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Uridine Rescue from the Lethal Toxicity of 5-Fluorouracil in Mice

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Summary. *To determine the relationship between 5-fluorouracil (FUra) toxicity and its RNA- and DNA-directed actions we examined the ability of continuous SC infusion with uridine (Urd), thymidine (dThd), or deoxyuridine (dUrd) to rescue mice from the lethal toxicity of FUra. Male B6D2F$_1$ mice were treated with a single IP injection of FUra (800 mg/kg) followed in 24 h by a 5-day infusion with either 0.9% NaCl or Urd (0.1, 1, 5, or 10 g/kg/day). Survivors were then followed up for 30 days after FUra treatment. Urd (1, 5, or 10 g/kg/day) rescued mice from the lethal toxicity of FUra, whereas Urd (0.1 g/kg/day) was as ineffective as 0.9% NaCl as a rescue agent. With variable doses of FUra followed in 24 h by a Urd infusion (5 g/kg/day) for 5 days, Urd rescued mice treated with FUra (400, 600, or 800 mg/kg) but was ineffective against higher doses of FUra (1,000 or 1,200 mg/kg). Mice treated with FUra (800 mg/kg) followed in 24 h by a 5-day infusion with either dThd (1, 5, or 10 g/kg/day) or dUrd (1 or 5 g/kg/day) could not be rescued from the lethal toxicity of FUra. In all experiments deaths occurred between 6 and 12 days after FUra. These results, which demonstrate a specificity for Urd, but not for either dThd or dUrd, for rescuing mice from the lethal toxicity of FUra, suggest the importance of the RNA- rather than the DNA-directed actions of FUra as a determinant of its toxicity in mice.*

Introduction

Following treatment with 5-fluorouracil (FUra) the formation of two anabolites, 5-fluorodeoxyuridine-5′-monophosphate (FdUMP) and 5-fluorouridine 5′-triphosphate (FUTP) lead to major disruptions in DNA and RNA synthesis, respectively [8]. FdUMP competes with 2′-deoxyuridine 5′-monophosphate (dUMP) and in the presence of 5,10-methylenethetrahydrofolate forms a ternary covalent complex with thymidylate synthetase, which inactivates the enzyme. The resultant depletion of the deoxythymidine 5′-monophosphate pool inhibits DNA synthesis [14, 20]. FUTP is incorporated in place of uridine 5′-triphosphate into mRNA, tRNA, and rRNA and also interferes with rRNA maturation [16, 25].

In different tumor cell lines, the cytotoxic actions of FUra are related predominantly to either its DNA- or its RNA-directed effects, since depending upon the particular cell line there is a specificity for thymidine (Thd), uridine (Urd), or 2′-deoxyuridine (dUrd) (which are anabolized to dTTP, UTP, or dUMP, respectively) for reversing the cytotoxicity of FUra [2, 5, 24]. With respect to normal host tissues, the importance of the RNA-directed action of FUra, 5-fluorouridine or 5-fluorodeoxyuridine to their dose-limiting gastrointestinal toxicity in mice correlated best with incorporation of these fluorinated pyrimidines into RNA rather than with levels of FdUMP [9]. To further define the relationship between FUra toxicity and its RNA- and DNA-directed actions, we examined the ability of continuous SC infusion of Urd, dThd, or dUrd to rescue mice from the lethal toxicity of FUra.

Materials and Methods

Drugs. FUra and dThd were obtained from Dr. V. L. Naryanan, Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. Urd and dUrd were obtained from Sigma Chemical Co., St. Louis, MO, USA. FUra was prepared in 2% $NaHCO_3$ just prior to use, at a final concentration that permitted an injection of 0.01 ml/g mouse body weight. Urd, dThd, and dUrd were each prepared in 0.9% NaCl, and all solutions were sterilized by passage through 0.22 μ

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Millipore filters just prior to use. Sodium pentobarbital was obtained from Veterinary Laboratories, Inc., Lenaxa, Kansas, USA.

Animals. Male C57BL/6 x DBA/2 F_1 (hereafter called B6 $D2F_1$) mice weighing 20–23 g were obtained from Sprague-Dawley Laboratories, Madison, Wisconsin, USA.

Continuous Subcutaneous Infusion. Mice were anesthetized with a single SC injection of sodium pentobarbital (75 mg/kg) prior to insertion of an SC cannula. The technique for continuous SC infusion has been previously described [13]. In all experiments, animals not treated with a drug(s) received an equivalent volume of solvent.

