Chronotherapy with 5-Fluorouracil and Folinic Acid in Advanced Colorectal Carcinoma

Results of a Chronopharmacologic Phase I Trial

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Background. **Chronotherapy with antineoplastic drugs is a rather new strategy of reducing cytotoxic side effects. Because the circadian timing of 5-fluorouracil (5-FU) was reported to result in a higher efficacy and lower toxicity, the authors conducted a chronopharmacologic Phase I trial with 5-FU and folinic acid (FA).**

Methods. **Eight patients with advanced colorectal cancer received 5-FU (initial dose of 500 mg/m^2/day) and FA (20 mg/m^2/day) as a continuous intravenous infusion over 5 consecutive days. Using a portable, ambulatory drug delivery system, 75% of the daily dose of 5-FU and FA were given from 0h00–7h00, and the remaining 25% from 7h00–24h00. Treatment courses were repeated after 28 days. Dose escalations of 250 mg/m^2/day of 5-FU and 10 mg/m^2/day of FA per course were performed in the absence of any toxicity greater than WHO (World Health Organization) grade 2.**

Results. **Dose-limiting toxicity WHO grade 3 was observed at a dose of 750 mg/m^2/day of 5-FU and 30 mg/m^2/day of FA in five, and 1000 mg/m^2/day of 5-FU and 40 mg/m^2/day of FA in two patients, respectively. One patient tolerated 1000 mg/m^2/day of 5-FU and 40 mg/m^2/day of FA, but the treatment was stopped before further dose escalation because of rapid disease progression. Mucositis was the dose-limiting toxicity in seven patients and diarrhea in two. Disease stabilization occurred in three patients and disease progression in five. Compared with conventional Phase I/II trials using a 5-day infusion regimen, the maximal tolerated dose of 5-FU and FA was slightly higher but significantly lower than in a chronotherapeutic trial that used a different, sinusoidal mode of drug application.**

Conclusion. **Based on these results, the authors feel justified to caution that the circadian timing of 5-FU plus FA may not always allow the safe application of high dose levels. Future Phase I/II studies need to define whether specific drug delivery systems or schedules are necessary for chronotherapy with 5-FU and FA in patients with colorectal carcinoma. *Cancer* 1994; 73:2905–12.**

Key words: chronotherapy, chronopharmacology, circadian, 5-fluorouracil, folinic acid, colorectal carcinoma, toxicity, Phase I.

Colorectal carcinoma is one of the commonest neoplasias with an annual incidence of about 40 per 100,000 in Germany. Early stage patients may be cured by surgery, whereas all attempts to improve survival in advanced colorectal cancer have been frustrating. 5-fluorouracil (5-FU) is still the single most effective drug against colorectal cancer. Given as prolonged infusion, 5-FU induces remissions in 19–31% of the patients (mean, 25%); it is somewhat less potent given as conventional intravenous (IV) push (remission rate, 6–14 %; mean, 10%).[1] So far, combinations of 5-FU with other cytostatic drugs have failed to significantly improve these results or have resulted in an increased morbidity.[1]

Preclinical studies have shown that the antineoplastic activity of 5-FU can be potentiated by folinic acid (FA),[2–5] a strategy termed "biochemical modulation," because these agents enhance the intracellular biochemical effects and the antineoplastic activity of 5-FU in vitro.[5] Accordingly, early Phase I and II trials suggested that FA might increase the therapeutic activity of 5-FU in patients with advanced colorectal carcinoma.[6,7] Therefore, several Phase II trials with 5-FU plus FA have been performed that uniformly reported a higher rate of clinical remissions for 5-FU plus FA than for 5-FU alone.[8–14] In some studies, a small, but significant

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improvement of the overall survival was reported.[10,12,13] However, a metaanalysis of nine large randomized clinical trials revealed that the overall survival of patients with advanced colorectal cancer could not be prolonged by combining 5-FU with FA.[15] Nevertheless, the benefits of modulating the effects of 5-FU with FA in terms of tumor shrinkage seem to justify further research and clinical trials in that direction.[15]

