Pergamon

European Journal of Cancer Vol. 31A, Nos. 7/8, pp. 1264–1270, 1995

0959-8049/95 $9.50+0.00

0959-8049(95)00242-1

Chronomodulation of Chemotherapy Against Metastatic Colorectal Cancer

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Toxic effects of 5-fluorouracil (5-FU) and oxaliplatin (L-OHP), two active drugs against metastatic colorectal cancer, varied by 50% or more according to circadian dosing time in mice or rats. Adaptation of chemotherapy delivery to circadian rhythms (chronotherapy) was assessed in fully ambulatory outpatients, using multichannel programmable pumps. These devices allowed us to reliably test the clinical relevance of such a chronotherapy principle. First, single agent 5-day chronomodulated schedules were devised and assessed in Phase I and II trials with 5-fluorouracil (5-FU, peak delivery at 4:00 h) or oxaliplatin (L-OHP, peak at 16:00 h). Both schedules were then combined, folinic acid (FA) being added, synchronous with 5-FU infusion. This three-drug chronomodulated regimen (chrono-FFL) produced a 58% response rate (95% C.I.: 48–68%) in 93 patients with metastatic colorectal cancer, 46 of whom had previously received chemotherapy. In the first European randomised trial in 92 previously untreated patients, chronomodulated three-drug delivery achieved 53% response, as compared to 32% in those patients receiving flat infusion ($P = 0.038$). These respective figures were confirmed in a subsequent multicentre randomised trial involving 186 additional patients. Since the most active schedule was also the least toxic one by 2- to 10-fold, chrono-FFL was further intensified in three consecutive Phase II trials involving a total of 200 additional patients. Results suggest that both response rate and quality were further improved with such treatment intensification. Thus, chrono-FFL more than doubled the activity of chemotherapy against metastatic colorectal cancer in a multicentre European setting. As a result, it allowed us to attempt to surgically remove previously unresectable metastases in 25% of the 252 patients (55% previously treated) receiving chrono-FFL at our institution from 1987 to 1993. This overall strategy appears to exert a significant impact on long-term outcome.
Eur J Cancer, Vol. 31A, Nos 7/8, pp. 1264–1270, 1995

INTRODUCTION

5-fluorouracil (5-FU) has remained the main active drug against colorectal cancer. Its efficacy has been enhanced by modulating its cytotoxicity with folinic acid (FA) or by administering it as a continuous intravenous infusion. Both such regimens resulted in a 3- to 4-fold improvement of tumour response rate in patients with metastatic disease compared with standard 5-FU treatment. These figures, however, are still low (25%) and survival is affected only modestly [1–3].

Cisplatin, among other agents, is also able to modulate 5-FU cytotoxicity in experimental systems. It was shown to block methionine entry into tumour cells *in vitro* and increase both endogenous methionine synthesis and thymidylate synthetase activity. As a result, tumour cells became more susceptible to injury by 5-FU [4]. The severe clinical toxicity and/or the apparent lack of increased antitumour efficacy of chemotherapeutic regimens combining cisplatin and 5-FU with or without FA led most oncologists to avoid such combination chemotherapy in patients with gastrointestinal malignancies, despite initial encouraging results [5, 6].

Because oxaliplatin (1,2-diammino-cyclohexane (trans-1) oxalatoplatin (II); L-OHP), a new third generation platinum complex, is not associated with renal toxicity and has minimal haematological toxicity, this drug was considered to be a good candidate for further platinum modulation of 5-FU and FA cytotoxicity. The association of all three drugs was synergistic against murine L1210 leukaemia [7]. L-OHP also displayed activity against cisplatin-resistant or human colorectal cell lines [8].

Since single drug solutions of 5-FU, FA or L-OHP remained stable at ambient temperature and under normal lighting conditions for 5 days or more, this three-drug combination chemotherapy was further amenable to continuous ambulatory infusion.

