

CIRCADIAN RHYTHM-MODULATED CHEMOTHERAPY WITH HIGH DOSE 5-FLUOROURACIL AGAINST GASTROINTESTINAL CANCERS: EVALUATION AND CASE REPORT

HIROSHI KOJIMA, JUNICHI SAKAMOTO and MITSUNORI YASUE

Department of Surgery, Aichi Prefectural Hospital, Okazaki, Japan

ABSTRACT

Circadian variations in chemotherapy toxicity and antitumor effects were investigated in experimental and clinical studies. In the experimental study, Balb/c mice bearing murine colon carcinoma Colon 26 were treated with 4 injections of 5-fluorouracil (5-FU) (80 mg/kg) at 0000 Hours After Light On (HALO), 0600 HALO, 1200 HALO and 1800 HALO. The antitumor effect of treatment at 0000 HALO (early resting phase) group was significantly better with lower toxicity than the 1200 HALO (early activity phase) group, resulting in significantly longer survival ($p < 0.05$). In the clinical study, the effect of circadian rhythm-modulated 5-FU plus leucovorin therapy was evaluated in an end-stage patient with recurrent gastric carcinoma. After continuous weekly infusion of 5-FU (1000 mg/m²/day \times 2) was stopped because of its gastrointestinal toxicity, circadian rhythm-modulated chemotherapy (CRMC) was performed changing the dose of 5-FU to 666 mg/m²/day during the daytime (0500 to 1700) and to 1333 mg/m²/day from evening to night (1700 to 0500). The patient persevered with the 23 CRMC course without any signs of severe side effects and survived for nearly a year, suggesting the potential effect of CRMC in minimizing toxicity and prolonging survival.

Key Words: circadian rhythm-modulated chemotherapy, 5-fluorouracil, recurrent gastric cancer, Hours After Light On

INTRODUCTION

To date, chronobiologic investigations have shown that the therapeutic indices of drugs can be affected by varying circadian drug timing. This phenomenon may be particularly important in anticancer chemotherapy. Powerful chemotherapeutic agents can kill cancer cells but also kill or severely injure cells of normal tissues. Since the susceptibility of the normal tissues is rhythmically variable during the circadian cycle, whereas that of the malignant tissues is less so, the timing of chemotherapy may be an important element in creating greater therapeutic specificity.

5-fluorouracil (5-FU) has remained the main active drug against gastrointestinal malignancies. Its tolerability and/or its efficacy have been shown to increase in combination with folic acid (FA)¹⁻³⁾ or administration by continuous venous infusion.⁴⁾ Both such regimens usually resulted in a threefold to fourfold improvement in the tumor response rate in patients with advanced gastrointestinal cancer as compared with standard 5-FU treatment. These

Correspondence and reprint requests to: Junichi Sakamoto, M.D., Department of Surgery and Laboratory of Clinical Oncology, Aichi Prefectural Hospital, 18 Kuriyado, Kakemachi, Okazaki 444-0011, Japan

figures, however, were still low and affected survival modestly.¹⁻⁴⁾ In addition, severe gastrointestinal toxicity can be a limiting factor in therapy with 5-FU or 5-FU + FA, since the intestinal epithelium, being a rapidly proliferating tissue, is highly susceptible to damage from these agents.⁵⁾ Since a dose-response relationship characterizes the antitumor efficacy of 5-FU against gastrointestinal cancer, a reduction in treatment toxicity would be beneficial to achieving a high dose intensity and therapeutic effect.

Therefore, in the present study, we attempted to reduce the toxicity of 5-FU by altering the drug administration time. 5-FU was injected into tumor-bearing mice at four equidistant time points, i.e., 0, 6, 12, and 18 hours after lights on (HALO), and its antitumor effects, toxicity, and effects on survival were evaluated. We also performed a circadian rhythm modulated chemotherapy (CRMC) using 5-FU and FA in a patient suffering from recurrent gastric cancer, which led to results showing apparent antitumor efficacy and survival benefit.

