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ORIGINAL PAPER

Chronotherapeutics: the relevance of timing in cancer therapy

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Abstract
Background Cell physiology is regulated along the 24-h time scale by a circadian timing system composed of molecular clocks within each cell and a central coordination system in the brain. The mammalian molecular clock is made of interconnected molecular loops involving at least 12 circadian genes. The cellular clocks are coordinated by the suprachiasmatic nuclei, a hypothalamic pacemaker which also helps the organism adjust to environmental cycles. The rest–activity rhythm is a reliable marker of the circadian system function in both rodents and man. This circadian organization is responsible for predictable changes in the tolerability and efficacy of anticancer agents, and possibly also in tumor promotion or growth.
Methods Expected least toxic times of chemotherapy were extrapolated from experimental models to human subjects with reference to the rest–activity cycle. The clinical relevance of the chronotherapy principle, i.e. treatment administration as a function of rhythms, has been demonstrated in randomized multicenter trials.
Results Chronotherapeutic schedules have been used to safely document the activity of the association of oxaliplatin, 5-FU and leucovorin against metastatic colorectal cancer and to set up a new medicosurgical management of this disease which achieved unprecedented long term survival.
Conclusion The chronotherapy concept offers further promises for improving current cancer treatment options as well as for optimizing the development of new anticancer or supportive agents

Keywords Circadian · Rhythms · Chronopharmacology · Chronotherapy · Quality of life · Survival

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The circadian timing system

The biological functions of most living organisms are organized along an approximate 24-h time cycle or circadian rhythm. The endogenicity of the circadian rhythms has been demonstrated in microorganisms, in plants and in all kinds of animal species including man. These endogenous rhythms govern daily events like sleep, activity, hormonal secretion, cellular proliferation and metabolism [1].

Circadian rhythms are genetically fixed. For instance, mutations of the circadian genes *per* in *Drosophila*, in mouse or in humans result in severe disturbances of the rest–activity circadian cycle, which translate into modifications of the period, amplitude or acrophase pending upon experimental conditions [1–5].

The light perceived by the visual pathways and the secretion of melatonin, a hormone released by the pineal gland during darkness, help to reset the internal clock that regulates the timing of different body functions. A hypothalamic structure, the suprachiasmatic nuclei (SCN), plays a key role in the coordination of circadian rhythms (Figure 1) [1, 6].

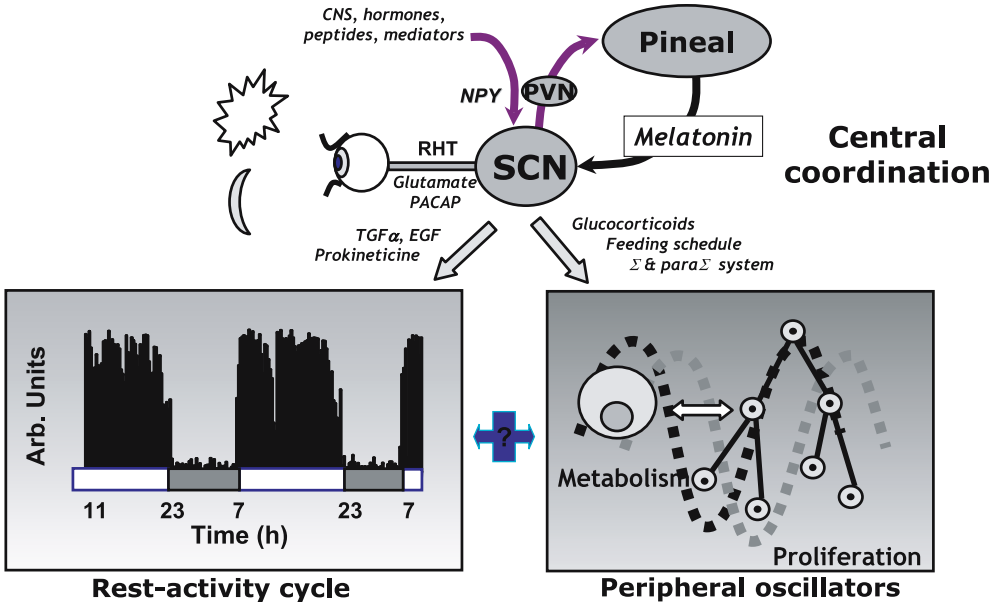

Fig. 1 Schematic view of the circadian system. The suprachiasmatic nuclei (SCN) is a biological clock located at the floor of the hypothalamus. Its period (cycle duration) is calibrated by the alternation of light (L) directly and darkness (D) through melatonin secretion by the pineal gland. The SCN controls or coordinates the circadian rhythms in the body. The main overt 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. Several chemical and neuro–anatomic pathways that convey the synchronization information from the SCN to the periphery have been identified (Abbreviations: PVN, paraventricular nucleus; IGL, intergeniculate leaflet; RHT, retinohypothalamic tract; NPY, neuropeptide Y; 5-HT, 5-hydroxytryptamine, or serotonin; PACAP, pituitary adenylate cyclase peptide; TGFα, transforming growth factor α; EGF, epidermal growth factor; Σ, sympathetic)

This temporal organization makes it possible to predict the rhythmic aspects of cellular metabolism and proliferation. Synchronized individuals display circadian rhythms with predictable times of peak and trough. These rhythms may influence the pharmacology and the tolerability of anticancer drugs and/or their antitumor efficacy. Conversely, a lack of synchronization, or an alteration of circadian clock function makes rhythm peaks and troughs unpredictable, and may require specific therapeutic measures to restore normal circadian function.