A dose–response relationship characterised the antitumour efficacy of 5-FU against colorectal cancer [9]. Furthermore, recent experimental results suggested that high doses of cisplatin or carboplatin were necessary to achieve biochemical modulation of 5-FU cytotoxicity *in vivo* [10–14]. This may explain why the

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association of L-OHP with a standard 5-FU and FA schedule only achieved a 25% response rate in 25 patients with previously untreated metastatic colorectal cancer [7]. To achieve a high dose intensity, we aimed at reducing treatment toxicity as much as possible so that doses could be increased compared with standard chemotherapeutic schedules. For this purpose, we used a strategy based on circadian biological rhythms with an approximately 24 h period [14]. We hypothesised that high doses of all three drugs and proper circadian scheduling of drug delivery were needed to achieve clinical synergy. Figure 1 summarises our knowledge on the circadian system and the current chronopharmacological approach to chemotherapy optimisation.

We will briefly review the main results from this approach along the lines of experimental, clinical and technological prerequisites, and Phase I, II and III clinical trials in order to define the role of chronotherapy in the medicosurgical management of patients with metastatic colorectal cancer.

EXPERIMENTAL AND CLINICAL PREREQUISITES

Experiments in mice have indicated large, reproducible, and thus predictable changes in the toxic effects of 5-FU and L-OHP, like other platinum complexes, depending on the circadian time of drug administration (Figure 2) [15–21]. Mechanisms included 24 h changes in the activities of several enzymes involved in 5-FU catabolism or in the anabolism of its cytotoxic forms [20, 21]. These rhythms possibly accounted for circadian changes in plasma 5-FU levels, despite constant release in mice [22]. Similarly, the pharmacokinetics of L-OHP in blood or tissues also varied according to dosing time [18, 23]. Furthermore, the proliferative activity of human bone marrow, oral and intestinal mucosa exhibited a 50% increase between midnight and 4:00 h (trough) to 12:00 to 20:00 h (peak) (Figure 3) [24–26].

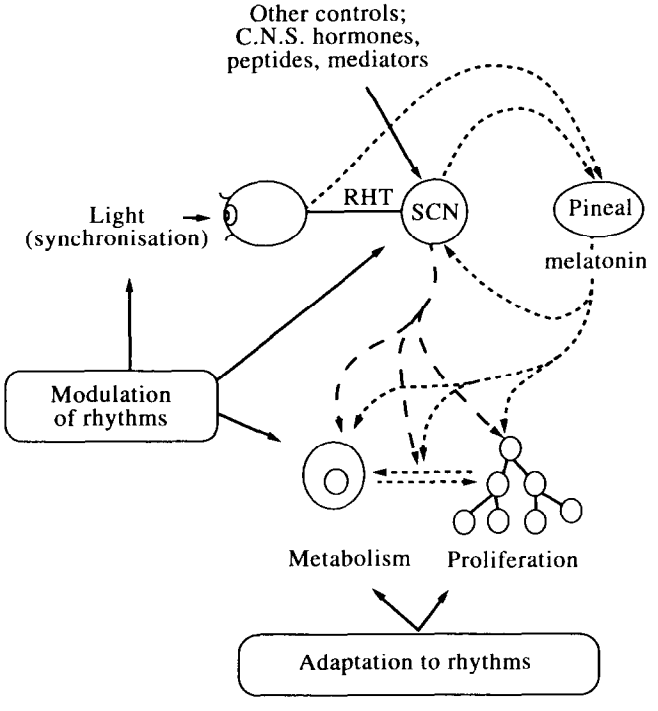

Figure 1. Schematic view of the circadian system. The suprachiasmatic nucleus (SCN) is a biological clock located at the floor of the hypothalamus. It is able to maintain a ~24 h cycle in its electrical activity *in vitro*. Its period (cycle duration) is calibrated by the alternation of light (directly) and darkness (through melatonin secretion by the pineal gland). The SCN controls and/or co-ordinates the circadian rhythms in the body. The main circadian rhythm is the rest–activity cycle. Cellular metabolism and proliferation also display rhythms in normal tissues, which may be keyed to the rest–activity cycle. Chronopharmacological intervention can consist of the adaptation of drug delivery to circadian rhythms and/or in the modulation of circadian mechanisms. The former approach was the only one used thus far for the chronomodulation of cancer chemotherapy. In this case, chemotherapy delivery was increased when normal tissue proliferation was at its low point along the 24 h time course.