MATERIALS AND METHODS

1. Experimental Study of Chronotherapy

Chemotherapeutic agent

5-FU was obtained from Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan) and formulated as a 250 mg/10 ml solution in 0.85% saline. Before administration to mice, this stock solution was diluted with pyrogen-free 0.85% saline to a concentration of 100 mg/10 ml. A total of 320 mg/kg of 5-FU was injected intraperitoneally; i.e. 80 mg/kg was administered twice a week for 2 weeks. Injection volume of 5-FU varied between 0.15 and 0.25 ml according to the weight of the mice.

Mice

Balb/c mice were obtained at 6 weeks of age and were kept in a controlled area for at least 10-14 days prior to the beginning of the experiment. The area had a standardized light-dark cycle (light, 0600; dark, 1800). Mice had access to food and water ad libitum. The murine colon carcinoma Colon 26 was obtained from Dr. T. Watanabe of the Second Department of Surgery, Nagoya University, Nagoya, Japan.

Toxicity Study

Eighty Balb/c mice were grouped for different time points. Four time points: 0, 6, 12, and 18 HALO were arbitrarily chosen for evaluation of toxicity. Whereas HALO stands for "Hours After Light On", in our study 0 HALO corresponded to 0600, 6 HALO with 1200, 12 HALO with 1800 and 18 HALO with 0000 local time. Each group of mice consisted of 20 mice. The hematologic toxicity of 5-FU was assessed by measuring the leucocyte count in these non-tumor bearing Balb/c mice. Blood samples were obtained with heparinized Ht capillaries using retroorbital puncture. Blood sampling was performed before the first administration of 5-FU and continued weekly for 4 weeks. The intestinal toxicity of 5-FU was evaluated by the incidence of watery diarrhea observed over 2 days following each injection.

Therapeutic efficacy against murine colon cancer

Murine colon cancer Colon 26 was maintained in Balb/c mice. The growth characteristics and sensitivity of the cell line have been described previously.^{6,7)} Tumors were implanted as small fragments of 1-5 mm in both flanks subcutaneously. Growth of the tumor was determined by caliper measurement (length × width × thickness × 0.5) once a week. The volume of the tumors was calculated relative to that on the first day of treatment (day 0). Before treatment, 50 tumor bearing Balb/C mice were randomized in five groups, one group as a control

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and four groups for 5-FU treatment at different HALO. Treatment was started when tumor volume was between 50-150 mm (approximately after 10 days). Survival time and the ratio of treated/control tumor size (T/C) was used for evaluation of the results.⁸⁾

Statistical analyses were performed using Student's t test for paired and unpaired data in leucocyte count and antitumor effect. A chi-square test was performed with regard to the incidence of diarrhea in different HALOs.

2. CRMC with 5-FU in a patient with recurrent gastric cancer

Chronomodulated chemotherapy using 5-FU plus leucovorin was performed in a 63-year-old man with peritoneal recurrence of gastric carcinoma (Fig. 1).

The patient underwent gastrectomy and D3 dissection of the lymph nodes for gastric carcinoma (pathology T3, n1) on March 15, 1991. On April 30, 1993, the patient was operated for recurrent carcinoma in the abdominal wall and at the duodenum and both lesions were resected. Continuous infusion of 1000 mg/m²/day of 5-FU and 20 mg/m²/day of leucovorin was started twice a week on May 26, 1993. However the administration was interrupted after 2 weeks by severe gastrointestinal toxicities; i.e., WHO grade III watery diarrhea, nausea and vomiting.

Oral Tegafur was then administered from June 9, 1993 for 2 months; however, the patient underwent a third operation for multiple peritoneal recurrence on October 8, 1993. During the operation, a twelve focus from 1.0 cm to 4.5 cm in diameter was found in the peritoneal cavity, and one metastasis of 5.5 cm in the upper abdomen was noticed. A palliative ileo-sigmoidostomy was constructed. 5-FU and leucovorin injection using the same doses as May 26, 1993 was tried again, but this time adopting a circadian rhythm shaped administration protocol (Fig. 2).

