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Report

Biologic Activity of 5′-Deoxy-5-fluorouridine by Rectal Administration

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5′-Deoxy-5-fluorouridine (dFUR) is used orally to treat human malignancies. This study compared the antitumor activity and toxicity of rectally and orally administered dFUR. A 7-day treatment of dFUR (350 or 700 mg/kg/day) was infused rectally over 30 min or administered by oral gavage (500 mg/kg/day) to rats bearing transplanted dimethylhydrazine-induced colon tumors. The oral treatment was previously shown to produce a 82% cure of the tumor-bearing animals. The tumor weight after 7 day treatment was compared to that before treatment. The size of the tumor in the saline-treated control group ($N = 6$) increased by 55%. The maximum tumor size reductions by drug treatments were 40% for the 350-mg/kg rectal dose ($N = 5$), >99% for the 700-mg/kg rectal dose ($N = 10$), and 100% for the 500-mg/kg oral dose ($N = 4$). The 350-mg/kg rectal dose did not produce any cures, while the 700-mg/kg rectal dose produced 80% cures and the 500-mg/kg oral dose 100% cures. The cured animals remained tumor-free during the observation period of 163 to 243 days. The tumor-bearing rats were euthanized between 46 and 132 days when they appeared moribund or when the tumor began to ulcerate. The 700-mg/kg rectal and 500-mg/kg oral treatments produced greater weight loss than saline, suggesting a drug-induced intestinal toxicity. After rectal drug treatment, the animal weight returned to pretreatment level within 3 days, indicating a rapidly reversible intestinal toxicity. The oral group suffered a greater weight loss than the rectal group and took more than 10 days to recover. This suggests that the intestinal toxicity of the rectal treatment was less severe than the oral treatment. The leukocyte and thrombocyte counts after drug treatments were not significantly different from the pretreatment levels, which suggests an absence of myelosuppression. In summary, these results indicate that dFUR by rectal administration had antitumor activity with minimal host toxicity.

KEY WORDS: 5′-deoxy-5-fluorouridine; 5-fluorouracil prodrug; antitumor activity and toxicities; colon tumors in rats; oral and rectal administration.

INTRODUCTION

5′-Deoxy-5-fluorouridine (dFUR) is a fluorinated pyrimidine and a metabolic prodrug of 5-fluorouracil (1). dFUR has antitumor activity after oral and intravenous (iv) administrations. dFUR is currently under clinical trials in Europe and Japan (2–4). The route and rate of administration affect its disposition, pharmacologic activity, and toxicity. After iv injection, the dose-limiting toxicities are myelosuppression and neurotoxicity (1,2). The drug is active by the oral route. Oral administration offers advantages over the iv route in that it does not produce myelosuppression and it is convenient, especially for long-term therapy. However, after oral administration, the gastrointestinal toxicity of dFUR becomes dose limiting and life threatening (4). We speculate that the more pronounced intestinal toxicity is due partly to the dispersion of the entire dose in the small intestine, giving a high local drug concentration/exposure in this sensitive tissue. From this consideration, the rectal route of administration can be an effective alternative. The high local concentration of dFUR in the small intestine after oral dosing could be avoided by rectal treatment, and the treatment can continue even if nausea and vomiting occur.

Previous studies in our laboratory showed that oral and iv treatments of dFUR produced a cure rate of 80–90% in rats bearing transplanted dimethylhydrazine-induced colon tumors (5–7). The present study investigated the antitumor activity and the bone marrow and intestinal toxicities of dFUR after rectal administration to rats and compared the relative biologic activity of dFUR by oral and rectal administration routes.

MATERIALS AND METHODS

Chemicals. dFUR (MW 246.1 g, Lot No. 305001) was provided by Hoffman-La Roche Inc. (Nutley, N.J.). All other chemicals and solvents were of reagent grade or spectro quality and were obtained from Sigma Chemical Co. (St. Louis, Mo.) and Fisher Scientific Co. (Cincinnati, Ohio). The concentration of dFUR solution was 140 mg/ml saline, and the pH was adjusted to 7 with 1 *N* NaOH.