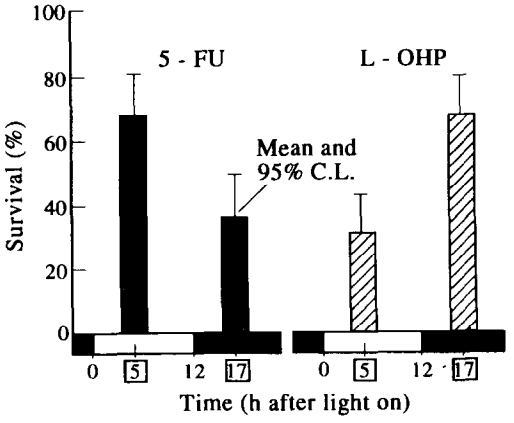

Figure 2. Relationship between circadian dosing time and lethal toxicity of 5-FU and L-OHP in mice. Time is expressed in "hours after light onset" (HALO) rather than in local clock hours, since light onset is the main signal which resets the circadian cycle in these nocturnally active animals. The circadian times when 5-FU and L-OHP were least toxic were located 12 h apart, being 5 HALO (±2 h) and 17 HALO (±2 h), respectively. Tolerability was doubled, by injecting either agent at its "best" rather than at its "worst" dosing time, differences being largely statistically significant. 5-FU (200–600 mg/kg i.p.) in a total of 50 healthy male CD_1 mice at each circadian time (after Burns and Beland, 1984 [15]); L-OHP (17 mg/kg i.v.) in a total of 60 healthy male $B_6D_2F_1$ mice at each circadian time (after Boughattas *et al.*, 1989 [23]).

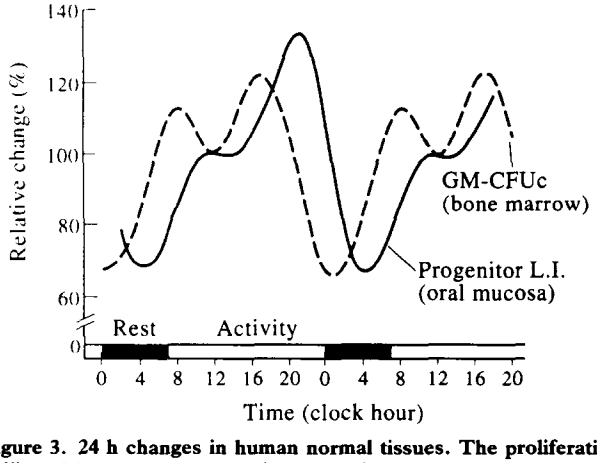

Figure 3. 24 h changes in human normal tissues. The proliferative ability of bone marrow granulomonocytic precursors was assessed from 4 hourly bone marrow aspirations for 24 h in 19 healthy volunteers (after Smaaland *et al.*, 1992 [24]); that of oral mucosa progenitors was documented with a similar sampling scheme in 11 healthy subjects (after Warnakulasuriya and MacDonald, 1993 [26]). A 4-h span, located between midnight and 4:00 h corresponds to a low point in the proliferative activity of both of these tissues in man. This also applies to the proliferative activity of rectal mucosa, although its peak occurred near 8:00 h in 24 healthy men (not shown; Buchi *et al.*, 1991 [25]).

