

REVIEW

Implications of circadian clocks for the rhythmic delivery of cancer therapeutics

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The circadian timing system (CTS) controls drug metabolism and cellular proliferation over the 24 hour day through molecular clocks in each cell. These cellular clocks are coordinated by a hypothalamic pacemaker, the suprachiasmatic nuclei, that generates or controls circadian physiology. The CTS plays a role in cancer processes and their treatments through the downregulation of malignant growth and the generation of large and predictable 24 hour changes in toxicity and efficacy of anti-cancer drugs. The tight interactions between circadian clocks, cell division cycle and pharmacology pathways have supported sinusoidal circadian-based delivery of cancer treatments. Such chronotherapeutics have been mostly implemented in patients with metastatic colorectal cancer, the second most common cause of death from cancer. Stochastic and deterministic models of the interactions between circadian clock, cell cycle and pharmacology confirmed the poor therapeutic value of both constant-rate and wrongly timed chronomodulated infusions. An automaton model for the cell cycle revealed the critical roles of variability in circadian entrainment and cell cycle phase durations in healthy tissues and tumours for the success of properly timed circadian delivery schedules. The models showed that additional therapeutic strategy further sets the constraints for the identification of the most effective chronomodulated schedules.

Keywords: cancer; circadian; cell cycle; chemotherapy; chronotherapeutics; mathematical models

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1. Introduction

Most biological functions in living organisms display rhythmic variations across a wide array of periods, ranging from milliseconds to years. These rhythms are found in single-cell organisms, such as cyanobacteria, as well as in plants, flies, fish, rodents, humans, etc., and are endogenous, i.e. they are not the mere reflection of environmental changes but rather are internally driven. Their frequency domain has been the basis for their classification as ultradian, with a period less than 20 hours, circadian, with a period ranging between 20 and 28 hours, and infradian, with a period more than 28 hours (Smolensky & Peppas 2007). Rhythms with different periods can modulate the same biological function. For instance, the secretion of cortisol by the adrenal gland displays rhythms with periods of approximately 3 hours, 24 hours and 1 year in humans (Weitzman *et al.* 1971). Chronic disruption of cardiac, neuronal or hormonal rhythms with different periods can translate into diseases. Thus, the restoration of physiological rhythms constitutes a therapeutic objective for several cardiac or endocrine disorders, among others. Cellular proliferation is ensured by the cell division cycle, a biological rhythm with its own molecular regulations. Along four successive phases (G₁, S, G₂ and M), the cell will duplicate its genome (during the DNA synthesis phase, S-phase) and then divide into two cells (during mitosis, M-phase). Disruption of the cell cycle is a core mechanism in malignant transformation and cancer progression (Hanahan & Weinberg 2000). Cyclin-dependent kinase inhibitors represent a promising class of drugs that inhibit the protein assemblies that gate transitions from one cell cycle phase to the next and can halt or regulate the cell division cycle (Iurisci *et al.* 2006).

Chronotherapeutics aim at improving the tolerability and/or the efficacy of medications through the administration of treatments according to biological rhythms (Lemmer 2007; Smolensky & Peppas 2007). This consists in the adequate adjustment of treatment delivery to physiological rhythms and/or in the restoration or the induction of physiological rhythms. The relevance of chronotherapeutics has been mostly studied along the circadian time scale for the main causes of mortality and morbidity worldwide, including malignant, cardiovascular, cerebrovascular, neurological, respiratory, infectious and metabolic diseases (Smolensky & Peppas 2007).

Here we consider the relevance of the circadian timing system (CTS) and its dynamic interactions with the cell division cycle and drug metabolism pathways for cancer chronotherapeutics. Indeed, an 8 hour shift in dosing time accounted for up to an eightfold increase in tolerability for over 30 anti-cancer drugs in experimental models (Mormont & Lévi 2003). These large differences resulted from the coordinated circadian rhythms that modify drug pharmacokinetics (PK) and pharmacodynamics (PD) across the 24 hours (Lévi & Schibler 2007). These findings led us to develop a chronomodulated circadian delivery schedule of three anti-cancer drugs that produced fivefold less severe toxicity when compared with wrongly timed or constant-rate infusion (Mormont & Lévi 2003; Lévi *et al.* 2007b). However, gender was responsible for large differences in both the tolerability and the survival of patients on cancer chronotherapeutics (Giacchetti *et al.* 2006). Here, a better understanding of the underlying circadian determinants of the success of cancer chronotherapeutics is provided using two

mathematical approaches, a cell cycle automaton model synchronized by the circadian clock, and a deterministic model of anti-cancer drug chronopharmacodynamics. *In silico* testing further revealed key roles for variability in cell cycle phase durations and circadian entrainment, and identified novel and non-intuitive optimal circadian schedules for anti-cancer drug delivery as a function of the therapeutic strategy set for a given patient. Therefore, the integration of a computational approach into translational research of cancer chronotherapeutics should prove of great help for the personalization of optimal circadian delivery schedules of cancer treatments.

2. The circadian timing system

The CTS coordinates physiology and cellular functions across the 24 hours and the endogenous circadian rhythms to the regular alternation of light and darkness over the 24 hour day as well as to other environmental or sociocultural cycles (figure 1). The adaptation of this circadian time structure to the environmental synchronizers can be viewed as an optimization of the efficiency of energy usage. Environmental synchronizers, such as the alternation of day and night over the 24 hours, socio-professional routine and meal times, entrain and calibrate at precisely 24 hours the period of the CTS (figure 1a). Endogenous circadian rhythms with periods differing from precisely 24 hours have long been known to characterize all aspects of mammalian physiology. In human beings synchronized with usual light-dark, socio-professional and feeding synchronizers, motor activity is high during daytime and low at night, body temperature reaches a maximum in the early evening, cortisol secretion by the adrenal gland rapidly rises from a nadir near 02.00 to a maximum near 08.00 and melatonin secretion by the pineal gland mostly occurs at night, with a maximum near 02.00. This circadian physiology (figure 1b) is generated or controlled by a central pacemaker, the suprachiasmatic nuclei (SCN) in the hypothalamus. The circadian period of the SCN neurons is calibrated to 24 hours through the perception of synchronization signals, namely light and darkness via the retinohypothalamic tract using glutamate and PACAP (pituitary adenylate cyclase activating peptide) as neuromediators, and other brain areas via neuropeptide Y fibres. The SCN generate circadian physiology through diffusible signals (transforming growth factor α (TGF α), epidermal growth factor (EGF), prokineticin-2 and cardiotrophin-like cytokine) and neuroanatomic sympathetic and parasympathetic pathways. Circadian physiology and other signals directly or indirectly emanating from the SCN coordinate molecular clocks in each cell (figure 1c). The molecular clock rhythmically controls many cellular functions that are relevant for cancer treatment, including cellular proliferation, DNA damage sensing and repair, apoptosis and drug metabolism, as will be discussed later.

The periodic resetting of the circadian time structure by these external 24 hour cycles allows for the prediction of the times of the peaks and troughs of circadian rhythms in rodents and humans. In particular, this applies to the rhythms that modify anti-cancer drug pharmacology and cellular proliferation (Lévi & Schibler 2007). Conversely, a lack of external synchronizers, i.e. a defect in the perception of environmental time cues through blindness, for instance, or an alteration of the circadian physiology, molecular clock or clock-controlled pathways, results in the deregulation of the circadian time structure. In turn,