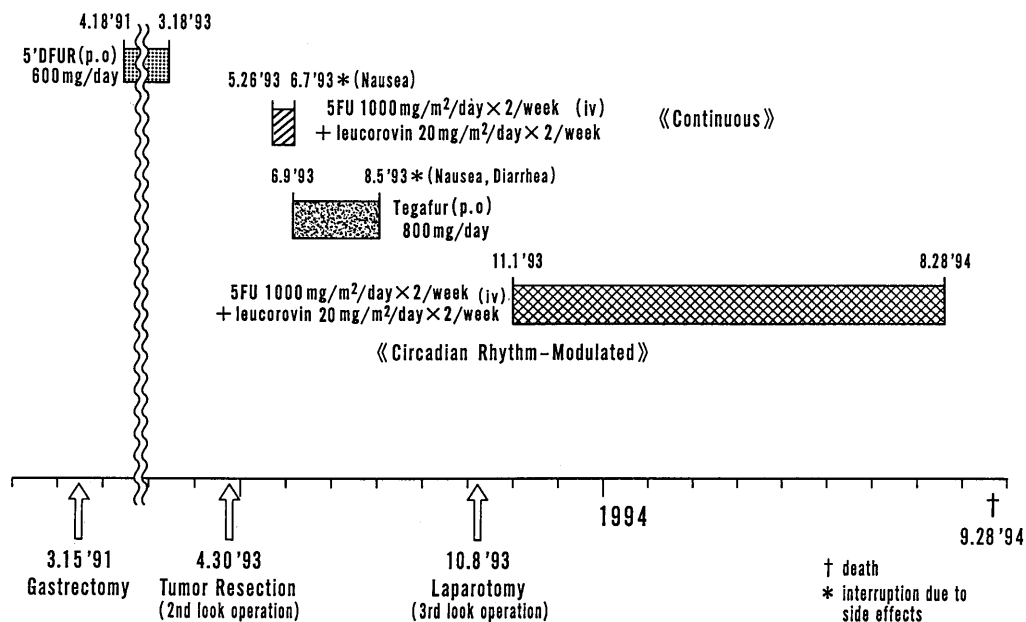


Figure 1. Treatments performed in a patient with recurrent gastric carcinoma, who was enrolled in the chronomodulated chemotherapy schedule after the third operation.