The rest–activity circadian rhythm, a window on the circadian timing system

Locomotor activity reliably reflects circadian clock function in several animal species. Its endogenicity was demonstrated by its persistence in constant environmental conditions in flies, rodents and humans. This rhythm is controlled by the molecular clock genes in mammals. Direct pharmacologic actions targeted at the SCN in rodents translate into a phase shift of the rest–activity rhythm of the animals. In rodents, the physical destruction of the SCN results in a complete suppression of the rest–activity rhythm, while the transplantation of SCN restores circadian rhythmicity. These experimental facts clearly demonstrate the dependency of this rhythm upon SCN function [6].

In man, the rest–activity rhythm is considered and used as a marker of the circadian timing system in isolation studies, in phase shift studies, and in psychiatry [7]. The rest–activity rhythm can be easily measured using a small-size instrument worn on the wrist, and called an actigraph. As wrist monitoring of activity is totally non-invasive, there is no restriction to its use in cancer patients, even in an ambulatory setting. The easy recording of rest–activity has further supported its use as a reference rhythm for the circadian timing of medications and for the evaluation of circadian clock function.

The molecular circadian clock control of cell cycle, apoptosis and repair

The complex machinery of the molecular clock was recently shown to exert a negative control on the transcriptional activity of some key genes involved into cell cycle regulation, thereby suggesting that the circadian clock could regulate cell proliferation. Circadian rhythms have been extensively reported for cell cycle phase distribution in healthy or malignant mammalian tissues [8–11]. Two

recent studies have further identified *c-myc*, *p53* and *wee1* as being clock-controlled genes [12, 13]. *C-myc* and *wee1* respectively promote cell cycle progression from G1 to S and from G2 to M. Furthermore, *c-myc* can also exert proapoptotic effects through p53-dependent or independent pathways [14–16].

Many other cell cycle-related genes display 24-h rhythms in mRNA and/or protein expression in healthy tissues from rodents and/or humans, also equipped with molecular clock components. This is the case for the expression pattern of genes which control cell cycle checkpoints, such as *cdk2, cyclins A, B1, D, E* or *mdm2*, or which regulate apoptosis, such as *gadd45α, bcl2* and *bax* [9, 12, 13, 17]. Finally, clock genes *cry* are members of the photolyases family, which is involved into the repair of UV DNA damage, indicating a close link between both systems [18].

Experimental chronotherapeutics of cancer

Circadian dosing time influences the extent of toxicity of ~30 anticancer drugs, including cytostatics and cytokines, in mice or rats (Figure 2). For all these drugs, survival rate vary by 50% or more according to circadian dosing time of a potentially lethal dose. Such large difference is observed irrespective of injection route – intravenous oral or intraperitoneal – or number of injections – single or repeated [19]. For instance, platinum complex analogs – cisplatin, carboplatin, and oxaliplatin (l-OHP) – are all best tolerated near the middle of the nocturnal activity span of mice or rats, although the respective target tissues for toxicity of these compounds differ: cisplatin is chiefly toxic to both kidney and bone marrow, carboplatin to bone marrow and colon mucosa and l-OHP to jejunal mucosa and bone marrow [20–21]. Yet, 5-fluorouracil (5-FU) and floxuridine, two antimetabolite drugs which can convert into one another are best tolerated at opposite circadian dosing times [22].

Quite strikingly, the administration of a drug at a circadian time when it is best tolerated has usually achieved best antitumor activity. This was found for antimetabolites, such as arabinofuranosylcytosine, 5-FU or FUDR, for intercalating agents such as doxorubicin, for alkylating drugs such as melphalan, cisplatin or oxaliplatin, as well as for antimitotic drugs such as docetaxel or vinorelbine [11, 19, 22–25]. The reproducible coincidence between times of highest efficacy and least toxicity for most anticancer agents suggest that common mechanisms are involved.

Clinical chronopharmacology of anticancer drugs

Short intravenous infusions of cisplatin, carboplatin, doxorubicin, 5-FU or methotrexate, or oral intake of busulfan or 6-mercaptopurine but not methotrexate were associated with modifications of plasma and/or urinary pharmacokinetics according to dosing time. Continuous intravenous infusion of 5-FU, doxorubicin or vindesine also resulted in circadian changes in plasma drug levels, despite a flat infusion rate [22, 26, 27]. Interpatient vari-