The efficacy of 5-FU against colorectal cancer is characterized by a dose—response relationship.[16] Therefore, several strategies have been tested to overcome the dose-limiting toxicity of 5-FU or to increase the local concentration of the drug at the tumor site. These strategies include chemotherapy regimens with 5-FU "loading" doses, continuous IV infusion ranging from 24 hours to 14 or 70 consecutive days, and the local drug application by implantable delivery systems.[1,17-24]

The circadian timing of antineoplastic drugs has been introduced as a strategy of reducing cytotoxic side effects.[25-29] This strategy is based on the principle that all functions of the human organism are organized in a time-dependent way. For example, the numbers of circulating leukocytes, lymphocytes, erythrocytes, and platelets are not constant, but show considerable diurnal fluctuations.[30-32] Circadian rhythms have also been demonstrated for the mitotic activity of the bone marrow, the number of hematopoietic precursors in human blood and bone marrow, and the phagocytic activity of peripheral blood granulocytes.[31,33-35] It has been reported both in experimental animal systems and in clinical trials that the toxic side effects of 5-FU can be decreased by administration of the drug during the rest span (i.e., nighttime in human beings).[36] Preliminary results of a Phase I study in 34 patients with advanced colorectal cancer indicated that the circadian modulation of the drug application allowed the safe delivery of a 7.5 g/m^2/course of 5-FU (mean dose over 5 consecutive days) with surprisingly low toxicity.[37] In that study, the delivery rate of 5-FU varied sinusoidally with a peak flow rate at 4h00. A dose escalation of 1g/m^2/course was performed in each patient. Side effects such as stomatitis, diarrhea, leukopenia, and anemia WHO grade 2 were seen in less than 10%. Partial remission was achieved in 10 patients, and disease stabilization in 15 patients.[37] In another trial, the circadian modulation of the delivery of 5-FU, FA, and oxaliplatin in 93 patients with advanced colorectal cancer allowed a relatively high dose intensity with low toxicity, thereby achieving a response rate of 54% (partial or complete remissions).[38] Although the mechanisms underlying these circadian changes of 5-FU cytotoxicity are not fully understood, it has been suggested that the metabolic degradation of 5-FU by hepatic enzymes is regulated in a time-dependent way.[39] Accordingly, constant infusions with 5-FU do not result in constant 5-FU plasma concentrations but follow a circadian rhythm.[40]

The circadian timing of 5-FU in combination with FA has not been tested so far. Stimulated by the abovementioned observations of a higher efficacy and lower toxicity of 5-FU by an appropriate circadian drug timing, we, therefore, asked whether the circadian modulation of the combination of 5-FU plus FA would improve the tolerance and/or efficacy of this drug combination. For that purpose, we conducted a chronopharmacologic Phase I trial in eight patients with advanced colorectal cancer who received 75% of the daily dose of 5-FU and FA as a continuous IV infusion during night hours (from midnight to 7h00) and the remaining 25% during the rest of the day. The results presented suggest that the circadian timing of 5-FU and FA as performed in this study does not allow the application of higher dose levels as compared with constant delivery or a bolus regimen.

Patients and Methods

Patients

Between 1990 and 1992, eight patients (aged 41–71 years; three women, five men) treated at the outpatient clinic of the Department of Hematology and Oncology, Medical Clinic, Klinikum Innenstadt, University of Munich, Germany, were included in the study. Inclusion criteria were the existence of progressive, histologically proven, measurable recurrent, or metastatic colorectal carcinoma not amenable to surgical resection and causing severe symptoms, age under 75 years, a performance status (Karnofsky index) higher than 60%, and an adequate bone marrow function as defined by a leukocyte count of greater than or equal to 3×10^9/l and a platelet count of greater than or equal to 100×10^9/l. Patients with second malignancies were excluded. Informed written consent was obtained from each patient according to the Helsinki declaration of human rights.