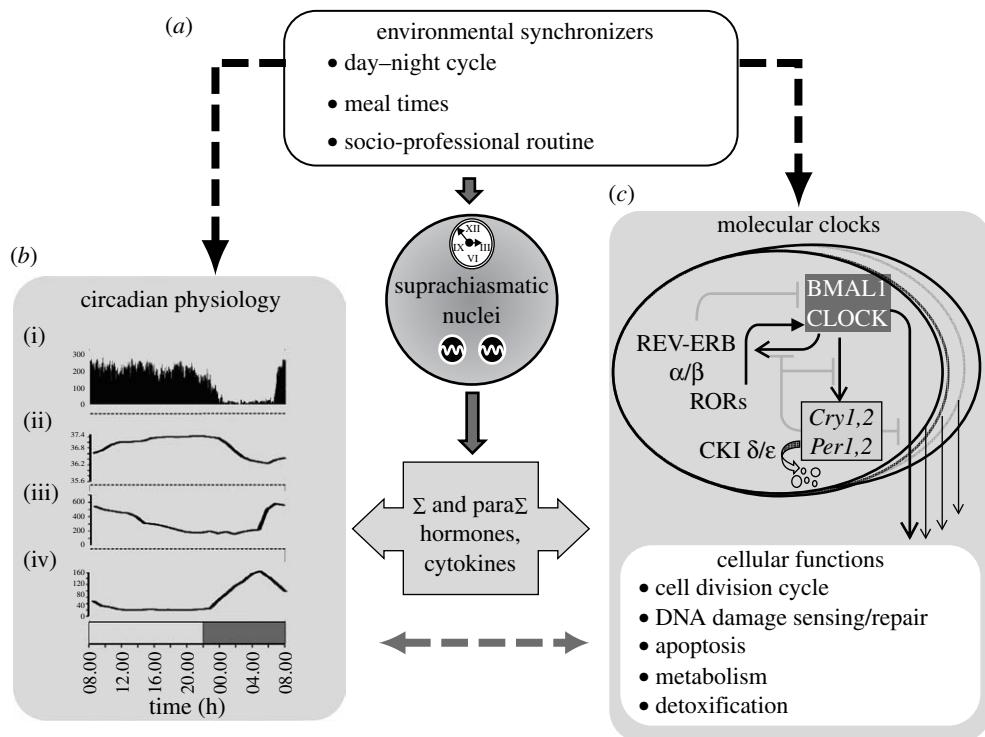


Figure 1. Schematic of the CTS. (a) The SCN are located at the floor of the hypothalamus. Their periods are calibrated to precisely 24 hours by the alternation of light and darkness as well as sociocultural and feeding synchronizers (meal times). (b) The SCN generate or control cellular physiology including rhythms in (i) locomotor activity (mvts min^{-1}), (ii) core body temperature ($^{\circ}\text{C}$) and (iii) serum cortisol (nM l^{-1}) and (iv) serum melatonin (pg ml^{-1}) secretions. Other circadian signalling pathways from the SCN involve the sympathetic (Σ) and parasympathetic (para Σ) systems as well as cytokines, in particular TGF α and EGF. (c) These rhythms coordinate molecular clocks that are located in all peripheral cells and involve interacting transcription/translation feedback loops, where BMAL1:CLOCK protein dimers play a central role. The molecular clocks rhythmically control most cellular functions. Circadian physiology can further redundantly regulate clock-controlled cellular functions.

relevant 24 hour rhythms become damped, ablated or phase shifted, with an unpredictable timing during the day and night of the peaks and troughs if the circadian period is lengthened or shortened. In such cases, specific therapeutic measures may be required to restore proper circadian function or coordination (Liu *et al.* 2007).

3. Mechanisms of circadian rhythms and therapeutic implications

A dozen specific clock genes constitute the core of the molecular clock in mammals. These genes are involved in transcriptional and post-transcriptional activation and inhibition regulatory loops that result in the generation of the circadian oscillation in individual mammalian cells. The CLOCK:BMAL1 or

NPAS2:BMAL1 protein dimers, in particular, play a key role in the molecular clock through the activation of the transcription of the clock genes *Per* and *Cry* (Lévi & Schibler 2007; Liu *et al.* 2007). This protein dimer also exerts a negative control on the cell cycle, both through the repression of *c-myc* and *p21*, two important players in cellular proliferation and apoptosis, and through the activation of *p53*, a pro-apoptotic gene, and *wee1*, whose protein gates cell cycle transition from G2 to mitosis (Matsuo *et al.* 2003; Chen-Goodspeed & Lee 2007; Okyar & Lévi 2008). In proliferating cells, this results in the circadian control of the transition from G1 to S and from G2 to M (Matsuo *et al.* 2003; Lévi *et al.* 2007a). Recent data have further shown the circadian regulation of apoptosis through the rhythmic expressions of anti-apoptotic BCL-2 protein and pro-apoptotic BAX protein (Granda *et al.* 2005), that of DNA damage sensing through molecular interactions of ATM/ATRIP with clock proteins PERs, CRYs and TIM (Gery *et al.* 2006; Okyar & Lévi 2008) and that of DNA repair through rhythmic activities or levels of O₆-methylguanine DNA methyltransferase, a protein that excises lethal DNA alkylated lesions produced by nitrosourea anti-cancer drugs (Marchenay *et al.* 2001).

Clock genes *Per1*, *Per2*, *Bmal1* and *Rev-erba* are usually expressed in experimental tumour models, although the overall level of mRNA expression seems to be lower than that in the liver or the original tissues from which these tumours derive. In addition, the mRNA expression patterns over the 24 hours appear to be maintained, damped or ablated depending on the type of tumour and/or its developmental stage (Koyanagi *et al.* 2003; Filipski *et al.* 2005; You *et al.* 2005; Iurisci *et al.* 2006). An alteration of the molecular clock in human tumours is further supported by decreased expressions of the *Per1*, *Per2* or *Per3* genes in comparison with reference tissues (Okyar & Lévi 2008). These data are in rather good agreement with the occurrence of rhythms with periods of approximately 24 hours or less in more than a dozen murine tumour models (Granda & Lévi 2002). Thus, the circadian periodicity in metabolic activity or cellular proliferation is usually retained in slow-growing or well-differentiated tumours, although with a 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 tumours. This also characterizes human cancers (Smaaland *et al.* 2002).

The CTS also controls the main pathways that are responsible for the PK and the cellular metabolism of anti-cancer medications, resulting in the chrono-pharmacology of these agents, i.e. circadian time-dependent PK and PD (Lévi & Schibler 2007). Thus, circadian rhythms characterize most detoxification processes at the transcription, protein and enzymatic levels in the liver, the chief drug-metabolizing organ, as well as in intestine, kidney, lung, etc. (Gachon *et al.* 2006). As a result, the circadian dosing time influences the extent of the toxicity of more than 30 anti-cancer drugs, including cytostatics, cytokines and ‘targeted biological agents’ in laboratory mice or rats (Koyanagi *et al.* 2003; Mormont & Lévi 2003; Iurisci *et al.* 2006). For all these drugs, the survival rate varies by 50 per cent or more according to the circadian dosing time of a potentially lethal dose. Such large differences in drug tolerance were observed irrespective of administration route or drug class (Mormont & Lévi 2003; Lévi *et al.* 2007a,b).

4. Experimental chronotherapeutics with 5-fluorouracil and oxaliplatin

Investigations of circadian dependences in drug effects require the standardization of the light–dark schedules that synchronize the CTS of the experimental animals. Usually, mice or rats are exposed to the regular alternation of 12 hours of light and 12 hours of darkness (12 D : 12 L) for three weeks prior to drug dosing. Time is referred to light onset, through its expression in hours after light onset (HALO) or as zeitgeber time, with 0 HALO being ZT0 and 12 HALO being ZT12.

The antimetabolite drug 5-fluorouracil (5-FU) substitutes for uracil in its physiological reactions and kills cells through that mechanism. The drug has a 10–20 min half-life in the plasma. The tolerability of a potentially lethal dose of 5-FU was three- to eightfold better in mice dosed in the early light span when compared with those receiving the drug at night. The best and worst dosing times were rather consistent among the different studies and investigators, and corresponded to the early stage of the rest span and the middle of the activity span of the rest–activity circadian rhythm of the mice, respectively (Peters *et al.* 1987; Mormont & Lévi 2003; Wood *et al.* 2006).