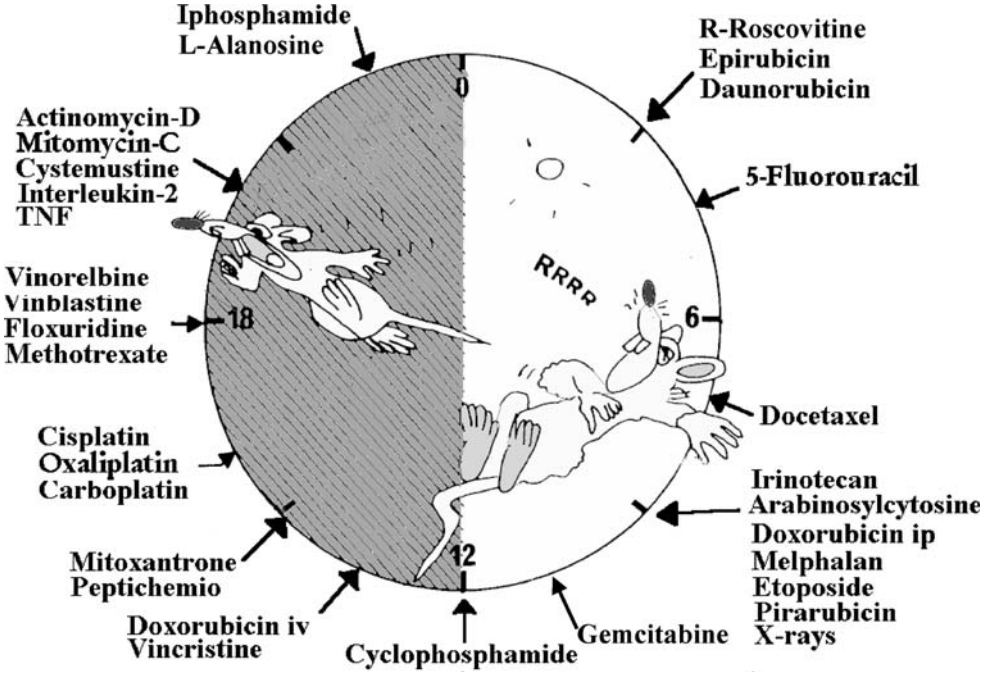

Fig. 2 Circadian rhythms in anticancer drug tolerability in laboratory mice or rats. The least toxic dosing time is indicated for each cytostatic or immunologic agent as a function of the rest–activity cycle

ability in circadian time-dependent pharmacokinetics were also observed [19, 22].

The activity of dihydropyrimidine dehydrogenase (DPD), the initial enzyme for the catabolism of 5-FU, was studied around the clock in peripheral blood mononuclear cells of patients suffering from a gastrointestinal tumor, with higher DPD activity at early night, near midnight or 4:00 h [22, 28].

Cell proliferation is also likely to be one mechanism involved, as cells which are engaged into DNA synthesis usually display an increased susceptibility to antimetabolites or intercalating agents. The proportion of bone marrow, gut, skin and oral mucosa cells engaged in the S-phase of the cell division cycle vary by 50% or more along the 24-h time scale in healthy human subjects. For all these tissues, lower mean values occur between midnight and 4:00 during the night, and higher mean values between 08:00 and 20:00 [29–31].

These mechanisms of anticancer drug chronopharmacology display a similar phase relationship with the rest–activity cycle in mice and in humans, despite the fact that the former are active at night and the latter during daytime. Thus, DPD activity peaks during early light in mice or rats and at early night in humans. Similarly, the proportion of S-phase bone marrow cells peaks in the second half of darkness in mice [32] and near 16:00 in humans [29]. In addition, constant rate infusion of 5-FU results in a circadian rhythm in plasma level both in mice and in cancer patients. Peak concentration in 5-FU occurs in the early rest span in both species, if the drug is infused continuously over 1 week or less [22].

Chronotherapeutics: principles and methods

The apparent coupling between the circadian rest–activity cycle and several chronopharmacology mechanisms across species has been the basis for the chronotherapy schedules which have been given to cancer patients. As a working hypothesis, expected times of least toxicity in human patients were extrapolated from those experimentally demonstrated in mice or rats, by referring them to the respective rest–activity cycle of each species, e.g. with ~12 h time lag. For instance, least toxicity of 5-FU occurred near 5 HALO in mice and was predicted to correspond to 04:00 h in human subjects, resting from 23:00 h to 07:00 h [22].

Several strategies have been devised to take advantage of the adjustment of chemotherapy delivery to the circadian timing system (Figure 3).

Multichannel programmable in time pumps have allowed a test of the clinical relevance of the chronotherapy principle in fully ambulatory patients. For this purpose, the same

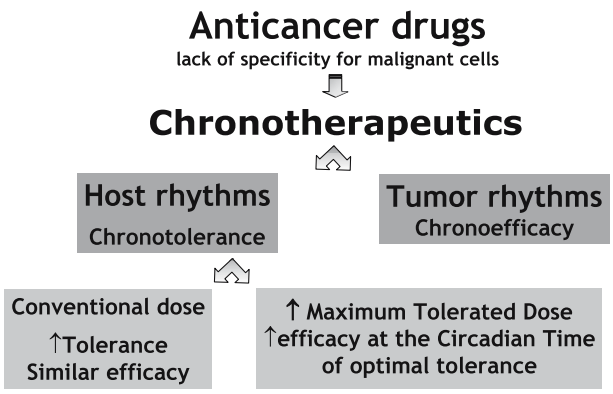

Fig. 3 Possible strategies for cancer chronotherapeutics. The circadian rhythm in anticancer treatment tolerability results in a predictable variation in maximum-tolerated dose (MTD), an endpoint that is determined usually in clinical Phase I trials. If a standard dose is given in the 'best' circadian schedule, tolerability and quality of life are expected to improve, while efficacy remains similar to that of conventional treatment modalities. Conversely, if the dose delivered in the 'best' schedule is increased to MTD in this very schedule, efficacy should be enhanced, while tolerability remains similar to that of conventional treatment modalities

chronomodulated schedule is applied to all cancer patients registered in each protocol. Today, the sinusoïdal delivery of up to 4 anticancer drugs can be routinely performed in the patients' home or during their usual activities.