24 h changes in bone marrow proliferation (GM-CFUc) and dihydropyrimidine dehydrogenase activity (DPD), among other relevant circadian rhythms, were approximately 12 h out of phase in nocturnally active rodents and in diurnally active humans [19, 20, 24, 27, 28]. The mechanisms responsible for the chronopharmacology of drugs may thus be linked to the sleep–wakefulness cycle across species. The stability of circadian metabolic rhythms was indicated by the demonstration of greater than 50% rhythmic changes in 5-FU plasma levels both in mice and in patients receiving constant drug infusion either alone, or co-administered with FA and L-OHP [22, 27, 29, 30].

TECHNOLOGY AND LOGISTICS

The availability of programmable-in-time ambulatory pumps was indispensable for testing the relevance of chronotherapy principles. Programmable-in-time single reservoir pump prototypes (AS 20C-Chronopump, Autosyringe-Baxter Travenol, Hooksett, U.S.A.; Zyklomat, Ferring, Germany) were first used for pilot or Phase I–II trials of single drug chronomodulated regimens. We mostly used a multichannel programmable pump (IntelliJect®, Aguettant, Lyon, France) since 1987.

This pump is equipped with four 30 ml disposable syringes. In case of chemical incompatibilities between drugs, one, two or three of the reservoirs (each one corresponding to a different drug solution) can be connected to a single central venous line by a manifold. The remaining channel(s) can be connected to a second separate central venous line. This setting was used in all our multidrug chronotherapy protocols, in particular, those involving 5-FU and FA, with or without oxaliplatin. In the course of our studies, we learned that contact exposure between 5-FU solution, which is basic and L-OHP solution resulted in a gradual chemical and biological inactivation of L-OHP. Its extent depended on both temperature and flow rate. This reaction was slower than that observed with other platinum complexes—cisplatin or carboplatin ([31], Hecquet B. and Fournier C., personal communication). Thus, two separate lines, 5-FU in one, FA and oxaliplatin in the other, were connected to a double lumen venous side port (Figure 4).

The plunger of each syringe of the pump was driven independently by a step motor. The rotation rate of the step motor varies in time according to the programme that has been written in a programmable read-only memory chip located in the pump; this chip was programmed by an International Business Machines personal computer using Intellimed software (Aguettant, Lyon, France). Two 9 V batteries permit 15 days of operation of the pump using either a flat or a chronomodulated programme, which was far beyond what was needed for one course. In the course of the development of chronotherapy, we organised specific training sessions for medical staff and nurses from each centre and from their related home care organisations to programme and manipulate the devices and to check their built-in safety systems. Training sessions were performed both in Villejuif and at each site. Each centre participating in multicentre trials was provided with a diskette containing several reference profiles of each schedule, differing by hour of treatment onset.

PHASE I AND II TRIALS OF CHRONOMODULATED INFUSIONS

On the basis of these prerequisites, circadian timing or modulation of drug delivery was hypothesised to permit a 20% or greater increase in drug doses. Phase I and II trials were conducted for 5-FU, 5-FU–FA and L-OHP (Table 1).

Drug delivery in the chronomodulated schedules varied sinusoidally along the 24 h time scale. Times of maximum flow rate were extrapolated from murine experiments. For these reasons, peak delivery was scheduled at 4:00 h for 5-FU (±FA) and at 16:00 h for L-OHP (Figure 4).

In brief, 5- or 14-day chronomodulated schedules proved less toxic and allowed us to deliver higher dose intensities by 20% or more as compared to flat infusions [32–35]. In Phase II trials, the antitumour activity of the circadian schedules were encouraging for 5-FU alone or 5-FU–FA [32, 33, 36]. A Phase II trial is ongoing with chronomodulated 5-FU–FA at the recommended doses of 900 mg/m²/day of 5-FU and 150 mg/m²/day of l-FA. Chronomodulated L-OHP displayed a 10% response rate in 29 previously treated patients with metastatic colorectal cancer [37]. A similar activity was achieved with a 2-h infusion of L-OHP [38]. However, the most exciting results came from the Phase II trial of the three-drug chronomodulated combination [39].