Effect of Infusion with Urd, dThd, or dUrd on Gross Toxicity in Mice. Male $B6D2F_1$ mice, in groups of six each, received a single IP injection of Urd (50, 250, or 500 mg/kg) as a loading dose. An SC infusion of Urd (1, 5, or 10 g/kg/day, respectively) was immediately begun and continued for 5 days, after which time the mice were removed from the infusion apparatus. Individual body weights were determined on days 0, 5, 6, 7, and 8, and survivors were checked daily for 30 days after the beginning of the infusion. Similar experiments were also done with either dThd or dUrd at the same doses as described for Urd.

Effect of Urd Infusion to Rescue Mice from the Lethal Toxicity of FUra. Mice were given a single IP injection of FUra (800 mg/kg). Twenty-four hours later the mice were randomly distributed into groups of six each. They then each received a single IP injection of Urd, after which an SC infusion of Urd (0.1, 1, 5, or 10 g/kg/day) was immediately begun and continued for 5 days. Survivors were determined daily for 30 days after FUra. The loading dose of Urd was 5, 50, 250, or 500 mg/kg for a Urd infusion of 0.1, 1, 5, or 10 g/kg/day, respectively.

In another experiment mice were given a single IP injection of FUra (200, 400, 600, 800, 1,000, or 1,200 mg/kg). Twenty-four hours later the mice were randomly distributed into groups of eight each. They were then each given a single IP injection of Urd (250 mg/kg), after which an SC infusion of Urd (5 g/kg/day) was begun and continued for 5 days. Survivors were determined daily for 30 days after FUra.

Effect of Either dThd or dUrd Infusion to Rescue Mice from the Lethal Toxicity of FUra. Mice were given a single IP injection of FUra (800 mg/kg). Twenty-four hours later the mice were randomly distributed into groups of six each. They were each given a single IP injection of dThd, after which an SC infusion of dThd (1, 5, or 10 g/kg/day) was immediately begun and continued for 5 days. Survivors were determined daily for 30 days after FUra. The loading dose of dThd was 0, 50, 250, or 500 mg/kg for a dThd infusion of 1, 5, or 10 g/kg, respectively. A similar experiment was also done in which mice were given a single IP injection of FUra (800 mg/kg) followed in 24 h by a dUrd infusion for 5 days, except that the doses of dUrd were 1 and 5 g/kg/day.

Results

Effect of Infusion with Urd, dThd, or dUrd on Gross Toxicity in Mice

To determine the gross toxicity to mice of an SC infusion of Urd, dThd, or dUrd each nucleoside was administered to mice for 5 days. Urd given at a dose of 1, 5, or 10 g/kg/day to groups of six mice each did not cause any marked gross toxicity, as there were only small decreases in mean body weights on day 5 (6% or less compared with day 0 body weights) for all groups. All mice returned to day 0 body weights on day 6 and were alive on day 30 after the infusions were begun. dThd at 1, 5, or 10 g/kg/day caused decreases in mean body weights on day 5 of 8%, 9%, and 12%, respectively. Weight loss was recovered within a few days and all dThd-treated mice were alive on day 30. In contrast, dUrd infusion at 10 g/kg/day resulted in two deaths in a group of six mice, while dUrd at 1 or 5 g/kg/day caused only slight losses in mean body weights on day 5 and all animals were alive on day 30. Control mice which received a 0.9% NaCl infusion for 5 days had a decrease in mean body weights of 3% on day 5. Weight loss was recovered by day 6 and all mice were alive on day 30 after the infusion was begun.

Table 1. Effect of Urd infusion to rescue $B6D2F_1$ mice from the lethal toxicity of FUra[a]

Experiment	Treatment		Individual days of death	Day 30 survivors/ total treated
	Day 0 Drug FUra dose (mg/kg)	Days 1–5 Drug Urd dose (g/kg/day)		
I	800	–	6, 7, 8, 9, 10, 11	0/6
	800	0.1	6, 6, 7, 8, 11, 11	0/6
	800	1	9, 10	4/6
	800	5	–	6/6
	800	10	6, 10	4/6

[a] Groups of six mice each, as indicated, were given a single IP injection of FUra followed in 24 h by a Urd infusion, as indicated, for 5 days. Survivors were checked daily for 30 days