Clinical trials of chronotherapeutics

Early clinical trials had suggested significant clinical benefits from specific circadian timing of chemotherapy (combined vinblastine, cyclophosphamide and methotrexate or 5-FU) or radiotherapy [33].

The survival rate of children with acute lymphoblastic leukemia (ALL) differed markedly depending on the time of maintenance chemotherapy [34]. Thus 80% of the patients dosed with 6-mercaptopurine and methotrexate in the evening were alive and disease-free 5 years after disease onset, as compared with 40% of the children receiving the same drugs in the morning ($p < 0.001$). These findings suggested that residual malignant lymphoblasts might be more susceptible to antimetabolites in the evening than in the morning. The results have been confirmed independently [35]. Although these studies were not randomized, the magnitude of the time-related difference were so impressive that circadian-timed maintenance therapy is currently a consensus treatment for childhood ALL.

Two clinical trials compared the toxicity of anthracyclines and cisplatin given at one of two dosing times to 30 patients with advanced ovarian cancer. Both randomized studies have demonstrated that doxorubicin or

theprubicin near 06:00 and cisplatin between 16:00 and 20:00 produced significantly fewer severe events of hematologic suppression and renal toxicity than treatment given 12 h apart [36, 37].

Chronomodulated chemotherapy using programmable pumps

The clinical relevance of the chronotherapy principle was tested in a large population of patients with metastatic colorectal cancer, using the standard methodology of clinical trials [38, 39]. Metastatic colorectal cancer is the second most common cause of cancer deaths in both genders, and its conventional treatment methods did not offer many therapeutic possibilities other than the reference combination chemotherapy of 5-FU and leucovorin (LV) until the mid-90s. The chronomodulated protocols involved the time-qualified infusion of 5-FU and LV, eventually associated with l-OHP, an active drug more recently recognized. Maximum delivery rate of 5-FU and LV was scheduled at 04:00 at night, and that of l-OHP at 16:00, based upon an extrapolation from experimental data (Figure 4). Courses lasted 4 or 5 days and were repeated every 2 or 3 weeks depending on study.

The tolerability, maximum dose intensities and antitumor activity of these chronotherapy schedules were evaluated in Phase I, II and III clinical trials, involving over 2000 patients with metastatic colorectal cancer. In a phase II single institution trial, 93 patients, 46 of whom had received previous chemotherapy, were treated with the chronomodulated combination of 5-FU, LV and l-OHP during 5 days every 3 weeks: this treatment resulted in a 58% response rate (95% confidence interval: 48–68%) [40]. A first randomized multicenter study in 92 previously untreated patients compared the constant rate infusion to chronomodulated administration of 5-FU, LV and l-OHP. The chronomodulated regimen achieved 53% objective responses, as compared to 32% in patients receiving flat infusion (p=0.038) [41]. These figures were confirmed in a subsequent multicenter trial involving 186 patients: chronotherapy reduced the incidence of severe mucositis fivefold, halved that of functional impairment from peripheral sensory neuropathy and reduced threefold the incidence of grade 4 toxicity requiring hospitalization, as compared to the flat infusion regimen. This improvement in tolerability was accompanied with a significant increase in objective response rate from 29% to 51% (Figure 5) [42]. The good tolerability of chronotherapy further allowed its dose-intensification by administering a 4-day course every 2 weeks, and by increasing the dose of 5-FU. This was achieved first in a phase II study involving 50 patients and confirmed in a multicenter study, with apparent improvements in both objective response rate and survival, which were respectively increased to 66% and 18.5 months [43]. Both figures rank among the highest ones that have been reported for the treatment of metastatic colorectal cancer in a multicenter setting. Taken together, the results from chronotherapy multicenter trials were suggestive of a survival improvement. A randomized trial has been undertaken in 564 patients with metastatic colorectal cancer by the Chronotherapy Group of the European Organization for Research and Treatment of Cancer (EORTC). This study compared the two most active schedules administering 5-FU, LV and l-OHP near maximum tolerated dose: chronomodulated infusion of the 3 drugs over 4 days (so called,