In a pilot randomised trial involving 9 patients, we first compared the tolerability of chronomodulated infusion to that

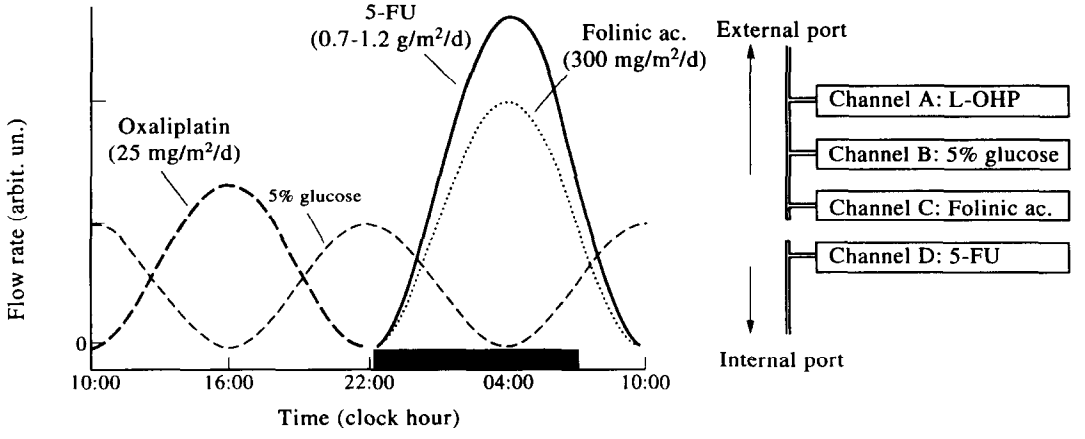

Figure 4. Ambulatory chronomodulated schedule of 5-fluorouracil (5-FU), folinic acid (FA) and oxaliplatin (L-OHP) (chrono-FFL - left) and reservoir connections in the multichannel pump (IntelliJect®) that were devised for avoiding chemical alterations between drugs (right). Both lines were connected to a double-lumen implanted venous side port. Respective daily doses are shown for each drug. In the 5 day every 21 day chrono-FFL regimen, recommended 5-FU dose was 700 mg/m²/day. In the 4 day every 14 day chrono-FFL regimen, intrapatient dose escalation could be performed up to 1200 mg/m²/day in some patients.

Table 1. Summary of Phase I or II clinical trials of 5 day chronomodulated infusions of 5-FU, 5-FU–FA, L-OHP and 5-FU–FA–L-OHP

Phase	Trial design	Drug	Dose and schedule	No. of patients (*n* pretreated)	Main conclusions	Reference
I–II	Intrapatient dose escalation if toxicity <gr.2	5-FU	800–1900 mg/m²/day (peak at 4:00 h) × 5 day every 21 days	35 (15)	Dose limiting toxicities: mucositis, diarrhoea, hand–foot syndrome Recommended dose: 1400 mg/m²/day × 5 day every 21 days Objective responses: 3/15 pretreated (20%) 7/20 naive (35%)	Lévi [32]
I	Inter- and intrapatient dose escalation if toxicity <gr.2	5-FU and l-FA	600–1100 mg/m²/day 150 mg/m²/day (peaks at 4:00 h) × 5 day every 21 days	34 (17)	Dose limiting toxicities: mucositis, diarrhoea Recommended dose of 5-FU: 900 mg/m²/day × 5 day every 21 days Objective responses: 3/17 pretreated (18%) 6/16 naive (38%)	Garufi [33]
I	Interpatient dose escalation	5-FU and dl-FA	200–300 mg/m²/day 5–20 mg/m²/day (peaks at 4:00 h) × 14 day every 28 days	14 (7) (3 with colorectal cancer)	Dose limiting toxicities: mucositis, hand–foot syndrome MTD: 250 mg/m²/day of 5-FU and 20 mg/m²/day of FA	Bjarnason [34]
I	Intrapatient dose escalation randomised	L-OHP	25–40 mg/m²/day chrono versus flat (peak at 16:00 h) × 5 day every 21 days	23 (16) (none with colorectal cancer)	Dose limiting toxicities: neutropenia, vomiting, peripheral sensitive neuropathy (cumulative) Recommended dose: chronomodulated: 35 mg/m²/day × 5 day flat: 25 mg/m²/day × 5 day	Caussanel [35]
II	Multicentre (France, 2; Belgium, 1; Italy, 1)	5-FU and dl-FA	600–800 mg/m²/day 300 mg/m²/day (peaks at 4:00 h) × 5 day every 21 days	36 (17)	No toxicity >gr.2 Objective responses: 1/17 pretreated 6/19 naive (35%)	Chollet [36]
II	Multicentre (France, 3; Belgium 1; Italy, 1)	L-OHP	30–40 mg/m²/day (peak at 16:00 h) × 5 day every 21 days	29 (26)	Median dose: 35 mg/m²/day × 5 days Objective responses: 3 (10%)	Lévi [37]
II	Single institution	5-FU dl-FA L-OHP	700 mg/m²/day 300 mg/m²/day (peaks at 4:00 h) 25 mg/m²/day (peak at 16:00 h) × 5 day every 21 days	93 (46)	Dose limiting toxicities: diarrhoea, vomiting, peripheral sensitive neuropathy (cumulative) Objective responses: 54 (58%)	Lévi [39]