The 5-FU chronotolerance results from multiple rhythms in healthy target tissues, such as those in bone marrow, gut, skin and liver, which are coordinated by the CTS. Circadian rhythms have been shown for the enzymatic activities of dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme that catabolizes 5-FU, orotate phosphoribosyl transferase, uridine phosphorylase and thymidine kinase, which are involved in the generation of the cytotoxic forms of 5-FU, and thymidylate synthase (TS), the main target enzyme of this antimetabolite (Naguib *et al.* 1993; Porsin *et al.* 2003; Wood *et al.* 2006). Since TS is required for DNA synthesis, its activity reaches its acme during the S-phase of the cell division cycle. As a result, the cell-kill potential of 5-FU is by far the greatest for S-phase cells. Interestingly, the proportion of S-phase cells in mouse bone marrow is the highest during darkness, corresponding to the usual span of mouse activity (Granda *et al.* 2005). Mechanisms of cell death result from P53-dependent apoptosis, a process that involves several rhythmic components (Granda *et al.* 2005; Gery *et al.* 2006). Furthermore, both P53 expression and apoptosis were downregulated by circadian disruption through *Per2* mutation, *Per1* knockout cells or chronic jet lag (Filipski *et al.* 2005; Gery *et al.* 2006; Chen-Goodspeed & Lee 2007). Thus, the molecular interactions between the circadian clock and the cell cycle and its related apoptosis pathways represent a major determinant of 5-FU chronotolerance (figure 2). Indeed, as shown in figure 2 for rodents whose CTS is synchronized with a regular alternation of 12 hours of light and 12 hours of darkness, the least toxicity of 5-FU corresponded to drug dosing in the early rest span, when healthy tissues best catabolize the drug through high DPD activity, are the best protected against drug-induced apoptosis through high BCL-2 and low BAX expressions, and display fewer 5-FU-sensitive S-phase cells.

On the contrary, oxaliplatin is an alkylating agent that forms DNA adducts, which in turn are responsible for cell death. Following administration, oxaliplatin irreversibly binds to plasma proteins while the free (unbound) fraction crosses the cellular membranes within minutes, resulting in triphasic plasma PK (Lévi *et al.* 2000). Oxaliplatin tolerability was enhanced approximately threefold in mice through drug administration near the middle of the dark span rather than

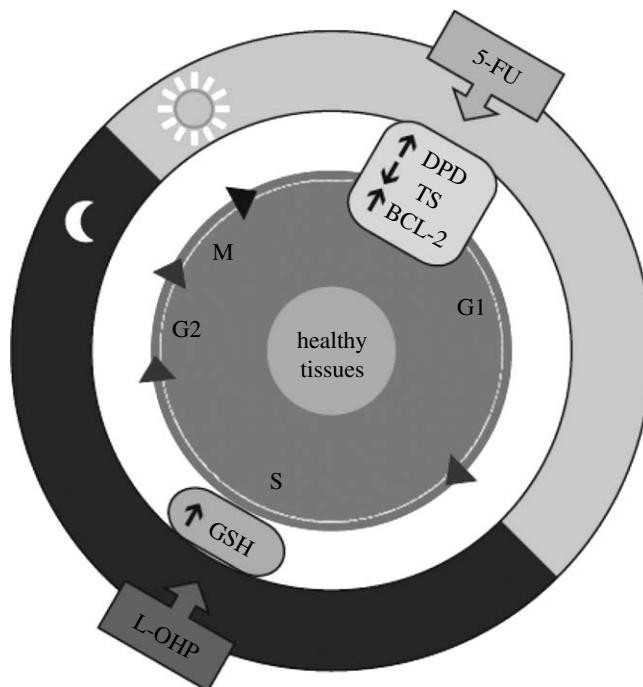


Figure 2. Multiple coordinated mechanisms in the detoxification of anti-cancer drugs 5-fluorouracil (5-FU) and oxaliplatin (L-OHP) in healthy tissues of nocturnally active mice. Following rapid cellular entry, 5-FU is rhythmically catabolized by DPD. The active metabolites that are rhythmically formed through pathways not shown here suppress DNA synthesis through the inhibition of TS, an enzyme with rhythmic activity, which peaks during the DNA synthesis (S) phase of the cell division cycle. 5-FU can also elicit apoptosis, a process that is antagonized by BCL-2, an anti-apoptotic protein that is also rhythmic in bone marrow, a main toxicity target for 5-FU. Finally, proliferating cells are more sensitive to 5-FU following exposure during the S-phase when compared with other stages of the cell cycle. After fast cellular uptake, L-OHP interacts with GSH and other thiol-containing peptides and proteins, a process that reduces the formation of DNA cross-links. GSH and thiol contents in many organs are rhythmic, a process that participates in the detoxification rhythms of many drugs, including oxaliplatin. These cellular rhythms determine a several-fold improvement in tolerability through the delivery of 5-FU during the early light span, when the mice are resting, and L-OHP near the middle of darkness, when the animals are active. (Adapted from Lévi & Schibler 2007.)

at daytime. The best and worst dosing times corresponded to mid-activity and mid-rest in the circadian rhythm in rest–activity, respectively (figure 2; Lévi *et al.* 2000). While no cell cycle phase specificity characterizes the cytotoxicity of oxaliplatin, the drug mostly arrests cycling cells at the G2/M transition, before they enter mitosis, resulting in cell cycle delay or cell death (Voland *et al.* 2006). Following intracellular entry, oxaliplatin irreversibly binds to thiol groups, such as reduced glutathione (GSH), a tripeptide that is present in the cytoplasm of most cells and shields the intracellular milieu from exposure to many toxicants, including oxaliplatin. Thus, the pronounced circadian rhythm in GSH is a major determinant of chronotolerance for oxaliplatin and other Pt complexes, and the highest values are found in the dark span in mice or rats (Li *et al.* 1998).

Quite strikingly, the administration of a drug at the circadian time when it is best tolerated usually achieves the best anti-tumour activity, as demonstrated for 10 anti-cancer agents belonging to various classes (Lévi *et al.* 2007b). This principle also applies to 5-FU and oxaliplatin. Thus, the best anti-tumour efficacy was achieved in tumour-bearing mice receiving 5-FU in the early light (rest) span or oxaliplatin near the middle of the dark (activity) span (Peters *et al.* 1987; Granda *et al.* 2002). These experimental prerequisites have warranted the clinical development of chronotherapeutics with 5-FU and oxaliplatin.

5. Clinical chronotherapeutics with 5-FU and oxaliplatin

(a) Chronopharmacokinetics

Human PK of 5-FU and oxaliplatin are also controlled by the CTS, resulting in 24 hour changes in the exposure of target tissues and tumours to these drugs (Nowakowska-Dulawa 1990; Lévi *et al.* 2000). Circadian variations in plasma drug levels were found despite continuous, constant-rate intravenous infusion of 5-FU with superimposed inter-patient variability (Lévi & Schibler 2007). Of interest is the finding that the activity of DPD, the initial enzyme for the catabolism of 5-FU, in the peripheral blood mononuclear cells of diurnally active cancer patients varies significantly during the 24 hour time period, with DPD activity being the greatest between midnight and 04.00 (Harris *et al.* 1990; Zeng *et al.* 2005). Similarly, plasma GSH concentration also displayed a 24 hour rhythm in cancer patients, with a maximum occurring near noon (Zeng *et al.* 2005). These results are consistent with the prior ones on GSH concentration in human bone marrow (Smaaland *et al.* 2002). The GSH rhythm probably contributes to reduced oxaliplatin toxicity in the early afternoon.

(b) Cell cycle rhythms in humans

Cell proliferation is also likely to be responsible for the chronotolerance to 5-FU. The proportion of bone marrow, gut, skin and oral mucosa cells engaged in the S-phase of the cell division cycle varies by 50 per cent or more along the 24 hour time scale in healthy human subjects. For all these tissues, the probability of cells being in the S-phase is the lowest between midnight and 04.00 and the highest between 08.00 and 20.00 in persons adhering to a routine of diurnal activity alternating with night-time sleep (figure 3*a*; Bjarnason & Jordan 2002; Smaaland *et al.* 2002).