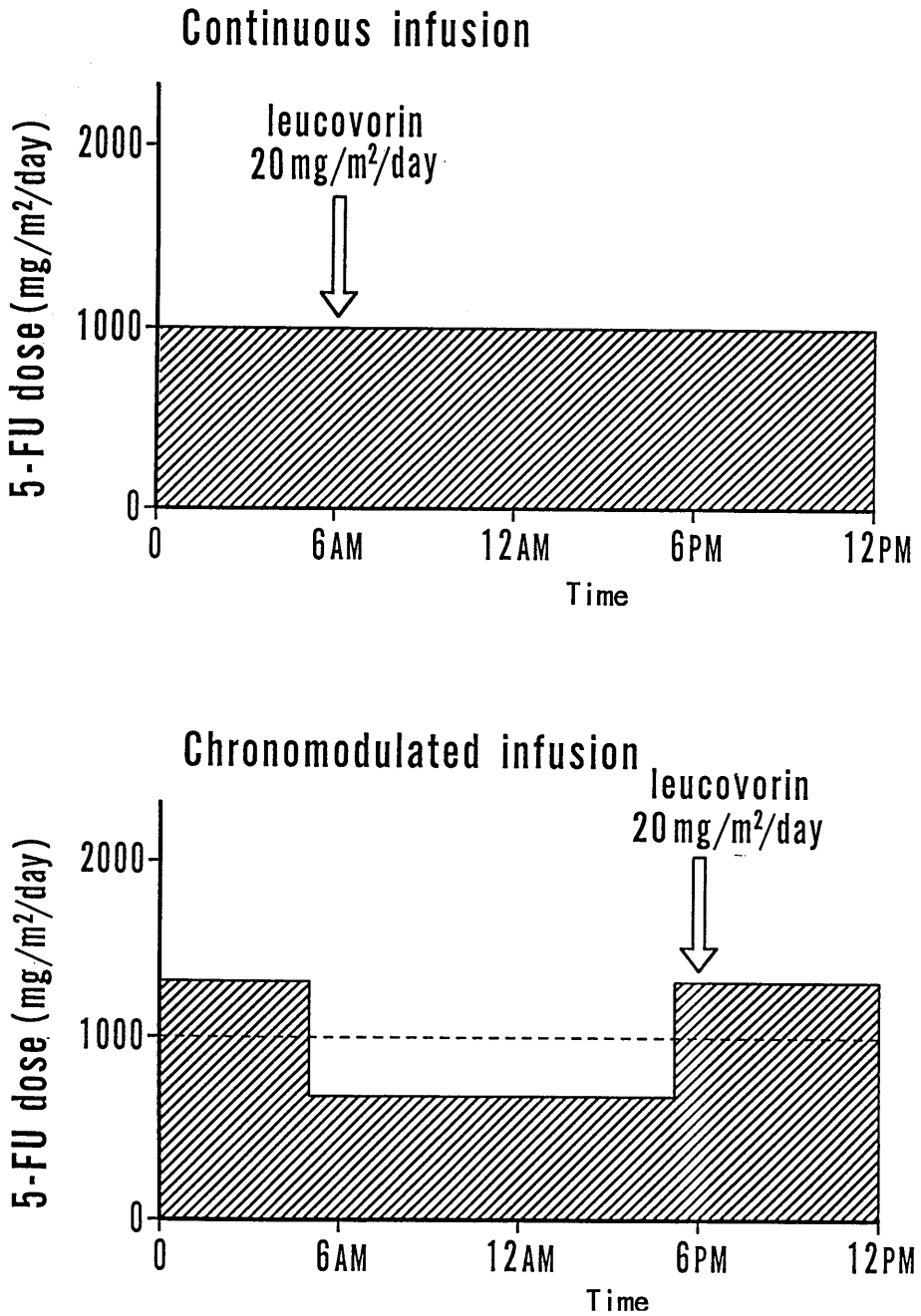


Figure 2. Daily schedules of continuous and chronomodulated (circadian rhythm shaped) chemotherapy using 5-FU and leucovorin in a patient with recurrent gastric carcinoma.