Device for Rectal Administration. Other investigators

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have used catheters surgically implanted in the colon to examine the absorption of rectally administered drugs (8,9). To avoid the possible interference of surgery-related complications on the long-term biologic effect, we developed a nonsurgical drug delivery method. A suppository, in order to contain a sufficient dose of dFUR, required a large size due to the limited solubility of the drug and could not be easily inserted and retained in the rectum. A rectally inserted infusion catheter, which was held in place by an inflatable balloon surrounding the catheter, caused a reduction in urinary output, probably due to a mechanical obstruction of the urinary tract. Upon repeated usage the balloon plug weakened the colon muscle wall and resulted in hemorrhage, infection, and death. The device that was selected for the study was made with a RT200 pipette tip (Rainin Instruments, Emeryville, Calif.), a 250-μl Eppendorf centrifugation tube (VWR Scientific, Chicago, Ill.), and a 12-cm piece of PE-60 polyethylene tubing. The two ends of the pipette tip and Eppendorf tube were trimmed to size. The pipette tip was inserted in the Eppendorf tube. A hole was bored through the center of the base of the Eppendorf tube for the PE-60 tubing to pass through. The PE-60 tubing, pipette tip, and Eppendorf tube were cemented together using Krazy Glue. The plug was covered with soft rubber tubing (o.d., 6 mm; i.d., 3 mm) which provided a smooth outer surface and, further, provided some adhesion to the rectal mucosal lining. The junction point of the Eppendorf tube and pipette tip formed a ridge that allowed the anal sphincter muscle to close and form a seal around it. The proximal end of the PE-60 tubing (inserted in rectum) was extended 5 mm with a flexible Silastic medical-grade tubing (o.d., 1.20 mm; i.d., 0.64 mm; Dow Corning Co., Midland, Mich.). The distance between the ridge which lodged at the anus and the tip of the Silastic tubing was 3.7 cm. The distal end of the PE-60 tubing was connected to an infusion pump.

Animal Protocols. We have studied the pharmacology of fluorinated pyrimidines in female Fischer rats bearing transplanted colon tumors (5–7). Female Fischer rats (Charles River Breeding Laboratories, Kingston, N.J.; 5 months old) were also used in the present study. The rats were 128.7 ± 23.0 days old (range, 81–147 days) and had a body weight of 156.1 ± 14.3 g (range, 107.0–172.7 g). The rats were housed in metabolism cages 2 days before and throughout the experiment. During the 7-day treatment, food was withheld from midnight until 2 hr after treatment ended. Treatment was administered between 10:30 AM and 6 PM. The transplantable dimethylhydrazine-induced colon tumor was excised from a host under ether anesthesia, and the nonnecrotic portion was used for transplantation. Tumor fragments of 30 or 100 mg were transplanted by trocar subcutaneously in the right iliac region of new hosts 34 (30 mg) or 22 (100 mg) days before treatment. The tumor weight was measured as one-half of the product of (length of tumor mass) and (squared width of tumor mass) and ranged from 1.2 to 9.5 g on the first day of treatment. The animals were randomly assigned to control and different treatment groups. The average tumor size was 4.8 ± 1.4 g (mean ± SD) for the control group (both oral and rectal; $N = 6$), 7.9 ± 2.0 g for the group which received a rectal dose of 350 mg/kg ($N = 5$), 3.1 ± 1.3 g for the 700-mg/kg rectal dose ($N = 10$), and 3.8 ± 1.6 g for the 500-mg/kg oral dose ($N = 4$). The mean tumor weights in the different groups were in the following order: 350 mg rectal > control > 750 mg rectal = 500 mg oral. It should be noted that there is no correlation between the size of this transplanted colon tumor and the treatment outcome (6).

