

TOXICITY AND ANTITUMOR EFFECT OF 5-FLUOROURACIL

AND ITS RESCUE BY URIDINE

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INTRODUCTION

The pyrimidine analog 5-fluorouracil (5FU) has been used in the treatment against several solid carcinomas, mainly of the colon, for several decades (1). However, overall response rate is only about 20%, while gastrointestinal and myeloid toxicity are dose-limiting (1). Research on 5FU has been directed towards enhancing 5FU anabolism by combination with e.g. methotrexate (2) or thymidine (3) and towards the development of several analogs of 5FU. One recent analog, Doxifluridine (5'-deoxy-5-fluorouridine, 5'dFUR) showed an improved therapeutic index against a broad range of murine and rat tumors (4,5) and therapeutic activity in human advanced rectosigmoid adenocarcinoma (6).

Martin et al. (7) and Klubes et al. (8) used delayed uridine administration to "rescue" mice from 5FU toxicity. Martin et al. (7) combined 5FU with repeated i.p. injections of high-dose uridine, while Klubes et al. (8) combined 5FU with long-term subcutaneous moderate-dose uridine infusions. Both schedules permitted the use of high doses of 5FU, which would be lethal in the absence of uridine. Antitumor activity could be enhanced against several 5FU resistant tumors such as Colon 26 (7) and B16 melanoma (9) but not against L1210 leukemia (9). However, it was not clear whether the response rate of 5FU-sensitive tumors would be affected by the combination with uridine. This is of special interest since in clinical trials with 5FU followed by delayed uridine, it is not known if the tumor will be sensitive or resistant. Therefore, we evaluated the antitumor effect of 5FU and uridine on the murine colon carcinomas Colon 38 (sensitive) and against the murine colon carcinoma Colon 26, (less sensitive) (10). The results were compared with the antitumor effect of 5'dFUR. In C57Bl mice we investigated the time course of the hematological toxicity of 5FU and its "rescue" by uridine. In addition we investigated the toxic effects of uridine itself.

EXPERIMENTAL

For all experiments 2-month-old female mice were used. Subcutaneous transplants of Colon 38 and Colon 26 were maintained in both

flanks of C57Bl/6 and Balb-c mice, respectively. The size of the tumor was determined by caliper measurement (11) every 3-4 days. Treatment was started when tumor volume was between 50 and 150 mm³. Mice were treated with 5FU or 5'dFUR (Hoffman-La Roche, Mijdrecht, Netherlands) by intraperitoneal injection once a week. Uridine was administered at 2 and 20 hr after 5FU. Tumor size was calculated relative to that of the first day of treatment (11). Therapeutic effectiveness was evaluated by calculations of T/C values (tumor size of treated animals divided by tumor size of untreated controls) at various days after treatment. In each experiment the control group consisted of 4-6 animals, and the treated group consisted of 5-6 animals.

Toxicity of 5FU and of the combination of 5FU and uridine were evaluated in C57Bl/6 mice. Blood samples were obtained weekly by retro-orbital bleeding and the number of leucocytes and thrombocytes and the hematocrit value were determined. Body temperature of the mice was monitored rectally with a thermosensitive probe. Statistical analyses were performed by Student's t-test.

RESULTS

Colon 38 and Colon 26 are both chemically induced murine colon carcinomas; Colon 38, an adenocarcinoma and Colon 26, an undifferentiated carcinoma with local fibrosarcoma structures (12). Colon 38 reached a volume of 100 mm³ at about 20 days after transplant and the doubling time was 5.2 days. The take rate was about 90%. Mice tolerated a large tumor load and the median life-span was longer than 40 days. Colon 26 reached a volume of 100 mm³ after about 13 days and the doubling time was considerably shorter, 1.9 days. The take rate was almost 100%. The tumor appeared to be aggressive since the median life span was only 18 days.