The characteristics of these patients are shown in Table 1. All patients had undergone surgical resection of their primary lesion (four rectal carcinoma and four colon carcinoma). Primary staging at diagnosis was performed according to Dukes.[41] Six patients were assigned to Dukes stage C, one to stage A, and one to stage D. Six patients had received systemic chemotherapy previously, one patient local chemotherapy of liver metastases, and four patients pelvic radiation. The total dose of systemic 5-FU applied before the study ranged from 6 to 60 g (Table 1).

The indications for systemic chemotherapy were liver metastases in two patients, local relapses in two

Table 1. Study Patients

Patient no.	Sex	Age (yr)	Previous chemotherapy: Drugs	Previous chemotherapy: Total 5-FU dose (g)	Previous chemotherapy: Response	Primary lesion: Site	Primary lesion: Duke's stage	Site of relapse
1	M	41	5-FU 5-FU + IFNα	30	PD	Rectum	A	Liver
2	F	65	5-FU	19	PD	Sigmoid	C	Local
3	M	61	5-FU	14	PD	Sigmoid	D	Local, liver
4	F	63	5-FU	40	PD	Rectum	C	Local, liver
5	F	71	5-FU	9	PD	Colon	C	Liver
6	M	47	5-FU + IFNα	60	PD	Colon	C	Lung, liver
7	M	48	None	—	PD	Rectum	C	Liver, inguinal lymph nodes
8	M	52	None	—	PD	Rectum	C	Local

5-FU: 5-fluorouracil; PD: progressive disease; IFNα: interferon-α.

patients, the combination of local relapse and liver metastasis in another two patients, lung metastasis in one patient, and a combination of metastases in liver and inguinal lymph nodes in another patient. All eight patients were evaluable for response and toxicity, whereas the evaluation of survival was possible in seven patients only, because one patient was lost to follow-up.

Eligible patients underwent a complete workup including complete history and physical examination. Weight and height, complete blood cell count, serum bilirubin, creatinine, urea, electrolytes, calcium, total proteins, alkaline phosphatase, serum liver transaminases, gamma-glutamyltransferase, lactate dehydrogenase, carcinoembryonic antigen, and CA 19–9 were determined. Initial radiologic examination examinations included chest radiography, computed tomography of the abdomen, and abdominopelvic ultrasonography. Colonoscopy was done routinely before treatment onset. Clinical examination and all biologic determinations were done before each therapy course, and radiologic examinations after the second, fifth, and eighth treatment course.

Study Design and Chemotherapeutic Regimen

All patients received a central venous access port (Port-a-Cath, Pharmacia, Freiburg, Germany) to permit ambulatory IV infusion of cytostatic treatment. One therapy course consisted of five days. All treatment courses started with a loading dose of 200 mg of FA (Leucovorin, Lederle Arzneimittel, Wolfratshausen, Germany) given as IV bolus on Day 1, followed immediately by continuous, time-modified IV infusion of 5-FU (Fluroblastin, Farmitalia Carlo Erba, Frieburg, Germany) and FA delivered by a one-channel, programmable pump (Chronomat, Fresenius, Germany). For this treatment, 5-FU and FA were mixed in the reservoir of the pump for a maximum of 48 hours. Previous investigations had proven the stability of both drugs for more than 72 hours when combined in one solution (Dr R. Schenk, Lederle, Wolfratshausen, Germany, personal communication, 1989). The pump was programmed to infuse 75% of the total daily dose between 0h00 and 7h00, and the remaining 25% between 7h00 and 24h00 (Fig. 1). The first treatment course was always started at a