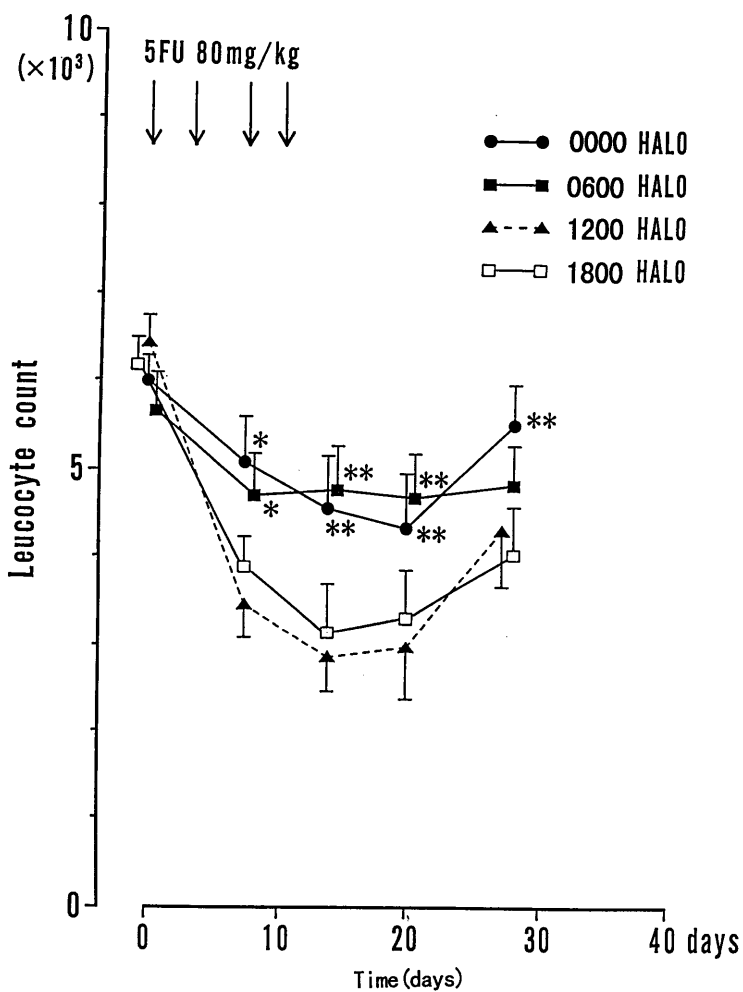
RESULTS

1. Experimental Study of Chronotherapy

Hematologic toxicity

Treatment of Balb/c mice with 5-FU at 1200 or 1800 HALO, resulted in leucopenia at days 7, 14 and 21 and significant difference was observed compared to the groups treated at 0000 and 0600 HALO (Fig. 3).

Appearance of hematologic toxicity was significantly reduced when mice were treated during their early resting phase (0000 HALO), or in the middle resting phase (0600 HALO) compared to their early active phase (1200 HALO) or mid-active phase (1800 HALO).



*, ** Significance of difference from 1200 and 1800 HALO

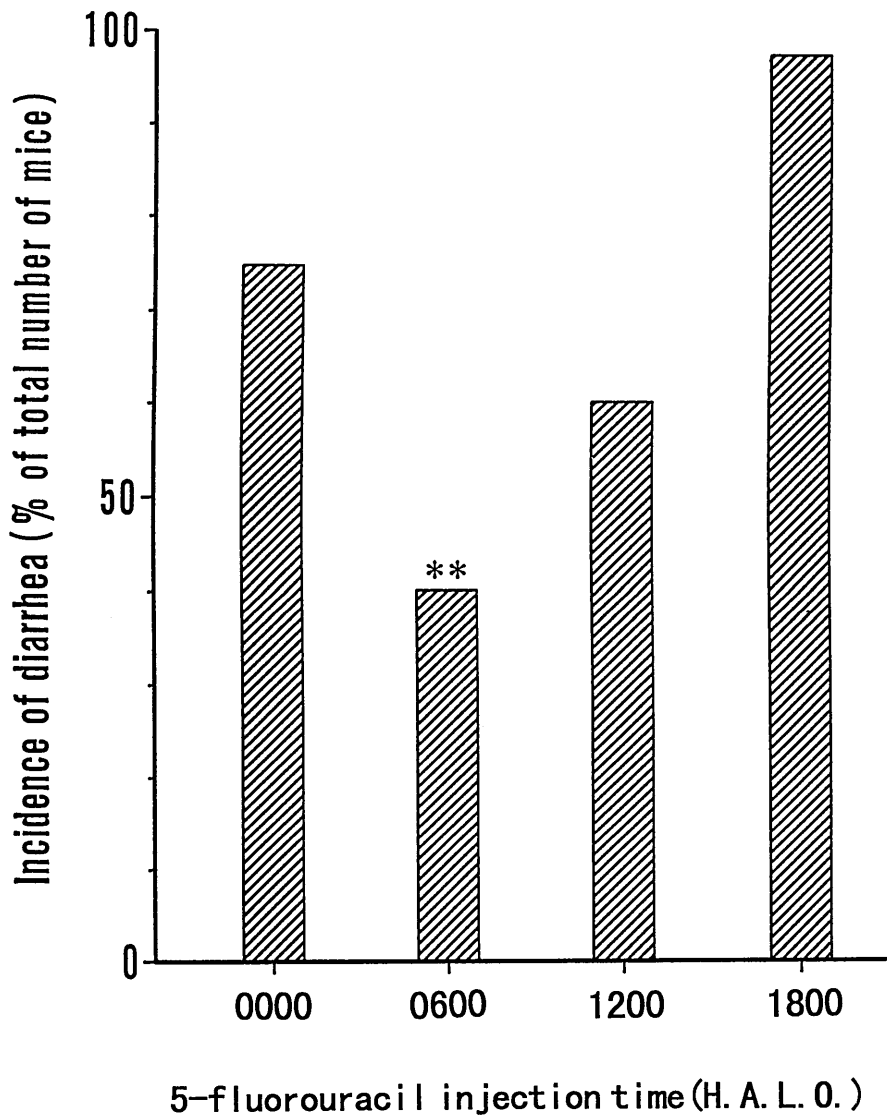
(*: $p < 0.05$, **: $p < 0.01$)

Figure 3. Leucocyte count in Balb/c mice treated with 5-FU at different HALOs. Values are means \pm SE of 5 mice which were used for repeated blood sampling throughout the course of the experiment. Arrows indicate days of 5-FU treatment.