All studies were performed in patients with metastatic colorectal cancer, except where indicated. MTD, maximum tolerated dose.

of flat delivery of 5-FU (600 mg/m²/day), FA (300 mg/m²/day) and L-OHP (20 mg/m²/day). Severe oral mucositis (WHO grade 3 or 4) was encountered in 3/4 patients receiving constant infusion, as compared with 0/5 patients treated with the chronomodulated schedule. The relationship between plasma 5-FU time course and mucosal toxicity is illustrated in Figure 5 [30].

The latter regimen involved the daily administration of 5-FU and FA (with maximal delivery at 4:00 h at night) and of L-OHP (with maximal delivery at 16:00 h during the day) (chrono-FFL) for 5 consecutive days and was tested in a Phase II trial. Courses were repeated every 21 days. A 58% objective response rate (95% CI 48–68%) was obtained in 93 patients with metastatic colorectal cancer; 46 of these patients had received previous chemotherapy. Moreover, all treatments were administered on

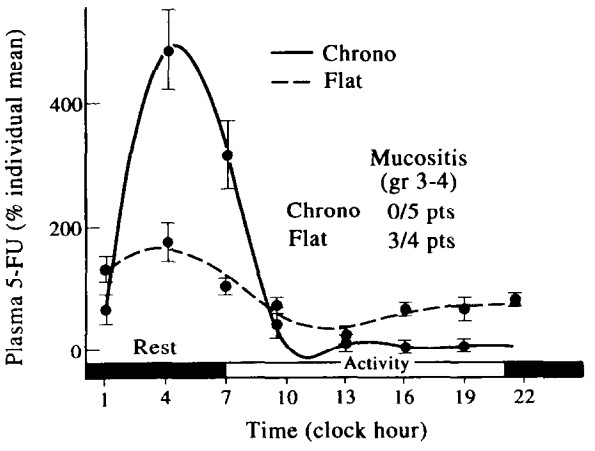

Figure 5. Imposed amplification of circadian rhythmicity in 5-FU plasma level, through chronomodulation of drug delivery, abolished its oral mucosa severe toxicity. The figure shows mean relative variation of plasma 5-FU along the 24 h time scale during flat or chronomodulated three-drug 5-day infusion in 9 patients with metastatic colorectal cancer (5-FU: 600 mg/m²/day; folinic acid: 300 mg/m²/day; oxaliplatin: 25 mg/m²/day). In the chronomodulated schedule, peak delivery was at 4:00 h for 5-FU and FA and at 16:00 h for L-OHP. 24 h mean concentration of plasma 5-FU (here designated as "100%") was 470 μmol/l. An apparent relationship exists between extent of 24 h change in 5-fluorouracil (5-FU) plasma concentration and toxicity to the oral mucosa (after Metzger *et al.*, 1994 [30]).