During rectal treatment, saline enemas were given at midnight and 2–3 hr before treatment. The purpose of administering the enemas was to minimize the fecal materials stored in the colon and hence reduce the adsorption of dFUR by fecal material. Rats were treated for 7 days with a daily rectal dose of 350 (approximately 0.4 ml) or 700 mg/kg (approximately 0.8 ml) or normal saline (0.4 to 0.8 ml). The infusion plug was inserted in the anus and affixed to the base of the tail with adhesive tape. Drug solution was infused over 30 min. The plug was left in the rectum for an additional 90 min to prevent leakage. The rat body weight and food intake were measured daily during treatment and at 1- to 4-day intervals for 3 additional weeks to monitor gastrointestinal side effects (10,11). Other nonspecific parameters, i.e., water intake and urine and feces output, were measured to monitor possible mechanical damage to the urinary tract and the lower bowel by the infusion plug and the general well-being of the animals. Blood counts were determined using a hemacytometer. Samples for blood counts were obtained by tail vein punctures between 12 and 4 PM, before treatment, and at different intervals until day 28.

We previously showed that dFUR is active orally. A 7-day treatment of 500 mg/kg/day of dFUR produced a 82% cure in tumor-bearing rats (7). In the present study, the activity of oral dFUR was reexamined in a small group of animals and compared to the activity of rectal dFUR. Tumor-bearing rats were given a 7-day treatment by oral intubation of 500 mg/kg/day of dFUR solution.

The rectal doses were 350 and 700 mg/kg, which were different from the 500-mg/kg oral dose. The 700-mg/kg rectal dose gave the same antitumor activity as the 500-mg/kg oral dose.

Data Analysis. The tumor weight in individual animals after treatment was compared to that before treatment, i.e., the weight at different intervals after treatment was divided by the pretreatment weight. The net body weight data (i.e., animal weight minus tumor weight) were calculated as the percentage deviation from the pretreatment level. The food and water intake and urine and feces output were standardized to the animal net body weight and calculated as the percentage deviation from the pretreatment level. The pretreatment level was the baseline value averaged over 3 days before treatment. Because of the different group sizes and variances, an approximate unpaired two-tailed t test was used in the statistical calculations (12). A P value of 5% was considered statistically significant.

RESULTS

Antitumor Effect. Figure 1 shows the time course of tumor growth in the control group and the growth inhibition and tumor regression due to 7-day dFUR treatments. In the control group, the size of the tumor increased by 55 ± 20% (mean ± SD) at the end of the treatment and by 244 ± 139% on day 28, and there were no spontaneous cures. The 350-mg/kg/day rectal dose produced a maximum tumor re-

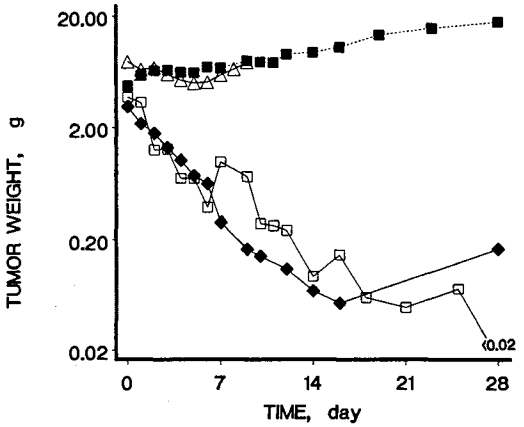

Fig. 1. Effect of dFUR treatment on tumor growth. The tumor-bearing rats were treated for 7 days. Day 0 is the first day of treatment. The control group received saline rectally or orally (■; N = 6). The dFUR-treated groups received 350 mg/kg/day rectally (△; N = 5), 700 mg/kg/day rectally (◆; N = 10), or 500 mg/kg/day orally (□; N = 4). Tumor weight was calculated as ½ × (length × squared width). The weight of inpalpable tumors (<20 mg) was entered as zero. Data represent the mean of each group.

