objective; *F* was captured through a ×40 objective.

abine (26). 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 (6) may result in an impairment of radiation-induced DNA damage repair and may be an important determinant of radiosensitization with FMdC (8). This has been shown in other RR inhibitors such as hydroxyurea (13) and gemcitabine (11, 12). 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. 27–31) and the thymidine analogues, bromodeoxyuridine and iododeoxyuridine (depletion of dCTP and TTP pools; Refs. 10 and 32). Thus, the antitumor and radiosensitizing effects of FMdC may be associated with the depletion and intracellular imbalances of dNTP pools. The reduced availability of DNA precursors or perturbation of dNTP pools may also induce apoptotic or programmed cell death (33) and induce p53-mutated cells to progress into S phase (27, 30, 31, 34, 35). Lawrence hypothesizes that S phase progression seems to be a key factor in the cytotoxicity and radiosensitization of a variety of S phase-active agents, because if tumor cells were treated with aphidicolin, S phase progress is arrested and the cytotoxicity and radiosensitization does not occur (10, 30, 31). Regardless of the precise mechanism, the above studies show compelling evidence that FMdC may be an effective antitumor and radiosensitizing agent for treatment of human cervix cancer and glioblastoma and probably also for other tumors.

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