(c) From mouse to human cancer chronotherapeutics

The mechanisms of anti-cancer drug chronopharmacology display a similar phase relationship with the rest–activity cycle in mice and humans, despite the fact that the former is active during the night and the latter during the daytime. Thus, DPD activity peaks during the early light span (rest phase) in mice or rats and early night in human beings. The proportion of S-phase cells in the bone marrow peaks in the second half of the dark span (activity phase) in mice and at approximately 16.00 in humans. In addition, constant-rate 5-FU infusion results in a circadian rhythm in its plasma level, both in mice and in cancer

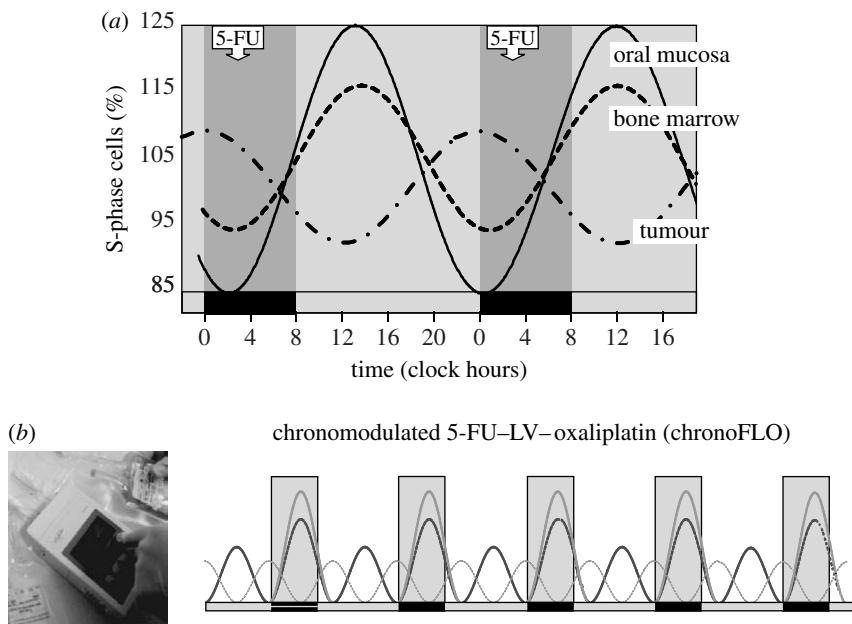


Figure 3. Rationale of circadian-based delivery of 5-FU and oxaliplatin, chronomodulated drug-delivery schedule and main results in cancer patients. (a) Circadian organization of cell cycle phase distribution in humans. In healthy human beings, the proportion of S-phase cells rises and reaches a maximum near 16.00 daily in oral mucosa as well as in bone marrow. Since 5-FU is most toxic for S-phase cells, exposure at this time should be avoided in order to reduce toxic effects for both of these proliferating healthy tissues, which represent the main toxicity targets of this drug. (b) ChronoFLO schedule. Chronomodulated sinusoidal delivery schedules have been designed based on experimental data in mice as well as the rhythms in cell cycle phase distribution and detoxification processes shown in humans. Here, the reference chronoFLO5 consists in the sinusoidal delivery of 5-FU and leucovorin, for 12 hours with a peak at 04.00 in alternation with oxaliplatin for 12 hours with a peak at 16.00 for five consecutive days every three weeks. This schedule has been administered with programmable-in-time multichannel electronic pumps and validated in patients with metastatic colorectal cancer registered in phase I, II and III clinical trials. (Adapted from Bjarnason *et al.* (2001), Bjarnason & Jordan (2002), Smaaland *et al.* (2002), Lévi (2001) and Lévi *et al.* (2007b).)

patients. The peak concentration of 5-FU occurs in the early rest span in both species when the drug is infused continuously at a constant rate for up to a one-week span (Lévi & Schibler 2007).

The apparent coupling between the circadian rest–activity cycle and the specific mechanisms that give rise to 24 hour rhythms in the PK and PD of medications across species has been the basis for the chronotherapeutic schedules given to cancer patients. As a working hypothesis, the expected times of least toxicity in cancer patients were extrapolated from those experimentally demonstrated in synchronized mice or rats (i.e. animals that were housed under a regulated, typically 12 hour light–12 hour dark environment), by referring them to the respective rest–activity cycle of each species, e.g. with approximately 12 hour time lag. For instance, in mice the least toxicity of 5-FU occurs approximately 5 hours after light onset in the animal quarters, and this was predicted to correspond to 04.00 in human beings, adhering to sleep between 23.00 and 07.00 alternating with activity between 07.00 and 23.00.

The availability of portable multichannel programmable-in-time pumps constituted a key technological step for the implementation of cancer chronotherapy. Indeed, these electronic devices make possible the continuous chronomodulated delivery of up to four different medications in the patients' usual activities in- or outside the home. The clinical relevance of the chronotherapy principle was first tested in a large population of patients with metastatic colorectal cancer, using standard methods of clinical trials.

(d) *Chronotherapeutics of colorectal cancer*

Metastatic colorectal cancer is the second most common cause of cancer deaths in both men and women. Until the early 1990s, conventional treatment methods offered few therapeutic options other than the reference combination chemotherapy of 5-FU-leucovorin (LV). The chronomodulated protocols involved the time-qualified infusion of 5-FU and LV, eventually associated with oxaliplatin, an active drug that was first recognized as such through chronotherapeutic development (Lévi *et al.* 1992). The maximum delivery rate of 5-FU-LV was scheduled during sleep at 04.00 and of oxaliplatin at 16.00, based upon the extrapolations from experimental laboratory rodent modelling data. Courses lasted 4 or 5 days and were repeated every second or third week (figure 3b). The tolerability, maximum dose intensities and anti-tumour activity of this chronotherapeutic schedule were evaluated in phase I, II and III clinical trials involving over 2000 patients with metastatic colorectal cancer. In a first 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 oxaliplatin for 5 days every three weeks. This new treatment achieved a 58 per cent response rate, a figure that was approximately fourfold higher than that produced by the conventional daily bolus of 5-FU-LV for 5 days every three weeks (Lévi *et al.* 1992).

Two consecutive randomized trials in a total of 278 previously untreated patients compared the constant-rate infusion to the chronomodulated infusion of 5-FU-LV and oxaliplatin (Lévi *et al.* 2007b). Chronotherapy reduced the incidence of severe mucositis fivefold, halved the incidence of functional impairment from peripheral sensory neuropathy and reduced the incidence of grade 4 toxicity requiring hospitalization by threefold when compared with the flat infusion regimen. This improvement in patient tolerability to the cancer medications was accompanied by a significant increase in the objective response rate to the cancer chemotherapy, from 29 to 51 per cent (table 1; Lévi *et al.* 2007b).

The relevance of peak time of drug delivery for early toxicity was investigated in a subsequent study in 114 patients with metastatic colorectal cancer receiving chronomodulated 5-FU-LV and oxaliplatin (Lévi *et al.* 2007b). Patients on this trial were offered to participate in the study after they had failed a conventional chemotherapy regimen. They received then one of eight differently timed schedules. In each schedule, however, the sequence and intervals between the drugs were kept similar, since the delivery rate of 5-FU-LV and that of oxaliplatin peaked 12 hours apart. The incidence of severe toxicity (grades 3–4 on the WHO scale) was 16.6 per cent in the patients receiving maximum infusion rate at 01.00 or 04.00 for 5-FU-LV and at 13.00 or 16.00 for oxaliplatin. Conversely, 40–60 per cent of the patients treated 6 or 9 hours before or after these times displayed severe toxicity, while this was the case for 80 per cent of

Table 1. Main toxicity and efficacy outcomes in cancer patients receiving infusional 5-fluorouracil, leucovorin and oxaliplatin with chronomodulated or constant delivery rate. The reference chronoFLO schedule depicted in figure 3b was compared to constant-rate infusion of the same three drugs in phase III trials. The main results revealed that oral mucosa tolerability was improved fivefold by chronoFLO when compared with constant infusion, while anti-tumour efficacy, as assessed by tumour response rate, was nearly twice as high with chronoFLO5. Similar differences in tolerability and anti-tumour efficacy were noted between the reference chronoFLO and chronomodulated schedules with peak times of delivery rate occurring at 16.00 for 5-FU-LV and at 04.00 for oxaliplatin in patients refractory to a first conventional chemotherapy regimen. (Adapted from Lévi *et al.* 2007b.)

delivery schedule	percentage of 278 patients without any previous chemotherapy		percentage of 114 patients failing prior chemotherapy	
	chronoFLO	constant rate	chronoFLO	'opposite' chronoFLO
severe toxicity (grade 3–4)	14	76	16	80
major tumour responses	51	30	30	12

the patients receiving peak 5-FU-LV at 16.00 and peak oxaliplatin at 04.00. Tumour response occurred in 30.4 per cent of the patients receiving either one of the best tolerated modalities, as compared to 12.5 per cent of the patients given either one of the worst tolerated modalities (table 1; Lévi *et al.* 2007b).