Gastrointestinal toxicity

The incidence of watery diarrhea observed over 2 days immediately after single injections of 5-FU at various HALOs was investigated in Balb/c mice (Fig. 4). The least incidence of diarrhea occurred after injection at 0600 HALO. Administration of 5-FU at 1800 HALO was associated with a significantly high incidence of diarrhea.

Gastrointestinal toxicity of 5-FU was also reduced when it was administered during the resting phase.



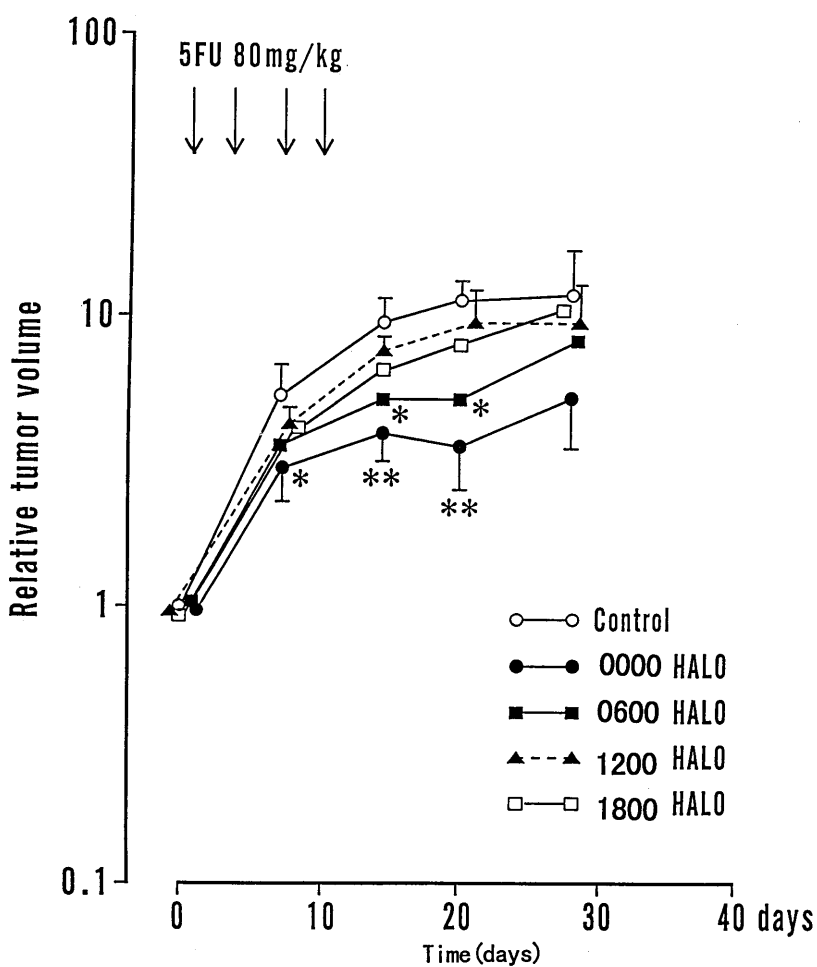
** Significance of difference from 1800 H.A.L.O. ($p < 0.01$)

Figure 4. Incidence of diarrhea in Balb/c mice observed over 2 days following single injections of 5-FU at different HALOs.

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Therapeutic efficacy against murine colon cancer

Antitumor activity of 5-FU administered at various HALOs against Colon 26 was investigated (Fig. 5). Treatment at 0000 and 0600 HALO of Balb/C mice bearing Colon 26 resulted in a significant tumor growth delay. Antitumor activity was the greatest when the mice were treated at 0000 HALO. The difference between tumor volumes at days 7, 14 and 21 was significant compared to the untreated control. No important weight loss (>5%) was observed after treatment in any of the groups. Median survival time was 22 days in the control group, 25 days in 1800 HALO group, 27 days in 1200 HALO group, 28 days in 0600 HALO group, and 31 days in 0000 HALO group. A significant increase in survival time ($p < 0.05$) was observed in the 0000 HALO group as compared to the control group.