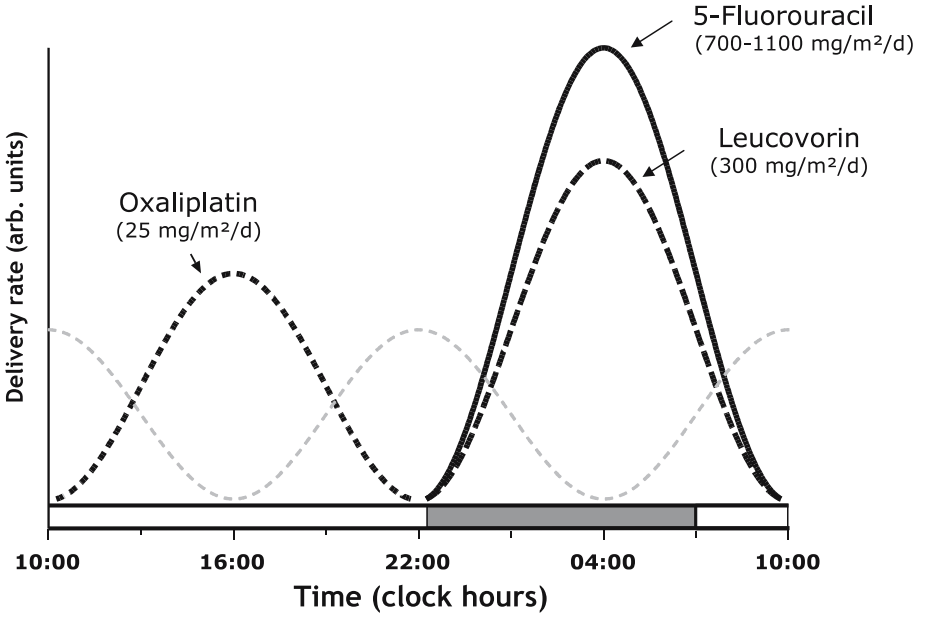

Fig. 4 Profile of the chronomodulated infusion of 5-FU, folinic acid, and oxaliplatin (l-OHP) over 24 h. This cycle is usually repeated automatically for 4 or 5 consecutive days using a programmable in time multichannel pump. This treatment schedule is used to treat patients with colorectal malignancies at home or during their usual activities

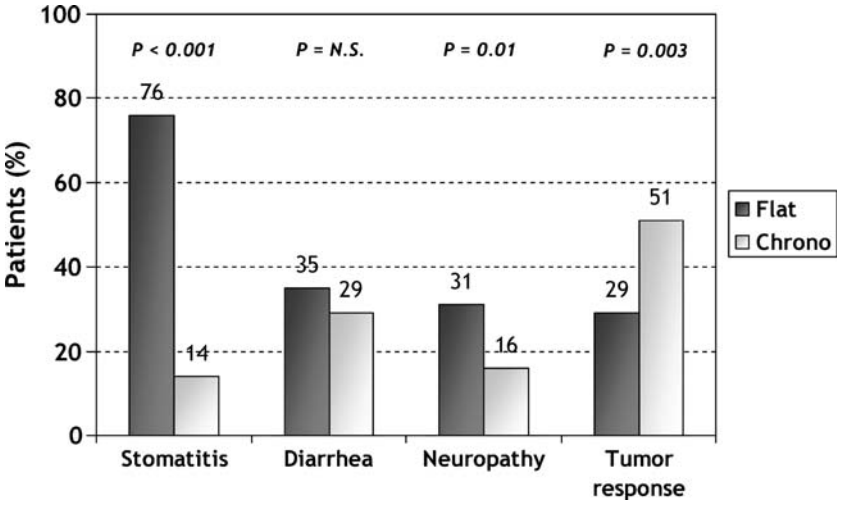

Fig. 5 Main results of a randomized Phase III trial comparing chronomodulated infusion of 5-FU, leucovorin and oxaliplatin (scheme depicted in Figure 4) with constant rate infusion of the same drugs for the same 5 days' duration. In 186 registered patients, chronotherapy was significantly better than flat delivery with regard to both the incidence of severe toxicities (grade 3 or 4, according to the World Health Organisation grading system) and the antitumor activity (rate of patients whose tumor regresses by 50% or more) (After Lévi *et al.* [42])

chrono FFL4–10) vs. 44-h infusion of 5-FU on days 1 and 2 and 2-h infusions of oxaliplatin and leucovorin on day 1 and leucovorin on day 2 (FOLFOX2). A first report indicates excellent survival in both schedules, yet no significant difference between them [44]. Further analyses are identifying the patient characteristics which predict for best efficacy of the current chronomodulated schedule.

Chronotherapy has also played a major role in the development of a new medico-surgical strategy with curative intent for patients with initially unresectable liver metastases. None of these patients usually survive beyond 3 years. Since the very early stage of chronotherapy development in colorectal cancer, patients with initially unresectable colorectal cancer metastases were reassessed for liver surgery after responding to chronomodulated chemotherapy with 5-FU, LV and l-OHP. In a group of 151 patients, retrospective analysis showed that 77 patients could benefit from surgery after responding to chronotherapy, and that among them, 58 patients could undergo a complete resection of their metastases; the survival rate of the operated patients was 50% after 5 years [45]. These results clearly show that chronotherapy, either alone or associated with surgery, improves the therapeutic index of the chemotherapy of metastatic colorectal cancer (Figure 6). Our group has published a recent enlarged series on the long-term efficacy of this strategy [46].