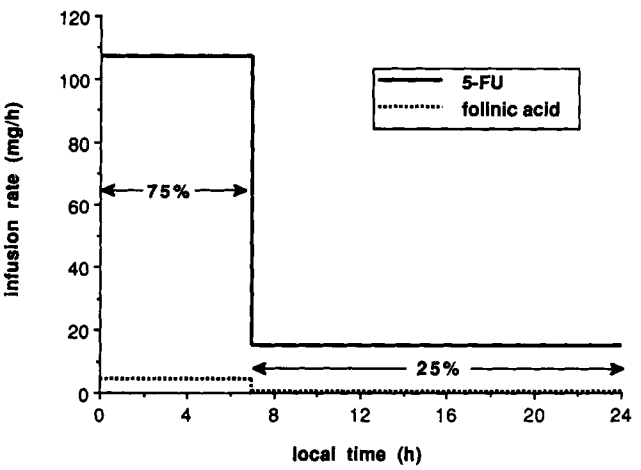

Figure 1. Drug delivery schedule as automatically administered for 5 consecutive days to outpatients with the use of a one-channel programmable ambulatory pump (Chronomat, Fresenius, Germany). In this example, 1000 mg of 5-fluorouracil (5-FU) and 40 mg of folinic acid (FA) were given every 24 hours. Seventy-five percent of this dose was applied between 0h00 and 7h00, and 25% between 7h00 and 24h00. Therefore, the pump was programmed to deliver 5-FU at infusion rates of 107 mg/hour between 0h00 and 7h00, and 15 mg/hour between 7h00 and 24h00. FA was delivered at 4.3 mg/hour between 0h00 and 7h00, and 0.6 mg/hour between 7h00 and 24h00. In addition to the daily dose of FA, a single-loading dose of 200 mg of FA was given as IV bolus on Day 1, before beginning the infusion (not shown).

dose of 500 mg/m^2/day of 5-FU for 5 days and 20 mg/m^2/day of FA for 5 days. The initial dose level was chosen under the influence of a report by Lévi and coworkers who did observe little WHO grade 3 or 4 toxicity at comparable doses.[38] In absence of any toxicity WHO grade 3 or 4, the dose of 5-FU was increased by 250 mg/m^2/day and the dose of FA by 10 mg/m^2/day in the following treatment course, respectively. The maximal dose planned in the study protocol was 1500 mg/m^2/day of 5-FU and 60 mg/m^2/day of FA (in the absence of any toxicity greater than WHO grade 2). In case of dose-limiting toxicity, the treatment with 5-FU and FA was reduced to the dose level of the previous treatment course and continued until disease progression occurred. The maximal tolerated dose was defined as a dose without side effects greater than WHO grade 2.

The following criteria were established for treatment discontinuation: tumor progression by more than 25% after two treatment courses, appearance of new metastatic lesions, toxicity WHO grade 4 even after dose reduction, manifestation of secondary malignancies, and refusal of the patient to continue the treatment.

The following endpoints were evaluated: maximal tolerated dose of 5-FU and FA (i.e., toxicity less than WHO grade 3); side effects of cytotoxic drugs according to WHO criteria; clinical response and duration of clinical remission using Union Internationale Contre le Cancer criteria; and survival. Regular follow-up examinations were performed in all patients after discontinuing study treatment.

Results

Circadian Timing of 5-FU and Folinic Acid Does Not Reduce the Cytotoxic Side Effects