6. Probing the relevance of circadian delivery of 5-FU using a cell cycle automaton model

Assessing the effectiveness of various temporal schedules of drug delivery is central to cancer chronotherapy. Modelling tools can help to optimize time-patterned drug administration to increase effectiveness and reduce toxicity (Goldbeter & Claude 2002). Probing the effect of circadian delivery of anti-cancer drugs by means of modelling and numerical simulations requires a model for the cell cycle. Detailed kinetic models have been proposed for the embryonic and yeast cell cycles and for the mammalian cycle. An alternative approach is to rely on a simple phenomenological description of the cell cycle in terms of an automaton, which switches between sequential states corresponding to the successive phases of the cell cycle. In this model, cell cycle progression or exit from the cycle is affected by the presence of anti-cancer medications. The cell cycle automaton model is based on the perspective that the transitions between the various phases of the cell cycle possess a random nature (Smith & Martin 1973; Brooks *et al.* 1980; Cain & Chau 1997). The model allows us to readily investigate how different temporal patterns of drug administration affect cell proliferation.

Anti-cancer medications generally exert their effect by interfering with the cell division cycle, often by blocking it at a specific phase. As previously reviewed, 5-FU is primarily toxic to cells that are undergoing DNA synthesis, i.e. during the S-phase. Conversely, alkylating agents such as oxaliplatin do not display any

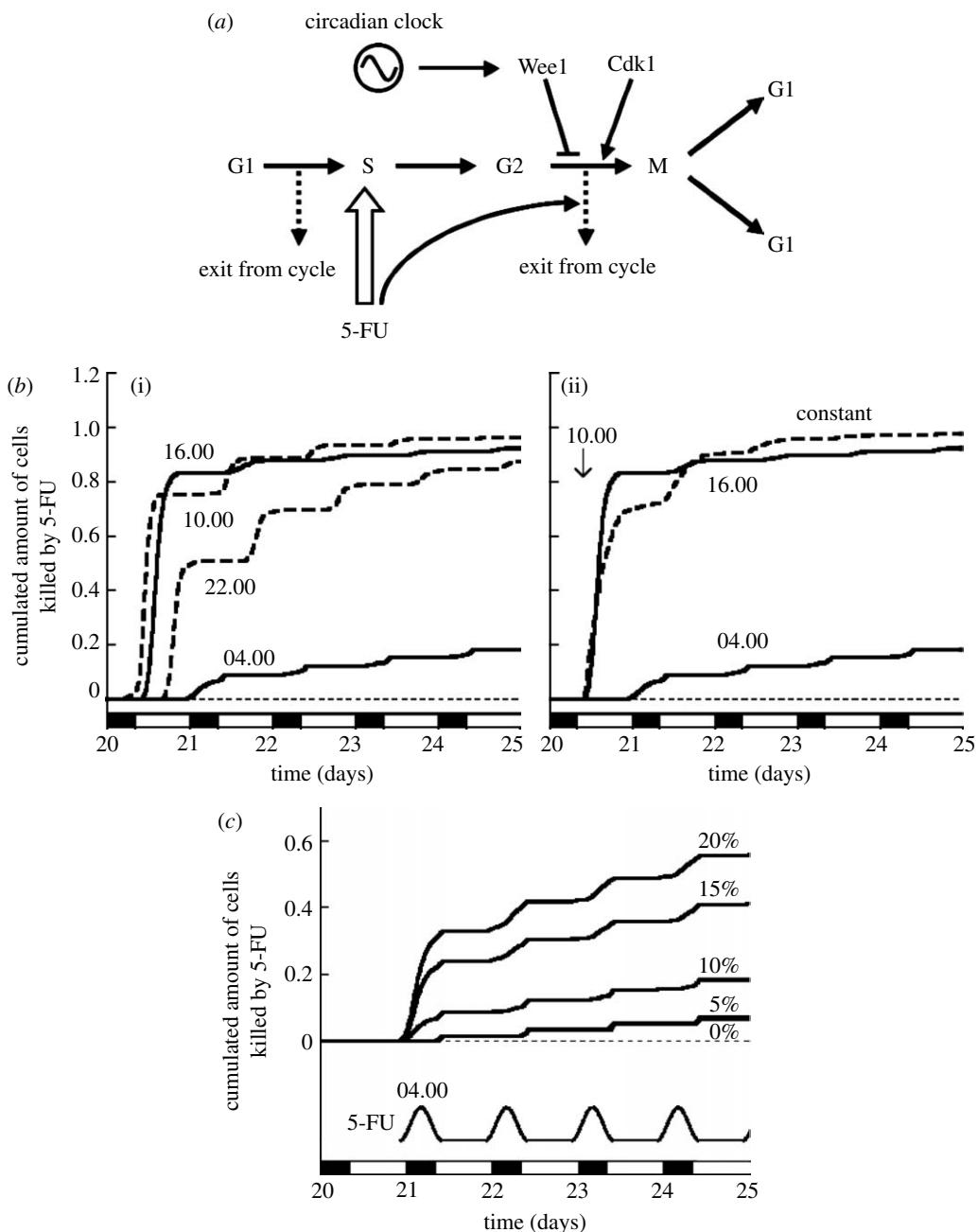


Figure 4. (Caption opposite).

cell cycle phase specificity. To illustrate the use of the cell cycle automaton model, we focus here on the chronotherapeutic scheduling of 5-FU. The half-life of this medication is 10–20 min; thus, the exposure pattern will be the only one considered here since it matches rather well the corresponding chronotherapeutic drug-delivery schedule.

Figure 4. (*Opposite.*) The automaton model. (a) Scheme of the automaton model for the cell cycle. The automaton switches sequentially between the phases G1, S, G2 and M. At the end of the M-phase, the automaton cell divides into two cells that enter a new G1-phase. Switching from one phase to the next one occurs in a random manner as soon as the end of the preceding phase is reached, according to a transition probability related to a duration distribution centred for each phase around a mean value D and a variability V (see text). Exit from the cell cycle occurs with a given propensity at the G1/S and G2/M transitions. Coupling to the circadian clock occurs through the kinases Wee1 and cdc2 (Cdk1), which, respectively, inhibit and promote the G2/M transition. We incorporate into the model the mode of action of the anti-cancer drug 5-FU by assuming that cells exposed to 5-FU while in S-phase have a higher propensity of exiting the cell cycle at the next G2/M transition. (b) Drug toxicity as a function of peak time of circadian 5-FU administration. (i) Cytotoxicity for circadian schedules of 5-FU delivery peaking at various times (04.00, 10.00, 16.00 and 22.00), when variability V is equal to 10%. (ii) The circadian patterns peaking at 04.00 or 16.00 are compared with the continuous delivery of 5-FU, which begins at 10.00 on day 20 (vertical arrow). The curves show the cumulated cell kill (in units of 10^4 cells) for days 20–25, in the presence of entrainment by the circadian clock. Prior to entrainment, the cell cycle duration is 22 hours. (c) Cytotoxicity of chronomodulated 5-FU: effect of variability of cell cycle phase durations. Shown is the cumulative cell kill (in units of 10^4 cells) when 5-FU is delivered in a circadian manner with a peak at 04.00, in the presence of entrainment by the circadian clock, for different values of variability V indicated on the curves. Prior to entrainment, the cell cycle duration is 22 hours. (After Altinok *et al.* 2007*a,b.*)

(a) An automaton model for the cell cycle

(i) Rules of the cell cycle automaton

The automaton model for the cell cycle (figure 4*a*) is based on the following assumptions. The cell cycle consists of four successive phases along which the cell progresses: G1, S (DNA replication), G2 and M (mitosis). Upon completion of the M-phase, the cell transforms into two cells that immediately enter a new cycle in G1 (the possibility of temporary arrest in a G0-phase is not considered here). Each phase is characterized by a mean duration D and a variability V . As soon as the prescribed duration of a given phase is reached, the transition to the next phase of the cell cycle occurs. The time at which the transition takes place varies in a random manner according to a distribution of durations of cell cycle phases. In the case of a uniform probability distribution, the duration varies in the interval $[D(1 - V), D(1 + V)]$. At each time step in each phase of the cycle, the cell has a certain probability to be marked for exiting the cycle and dying at the nearest G1/S or G2/M transition. To allow for homeostasis, which corresponds to the maintenance of the total cell number within a range in which it can oscillate, we further assume that cell death counterbalances cell replication at mitosis. Given that two cells in G1 are produced at each division cycle, the propensity P_0 of exiting the cycle must be of the order of 50 per cent over one cycle to achieve homeostasis. When the propensity of exiting the cycle is slightly larger or smaller than the value yielding homeostasis, the total number of cells increases or decreases in time, respectively, unless the propensity of quitting the cycle is regulated by the total cell number. We have used these rules to simulate the dynamic behaviour of the cell cycle automaton in a variety of conditions, with or without entrainment by the circadian clock.