*, ** Significance of difference from untreated control
(*: $p < 0.05$, **: $p < 0.01$)

Figure 5. Antitumor activity of 5-FU administered at different HALOs against Colon 26 mouse colon tumor xenograft. Values represent means \pm SE of 10 tumors in each group of mice.

2. Chronotherapy in a recurrent gastric cancer patient

No severe diarrhea, nausea or vomiting, experienced at the time of previous continuous injection of 5-FU, was observed in the patient treated by chronotherapy. Only mild transient anorexia of WHO Grade I was noticed. However, the patient tolerated the biweekly circadian rhythm-shaped 5-FU therapy for 23 courses. Oral food intake recovered after the 4th course and tumor regression was confirmed both by physical examinations and by the CT image at that time. The tumor stayed stable for nearly one year.

However, in August 1994, the tumor suddenly started to regrow rapidly. Any attempt to stop the tumor was in vain, and the patient died at the end of September 1994.

DISCUSSION

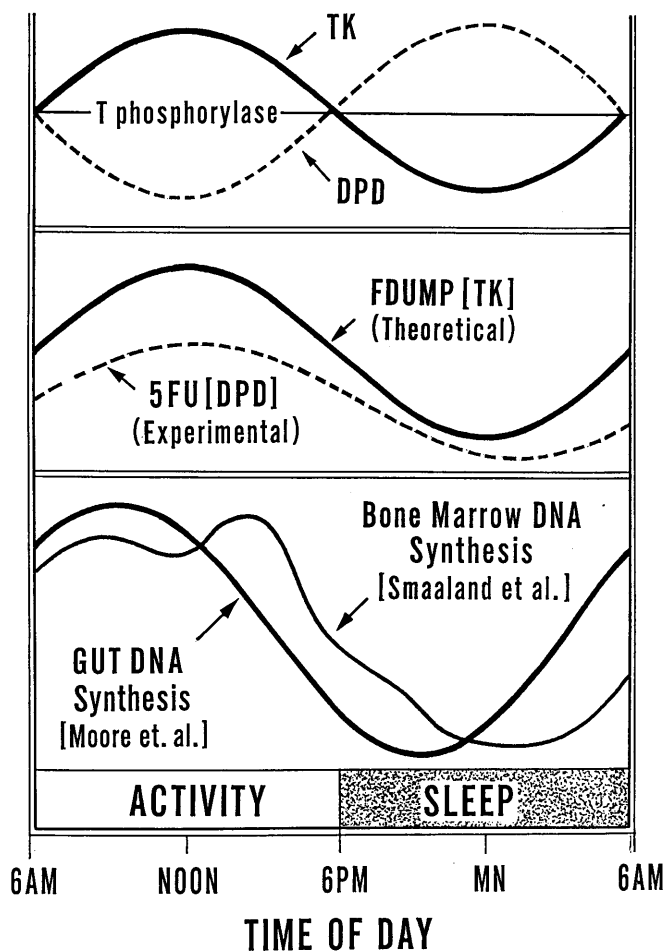
Since cancer chemotherapy has its limitations due to the toxic effects it produces, several attempts have been made to schedule a suitable time for drug administration to achieve an optimal drug tolerance and to obtain the maximum therapeutic effect.⁹⁾ To date, several animal experiments have shown that host tissues are affected by chemotherapeutic agents according to a diurnal time dependent pattern called circadian rhythm.¹⁰⁾ These studies have suggested that the pharmacologic and toxicologic effects of a drug may vary according to dosing time. These mechanisms involve 24-hour changes in drug disposition and/or in the susceptibility of target tissues.