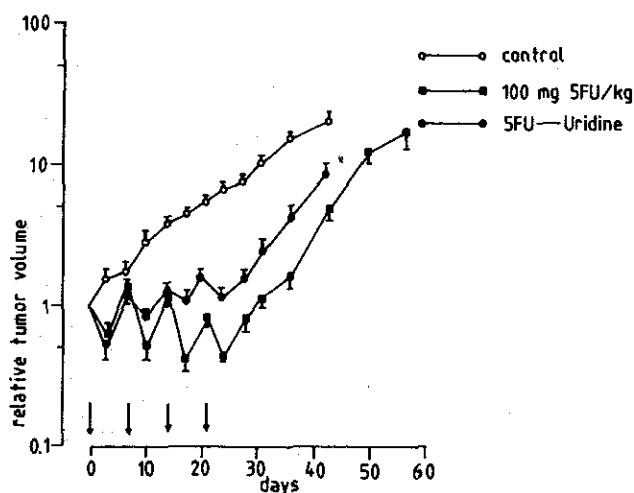


Fig. 1. Antitumor activity against Colon 38 of 5FU (100 mg/kg) and of 5FU (100 mg/kg) followed by uridine (3500 mg/kg) after 2 and 20 hr. Values are means \pm SEM of 8-12 tumors. Arrows indicate the day of 5FU administration.

Colon 38 was sensitive to 5FU. At 60 mg/kg a significant tumor growth delay was observed (data not shown) when 5FU was administered for 4 weeks. Toxicity was moderate. At 100 mg 5FU/kg, also administered for 4 weeks, the tumor did not grow during treatment. A representative experiment is shown in Fig. 1. Administration of uridine after 5FU did not significantly alter the tumor growth rate (Fig. 1).

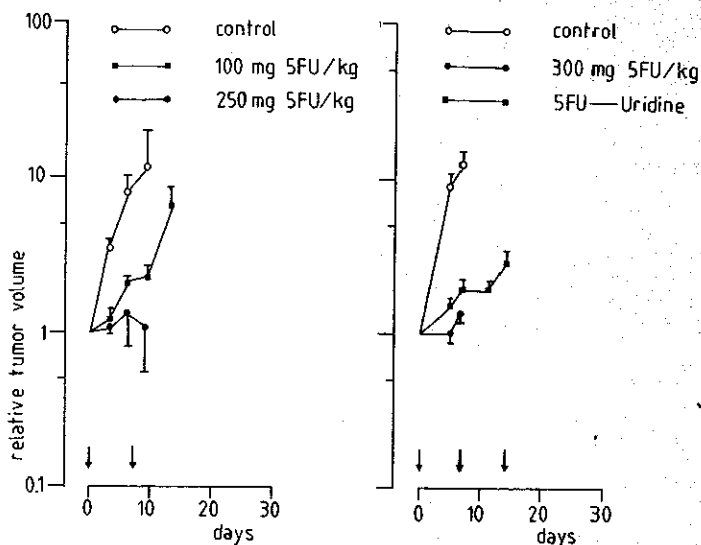


Fig. 2. Antitumor activity against Colon 26 of 5FU at various doses and of 5FU followed by uridine (3500 mg/kg) after 2 and 20 hr. values are means \pm SEM of 8-12 tumors. Arrows indicate the day of 5FU administration.

Colon 26 appeared to be relatively resistant to 5FU at 100 mg/kg (Fig. 2). At 250 and 300 mg/kg tumor growth was arrested, but the mice died due to toxicity of 5FU. Administration of uridine increased the life-span of the mice but the tumors did not regress.

Doxifluridine (5'dFUR), a prodrug of 5FU, is considered to be less toxic than 5FU. With mice bearing Colon 38 and treated with 800 and 1000 mg/kg, a significant tumor growth delay could be observed (Fig. 3). The antitumor effect was comparable to that observed with 100 mg 5FU/kg. With mice bearing Colon 26 a significant tumor growth delay was also observed at 800 and 1000 mg 5'dFUR/kg. The antitumor effect was again comparable to that observed with 100 mg 5FU/kg, but treatment with 5'dFUR resulted in a considerable increase in life-span. The results are summarized in Table 1. With Colon 26 high T/C values were obtained after treatment with high, very toxic doses of 5FU. At 300 mg/kg a mean weight loss of almost 10% was observed. With Colon 38 maximal T/C values were obtained with 100 mg 5FU/kg, but with 5'dFUR the weight loss was less. None of these mice died due to toxicity from these drugs.