The effect of 5-FU can be incorporated into the automaton model by assuming that cells exposed to 5-FU while in S-phase have an enhanced propensity to quit

the cycle at the next G2/M transition. This propensity to exit the cycle is taken as being proportional to the amount of 5-FU. We wish to compare two kinds of temporal profile of 5-FU. Either 5-FU remains constant in time, or it is delivered in a circadian, semi-sinusoidal manner (table 1), with a peak time that will vary along a 24 hour span.

(ii) Dynamics of the cell cycle automaton

The variability in the duration of the cell cycle phases is responsible for progressive cell desynchronization. In the absence of variability, if the duration of each phase is the same for all cells, the population behaves as a single cell. Then, if all cells start at the same point of the cell cycle, e.g. at the beginning of G1, a sequence of square waves bringing the cells synchronously through G1, S, G2, M and back into G1 occurs. As soon as some degree of variability of the cell cycle phase durations is introduced, the square waves transform into oscillations of the fractions of cells present in the various cell cycle phases. The amplitude of these oscillations diminishes as the variability increases. In the long term, the oscillations dampen as the system settles into a steady-state distribution of cell cycle phases.

To determine the effect of circadian rhythms on anti-cancer drug administration, it is important to incorporate the link between the circadian clock and the cell cycle. Entrainment by the circadian clock can be included in the automaton model by considering that the protein Wee1 undergoes circadian variation due to induction by the circadian clock proteins CLOCK and BMAL1 of the expression of the *Wee1* gene (Matsuo *et al.* 2003; Hirayama *et al.* 2005; Reddy *et al.* 2005; figure 4a). Wee1 is a kinase that phosphorylates and thereby inactivates the cyclin-dependent kinase Cdk1 that controls the G2/M transition. When modelling the link between the cell cycle and the circadian clock in humans, we will consider a 16:8 light-dark cycle (16 hours of light, from 08.00 to 00.00, followed by 8 hours of darkness, from 00.00 to 08.00). In agreement with the observations in human cells (Bjarnason *et al.* 2001), the rise in Wee1 should occur at the end of the activity phase, i.e. with a peak at 22.00. The decline in Wee1 activity is followed by a rise in the activity of the kinase Cdk1, which enhances the probability of transition to the M-phase. We thus consider that the rise in Wee1 is immediately followed by a similar rise in Cdk1 kinase. Upon entrainment by the circadian clock, cells become more synchronized than in the absence of entrainment. In contrast to the progressive dampening of the oscillations in the absence of entrainment, when the cell cycle automaton is driven by the circadian clock, oscillations appear to be sustained.

(b) Circadian versus continuous administration of 5-FU

We consider a circadian profile of 5-FU, which is similar to that used clinically (figure 3b). Over the 24 hour period, the 5-FU level is zero between 10.00 and 22.00, and rises in a semi-sinusoidal manner between 22.00 and 10.00 with a peak at 04.00. Keeping the same waveform, we have varied the time of the peak over a 24 hour time span. When measuring the cytotoxic effect of the drug by the normalized, cumulated number of cells killed by 5-FU, a minimal toxicity area appears when the 5-FU peak time occurs near 04.00. The fact that a peak time at 04.00 leads to minimum toxicity in the simulated cell population supports the clinical use of this pattern of drug administration to achieve the best tolerance.

We further compared the effect of the continuous administration of 5-FU with various circadian patterns of 5-FU delivery peaking at 04.00, 10.00, 16.00 or 22.00 in the presence of entrainment by the circadian clock (figure 4b). Several conclusions can be drawn from this comparison. First, the various circadian patterns of 5-FU delivery have markedly different cytotoxic effects on diurnally active cancer patients: the least toxic pattern is that peaking at 04.00, while the most toxic one is that which peaks at 16.00. The other two patterns peaking at 10.00 or 22.00 exert intermediate cytotoxic effects. Conventional continuous infusion of 5-FU is nearly as toxic as the circadian pattern of 5-FU delivery peaking at 16.00 (figure 4b).

The cell cycle automaton model permits us to clarify the reason why circadian delivery of 5-FU is the least or most toxic when it peaks at 04.00 or 16.00, respectively (Lévi *et al.* 2007b). Indeed, the model allows us to determine the relative positions of the peaks in S-phase cells and in 5-FU (Altinok *et al.* 2007a,b). 5-FU is the least cytotoxic when the fraction of S-phase cells (peak at 16.00 and trough near 04.00) oscillates in antiphase with 5-FU (peak at 04.00) and most toxic when both oscillate in phase (peak of 5-FU at 16.00). Intermediate cytotoxicity is observed for other circadian patterns of 5-FU (peak at 10.00 or 22.00), for which the peak of 5-FU partially overlaps with the peak of S-phase cells. For the continuous infusion of 5-FU, the peak in S-phase cells necessarily occurs in the presence of a constant amount of 5-FU. Hence, the constant delivery pattern is nearly as toxic as the circadian pattern peaking at 16.00, a finding that also confirms the clinical results summarized in table 1 (Lévi *et al.* 1997, 2007b).

(c) Circadian administration of 5-FU: effect of variability of cell cycle phase durations

Another parameter that markedly influences the effects of 5-FU on the dynamics of the cell population is the variability of the durations of cell cycle phases. Shown in figure 4c as a function of variability V is the cytotoxic effect of the 5-FU circadian profile, with a peak at 04.00, in the presence of entrainment of the 22 hour cycle by the circadian clock. The cumulated cell kill increases when V rises from 0 to 20 per cent. For this circadian schedule of 5-FU, which is the least toxic to the cells (see above), we see that the better the synchronization between cells, the smaller is the number of cells killed. In the presence of entrainment, a larger increase occurs between $V \leq 10\%$ and $V \geq 15\%$ in the number of cells killed by the drug (Altinok *et al.* 2007a,b).

(d) Differential effect between cell populations

The goal of anti-cancer chronotherapeutics is to maximize the cytotoxic effect of medications on the tumour while protecting healthy tissues to achieve better tolerance. For reasons that are still unclear, the maximum 5-FU cytotoxicity to tumour cells occurs at the same time as the best tolerance to 5-FU, i.e. when the drug has minimal cytotoxicity to healthy tissues. In anti-cancer treatment, 5-FU is therefore administered according to a semi-sinusoidal pattern with peak delivery at 04.00 (Lévi *et al.* 1992, 1997). The question arises as to how the above results might be used to predict the differential effect of an anti-cancer drug such as 5-FU on normal and tumour cell populations. This issue relates to the

ways in which normal and tumour cells differ (Granda & Lévi 2002). Such differences may pertain to the characteristics of the cell cycle, e.g. duration of the cell cycle phases and their variability, or to entrainment of the cell cycle by the circadian clock.

For the sake of clarity, let us focus on the case of two cell populations, one that corresponds to tumour and the other to healthy tissue. Let us assume that the two cell populations have the same durations of the cell cycle phases, but differ in their variability, which is equal to 5 per cent (population 1 of healthy cells) or 15 per cent (population 2 of tumour cells). We will compare the effect of the circadian pattern of 5-FU delivery that peaks at 04.00, when the two populations are entrained by the circadian clock or when only the healthy population 1 is entrained (Altinok *et al.* 2007*a,b*). The two situations have been encountered in experimental tumour models (Granda *et al.* 2005; You *et al.* 2005).

The results of figure 5*a* indicate that, when the circadian delivery of 5-FU peaks at 04.00, the differential effect (iii) of the drug on the two cell populations is the largest when population 1 ($V=5\%$) is entrained by the circadian clock, while population 2 ($V=15\%$) is not entrained. An intermediate enhancement of cytotoxicity is observed, the difference marked (ii), when the two populations are entrained by the circadian clock. In both cases, the cytotoxic effect of 5-FU on tumour cells, characterized by the largest variability, is much stronger. If we compare two cell populations with the same variability, we observe that cytotoxicity can markedly increase in the population that is not entrained by the circadian clock, the difference marked (i). Thus, as previously noted, synchronization of the cells minimizes cytotoxic damage when the circadian 5-FU modulated delivery pattern peaks at 04.00. These differential effects disappear when 5-FU is administered as a constant infusion (figure 5*b*). Thus, only the chronoadministration of 5-FU with a peak at 04.00 gives the opportunity to potentially differentiate healthy and tumour cell populations. These results yield insights into the cellular bases of tolerance and efficacy in anti-cancer chronotherapy.