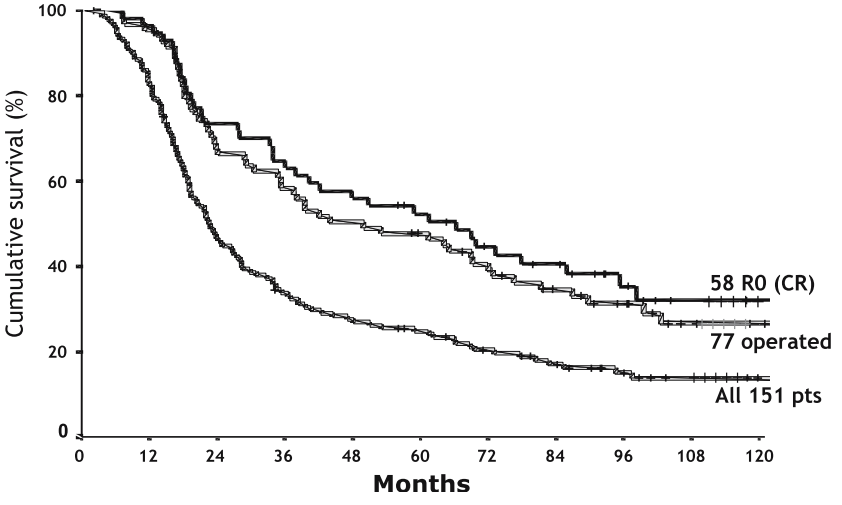

Fig. 6 Survival curves of 151 patients with liver-only metastases from colorectal cancer receiving chronomodulated chemotherapy with 5-FU, leucovorin and oxaliplatin at P. Brousse hospital, with a minimum follow up of 5 years. Chronotherapy was administered as first, second or third line chemotherapy for metastatic disease, which further emphasizes the poor prognosis of this patient population, whose survival beyond 3 years was currently less than 5% (Updated results from a series reported by Giacchetti et al. [45])

Circadian rhythms in cancer patients

Rhythms with periods of about 24 h or shorter (ultradian rhythms, with a 12-h or an 8-h period for instance) have been documented in more than a dozen murine tumor models. These studies have indicated that the circadian periodicity in cellular proliferation indices or metabolic activity is usually retained in slow-growing or well-differentiated tumors, yet with reduced amplitude and sometimes a shift in phase. Conversely, the circadian organization tends to be lost and possibly replaced with an ultradian periodicity in rapidly growing or advanced stage tumors. Similar data seem to characterize human cancers [47].

The mean proportion of bone marrow cells in S-phase was significantly greater at noon as compared to midnight both in 19 healthy subjects and in 11 patients with advanced cancer [29–48]. A concurrent disruption of the normal rhythms in cortisol secretion and in bone marrow S-phase cells was found in 4 cancer patients [48]. This supports the occurrence of altered circadian coordination in some cancer patients.

Studies on cortisol and other blood circadian rhythms were performed in 51 patients with advanced or metastatic ovarian, breast or colorectal cancer. A circadian rhythm was statistically validated for each group of patients. Nevertheless, the 24-h rhythms in plasma cortisol and other variables were prominent in some patients and apparently suppressed in others [49]. These rhythm alterations were mostly found in patients with poor performance status (graded as 2–4, according to the W.H.O. scale) and/or large tumor burden. A recent study in 104 breast cancer patients found that abnormalities in the diurnal cortisol rhythm are associated with reduced survival [50]. The mechanism by which altered circadian rhythms may impact on performance status and survival in breast cancer patients is unclear.

In a population of 200 patients with metastatic colorectal cancer eligible for clinical trials, i.e. with a performance status ≤ 2, a study was undertaken to estimate the frequency of circadian system alterations, as assessed from rest–activity and cortisol rhythms. This circadian system assessment was as little invasive as possible, and did not require hospitalization. Motor activity was continuously monitored for 3 days, using an actigraph worn on the wrist. The strength of the circadian component was assessed with two robust parameters, an autocorrelation coefficient at 24 h (r24) and a dichotomy index comparing amounts of activity when in bed and out of bed ($I < O$) [51]. For cortisol assessment, a blood sample was obtained at 8:00 and at 16:00 on two consecutive days in each patient, because the relative difference between cortisol levels at 8:00 and at 16:00 had been shown to be a good estimator of the circadian amplitude of this rhythm, with 40% being the lower limit of normal [39]. Thirty percent of the 200 patients had an abnormal cortisol rhythm using this criterion. The rest–activity pattern ranged from marked to completely disrupted 24-h rhythmicity. Approximately 30% of the patients displayed a profoundly disturbed cycle, with $r24 < 0.30$ (Figure 7) [51]. Nevertheless, only a weak correlation was found between cortisol rhythm and rest–activity cycle alterations. This suggests that the two rhythms are controlled by different circadian oscillators and/or circadian clock pathways.

The latter study prospectively investigated the relevance of circadian system function for survival and quality of life outcomes. The circadian rest–activity rhythm appeared as a strong predictor of both tumor response and survival in patients with metastatic colorectal cancer. Each of the rest–activity-related variables provided additional prognostic information on maximum response to treatment and survival, to that of the other well-known clinical factors, reflecting tumor burden and general condition. The patients with poor circadian rhythmicity, i.e. with r24 or $I < O$ in the lowest quartile had a five-fold higher risk of dying within 2 years than the patients with better circadian rhythmicity (Figure 8). Furthermore, the prognostic value of $I < O$ remained statistically significant in the subgroups of patients with PS=0 or PS=1, both by univariate and multivariate analysis. This result demonstrated that low rest–activity rhythm parameters did not merely reflect poor PS [51]. The circadian distribution of activity was also correlated to several quality of life parameters from the EORTC QLQ-30 questionnaire [52]. The significant relations between the rest–activity circadian rhythm and both quality of life and survival of patients with colorectal cancer metastases have just been confirmed in a multicenter trial of the EORTC [53, 54]. These results support an important role for the circadian timing system in cancer control and progression.

Indeed mouse experiments have demonstrated an acceleration of malignant growth in mice with markedly altered or suppressed rest–activity and corticosterone rhythms as a result of SCN ablation or chronic jet lag exposure [55–57].

Discussion and perspectives

Malignant tumors and cancer-bearing hosts may exhibit nearly normal or markedly altered circadian rhythms. Rhythm alterations seem to depend upon tumor type, growth rate and level of differentiation, both in animal and human tumors, and they usually worsen along the course of cancer progression.