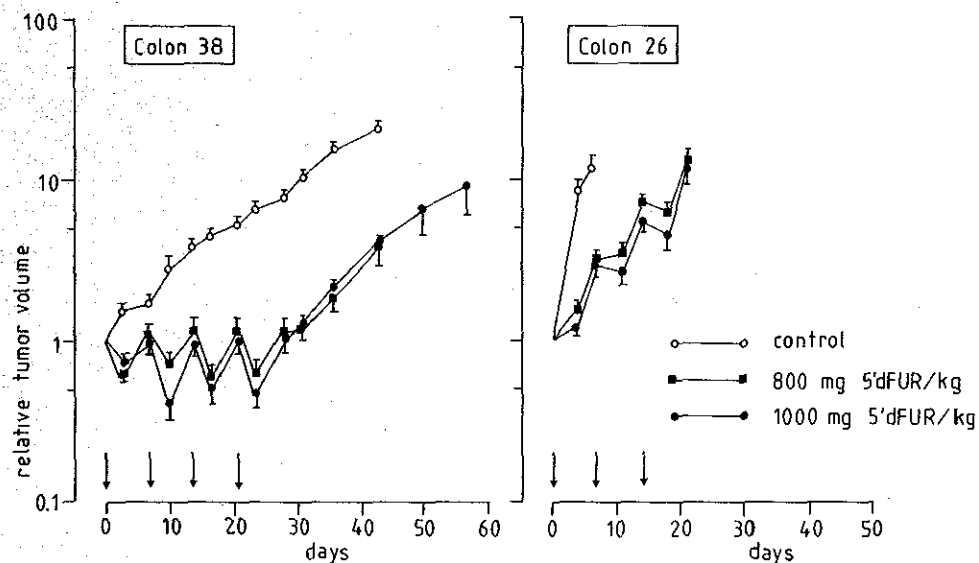


Fig. 3. Antitumor activity of 5'dFUR against Colon 38 and Colon 26. Values are means \pm SEM of 8-12 tumors. Arrows indicate the day of 5'dFUR administration.

Table 1. Antitumor activity against Colon 38 and Colon 26.

Drug	Dose (mg/kg;ip)	Days of treatment	Maximal T/C % (day) ^a	Median life span ^b	Weight loss ^c
<u>Colon 26</u>					
5FU	100	0,7	17.1 (9)	13	1.8
	250	0,7	9.2 (7)	8	4.3
	300	0,7	10.6 (7)	7	9.8
5FU-UR	300-3500	0,7	14.9 (7)	13	7.1
5'dFUR	800	0,7,14	24.6 (7)	24	5.4
5'dFUR	1000	0,7,14	23.3 (7)	17	6.2
<u>Colon 38</u>					
5FU	100	0,7,14,21	6.9 (24)	> 40	2.4
5FU-UR	100-3500	0,7,14,21	17.1 (24)	> 40	3.0
5'dFUR	800	0,7,14,21	9.0 (24)	> 40	0.8
5'dFUR	1000	0,7,14,21	7.2 (24)	> 40	0.7

^a The day at which difference between T and C was maximal. All values are significantly different ($p < 0.05$) from control values.

^b Days after first treatment.

^c Mean percentage weight loss, one day after treatment.

UR; uridine.