Effect of Urd Infusion to Rescue Mice from the Lethal Toxicity of FUra

We first examined what effect varied doses of Urd by infusion would have on FUra-treated mice. As can be seen, treatment of mice with a single IP injection of FUra (800 mg/kg) followed in 24 h by a 5-day infusion with Urd (0.1 g/kg/day) resulted in no day 30 survivors out of a group of six, i.e., it was as ineffective as an infusion with 0.9% NaCl. In contrast, infusions with Urd at 1, 5, or 10 g/kg/day were all effective in rescuing mice from the lethal toxicity of FUra (Table 1). We next examined the effect of varied doses of FUra followed in 24 h by Urd infusion (5 g/kg/day) for 5 days. Urd at 5 g/kg/day was used, since according to the results shown in Table 1 it was the optimal dose for rescue from the lethal toxicity of FUra. As can be seen in Fig. 1, Urd (5 g/kg/day) rescued mice that had been treated with 400, 600, or 800 mg FUra/kg. However, Urd (5 g/kg/day) was only slightly effective in rescuing mice treated with FUra, 1,000 mg/kg, and it was completely ineffective after FUra, 1,200 mg/kg. In contrast, FUra at 400 mg/kg followed by infusion with 0.9% NaCl resulted in only a 25% survival rate, while higher doses of FUra (600, 800, 1,000, or 1,200 mg/kg) followed by 0.9% NaCl infusion were uniformly lethal to all mice. These results indicate that the approximate LD_{10} of FUra was more than tripled by Urd rescue. In the experiments shown in Table 1 and Fig. 1, deaths occurred usually between 6 and 12 days after FUra. This is consistent with death being due to gastrointestinal toxicity rather than myelotoxicity [9]. Having established that Urd could rescue mice from FUra toxicity, we then compared the effectiveness of dThd and dUrd to rescue mice from FUra toxicity.

Effects of dThd Infusion to Rescue Mice from the Lethal Toxicity of FUra

Treatment of mice with a single IP injection of FUra (800 mg/kg) followed in 24 h by a 5-day infusion with dThd at 1, 5, or 10 g/kg/day did not rescue mice from the lethal toxicity of FUra; the results with dThd were no different than those obtained with a 0.9% NaCl infusion (Table 2).

Effect of dUrd Infusion to Rescue Mice from the Lethal Toxicity of FUra

Treatment of mice with a single IP injection of FUra (800 mg/kg) followed in 24 h by a 5-day infusion with

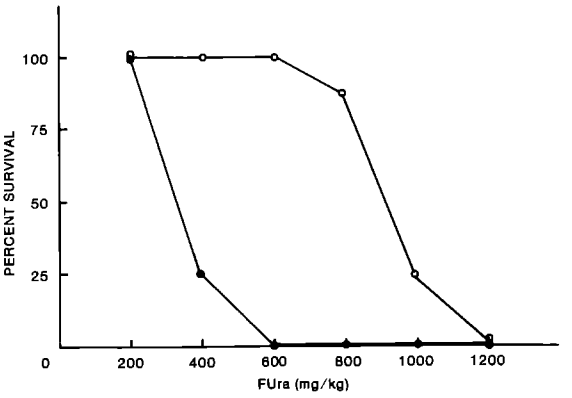

Fig. 1. Effect of Urd infusion to rescue mice from the lethal toxicity of varying doses of FUra. Groups of eight mice each received a single IP injection of FUra (200–1,200 mg/kg) followed in 24 h by an infusion with either Urd (5 g/kg/day) or 0.9% NaCl for 5 days. Survivors were followed up for 30 days after FUra. ●——●) FUra (day 0), saline infusion (days 1–5); ○——○) FUra (day 0) Urd infusion (days 1–5)

Table 2. Effect of dThd infusion to rescue B6D2F$_1$ mice from the lethal toxicity of FUra[a]

Treatment		Individual days of death	Day 30 survivors/ total treated
Day 0 Drug FUra dose (mg/kg)	Days 1–5 Drug dThd dose (g/kg/day)		
800	–	5, 5, 6, 6, 8, 8	0/6
800	1	6, 9, 11, 12, 13	1/6
800	5	6, 7, 7, 9, 12, 12	0/6
800	10	5, 5, 6, 6, 7, 8	0/6

[a] Groups of six mice each were given a single IP injection of FUra followed in 24 h by a dThd infusion, as indicated, for 5 days. Survivors were checked daily for 30 days