Possibly, the tumor itself is able to alter the circadian timing system of the host via the release of cytokines or growth factors. Indeed, patients with abnormal rest–activity cycle had elevated levels of TGFα, TNFα and IL-6,

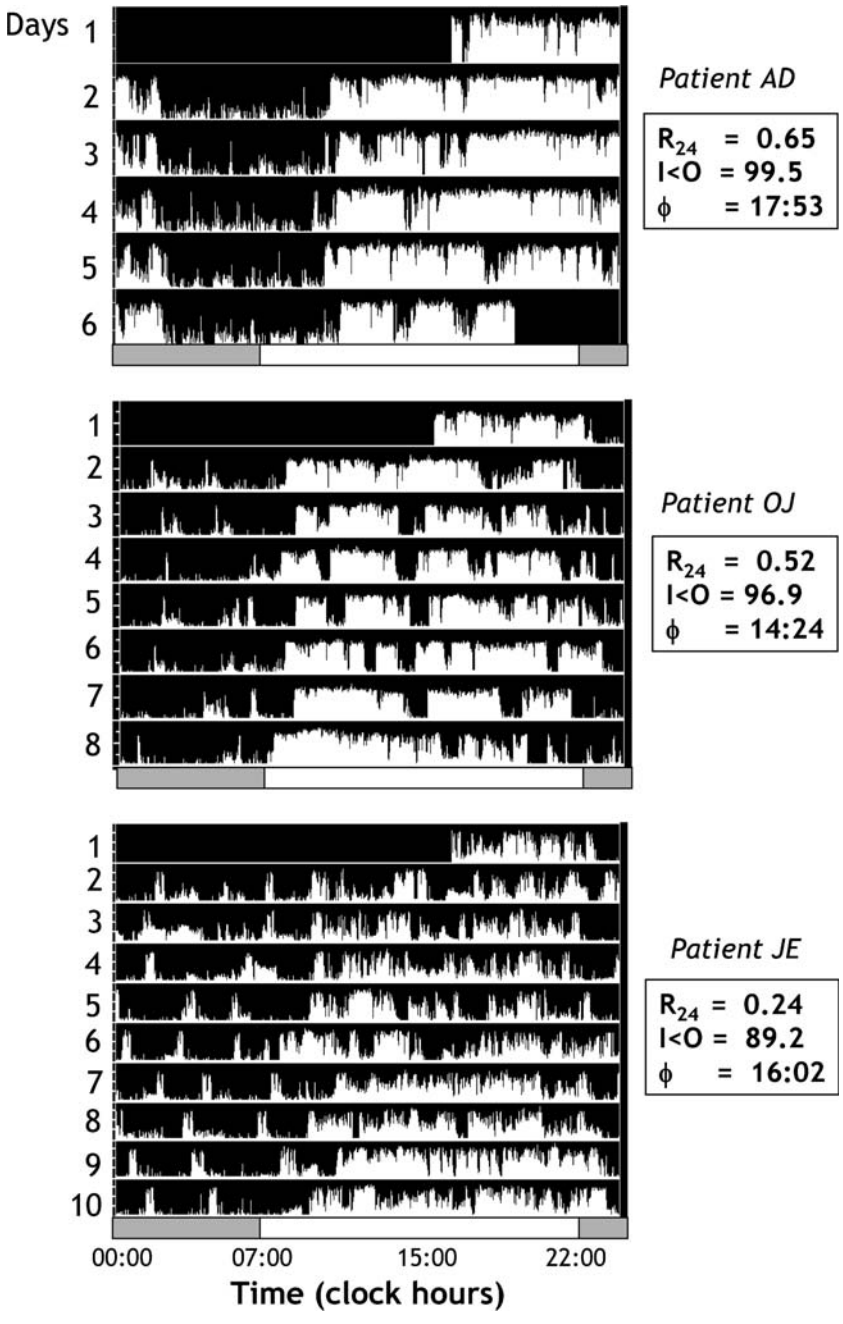

Fig. 7 Example of individual actigraphy records of 3 patients with metastatic colorectal cancer. The subject on the top of figure (AD) displayed a high activity level during the day, which decreased during the night (rest) period: he had thus high rhythm parameters [autocorrelation coefficient at 24 h (r24) and dichotomy index (I < O)*]. The subject in the middle panel (OJ) also had a regular alternation of rest and activity as indicated by r24 > 0.50 yet she displayed minor alterations of the rest period as indicated by a lower I < O. The subject in the lower panel (JE) had a markedly irregular rest–activity circadian pattern with both low r24 (i.e. his activity pattern was not reproducible from one day to the next) and low I < O (he slept poorly when he was in bed). In addition the acrophase (estimated maximum of activity along a 24 h period differed between the three patients (*The dichotomy index I < O, is the percent of the activity counts measured when the patient is in bed, that are inferior to the median of the activity counts measured when the patient is out of bed)

which are known to alter circadian clock function [58, 59]. Specific circadian-targeted support therapy will stem out of a better understanding of the mechanisms through which these substances affect the circadian timing system.

Some of the available experimental and clinical papers provide interesting tracks for understanding the relevance of biological rhythms for cancer treatment. Comparative human studies [60, 61] evidenced a phase opposition between DNA synthesis rhythms in healthy target organs and in tumor; this suggests an involvement of cell cycle regulation mechanisms for the chronopharmacology of anticancer drugs.