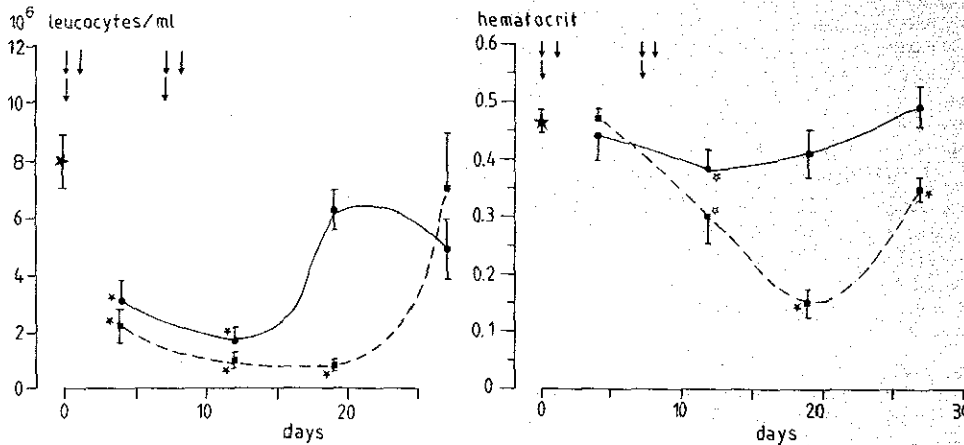


Fig. 4. Leucocyte count and hematocrit value in C57Bl/6 mice after treatment with 5FU (100 mg/kg; broken line) and in mice treated with 5FU followed by uridine (3500 mg/kg; solid line). Value at day 0 is of 18 mice, other values are of 5-7 mice and represent mean \pm SEM. Closed asterisk, $p < 0.01$; open asterisk, $0.02 < p < 0.05$; differences are that between treated and control group.

Myeloid toxicity was studied by weekly monitoring of several hematological parameters. As expected 5FU caused a severe leukopenia which was more pronounced after the second treatment (Fig. 4). The leukopenia reached its nadir 18 days after the first administration of 5FU. One week thereafter the leucocyte count was within the normal range. With the combined 5FU-uridine treatment the nadir of the leukopenia was found after 12 days and was less severe than with 5FU alone. Furthermore, the mice recovered much more rapidly from the treatment than with 5FU alone. 5FU also seriously affected the hematocrit value. Again the nadir was after 18 days which is 11 days after the last treatment (Fig 4). With the combined treatment only a moderate effect on the hematocrit value was observed shortly after the second treatment. A modest thrombocytopenia was only observed in the 5FU treated group 4 days after the first treatment. On subsequent days thrombocyte count was in the normal range, but after 27 days an overshoot was observed (about twice the normal values), which was long-lasting, up to 68 days after the first treatment (data not shown).

The toxicity of uridine was limited to a pronounced effect on body temperature. At a dose of 3500 mg/kg temperature decreased very rapidly (Fig. 5). Hypothermia was accompanied by spasms, solitary behavior, discolored fur, etc. However, as soon as temperature increased, their behavior normalized. After 6 hr all mice recovered. At a lower dose the symptoms were less severe. At 1000 mg/kg no hypothermia was observed, but at 500 mg/kg a small but significant fall in body temperature occurred.

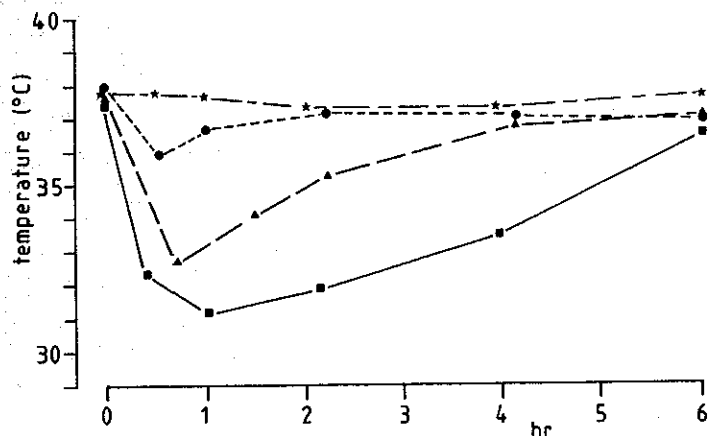


Fig. 5. Effect of uridine on body temperature of C57Bl/6 mice. Values are means of 6-12 mice. ■—■, 3500 mg/kg; ▲—▲, 2000 mg/kg; *—*, 1000 mg/kg; and ●—●, 500 mg/kg.