7. Identification of optimal circadian delivery schedules of oxaliplatin

In recently published articles, we have presented an alternative approach to determine optimal patterns of cancer chronotherapy delivery. Thus, we proposed a simplified PK–PD deterministic model to theoretically define an optimal infusion flow rule with the objective to hit a maximum of tumour cells under the constraint of strictly limiting the treatment toxicity to healthy cells, here jejunal mucosa villi cells (Clairambault *et al.* 2003; Basdevant *et al.* 2005; Clairambault 2007). This model takes into account the impact of circadian clocks on anti-tumour efficacy and unwanted toxic side effects on healthy cells, by assuming a cosine-like time modulation of the dose–response functions. These functions thus depend on both time and drug concentrations in the tissues. They correspond to an instantaneous death term for the tumour cell population and an additive reinforcement of a negative feedback. The latter represents an autoregulation function of epithelial cell proliferation, on the production of young cells from the jejunal crypts.

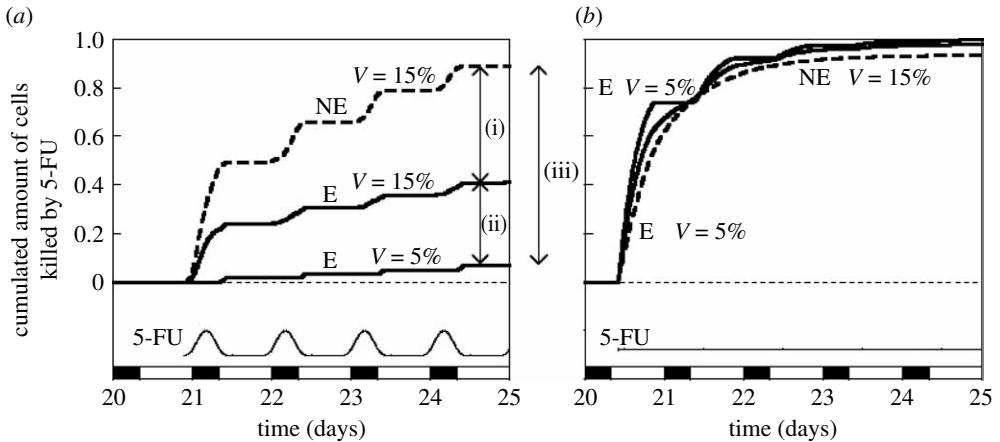


Figure 5. Possible sources of differential effect of 5-FU on normal and tumour cells. (a) Comparison of cumulative cell kill (in units of 10^4 cells) by circadian delivery of 5-FU with a peak at 04.00 for two cell populations differing by variability V and by the presence or absence of circadian entrainment. (i) The variability is 15% but one population is entrained (E) and the other not (NE). (ii) Both populations are entrained by the circadian clock but the variabilities differ. (iii) Here, the largest differential effect is observed when the first population is not entrained and has a variability of 15%, while the second population is entrained by the circadian clock and has a variability of 5%. The two cell populations have the same cell cycle duration of 22 hours in the absence of entrainment. (b) The differential effects of variability and entrainment observed in (a) disappear in the case of constant infusion of 5-FU.

The system of ordinary differential equations runs as follows. For first-order pharmacokinetic equations:

$$\frac{dP}{dt} = -\lambda P + \frac{i(t)}{V}, \quad (7.1)$$

$$\frac{dC}{dt} = -\mu C + \xi_C P, \quad (7.2)$$

$$\frac{dD}{dt} = -\nu D + \xi_D P. \quad (7.3)$$

Variables P , C and D stand for drug concentrations in the plasma, healthy tissue and tumour, respectively. Function i is the continuous drug flow function, instantaneous flow of an intravenous infusion in the plasma compartment, to be optimized, i.e. to be determined by its shape so as to give the best therapeutic results. For cell population dynamics:

$$\frac{dA}{dt} = Z - Z_{eq}, \quad (7.4)$$

$$\frac{dZ}{dt} = -[\alpha + f(C, t)]Z - \beta A + \gamma, \quad (7.5)$$

$$\frac{dB}{dt} = -\left[a \ln\left(\frac{B}{B_{max}}\right) - g(D, t) \right] B. \quad (7.6)$$

Variables A and Z represent villi cell population and flow from crypts, respectively, and B , following Gompertz growth without treatment, is the

tumour cell population (located immediately under the skin in the experimental settings used for model parameter identification in tumour-bearing mice), assumed to be without any contact with healthy jejunal cells. The parameters are chosen from literature data, such that without treatment the healthy cell population shows a stable equilibrium point ($A_{\text{eq}}, Z_{\text{eq}}$), a ‘sink’ in dynamical systems language, towards which the variables A and Z normally converge with damped oscillations following a perturbation, reflecting the stable, or homeostatic, nature of the jejunal mucosa population.

The *chronopharmacodynamic* functions f and g have, with different parameters, the same Hill-like form (where $\gamma \geq 1$ is the Hill coefficient), with 24 hour periodic modulation

$$f(X, t) = f_{\max} \cos^2\{\pi(t - \varphi)/24\} \frac{X^\gamma}{(K^\gamma + X^\gamma)}.$$

Parameter φ is the phase (modulo 24 hours) of the maximal action of the drug on the cell population under consideration. It differs by approximately 12 hours between the healthy and tumour cell populations, and in this model we take this phase difference as the basis of the difference in behaviours for the two cell populations. It will allow (as has been observed in the clinic) the drug infusion flow to be simultaneously maximally efficient on tumour cells and minimally toxic on healthy cells.

Within the frame of this simple ‘PK–chronoPD’ model, we were able to tackle several optimization problems for the drug infusion flow function i . The simplest consists in mimicking clinical habits that are usual in chronotherapeutic regimens, delivering the drug on the basis of a 24 hour periodicity of the infusion flow, during each of the four or five consecutive days of the chemotherapy course. It has been shown for instance (by trial and error) that the periodic infusion regimen i leading to the best anti-tumour efficacy was, among a small dictionary of simple shapes, a sinusoid-like regimen lasting 5 hours within the 24 hour span.

Another way of dealing with the optimization problem of chronotherapy is to get rid of the 24 hour periodicity constraint, knowing that drug-delivery pumps that are programmable on a time range of several weeks are now available in clinical settings, so that there are no longer necessary constraints linked to the habits of the health care personnel. We thus submitted the infusion profile function i to an optimization procedure with no constraint on it other than to be continuous, with the objective to kill as many tumour cells as possible, under the constraint to preserve the healthy tissue over a prescribed level, here a percentage of the equilibrium value for the villi cell population.