Table 3. Effect of dUrd infusion to rescue B6D2F$_1$ mice from the lethal toxicity of FUra[a]

Treatment		Individual days of death	Day 30 survivors/ total treated
Day 0 Drug FUra dose (mg/kg)	Days 1–5 Drug dUrd dose (g/kg/day)		
800	–	6, 6, 7, 8, 9, 9	0/6
800	1	7, 8, 8, 9, 9, 9	0/6
800	5	9, 9, 10, 10, 11, 11	0/6

[a] Groups of six mice each were given a single IP injection of FUra followed in 24 h by a dUrd infusion, as indicated, for 5 days. Survivors were checked daily for 30 days

dUrd at 1, 5, or 10 g/kg/day also failed to rescue mice from the lethal toxicity of FUra; the results with dUrd were no different than those obtained with a 0.9% NaCl infusion (Table 3).

Discussion

Using a long-term continuous SC infusion technique in unrestrained mice, we have shown that mice can be rescued from the lethal toxicity of a single IP injection of FUra with a Urd infusion begun 24 h after FUra and continued for 5 days. In contrast, infusions with either dThd or dUrd failed to rescue mice from the lethal toxicity of FUra. Our experiments support the concept that the RNA- rather than the DNA-directed action of FUra is an important determinant of its toxicity in mice [9]. A possible explanation for our results is related to the fact that administration of Urd would presumably expand the intracellular uridine ribonucleotide pool in sensitive normal tissues [10]. Increased levels of uridine ribonucleotides would then allow normal RNA synthesis to resume despite the known persistence of FUra ribonucleotides in tissues for up to 3 days after FUra [4, 23]. This hypothesis remains to be tested.

In mice the plasma half-life of a therapeutic dose of FUra is about 15 min [4]. The rate-limiting step for FUra catabolism is reduction by dihydrouracil dehydrogenase, an enzyme that also catabolizes uracil and thymine [19]. Concurrent administration of FUra plus dThd decreases the clearance of FUra from plasma, while at the same time the toxicity of FUra is increased. This effect is probably due to competition for dihydrouracil dehydrogenase between FUra and thymine, the latter of which is derived from dThd [12, 26]. In our study, reported herein, it is likely that the 24 h delay between administration of FUra and the beginning of a Urd infusion may avoid any pharmacokinetic interaction between FUra and uracil.

There is considerable evidence that the effects of FUra on RNA metabolism contribute to its cytoxic actions. For example, the responsiveness of several animal tumors to FUra is related to its incorporation into RNA [1, 11, 15, 17]. rRNA processing is especially sensitive to FUra, and this effect is proportional to the incorporation of FUra into rRNA and the sensitivity of a tumor to FUra [24]. Although FUra is also incorporated into tRNA and poly (A)-containing mRNA, impairment in their synthesis is relatively insensitive to FUra [6, 7]. Co-administration of either Urd or dThd with FUra to the S-49 mouse lymphoma cell line in culture increased the incorporation of FUra into RNA, so that in this system the RNA-directed effects of FUra appear to be the principal determinant of growth inhibition [18, 22]. The enhanced action against a mouse mammary carcinoma due to co-administration of dThd plus FUra is related, at least in part, to a selective incorporation of FUra into tumor nuclear RNA [21]. In rat regenerating liver co-administration of dThd intensified the effect of FUra on interference with the processing of rRNA from precursor RNA [3].

When used clinically, FUra is given as a single IV bolus injection, in five IV bolus injections daily, or by continuous IV infusion [19]. Our study indicates that Urd rescue is effective when FUra is administered as a single bolus injection. It remains to be determined in our system whether Urd infusion would also be effective in rescuing mice when FUra is administered either by repeated daily bolus injections or by continuous infusion.

The use of a Urd infusion to rescue mice from the lethal toxicity of FUra might provide a selective biochemical difference that could be exploited. For example, treatment with a high-dose FUra followed by delayed Urd infusion against a tumor that is inhibited predominantly by the DNA-directed action of FUra would allow this action of FUra against the tumor to continue, while Urd would rescue normal tissues. Furthermore, the antitumor effect of FUra at high doses may be quite different from those at low, i.e., conventional doses of FUra [24]. Therefore, a tumor that was unresponsive to conventional doses of FUra might respond to high-dose FUra, while Urd would rescue host normal tissues. We now intend to examine whether delayed infusion with Urd can increase the therapeutic efficacy of Fura against tumor-bearing mice.

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