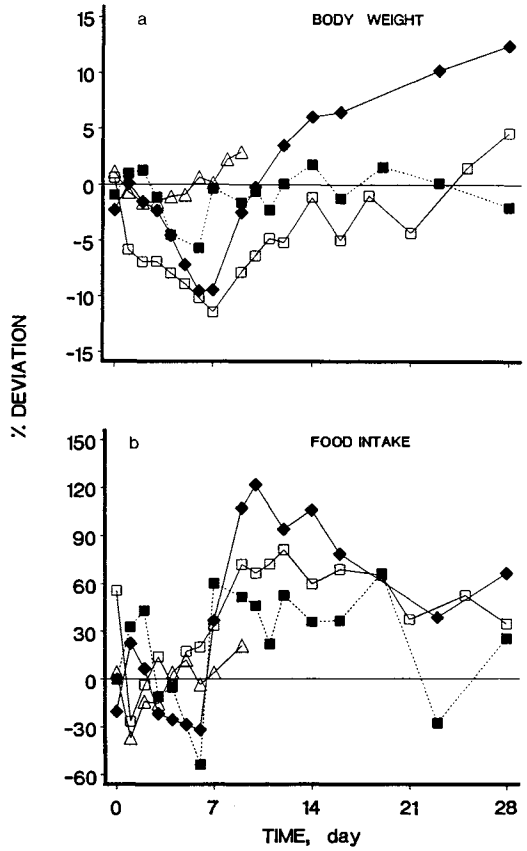

Fig. 2. Gastrointestinal toxicity of dFUR. The animal net body weight (a) and food intake (b) are expressed as the percentage deviation from the pretreatment level. The net body weight is the animal weight minus the tumor weight. The control group received saline rectally or orally (■; N = 6). The dFUR-treated groups received 350 mg/kg/day rectally (△; N = 5), 700 mg/kg/day rectally (◆; N = 10), or 500 mg/kg/day orally (□; N = 4). Tumor weight was calculated as ½ × (length × squared width). The weight of inpalpable tumors (<20 mg) was entered as zero. Data represent the mean of each group.

gression of 40 ± 26%, but tumor growth resumed after treatment. For the two groups which received 700-mg/kg/day rectal and 500-mg/kg/day oral doses, tumor regression continued for several weeks. The maximum regression was >99% for the 700-mg/kg rectal group and 100% for the 500-mg/kg oral group. The 350-mg/kg rectal dose did not produce any cures, while the 700-mg/kg rectal and 500-mg/kg oral doses cured 8 of 10 and 4 of 4 treated rats, respectively. Rats that were cured by dFUR treatment remained tumor-free during the observation period of 163 to 243 days. The remaining animals were euthanized when they appeared moribund or when the tumors began to ulcerate. This happened between 46 and 132 days after treatment.

Toxicity. Figure 2a shows the changes in net body weight with time. The maximum weight loss was 5.7 ± 4.8% for the control group, 1.7 ± 4.7% for the 350-mg/kg rectal group, 9.6 ± 5.9% for the 700-mg/kg rectal group, and 11.4 ± 2.8% for the 500-mg/kg oral group. The 350-mg/kg rectal group lost significantly less weight than the control group on days 4 and 6 and started to gain weight by day 6. The following comparisons pertain to the control, 500-mg/kg oral, and 700-mg/kg rectal groups. The body weight in the control group was significantly higher than those in the rectal group on days 5 and 7 and in the oral group on days 1 through 10. The control animals lost weight from the beginning of the treatment, started to recover after treatment, but again lost weight rapidly after day 14 when the tumor size doubled. The body weight of the rectal group returned to the pretreatment level on day 10 or 4 days after treatment and continued to increase. The oral group regained the pretreatment weight between day 16 and day 28. Compared to the control group, the rectal group had a greater body weight from day 16 onward, and the oral group on day 28. Compared to the oral group, the rectal group lost significantly less weight on days 1 to 4 during treatment, recovered much more rapidly, and had a greater body weight from day 9 onward. The food consumption followed a similar pattern (Fig. 2b). From day 9 onward the drug-treated groups consumed more food than the control group, and the rectal group consumed more food than the oral group. The rectal group also produced significantly more feces and urine than the oral and control groups from day 10 onward, while there was little difference between the oral group and the control group. There was no difference in water intake between the control and the drug-treated groups. Diarrhea occurred on days 4 to 7 in all rats treated with 700 mg/kg of dFUR rectally. But no diarrhea was seen in the control, the 350-mg/kg rectal, or the 500-mg/kg oral group.