The number of treatment courses applied, and the maximal tolerated dose of 5-FU and FA are shown in Table 2. One patient received nine, two patients six, three patients three, and two patients two complete treatment courses with 5-FU and FA. At the initial dose of 500 mg/m^2/day of 5-FU and 20 mg/m^2/day of FA, this treatment could be given to all patients without major toxicity. However, dose escalation had to be stopped due to dose-limiting toxicity in the majority of patients (seven out of eight; Table 2). Dose-limiting side effects (WHO grade 3) occurred at 750 mg/m^2/day of 5-FU and 30 mg/m^2/day of FA in five patients and at 1000 mg/m^2/day of 5-FU and 40 mg/m^2/day of FA in two patients. Only in one patient (Patient 2), 1000 mg/m^2/day of 5-FU and 40 mg/m^2/d of FA were tolerated with acceptable toxicity (burning-hand-and-feet syndrome, WHO grade 2), but further dose escalations were not possible because disease progression occurred at the site of relapse, which required a surgical resection of the sigmoidal tumor mass. Thus, the maximal tolerated dose of 5-FU and FA was 2500 mg/m^2/course and 300 mg/m^2/course, respectively, in five patients, 3750 mg/m^2/course and 350 mg/m^2/course, respectively, in two patients, and greater than or equal to 5000 mg/m^2/course and 400 mg/m^2/course, respectively, in one patient (median of 5-FU, 2500 mg/m^2/course and of FA 300 mg/m^2/course) (Table 2).

Mucositis was the most frequent and severe side effect. Seven out of eight patients had mucositis WHO grade 3 (Table 3), which was, therefore, the major dose-limiting factor according to the definitions in the study protocol. In one of these patients, the mucositis affected the colostomy. In two patients, severe diarrhea (WHO grade 3) was also observed. Nausea (WHO grade 2) occurred in one patient that could be controlled by the oral administration of a single dose of 10 mg of metoclopramide. Mild skin problems (WHO grade 1–2) occurred in three patients, two of which were burning-hand-and-feet syndromes. No severe side effects (greater than WHO grade 1) on the central or peripheral neural system or on the hematopoietic system were seen.

Because severe side effects (mucositis and diarrhea, WHO grade 3) were seen in the majority of patients at dose levels only slightly higher or similar as compared with conventional Phase I/II studies using 5-day infusions of 5-FU and FA at a constant rate,[42] this study was closed after including eight patients.

Response to Treatment and Survival

In five patients, the time-modified chemotherapy with 5-FU and FA proved to be inactive and had to be discontinued because of disease progression under treatment (Patients 2, 5, 6, 7, and 8). These patients were referred to a third-line chemotherapy (Patients 6, 8), or to a surgical resection of the local tumor mass at the relapse site (Patient 2), or were stopped from chemotherapeutic treatment because of proven inefficacy (Patients 5 and 7). In all these patients, treatment was discontinued very early, i.e., after two or three treatment courses (Patients 2, 5, 6, 7, and 8). With one exception (Patient 2), all these patients received 5-FU and FA at the maximal tolerated dose (Patients 5, 6, 7, and 8). In the three remaining patients, chemotherapy with 5-FU and FA at the maximal tolerated dose induced an intermittent disease stabilization that lasted 4 months (i.e., four treatment courses) in two patients (Patients 3 and 4) and 8 months (i.e., eight treatment courses) in one patient (Patient 1). No partial or complete remission was seen.

Table 2. Maximal Tolerated Dose of 5-FU and FA, Response to Treatment, and Survival

Patient no.	Treatment courses given	Highest dose tolerated (5-FU/m²/5 days)	Highest dose tolerated* (FA/m²/5 days)	Dose-limiting toxicity (WHO Grade 3)	Response and duration	Survival since beginning (mo)
1	9	2500 mg	300 mg	Mucositis	SD for 8 mo	12
2	3	5000 mg	400 mg	None†	PD	17+
3	6	3750 mg	350 mg	Mucositis	SD for 4 mo	9
4	6	3750 mg	350 mg	Muc. + diarrh.	SD for 4 mo	12
5	2	2500 mg	300 mg	Muc. + diarrh.	PD	12+
6	3	2500 mg	300 mg	Mucositis	PD	6
7	3	2500 mg	300 mg	Mucositis	PD	Not known
8	2	2500 mg	300mg	Mucositis	PD	7+

5-FU: 5-fluorouracil; FA: folinic acid; WHO: World Health Organization; muc. + diarrh.: mucositis and diarrhea; PD: progressive disease; SD: stable disease.