DISCUSSION

In previous reports (7-9) it has been demonstrated that 5FU in combination with delayed uridine administration can be used for the treatment of 5FU resistant tumors. Our results demonstrated that 5FU sensitive tumors can also be treated with 5FU combined with delayed uridine, without affecting the antitumor effect significantly. The 5FU resistant tumor Colon 26 can be treated by 5FU in combination with uridine, resulting in an increased life-span. The main advantage of the delayed uridine administration actually appears to be a prevention of toxicity, rather than a rescue (7,8).

The antitumor effects of 5FU on Colon 38 and Colon 26 are comparable to that described previously (12,13) for these tumors. Delayed administration of uridine does not affect the differences in sensitivity between the tumors. The Colon 26 is relatively resistant to 5FU treatment and mice still die due to toxic effects of 5FU, although uridine increased the life-span. Doxifluridine, a precursor of 5FU, appeared to be as effective against Colon 38 as 5FU. Although a higher T/C value was found for the 5FU-uridine treatment, the difference with Doxifluridine was not significant. However, toxicity of Doxifluridine measured by weight loss, was less severe than for 5FU or 5FU-uridine. The T/C value for Doxifluridine in Colon 26 was higher than for 5FU alone or 5FU combined with uridine, but with Doxifluridine a significant increase in life-span was observed and the mice only died when the tumor load was comparable to that of untreated mice at the time of death. It appears that Doxifluridine has a better therapeutic index in the treatment of Colon 26 and to a lesser extent also for Colon 38 than treatment with 5FU alone. However, in clinical trials it appears that toxicity of Doxifluridine is more severe than expected (6,14). The possibility remains that the use of longterm infusions or other modes of administrations may enhance the therapeutic index of this new fluoropyrimidine.

The time course and extent of leukopenia is comparable to that described previously (15). However, the toxic effects of 5FU on the hematocrit were not reported (15), nor the protecting effect of uridine on the hematocrit value (7,8). 5FU did not cause severe thrombocytopenia, but the overshoot indicates that synthesis of thrombocytes was affected during 5FU therapy. The absence of an overshoot in the combination therapy, therefore, indicated that uridine also prevented toxic effects of 5FU on thrombocytes.

The mechanism of uridine rescue of 5FU toxicity is not totally clear, but there is evidence that toxicity in bone marrow is mainly mediated by incorporation of 5FU into RNA (7). Expansion of the uridine nucleotide pool by uridine administration would enhance the replacement of FUTP in RNA by UTP, preventing a further disturbance in the proliferation of hematopoietic progenitor cells. The selectivity of the rescue for bone marrow could be related to the relative large expansion of the uridine pool in blood compared to tissues (16).

The effect of uridine on body temperature indicates that uridine or its metabolites directly or indirectly interfere with the thermoregulatory function of the hypothalamus. This effect of uridine on body temperature has not been described previously. The mechanism is not clear but uracil or another catabolite might be involved since inhibition of uridine phosphorylase partially prevented the fall in body temperature (data not shown). Furthermore, the lowest temperature was observed after 30-60 min when plasma concentrations were highest (15-20 mM); temperature increased when uridine concentrations decreased. In contrast, high-dose uridine administration resulted in fever in both rabbits and patients (17). In patients this fever was only observed after long-term continuous infusions, but not with one-hr infusion (18) or with an intermittent administration schedule. With the latter schedule a reversal of 5FU induced toxicity was observed in two patients up to now (19).

In conclusion, delayed uridine administration prevents 5FU induced myelosuppression. The sensitivity of Colon 38 to 5FU is not or only slightly affected by delayed uridine administration. Colon 26 is relatively resistant to 5FU, but a higher dose of 5FU can be administered in combination with uridine, resulting in an increased life-span. However, Doxifluridine appears to have a better therapeutic effectiveness against Colon 26. Uridine has severe side-effects on body temperature, but they are not long-lasting or lethal. Preliminary clinical trials indicated that uridine can be given safely to patients by adjusting the administration schedule.

ACKNOWLEDGEMENTS

This work was supported by the Dutch Cancer Foundation, grant IKA 83-16. We acknowledge the Department of Hematology for their assistance and Ms C. Vanderlans for typing the manuscript. We also thank Hoffmann-La Roche, Mijdrecht, The Netherlands, for financial support.

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