5-FU has remained the reference drug against gastrointestinal cancers despite its rather modest antitumor activity. Circadian studies have been carried out with 5-FU in mice, and the LD₅₀ of 5-FU was demonstrated to be higher in the resting phase than in the active phase of the animal.¹¹⁾ Also, gastrointestinal toxicity of 5-FU in rats was reported to vary according to the time of day administered.¹²⁾

Results of our present study were in line with the above-described investigations. We injected 80 mg/kg of 5-FU which corresponds to approximately one fifth of the LD₅₀ dose when administered as a bolus injection. As for the toxicity of 5-FU in our experiments, hematologic and intestinal involvement varied markedly with the time of injection. The least toxicity estimated both by the leucocyte count and the incidence of diarrhea, was observed in Balb/c mice which had been injected with 5-FU at 0000 HALO and 0600 HALO. We also investigated the therapeutic efficacy of 5-FU in murine colon carcinoma autograft. In this experiment likewise, injection of 5-FU at 0000 HALO significantly reduced tumor growth compared to the untreated control, while no obvious difference was observed between the 5-FU treated group at 1800 HALO. Since the resting phase for rodents is in daylight and their active phase is during the night, the effect of 5-FU increased and its toxicity decreased when it was administered in the early to mid-resting phase of the animal.

The circadian rhythm dependent effect of 5-FU proven with experimental animals might also be exploitable in patients with gastrointestinal cancers. The human bone marrow cells and the human gut epithelial cells synthesize DNA nonrandomly within the day. The greatest DNA synthesis occurs in both tissues between the early morning hours and the late afternoon.^{13,14)} In addition to this coordinate DNA synthetic capacity, the biochemical pathways responsible for fluoropyrimidine catabolism and anabolism are also coordinated in circadian timing within both the liver and other cells of the body, including toxicity targets such as bone marrow and the gut.¹⁵⁾ During the past year, the circadian dynamics of many enzymatic pathways were investigated and circadian rhythmic patterns of the activities of 3 key enzymes in fluoropyrimidine metabolism was elucidated (Fig. 6). Dehydropyrimidine dehydrogenase (DPD) catabolizes 5-FU to noncytotoxic metabolites; thymidine phosphorylase (TP)

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(Hrusheski, Abstract Chemotherapy Foundation Symposium, 1994)

Figure 6. Circadian rhythmic patterns of the 3 key enzymes in fluoropyrimidine metabolism (top), pharmacokinetics of 5-FU and FDUMP levels of constant rate 5-FU infusion (middle), and circadian patterns of gut and bone marrow DNA synthesis in human beings (from Hrushesky W.J.M. abstract of Cancer Chemotherapy Foundation meeting, 1994)

converts 5-FU to its nucleotide fluorodeoxyuridine (FUDR); thymidine kinase (TK) converts FUDR to fluorodeoxyuridil monophosphate (FDUMP), which binds to thymidilate synthase (TS), blocking DNA synthesis by starving the cell for thymidine. DPD activity peaks about midnight; TP activity does not vary during the day; TK activity peaks around noon.

In the case of constant-rate 5-FU infusion, DPD removes 5-FU rhythmically during the day, making much more 5-FU available in the early daytime hours; TP converts 5-FU to FUDR at a constant rate but the higher substrate levels in the morning result in higher FUDR levels in the morning. TK activity is higher in the morning, converting more of the higher levels of FUDR to FDUMP at that time of day. According to the circadian dynamics of these enzymatic pathways, much more DNA synthesis is ongoing in the normal tissues at the time of the day associated with highest FDUMP levels. This coincidence of enzyme activities in gut and bone marrow DNA synthesis results in the experimentally observed high amplitude re-

producibile circadian rhythm in myeloid and gastrointestinal susceptibility to 5-FU.^{16,17)} Therefore, changing the circadian pattern of the 5-FU infusion so that most of the daily continuous infusion is given in the evening would change the resultant fluoropyrimidine pharmacokinetics and pharmacodynamics to one with a more favorable toxic therapeutic profile.

Our preliminary data of “within patient comparison” in the case of one gastric cancer patient, encourages us to plan a new clinical study of this chronotherapy against gastrointestinal cancers. Further investigations are essential to develop optimal temporal schedules relative to internal biological rhythms to steadily improve our ability to control cancer.

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