an outpatient basis and less than 10% of the 784 courses given were associated with severe toxicity [39]. These results compared favourably with those achieved by standard 5-FU–FA combination chemotherapy, the reference treatment for this disease. According to a meta-analysis, 5-FU–FA yielded 23% objective responses, and an approximate 12-month median survival in previously untreated patients [1]. These figures appeared to be independent of the 5-FU–FA ratio and scheduling, among the several regimens in current clinical use [1]. Similar results stemmed from a 7-arm multicentre trial performed by the Intergroup in the U.S.A. [3]. Two factors probably accounted for the high antitumour efficacy of chrono-FFL: a new active drug, L-OHP, and chronomodulation, which allowed safe delivery of high drug doses.

PHASE III TRIAL OF THREE-DRUG CHRONOMODULATED INFUSION

A randomised, multi-institutional trial was then undertaken in patients with previously untreated metastatic colorectal cancer in order to assess the role of chronomodulation. From May 1990 to May 1991, 92 consecutive patients with metastatic colorectal cancer were registered in the first stage of this Phase III evaluation. Seven centres participated in this trial: four in France (Hôpital Paul Brousse, Villejuif; Clinique Hartmann, Neuilly; Hôpital Bellevue, Saint-Etienne and Centre Jean Perrin, Clermont-Ferrand), two in Italy (Ospedale San Luigi Gonzaga, Torino and Cattedra di Oncologia Medica, Universita G. D'annunzio, Chieti) and one in Belgium (Centre Hospitalier Saint-Joseph-Espérance, Liège) [40].

Treatment consisted of a 5-day course of continuous intravenous infusion of 5-FU (600 mg/m²/day), FA (300 mg/m²/day) and L-OHP (20 mg/m²/day), which was repeated every 21 days (after a 16 day interval). In the absence of toxicity greater than Grade 1, the daily doses of 5-FU and that of L-OHP were increased up to 700 mg/m² and 25 mg/m², respectively.

Two schedules of drug delivery were compared: 5-FU, FA and L-OHP automatically delivered to outpatients either at a flat rate (schedule A) or a chronomodulated one (schedule B). Both complex delivery schedules of these three drugs were administered using a programmable in-time multichannel ambulatory pump in an outpatient setting (IntelliJect, Aguettant, Lyon, France).

Of the 92 registered patients, 17 patients (18%) had received previous adjuvant chemotherapy and/or radiotherapy, 9 patients (10%) had a performance status of 2, 43 patients (47%) had two or more metastatic sites and 80 patients (87%) had liver involvement. Per course, stomatitis was by far the most frequent acute dose-limiting toxicity of this protocol. Grade 3 or 4 stomatitis occurred 8.7 times as often on schedule A as compared to schedule B ($\chi^2 = 82$; $P < 0.0001$). The incidence of severe toxic symptoms (grade 3 or 4) was less than 5% for diarrhoea, for nausea or vomiting or for skin toxicity. No haematological suppression greater than grade 2 was observed.

The proportion of patients experiencing grade 3 or 4 toxicity was 5-fold higher in schedule A than in schedule B for stomatitis (89% versus 18%; $P < 0.0001$) and 2.5-fold higher for hand–foot syndrome (11% versus 4%; N.S.). It was similar for diarrhoea (24% versus 20%). Schedule B, however, produced grade 3 or 4 nausea or vomiting in 2.5 times as many patients than schedule A (24% versus 9%; $P = 0.05$), and grade 2 peripheral sensitive neuropathy in 4 times as many patients than schedule A (27% versus 7%; $P = 0.02$). The median dose of 5-FU was 700 mg/m²/day on schedule B and 500 mg/m²/day on schedule A. The median dose intensity of 5-FU was approximately 22% higher in schedule B than in schedule A ($P < 0.0001$).