* Includes 200 mg FA bolus i.v. on day 1 (loading dose).

† In patient 2, dose escalation was stopped before achieving dose-limiting toxicity, because disease progression occurred under treatment.

Note: The maximal tolerated dose was defined as dose at which no side effects > WHO Grade 2 were seen (see Methods section).

Four patients died within 12 months after the treatment was stopped. One patient did not return to the hospital and, therefore, was lost to further follow-up examinations. Three patients were still alive 7–17 months after completing the study treatment.

Discussion

Chronotherapy with antineoplastic drugs is a relatively new strategy to reduce cytotoxic side effects and/or to increase antineoplastic activity of cytostatic drugs.[25–29] This approach is derived from experimental evidence that both toxic side effects and therapeutic efficacy of drugs may vary in a time-dependent way.[43] With regard to the use of cytostatic drugs and cytokines, it has been shown that parameters such as DNA synthesis in different tissues including intestinal mucosa, skin, bone marrow, spleen, testis and thymus,[33,34,44,45] function and number of different lymphocyte subsets or granulocytes in peripheral blood,[31,46] or numbers of granulocyte-macrophage colony-forming units derived from the bone marrow[35] show significant circadian variations in rodents and human beings. This has led to the development of time-modulated therapeutic regimens for cytostatic drugs. Circadian timing of the pyrimidine analog 5-FU with peak flow rates at 4h00 has resulted in particularly promising results allowing the application of high concentrations of the drug with surprisingly low toxicity.[37]

Table 3. Dose-limiting Toxicity

Toxicity	Toxicity grade (WHO) 1	2	3	4	Effect on dose
Mucositis	1	0	7‡	0	Limiting
Diarrhea	0	0	2	0	Limiting
Nausea	0	1	0	0	None
Skin	1*	2†	0	0	None
Blood	0	0	0	0	None
Neurologic	1	0	0	0	None

WHO: World Health Organization.

* Rash.

† Burning hand and feet syndrome.

‡ Mucositis affecting the colostoma in one patient.

Because the combination of 5-FU with FA has a higher antitumor efficacy in vitro and in vivo than 5-FU alone, we sought to apply the principle of chronotherapy to that combination. Eight patients with advanced colorectal carcinoma received a time-modulated chemotherapy with 5-FU and FA over 5 days in which 75% of the total daily dose was given between 0h00 and 7h00 (Fig. 1). Dose escalations in steps of 250 mg/m²/day per course of 5-FU and 10 mg/m²/day per course of FA were performed in the absence of any toxicity greater than WHO grade 2. In the majority of patients, dose escalation was stopped because of dose-limiting toxicities (WHO grade 3). These dose-limiting side effects were observed at 750 mg/m²/day of 5-FU and 30 mg/m²/day of FA in five patients, and at 1000 mg/m²/day of 5-FU and 40 mg/m²/day of FA in two patients (Table 2). One patient tolerated 1000 mg/m²/day of 5-FU and 40 mg/m²/day of FA, but disease progression under chemotherapy prevented further dose escalation. Mucositis was the dose-limiting toxicity in the majority of the patients (Table 3). Disease stabilization occurred in three patients and disease progression in the remaining five patients. Because it became evident that 5-FU and FA caused major side effects at dose levels of greater than or equal to 750 mg/m²/day (≥3750 mg/m²/course) and greater than or equal to 30 mg/m²/day

(≥350 mg/m^2/course, including a single 200-mg loading dose) respectively, without impressive beneficial effects on the clinical response, this chronopharmacologic Phase I trial was stopped after including eight patients.