The interactions between the circadian timing system, a growing tumor and anticancer treatments needs accurate study through experimental models. Currently available models include mutant mice with longer, shorter or ablated circadian period as compared with 'wild type' animals, light-induced functional rhythm alterations, lesions of the suprachiasmatic nuclei in mice, and computer simulations. Finally, the search for clock genes in healthy and tumor

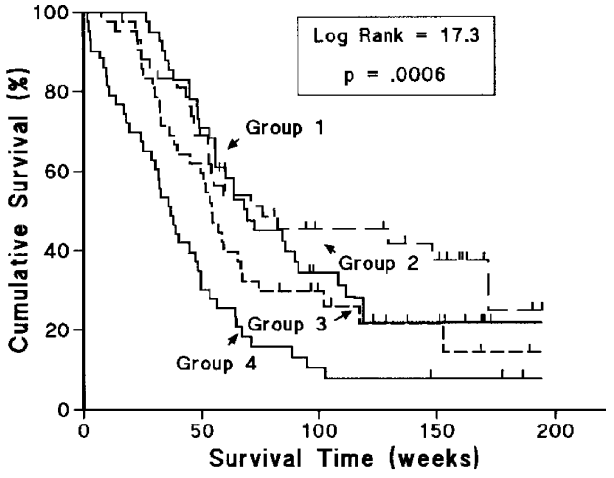

Fig. 8 Kaplan–Meier estimates of survival as a function of the 24-h rhythm parameters, *r*24 (a) and I < O (b), assigned to one of four categories according to quartiles: *group 1*, very high (>75% quartile); *group 2*, high (>50% and ≤ 75% quartile); *group 3*, low (>25% and ≤ 50% quartile); *group 4*, very low (≤ 25% quartile). Comparison of the survival curves with the log-rank test was statistically significant for either circadian rhythm parameter i.e. for *r*24 ($p < 0.0001$; not shown) and for I < O ($p < 0.0001$). The independent prognostic value of either circadian rest–activity parameter was shown in multivariate analyses and confirmed in subset analyses of patients with good performance status, indicating that rest–activity monitoring provides a prognostic information that is not obtained with the usual clinical or biological tests (After Mormont et al. [51])

tissues and the understanding of their crosstalks with cell cycle, apoptosis and DNA repair genes is one of the next challenging steps towards the understanding of the mechanisms of anti-cancer drug chronopharmacology, both in experimental models and in cancer patients [62].

In humans, the functioning of the circadian system is reflected by behavioral and physiological circadian rhythms, such as locomotor activity, body temperature and several neuroendocrine secretions. However, most of these rhythms cannot be evaluated in large-cohort studies. This is the case for measures of proliferation rhythms in tumor or healthy tissue, which imply repeated biopsies, or for the assessment of the rhythm in central temperature, which requires the patient to wear a rectal probe for at least 72 h. Sometimes, a two-time sampling procedure can provide an estimate of a given rhythm, if one of the time-points corresponds to the group maximum. This approach was validated in an analysis of cortisol circadian rhythm both in controls and in cancer patients, where blood sampling at 8:00 h and 16:00 h provided an accurate estimate for individual circadian amplitude. Similar conclusions were suggested for DNA synthesis in human bone marrow [39]. Nevertheless, studies with few time-points will not allow detection of short-period rhythms, which have often been suspected to replace circadian rhythms in late cancer stages. The collection of dense longitudinal time-series is a necessary step towards understanding cancer-associated circadian system alterations. So far, only the assessment of the rest–activity rhythm by wrist actigraphy meets such methodological constraints in cancer patients.

The rest–activity rhythm was a positive prognostic factor of both tumor response and survival in patients with metastatic colorectal cancer [51]. The same study also documented the existence of a link between the rest–activity rhythm and the quality of life of cancer patients. These results open novel perspectives towards understanding the impact of cancer-induced circadian system alterations on host physical and psychological balance.

The above-mentioned investigation also indicates that individual patients' circadian function may provide a pertinent explanation for interindividual differences in the outcome of patients with colorectal cancer metastases. The scope of application of this concept now needs to be assessed, with regard to other human cancers, and other chemotherapy schedules. These results also call for devising specific therapies to restore the circadian rest–activity rhythm: such therapies could include chronobiotics, like melatonin and its analogs, light therapy, sleep management, and psychosocial support. Such specific treatments for circadian dysfunctions may help to improve the status and/or the outcome of cancer patients, and contribute to enhancing the therapeutic efficacy of chemotherapy.

The concept of cancer chronotherapy, which consisted in the extrapolation of the least toxic times of chemotherapy from mice to cancer patients, has been validated in phase III clinical trials for the treatment of colorectal cancer metastases. Yet the benefit in terms of survival still remains to be fully demonstrated.

According to previous experience, there are two ways by which the chronomodulation of chemotherapy can be beneficial to the patients. On one hand, the dosing time-related reduction of chemotherapy toxicity may result in an improvement in quality of life, although high doses of chemotherapy are being delivered. On the other hand, the administration of a higher maximum tolerated dose at the least toxic circadian time, as compared to other dosing times, may result in an improvement in efficacy outcomes, i.e. tumor response and/or survival.

Additional phase III trials are needed to firmly establish chronotherapy in medical oncology, through multicentre cooperative groups, such as the Chronotherapy Group of the EORTC.

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