Drug-induced bone marrow toxicity was monitored by the leukocyte and thrombocyte counts. In female rats, the blood count fluctuates with the age and strain of the animal, the estrous cycle, and the circadian rhythm. The normal range is 3000–15,725/mm^3 for leukocytes and 500,000–1,000,000/mm^3 for thrombocytes (13,14). The baseline leukocyte and thrombocyte counts in the Fischer rats used in this study are within this range. Table I shows the mean values of pretreatment and nadir counts for leukocytes and thrombocytes. There were no significant differences in

Table I. Effect of 7-Day dFUR Treatment on Leukocyte and Thrombocyte Counts

Dose/route (mg/kg/day)	N	Leukocytes (cells/mm³)			Thrombocytes (× 10³ cells/mm³)		
			Nadir			Nadir	
		Pretreatment	Count	Day[a]	Pretreatment	Count	Day[a]
Control	6	7950 ± 3060	6963 ± 1679	4[b]	642 ± 176	442 ± 142[c]	10
350 rectal	5	4320 ± 1697	3120 ± 858	4	464 ± 92	464 ± 92	0
700 rectal	10	4833 ± 2189	4425 ± 1667	4	511 ± 396	416 ± 167	7
500 oral	4	7000 ± 2952	5075 ± 310	7	450 ± 248	425 ± 185	7

[a] Day nadir was observed.
[b] Another nadir count of 6775 ± 778 occurred on day 28 for this group.
[c] Another nadir count of 443,000 ± 147,000 occurred on day 28 for this group.

the changes of leukocyte and thrombocyte counts with time between the control and the drug-treated groups (Fig. 3) or between the pretreatment and the nadir values after different drug treatments (Table I).

DISCUSSION

Results of this study indicate that dFUR was active by rectal administration. The rectal dose of 700 mg/kg/day dFUR produced about the same cure rate as the iv and oral doses of 500 mg/kg/day, as shown in previous studies (5–7) and confirmed in the present study. A separate pharmacokinetic study showed that the absorption rate and the bioavailability of the rectal dose were lower compared to the other routes of administration (15). This explains the higher rectal dose needed to produce the same cure rate.