Now, one can give at least two meanings to the expression ‘as many tumour cells as possible’. It can mean ‘tend to bring the tumour cell population as close to zero as possible’ during a given one-shot chemotherapy course. We will call this option the ‘eradication strategy’, since it implicitly assumes that zero can be reached, otherwise when the treatment stops to allow the patient to recover from other non-represented toxicities, such as bone marrow hypoplasia, then there will be regrowth of the tumour cell population, possibly above the initial value if the tumour is very rapidly growing (as is the case for the murine tumour on which parameters were identified, Glasgow osteosarcoma). Such recovery times in the clinic classically last 16, 10 or even 5 days after 5-day, 4-day or 2-day chemotherapy courses, respectively. The objective function will then be

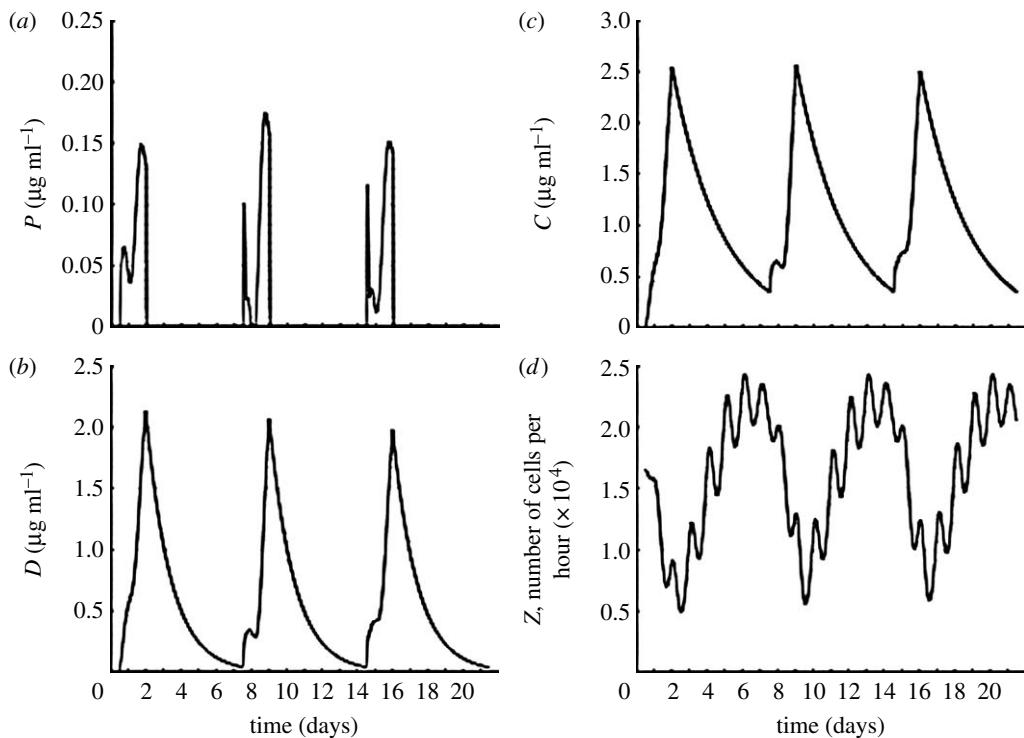


Figure 6. Computer simulation of the deterministic pharmacokinetic–pharmacodynamic model. Optimized profile of drug concentration in (a) the plasma compartment, with the simultaneously resulting concentrations in (b) the tumour and (c) the healthy jejunal mucosa tissue, and focus on the effect of the treatment on (d) the cell flow from crypts in the jejunum. One can see in (d) that, as soon as the drug concentration drops in the jejunal mucosa, due to intervals of recovery between chemotherapy courses, the crypt flow rises, with damped oscillations that would eventually make it converge to its equilibrium level without treatment (set here at 16 500 cells per hour for a mature villi cell population normalized to 1 000 000), to help the mucosa recover from the toxic insult. The constraint on the toxicity limit on villi cells (not shown) indirectly imposes a limit on the drug infusion flow so as to maintain a cell flow from crypts sufficient always to keep the villi cell population over a specified tunable level, here defined as 50% of its equilibrium value without treatment, i.e. 500 000 cells. (Adapted from Basdevant *et al.* 2005 and Clairambault 2007.)

to minimize the minimum of the tumour cell population, under the constraint to absolutely limit the healthy cell kill under a given level, to be determined by the clinician according to the patient's state of health.

But rather than minimizing the absolute minimum of the tumour cell population, it can be more realistic, though less ambitious, *to minimize its maximum over a given observation period* of chemotherapy followed by recovery, such periods being sequentially repeated. We call this option the ‘stabilization strategy’: it does not aim at eradication, but aims at *containment* of the tumour within limits that are compatible with the patient’s life, e.g. its weight remaining under 10 per cent of the initial weight at diagnosis. The objective function will then be to minimize the maximum of the tumour cell population in a given period of time, repetitive, since chemotherapy courses can be administered *a priori* (i.e. drug-resistance phenomena not being taken into account) without

any limit in time, under the same constraint as in the eradication case. In the example that is illustrated in figure 6, we assumed consecutive 2-day chemotherapy courses with 5 days of recovery after each course. These two strategies lead us to identify distinct optimal dynamic schedules of drug delivery (Basdevant *et al.* 2005; Clairambault 2007).

It is noteworthy that this model deals only with single-agent therapies, and this is one of its limitations. In particular, this is the reason why we are presently developing more elaborate models of cell proliferation, for healthy or tumour tissues. These new models take into account several physiological events in the cell cycle, which are targeted by anti-cancer drugs (Bekkal-Brikci *et al.* 2008; Clairambault 2008), so as to deal jointly with the circadian regulation of cell cycle determinants and therapeutic optimization of control flows for several drugs, such as 5-FU, oxaliplatin and irinotecan, which are used as triplets for treating colorectal cancer (Garufi *et al.* 2003; Falcone *et al.* 2007).

8. Discussion and perspectives

The CTS rhythmically controls many of the processes that are relevant for malignant processes and their treatments. While the molecular mechanisms of such controls are being uncovered, it is becoming increasingly clear that the cell division cycle and its related DNA sensing/repair and apoptosis/survival pathways closely interact with circadian clocks (Chen-Goodspeed & Lee 2007; Lévi *et al.* 2007a; Oklejewicz *et al.* 2008; Okyar & Lévi 2008). These dynamic interactions modify the pharmacologic effects of anti-cancer agents and result in temporal variations in therapeutic activity. While the CTS ensures the physiological adaptation to environmental 24 hour cycles and the coordination of cellular functions and their upstream molecular pathways throughout the 24 hours, the cell division cycle is responsible for maintaining the cellular composition of healthy tissues. Dysfunctions in these systems can, respectively, alter the main dimensions in quality of life and cause cancer (Mormont *et al.* 2000).

Chronotherapy takes into account the rhythmic systems whose interactions modify treatment activity (Lemmer 2007; Smolensky & Peppas 2007). Chronotherapeutic experiments in mice and clinical investigations in cancer patients have led to intuitive sinusoidal schedules of anti-cancer drug delivery in patients. Dedicated drug-delivery systems have been developed so as to allow for the chronomodulated delivery of anti-cancer agents without hospitalization constraints (Lévi *et al.* 2007b). Thus, a coordinated chronotherapeutic development has been implemented from mouse to cancer patients, which first identified the anti-tumour activity of oxaliplatin in combination with 5-FU-LV against colorectal cancer (Lévi *et al.* 1992) and then established the clinical relevance of circadian-based drug delivery in cancer patients through phase I, II and III clinical trials (Lévi 2001; Lévi *et al.* 2007b). However, gender, circadian physiology and clock gene or protein expressions in tumours were recently found to play a critical role in the success of a fixed chronotherapeutic schedule (Mormont *et al.* 2000; Giacchetti *et al.* 2006; Lévi *et al.* 2007b; Iacobelli *et al.* 2008). More specifically, the fixed chronotherapy schedule with peak delivery of 5-FU-LV at 04.00 and oxaliplatin at 16.00 was significantly more toxic and less effective in women when compared with men with metastatic colorectal cancer (Giacchetti *et al.* 2006; Lévi *et al.* 2007b). Indeed,

preclinical cancer chronotherapeutics had been established in male mice of the same strain with properly synchronized CTS. Taken together, the results indicated that increased clinical benefit should result from the adjustment of chronotherapeutic delivery of cancer treatments to the individual characteristics of the patient, including those of his or her CTS. Thus, an important current challenge for cancer chronotherapeutics is understanding the molecular and physiological mechanisms through which gender, age and lifestyle modify the dynamic crosstalk between the CTS, the cell division cycle and the relevant pharmacological pathways.

Mathematical modelling is beginning to provide some new insights into these issues, which will eventually lead to the personalized chronotherapeutics of cancer. In the meantime, the large body of experimental cancer chronotherapeutics can be used to greatly improve the tolerability and efficacy of anti-tumour medications, whether their delivery route is intravenous, intra-arterial or oral. We feel that biosimulation of the crosstalks between circadian clocks, cell cycle and pharmacological pathways should be integrated into translational chronotherapeutic research so as to offer optimal chronomodulated treatment delivery to each patient.

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NOTICE OF CORRECTION

The first paragraph of p. 3585 is now present in its correct form.

The equation on p. 3592 is now present in its correct form.

The first sentence of the second paragraph on p. 3595 is now present in its correct form.

A detailed erratum will appear at the end of volume 366.

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