Previous Phase I and II studies with the combination of 5-FU and FA given as continuous IV infusion showed that 5-FU could be given at doses up to 4800 mg/m^2/course in combination with different doses of FA (500–3000 mg/m^2/course) with acceptable toxicity.[42] However, considerable side effects were usually observed at 5-FU dose levels higher than 2500 mg/m^2/course. Most large randomized trials comparing 5-FU versus 5-FU plus FA used 5-day IV bolus schedules for both drugs.[15] In these Phase III trials, the total dose ranged from 1850 to 2000 mg/m^2/course of 5-FU (370–400 mg/m^2/day) and from 100 to 1000 mg/m^2/course of FA (20–200 mg/m^2/day).[15] In comparison, the maximal tolerated dose of 5-FU (median, 2500 mg/m^2/course) and FA (median, 300 mg/m^2/course) achieved with a circadian-modified treatment in this Phase I study seemed to be slightly higher. However, this modest benefit may be the consequence of the infusional application of 5-FU rather than of the chronotherapeutic treatment schedule.

In comparison with earlier chronopharmacologic studies that tried to reduce the cytotoxic side effects by circadian timing of 5-FU alone or 5-FU plus FA,[37,38] the benefit achieved with our 5-day continuous IV, chronopharmacologic treatment regimen was considerably smaller. This difference may be explained by schedule-dependent interactions of 5-FU and FA. This drug combination seems to be more toxic when given as a simultaneous, prolonged IV infusion. For example, a weekly IV bolus of FA does not reduce the tolerated dose of protracted infusional 5-FU[47] or of weekly 24-hour infusions of 5-FU (at 2600 mg/m^2/week).[48] However, when FA is given as continuous IV infusion and simultaneously with 5-FU, as little as 5 mg/m^2/day may significantly lower the tolerated dose of 5-FU.[49] Similarly, frequent oral administration of low-dose FA (q. 8 hours) seems to increase the toxicity of protracted 5-FU infusions.[50]

These observations may explain why our results appear somewhat contradictory to a recent report by Lèvi and coworkers who treated 93 patients with advanced colorectal carcinoma with a time-modulated chemotherapeutic regimen. Their treatment consisted of a 5-day continuous infusion of 5-FU (700 mg/m^2/day), FA (300 mg/m^2/day), and the platinum analogue, oxaliplatin (25 mg/m^2/day). Using a multichannel ambulatory programmable pump (Intelliject, Aguettant, Lyon, France), the infusion rate was modulated sinusoidally, with peak flow rates at 4h00 for 5-FU and FA and at 16h00 for oxaliplatin. Dose escalations were performed for 5-FU and oxaliplatin, if the toxicity was less than WHO grade 2. Despite adding oxaliplatin and giving more FA, only little toxicity was observed (≤10% WHO grade 2 toxicity for a total of 784 chemotherapy courses). Median doses were 3500 mg/m^2/course for 5-FU, 1500 mg/m^2/course for FA, and 125 mg/m^2/course for oxaliplatin.[38] The median dose of 5-FU and FA achieved in that study was higher than the maximal tolerated dose in this study. One cannot exclude the possibility that the application of 5-FU and FA in a sinus-wave model[37, 38] may be beneficial because this mode of application results in a more bolus-like application of 5-FU and FA (around the optimal peak at 4h00) than a 7-hour flat infusion from 0h00 to 7h00 as used in this study. This hypothesis is supported by the above-mentioned observations that continuous FA infusions seem to increase the toxicity of 5-FU and FA treatment schedules,[47–50] but it awaits verification by further studies with similar dosing schedules. Because the pump used by Lèvi and coworkers was not available to us at that time, we had to use a different device that allowed only a few, simple changes of the infusion rate over a 24-hour period (Fig. 1 and Methods). Therefore, we had to choose an approximate imitation rather than an exact reproduction of the delivery protocol published previously by the French group.[37,38]

Based on results reported here, we feel justified to caution that the circadian timing of 5-FU plus FA may not always allow the safe application of high doses in patients with advanced colorectal carcinoma. Future Phase I/II studies will have to define which drug delivery systems or schedules are required for an optimized chronotherapy with 5-FU and FA.

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