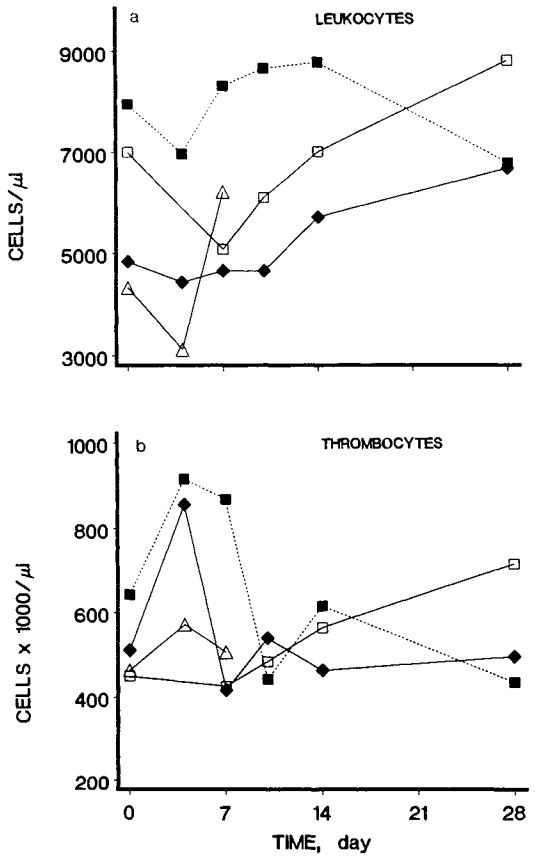

Fig. 3. Effect of dFUR treatment on leukocyte (a) and thrombocyte (b) counts. The control group received saline rectally or orally (■; $N = 6$). The dFUR-treated groups received 350 mg/kg/day rectally (△; $N = 5$), 700 mg/kg/day rectally (◆; $N = 10$), or 500 mg/kg/day orally (□; $N = 4$). Tumor weight was calculated as ½ × (length × squared width). The weight of inpalpable tumors (<20 mg) was entered as zero. Data represent the mean of each group.

The 350-mg/kg rectal dose did not result in adverse effects but produced only suboptimal antitumor activity. The 700-mg/kg rectal and 500-mg/kg oral doses induced body weight loss during treatment but the weight was regained after treatment, indicating an acute and reversible gastrointestinal toxicity. The rectal group recovered more rapidly than the oral group; the rectal treatment also produced less weight loss and greater food consumption in the beginning of treatment and during recovery. These data suggest that the gastrointestinal toxicity by the rectal route was less severe than by the oral route. This may be because the rectal route avoids the high local concentration in the small intestine. On the other hand, the rectal dose of 700 mg/kg produced diarrhea, while the oral route did not. Diarrhea can be due to local irritation to the large bowel and/or drug-induced malabsorption in the small bowel secondary to intestinal crypt cytotoxicity (11,16). The rectal treatment produced less weight loss than the oral route, suggesting a less severe malabsorption problem. It follows that the diarrhea after the rectal treatment may be due to a local irritation in the large bowel.

The tumor-bearing rats appeared lethargic and ungroomed and exhibited ataxia, while the rats cured of the tumors or with reduced tumor burden were active, responsive, and groomed. The higher body weight and food intake of the drug-treated groups after treatment, as compared to the control group, are likely a consequence of the general well-being of the drug-treated rats, which either were cured or had a substantially reduced tumor burden. dFUR treatments did not reduce the leukocyte and thrombocyte counts and, hence, were not myelosuppressive. In conclusion, these data show that rectal administration of dFUR was effective with minimal host toxicity.

This study used a tumor transplanted subcutaneously in the hind limb of a rat. The rectally administered drug must enter the systemic circulation in order to reach the tumor site. Because the colon is the first-pass tissue, a colorectal

cancer located in close proximity to the rectal administration site may receive a higher drug exposure. It is possible that rectal dFUR can produce a greater antitumor effect against a tumor located in the colorectal region (as opposed to the tumor implanted in the hind limb).

Several recent reports indicate that the portal vein infusion of 5-fluorouracil is effective in the adjuvant therapy of colorectal carcinomas (17,18). Because of its extensive presystemic metabolism, 5-fluorouracil has a very low and erratic oral bioavailability (1) and must be administered parentally. In contrast, dFUR is readily absorbed from the colon, with a systemic bioavailability of about 30% and a relatively small coefficient of variation (<8%) (15). In this study, dFUR was administered by infusion over 30 min. A commercially available constant-infusion device such as the suppository-size osmotic pump (Alza, Palo Alto, Calif.) can be inserted into the rectum and used for this purpose. Rectal administration of dFUR using this device can be a noninvasive and convenient alternative to the portal vein infusion of 5-fluorouracil.

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