Of the 47 patients on schedule A, 15 had an objective response (two being complete). Of the 45 patients on schedule B, 24 had an objective response (three being complete). Thus response rates of all registered patients were 32% (95% confidence limits: 18%; 46%) for schedule A and 53% (38%; 68%) for schedule B ($\chi^2 = 4.3$; $P = 0.038$). All maximal responses were achieved over the 9 initial courses. Median progression-free survival of all patients was 8 months on schedule A and 11 months on schedule B (N.S.). The median survival of all patients was 14.9 months (95% confidence limits: 12.1, 17.8) on schedule A and 19 months (14.8, 23.2) on schedule B (P from log-rank = 0.03). Logistic regression for response and Cox analysis for survival isolated the same two significant factors associated with improved outcome: treatment schedule and number of metastatic sites.

A risk of partial chemical inactivation of L-OHP with the basic pH of 5-FU in the flat schedule was, however, documented and prompted an early termination of this trial. A new multicentre trial was undertaken in which any risk of chemical drug interaction was avoided (Figure 4). Accrual of 186 patients to this trial was completed in February 1993. Results support the present main findings and conclusions ([41]; Lévi *et al.*, in preparation).

Both of these trials, involving a total of 278 patients with metastatic colorectal cancer, have confirmed that 5-day chronomodulated infusion of 5-FU, FA and L-OHP produced approximately twice as many objective responses as current chemotherapeutic schedules, or flat three-drug infusion. Furthermore, in this European multicentre randomised setting, the most active chronomodulated schedule was also the least toxic one [40, 41].

Because of the high activity and good tolerability of this regimen, two areas of investigation have been actively explored: (1) Can three-drug chronotherapy be further intensified and does this further improve efficacy? (2) Can patients, with previously unresectable metastases, undergo surgical resection of

their metastases after effective chronotherapy, and does this combined approach impact on survival?

Early results and retrospective analysis suggest positive answers to these questions [42, 43]. We performed a preliminary analysis of the outcome of 252 patients with previously unresectable metastatic colorectal cancer receiving chrono-FFL at our institute between 1987 and 1993. Standard chemotherapy had been administered to 55% of these patients before they received chrono-FFL. An attempt to resect metastases after effective chrono-FFL was performed in 25% of patients. An impact of this combined strategy on long term outcome was suggested since median projected survival of the whole population was 18 months. This figure exceeds by more than 6 months that usually obtained in a similar patient population.

Ongoing or future developments of chronotherapy of colorectal cancer involve (1) examination of the respective roles of chronomodulation versus circadian peak time of drug delivery; (2) further assessment of the possible role of L-OHP schedule; (3) assessment of the relevance of a "normal" circadian system for a favourable outcome after chronotherapy; and (4) further evaluation of the relevance of this approach for improving survival, both in metastatic disease and in adjuvant situations.

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Acknowledgements—The authors are indebted to S. Brienza, P. Deprés-Brummer, G. Gruia, M. Itzhaki, F. Kunstlinger, C. Massari, C. Jasmin and H. Bismuth (Hôpital P. Brousse, Villejuif, France), F. Cvitkovic (Centre R. Huguenin, St-Cloud, France), P. Chollet (Centre Jean Perrin, Clermont-Ferrand, France), R. Faggiuolo and L. Dogliotti (Ospedale San Luigi, Orbassano, Torino, Italy), C. Garufi and L. Terzoli (Istituto Regina Elena, Rome, Italy), C. Focan (Hôpital St-Joseph-Espérance, Liège, Belgium), A. Le Rol (Hôpital Notre Dame du Perpétuel Secours, Eaubonne, France), B. Perpoint (Hôpital Bellevue, St-Etienne, France), J.M. Vannetzel (Clinique Hartmann, Neuilly, France) who actively contributed to the design, implementation, analysis and interpretation of most of the clinical studies herein reported. We thank M. Lévi for excellent editorial assistance. This work was supported in part by the Association pour la Recherche sur le Temps Biologique et la Chronothérapie, Hôpital Paul Brousse, 94800 Villejuif, France.